Alkaloids: Chemical and Biological Perspectives

S. William Pelletier
Editor

Alkaloids: Chemical and Biological Perspectives

Volume 8

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Dedicated to
Derek Harold Richard Barton
(1918–)

In 1953, Barton realized that on theoretical grounds the formula of Pummerer's ketone, and hence the biosynthesis of morphine according to Robinson, must be wrong. He then developed (with T. Cohen, 1957) a rational theory for the biosynthesis of all phenolic alkaloids. Work in the greenhouse eventually confirmed almost all the predictions starting with morphine [in association with W. Steglich (1963) and partly with A.R. Battersby (1965)], alkaloids, sinomenine, *Amaryllidaceae* alkaloids, especially galantamine (G.W. Kirby, 1960–1963), proaporphine alkaloids like crotonosine (1963) and finally the *Erythrina* alkaloids (1966 and 1968) where the simplest phenolate radical coupling had to be replaced by a more complex one (1974). He was also the author of the first paper on conformation analysis (1950) which applies to all classes of molecules. In later life (1960–), he was mainly concerned with the discovery and invention of new chemical reactions for use in synthesis, including manipulation and synthesis of alkaloids.

Preface

Volume 8 of this series presents four timely reviews on alkaloids: Chapter 1 is a magnificent and monumental review of curare, "a group of dart and/or arrow poisons varying in composition and featuring muscle relaxation as their basic pharmacological action." The fascinating history of curare is recounted, beginning with early encounters by the Spanish Conquistadores through its use as arrow poisons by the forest tribes in hunting and warfare, its chemistry, ethnography, botany and pharmacology. A terminal section of this chapter treats the development of modern muscle relaxants. This chapter thus traces how curare—initially only a crude plant extract—has given rise to the widely used and very important neuromuscular blocking agents of today.

The precise role of plant secondary metabolites and their interactions with insect herbivores have been focal points for research by chemists, botanists and entomologists for many years. Alkaloids and their glycosides are frequently involved as feeding deterrents. Chapter 2 treats the relationships between the chemistry of alkaloids in host plants and the effects that these compounds may have on insect herbivores. Interestingly, an alkaloid produced by a plant may manifest different effects on different insects.

Members of the yohimbine family of alkaloids exhibit a wide range of medicinal properties. The diverse biological activity of these compounds and their intriguing structures have stimulated the interest of synthetic organic chemists. Several of these complex alkaloids, such as reserpine and yohimbine, present challenging synthetic targets. Chapter 3 reviews developments in the synthesis of yohimbine alkaloids over the past twelve years, focusing particularly on the approaches used to synthesize target molecules.

Chapter 4 summarizes available knowledge about the loline-type pyrrolizidine alkaloids. These alkaloids have been reported from only a few genera of grasses (Poaceae). Their occurrence in grasses is closely associated with the presence of endophytic fungi. The chapter treats isolation, characterization, synthesis and the biological activities of these alkaloids.

Each chapter in this volume has been reviewed by at least one expert in the field. Indexes for both subjects and organisms are provided.

The editor invites prospective contributors to write him about topics for review in future volumes in this series.

S. William Pelletier
Athens, Georgia
August 1991

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1
Curare

NORMAN G. BISSET

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Prologue

When, on Monday, 12th June 1514, the fleet carrying Pedrarias Dávila, the first Governor of the Tierra Firme (the mainland territories), arrived at Santa Marta on the northern coast of present-day Colombia, it met with a hostile reception and showers of arrows rained down upon the Spaniards when they attempted to land. Among the accompanying retinue were Martín Fernández de Enciso and Gonzalez Fernández de Oviedo y Valdés, both of whom later committed their impressions to paper.

In his *Suma de geografia* of 1519 (1), Fernández de Enciso wrote:

The people are war-like and fierce. [They have] bows and arrows that are slightly larger than darts and they smear them with a preparation [*yerva*] that is so poisonous that the man who escapes on being wounded [with it] is fortunate. One of the things with which they make the poison are apples [*mançanas*] which they have in this land and... the tree that yields them is like a pear tree; and after a man eats one of these [fruits], in the body it turns to worms that grow very fast and in eating the body kill it. I know that this is so, because I made an experiment in which I gave one [fruit] to a dog to eat. We found that after four hours, when we opened up the dog to see the effects of the *mançana*, it had turned to worms. If a man lies in the shade of one of these trees, after a time he starts to get a headache. If he stays too long, the face swells up and the eyesight is disturbed; and, if by chance, he goes to sleep underneath [the tree], afterwards he loses his sight. All this I have seen with my own eyes. (**A**—see the Notes at the end of the Prologue)

In the *Historia general y natural de las Indias*, first published along after the author's death, Fernández de Oviedo (2) told of the following incident: "... while at my side, one of my companions, Hernando de Arroyo,... received an arrow in the shin of one of his legs; and the wound was so small that in causing it the arrow fell to the ground. But the poison was such that he immediately fainted and it was seen to be mortal... on the third day he died maddened with pain [*rabiando*]."

He subsequently described the poison in the *Sumario de la natural y general historia de las Indias* (3) in the following terms:

... it is a marvel if a wounded man escapes—rather, he dies crazed with pain, devouring pieces of earth... and up to now the remedy against this poison has remained unknown, although many Christians [i.e. Spaniards] have died from it:... According to what several Indians have told me, the poison that they use they make from a kind of fragrant little apple [*mançanilla*] and certain large ants... and vipers and scorpions and other poisons which they mix with them; and it is as black as pitch. (**A**)

In the later *Historia general de las Indias* of 1535, Fernández de Oviedo (4) discussed the poison in even more extreme terms: "... the Carib bowmen... shoot with a poison that is so pestilential and excruciating as to be incurable: and men wounded with it die raving mad, in their frenzy biting their own hands and flesh from the extreme pain...."

Evidently, the poisons used by the Indians along the northern coasts of South America, especially at Santa Marta, made a profound and lasting impression on the Spaniards, and they are mentioned in other writings as well. The continuing and mounting fear of these poisoned arrows stands out clearly in the following extract from the *Historia de Santa Marta y Nuevo Reino de Granada*, which was written before 1575 (5):

The poison... reaches the heart within twenty-four hours, where its active principle acts with greater force, causing trembling and convulsions of the body and loss of reason in the victims, so that men say dreadful things and those dying doubt their faith. In the end they die in such a desperate state that the living are often induced to commit suicide rather than allow themselves to suffer such a fate.

The relevant passage by Sir Walter Ralegh in the narrative of his 1595 voyage to the Orinoco and along the coast as far as Santa Marta, *The discoverie of the large, rich, and bewtifvl Empyre of Gviana* (6), is every bit as graphic as the Spanish accounts:

... [the] *Aroras*... are very valiant, or rather desperate people, and haue the most strong poison on their arrowes, and most dangerous of all nations, of which poison I will speak somewhat being a digression not vnnecessary.

There was nothing whereof I was more curious, than to finde out the true remedies of these poisoned arrowes, for besides the mortalitie of the wound they make, the partie shot indureth the most insufferable torment in the world, and abideth a most vglie and lamentable death, sometimes dying starke mad, sometimes their bowels breaking out of their bellies, and are presently discolored, as blacke as pitch, and so vnsauery, as no man can endure to cure, or to attend them.... (**B**)

Ralegh did not accompany the next expedition, and the over-all commander on that occasion, Lawrence Keymis, noted in his report, *A Relation of the Second Voyage to Guiana. Perfourmed and written in the yeare 1596* (7), that the Ipaios:

... are but few, but verie cruell to their enemies. For they bind, and eat them aliue peecemeale. This torment is not comparable to the dedlie pains that commeth of

hurtes, or woundes, made by those arrowes that ar[e] inuenomed with the iuice of the hearbe *Wapototo*. These Indians because they eat those whom they kill, vse no poyson. (**C**)

Compare these foregoing descriptions with those that follow.

According to the *Historia de la Conquista, y Población de la Provincia de Venezuela*, written by Oviedo y Baños and published in 1723 (8), while exploring the Lake Maracaíbo region of western Venezuela in 1548 the Spaniards led by Alonso Pérez de Tolosa came across:

... near the entrance to these *llanos* a village of the Babures Indians, a friendly nation and little given to war. Their only instruments of war are blowguns [*cerbatanas*] from which, with a puff of breath, they discharge small darts tipped with a certain poison [*yerva*] having the very strange property that when a person is wounded he at once appears as if dead, deprived of his senses for two or three hours, which is the length of time [the Indians] need to escape from danger. And after this time the wounded person returns to his senses, without any other injury or hurt. (**D**)

In an account of the Indians of Lake Maracaíbo, dating from 1573/75, which included a discussion of their offensive and defensive weapons, Diego Sánchez Sotomayor (9) wrote: "These Indians have little poison [*yerua*]; and it does not cause dreadful suffering, even though it is indeed mortal." (**E**)

Keymis' *A Relation of the Second Voyage to Guiana*, already quoted above, probably has the first mention of the word that we know today as *curare*. He included the word *ourari* in a list of "Names of poysoned hearbes", but made no mention of a connection with arrow or dart poison. (**F**)

The barefoot Carmelite monk Vázquez de Espinosa wrote a *Compendio y descripción de las indias occidentales*. Though permission for it to be printed was granted in 1629, the account did not see the light of day until the present century (10). He noted that in the Río Caqueta/Putumayo region of Colombia: "... their arms are lances, and darts made from palms and having points of fishbone; and on them they put poison [*yerba*] which is not mortal but only intoxicates [*embriaga*] the wounded for 24 hours...." (**G**)

As the foregoing passages clearly demonstrate, in the northern part of South America the Spaniards experienced two entirely different types of poison:

(1) A war poison, used by the inhabitants of the northern coasts with the bow and arrow, that caused terrible suffering before death intervened usually after several days.
(2) A poison, used by tribes further inland and mostly put on blowgun darts, that seems to have caused some kind of paralysis.

Both the toxicologist Lewin (11) and the ethnologist Nordenskiöld (12) understood the difference, and the medical ethnographer Vellard (13, 14: pp. 15–34), in particular, has clearly discriminated between the two types of poison. The distinction is emphasized here, for in several fairly recent publications (15–19) it is still not fully appreciated. It is the second group of poisons,

the paralysing poisons, which were probably curares in the modern sense, that concern us here.

Notes

(A) The *mançanas* (apples) or *mançanillas* (little apples), from which the poison was made, came from the ill-reputed machineel tree, *Hippomane mancinella* L. (Euphorbiaceae). Especially noteworthy is the attempt by Enciso to determine the effects of the poison—it is probably one of the earliest experiments reported in the literature that may properly be termed pharmacological. The unpleasant nature of the plant is due to the presence in its latex of a complex mixture of potent pro-inflammatory and tumor-promoting esters of various diterpene alcohols (20, 21).

Bryn Thomas (22) suggested that the "venomous apples" of Oviedo might rather have been the fruits of a *Strychnos* species. This is very unlikely, for, as he himself pointed out, curare is made from bark and not fruits and Oviedo described a seaside tree and not a jungle liane; moreover, the poison Oviedo described was being used by coastal tribes. There is no need to suppose that the Spaniards were such inaccurate observers. Being shot by one of these poisoned arrows would be equivalent to an intraparenteral injection of *Hippomane* latex and the consequences could no doubt be serious enough to account for some of the less extreme descriptions of the effects. See also Note **B**.

(B) It may be noted that the passage in Ralegh's narrative is a good description of an advanced case of clostridial myonecrosis (gas gangrene)—infection by anaerobic bacteria like *Clostridium perfringens* and/or *C. novyi* and/or *C. septicum*—resulting from the trauma of a deep punctured wound (23, 24). Given the general lack of hygiene of the period, it is hardly surprising that such wounds could become dangerously infected, the more so as the Indians often re-used arrows without cleaning them.

(C) The intention seems to be to emphasize the frightful nature of the poison as compared with merely being eaten "aliue peecemeale"! Unfortunately, the *wapototo* plant that yielded this poison has not been identified—it might have been *Hippomane mancinella* or *Hura crepitans* or some related species.

(D) Pedro Simon (1627) and Fernández de Piedrahita (1688) mentioned the weapons of the Babures (more correctly Bobures). But Oviedo y Baños has the best account and he recognized more clearly than his predecessors the unusual nature of the poison, so unlike the devastating products of the coastal tribes. Vellard (14: p. 47) has pointed out that the rapid, almost instantaneous effect, preventing the wounded person from moving or speaking, and the subsequent recovery without leaving any traces accord well with what is known about curare. The blowgun is rarely mentioned in the early Spanish accounts and it has not often been used in warfare (12, 25).

(E) Breton (26) at this point completely misunderstood the Spanish text

and translated the crucial words *no es rrauioza* as "it does not cause rabies." Here, the word *rrauioza* (*rabiosa*) has nothing to do with rabies—it refers instead to the terrible suffering caused by the arrow poisons of the coastal tribes, alluded to above.

(F) The story that Sir Walter Ralegh was the first to bring back curare to Europe is still being told, even though McIntyre (27) pointed out over 40 years ago that it is not true. A careful reading of Ralegh's own writings on the voyages to Guiana and the Orinoco yields no evidence to support the idea. True, he was interested in the arrow poisons of the tribes in Guiana, but, as already seen, what he came across were the fearsome war poisons of the coastal tribes and not the hunting poisons of the forest tribes.

Cayley (28) in his biography of Ralegh reproduced Keymis' account of the second voyage and placed the accompanying table of rivers, towns, tribes, etc., in which the word *ourari* is to be found, in an Appendix. This may have led to the mistaken belief that Ralegh had come across the word or had brought back the actual poison. Certainly, Humboldt (29) was already confused. When he published his own discoveries about the preparation of curare, he included a mangled reference to Cayley's biography and stated that: "Déjà, vers la fin du seizième siècle, Ralegh avoit entendu prononcer le nom d'*Urari* comme celui d'une substance végétale avec laquelle on empoisonne les flèches." (As early as the end of the 16th century, Ralegh had heard the name *ourari* as that of a plant substance with which arrows are poisoned.)

In due course, when writing about "woorara", Münter (30) stated baldly: "Der Erste, welcher ein Pfeilgift unter dem Namen Ourari nach Europa brachte, war der Admiral *Walter Raleigh* selbst, der das El Dorado suchend, im Jahre 1595 auf seiner phantastischen Expedition, den Orenoko herauf, von den Amazonen vergiftete Pfeile mitbrachte...." (The first to bring back an arrow poison named *ourari* to Europe was the Admiral Walter Ralegh himself, who, when searching for the El Dorado, in 1595 during his fantastic expedition up the Orinoco brought poisoned arrows from the Amazons....)

Münter here seems to be mixing up the Orinoco and Amazon rivers, but, as is evident from what has been written above, Keymis obtained the word *ourari* during one of the Guiana/Orinoco voyages which Ralegh did ***not*** accompany. Indeed, while the second voyage to Guiana was taking place, Ralegh was heavily involved, together with the Earl of Essex, in organizing and carrying out the raid on Cadiz in June and July of the same year, 1596, which was in retaliation for the defeat of Sir Richard Grenville's ship *Revenge* in 1591.

(G) In his English version, Clark (31) has "paralyzing". Though perhaps the most appropriate rendering, it strictly goes further than the Spanish text allows, for "*embriaga*", the word used, means no more than "drunk" or "intoxicated."

1.1. Introduction

Curare! Evocative, on the one hand, of South American Indian hunters moving stealthily through the jungle with blowgun and poisoned dart and, on the other hand, of the patient on the operating table with muscles relaxed and the surgeon poised, scalpel in hand, ready to make the first incision or of the mysterious South American dart poison used by the murderer in more than one detective story—together, the epitome of one of the most fascinating and interesting of poisons and a successful example of the transformation of a plant product of ethnic origin into an essential aid in the practice of modern medicine.

To outline how this has come about, in other words, to examine the rôle of curare in the development of modern muscle relaxants, is the aim of the present review. But it is also concerned with the plants that are the sources of activity and with the substances that are their active principles. Certain aspects of the pharmacology, including the mode of action, of these compounds and the development of the muscle relaxants derived from them are also outlined.

Having seen in the Prologue that the Spaniards in South America came across two dart/arrow poisons with entirely different effects, it is desirable at this point to consider what we nowadays understand by the term **curare**:

(a) To the anthropologist or the ethnographer it is a group of dart/arrow poisons prepared by the Indians of tropical South America, the characteristic feature of which is to bring about paralysis.
(b) To the pharmacologist it is a dart/arrow poison acting at the neuromuscular junction to bring about relaxation or paralysis of the musculature through blockade by a nondepolarizing, competitive mechanism, the effects of which can be reversed by small doses of neostigmine (or other anticholinesterase drugs).
(c) To the anaesthetist it is often simply the muscle-relaxant alkaloid tubocurarine.

What, or which, definition should one choose? This, as the three foregoing definitions imply, may be made to depend on the purpose. Bauer (32, 33), for example, who has carried out a lengthy series of chromatographic and pharmacological investigations on museum specimens of curare, accepted a pharmacologically based definition (cf. (b), above), one that is implicit in the currently used verb "to curarize," with its derived noun "curarization" and adjective "curarizing." It is the one adopted here[1] and it should be abundantly clear that the poisons produced by the inhabitants of Hispaniola and the

[1] Schultes and Raffauf (34) in their recent book dealing with the medicinal and toxic plants of Northwest Amazonia use the word *curare* in a broad sense, and they include poisons made solely from species of *Lepianthes*, *Piper*, or *Schoenobiblus*. None of these three genera is known to have representatives with muscle-relaxant activity and in the present context the poisons derived from them are not considered to be true curares.

northern coasts of South America, the devastating effects of which are described in the passages quoted in the first part of the Prologue, cannot have been due to a type of poison so defined.

Curare, then, is a group of dart and arrow poisons varying in composition and featuring muscle relaxation as their basic pharmacological action. Considered thus, it can no longer be looked upon as a purely South American product, for poisons having the same effects, and active constituents, are now known to have been made in Central Africa and South-East Asia. It also entails consideration of another South American arrow poison, *guachamacá*, a highly reputed, though rather mysterious, Venezuelan poison that indirectly has played a small, but significant rôle in the story of modern muscle relaxants. See further Section 1.2.1.2.

The literature on curare and associated subjects is vast. A bibliography (35) prepared for the International Symposium on Curare and Curare-like Agents held in Rio de Janeiro in 1957 (36) contained almost 3000 references and was known to be far from complete. Many hundreds, if not thousands, of papers have appeared since then. The choice of material for discussion must therefore be highly selective. In addition to the Symposium Proceedings just mentioned, there is a number of general and historical accounts which will help to orient the interested reader, among them: Perrot and Vogt (37), Lewin (11), McIntyre (27, 38), Granier-Doyeux (39), Biocca (40), Bryn Thomas (22), Vellard (14), Curare-Symposion (41), Burnap and Little (42), and Bisset (43).

Aspects of the ethnographic background are dealt with briefly in Section 1.2, in an attempt to set the poison in its original context and to lay a basis for the material discussed in the subsequent sections. Botanical studies on the plant sources are considered in Section 1.3. Recent chemical work on the active principles is outlined in Section 1.4. Some pharmacological points relating to the poison and its alkaloids, as well as derived synthetic products, are touched on in Section 1.5, and the story is brought to a close with a look at the development of modern muscle relaxants in Section 1.6. This chapter thus traces how curare—to begin with, no more than a crude plant extract—has given rise to the widely used neuromuscular blocking agents of today.

1.2. Ethnographic Background

1.2.1. Geographical Distribution

1.2.1.1. South America

The region throughout which curare has been used is a wide, more or less semicircular, zone stretching from French Guiana to the Mato Grosso and covering the northern and western parts of the Amazon basin, the middle and upper parts of the Orinoco basin, the Montaña of Ecuador and Peru, and northeastern Bolivia and adjacent parts of the central Brazilian plateau

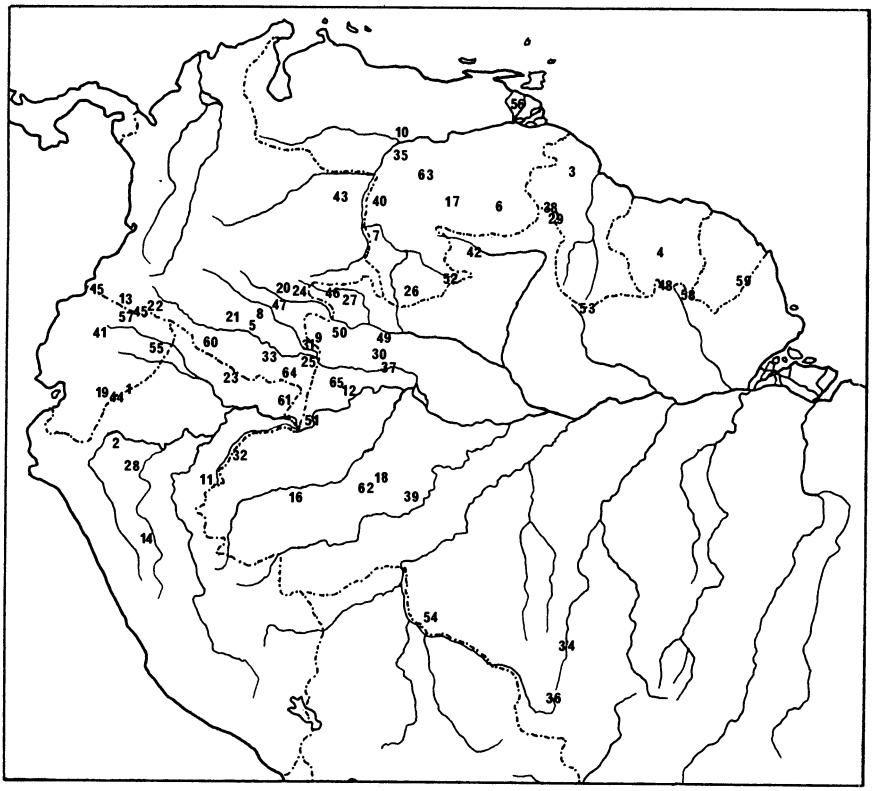

FIGURE 1.1. Approximate locations of tribes mentioned in the text. See also Figure 1.2. (1) Achuara. (2) Aguaruna. (3) Akawai. (4) Akoerio (Wama). (5) Andoke. (6) Arekuná. (7) Baníwa. (8) Barasana. (9) Baru-Makú. (10) Caberre. (11) Cahuapanan Balzacho. (12) Caixána (Cauichana, Kauichana, Kawichanes). (13) Canelo Quechua (Ketchwa). (14) Cholón. (15) Coaiquer. (16) Dení. (17) Hóti (Waruwádu, Yowana). (18) Jarauára (Jarawara). (19) Jívaro (Jíbaro). (20) Karapaná. (21) Karijóna. (22) Kofán. (23) Koto (Orejone). (24) Kubeo. (25) Kueretú. (26) Kunipúsana. (27) Kuripako (Pakú-Tapuya). (28) Lamista Quechua (Lama, Chazuta, Motilón). (29) Macushi. (30) Makú. (31) Makúna. (32) Mayoruna. (33) Miranyá (Miranha). (34) Nambikwára. (35) Panáre (E'ñiepá). (36) Paresí. (37) Pasé. (38) Patamona. (39) Paumarí. (40) Piaróa (De'áruwa). (41) Quijo. (42) Sanama(-Waiká). (43) Sáliva. (44) Shuara. (45) Siona. (46) Siusi. (47) Taiwano. (48) Tirió (Tiriyo). (49) Tukano. (50) Tukána. (51) Tikuna (Ticuna). (52) Waiká (Yanomamo). (53) Wai-wai. (54) Wanyam (Huanyam). (55) Waorani (Auca). (56) Waraú. (57) Warekéna (Arekena). (58) Wayána (Oajana, Oayana, Roucouyenne). (59) Wayapí. (60) Witóto (Huitoto). (61) Yagua (Java, Peba). (62) Yamamadí. (63) Yekuaná (Mayongong, Makiritáre). (64) Yukúna (Yacuna). (65) Yurí.

(Fig. 1.1). The tribes involved are mostly hunter–gatherers in the tropical rain-forest area; some marginal tribes such as the Guaharibo on the Upper Orinoco and the Nambikwára and Paresí on the central Brazilian plateau are also included. Tables 1.1 and 1.2 record the species of Loganiaceae and Menispermaceae that have been documented, usually by means of herbarium specimens, as components of curare; the localities where used and some of the tribes that are known to have employed the plants are also indicated.

The tribes using curare have been surveyed in a number of earlier publications, most extensively by Lewin (11), but also by Perrot and Vogt (37) and Vellard (14). Although now often out-of-date, dispersed throughout the volumes of the *Handbook of South American Indians* (44) is a fund of information about the various groups of tribes who are known to have used, and still do use, curare. The account by Ribeiro and de Melo Carvalho (45) is in the nature of a mid-century status report on the tribes that were still making curare during the first half of the twentieth century. An important paper by Schultes and Raffauf (46), embodying the findings of long personal experience by the doyen of ethnobotanists, surveys the use of curare among the tribes of Northwest Amazonia. The following paragraphs include notes on other recent studies, but do not aim at complete coverage, and they are followed by brief accounts of some curarizing poisons from outside South America.

In the Guyana Highlands, it was the **Tirió**, living along the Upper Rio Parú and Rio Jarí, south of the Sierra de Tumucumaque, who prepared curare (47, 48) and from them the **Wayána** (Oajana, Roucouyenne) of French Guiana and Surinam learned how to make it (49, cf. 50). The **Akoerio** (Wama) of southeastern Surinam are a group of ca. 40–50 people, possibly belonging to the Tirió, with whom contact was made in the late 1960s. Some of them are still nomads and they put curare on their arrow points (51). Various tribes in the region between the Guyana Highlands and the Orinoco use the poison. The **Macushi**, who live in southern Guyana and the adjacent part of Brazil, gave up making curare over 50 years ago, and nowadays it is from the c.7000 strong **Piaróa** (De'áruwa), who live near the middle reaches of the Orinoco, on the Venezuelan side, that most of the supply appears to originate (52). Trade in the poison is discussed in Section 1.2.5.1.

In Roraima (Brazil), curare is still being widely made and used. Among the tribes able to prepare it are the **Sanama**, a subgroup of the Waiká (cf. 53), and **Yekuaná** (Mayongong, Makiritáre), but the latter prefer the stronger Piaróa poison (201, 202, 205).

The **Hóti** (Waruwádu, Yowana) comprise between 300 and 400 people located in the mountainous border region between the Venezuelan States of Bolívar and Amazonas. Their nearest neighbours are the Panáre (E'ñiepá) to the northwest and north, the Piaróa to the southwest and south, and the Makiritáre (Yekuaná) to the south and east. The limited information available about the tribe has been gained mostly during the last 20 years (54). The southern Hóti produce their own curare, part of which is destined for trade,

while the northern Hóti obtain theirs from the **Panáre**—a situation perhaps reflecting ecological (floristic) and possibly also social differences.

In the frontier region between Brazil and Venezuela are the **Waiká**, better known as the Yanomamo. In the past, feuding has been a way of life among the various subgroups. The preparation of their curare has been recorded by several observers (55–57) and is described briefly in Section 1.2.4.

The Kuripako (Pakú-Tapuya, Curipaco), a small group of just over 300 people, and the Warakéna (Arekena), who are situated between the upper reaches of the Orinoco and the Río Guiania on the border between Venezuela and Colombia (33), still obtain curare from the **Baníwa** who live to their south.

Field work in the northwest Amazon region (34, 46, 58–61) shows that the **Kofán, Witóto**, and many other smallish tribes of Colombia, Ecuador, and Peru (Tables 1.1 and 1.2) make effective hunting poisons. The Kofán, however, may also get curare from the **Achuara** (33). Cf. Section 1.2.5.2. There is recent information about the poison produced by the c.700-strong **Waorani** (Auca) of eastern Ecuador (33, 62, 63).

The **Tikuna** live on the Upper Rio Solimões and between the Rio Içá (Putumayo) and the Rio Javarí, and it was with a sample of their curare, *ticunas*, that the first experiments in Europe were carried out in the eighteenth century (64, 65). Now, their once eagerly sought after poison has been displaced by that of the **Lamista Quechua** (Lama) of the middle Río Huallaga in Peru. There are, nevertheless, Tikuna medicine men on the Río Loretoyacu (near Leticia, Colombia) who still prepare curare.

The plants used by the **Paumarí, Yamamadí, Jarauára** (Jarawara), and **Dení** for making curare have also been investigated. These tribes live in the region of the Rio Purús, a major tributary of the Amazon in Brazil (66). See further Table 1.1 and Section 1.2.3.1.

Groups living further to the south also produced arrow poisons. Thus, in Rondônia, near where the Rio Guaporé (Iténez) and Río Mamoré join, the **Wanyam** (Huanyam) made a curare, possibly through a skill acquired from the Palmella who may have fled from Guiana in post-Conquest times (12, 67). It is not clear how far the use of curare extended among the tribes of eastern Bolivia (12, 68). Of the tribes located in the Mato Grosso savanna, the **Paresí** (69–71) and the **Nambikwára** (12, 14: pp. 89–104) are known to have prepared the poison, but neither the Paresí nor some of the Nambikwára groups do so any longer (71, 72). See also Section 1.2.4.

1.2.1.2. South America—*Guachamacá*

Another South American poison that has played a small but significant part in the development of present-day muscle relaxants is *guachamacá*. Most of what is known about this poison, which has had the reputation of being particularly deadly, has come from different parts of Venezuela (73, 74). There is, however, also a report by Crevaux (47), based on hearsay information that he obtained in 1881, to the effect that **Sáliva** Indians located on a tributary of

TABLE 1.1. *Strychnos* species used in the preparation of curare.

Species	Locality	Tribe	References
Section **Strychnos**			
6. *S. rondeletioides*	Colombia: Vaupés	?	89, cf. 34, 46; US[a]
	Venezuela: Bolívar, Amazonas		89, 90; US
	Brazil: Amazonas	Miránya,[b] Paumarí	91, 92; US, K, cf. 66
10. *S. brachiata*	Colombia: Putumayo	Kofán	58, 61, 89, 93; US
19. *S. toxifera*	Panama	?	94
	Colombia: Vichada		93
	Venezuela:	Kunipúsana, Piaróa	95, 96, cf. 97; K, P
	Bolívar	Panáre	98
	Colombia/Ecuador: Oriente	Kofán, etc.	34, 46
	Ecuador: Pastaza	Canelo	99, 100; US
	Guyana: Essequibo	Macushi	101; K, IAN
	French Guiana	Wayapí, Tiriyo, Wayána	50
	Brazil: Amazonas	Tikuna, Miránya	92, 93, 99, 102, 103; K
20. *S. tomentosa*	Guyana: Essequibo		K
	French Guiana	Wayapí, Tiriyo, Wayána	50
	Brazil: Roraima		104; K
21. *S.* cf. *diaboli*	Guyana: Essequibo	Patamona	K[c]
22. *S. javariensis*	Colombia: Putumayo	Kofán	61, 105, 106
	Peru/Brazil: Amazonas	Yagua (Java)	99, 102, 107
23. *S. sandwithiana*	Brazil: Amazonas		108; 109; K, RB
24. *S. jobertiana*	Colombia: Putumayo	Kofán	58, 61, 93; K
	Caquetá	Miránya, Kueretú	47, 110; [FP]
	Vichada	Piaróa	47
	Amazonas	Tikuna	34, 46
	Ecuador: Pastaza	Canelo	89, 99, 100
	Peru/Brazil: Amazonas	Yagua	99, 102
		Caixána (Kawichanes)	112–114

(*cont.*)

TABLE 1.1. (*cont.*)

Species	Locality	Tribe	References
27. *S. amazonica*	Colombia: Putumayo	Kofán	34, 46
	Amazonas	Andoke[d]	34, 46
28. *S. solimoesana*	Brazil: Amazonas	Caixána (Cauichana)[e]	108; K, IAN, R, RB
		Jarauára	66; US
		Yamamadí	66, 115
31. *S. peckii*	Colombia: Guiania	Kuripako	34, 46
	Putumayo	Kofán	58, 61, 89, 93
	Amazonas	Karapaná	61, 89
	Ecuador: Napo	Kofán	105
	Pastaza	Canelo	89, 99, 100
	Brazil: Amazonas	Tikuna	89, 99, 102, 108
32. *S. erichsonii*	Colombia: Vaupés	Makú, Makúna	34, 46, 61, 116
	Putumayo	Kofán	93, 106
	Amazonas	Karijóna, Tikuna	34, 46
	Guyana: Essequibo	Patamona	K, MG
	Surinam		U
35. *S. bredemeyeri*	Guyana: Essequibo	Macushi, Arekuná	89, 105, 112, 117, 118
	Brazil: Roraima	Mayongong, Sanama	115; US, K
S. mitscherlichii			
36a. var. *mitscherlichii*	Colombia/Brazil	Tikuna	34, 46
	Colombia: Putumayo	Siona	61, 89, 105
	Ecuador: Pastaza	Canelo	89, 99, 100
	Guyana: Essequibo	Akawai, Arekuná	117, 119; K
36b. var. *pubescentior*	Colombia: Vaupés	Kubeo	34, 46
	Amazonas	Makúna	89, 104; US
37. *S. solerederi*	Colombia: Amazonas	Yukúna, Tikuna (?)	34, 46
	Brazil: Amazonas	Caixána (Cauichana)	91, 99, 101; US, IAN, RB
38. *S. darienensis*	Ecuador: Napo	Kofán	131

Section **Rouhamon**			
39. *S. guianensis*	Colombia: Putumayo	Kofán, Siona	61, 89, 106; US
	Amazonas	Tikuna	34, 46
	Venezuela: Bolívar	Piaróa	89
	Guyana: Essequibo	Wai-Wai	117, 120, 121; US, K, P
	Surinam		Cf. 99, 122
	French Guiana	Wayapí, Tiriyo, Wayána	50
	Ecuador: Pastaza	Canelo	99, 100
	Brazil: Roraima	Mayongong	101, 106; US, K, cf. RB, K
	Amazonas		89, 123
	Mato Grosso	Nambikwára	69, 70, 105; R
40. *S. glabra*	French Guiana	Wayapí, Tiriyo, Wayána	50
	Brazil: Amazonas	Baníwa	101, 107; K, U
	Pará	Tiriyo (Tirió), Wayána	47, 114, 124, 132; [FP]
41. *S. subcordata*	Colombia: Putumayo	Kofán	61, 89, 106
	Brazil: Amazonas	Caixána	89, 99, 113, 114; cf. US
43. *S. panurensis*	Colombia: Vaupés/Amazonas	Kubeo	34, 46, 61
	Venezuela		89, 90, 99; P
47. *S. cogens*	Colombia: Amazonas	Tikuna (Tukuna)	61, cf. 34, 46
	Venezuela: Bolívar	Panáre	US, K
	Guyana: Essequibo		K
	Brazil: Amazonas	Yamamadí	US, MG
48. *S. melinoniana*	Guyana: Essequibo	Waraú	89; K[f]
	French Guiana (?)		125[g]
Section **Breviflorae**			
50. *S. castelnaeana*	Peru: Loreto	Yagua (Peba)	99, cf. 14, 46; [FP], P, US
		Tikuna (Ticuna)	P
	Peru/Brazil: Amazonas	Yagua (Java)	102
	Brazil: Amazonas	Yagua	105; M, P
		Tikuna (Ticuna)[h]	105, 126, 127; K, M, IAN, R
		Miranha	90
		Orejone	105; M
51. *S. progeliana*	Brazil: Amazonas	Yurí	105, 128–130; M

(cont.)

TABLE 1.1 (*cont.*)

[a] [FP] = Herbarium, Faculté de Pharmacie, Paris; **IAN** = Herbário, Centro de Pesquisa Agropecuária do Trópica Úmido, EMBRAPA, Belém; **INPA** = Herbário, Instituto Nacional de Pesquisas da Amazônia, Manaus; **K** = The Herbarium, Royal Botanic Gardens, Kew; **M** = Herbarium, Botanische Staatssammlung, Munich; **MG** = Herbário, Museu Paraense Emilio Goeldi, Belém; **P** = Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie, Paris; **R** = Herbário, Departamento de Botânico, Museu Nacional, Rio de Janeiro; **RB** = Herbário, Jardim Botânico do Rio de Janeiro, Rio de Janeiro; **U** = Institute of Systematic Botany, University of Utrecht, Utrecht; **US** = United States National Herbarium, Smithsonian Institution, Washington.

[b] Barbosa Rodrigues [92] also stated that the Miránya Indians used *S. parviflora* Spruce ex Benth., a species not otherwise recorded as a curare plant.

[c] The note on the herbarium sheet, *Altson 487*, collected 1926, merely says: "*urali* of the Patamona".

[d] Several other species which have not been identified are also used.

[e] Barbosa Rodrigues [103] indicated that the Cauichana employed the three species *S. kauichana*, *S. lethalis* (supposedly the strongest component—hence the name), and *S. tonantinensis* in their curare. No specimens are known to be extant and their identities cannot be established [cf. 108]. Material thought to represent *S. lethalis* and examined chemically by de Berrêdo-Carneiro [133, 134] is now known to have been of *S. solimoesana* [101, 109]. At the time of Barbosa Rodrigues' visit, if *S. lethalis* was not available the Cauichana Indians used *S. solerederi (mitscherlichii)* instead. Ducke [101] confirmed this and noted that *S. sandwithiana (diaboli)* was also utilized.

[f] The annotation to this collection, *Jenman (Im Thurn) 5198*, obtained in 1888, is simply: "Warrou's ourali".

[g] The specimen, *Mélinon s.n.*, collected about 1880, has no annotation; when Baillon [125] described it as a new species, he suggested that it was probably a curare plant. There has been no confirmation of this.

[h] Besides the species mentioned in the table, Schultes and Raffauf [34, 46], following indications by Ducke [91], suggest that the Tikuna may also have used *S. diaboli*, *S. duckei* Krukoff et Monachino, *S. peckii*, *S. poeppigii* Progel (*S. longisepala* Krukoff), *S. sandwithiana*, and *S. solimoesana*.

TABLE 1.2. Menispermaceae used in the preparation of curare.

Species	Locality	Tribe	References
Chondrodendron			
C. tomentosum	Colombia: Putumayo	Siona	34, 46, 135
	Ecuador: Napo, Pastaza, Morona Santiago	Canelo Quechua (Ketchwa), Kofán, Quijo, Shuara	100, 131
	Peru: Loreto	Lamista Quechua (Lama,	34, 46, 135, 136
	San Martín	Chazuta)	135; K,[a] INPA, R
Curarea			
C. toxicofera	Colombia: Amazonas	Witóto	58, 135
	Vaupés	Karijóna	34, 46, 61, 116, 135, 137, 138, 140
	Peru: Loreto	Yagua, Koto (Orejone)	135, 139, 140
	Brazil: Amazonas	Yagua (Peba), Makú	135, 140
		Jarauára	66, 141; US, K, INPA
		Yamamadí	66; K, INPA
C. candicans	Guyana: Essequibo	Waraú	100, 135, 139
	Brazil: Amazonas	Tikuna	Cf. 139
C. tecunarum	Colombia: Vaupés	Bara-Makú[b,c]	34, 46, 61, 116, 137, 138
	Amazonas	Witóto	142, cf. 143
	Putumayo	Kofán[b,d]	34, 46, 58, 61, 135, 140, 144
	Ecuador: Carchi, Napo, Pastaza, Morona Santiago, El Oriente	Coaiquer, Kafán, Quijo, Canelo, Waorani, Achuara, Shuara	34, 46, 62, 63, 100, 131, 135, 140, 145, 146
	Brazil: Amazonas	Tikuna	102, 135, 139, 140, 142

(*cont.*)

TABLE 1.2 (*cont.*)

Species	Locality	Tribe	References
Scladotenla			
S. peruviana	Peru: Amazonas		146
S. toxifera	Colombia: Amazonas	Witóto	147
	Ecuador: Napo, Pastaza, Morona Santiago	Coaiquer, Kofán, Quijo, Canelo, Shuara	100, 131, 135, 146
	Peru: San Martin		135; K
Abuta			
A. grandifolia	Colombia: Putumayo	Siona	34, 46
	Amazonas	Andoke, Karijóna, Makúna	34, 46
A. grisebachii	Brazil: Roraima	(Waiká-)Sanama	146, 148; INPA
	Amazonas	Tikuna (?),[e] Tukána	34, 146
A. imene	Colombia: Vaupés	Taiwano, Makúna[b,f]	34, 46, 146
	Brazil: Amazonas	Yurí	129, 135, 139
A. pahni	Peru: Loreto	Bora	34
	Brazil: Amazonas	Yurí	140, 142; cf. M, MG
A. rufescens	Colombia: Putumayo	Kofán	60, cf. 135, 144, 149
(A. splendida)	Amazonas	Yukúna (Yacuna), Tanimuka	34, 116, 140
	Ecuador: Napo	Kofán	131, 135, 140
	Peru	Shuara-Achuara	131
	Brazil: Roraima	(Waiká-)Sanama	148, 149; INPA
	Amazonas	Yurí, Tukano, Yamamadí	139, cf. 46, 140; INPA
A. sandwithiana	Brazil: Amazonas	Jarauára (Jarawara)	148; INPA
A. species	Ecuador: Carchi	Coaiquer	131
A. vaupesensis	Colombia: Vaupés	Makú, Barasana[b,g]	34, 46, 60
Anomospermum			
A. chloranthum	Colombia: Putumayo	Siona	34, cf. 140
A. grandifolium	Ecuador: Pastaza		140, 146
A. reticulatum			
subsp. *reticulatum*	Colombia: Amazonas	Tikuna	60
	Peru		34, 46
A. species	Brazil: Amazonas	Waiká (Yanomamo)	57

Orthomene			
O. schomburgkii	Colombia: Putumayo	Kofán	34, 46
	Amazonas	Tikuna	34, 46
Telitoxicum			
T. duckei	Colombia: Vaupés	Barasana[b,h]	60
T. minutiflorum	Colombia: Vaupés/Amazonas, Río Apaporis		34, 46
	Peru	Shuara-Achuara	131
	Brazil: Amazonas	Tikuna	102, 135, 139
T. peruvianum	Colombia: Vaupés	Makúna	116, 137
	Peru: Loreto	Witóto	135; K
Cissampelos			
C. andromorpha	Colombia: Amazonas	Karijóna, Makúna	34, 46
C. fasciculata	Colombia: Vaupés	Taiwano	34, 46
C. ovalifolia	Guyana: Essequibo	Macushi	43; K
C. pareira	Ecuador: Napo, Pastaza, Morona Santiago	Coaiquer, Kofán, Quijo, Canelo, Shuara	34, 46, 100, 131, 135, 146

[a] See Table 1.1 for details of the herbarium abbreviations.

[b] The fact that in the course of time Krukoff changed the identifications of certain collections bearing annotations relating to their use in curare has led to some confusion in the ethnobotanical literature. Since, in the present chapter, the botany of the curare plants is to a large extent based on Krukoff's work, for the sake of consistency the writer has preferred to accept his later identifications, where available. It is worth emphasizing, yet again, that with a dioecious family, like the Menispermaceae, identification of the often scanty and incomplete herbarium material may be difficult even for the specialist. It is well to bear in mind, therefore, that there is an element of uncertainty about many of the reported identities.

[c] The relevant collection has also been listed under *C. toxicofera* [61].

[d] The documenting collection has also been included with *C. toxicofera* [34, 46, 58, 61].

[e] Von Reis and Lipp [146] list a collection *Krukoff 7572* with the annotation "used in curare". It is not mentioned in Krukoff and Smith [102].

[f] Collections have also been cited under the names *Sciadotenia duckei* Moldenke and *Abuta selloana* Eichler [34, 46]. Barneby and Krukoff [141] point out that this latter taxon could also be considered a geographical subspecies of *A. imene*.

[g] The same documenting collections have also been assigned to *A. obovata* Diels [34, 46].

[h] Barneby and Krukoff [141] identified the relevant specimen **with reservations** as this species. It has also been placed under *T. peruviana* [116].

the Río Vichada in Colombia used *guachamacá* on their arrows. (The normal arrow poison of the Sáliva Indians is said to have been curare (75).) Towards the end of the nineteenth century, the source of Venezuelan *guachamacá* was tentatively identified as a *Malouetia* species. Later gatherings of the plant(s) concerned belong to the two apocynaceous genera *Malouetia* and *Tabernaemontana* (73, 74–77). See further Sections 1.4.9 and 1.6.4.1.

More recently, De Civrieux (78) found that the Makiritáre of the Upper Orinoco in Venezuela know *wachImakA* (*guachimaca*) as a poisonous plant, identified as a species of *Tabernaemontana* (*Bonafousia*).

1.2.1.3. Central Africa—*Umuhoko*

During the course of research into the medicinal plants of the Central African country Rwanda in 1970, Angenot visited the **Banyambo**, a small tribe of hunters living in the somewhat isolated marshy region along the Akagera river which forms the frontier between Rwanda and Tanzania. He was able to observe how they made their poison, which is used with the bow and arrow, chiefly for hunting large game. Examination of the quaternary alkaloids from the prepared poison and from the *umuhoko* plant itself showed that they both had a genuine curarizing action (79, 80). See also Section 1.2.3.1 and Table 1.4.

1.2.1.4. Western Malaysia—*Ipoh akar* and *lampong*

In 1891, Leonard Wray Jr. (81, 82) obtained dart-poison materials when visiting **Semai Senoi** aborigines living in southern Perak, Western Malaysia. Subsequent pharmacological testing with frogs suggested that the prepared poisons, *ipoh akar* and *lampong*, had both cardiotonic and curare-like activities (83, 84, cf. 85). The curarizing activity, probably derived from the inclusion of a *Strychnos* species, has been confirmed in a reexamination of some of Wray's materials (86). Studies on another Perak dart poison, but one of unknown origin, have also demonstrated some curare-like activity (87, 88). See Section 1.2.3.1 and Table 1.4.

1.2.2. Weapons Associated with Curare

Ribeiro and de Melho Carvalho (45) have reviewed the various types of weapon to which curare has been applied. The poison is chiefly associated with use of the **blowgun and dart** by hunting tribes living in the forest. As a weapon, the lightweight blowgun dart is not suited to the open savanna, where any wind blowing will make it veer away from its target. Mostly, it is used for hunting small animals such as monkeys and other mammals and birds, but it is also employed against much larger game when a succession of darts can be fired at the animal. The weapon has the advantage that, unlike a shotgun, it does not make a loud noise which scares away the potential victims, and several animals can be shot before they take fright and run or fly

away; moreover, the relaxation of the muscles occasioned by curare prevents monkeys and birds from hanging in the trees instead of falling to the ground (14: pp. 128 et seq.; cf. 150). The meat of animals killed with curare is harmless, since little of the poison is absorbed on ingestion. It is also said to be tender and of excellent flavor, which again is due to the muscle relaxation engendered by the curare; this leads to the retention of both glycogen and ATP in the muscles, thus maintaining flavor and delaying rigor mortis and subsequent toughness (151, 152).

The 3-m long Waorani (Auca) blowgun, for example, has a useful range of between 30 and 40 m when pointed near the vertical; accuracy decreases to about 25 m when it is held near the horizontal position. These Indians use unpoisoned darts to hunt small birds and mammals up to about 80 g in weight, but they may take with them as many as 300 poisoned darts. When he comes across a potential target, the hunter simply notches the dart behind the poison and winds some kapok on to it near the butt end before inserting it in the blowgun. A woolly monkey fell from the trees after 15–20 minutes, while a puma needed 11 darts to bring it crashing to earth after more than 30 minutes—these times suggest that the curare was not particularly strong (150).

Though shotguns are becoming increasingly popular, the blowgun, as indicated above, is still frequently used for hunting small animals (52, 150, 153–155). With certain tribes, like the Lamista Quechua, Jívaro, and others, the blowgun and poisoned dart appear to some extent to have renewed their popularity as a weapon, among other things because of the increasing cost of shotgun ammunition (154, 156, cf. 14: p. 112).

The **curabí** is a type of arrow, different from the normal one, in which a long wooden point, which may be detachable, is fixed in the end of a reed; they are usually carried in bundles of six or seven. The points are coated with curare and are often protected with a small bamboo sheath. Use of such curare-poisoned curabís and of ordinary arrows has been widespread but scattered, in many cases by tribes who do not have the blowgun and dart. Curabís have been employed in hunting larger animals, such as tapir, wild boar, deer, peccary, leopards, and other felines, etc., and in intra- and intertribal warfare (14, 55, 57, 71, 154, 157).

The curare-poisoned **murucú**, or spear, is another widespread but scattered weapon for hunting larger game and for warfare. Some tribes, e.g. the Yagua and Jívaro, believed that curare would lose its power if used against man and the weapon was not poisoned. Murucús are found mainly among tribes in Peru and the far western part of Amazonian Brazil. They may be kept in bundles of two to five, and each point, which may be detachable, is covered with a bamboo sheath (45).

Nimuendajú (158) records that the Tikuna protected the routes leading to their villages with strips of the *paxiúba* or rasp palm (*Iriartea* sp.). These bear sharp thorns which they smeared with curare. A similar ploy is mentioned for a number of other tribes (45).

1.2.3. Composition

The activity of curare comes from plants belonging to two very different families:

(a) Loganiaceae. Asian members of the genus *Strychnos* are well known as having provided lignum colubrinum (snakewood) from *S. wallichiana* Steud., *S. minor* Dennst., and *S. lucida* R. Br.; nux vomica from *S. nux-vomica* L.; and fabae St. Ignatii from *S. ignatii* P. Bergius. These last two species yield strychnine and brucine, and have been used as sources of dart and arrow poisons in South-East Asia. *S. icaja* Baill., among others, has furnished arrow poison in West and Central Africa (159, 161, 162).
(b) Menispermaceae. This family has also provided drugs for the medical armamentarium, e.g. cocculus indicus from *Anamirta paniculata* Colebr. (168); pareira brava from *Chondrodendron platiphyllum* (A.St.Hil.) Miers, *C. microphyllum* (Eichler) Mold. (169), and perhaps also *Cissampelos pareira* L (170); and calumba root from *Jateorhiza palmata* (Lam.) Miers (168).

Strychnos is the only genus of the Loganiaceae whose species are involved in the making of curare (Table 1.1).[2] On the other hand, representatives of several genera of Menispermaceae have been utilized—not only species of *Chondrodendron* and *Curarea*, which are known to be active, but also species of *Sciadotenia*, *Abuta*, *Anomospermum*, *Telitoxicum*, as well as *Cissampelos* (Table 1.2); there is a dearth of information about the activity of these latter genera.

1.2.3.1. Variation

Some tribes make curares from a single plant, e.g. the Nambikwára and Piaróa use only *Strychnos*, the Waiká-Sanama may use *Abuta* on its own, the Lamista Quechua make a curare solely from *Chondrodendron*, and the Waorani produce one just from *Curarea* (Section 1.2.4). However, most accounts describing the preparation of curares reveal a greater complexity in

[2] In a series of four papers, Freise (163–166) discussed his work on the source plants and adjuncts of curares prepared by Brazilian Indian tribes. While it may well be that some, or even much; of his information is good, certain parts of it are suspect. For example, he begins (163): "Zur Curareherstellung werden, wie durchaus allgemein bekannt, Rinden verschiedener Strychnos-Arten verwendet; bei den Uaupés-Indianern ... einem der Stämme, die als hervorragende Giftbereiter gelten, kommen vornehmlich Str.lethalis Barb.Rodr., Str.Icaja Baill. und Str.lanceolaris Benth.... vor." But *S. icaja* is a West African species and *S. lanceolaris* comes from Sumatra and is a form of *S. ignatii*....! Freise (165) also indicated that *Macoubea guianensis* Aubl. (Apocynaceae) provided a non-loganiaceous curare used by the Chavante; however, none of the other references consulted by the writer suggests that this tribe has ever used poison on its weapons. Moreover, *M. guianensis* is a well-known fruit plant and it seems unlikely that its latex will be toxic. See also: Anderson et al. (167). Many of Freise's reported phytochemical results appear improbable in the light of current knowledge. Care is required in making use of his publications.

their composition. This is illustrated by the following two examples, which also provide rare quantitative data. The first was reported by Krukoff and Smith (102) and the second is one of several Canelo Quechua formulas obtained by Gill (in: 100). The identifications of the Menispermaceae have been amended in accordance with Krukoff's later work:

Tikuna curare

Strychnos castelnaeana	2 lb outer stem bark
Curarea tecunarum *Telitoxicum minutiflorum*	0.5 lb bark
2 *Piper* spp. (Piperaceae)	0.25 lb roots
Aristolochia sp. (Aristolochiaceae)	1 tuber
Cucurbitacea	1 tuber
Annona ambotay (Annonaceae)	0.1 lb root bark
Petiveria sp. (Phytolaccaceae)	0.1 lb roots

Canelo Quechua curare

Curarea tecunarum (*yana pala ango*)	6.5 lb stems
Curarea tecunarum (*tonispa pala ango*)	11 lb stems
Sciadotenia toxifera	0.25 lb root bark
Cissampelos pareira	4.5 lb stems
Strychnos toxifera	2.5 lb stem bark
Strychnos peckii	0.5 lb stem bark
Unidentified	1 lb branch bark
Strychnos jobertiana	0.13 lb root bark

Strychnos and *Curarea* supplied about 85% of the plant material in the case of the Tikuna curare and about 78% in the case of the Canelo Quechua product. It may well be that the other Menispermaceae employed—the *Sciadotenia*, *Telitoxicum*, and *Cissampelos*—contributed additional active substances, but with the current incomplete knowledge of their alkaloids the point remains uncertain (cf. Sections 1.4.6.3, 1.4.6.8, 1.4.6.9).

During their ethnobotanical researches, Prance and his coworkers (66, 171) have noted the ingredients of the curares prepared by a number of tribes about whom there has so far been little information. Two of the formulas they were able to acquire are given below. The bulleted plant names have been kindly supplied by Prof. Prance.

The **Yekuaná** (Mayongong) near Auaris in the Brazilian Territory of Roraima make a curare, the components of which are as follows (115, herb. K and US):

Strychnos guianensis or *Strychnos bredemeyeri* stem bark
- *Strychnos diaboli*
- *Dalbergia monetaria* L.f. (Papilionaceae)
- *Virola theiodora* (Spruce ex Benth.) Warb. (Myristicaceae)
- Unidentified
- Unidentified

The **Yamamadí**, who live along the middle reaches of the Rio Purús, one of the main tributaries entering the Rio Solimões from the south, make a curare from a mixture of the stem barks of the following ingredients (66):

Strychnos solimoesana
- *Curarea toxicofera* + *Abuta rufescens* (*splendida*)
- *Duguetia asterotricha* Diels (Annonaceae)
- *Guarea* cf. *grandifolia* C.DC. (Meliaceae)
- *Picrolemma sprucei* Benth. (Simaroubaceae)
- *Guarea carinata* Ducke (Meliaceae)

The next two formulas are of muscle-relaxant poisons made in other parts of the world and offer a comparison with those of South America. In the mixture utilized by the Central African **Banyambo** there are eight components (80):

Strychnos usambarensis (*umuhoko*)	roots, leaves
Euclea schimperi (DC.) Dandy (Ebenaceae)	roots
Ipomoea wightii (Wallich) Choisy var. *wightii* (Convolvulaceae)	tubers
Opuntia ficus-indica J. Miller (Cactaceae)	leaves
Aloe laterita Engler (Liliaceae)	leaves
Capsicum frutescens L. (Solanaceae)	fruits
Synadenium grantii Hook. (Euphorbiaceae)	stems
Euphorbia tirucalli L. (Euphorbiaceae)	leafy stems

The *lampong* poison obtained from the Batang Padang **Semai Senoi** of Western Malaysia (86), whose curarizing activity was found to be rather weak, appears to have consisted essentially of:

Strychnos ignatii (*lampong*)	root bark
Antiaris toxicaria (Pers.) Leschen. (Moraceae)	latex
Coptosapelta tomentosa (Blume) Valeton ex K. Heyne (Rubiaceae)	root bark (?)

This last poison is a two-pronged one, for the *Antiaris* latex is a second source of powerful active principles, viz cardiotonic glycosides, which are even more toxic than the alkaloids of the *Strychnos*.

1.2.3.2. Additives

As is evident from the above formulas, besides the essential components, a variety of other plants may be added during the preparation of the poison. Material of species from well over 60 genera are known to have been included as ingredients in curare formulations; the Kofán Indians of Colombia/Ecuador, for example, have utilized more than 75 species in making their various arrow and dart poisons (172). These plant additives, many of which are not known to contain any obviously toxic substances, may be included for a variety of reasons: to thicken or harden the extract and enable it to adhere to the dart or arrow; or to reinforce the effects of the poison; or for

magical purposes; or to hide the real composition of the poison. Poisonous animals of various kinds, or their toxins, may also be added, supposedly in order to augment the potency of the final product, but without its being understood that the method of preparation would largely destroy the sought for toxic properties (14: pp. 144–156). Much information on the plants used as additives in the curares of Northwest Amazonia is given by Schultes and Raffauf (34, 46).

It is not possible within the scope of the present chapter to consider the wide range of additives in detail. Table 1.3 lists a selection of the plants by families and genera. Some genera will be recognized as harbouring biodynamically active molecules. The alkaloid-containing genera are asterisked; some of their constituents are related to the active principles of the poison and are discussed briefly in Section 1.4.9. However, owing to the general lack of data relating to the proportions of the various ingredients incorporated into the poisons, it is difficult, if not impossible, to assess their contribution to the over-all efficacy of the final product. For many of the other genera concerned, including those which belong to families like the Burseraceae, Connaraceae, Gesneriaceae, Violaceae, etc., which are not usually considered as having strongly biodynamic constituents, there is little or no information about their chemistry.

The Annonaceae mentioned are all known to contain isoquinoline alkaloids (Section 1.4.9). *Guatteria calva* R.E. Fries and *G. dura* R.E. Fries are among the ingredients formerly used by the Kubeo Indians of Colombia in making curare (60, 116). *Unonopsis* (*Guatteria*) *veneficiorum* (C. Martius) R.E. Fries was first reported as an ingredient of arrow poisons made by the now extinct Yurí, Miránya, and other tribes who were then living in the Colombia/Brazil border region (129, 160). More recent reports indicate that its root bark has been used by the Kofán and Barasana, tribes situated further to the west, in preparing their curare (34, 46, 59, 60, 116).

Species of *Dieffenbachia* (Araceae) are known as "dumbcane," for when fresh plant material is chewed or when the juice comes into contact with the mucous membranes of the tongue and mouth the throat swells painfully causing difficulty in swallowing and temporary loss of speech; the mechanism of action is still not clear, but may be related to a combination of mechanical injury caused by needle-sharp crystals of calcium oxalate and oxalic acid poisoning (183).

According to Krukoff and Smith (102), the Yagua (Java) considered the bark and sapwood from the stems of *Capparis sola* J.F. McBr. (Capparidaceae) to form a very important constituent of their curare.

Most species of Euphorbiaceae and Moraceae contain a milky latex which can act as an effective adhesive, but the genera of Euphorbiaceae listed in the table are also well known for the presence in them of long-chain fatty acid esters of diterpene alcohols whose properties may include powerful pro-inflammatory and/or cocarcinogenic and other effects. Two species of the small genus *Schoenobiblus* C. Martius, which belongs to the Thymelaeaceae,

TABLE 1.3. Genera which supply some of the plant additives in South American curares.

Family	Genus	Approx. no. of species[a]	Notes	References
Annonaceae	*[b]*Anaxagorea*	30	Mucilage common in family. Chemistry of genus poorly known	34, 46, 116
	**Annona*	100	Isoquinoline alkaloids present; see Section 1.4.9	100, 102
	**Duguetia*	70	Isoquinoline alkaloids present; see Section 1.4.9	66, 102, 171
	**Guatteria*	250	Isoquinoline alkaloids present; see Section 1.4.9	34, 60, 116
	**Unonopsis*	27	Isoquinoline alkaloids present; see Section 1.4.9	11, 37, 59, 60, 116
	**Xylopia*	100	Isoquinoline alkaloids present; see Section 1.4.9	95
Apocynaceae	*Spongiosperma*	6	Chemistry unknown, contains latex	46
	**Tabernaemontana*	100	Latex present; also pharmacologically active monoterpenoid indole alkaloids	11, 29, 37
	**Malouetia*	30	Steroidal alkaloids present; see Section 1.4.9	34, 36
	Mandevilla	114	Chemistry unknown	34
Araceae	*Dieffenbachia*	25	"Dumbcane"; oxalic-acid poisoning, coupled with mechanical injury caused by calcium-oxalate raphides; other toxic substances may be present	11, 37, 102
	Philodendron (?)	500	As for *Dieffenbachia*	37, 173, 174
	Urospatha	20	Chemistry unknown	34, 46
Aristolochiaceae	**Aristolochia*	300	A range of alkaloid types present; see Section 1.4.9	11, 37, 102, 136
Bignoniaceae	*Distictella*	10	Chemistry unknown	46
	Martinella	5	Alkaloidal (?)	46
Bombacaceae	**Quararibea*	50	Pyrrole alkaloids and furanones present; chemistry poorly known	546
Burseraceae	*Protium*	85	Mucilaginous oleo-resins are present in the family; phenols also occur in *Protium* species	102
Capparidaceae	**Capparis*	250	Sulphur-containing glycosides and curarizing alkaloid-like substances present in some species; see also Section 1.4.9	102

Caryocaraceae	*Anthodiscus*	12	Chemistry unknown; toxic properties common in the family	34, 46
Clusiaceae (Guttiferae)	*Caraipa*	21	Resinous materials present	11, 37
Connaraceae	*Connarus*	100	Chemically, a poorly studied family; no obviously toxic constituents	174
Convolvulaceae	*Ipomoea*	500	Drastic purgative resins present; lysergic acid derivatives found	102
Cucurbitaceae	*Cucurbita*	27	Triterpenoid (steroidal) bitter substances (cucurbitacins) and saponins present, also alkaloids and alkaloid-like substances	102
Euphorbiaceae	*Euphorbia*	1600	Caustic latex in many species, due to presence of highly irritant pro-inflammatory, cocarcinogenic diterpene derivatives	11, 37, 163
	Hippomane	5	Highly irritant, pro-inflammatory, cocarcinogenic diterpene derivatives present	11, 37
	Hura	2	As for *Hippomane*; lectins have also been isolated	11, 37
Fabaceae (Leguminosae)	**Acosmium*	12	A sparteine derivative occurs in one species	34, 46, 116, 137
	Dalbergia	100	Various flavonoids, benzophenones, styrenes, furans, etc., present	66, 178
	Enterolobium	10	Saponins and possibly alkaloids present	46
	Lonchocarpus	150	Toxic rotenoids present; species often used as fish poisons	100
	**Ormosia*	100+	Moderately toxic lupinine-type alkaloids present in some species	34, 46, 173
Flacourtiaceae	*Euceraea*	1	Cyanogenetic glycoside (?)	34, 46, 166
	Mayna	20	Chemistry largely unknown; possibly cyanogenetic. *Ryania* has reputedly toxic members with nitrogenous insecticidal substances	46, 137
Gentianaceae	*Adenolisianthus*	2	Bitter substances (presumably iridoid glycosides) present	181
Gesneriaceae	*Nautilocalyx*	50	Chemistry of family poorly known	34, 46
Icacinaceae	*Emmotum*	12	Chemistry largely unknown	34, 46

(*cont.*)

TABLE 1.3 (*cont.*)

Family	Genus	Approx. no. of species[a]	Notes	References
Lauraceae	**Ocotea*	200	Isoquinoline alkaloids present; see Section 1.4.9	175
Lecythidaceae	*Gustavia*	45	Triterpenoid saponins present in the family	34, 61, 163
	Grias	6	Chemistry unknown	34, 61
Malpighiaceae	*Mascagnia*	65	Saponins may be present in the genus	34, 46, 176
	Tetrapterys	100	Chemistry unknown	34, 46
Meliaceae	*Guarea*	40	Triterpenes and bitter substances occur in the family	66, 171
Moraceae	*Ficus*	800	Primarily latex-containing plants; some species contain alkaloids	11, 46, 100
	Naucleopsis	20	Genus contains latex and cardiac glycosides	34, 46
Myristicaceae	*Virola*	60	Resin a well-recognized hallucinogenic material; tryptamine derivatives present; see also footnote 3	34, 66
Phytolaccaceae	*Petiveria*	1–2	Saponins common in the family; sulphur-containing substances also present	11, 37, 102
Piperaceae	*Lepianthes (Pothomorphe)*	10	Unusual in that the plant is said to be used on its own or mixed to make arrow poison; no curarizing constituents known in the family	34, 37, 46
	**Piper*	1200+	Pungent-tasting amide derivatives and essential oils present	11, 37, 102, 177
Proteaceae	*Roupala*	50	Chemistry unknown	102
Rubiaceae	**Psychotria*	800–1400	Chemistry poorly known; some species have alkaloids	34, 46, 100
	**Guettarda*	60	Some species contain monoterpenoid indole alkaloids	43
Rutaceae	*Erythrochiton*	8	Chemistry not studied	34, 46, 100
	**Zanthoxylum (Fagara)*	200	Alkaloids and coumarins common in the family and in the genus	152, 179
Sapotaceae	*Pouteria*	50	Resin and latex common in the family; saponins and alkaloids may be present in the genus	11, 37
Simaroubaceae	*Picrolemma*	3	Bitter alkaloids may be present	66

Solanaceae	**Capsicum*	10	Highly pungent capsaicinoids present; see Section 1.4.9	11, 34, 37, 102
	**Solanum*	1400	Toxic steroidal alkaloids present	11
Theophrastaceae	*Clavija*	55	Polyphenols present in at least one species	102
	Jacquinia	30	Chemistry not well known; triterpenoid saponins in some species	37
Thymelaeaceae	*Schoenobiblus*	12	Not studied chemically. Other genera in the family (*Daphne*, *Gnidia*, etc.) have pro-inflammatory, co-carcinogenic diterpenes	34, 58–60, 111, 114, 180
Violaceae	*Corynostylis*	4	Seemingly toxic, but chemistry unknown	46
Vochysiaceae	*Vochysia*	100	Little or nothing known about the chemistry of this family	34, 46, 60, 173

[a] Data from: Mabberley [182] and Schultes and Raffauf [34].
[b] The asterisk denotes a recognized alkaloid genus.

have been reported as additives in dart poisons: *S. daphnoides* Sieb. et Zucc. used once upon a time by the Yurí in western Brazil (114) and *S. peruvianus* Standley employed by the Kofán in Colombia/Ecuador (58, 143, 180). Chemical investigations have not yet been carried out, but other members of the family contain highly active esters related to those present in Euphorbiaceae. Some of the esters have become widely employed as pharmacological probes (184, 185).

Capsicum L. (Solanaceae) comprises between 10 and 20 species of herbs, with many cultivated forms arising from the widespread use of their fruits, "red peppers," as a food additive (186).

Virola Aublet (Myristicaceae) is a genus of about 60 species. Several of them, in addition to being exploited commercially for their wood and being used locally for the oil in their seeds, produce a resin which is used by the Indian tribes as a hallucinogenic snuff. It is surprising and of great interest that the resin is also employed among the Waiká as what is said to be a slow-acting arrow poison (53, 187). See footnote 3.

It has long been a point of controversy whether the "whimsical additions" or "frivolous articles" of Bancroft and others: snakes, lizards, frogs, beetles, ants, etc., really were added during the preparation of curare. Yet there seems little doubt that these items could be incorporated, for such reliable observers as, for example, Martius (128), Nimuendajú (158), and Vellard (14: pp. 144–156), who also reviews the literature on the question, have noted their use. Boehm (188, 189), after a rather superficial microscopical examination of many curare samples, merely reported the occasional presence of plant fragments (in addition to crystals of various kinds). Bauer and Fondi (190) and Bauer (32, 191), on the other hand, in a search specifically for insect fragments observed them as an integral constituent in museum samples of Yurí and Baníwa curare.

1.2.4. Preparation

When, as the first scientists, Humboldt and Bonpland at long last actually witnessed the preparation of curare, at Esmeralda on the Upper Orinoco in May 1800, Humboldt (29) commenced his account with the words: "This chemical operation, to which the *maître du curare* attached such importance, appeared to us [to be] very simple." And in stripping away most of the mystery surrounding the manufacture of the poison, they revealed that the process consisted merely in percolating with cold water a quantity of the ground-up **bark** and part of the **sapwood** from the fresh or dried **branches** of the *bejuco de mavacure* contained in a funnel made of a rolled-up banana leaf placed inside another funnel made from palm leaves. This was followed by concentrating the filtrate in a clay pot over a fire and adding juice from a large-leafed tree, *kiracaguero*, to give the poison body and enable it to adhere to the arrows (darts). Humboldt recognized *mavacure* as a plant related to

Strychnos. He noted that curare was also made from the **roots** but that the product was supposedly weaker and therefore less esteemed.

Humboldt contrasted this simple method of preparation with the more complicated procedure used to prepare the Mayobamba poison, a Lamista product, on the Río Huallaga in northern Peru. Here, the **juice** from the *bejuco de ambihuasca*, the main ingredient, was mixed with chilli (*Capsicum*), tobacco (*Nicotiana*), barbasco (*Jacquinia*), sanango (*Tabernaemontana*), and the latex of several other Apocynaceae. *Ambihuasca* is now known to be a species of *Chondrodendron* or *Curarea*.

Since Humboldt and Bonpland first saw the preparation of curare, there have been many descriptions, some more detailed than others, of the procedures used by the Indians. Inevitably, there is considerable variation in the methods, but they all have in common: extraction of the plant material that provides the active principles, followed by concentration of the extract; depending on their supposed properties, other ingredients may be added before the extraction is carried out or added as such, or as an extract, in the course of the concentration phase. Several methods of preparation are described below and they make clear, when the sensitivity of the active principles to the effects of heat, light, air, and pH is taken into account, why *Strychnos*-based curares contain such highly complex mixtures of alkaloids. See further Sections 1.4.2 and 1.4.3.

Piaróa. According to Grelier (153), while all the Piaróa (De'áruwa) hunters knew how to make the poison, only a few were considered expert. Several *Strychnos* species were used, and the soil in which the plants grew and the time of collection (at the end of the dry season) were believed to be important factors in ensuring the quality of the product. The Piaróa emphasized that it was the **fibrous inner bark of the stem** that had the poison in the most concentrated form; they tested a sliver of it on a frog before selecting the material to be used, which was then carefully decorticated on the spot. The extraction was carried out with cold water, and this was followed by filtration and concentration over a moderate fire, which could take 24 hours or more, until it became a thick, syrupy paste. While still hot, it was poured into small calabashes about the size of a duck's egg. The poison—the *curare fuerte* of the Piaróa—solidified to a brittle resin which had to be moistened before it could be coated on the darts.

Kunipúsana. Spruce (95) observed that the Kunipúsana of the Rio Pacimoni, a tributary of a tributary of the Rio Negro, made their curare by percolating the **thin outer bark** of a *Strychnos* species with water. After boiling down the liquid obtained, they added mucilage, by squeezing out the juice of various species of *Xylopia* or *Guatteria*, in order to ensure that the curare adhered to the arrow. According to Spruce, good curare "should adhere when applied to [the] arrow and quickly dry as a black varnish, but its strength can only be

ascertained by trial and [the] Indians are accustomed to try it on a bird or on one of [the] large lizards. If it only stupefies without killing, or if it does not kill speedily, seasonings (*temperos*) are added of 4 sorts, all derived from poisonous plants...." Tests of this kind are carried out by most tribes, in order to gauge the efficacy of the product.

Waiká (*Yanomamo*). Groups visited by Lizot (57) had a rather unusual way of drying the plant material before making their curare. The shaman piled scrapings of *mamakori* (*Strychnos* species) into a pyramid on a leaf and dried them by setting alight to some dried twigs placed on the upper part of the pyramid and stirring the mass. Scrapings of *mamakori sina* (*S. peckii*), after drying, were then mixed in and the whole rubbed between the hands to a coarse powder. It was next transferred to a leaf filter and water that had been simmering was then poured on top of the powder in small portions; the percolation took about 50 minutes. The men collected a little of the coffee-colored percolate in a small calabash to paint on their 20–25 cm long arrow tips, *pé ke namo*, made from the wood of various palms; this was a slow and painstaking process, for many layers were applied and each one slowly dried before the next one was painted on. Among the other ingredients noted by Lizot were species of *Anomospermum* (?), *Tabernaemontana*, *Clathrotropis*, *Piper*, and a Malpighiacea.[3]

Biocca et al. (55) described a similar procedure for another Waiká group living on a tributary of the Rio Cauaburí near the Brazil/Venezuela frontier. In this case, the percolation was carried out with cold water. The essential ingredient was again a *Strychnos* species, perhaps the **roots** of *S. toxifera*, but chromatographic analysis (192, 193) showed that material from a *Chondrodendron* species must also have been used.[4]

According to the observations of Seitz [56], the Waiká of the Upper Rio Marauiá packed **root-bark** scrapings (removed while the roots were still fresh) in leaves and stored them under the palm-leaf roof of the hut to dry; they were kept there till required. A1:3-mixture of the **root bark** of *mamakotomá*, a *Strychnos* species, and the **root** or **stem bark** of *mamakoríma*, possibly another

[3] Galeffi et al. (198) examined the poison on a bundle of Waiká darts and found curarizing alkaloids to be absent. Instead, the poison on them contained about 8% (c. 12 mg/dart) of *O*-methylbufotenine (5-methoxy-*N*,*N*-dimethyltryptamine), the only basic substance present. This finding indicates the use of the resin from a *Virola* species (Myristicaceae). At a dose level of 0.05 mM/kg, the compound isolated is more active in causing behavioral disturbances than other tryptamine derivatives and accounts for the use of the resin in hunting; its toxicity is low: LD_{50} 0.5 mM/kg. About this interesting and unusual type of hunting poison, see further: Schultes (542) and Schultes and Holmstedt (53, 187).

[4] It is not known how Biocca's materials were identified—voucher specimens are not on deposit in any recognized herbaria.

Strychnos species, was percolated with hot water. The percolate was not concentrated but painted directly on the tips of the arrows, and any left over was thrown away.

About the poisons prepared by the Sarára, a Waiká group living between the Rio Aracá and Rio Demini, tributaries entering the Rio Negro from the north, see Becher (541).

Makú of the Rio Tiquié, a tributary of the Rio Uaupés, hunt with curare-coated darts and arrows. Biocca witnessed their preparation of a mixed Loganiaceae/Menispermaceae poison (40, 194–196); the plants used included the *Chondrodendron bioccai* of Lusina (197), which is probably identical with *Curarea toxicoferum* (140), and perhaps *Abuta grisebachii*.[4] The **bark** and **root** scrapings of the eight component plants were each macerated separately in water and, after filtration, the macerates were slowly concentrated portion by portion by gentle boiling in the same pot.

Tikuna. In 1937 Krukoff and Smith (102) described in detail the preparation of curare as remembered by a then very old Tikuna Indian (see Section 1.2.3.1). The main ingredient, shavings of the **outer stem bark** (the inner bark and wood supposedly had no poison) of *S. castelnaeana*, together with **bark** shavings of *Curarea tecunarum* and *Telitoxicum minutiflorum*, were packed in a bundle of palm leaves and subjected to repeated slow percolation with cold water. Each percolation took up to half an hour and required about 2 liters of water; the final percolations lasted over an hour. The percolates were boiled down over a fairly strong fire in the course of about 8 hours. The next day, the partially concentrated extract was strained and collected in a clay pot placed on a low fire for further gentle boiling. The other ingredients—roots of *Piper*, tubers of *Aristolochia*, roots of *Petiveria*, stem and leaves of *Dieffenbachia*—were extracted and strained and then added to the concentrate and the boiling process continued until the product became a thick syrup. After testing its potency, the poison was filled into a small clay pot and allowed to become hard.

Nimuendajú (158) observed the preparation of Tikuna curare four times. The principal ingredients were evidently similar to those indicated by Krukoff, but the making took over a week, for during the concentration phase the extract was not allowed to come to the boil as this would result in the loss of strength. Just before the final stage of the concentration, 6–8 black beetles were thrown into the pot where they exuded a foetid yellow secretion; they were removed after about an hour.

Canelo Quechua. Gill (in: 100) found that the Canelo Quechua simply mixed and macerated the ingredients, chiefly the **whole stems** of *Chondrodendron/Curarea*, with water in large earthenware containers. The solution was then

concentrated by boiling, the liquid occasionally being strained and the scum removed, and finally transferred to a smaller container and reduced over a slow fire to a thick syrup.

Waorani (Auca). A group of Waorani, a linguistically isolated hunting tribe of eastern Ecuador, have a very simple technique for making their curare. Yost and Kelley (150: p. 200) describe it as follows: "The Waorani manufacture their own poison and have no memories of ever having traded with other groups either to obtain or to give poison. The only ingredient in the poison is the bark of the large, flat vine *Curarea tecunarum.* The watery resins are compressed from the bark, heated until a surface scum forms and then spread onto the tips of the darts as a thick, jet-black lacquer that hardens like tar. The process is not extremely time-consuming; the poison for several hundred darts can be prepared and applied in less than 2 hours, excluding the time it takes to find the vine in the forest and carry it home. Once the darts are prepared, they will maintain their potency for several months if they are kept dry and reasonably cool." See also Section 1.2.2.

Lamista Quechua. About the large-scale preparation of and trade in Lamista curare, see Section 1.2.5.2.

Nambikwára. A western group of Nambikwára, living near the Rio Juruena in the Mato Grosso, used a very simple procedure: handfuls of **root bark** scrapings of a *Strychnos* species were placed in about 2 liters of water and the container was then heated over a fire until the water became too hot to bear; this took about 10 minutes. After that, the material was squeezed out between the hands and discarded. The extract was concentrated by vigorous boiling, taking care to remove the foam, and at the end of about one and a half hours there remained c.15 ml of a very thick, almost black, syrup (14: pp. 97–103, 71).

An interesting point in the foregoing descriptions is the differing opinions of the curare makers as to whether the inner or outer stem bark provides the most active raw material: inner bark according to the Piaróa, outer bark according to the Kunipúsana and Tikuna. For the rest, the root bark or whole roots, or the stems (or branch sapwood) are all used. There appears to be no preference for the alkaloid-rich root bark as a source of poison. Humboldt's point, already noted above, that curare poison prepared from the roots was thought to be weaker than that made using bark/sapwood, is also worth remembering.[5]

[5] Freise (164) emphasised that only stem and branch bark was used, and never root bark, but he did not specify the tribe(s) to which his remark applied. The cork layer of older bark was the preferred material for preparing the curare.

Boehm (189) examined materials evidently derived from *Strychnos* species, possibly including *S. toxifera,* and established microscopically that some of them consisted

1.2.5. Curare as a Trade Item

Curare has been, and to a considerable extent still is, an item of intertribal trade. Its manufacture has tended to be concentrated in the hands of tribes who in certain regions have acquired a reputation for making a particularly effective product. For example, in the middle of the eighteenth century the now extinct tribe of the Caberre, then probably living near the Río Manapire, an affluent of the Orinoco in Venezuela, held special annual markets at which they sold their curare to the surrounding tribes (200). For more about the eighteenth- and nineteenth-century trade in and distribution of curare, see (564).

1.2.5.1. *Strychnos*-Based Curare

Esmeralda, on the Upper Orinoco, where Humboldt and Bonpland first witnessed the preparation of curare in 1800, was famed as a center of curare making (29). Forty years later, Robert Schomburgk (151) found it inhabited by a single Indian family who bought their poison from the Guinaú and Mayongong living to the north along the Río Padamu and Río Ventuari. These tribes called their poison *cumarawa* and *makuri*, but did not consider it as good as *urari*, which they preferred and got by barter from the Macushi and Arekuná. In exchange, they gave lengths of *curata*, from the bamboo *Arundinaria schomburgkii* Bennett which does not grow in Macushi country, to be used in making blowguns; the internodes can be up to 5–6 m long.[6]

During his explorations of the Roraima/Orinoco region in 1911–1913,

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solely of periderm. He obtained large amounts of his amorphous curarizing base "curarine" by actual isolation and estimated the amount present in one sample to be about 7%. Other samples comprised periderm and phloem, and on examination proved to contain not only "curarine" but also the amorphous noncurarizing tertiary base "curine."

Sauvan (199) reported being able to detect "curarine" microchemically in several South American *Strychnos* species by means of the violet-red color obtained with Erdmann's reagent (20% w/v aqueous sulphuric acid containing a few drops of conc. nitric acid). He examined sections of materials derived from *S. bredemeyeri* (*S. pedunculata*), *S. castelnaeana*, *S. cogens*, *S. glabra*, *S. gubleri*, *S. guianensis*, *S. toxifera*, *S. trinervis* (*triplinervis*), and *S. yapurensis*. Supposedly, the alkaloid was localized in: (a) the cortex and phloem of the stem and root; (b) in the case of the young stem, also in the epidermal cells; and (c) throughout the parenchyma, in the phloem, and in the epidermal cells of the leaves. It is not at all clear what substances Sauvan was actually detecting.

[6] A major difficulty in following the ethnological/anthropological literature is the multiplicity of names that the tribes appear to have—not only the name they give themselves, but also the various names, which are often pejorative, given to them by their neighbors. The requisite knowledge is not easy to come by either from the literature or in the field. Colson (201: pp. 18–19) illustrates the problem with the following example:

"A cassave grater could be said to arrive in Waika country having derived from the Pawana and travelled via the Pötsawugok and the Ingariko. An alternative way of describing this particular route would be to say that a cassava grater arrived in

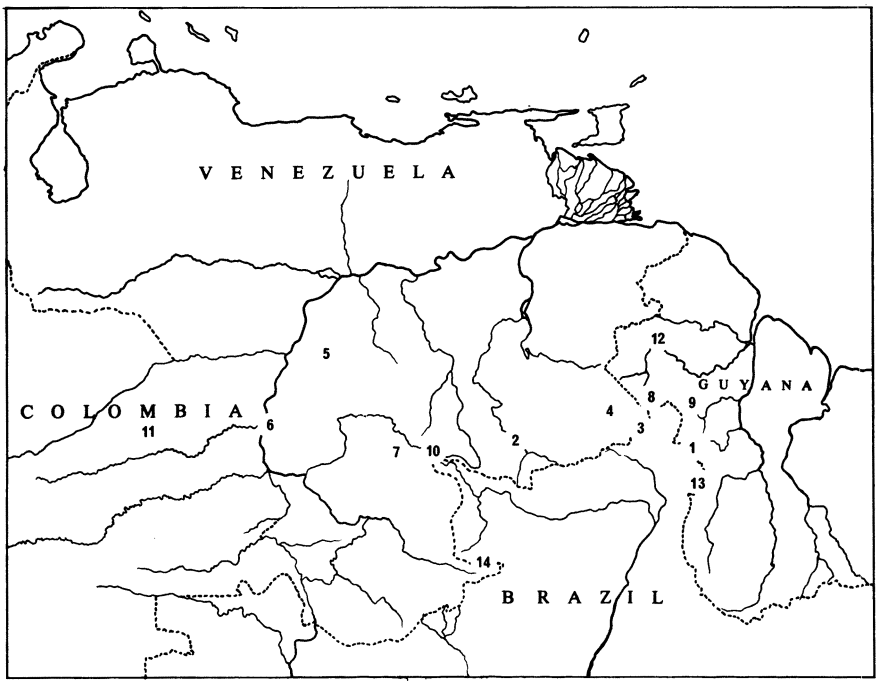

FIGURE 1.2. Approximate locations of some of the tribes concerned with intertribal trade in *Strychnos*-based curare. (For details, see: Section 1.2.5.1). (1) Macushi. (2) Arekuná. (3) Taulipáng (Pemong). (4) Kamarakoto. (5) Panáre. (6) Piaróa. (7). Yekuaná (Makiritáre, Mayongong). (8) Ingarikó. (9) Patamona. (10) Guinaú. (11) Guahibo. (12) Akawai. (13) Wapishiana. (14) Guaharibo.

Koch-Grünberg (203) was able to follow the trade pattern in more detail; for the approximate tribal locations, see: Figure 1.2. The Taulipáng hunted mainly with the blowgun and curare-coated dart. They did not make the *Arundinaria*-based blowguns themselves, but obtained them, via the Arekuná, from the Yekuaná and Guinaú, in whose land the bamboo grew. The Taulipáng got their poison from the Macushi and sold it to the Arekuná who used it to buy ready-made blowguns, or internodes of *Arundinaria*, from the

Akawaio country, having derived from the Makiritare and travelled via the Taulebang and the upper Cotinga Akawaio...."

"A strictly correct account, utilizing the proper names for themselves, would perhaps state that a grater from the *Yekuana* (Makiritare) arrived among the *Kapong* (Akawaio or Patamona) via the *Pemong* (Kamarakoto, Arekuna, Taulebang, or Makusi). But this bald statement is not so descriptive or as exact as the other, since the tribal groupings to which they refer are too large...." See also: Thomas (202).

Yekuaná and the Guinaú. So the two trade articles made the long journey from the Rupununi to the Upper Orinoco—curare traveling east to west and blowgun material in the opposite direction. It is not surprising that the poison was expensive.

The small calabashes in which the Piaróa of the Lower Ventuari and Middle Orinoco put up their curare (Section 1.2.4) have been used over a wide area as the equivalent of monetary units of payment. The Piaróa do not make pottery, which they require for cooking as well as for making curare, but get it in exchange for their poison from the neighbouring Guahibo, and ultimately the product reaches most tribes of the Guiana Shield (153). According to studies carried out in 1975/76 (204), the Panáre, who live to the northeast of the Piaróa, obtained blowguns from them (and the Yekuaná) in exchange for resin and unexpectedly, in view of the reputation of the Piaróa product (cf. above), curare.

As a response to the cessation of curare-making by the Macushi in the 1920s, the pattern of trade altered. The Piaróa, on the Colombia/Venezuela border, became the chief source of curare in the region, so that, like the material for blowguns, curare also tended to move from west to east. Extensive fieldwork carried out between 1951 and 1972 (201, 202, 205) has enabled the picture of the intertribal system of trade to be redrawn.

In the 1950s the Akawai Indians of the Upper River Mazaruni in the Guiana Highlands knew that blowguns, quivers, curare, etc., came from the far distant Mayongong (Pawana, Makiritáre). But this latter tribe was little more than a name and they actually obtained the goods from the Arekuná (Pemong), one of their western neighbors.

The Arekuná (Pemong) are located in the southeastern part of the Venezuelan State of Bolívar and in the 1970s still undertook trading trips to their eastern neighbors, including the Akawai (above), and also to the Ingarikó, who live where the boundaries of Venezuela, Brazil, and Guyana meet, and to the Macushi further to the south. Among the items traded were blowguns made by the Mayongong (Makiritáre) and also by the Arekuná themselves; in the case of the Ingarikó, one large clay pot was bartered for one blowgun. However, the blowgun is now relatively unimportant as a trade article, and shotguns have become an integral part of the hunting system.

The Yekuaná (Makiritáre, Mayongong) inhabit the region from the Upper Orinoco to the Lower Río Paragua. In the 1840s, their commercial relationships extended almost as far as Georgetown in Guyana and the journey, mainly by river, took about a year. Up to the 1950s, curare was one of their most important articles of export. Nowadays, the Yekuaná maintain trade relations with, among others, the Piaróa to the west, exchanging blowguns for curare, but this is diminishing in importance as acculturation proceeds apace, and with the Pemong to the east, who are both primary producers (of iron arrowheads, for example) and, as already seen, intermediaries in the movement of blowguns.

1.2.5.2. Menispermaceae-Based Curare

Curare made in the regions where the dominant sources of the active principles are species of Menispermaceae has also long been an item of intertribal trade.

Martius (129) noted that curare, mostly in small pots but occasionally in calabashes, made by the Yurí, Pasé, Miránya, and Tikuna living on the Rio Japurá and Upper Rio Solimões, went from hand to hand by exchange as far as tribes in central Ecuador. Some materials for making blowguns were acquired by trade with western neighbors. Among those who preferred Tikuna curare were the Chébero (Xébero), but later, as its quality declined, they got their poison from the Lamista Quechua (Lamista, Lama, Chazuta, Motilón), who supplied much of the central Montaña region (206).

In the Río Pastaza/Río Zamora area, on the Ecuador/Peru border, are several Jívaroan tribes: The Jívaro (Shuara) proper, notorious for their *tsantsa* or shrunken heads, divided into the frontier Jívaro, who live west of the Cordillera de Cutucú and who are in touch with the outside world, and the interior Jívaro, to the east of the Cordillera; the Achuara, who are based on the Río Pastaza and who also have contact with the outside world; and the Aguaruna, who sit astride the Río Marañon. Throughout the region there are chains of trading partners which stretch from the Andes and the Río Napo as far as the Río Marañon and connect the frontier and interior Jívaro with the Canelo Quechua to the north and the Achuara to the east and southeast. The Achuara have a near monopoly of the manufacture of blowguns and curare. The interior Jívaro trade a pot of salt (or a machete or axe head) for a blowgun or blowgun with gourd of poison (154, 207). See also: Bauer (33).

Upstream, along the middle reaches of the Río Huallaga, the Lamista Quechua formed another center of curare making and their product has long been traded throughout the region, especially to their Chébero (Xébero) neighbors, who gave blowguns, of which they were considered the best makers, in return (208). During field work in 1975/6 it was noted that the Lamista Quechua exchanged their curare for blowguns with the Aguaruna and Achuara to their north, as well as with the Balzacho; curare also went to the Cholón. This curare-blowgun exchange reflects the past existence of trade in goods manufactured by specialist producers, but it is now much reduced and the exchange fulfils rather the social function of maintaining relations between partners. The curare is filled into bamboo tubes of various sizes, which, like tobacco, constitute easily stored and transported units of exchange. These two items are the most general means of exchange and the traditional rate has been 3 or 4 of these tubes for a blowgun, which nowadays is of Chébero or Jívaro manufacture (156).

There is one important aspect in which Menispermaceae-based curare differs from that made with *Strychnos* and that is what amounts almost to the industrialization of its production in the valley of the Río Huallaga.

Ramón Ferreyra (209) described the preparation by the Lamista Quechua

living in the valleys of the Ríos Cumbaza, Mayo, and Sisa, tributaries of the Río Huallaga in the Peruvian Department of San Martín. They know two kinds of *ampihuasca*: in one the **stem** is red and in the other white; both are utilized and they both belong to the same species, *Chondrodendron tomentosum* (Table 1.2). 50–60 Kg loads of the liane are broken up into small pieces and crushed; then extracted with boiling water for several hours; and after removal of the foam, filtered through cotton cloth when cold.[7] Finally, the extract is slowly concentrated over a low fire, which largely determines the quality of the product. The curare thus prepared is a dark red, thick liquid with a bitter taste. According to Vogel (in: 209), who visited the region in 1946, 3250 kg of fresh *Chondrodendron* yielded 160 kg (4.9%) of curare extract. It was estimated that several tonnes of extract a month could be made, usually containing 4–5%, but sometimes 6% or more, of active principles (14: p. 113)

Since 1970, an entrepreneur in Sisa has undertaken the production of curare for sale to the pharmaceutical firms represented in Lima and in 1975 in the *Ampi Urku* ("Hill of Curare") region of the middle Huallaga he controlled three itinerant workshops (transportable extraction facilities) which were sheds containing large iron cauldrons and firewood to which the Lamista Indians brought their loads of *Chondrodendron* vine (156). The crude product could then go by mule to airstrips and be flown out in light aircraft in half-tonne quantities before being shipped abroad (210). Most of the *Chondrodendron* is harvested in the valley of the Río Huallaga between Tarapoto and Tocache Nueva where it grows in abundance along rivers. If, however, the demand continues to increase, supplies of the plant may become depleted. Already, some people in the region have been making attempts to grow the vine on a small scale (211, cf. 156).

1.2.6. Classification

Boehm (188, 189), in his now classical researches on curares carried out towards the end of the last century, was led to classify them according to the type of container they were stored in—the three most important types being calabash, tube, and pot curare. But he emphasized that chemical examination was an even better way of distinguishing the various products. Nevertheless, the classification by container proved convenient in use over a period of more than 60 years, as it turned out that, generally speaking, curare in calabashes has mostly been prepared from Loganiaceae; curares in bamboo tubes have usually been derived from Menispermaceae; and pot curares have mostly been mixed Loganiaceae/Menispermaceae products. So-called arrow-tip curares have been encountered in a small region on either side of the Venezuela/Brazil frontier (55, 57, 192, 193). These are painted directly on to

[7] In this connection, it is worth noting that after extraction of *Strychnos* material by the Yagua there was less than 0.1% alkaloid left in the residual marc (13), so that even under the conditions in which the Indians prepare their curares it is possible to remove almost all the alkaloids present in the raw material.

the arrows and are not kept in a container. As far as their composition is concerned, they are usually mixed Loganiaceae/Menispermaceae products and they hardly merit recognition as a separate category. For museum specimens of curare the container can be used as an indicator of the region from which they originated but **not** of their composition (191, 214), which is governed largely by the plants available to the maker(s).

Problems arose through the fact that some tribes may keep their curare in more than one type of container, nowadays even in tin cans or bottles which happen to be conveniently at hand (14: pp. 138–140). In addition, since certain tribes have been renowned for the quality of their curare, there has been, and still is, a considerable trade in the product and samples often travel hundreds of kilometers from their place of origin (Section 1.2.5). With the gradual increase in knowledge of the botany and chemistry of curare, the essentially utilitarian, rather than scientific, classification by container has outlived its usefulness and the time has now come to give it a decent burial. It will not be referred to further in the present account.

Vellard (14: pp. 141–142) put forward a geographical classification based primarily on the botanical sources of the active ingredients and noted that it was closely paralleled by the differences in the chemical composition of the various preparations. Nevertheless, here again the long-distance trading gives rise to difficulties, which are compounded by the fact that the botanical origin of the product is often unknown.

Bauer (32, 33, 191, 212–215) has analyzed more than 70 museum samples of curare by means of paper chromatography (PC). He (191) classified his findings in terms of the main active alkaloids present (for the structures of these compounds, see Sections 1.4.2 and 1.4.6) and has divided curares into three major groups mainly produced in different regions (Fig. 1.3):

(1) Those in which the *Strychnos* alkaloids curarine/calebassine are the main alkaloids; in some, dihydrotoxiferine (C-alkaloid K) may also be present. These products originate mainly from the eastern Amazonian region (Guiana and the Rio Negro, including the Rio Içana and Rio Uaupés).
(2) Those in which the *Strychnos* alkaloid toxiferine predominates. C-Alkaloids A and E may also occur in large amounts. In some, diaboline is present; and occasionally there are traces of curarine/calebassine and related alkaloids. In some samples there are *Chondrodendron* bases. Mostly, these curares come from the western Amazonian region, mainly between the Rio Japurá (including the Río Apaporis) and the Río Napo and south of the Rio Solimões to the Rio Javarí. This encompasses part of the Japurá-Putumayo cultural region.
(3) Those in which only *Chondrodendron* alkaloids occur. Curares of this group are chiefly found in the north and central Montaña, but they extend into the Japurá-Putumayo cultural region.

All three regions, but especially regions 2 and 3, overlap. But even for region 1, which supposedly has curares made only from *Strychnos*, there is

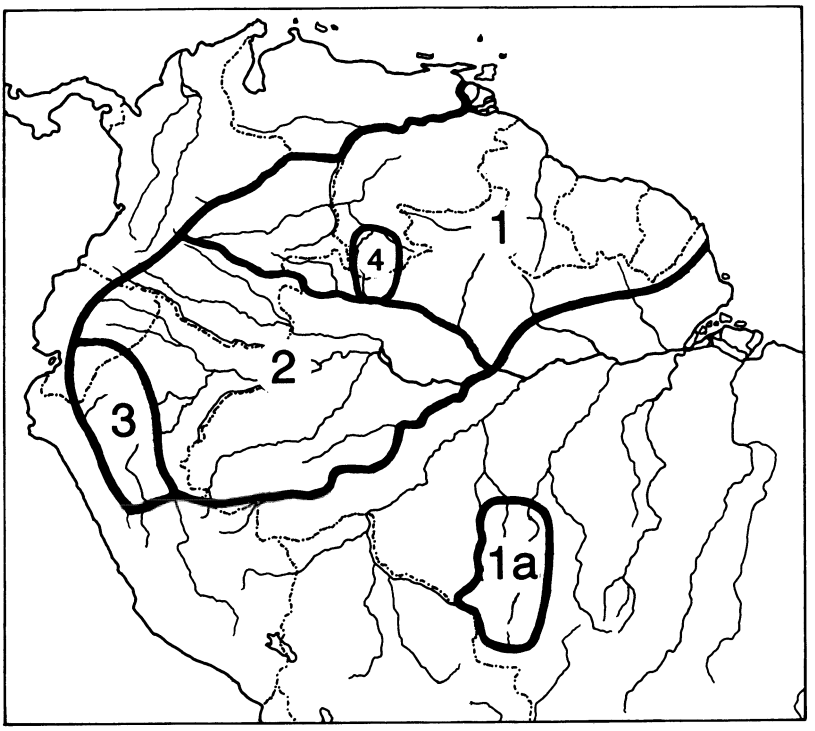

FIGURE 1.3. The curare-producing regions. (See further: Section 1.2.6). Region 1. Mainly *Strychnos*-based curares. Region 1a. Savanna *Strychnos*-based curares. Region 2. Principally *Strychnos*-based, but sometimes *Chondrodendron* alkaloids also present. Region 3. Essentially *Chondrodendron*-based curares. Region 4. Mixed *Strychnos-Chondrodendron* curares.

evidence that Menispermaceae have been incorporated into them—some Macushi curare makers of Guyana seem to have included material of a *Cissampelos* species (43) and, as already indicated in Table 1.2, the Sanama of the Brazilian Roraima make their poison from an *Abuta* species either on its own or included as one of a number of ingredients. Bauer places Siusi and Witóto products from the Brazil/Colombia frontier region in a separate category. Along with traces of curarine/calebassine and associated alkaloids, they contain several unidentified components. One possible ingredient in Witóto curare is a *Telitoxicum* species.

In a given region the composition will tend to be fairly constant; and because of the conservatism engendered by tradition, once a particular composition has been found to be effective, the tendency will be to fix by ritual and esoteric means the way in which the poison should be prepared. This is also evident from the very similar alkaloid composition of curares obtained recently and more than a century ago in both Ecuador and southern Venezu-

ela (33). Be that as it may, the same range of ingredients will not necessarily be used each time, and the fact that the composition appears to be similar throughout wide regions is due as much to lively intertribal trade in those curares which enjoy a particular reputation. Chemical analysis is essential in order to determine the composition and hence to obtain some idea of the botanical materials used.

Full understanding is hampered by current deficiencies in our knowledge of the chemical composition of the plants concerned—for tubocurarine has so far only been found in *Chondrodendron tomentosum*—yet, as demonstrated by the curare formulas given in Section 1.2.3.1, there are curares from regions 2 and 3 that do not have this species as an ingredient, so that their muscle-relaxant activity must be due to alkaloids from other plants, e.g. *Abuta* (cf. Section 1.4.6.4) or *Curarea* species.

1.3. Botany

Tables 1.1 and 1.2 (Section 1.2.1) list the species of Loganiaceae and Menispermaceae,[8] most of which are documented by annotated herbarium specimens, that are known to be, or to have been, utilized in making curare. The plants themselves are discussed briefly in the paragraphs below and, for completeness, information on those employed in the curarizing poisons of Africa and Malaysia is also included. Table 1.3 brings together a selection of the families and genera that have provided other components of curare.

1.3.1. Loganiaceae Associated with Curare

The only genus of this family whose members are used in the preparation of curare is *Strychnos* L. It comprises about 190–200 species found in three geographically separated regions: c.78 species in Central and South America, 75 species in Africa, and c.45 species in Australasia; there is one exception—the species *S. potatorum* L.f. occurs in both Africa and India. Mostly, the plants are lianes (climbers, bush-ropes) bearing tendrils or, less frequently, spines or both, and they can be as much as 120 m long, climbing 45 m high into trees; at the base, the stem may have a diameter of up to 30 cm. A few species are shrubs or small trees. The leaves are opposite and in the majority of species exhibit characteristic venation: 1-2(-3) pairs of distinct secondary veins arising at or up to 3 cm from the base and curving along the margin, which are usually very prominent on the lower surface. A few exceptional species have leaves that are pinnately-nerved. The few- to many-flowered inflorescences are axillary and/or terminal, with greenish yellow to white, relatively inconspicuous flowers. The fruit is a berry, 1–18 cm in diameter,

[8] The many supplements to the monographs on American Loganiaceae (*Strychnos*) (99) and Menispermaceae (139) by Krukoff and his collaborators can be traced through the final paper in each series—(221) and (235), respectively.

containing 1 to many, variously shaped, large seeds in a soft juicy, yellowish or white pulp (108, 216).

Leeuwenberg (216) divides *Strychnos* into twelve sections, and the American species belong to three of them: Strychnos, Rouhamon (Aublet) Progel, and Breviflorae Progel. Krukoff and Barneby (105) split this last section into two subsections: Breviflorae and Eriospermae Krukoff et Barneby, based primarily on supposed differences in the nature of the testa of the seeds; the distinction is not universally accepted and the point requires further study.

While *S. guianensis* is known from almost 300 collections and others such as *S. peckii*, *S. panamensis*, and *S. erichsonii* from about 200 (217), at the bottom end of the scale there are twenty-two species known from ten collections or less; in addition, the flowers of four and fruits of ten species are unknown. Among the poorly collected species are *S. solimoesana*, *S. diaboli*, and *S. progeliana* (217–221), all of which are recorded as sources of curare. Ducke (298) has written a general account of his many years of field experience with the genus *Strychnos* as it occurs in Brazil. Krukoff (217) has suggested that further species will be discovered in the peripheral parts of the Amazon basin and in Venezuela, regions that have still to be fully explored botanically.

An early, but still useful, summary of the use of *Strychnos* species in curare was put together by Planchon (110, 132, 222–227). Along with later work (298), it shows that at least fifteen species from South America have been described on the basis of collections made during explorations in connection with curare. Though only four of the taxa—*S. toxifera*, *S. jobertiana*, *S. cogens*, and *S. castelnaeana*—are still accepted as species in their own right, it is a measure of the stimulus given by the interest in curare to the collection and taxonomic study of the genus in that part of the world. Subsequently, Krukoff and his collaborators carried out research on South American *Strychnos* both in the field and in the herbarium over a period of more than 45 years, from 1935 to 1982, and this has meant that, as material and understanding of the genus have accumulated, there have been changes in the identification of certain collections. To facilitate consultation of Krukoff's many papers, in Table 1.1 the species are numbered and arranged in the order used by him in his later publications.

Use of more than twenty species in the preparation of curare is documented by herbarium specimens. Krukoff (108) indicated twenty-one species and one variety. He included *S. macrophylla* and *S. melinoniana*, but it is not clear from the original publications (192, 103, 125) that these two were actually used in curare. The *S. fendleri* Sprague et Sandw. reported by Henley (204) to have been used by the Panáre is not included, as later work by Boom (98) has shown that the plant they employ is actually *S. toxifera*. Krukoff did not list *S. amazonica* which has recently been added to the list of curare species (34, 46). *S. brachiata*, *S. castelnaeana*, *S. cogens*, *S. guianensis*, *S. peckii*, *S. solimoesana*, and *S. toxifera* have all been recorded as essential ingredients of curare. The paragraphs below discuss the individual taxa in more detail.

1.3.1.1. *Strychnos* Section Strychnos

S. rondeletioides Spruce ex Benth. is common in high forest on terra firme, where it grows as a huge bush-rope up to 50 m long, and on the shores of creeks, where it becomes a much smaller plant. It is widely distributed throughout the Amazon basin in Colombia, Venezuela, Brazil, Peru, and Bolivia; it is also known to occur in the Orinoco basin (108, 217, 220). Specimens documenting its incorporation into curare originate from Colombia, Venezuela, and Brazil.

S. macrophylla Barb. Rodr. A modest liane found mainly on terra firme in the vicinity of and to the northeast of Manáus in Brazil (108, 217). Barbosa Rodrigues (92, 103) said only that curare could be obtained from this species, not that it was actually used by the Indians to make the poison.

S. brachiata Ruiz et Pavón occurs widely as a large climber from sub-Andean Peru and Bolivia as far as Pará in Brazil. Its various habitats in Brazil include varzea and terra firme (108, 217, 220). It is recorded as a curare ingredient only from Colombia.

S. toxifera Rob. Schomb. ex Lindley (*S. syntoxica* Sprague et Sandw.) is a modest-sized liane up to 7 cm in diameter, with densely pubescent leaves. The species was first gathered by Robert Schomburgk in 1835 as a curare plant used by the Wapishiana (and Macushi) Indians in southern Guyana, and it is perhaps the most widely utilized species of *Strychnos* in curare—from Ecuador across the continent to Guyana. The plant has been frequently collected and is now known to grow in Panama, Colombia, Venezuela, Ecuador, and in Amazonian Brazil as well (217, 220, 221). It has not yet been found in Amazonian Peru or Bolivia, but is expected to occur there.

S. tomentosa Benth. (*S. rhexioides* Klotzsch ex Rich. Schomb., name only) is a huge liane, up to 10 cm in diameter, with very large fruits, also as much as 10 cm in diameter, found in various types of forest in the Guianas and across the northern part of Amazonian Brazil (108, 217, 221), where it is included in curares. Specimens from Venezuela formerly identified as belonging to this species have been transferred to the new taxon *S. davidsei* Krukoff et Barneby (219).

S. diaboli Sandw. is also a large climber, which is confined to Guyana and nearby parts of Venezuela (Amazonas) and Brazil (Roraima); it is known only from a few collections (108, 217). There is uncertainty about whether it has been a curare ingredient.

S. javariensis Krukoff is yet another huge liane and frequent in high forest on terra firme in the western part of the Amazonian basin (Brazil, Colombia). The distribution is like that of *S. castelnaeana* (108, 217). Collections document its inclusion in curares made in Colombia and the Peru/Brazil frontier region.

S. sandwithiana Krukoff et Barneby. Like the previous species, it is a large climber of high primary and secondary forest on terra firme, often over 35 m

long and up to 10 cm in diameter. It is found in Colombia, Peru, and across Amazonian Brazil as far as Pará (108, 217). Only one collection, from Amazonian Brazil, indicates that it is used in making curare.

S. jobertiana Baillon is a species similar in size and habitat to the previous one, but it enjoys a much wider distribution: Colombia, Venezuela, French Guiana, throughout a large part of Amazonian Brazil, Ecuador, and Peru (108, 217). The species is plentiful in northwest Amazonia and its roots are a preferred source of poison, especially in Colombia, but also in Ecuador and Brazil (34, 46). See also *S. yapurensis* (Section 1.3.1.4).

S. amazonica Krukoff is a fairly big liane widespread throughout Amazonian Brazil and also in Peru and Colombia, usually occurring in high forest on terra firme (108, 217). It has recently been reported as a component of curares made in Colombia (34, 46).

S. solimoesana Krukoff is a liane which can be over 50 m long and about 8 cm in diameter. The species is not well known botanically. Though originally described in 1942 on the basis of a number of sterile collections (99), it was not until 40 years later that it became possible to publish a description of the flowers (220); and even now, only one immature fruit of the species is known. So far, the plant has been found in the southern part of Amazonian Brazil, where it has been a curare ingredient, and in adjacent Colombia and Peru (217, 220).

S. peckii Robinson. In noninundated forest it is a huge bush-rope reaching the tops of the highest trees and sometimes attaining a length of more than 60 m and a diameter of up to 20 cm. When growing near creeks or rivers, the plant is much smaller. Its distribution is very wide—in Central America along the Atlantic coast and throughout northern South America as far south as La Paz in Bolivia and the Mato Grosso in Brazil (217, 220, 221). It has been used in curare in Colombia, Ecuador, and western Amazonian Brazil.

S. erichsonii Rich. Schomb. (*S. urbanii* Barb. Rodr., *S. bovetiana* Pires) is a gigantic bush-rope, larger even than *S. peckii*, and is known from Panama, Colombia, Venezuela, the Guianas, Amazonian Brazil, and Peru (108, 217, 220, 221). It is a very common species in northwest Amazonia and is said to be a major curare constituent in the region (34, 46), but its inclusion in curare is documented for Colombia, Guyana, and Surinam. Its investigation so far (Table 1.4) belies its reputation.

S. bredemeyeri (Schultes) Sprague et Sandw. (*S. pedunculata* (A.DC.) Benth.; *S. schomburgkiana* Klotzsch ex Rich. Schomb., name only) is a moderate-sized liane known from collections originating in Trinidad, Venezuela, Guyana, and Brazil (Roraima) (108, 217, 220, 221). It has been employed by the Macushi and other tribes of Guyana and also in Brazil.

S. mitscherlichii Rob. Schomb. var. *mitscherlichii* is another large climber reaching more than 30 m in length and a diameter of up to 10 cm; it occurs in a variety of habitats, including high forest on terra firme. It is found in Colombia, Venezuela, Guiana, and Amazonian and eastern Brazil (108, 217,

220, 221). Var. *pubescentior* Sandw. differs from the typical variety in the nature of the indumentum on the lower surface of the leaves and the corolla tube; it has been collected in Colombia, from where it is reported as a curare constituent, and the western part of Amazonian Brazil (108, 217).

S. solerederi Gilg is a huge liane which has been gathered in Colombia, French Guiana, and throughout a large part of Amazonian Brazil where it is found particularly in high forest on terra firme (108, 217). It is only from the western part of Amazonian Brazil that the plant is known to be a curare component.

S. darienensis Seemann is widespread in Central and South America, from Nicaragua as far south as Acre and Mato Grosso in Brazil. Mostly, it grows near watercourses and is rare in high forest (108, 217, 220, 221). It is recorded from Ecuador as a curare ingredient.

S. ignatii P. Bergius (*S. ovalifolia* Wallich ex G. Don) is a large liane (except in Borneo where it is occasionally a shrub or small tree) occurring throughout a large part of South-East Asia and on the island of Hainan. Morphologically it is rather variable, a feature extending to the seeds which are either lenticular or irregular in shape. Its fruits are large, with a very thick pericarp and a pulp that is eaten by monkeys and other animals. Unlike the South American species of the section Strychnos, *S. ignatii* has been extensively used in Asian traditional and folk medicine; it also found use as a medicament in eighteenth-century Europe (159, 160).

1.3.1.2. *Strychnos* Section Rouhamon

S. guianensis (Aublet) C. Martius (*Toxicaria americana* Schreber; *Lasiostoma curare* Kunth; *S. curare* (Kunth) Benth.). This is the most frequently collected member of the genus in South America and has been very widely used in curare—from Colombia to Surinam and in Ecuador and Brazil. It was the first *Strychnos* species to be described from South America, as *Rouhamon guianensis* from French Guiana (228), and it was also the first plant source of curare to be collected and identified botanically (29, 43, 122, 229). Usually a moderately-sized liane, the plant occurs in both primary and secondary forest, more especially along creeks and small rivers. It is found in the basin of the Middle and Upper Orinoco, and throughout the entire basin of the Amazon as far south as Beni in Bolivia and the Mato Grosso in Brazil (217, 220, 221).

S. glabra Sagot ex Progel (*S. crevauxiana* Baillon; *S. crevauxii* G. Planchon) is a large bush-rope which in many respects is similar to the previous species and has therefore not always been readily distinguished (cf. 108, 123). As at present understood, the species is found in Venezuela, Guyana, across the northern part of Amazonian Brazil, and in Peru (217), but it is only documented as a curare ingredient in Amazonian Brazil.

S. subcordata Spruce ex Benth. (*S. depauperata* Baillon) is a small woody

climber with flowers and inflorescences similar to those of *S. guianensis*. It is found in old clearings and in high forest on terra firme across northern Amazonian Brazil and also in Colombia and Peru (108, 217). Specimens recording its use in curare come from Colombia and Brazil.

S. panurensis Sprague et Sandw. is an erect shrub that later becomes scandent, forming a liane of modest dimensions. It ranges from Panama as far south as Peru (Junín) and Brazil (Acre) (108, 217). However, it is only known as a constituent of curare in the northwest Amazon and middle Orinoco regions. See also *S. gubleri* (Section 1.3.1.4).

S. cogens Benth. This is another big liane, reaching a length of 30 m or more and a diameter of about 11 cm. Like *S. toxifera*, it was originally obtained by Robert Schomburgk in Guyana as a constituent of curare and in due course described as a new species. Its distribution is now known to include Colombia, Venezuela, Guyana, French Guiana, part of Amazonian Brazil, and Bolivia (143, 217, 220, 222). Its inclusion in curare extends from Colombia to Guyana and Brazil.

S. melinoniana Baillon is a large climber found sometimes in rain forest and in high forest on terra firme in the Guianas and in the region of the Lower Amazon in Brazil (108, 217). It is not certain that this species is a genuine curare plant (cf. Table 1.1).

S. usambarensis Gilg ranges across tropical Africa from Guinea to Mozambique and as far south as Natal. It may be a shrub, a liane reaching 20 m in height, or a much branched small tree; its habitat varies from rain and secondary forest to evergreen bushland and it occurs at altitudes up to 2000 m. In West Africa, the Congo, and parts of East Africa the plant tends to be a climber; elsewhere, it is usually a small tree (216).

1.3.1.3. *Strychnos* Section Breviflorae

S. castelnaeana Wedd. (*S. castelnaei* Wedd. ex Benth.). This is one of the most famous of curare plants, but its scientific investigation has proved to be disappointing (cf. Section 1.4.1). It is a liane usually growing in noninundated forest and it may attain a length of about 12 m, with leaves that are unusually large for the genus and that can be as much as 30 cm long and over 20 cm broad. The plant is found chiefly in the western part of the Amazon basin, centered around the region where Colombia, Brazil, and Peru come together; however, it is not yet known to occur in Colombia (108, 217). This is one of the best documented curare species of *Strychnos*, being widely used in Peru and Brazil.

S. progeliana Krukoff et Barneby is only known from the original collection made by Martius in 1820 when on the Rio Japurá he observed curare-making by the now extinct tribe of the Yurí. The taxon was confused first with *S. guianensis* (128, 129) and subsequently with *S. castelnaeana* (130). It is a small tree with spines (105, 130).

1.3.1.4. Doubtful Species

Two *Strychnos* plants reported to be curare ingredients were described by Planchon from incomplete (sterile) material, viz *S. gubleri* and *S. yapurensis*, and they have never been satisfactorily identified. Such material as exists was not seen by Krukoff during his studies of South American *Strychnos*, so that renewed investigation is required in both cases.

Be that as it may, Planchon named sterile material of a species apparently used as an ingredient in the curare made by the Makiritáre and Piaróa of the middle Orinoco *S. gubleri* (*S. gubleriana*) (223, 224, 227, 230). In the herbarium of the Faculté de Pharmacie, Paris, there is an unnumbered Gaillard collection with the name *S. gubleri* which is almost certainly a duplicate of the sheet *Gaillard 16* kept in the herbarium of the Muséum National d'Histoire Naturelle, Paris (P); their annotations are very similar. The latter sheet has been determined as *S. panurensis* (90, 108) and it is likely that Planchon's *S. gubleri* should be identified with this species.

As regards the other taxon, in the herbarium of the Faculté de Pharmacie, Paris, there are two specimens obtained in 1879 and 1880. From the annotations, and the notes by the collector Crevaux (47: p. 372) and by Planchon (110, 227), it seems that they are the specimens to which Planchon gave the name *S. yapurensis*. Currently, they are filed under *S. jobertiana*, but whether they indeed belong to this species still has to be established.

1.3.2. Menispermaceae Associated with Curare

The family comprises about 70 genera with about 400 species distributed mostly in the palaeotropical and, to a lesser extent, the neotropical regions of the world. It is primarily one of twining woody vines and in rare cases erect shrubs or small trees. The leaves are alternate and mostly palmately veined, while the inflorescences of generally minute male and female flowers, whose parts are usually 3- or 6-merous, are borne on separate plants, and the fruit is a drupe or achene with or without a fleshy endosperm.

The subdivision of the family proposed by Diels (231) was based essentially on seed and flower characters and, in spite of all the advances made since then, Barneby and Krukoff (140) say that the arrangement appears still to be the most satisfactory way of organizing the family classification. Some of the characters differentiating the tribes of interest in the present context are as follows: The Triclisieae lack albumen and mostly have valvate inner sepals, while the Anomospermeae feature an albumen, ruminate throughout and formed of readily separable lamellae, which is folded round the embryo, and the endocarp shows little sculpturing. The Cocculeae, in contrast, have a continuous, rarely obscurely ruminate, endosperm, and the endocarp is more or less ribbed and sculptured.

Because in many cases the fruits were unknown, and staminate flowers tended to be better represented in herbaria than pistillate ones—a situation

unavoidable in a dioecious family—in practice the genera have tended to be assigned and delimited on the basis of their over-all appearance, coupled with characters of the staminate flowers. Barneby and Krukoff (140) have published a detailed reappraisal of the American genera of Triclisieae and Anomospermeae, taking into account the developments that have taken place since the appearance of the original monograph by Krukoff and Moldenke (139).

Table 1.2 lists the currently accepted identities of the various collections of Menispermaceae that bear annotations relating to curare.

1.3.2.1. *Chondrodendron* Ruiz et Pavón (Triclisieae)

Many botanical and chemical authors and others have followed the original spelling of Ruiz and Pavón, viz *Chondodendron.* But as Sandwith (232) has argued, this spelling is best treated as an unintentional error or, in terms of the International Code of Botanical Nomenclature, as an "orthographic error". It was intended to derive the first part of the word from the Greek *χονδρός*, meaning "granular" or "burred", and there is no root *χονδο-*in Greek or Latin. Krukoff accepted the spelling *Chondrodendron* in his later publications.

One of the consequences of the reappraisal by Barneby and Krukoff mentioned above was the partition of *Chondrodendron* as previously understood into *Chondrodendron sensu stricto* and a new genus appositely named *Curarea*, based on the correlation of differences in the perianth of the staminate flowers and in the carpophore and fruit of the two groups of species. There are also enough differences in leaf characters, so that it is usually possible to assign sterile material to one or the other group. This realignment leaves *Chondrodendron sensu stricto* with three species—*Chondrodendron tomentosum*, *Chondrodendron platiphyllum*, and *Chondrodendron microphyllum* —and *Curarea* (Section 1.3.2.2) with four species. Whether this partition is acceptable or whether the relationship of the two groups of plants is adequately expressed by considering them as sections of the same genus is a question that future work must answer. A recent study of the leaf anatomy of species from the two groups confirms their close relationship (233).

Chondrodendron tomentosum Ruiz et Pavón is a large liane, up to 20 m long, with yellow green flowers; its stems may be round or flat. As Table 1.2 makes clear, it is the species characteristic of curares prepared in the Montaña, from the Colombia/Ecuador border region as far south as Huánuco in Peru. Interestingly, the plant has a much wider distribution (140, 234) and it has been collected in Panama, Colombia, Ecuador, Peru, and Bolivia, and in Brazil (on the Rio Juruá in the western part of the State of Amazonas).

Chondrodendron microphyllum (Eichler) Mold. and *Chondrodendron platiphyllum* (A. St.Hil.) Miers, both apparently sources of the formerly much used drug pareira brava (169), have a very restricted occurrence. They are known only from the eastern and southeastern coastal regions of Brazil: the

former in Rio Grande do Norte and Bahia, the latter from Ceará as far south as São Paulo (234). Neither species is connected in any way with curare.[9]

1.3.2.2. *Curarea* Barneby et Krukoff (Triclisieae)

Regarding the taxonomy of this segregate genus, see above. Three of its four species are known to have been used in curares made primarily in the northwestern and western Amazon region.

Curarea toxicofera (Wedd.) Barneby et Krukoff (*Chondrodendron polyanthum* (Diels) Diels; *Chondrodendron iquitanum* Diels; ? *Chondrodendron bioccai* Lusina) ranges from Panama south to Bolivia and Brazil (western Amazonian region) (140, 234). The plant is a component of curares made in the area covering southern Colombia to northern Peru and parts of western Brazil.

Curarea candicans (Rich.) Barneby et Krukoff (*Chondrodendron candicans* (Rich.) Sandw.; *Chondrodendron limaciifolium* (Diels) Mold., type of basionym only), a big liane reaching into the forest canopy, has a more easterly spread: Venezuela (Bolívar), the Guianas, and Brazil (Amazonas, Pará) (234). About its probable use in curare, see Table 1.2.

Curarea tecunarum Barneby et Krukoff is a new name for the taxon largely covered by the old *Chondrodendron limaciifolium*. Since reexamination of the material on which this latter name was based showed that it belonged with *C. candicans*, the rest of the material formerly considered to be conspecific with it required a new name. In view of its widespread use by the Tikuna Indians in preparing curare, the species was given the appropriate name *C. tecunarum* (140). It is a large climber with an essentially western distribution: Colombia, Brazil (western Amazonian region), Ecuador, and Peru (234), and it has been used in all four countries for preparing curare.

1.3.2.3. *Sciadotenia* Miers (Triclisieae)

This genus comprises about eighteen species of dioecious woody vines and is divided into three sections (140). Because of the inadequate collections, the

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[9] Schultes and Raffauf (34, 46) quote the annotation to a herbarium specimen, *Plowman 7237*, identified as *Chondrodendron platiphyllum*, as follows: "said to be the curare of commerce." This collection documented plants that were being grown experimentally as part of a commercial project intended to ensure supplies of raw material for the extraction of tubocurarine in case of shortages. Professor Plowman later changed his mind (211) and no longer held the view that *C. platiphyllum* was the source of curare of commerce. As indicated, the natural distribution of the species is confined to regions well away from the vast area where hunting tribes have made and used curare.

De Lacerda (394) reported that he obtained paralysing effects with extracts of *Botryopsis platiphylla* and *Cocculus filipendula*. The material examined under the former name may have come from *Chondrodendron tomentosum* rather than *Chondrodendron platiphyllum* (cf. 139). *Cocculus filipendula* is now called *Ungulipetalum filipendulum* (C. Martius) Mold. and is the only representative of a poorly known genus (140); there has been no confirmation of its activity.

species are poorly known; it is not easy to match material having staminate inflorescences with that bearing fruits. A notable feature differentiating the genus from the related *Chondrodendron* is the considerable development of the carpophore after anthesis and although the structure is similar to that found in *Curarea* the reduced number of carpels in this latter genus serves to differentiate it from *Sciadotenia*. It is in secondary forest (capoeira) on terra firme that most *Sciadotenia* species are found, and some of them climb high in the trees and may reach a length of 30 m or more (135, 139, 140). They range from Panama and the Amazonian region of northern Peru, Ecuador, Venezuela, and the Guianas, as far as northeastern and eastern Brazil.

Two species, *S. peruviana* Krukoff et Barneby and *S. toxifera* Krukoff et A.C. Smith, are known to be constituents of curares produced in the Montaña.

1.3.2.4. *Abuta* Barrère ex Aublet (Anomospermeae)

This is a rather larger genus with at least 30 species of woody vines or clambering shrubs. In some species, e.g. *A. pahni* and *A. rufescens*, the wood is yellow and has a bitter taste. Barneby and Krukoff (140) point out that, in spite of the increased knowledge regarding its species, staminate flowers and fruits of several species remain unknown and there is too little information about intraspecific variation in the flower parts of the others. This means that excessive reliance must be placed on leaf characters, which is an inherently unsatisfactory situation from a taxonomic point of view. It will take many more years of collecting before enough material with flowers and fruits becomes available and the members of the genus can be adequately described.

The genus differs little from *Telitoxicum* in its fruit characters, but is readily distinguished by the apetalous staminate flowers and penninerved leaf blades. Details of the fruit structure and the apetalous flowers serve to differentiate it from *Anomospermum*. The fruit pericarp in *Abuta* is dry and firm. In some species, e.g. *A. imene* and *A. sandwithiana*, there is a considerable amount of pulpy mesocarp (140).

Seven species are known to have been used in curares prepared in the Montaña as well as the northern and western Amazon regions of Brazil.

A. grandifolia (C. Martius) Sandwith is a small shrub with round stems and reportedly edible fruit which has a very extensive distribution range covering the northern half of South America (135, 140, 234).

A. grisebachii Triana et Planchon is one of the largest American menispermaceous lianes and is found in Colombia, Venezuela, Surinam, French Guiana, throughout Amazonian Brazil, and in Peru (234).

A. imene (C. Martius) Eichler has a wider distribution, ranging from Colombia to French Guiana, as well as Peru, Bolivia, and Amazonian Brazil (234).

A. pahni (C. Martius) Krukoff et Barneby. This species, segregated from *A. rufescens*, is a large liane with yellow wood and root. Collections of it have been made in Venezuela, western Amazonian Brazil, Ecuador, and Peru (234).

A. rufescens Aublet (*A. splendida* Krukoff et Mold.) has flowers which are among the largest in the genus; even so, they measure only a few millimeters in length. Its distribution ranges from Colombia to French Guiana and also includes Ecuador, Peru, Bolivia, and Amazonian Brazil; but the species is also found as far north as Costa Rica in Central America (149, 234).

A. sandwithiana Krukoff et Barneby is a shrub becoming scandent above; it has yellow wood. Collections are known from the Guianas, across Brazil from Acre and Rondônia to Amapá, and in Bolivia (234).

A. vaupesensis Krukoff et Barneby is a woody vine known only from a few incomplete collections gathered in the basin of the Río Piraparaná, an affluent of the Río Apaporís in the Vaupés region of Colombia (135).

1.3.2.5. *Caryomene* Barneby et Krukoff (Anomospermeae)

The genus was first described during the revision of American Menispermaceae and comprises at least five species of large climbing shrubs resembling species of *Abuta* and *Anomospermum*. They are distinguished by having unusually large drupes with a hard, very thick-walled woody seed whose internal structure is entirely different from that of the two other genera; the venation and densely papillose undersurface of the leaves are also characteristic. The plants occur in Bolivia and Brazil, mostly along rivers but also in high forest on terra firme. Though not directly concerned with the curare story, the genus is mentioned here because the drupe of *C. foveolata* Barneby et Krukoff, from the basin of the Rio Tapajós (Brazil), is said to be poisonous (140).

1.3.2.6. *Anomospermum* Miers (Anomospermeae)

This is a genus of about eight species, for two of which—*A. chloranthum* and *A. reticulatum*—a number of subspecies have been described. There are considerable difficulties in identifying incomplete material, even for those well acquainted with the genus (140). Members of the genus are components of curare primarily in western Amazonia.

A. chloranthum Diels is widespread in the Amazon basin, but is found as far north as the Isthmus of Panama (140).

The report from Ecuador that *A. grandifolium* Eichler is used in preparing curare (Table 1.2) is unusual in that leaves are the part specified. The distribution of the plant is from the Guianas and Brazil (Pará, Amazonas, Acre) to Colombia, Ecuador, Peru, and Bolivia (140, 145, 234).

A. reticulatum (C. Martius) Eichler subsp. *reticulatum* occurs widely in Amazonian Brazil and is also found in Colombia (Amazonas) and Peru (Loreto) (140, 235).

1.3.2.7. *Orthomene* Barneby et Krukoff (Anomospermeae)

A small group of taxa formerly placed with *Anomospermum* but which exhibit major differences in the structure of their fruits has been removed to the new

genus *Orthomene* (140). *O. schomburgkii* (Miers) Barneby et Krukoff is found across a large part of northern South America, mainly along creeks and small rivers; it has also been collected in Trinidad. Unusually, the surface of the cut stem shows purple rings. Schultes and Raffauf (34, 46) record use of the plant as a curare ingredient in Colombia.

1.3.2.8. *Telitoxicum* Moldenke (Anomospermeae)

The genus is closely related to *Abuta*, particularly in its fruits, but differs in the presence of six petals in the staminate flower and in the pinnate venation of the leaf blades. There are probably about six species, but because of the paucity and inadequacy of the available specimens, the variability of the characters distinguishing the species is not sufficiently known. Collections assigned to the genus have been gathered in the Amazonian region of Peru, Colombia, the Guianas, and Brazil; but further material is needed to establish more certainly the distribution range of the genus and its species. While the pistillate inflorescences are reduced to a single flower, the staminate inflorescences, with their very small flowers, are highly complicated structures and are necessary for identification to species level (140).

Two, possibly three, species are known to have been incorporated into curares from western Amazonian Brazil and the Montaña of Peru, respectively:

T. duckei (Diels) Mold. is a taxon whose specific distinction from *T. minutiflorum* (below) is rather insecure (140). Specimens assigned to it come from Brazil (Pará, Amazonas, and perhaps Bahia) and, with reservations, Colombia (Vaupés—the specimen has also been cited as *T. peruvianum*) (60).

T. minutiflorum (Diels) Mold., a large woody climber reaching to the tops of the highest trees; it occurs in Peru and Amazonian Brazil and is confined to terra firme (234).

T. peruvianum Mold., a woody climber that has been collected only once; its fruits are unknown (234).

1.3.2.9. *Cissampelos* L. (Cocculeae)

This is a group of about 20 species which are mostly subherbaceous or suffrutescent lianes; *C. ovalifolia* is exceptional in that it is a perennial upright and unbranched herb. Members of the genus are distributed in North and South America, Africa, and Asia (236). The following four species are included in Table 1.2:

C. andromorpha DC., a shrubby liane growing to about 7 m, occurs in Central and northern South America, southwards as far as Peru (Huánuco) and eastwards to Guyana and throughout much of tropical and subtropical Brazil. *C. fasciculata* Benth., which is sometimes confused with it, is a longer suffrutescent liane growing out from deep-set rhizomes and is distributed from Mexico to Ecuador and in Brazil (Amazonas) (236). Both species are reported as components of curares made in northwest Amazonia.

C. ovalifolia DC. is found scattered from Colombia (Vichada) to Guyana

and in the south from Bolivia across Brazil to Minas Gerais and in the northeast Ceará and Paraíba (236). Its use in curare from Guyana has been documented.

Sap from the roots of *C. glaberrima* A. St.Hil. has been reported as a curare adjunct (163), but the observation requires confirmation. The vernacular name indicated, *orelha de onça*, is also applied to *C. ovalifolia*. See Section 1.2.3, footnote 2.

C. pareira L. is a small woody climber growing to a length of 8 m from a thickened root. It occurs widely throughout the tropics—in the western hemisphere: United States (Florida), throughout the Caribbean and Central America, and in South America as far as Argentina and Uruguay; in Africa: from Ethiopia and Kenya to Angola, Zimbabwe, and Tanzania, as well as Madagascar and Mauritius; in Asia: throughout the Indian subcontinent and eastwards as far as the Philippines, parts of Indonesia, and New Guinea. There is an impressive list of more than 60 synonyms (236, 237). Curare has been made from the plant in eastern Ecuador.

1.4. Chemistry

When Roulin and Boussingault (238) carried out their investigation of a sample of curare from the Rio Negro, in view of the then recent isolation of strychnine from *Strychnos nux-vomica* and of the indication by Kunth (229) that the plant from which the curare of the Casiquiare region was derived appeared to be related to *Strychnos*, they first of all looked for strychnine in their material. They were unable to detect it. Instead, they found that, although a "base alcaline végétale" was present, on basifying an aqueous solution containing it the substance did not appear in the precipitate but remained in the aqueous phase. Thus, Roulin and Boussingault had come up against the problem of how to isolate a water-soluble quaternary alkaloid. They solved the difficulty by precipitating the substance with a solution of "nut gall." Addition of oxalic acid, followed by boiling with magnesia, gave a residue which was almost entirely soluble in ethanol and which on concentration yielded a pale yellow, excessively bitter, hygroscopic syrup. It dried over concentrated sulphuric acid to a horny material that dissolved completely in ethanol and water (a property they recognized as differentiating it from other plant alkaloids), turned red litmus paper blue, and formed very soluble salts with acids.

At about the same time, Pelletier and Petroz (239) were examining the curare scraped from poisoned arrowheads given to them by Baron Larrey, Surgeon-in-Chief to Napoleon's Grande Armée. Their findings were almost identical with those of Roulin and Boussingault and they also pointed out that "c'est cette solubilité qui rend très-difficile l'obtention à l'état de pureté de certaines bases salifiables." Even now, with the facilities of a modern laboratory, it cannot be said that the investigation of quaternary alkaloids is easy.

The other factor that made the study of curare so problematical was the

great variation in composition between one sample and another, and no real progress was made until the detailed studies of Boehm during the 1880s and 1890s which led to the recognition of three main types of curare and of the fact that all three differed chemically from each other. But the products obtained by Boehm were still not pure, and it was not until 1935 that King (240) succeeded in isolating from museum samples of *Chondrodendron*-based curare a well-defined crystalline substance which he called (+)-tubocurarine. It appeared to be isomeric with (+)-curine ((+)-bebeerine) methochloride and, as a result of his chemical studies, he suggested that the two substances were bis-quaternary alkaloids built up from two norcoclaurine units. Thereafter, progress was rapid. King (240, 241, cf. 242, 243) extended his investigations to other samples of curare and to a number of *Strychnos* species from Guyana, including the widely used *S. toxifera*. The successful outcome of the work on *Strychnos*-based curare initiated in Germany (244–247) and continued in Switzerland (248, 249) owed much to the extensive use of the chromatographic techniques which had been developed in the 1930s and 1940s. Some of the compounds isolated by Wieland's group proved to be identical with alkaloids present in *S. toxifera* bark, and this was the first chemical demonstration that active principles in curare already existed in the plants used to make it.

The following sections deal in turn with the alkaloids of the Loganiaceae and Menispermaceae and with those isolated directly from samples of curare.

1.4.1. Alkaloid Composition of Central and South American *Strychnos* Species

Research on the *Strychnos* species associated with curare has very naturally placed the main emphasis on the alkaloids present in the stem bark and root bark, these being the plant parts most commonly used in the preparation of the poison. And indeed, screening small samples from 53 New World species for alkaloids has shown that, as with representatives from other continents, these parts tend to be a richer source than the leaves (250).

It is now well understood that the muscle-relaxant activity exhibited by extracts of *Strychnos* species is due primarily to the presence of bis-quaternary dimeric monoterpenoid indole alkaloids **(4d–f)**. Nevertheless, some bis-tertiary alkaloids, like bisnordihydrotoxiferine **(4c)** and caracurine V **(3)** (251), as well as monoquaternary bases like fluorocurarine **(2f)** and fluorocurine **(7)** (252) and monotertiary bases including diaboline **(9a)** (253), are able to bring about some muscle paralysis at high dosage levels. It appears that caracurine V and diaboline, at least, operate by a different mechanism from that of the bis-quaternary alkaloids, since they are not acetylcholine antagonists. But, when present in large enough amounts in curare, they could contribute to the over-all effects, although it must be admitted that so far there is no evidence that they do so; see further Section 1.5.3.

In the 1950s, investigation of *Strychnos* plant materials led to the isolation of very small amounts of many, often colored, alkaloidal products, which

were given names and about which little is known beyond certain of their chromatographic properties, their color reactions with cerium(IV) sulphate, and sometimes their UV spectra. Most of these are omitted from Table 1.4; available information on them can be found in several references (254–257). The fact that so many scantily characterized alkaloids have been isolated emphasizes how limited present knowledge of the alkaloids present in New World species of *Strychnos* is. The seeming complexity of the alkaloid composition is also affected by the reactivity of the alkaloids, especially under the conditions prevailing during the preparation of the poison. This point is discussed further below (Sections 1.4.2 and 1.4.3). When studying the quaternary alkaloids of *S. solimoesana* Marini-Bettòlo et al. (258) detected the presence of at least 40 different components. Schmid et al. (259) reproduce a two-dimensional paper chromatogram of the alkaloids from a sample of curare (no. N III) which, if anything, is even more complex. The 19 crystalline alkaloids isolated from yet another curare (no. N IV), which had a much simpler composition, are listed in Table 1.5. There are indications that in certain species the alkaloid composition may vary from one sample to another, though the available data relate chiefly to the tertiary rather than quaternary bases in *S. guianensis* (260), *S. amazonica* (261), and *S. jobertiana* (262), for example. No systematic studies of the question have yet been made.

14 of the species incorporated into curares come from section Strychnos, which has about 40 of the 80 or so taxa finally listed by Krukoff as occurring in the New World; five of the ten species in section Rouhamon have also been employed; just two of the twenty or so members of section Breviflorae have been used. Actual isolation of the many quaternary alkaloids that have been encountered during work on curare and the South American *Strychnos* species has only been accomplished from various samples of the poison (Table 1.5) and from *S. toxifera* (Table 1.4), the plant that has had the lion's share of the attention. Their reported presence in other members of the genus rests largely on extensive PC studies. It is no surprise, therefore, to find that it is mainly in species of the section Strychnos that recognized bis-quaternary bases have been detected; of the species in section Rouhamon, they are only known to be present in the African *S. usambarensis*; and no more than two members of the Breviflorae, *S. castelnaeana* and *S. parvifolia*, have them. In spite of the enormous amount of work that has been carried out over the last half century, Table 1.4 shows that little or nothing is known about the alkaloid composition of almost half the species said to be involved in the making of curares. Quetin-Leclercq et al. (250) have surveyed the occurrence of alkaloids in the New World species of *Strychnos*. Though it is mostly PC systems that have been utilized in analysing mixtures of quaternary *Strychnos* alkaloids, and *Strychnos*-based extracts and curares, suitable TLC systems have now been developed (263).

The chemistry of the muscle-relaxant *Strychnos* alkaloids has been reviewed several times (255, 264, 266–268) and will not be further discussed here. Table 1.4 records information about the curarizing activity of and the

TABLE 1.4. The curarizing activity and the occurrence of quaternary and other alkaloids of known structure in Central and South American *Strychnos* species.

Species no.[a] and name	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids	References
Section **Strychnos**					
1. *S. chlorantha*	Costa Rica	usb, msb		Quaternary bases absent; diaboline, henningsamine	269, cf. 219
		rb	weak	0.055% Quaternary bases with little or no dimers	
2. *S. ramentifera*	Brazil: Pará	sb, rb	weak		270
5. *S. romeu-belenii*	Brazil: Bahia	sb		0.21% 11-Methoxydiaboline	271, 324
*6. *S. rondeletioides*	Brazil: Mato Grosso	rb		Traces of quaternary bases; diaboline and a trace of Wieland-Gumlich aldehyde	272
		sb		An appreciable amount of alkaloid present	250
7. *S. macrophylla*	Brazil: Amazonas	b	moderate	0.45% Quaternary base chlorides (11 spots, 2D-PC), incl. **mavacurine**, fluorocurine	273, 274
*10. *S. brachiata*	Peru: San Martín	sb		11-Methoxydiaboline, Wieland-Gumlich aldehyde	261
11. *S. trinervis*	Brazil: Pernambuco	b	very strong	Activity stronger in jungle than in *capoeira* plants. 0.18% Quaternary base chlorides (23 spots, 2D-PC), incl. dihydrotoxiferine, calebassine, curarine, C-alkaloid H, florocurarine, fluorocurinine	274, 275, 277
	Brazil: Recife	br, r		Bisnordihydrotoxiferine, longicaudatine, normacusine B, trinervine	476, 477
	Rio de Janeiro	r	strong		270, 276
12. *S. panamensis*	Guatemala: Suchitepequez	usb		0.055% Quaternary bases: fluorocurine, dihydrotoxiferine, C-alkaloids F and G; 0.0023% diaboline	278
		lsb		0.067% Quaternary bases	

(*cont.*)

TABLE 1.4 (*cont.*)

Species no.[a] and name	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids	References
14. *S. divaricans*	Barzil: Pernambuco	b	weak	Total alkaloids (15 spots, 2D-PC), incl. calebassine, curarine, mavacurine, fluorocurarine	275, 279
	Recife	r		0.0035% divaricine (an 18-deoxy-Wieland-Gumlich aldehyde N^4-oxide/vellosimine-coupled bis-indole), 0.0016% vellosimine	547
18. *S. medeola*	Brazil: Pará	rb		Quaternary bases absent; 0.038% 11-methoxydiaboline	280
		sb		Quaternary bases absent; 0.033% normacusine B	
*19. *S. toxifera*	Guyana, Brazil	sb, rb	strong		240, 242, 243, 244
	Guyana	b		0.003% **Toxiferine I**, 0.0002% calebassine, 0.0021% toxiferine III, 0.0053% C-alkaloid A, 0.0007% caracurine II methochloride; hemitoxiferine I, macusines A and B; calebassine and dihydrotoxiferine absent	246, 265, 281, cf. 282
	Venezuela: Amazonas	b		0.045% Mavacurine, 0.025% fluorocurine, caracurines II and VI, 0.076% **caracurine V**, 0.064% caracurine VII, C-alkaloid Y, 0.058% bisnordihydrotoxiferine	283, 284, 326
*20. *S. tomentosa*	Brazil: Amazonas	b	weak to moderate	0.065% Quaternary base chlorides: (22 spots, 2D-PC), incl. C-alkaloid E, toxiferine, fluorocurarine, fluorocurinine, curarine	258, 270, 274, 275, 285–288
21. *S. diaboli*	Guyana	b	none to weak	0.2–0.6% Total alkaloid, incl. 0.126% diaboline	240, 242, cf. 108, 289–291, 329
	Brazil: Amazonas	sb	moderate		270
S. cf. *diaboli*	Guyana	sb ?	active	Alkaloid present	50, 242

*22.	*S. javariensis*	Brazil: Amazonas	sb	weak[d,e]		281
*23.	*S. sandwithiana*	Brazil: Amazonas	sb	moderate	Desacetyldiaboline	258, 274, cf. 108
*24.	*S. jobertiana*	Brazil: Amazonas	sb	weak		258, 270, 274, 281
			rb		0.25% Quaternary bases; 0.48% akagerine, 0.087% diaboline	262, cf. 272
		Brazil: Pará	rb		No quaternary bases; 0.061% diaboline, 0.027% jobertine	272
25.	*S. pseudo-quina*	Brazil: Brasilia	bb, tb, rb		Ca. 6% bisnordihydrotoxiferine	292
			l		Quaternary bases absent; 0.065% diaboline, 0.0032% 11-methoxydiaboline	293
*27.	*S. amazonica*	Brazil: Amazonas	sb	weak	0.26% Quaternary bases: mavacurine	274, 294
		Pará	sb		0.08% Impure macusine B; 0.10% 11-methoxydiaboline	261
			rb		0.08% Impure macusine B; 0.15% 11-methoxydiaboline	
			rb		0.34% Tertiary bases: nordihydrotoxiferine, 18-desoxy-Wieland-Gumlich aldehyde	295
*28.	*S. solimoesana*	Brazil: Amazonas	sb	active	0.52% Quaternary bases containing ca. 40 components (2D-PC), incl. fluorocurine, fluorocurarine, calebassinine, curarine, calebassine, C-alkaloids D-G, and 15 others	134, 296, 325, 327
			rb		0.5% **Calebassine**, 0.1% curarine; diaboline	297
29.	*S. froesii*	Brazil: Amazonas	b	weak to strong	0.3% Quaternary bases (14 spots, 2D-PC), incl. toxiferine, dihydrotoxiferine, C-alkaloid E, mavacurine, curarine, fluorocurinine	274, 275, 285
			rb		1.22% Tertiary bases: diaboline, Wieland-Gumlich aldehyde, 18-desoxy-Wieland-Gumlich aldehyde	295
*31.	*S. peckii*	Brazil: Amazonas	sb	none	Much alkaloid present	250, 281
			rb	strong		281
				weak	Alkaloids in traces	258, 274

(*cont.*)

TABLE 1.4 (*cont.*)

Species no.[a] and name	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids	References
*32. *S. erichsonii*	Guyana	sb	none	Alkaloids present	240, 242, cf. 329
	French Guiana	sb		Wieland-Gumlich aldehyde, diaboline, acetyldiabolines, etc.	50
	Brazil: Mato Grosso	rb	spasmolytic	Diaboline and related compounds	297
33. *S. gardneri*	Brazil: Rio de Janeiro		active		299
	Goias	rb		0.06% Crude quaternary bases (PC), incl. C-alkaloid H, traces curarine; diaboline	300
	Rio de Janeiro	rb		0.5% Quaternary bases; 0.045% 11-methoxydiaboline, 0.075% akagerine	262
*35. *S. bredemeyeri*	Guyana	b		Rich in alkaloids[f]	250
*36a. *S. mitscherlichii* var. *mitscherlichii*[g]					
	Guyana	sb	none; weak		240; 242
*36c. *S. mitscherlichii* var. *amapensis*					
	Brazil: Amazonas	b		Quaternary bases (23 spots, 2D-PC), incl. C-alkaloid D, calebassine, curarine, fluorocurine, mavacurine, fluorocurinine	279, 287, 288, 301
*37. *S. solerederi*[h]	Brazil: Pará	rb		Only a small amount of quaternary bases present; 0.017% diaboline, 0.009% Wieland-Gumlich aldehyde	300, cf. 108
*38. *S. darienensis*	Panama, Brazil	sb		Alkaloid present	250
[*S. nux-vomica*	Sri Lanka	rb	active	Quaternary bases of unknown nature; (also tertiary bases, incl. strychnine)	162, 302
S. ignatii	Malaysia	sb, rb	active	Unidentified quaternary bases present; (also tertiary bases usually incl. strychnine)]	86, 162, 303

Section **Rouhamon**					
*39. *S. guianensis*	Guyana, Brazil	sb, rb	at best weak		242, 270
	Brazil: Amazonas	sb	weak to strong	Quaternary bases (16 Spots, 2D-PC); retuline	258, 274, 275, 304, 305, 563
	Pará	sb		Absent (unusually old and large plant)	258, 260, 304
		rb		2% Quaternary bases (12 spots, 2D-PC), incl. curarine	
*40. *S. glabra*	Guyana	b	none;[i] active		306; 242
	Brazil: Pará	b	negligible[j]		258, 275, 287
	Amazonas		weak		274
*41. *S. subcordata*	Brazil: Amazonas	sb	strong	0.57% Quaternary bases (21 spots, 2D-PC), incl. fluorocurine, mavacurine, fluorocurarine; desacetyldiaboline	258, 274, 287, 288, 307
*43. *S. panurensis*	Brazil: Acre	r		Alkaloids present	250
46. *S. hirsuta*	Brazil: Pará	sb, rb	[k]	Only tertiary bases isolated	328
*47. *S. cogens*	Brazil: Amazonas	b	none		136
			weak		258, 274
48. *S. melinoniana*	Surinam	b		0.061% **Melinonine A**, 0.003% melinonine B	308
	Guyana		none	>0.014% **Melinonine A**, 0.0027% melinonine B, 0.0029% melinonine E, 0.00019% melinonine F, 0.00015% melinonine G, 0.001% mavacurine, 0.0011% fluorocurine	240, 242, 309
[*S. usambarensis*	Rwanda	rb	active	Quaternary bases incl. dihydrotoxiferine, calebassine, afrocurarine, curarine, fluorocurarine, macusine B]	251, 310–312
Section **Breviflorae**					
*50. *S. castelnaeana*	Brazil: Amazonas	b	active	0.2% Quaternary bases	306
		usb		Quaternary bases absent; 0.011% diaboline, 0.004% 3-hydroxydiaboline	313

(*cont.*)

TABLE 1.4 (*cont.*)

Species no.[a] and name	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids	References
		isb	active	Alkaloids present	281
		sw	none	Traces only	
		sb	active	Alkaloids present; a young plant	
		rb	active	Alkaloids present; a young plant	
		msb, lsb		Traces quaternary bases; diaboline, jobertine	314
		rb		0.082% Quaternary bases: C-alkaloid D; diaboline	
*51. *S. progeliana*	Brazil: Amazonas	b		—	Cf. Table 1
53. *S. fendleri*	Brazil: Roraima	sb		No quaternary alkaloids; 0.0125% henningsamine, 0.0075% diaboline, and other tertiary bases	297, 315, 316
54. *S. atlantica*	Brazil: Bahia	rb		Only tertiary alkaloids present	108
55. *S. rubiginosa*	Brazil: Bahia	sb		11-Methoxydiaboline, normacusine B	297, 317
		rb		0.052% 11-Methoxydiaboline, 0.04% normacusine B	
56. *S. parvifolia*	Brazil: Pernambuco	b[l]	moderate to strong	0.0125% Quaternary bases (16 Spots, 2D-PC), incl. calebassine, fluorocurinine, fluorocurarine, mavacurine	275, 285, 318
	Ceará	sb		Ca. 0.6% quaternary bases	319
	Bahia	rb		0.86% Quaternary bases; 0.65% akagerine	262, cf. 219
57. *S. fulvotomentosa*[m]	Brazil: Espirito Santo	b	strong		320
	Rio de Janeiro		moderate		274
57a. *S. recognita*	Brazil: Bahia	rb		Very small amount of tertiary alkaloids	219
58. *S. acuta*	Brazil: Rio de Janeiro	rb[n]	moderate		276
	São Paulo		none		318
59. *S. brasiliensis*	Brazil: Rio de Janeiro	rb[o]	weak		276
	Argentina: Misiones	sb		About 1% tertiary alkaloids present	321

61. *S. pachycarpa*	Brazil: Amazonas	b	weak	Only tertiary bases present	274
65. *S. mattogrossensis*	Brazil: Manaus	br, r		No quaternary alkaloids present; the tertiary bases include mattogrossine, an unusual Dragendorff-negative indolinic crypto-alkaloid	478
66a. *S. alvimiana*	Brazil: Bahia	b		Only tertiary bases present	322

[a] The asterisk indicates a species reported to be used in the preparation of curare. The number is that given to the species in Krukoff's later publications.
[b] b = bark, bb = branch bark, br = branch, isb = inner stem bark, l = leaves, lsb = lower stem bark, msb = middle stem bark, r = root, rb = root bark, sb = stem bark, sw = stem wood, tb = trunk bark, usb = upper stem bark.
[c] The curarizing activity has been determined by different authors using a variety of methods and the assessments are merely an indication—they are not strictly comparable.
[d] In contrast, an aqueous percolate, although containing alkaloids, was found not to have any curarizing activity.
[e] The material examined evidently comprised a mixture of the bark from the branches and the bark and cork from the roots, and the inner bark of the trunk.
[f] The bark extracted was from a sample of *yakki*, one of the components of Macushi curare, which has been identified as this species; but *Guettarda acreana* Krause (Rubiaceae) is another source of *yakki* [43].
[g] Kebrle *et al.* [323] examined bark from Brazil and found fluorocurinine, fluorocurarine, curarine, calebassine, C-alkaloid A, and alkaloids of the B, C, D group; alkaloids were absent from the wood. Krukoff and Barneby [112] explain that the identity of the material studied is doubtful.
[h] Data originally under the name *S. mitscherlichii.*
[i] The material, *F.D. 2467*, was originally identified as "near *S. guianensis* (Aubl.) Mart. and possibly a form or variety." Krukoff [108] later assigned this collection to the present species.
[j] The alkaloids are stated to have CNS activity [258, 287].
[k] Material identified as *S.* [near] *hirsuta* was reported to have curarizing activity [242, 328]. *S. hirsuta* does not occur in Guyana, where the material was obtained, and according to Krukoff and Barneby [105] and Krukoff [217] the incomplete collection concerned, *F.D. 2482*, together with several others, belongs to an as yet undescribed species related to *S. diaboli.* West [in: 89] found the pharmacology of *F.D. 2482* indistinguishable from that of *S. diaboli* and *S. erichsonii.*
[l] Data under the name *S. rubiginosa* Krukoff et Monachino.
[m] Data under the synonym *S. torresiana* Krukoff et Monachino.
[n] Data under the name *S.* aff. *albiflora.*
[o] Data under the synonym *S. breviflora* A.DC.

occurrence of quaternary alkaloids in Central and South American *Strychnos* species. It also includes data on tertiary bases that may have some muscle-relaxant activity at high dosage levels, mainly diaboline and derivatives (cf. **9**). In recent years, the curarizing dimeric compounds have received comparatively little attention and most studies have focussed on the accompanying range of monoquaternary bases and di- and monomeric tertiary alkaloids; these generally have little or no curarizing activity but they may have other pharmacological effects. Two aspects are of importance in dealing with the alkaloids present in the raw materials that furnish the curare and with the chemical changes that are presumed to take place while the poison is being prepared. Firstly, the relationships between the mono- and dimeric monoterpenoid indole alkaloids of *Strychnos* species are discussed, and this leads on to consideration of possible artefact formation during the making of curares. Finally, in this part, recent work on the indole alkaloids occurring in American *Strychnos* species is outlined.

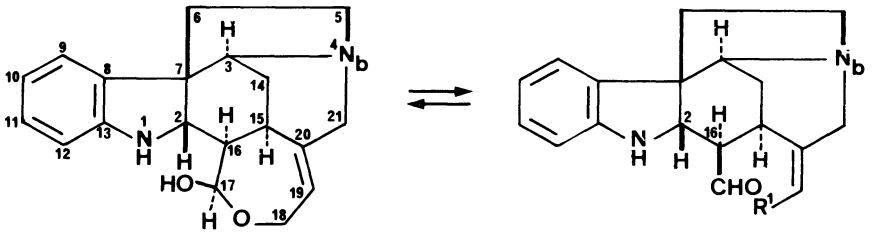
1.4.2. Interrelationships of the Monomeric and Dimeric Bases

The bis-tertiary bases **(4a–c)** arise from the three possible combinations of the monomeric units Wieland-Gumlich aldehyde[10] **(1/2a)** and 18-desoxy-Wieland-Gumlich aldehyde[10] **(2b)**, while the bis-quaternary alkaloids **(4d–f)** are derived from the combinations of their N_b-metho salts **(2d)** and **(2e)**. Wieland-Gumlich aldehyde itself **(2a)** has been obtained from several species, e.g. *S. brachiata*, *S. diaboli*, *S. solererederi*, and *S. subcordata*, and along with its N_b-metho salt[10] **(2d)** from *S. toxifera*. 18-Desoxy-Wieland-Gumlich aldehyde **(2b)** has been found in *S. amazonica* and *S. froesii*, but its N_b-metho salt **(2e)** is not known to occur naturally.

Wieland-Gumlich aldehyde **(1/2a)** is less reactive than the 18-desoxy compound **(2b)** since it is to some extent stabilized as the cyclic hemiacetal **(1)**. Nevertheless, heating it in acetic acid-sodium acetate or pivalic acid leads to the formation of mainly caracurine V **(3)** together with only a little bisnortoxiferine **(4a)**. On heating caracurine V in acetic acid in the absence of oxygen or its hydrochloride salt in distilled water at pH 6.7 it isomerizes to bisnortoxiferine; brief warming with methanolic hydrochloric acid reverses the process. The corresponding N_b-metho salts **(2d, 2e)** also undergo self-condensation and with dilute acetic acid an equilibrium is reached that lies predominantly on the side of the toxiferine **(4d)** and dihydrotoxiferine **(4f)**.

With dilute acid **4d** rapidly affords **2d,** but there is still a considerable amount of the starting material in the reaction mixture. In the case of **4f**, the equilibrium is almost entirely on the side of **2e**. Light is not necessary for this reaction. In the absence of oxygen, the monomers are obtained in high yield. In the presence of oxygen, however, the bis-quaternary dimers readily suffer further chemical change, depending on the conditions of pH, heat, etc. There are alterations in the oxygen level of the central diazacyclo-octadiene ring (see

[10] See the Appendix, where a chemical synonymy is set out.



1 Wieland-Gumlich aldehyde (closed form)

2a Wieland-Gumlich aldehyde (open form) $R^1 = CH_2OH$

2b 18-Desoxy-Wieland-Gumlich aldehyde $R^1 = Me$

2c Nor-fluorocurarine $R^1 = Me$, $\Delta^{2(16)}$

2d Wieland-Gumlich aldehyde metho salt $R^1 = CH_2OH$, N_b^+-Me

2e 18-Desoxy-Wieland-Gumlich aldehyde metho salt $R^1 = Me$, N_b^+-Me

2f Fluorocurarine $R^1 = Me$, N_b^+-Me, $\Delta^{2(16)}$

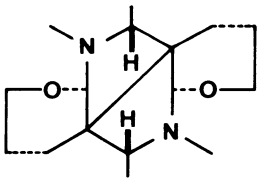
Scheme 1.1): **4d** gives rise to caracurine II dimetho salt, C-alkaloid E, and C-alkaloid A, while **4f** yields C-alkaloid D, curarine, and calebassine, as well as a number of further oxidation products of as yet unknown structure. **4e**, the hybrid C-alkaloid H, forms C-alkaloids G and F. Although, as indicated above, the parent dimers can be cleaved with acid into the corresponding monomers, their more highly oxidized derivatives are found to undergo a series of isomerizations instead.

On heating at 60°C with concentrated hydrochloric acid for 5 hours, curarine breaks down to, among other things, fluorocurarine **(2f)**, a frequent component of the alkaloid mixtures in *Strychnos* species, e.g. *S. trinervis*, *S. solimoesana*, *S. subcordata*, *S. tomentosa*, etc.

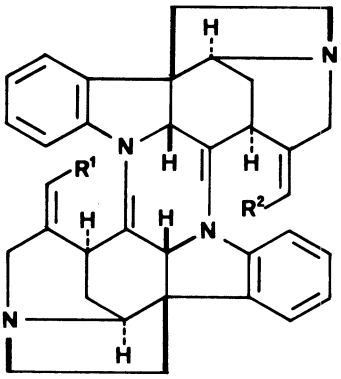
In addition to the mono- and dimeric bases already discussed, there is a range of other, mostly monomeric, alkaloids which have been isolated from various South American *Strychnos* species and from samples of curare. The occurrence of these compounds **(5–9, 13–15, 17)** is also recorded in Tables 1.4 and 1.5. They derive from the earlier stages in the biosynthetic evolution of *Strychnos* alkaloids (251), but some of them are considered to be artefacts (Section 1.4.3).

1.4.3. Artefact Formation

The various cleavages, oxidations, and isomerizations outlined in the previous section make it abundantly clear why the alkaloid composition of ex-



3 Caracurine V



Bis-tertiary bases:

4a Bisnor-toxiferine

$R^1 = R^2 = CH_2OH$

4b Bisnor-C-alkaloid H

R^1 = Me, $R^2 = CH_2OH$

4c Bisnor-dihydrotoxiferine

$R^1 = R^2$ = Me

Bis-quaternary bases:

4d Toxiferine

$R^1 = R^2 = CH_2OH$, 2 × N_b^+-Me

4e C-Alkaloid H

R^1 = Me, $R^2 = CH_2OH$, 2 × N_b^+-Me

4f Dihydrotoxiferine

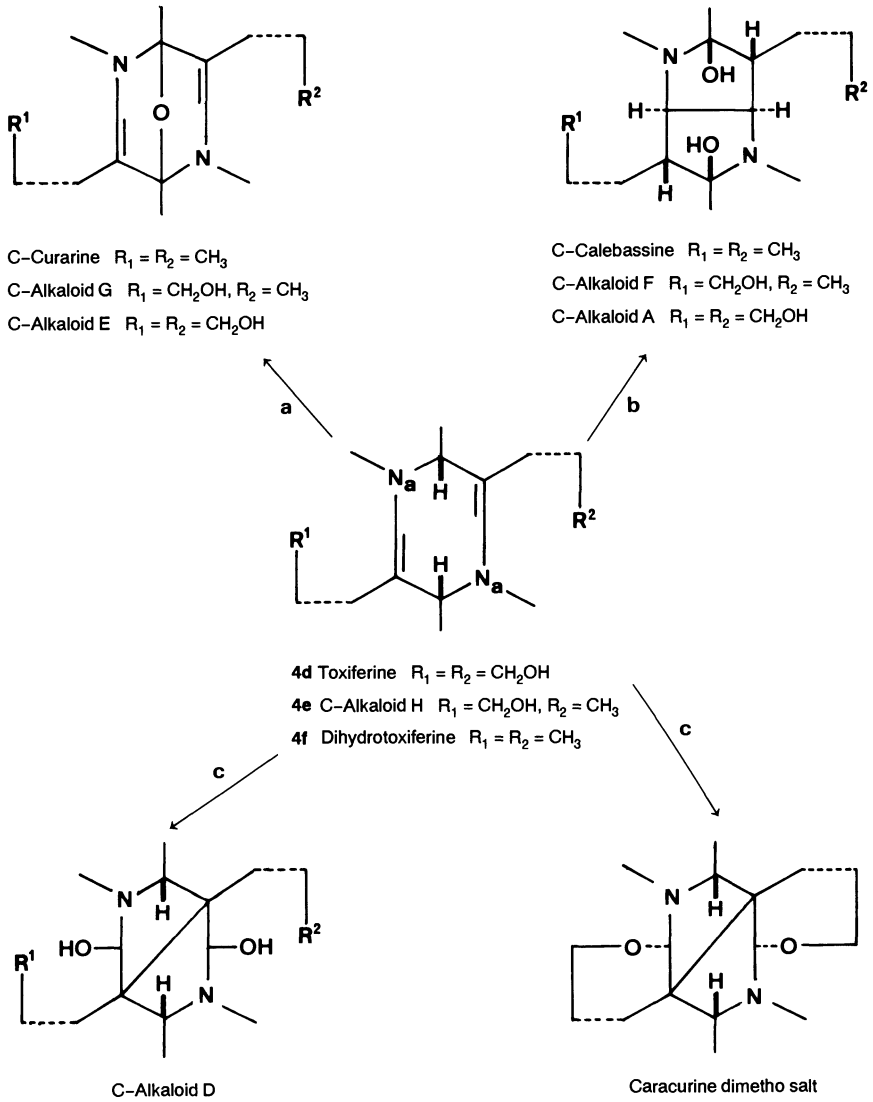
$R^1 = R^2$ = Me, 2 × N_b^+-Me

4g Alcuronium

$R^1 = R^2 = CH_2OH$, 2 × N_b^+-CH_2-CH=CH_2

tracts from *Strychnos* species and from curares prepared with them are so complex. Since, according to Bauer (32), aqueous solutions of curares tend to be acid, with pH values ranging from 4.5 to 6.0, under conditions of aerial oxidation it is not surprising that considerable alteration in the alkaloid composition takes place during the lengthy heating that most methods of preparation require for concentrating the poison.

As artefacts, dimers that are readily formed from monomeric precursors under mild conditions are obvious candidates, and the presence of acid during work-up can have far-reaching effects. In the bis-tertiary series, with ordinary methods of alkaloid extraction using acid, it is usually not possible to isolate bisnordihydrotoxiferine (**4c**), since it is highly labile and under such conditions rapidly isomerized and oxidized. However, Delle Monache et al.



SCHEME 1.1. Effects of heat, light, and acid in the presence of oxygen on the dimeric *Strychnos* alkaloids. Reagents: a. $O_2 + \Delta$, or $O_2 + h\nu$; b. $O_2 + h\nu$ + eosin, or O_2 + pyr/AcOH; c. O_2, H_3O^+.

TABLE 1.5. Alkaloids found in curare.

Origin	Alkaloids found	References
Colombia	Toxiferine, (−)-curine	246, 247
Venezuela	Dihydrotoxiferine, curarine, mavacurine, fluorocurine	423
Venezuela	0.76% Dihydrotoxiferine, 0.75% curarine, 2.06% **calebassine**, 0.024% fluorocurarine, and small amounts of C-alkaloids Q (macusine B), R, and S, and pseudofluorocurine	424
Middle Orinoco	**Curarine**, curarine II, dihydrotoxiferine, calebassine	244, 246, 247
Middle Orinoco (Piaróa ?)	**Curarine**, **calebassine**, toxiferine, norcurarine, fluorocurine, calebassinine, profluorocurine, fluorocurinine	248, 433, 434
Middle Orinoco (Panáre)	0.4% Panarine, 0.36% macusine B	425
Middle Orinoco (Hoti?)	1.1% **Venecurine**	426
Upper Orinoco (Piaróa)	1.54% Tertiary bases and 10.77% quaternary bases: 0.43% **curarine**, 0.15% calebassine, 0.023% fluorocurarine, 0.0077% dihydrotoxiferine, 0.0069% caracurine II, 0.015% fluorocurinine	427
Upper Orinoco, Río Ocamo	Macoline; peinamine (4.6 mg/arrow tip)	192, 193
Venezuela/Brazil border (Yanomamo)	(2D-PC): Curarine and 3 other bis-quaternary alkaloids of the toxiferine and calebassine groups, along with a small amount of (+)-tubocurarine	55
Brazil, Rio Uaupés (no. N III)	0.008% C-Alkaloid K (dihydrotoxiferine), 0.38% **curarine**, 0.801% **calebassine**, 0.096% C-alkaloid D, 0.002% C-alkaloid H, 0.032% C-alkaloid G, 0.009% C-alkaloid F, 0.022% toxiferine, 0.024% C-alkaloid E, 0.072% C-alkaloid A, fluorocurarine, fluorocurine, fluorocurinine, calebassinine; also 0.035% C-alkaloid B, 0.001% C-alkaloid C, 0.019% C-alkaloid I, 0.002% C-alkaloid J[a]	259
Rio Uaupés (nr. Jauareté)	1.57% **Curarine**, 0.48% calebassine, fluorocurarine, 0.71% C-alkaloid T (*O*-methylsarpagine),[b] C-alkaloid M (lochneram)[b]	428, 429
Rio Içá (nr. Brazil/Colombia border)	0.277% C-alkaloid O, 0.245% C-alkaloid P, 0.084% calebassine, 0.15% C-xanthocurine, 0.088% C-guianine	304
Brazil	Neoprotocuridine, protocuridine	306
Peru	(+)-Tubocurarine, chondrocurine, unknown base	430

TABLE 1.5 (*cont.*)

Origin	Alkaloids found	References
Peru	Ca. 16% (+)-Tubocurarine,[c] 1.7% (+)-chondrocurine, 1.5% (−)-curine, 0.65% (−)-nor-N_b-chondrocurine	431
Provenance unknown	**Curarine**	244, 245
Provenance unknown	2.72% **Curarine**, 0.86% calebassine, 0.28% C-alkaloid D, 0.17% dihydrotoxiferine, 0.06% C-alkaloid G, 0.04% C-alkaloid E, tr C-alkaloid A, 0.003% fluorocurarine, tr fluorocurine, mavacurine, 0.05% C-alkaloid BL, tr C-alkaloid I, 0.01% C-alkaloid V	330
Provenance not indicated (no. N IV)	2.8% **Curarine**, 1.84% **calebassine**, 0.1% fluorocurarine, 0.12% fluorocurinine, 0.068% C-alkaloid G, 0.02% toxiferine; also C-alkaloids B-E, fluorocurine[d]	259
Provenance unknown	4.7% (+)-Tubocurarine, 16% crude (−)-curine	240, 241
Provenance unknown	5.2% (+)-Chondrocurarine,[e] 3% (+)-protochondrocurarine, 0.5% (−)-isochondrocurarine,[e] 0.1% (+)-neochondrocurarine[e]	432

[a] Pooled fractions from several samples of curare also afforded mavacurine, fluorocurine, fluorocurinine, calebassinine, C-alkaloid Y (profluorocurine) and C-alkaloids L, M, and X [259, 283].
[b] These two alkaloids are not known to occur in *Strychnos* species and they pesumably came from an additive to the curare, perhaps a *Rauvolfia* species.
[c] Pharmacological evaluation of the curare showed that it contained 22.2% muscle-relaxant alkaloids calculated as (+)-tubocurarine.
[d] PC analysis of two further curares indicated that their alkaloid composition was very similar [323].
[e] These alkaloids were obtained after methylation and do not exist as such in the crude curare.

(292) were able to obtain a large amount of it from *S. pseudo-quina* bark by extraction with cyclohexane and subsequent chromatographic separation over florisil.

Among the examples of artefacts cited by Gorman et al. (267) are toxiferine **(4d)** and dihydrotoxiferine **(4f)**. As pointed out above, 18-desoxy-Wieland-Gumlich aldehyde metho salt **(2e)** is not known to occur naturally in *Strychnos* species, but on warming in the presence of acid it dimerizes very rapidly to **4f**, the isolation of which from an extract is no proof that it was present in the original material. However, the occurrence of calebassine (cf. Scheme 1.1) as a main alkaloid is taken to indicate the natural existence of **4f** (267).

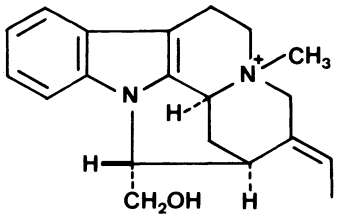
Curarine (Scheme 1.1), another alkaloid derived from dihydrotoxiferine, arises by mild oxidation; but it is also considered to have been isolated under conditions which do not suggest that it was an artefact. Biocca et al. (55), for

example, have identified curarine as one of the principal components in a sample of arrow-tip curare, a poison which is usually produced by fairly gentle means. C-alkaloid E (Scheme 1.1), an analogue of curarine, is formed in the same way from toxiferine.

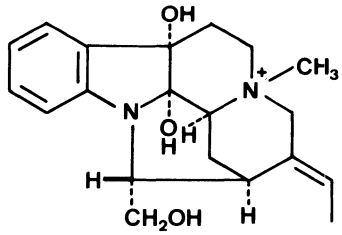
Jaeger et al. (330) analysed a 63-year old sample of *Strychnos*-based curare which yielded 12% crude quaternary alkaloids. They were able to isolate or detect at least 12 known bases, which together constituted about one-third of the fraction. Dihydrotoxiferine **(4f)** was a relatively minor component, and its associated derived bases curarine, calebassine, and C-alkaloid D together formed the bulk of the alkaloids; see Scheme 1.1 and Table 1.6. The analysis showed that, in spite of its age, the composition of the poison was similar to that of more recent samples.

According to the PC studies by Bauer (32, 191, 212, 215), in those poisons which had toxiferine **(4d)** as the principal base it was usually accompanied by its derivatives—almost as much C-alkaloid A and rather less C-alkaloid E. In other poisons, however, Bauer found calebassine and curarine to be the main alkaloids present and their parent base dihydrotoxiferine (C-alkaloid K) **(4f)** was usually no more than a minor component. These findings are in accord with the results of the analysis discussed in the previous paragraph and they also confirm the point made by Gorman et al. (267) that toxiferine is less reactive than dihydrotoxiferine, but much will depend on the circumstances under which the curares were prepared.

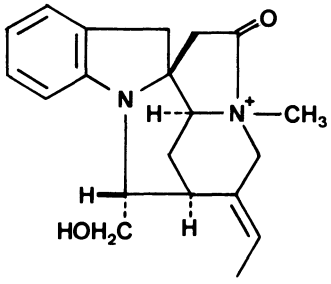
Experiments to test the basis of these ideas have not yet been carried out.



5 Mavacurine



6 Profluorocurine



7 Fluorocurine

It would also be of interest to compare the alkaloid composition of *Strychnos* starting materials and of curares made from them by some of the different procedures outlined in Section 1.2.4.

Several monomeric bases that have been isolated from plant materials and samples of curare are doubtless also artefacts. Thus, fluorocurine **(7)** is mavacurine pseudoindoxyl and will have been formed from mavacurine **(5)** through oxidation via profluorocurine **(6)**. C-Alkaloid O (Section 1.4.7.1) and calebassinine 1 (Section 1.4.7.2), both from curares, are further examples of presumed artefacts.

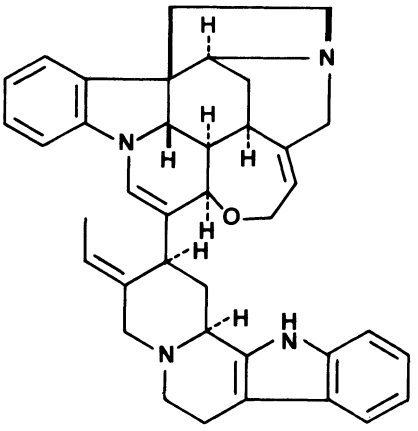
1.4.4. Dimeric Bases

1.4.4.1. X-Ray Crystallographic Studies

Jones and Nowacki (331) have provided decisive evidence for the structure of the central eight-membered ring and ether bridge in (C-)curarine di-iodide (cf. Scheme 1.1). The distance between the quaternary nitrogen atoms is 0.85 nm.

Making use of the dimethochloride octahydrate salt, Bourne et al. (332) have carried out an accurate determination of the crystal structure of caracurine II (Scheme 1.1). The fused rings of the molecule give it a rigid box-like structure, but much of the strain in and around the central ring is relieved by the lengthening of certain bonds: C(16)—C(16′), N(1)—C(2), C(2)—C(16), C(2)—C(7), C(15)—C(16), C(15)—C(20), and by the adjustment of certain bond angles. The N(4)—N(4′) distance is 0.88 nm, somewhat longer than in the corresponding methiodide salt where it is 0.85 nm (333) and almost identical with that for curarine di-iodide (above) See further, Section 1.5.2.

Fehlmann et al. (334) have carried out a X-ray crystallographic analysis of calebassine which confirms the structure established by degradative chemistry coupled with NMR studies (Scheme 1.1). The N(4)—N(4′) (inter-onium)



8 Longicaudatine

distance appears to be about 0.86 nm, as a result of deformation brought about by the presence of the central C(17)—C(17′) bridge (252).

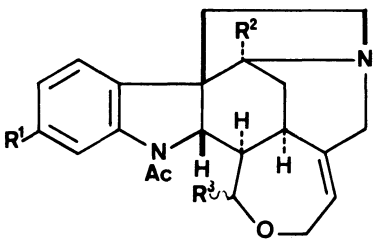
1.4.4.2. Longicaudatine

The quasidimer longicaudatine **(8)**, with a corynanthean-strychnan skeleton, is present in a variety of African and Asian *Strychnos* species and has now been found for the first time in an American species, *S. trinervis* (Table 1.4). Its structure is based largely on interpretation of its NMR spectra, especially the ^{13}C spectrum, which is very similar to those of the relevant parts of geissoschizine and strychnine. The compound is presumed to be derived via an enamine coupling between Wieland-Gumlich aldehyde and geissoschizal (475, 479).

1.4.5. Monomeric Bases

1.4.5.1. Wieland-Gumlich Aldehyde and Related Alkaloids

It is evident from Table 1.4 that Wieland-Gumlich aldehyde and related bases are among the most widely distributed alkaloids in South American *Strychnos* species, though to what extent they are genuine plant constituents rather than artefacts is difficult to make out. This is particularly true of 18-deoxy-Wieland-Gumlich aldehyde **(2b)** and Wieland-Gumlich aldehyde **(2a)** and their immediate derivatives, which, as already seen in Sections 1.4.2 and 1.4.3, may arise at least in part from degradation of the dimeric bases present. However, the compounds most often encountered are the N_a-acetylated diaboline **(9a)** and 11-methoxydiaboline **(9g)** and their *O*-acetylated derivatives. As far as known, these are not directly involved in the biosyn-



9a Diaboline
$R^1 = R^2 = H$, $R^3 = 17\beta$-OH

9b 3-Hydroxydiaboline
$R^1 = H$, $R^2 = OH$, $R^3 = 17\beta$-OH

9c α-*O*-Acetyl-3-hydroxydiaboline
$R^1 = H$, $R^2 = OH$, $R^3 = 17\beta$-OAc

9d Jobertine
$R^1 = R^2 = H$, $R^3 = 17\alpha$-OAc

9e Henningsamine
$R^1 = R^2 = H$, $R^3 = 17\beta$-OAc

9f Diaboline 17β-ethyl ether
$R^1 = R^2 = H$, $R^3 = 17\beta$-OEt

9g 11-Methoxydiaboline
$R^1 = OMe$, $R^2 = H$, $R^3 = 17\beta$-OH

9h Isocondensamine
$R^1 = OMe$, $R^2 = H$, $R^3 = 17\alpha$-OAc

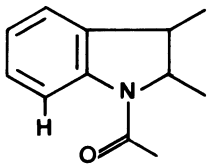
thetic processes and they probably represent storage forms to be used as and when required by the plant.

Wieland-Gumlich aldehyde and its allies present some interesting chemical features. Although it is possible to write "open" and "closed" structures for Wieland-Gumlich aldehyde **(1/2a)** and its N_a-acetyl derivative diaboline, NMR studies (339) have shown that they exist almost entirely in the "closed" form, with the seven-membered ring in a quasichair conformation; the anomeric H(17) has the α-configuration and its NMR signal is found at about δ 5.35 ($J \approx 2$ Hz). Acetylation leads to the formation of two epimeric N_a,*O*-diacetyl compounds: *O*-acetyldiaboline A, where the signal for the H(17) proton has moved downfield to δ 5.78 ($J \approx 1$ Hz), corresponding to an α-axial H(17), and *O*-acetyldiaboline B, with the signal located at δ 6.13 ($J \approx 2$ Hz) and indicating a β-equatorial H(17). The two substances are thus readily distinguished and, as Table 1.4 shows, they have both been isolated from South American *Strychnos* species under the names henningsamine **(9e)** (269, 315) and jobertine **(9d)** (272, 314), respectively.

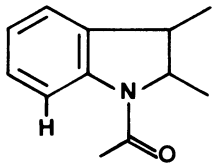
Treatment of Wieland-Gumlich aldehyde and diaboline with boiling ethanol/hydrochloric acid (339) or ethyl orthoformate/ammonium chloride (314) yields the corresponding 17β-ethyl ethers. Delle Monache et al. (314) have reported the isolation of diaboline 17β-ethyl ether **(9f)** from the root bark of *S. castelnaeana*. At first sight, the substance would seem to be nothing more than an artefact produced from the base and ethanol present in the chloroform used in the work up, but it is not formed under the conditions of the isolation procedure (ambient temperature) and it was not found in extracts from other diaboline-containing parts of the plant. Moreover, it was methanol that was used for the extraction and no diaboline methyl ether was detected. Nevertheless, it must be presumed that the ether is an artefact, though the conditions under which it is produced are obscure.[11]

Subsequently, Marini-Bettòlo et al. (271) obtained 11-methoxydiaboline **(9g)** from *S. romeu-belenii* and it has since been found in numerous other *Strychnos* species, both South American (Table 1.4) and African (251). NMR studies again show that essentially only one epimer is present, that with an α-axial H(17) (δ 5.25, $J \approx 2$ Hz). However, doubling of the signal for H(12), and broadening of others, also reveals the presence of cisoid (δ 7.57, $J \approx$ 2.5 Hz) **(10a)** and transoid (δ 7.08, $J \approx 7.8$ Hz) **(10b)** rotamers (conformers), brought about by restricted rotation of the N_a-acetyl group whose strong

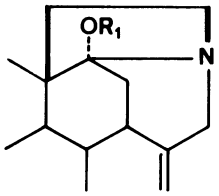
[11] The 17-ethyl ethers of diaboline and 11-methoxydiaboline have been isolated from the stem-bark alkaloids of *S. diaboli* (340). Similarly, the formation of very small amounts of what appear to have been two (epimeric ?) diaboline ethyl ethers has been noted during an investigation of *S. lucida* R. Br. from Thailand. In this case, they were isolated from the diaboline-rich mixture of alkaloids present in the stem bark, and it was assumed that they had arisen as a result of reaction with traces of ethanol still present in the redistilled chloroform used during work up. Again, the exact conditions under which the substances were formed are unclear (Bavovada and Bisset, unpublished observation).



10a Cisoid rotamer

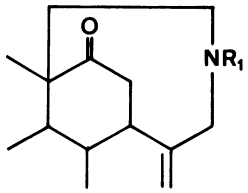


10b Transoid rotamer



11a Carbinolamine form
$R^1 = H$

11c *O*-Methyl ether
$R^1 = Me$



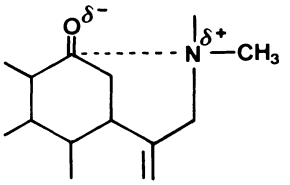
11b Ketoamine form
$R^1 = H$

11d N_b-Methyl derivative
$R^1 = Me$

magnetic anisotropy causes a downfield shift of the H(12) signal. Diaboline and congeners are known to exhibit the same phenomenon and it is also observed with other *Strychnos* alkaloids, notably members of the retuline/isoretuline (341) and spermostrychnine (315) series. Acetylation of (**9g**) at room temperature furnishes a 17-acetyl derivative (**9h**) which has the same stereochemistry as *O*-acetyldiaboline B (jobertine) (**9d**). It is therefore isomeric with condensamine, which occurs in an African species of *Strychnos*, and has been named isocondensamine (271).

From the upper trunk bark of *S. castelnaeana*, Galeffi et al. (313) have isolated small amounts of diaboline (**9a**) and 3-hydroxydiaboline (**9b**). The latter base, like 3-hydroxystrychnine (pseudostrychnine), exists almost entirely in the carbinolamine form (**11a**) and when treated with methanol forms an *O*-methyl derivative (**11c**). In contrast, when refluxed with methyl iodide the tautomeric equilibrium shifts and the compound behaves as a ketoamine (**11b**), yielding a N_b-methyl derivative (**11d**); further *N*-methylation, leading to the production of a classical quaternary base, is prevented by the transannular interaction between the N_b-methyl and 3-carbonyl groups (**12**).

In the ^1H NMR spectrum of **9b** the signal for the anomeric H(17) is a broadened doublet at δ 5.21 ($J \approx 2$ Hz). On *O*-acetylation, the signal becomes a singlet and moves to δ 6.07, this chemical shift indicating, as in the case of diaboline itself, a β-equatorial H(17) and thus an α-(pseudo-)axial configuration for the introduced acetyl group. Doubling of the signals for C(2), C(8),



12 Transannular interaction

C(10–13), and C(17) in its ^{13}C NMR spectrum again shows that the substance is a mixture of rotamers (313).

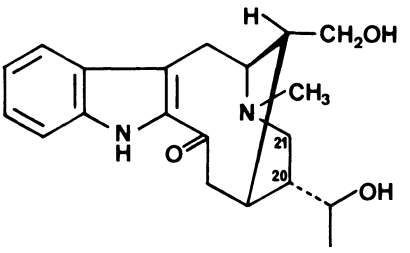
9b was synthesized in a series of reactions that parallels the strychnine/3-hydroxystrychnine (pseudostrychnine) rearrangement, i.e. treatment of the base *N*-oxide with aqueous potassium chromate; as with strychnine, the neutral 5-oxo derivative is a by-product of the reaction. This rearrangement, which is related to the Polonovsky reaction, also takes place in the presence of Fe^{3+} ions (313).

1.4.5.2. Erichsonine

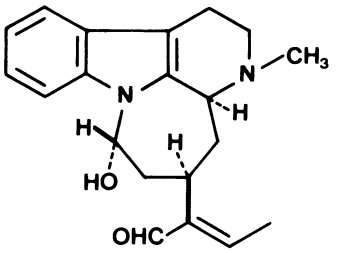
Spectral and X-ray crystallographic analysis has established the structure of the major alkaloid (0.11%) of *S. erichsonii* stem bark from French Guiana, erichsonine, as (**13**). It is a vobasine-type base, evidently related to 16-*epi*-affinine, and is of a type that has not previously been found in *Strychnos* species (338).

1.4.5.3. Melinonine E

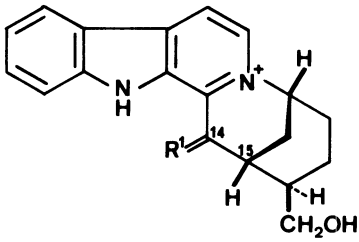
Borris et al. (335) have reinvestigated the structure of melinonine E, whose isolation was originally reported by Bächli et al. (309), with the aid of more modern techniques (335). The molecular formula of the base, determined by mass spectrometry, has been revised from $C_{20}H_{23}N_2O^+$ to $C_{19}H_{21}N_2O^+$. Detailed analysis of the ^1H and ^{13}C NMR spectra leads to structure **15a**, that of an anhydronium base with a new skeletal arrangement. Doubling of the



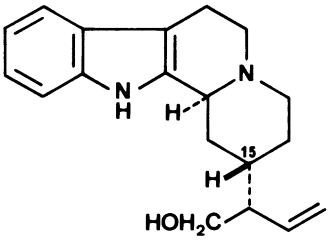
13 Erichsonine



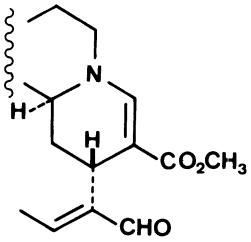
14 Akagerine



15a Melinonine E
$R^1 = H_2$
15b Strychnoxanthine
$R^1 = O$



16 Antirhine



17 Vallesiachotamine

NMR signals for C(15) and C(20) is thought to be due to the presence of two conformers originating in rotation about the bond joining C(20) and C(21).

At about the same time, Coune et al. (336) independently reported the isolation from roots of the African *S. gossweileri* Exell and structure determination of strychnoxanthine (**15b**), which is now seen to be the 14-oxo derivative of the curare alkaloid. The two substances belong to a group of compounds having the rather unusual H(15) β-configuration, the occurrence of which is explained by their presumed biosynthesis from antirhine (**16**) or vallesiachotamine (**17**) through an additional cyclization between C(17) and C(18) (336, 475). The presence of the latter base has not yet been demonstrated in South American *Strychnos* species.

1.4.6. Alkaloids of Menispermaceae Associated with Curare

In accord with the greater range of menispermaceous genera that are utilized as components of curares, chemical studies have shown that they contain a wide variety of isoquinoline and related alkaloids: bis-benzyltetrahydroisoquinolines, benzyltetrahydroisoquinolines, proaporphines, aporphines, oxoaporphines, azafluoranthenes, tropoloisoquinolines, and isoquinolobenzaze-

pines. One point that has emerged from recent studies of curare plants is that quaternary bis-benzyltetrahydroisoquinoline alkaloids other than (+)-tubocurarine may be important active principles in certain curares. Table 1.6 contains a listing of the Menispermaceae associated with curare, together with the alkaloids they contain.[12] More recent developments in the chemistry of some of the above-mentioned groups of alkaloids are dealt with in a number of reviews: azafluoranthenes and tropoloisoquinolines (342) and bis-benzyltetrahydroisoquinolines (343–347, 544) and obviate the need for extended discussion here. The following sections highlight some of the more interesting discoveries, but do not aim to treat the various topics in detail. Attention is also drawn to certain loose ends, i.e. to certain alkaloids that have been isolated but whose structures have yet to be fully established.

1.4.6.1. *Chondrodendron*

The alkaloids occurring in *Chondrodendron* species have long been recognized as benzyltetrahydroisoquinoline derivatives. A variety of bis- and monoquaternary and bis-tertiary bases has been found, in which the two halves are joined head-to-tail through two ether linkages: in the curine skeleton between 8–12′ and 11–7′ (cf. **18a**) and in the isochondrodendrine skeleton between 8–12′ and 12–8′ (**19a**). It is the quaternary alkaloids that are the main active principles.

No new work on *C. tomentosum* has been reported for the last 30 years, so that there has been little advance in our knowledge of its alkaloid composition. Available information on the chemistry of the species indicates that its quaternary alkaloids require more thorough investigation. *C. tomentosum* is still the only reported natural source of (+)-tubocurarine (**18j**). Wintersteiner and Dutcher (350) and Dutcher (351) isolated it from material from the Upper Amazon, and it proved to be identical with the alkaloid that King (241) had first obtained from a museum sample of curare originating from the Río Ucayali area of Peru (290, 396). King (354) examined other materials from Peru as well: stems of "*amphi huasca*" from Tarapoto yielded a little (−)-tubocurarine (**18l**) and no (+)-tubocurarine, while collections from Madre de Dios and Sisa afforded (+)-tubocurarine. In view of his previous findings with *C. microphyllum* and *C. platiphyllum*, which had yielded (+)-curine (**18d**) and

[12] In this connection it is of interest to note that Menispermaceae are also components of African arrow poisons; The roots of *Triclisia dictyophylla* Diels (*T. gilletii* (De Wild.) Staner) are reported to be used in the southwestern part of the Central African Republic (348). Phaeanthine (**20h**) and the bis-quaternary *N,N′*-dimethylphaeanthine (**20i**) are both present in the plant. In rabbits and rats, the latter base appears to have about one-tenth of the activity of (+)-tubocurarine (349); it seems unlikely that it will contribute much to the over-all effect of the arrow poison, which therefore cannot be classed as a curare.

Similarly, the roots of *Penianthus longifolius* Miers are an arrow-poison ingredient in the Central African Republic (348).

TABLE 1.6. The alkaloids of menispermaceous genera used in the preparation of curare.

Genus and Species[a]	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids present	References
Chondrodendron					
**C. tomentosum*	Peru: San Martín	sb	active	4.8% Tertiary bases; quaternary bases also present	136
	Peru/Ecuador: Upper Amazon	b + st[d]		**(+)-Isochondrodendrine**, cycleanine, (+)-chondrocurine, (−)-curine, (+)-chondrocurarine; **(+)-tubocurarine**, (−)-tomentocurarine	350–353
	Peru: Tarapoto, San Martín	st		5% **(−)-Curine**; 0.11% (−)-tubocurarine, 0.003% (−)-*O*-methyltubocurarine (after methylation)	354
	Peru: Madre de Dios	st	active	(−)-Curine, cycleanine, (+)-chondrocurine, (+)-isochondrodendrine; (+)-tubocurarine	355
	Peru: Sisa, San Martín	st		0.24% **(−)-Curine**, 0.21% (+)-chondrocurine, 0.18% (+)-isochondrodendrine, 0.013% (+)-tomentocurine; 0.53% **(+)-tubocurarine**[e]	355
				(−)-Norcycleanine, *N*-benzylphthalimide, (−)-curine, (+)-chondrocurine, (+)-tomentocurine, (+)-isochondrodendrine, possibly alkaloid B from *Curarea tecunarum* (*Chondrodendron limaciifolium*)	356
	Peru: San Martín	w		Isochondrodendrine, curine	357
C. microphyllum	Brazil: Bahia	r		2.03% **(+)-Isochondrodendrine**, 1.80% (+)-curine	169
C. platiphyllum	Brazil: Bahia	r		0.24% (+)-Isochondrodendrine, 7.89% **(−)-curine**	169
		st		5.26% **(−)-Curine**	
		l		0.013% (+)-Isochondrodendrine, 0.003% (−)-curine, 0.077% (−)-chondrofoline	
	Brazil: Rio de Janeiro	r		5.86% **(+)-Isochondrodendrine**, 1.05% (−)-curine	

Curarea					
**Curarea toxicofera*	Brazil: Manaus	st		0.19% Tertiary bases: 0.037% **(−)-curine**, 0.02% (−)-toxicoferine,[f] 0.0091% (+)-isochondrodendrine	358
**Curarea candicans*	Guyana	st		1.1% **(+)-Isochondrodendrine**, 0.33% (+)-curine	169
	Bolivia (?)	r		1.3% (−)-Limacine, 0.05% (+)-candicusine, 0.033% (+)-limacusine, 0.033% (−)-krukovine, 0.0013% (−)-limacine 2′β-*N*-oxide, 0.0009% (−)-limacine 2′α-*N*-oxide, 0.0005% (−)-limacine 2β-*N*-oxide	359
**Curarea tecunarum* (*Chondrodendron limaciifolium*)	Brazil: Amazonas	sb[g]	None; toxic	Aqueous extract; alkaloids present	281
			weak; toxic	Ethanolic extract; alkaloids present	
		sw	very weak; toxic	Ethanolic extract; alkaloids present	
	Brazil: Amazonas	sb	none; toxic	6.2% Tertiary bases	136
			none; toxic	Quaternary bases	
		w		4.13% Total alkaloids, incl 1.18% water-soluble bases; mostly **(+)-isochondrodendrine**, alkaloids A and B	356, 360
Sciadotenia					
S. eichleriana	French Guiana			0.04% Total alkaloids: **(+)-coclaurine**, **(+)-stepharine**, (−)-grisabine	361
		r		0.1% Total alkaloids: **(+)-coclaurine**, **(+)-stepharine**, (+)-2-nor-limacusine	
S. cayennensis	French Guiana			(+)-Launobine, (+)-actinodaphnine	361
**S. toxifera*	Peru: Cajamarca and San Martín	st		1.7% Tertiary bases: 0.08% (+)-isochondrodendrine, 1.00% **sciadenine**, 0.41% sciadoferine, 0.085% sciadoline	362, cf. 363, 364
	Peru: San Martín	w		(+)-*O*,*O*′-Dimethylcurine, (−)-isochondrodendrine, *epi*-norcycleanine	357

(*cont.*)

TABLE 1.6 (*cont.*)

Genus and Species[a]	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids present	References
Abuta					
**A. grandifolia*	Brazil: Acre	st		Grandirubrine	365; cf. 366[h]
**A. grisebachii*	Peru: Loreto	st		0.031% (−)-7-*O*-Demethylpeinamine, 0.041% (−)-macolidine, 0.08% (−)-peinamine, 0.068% (−)-*N*-methyl-7-*O*-demethylpeinamine, 1.1% **(−)-macoline**	367
				0.07 or 0.16% (−)-magnoline (grisabutine)	367, 368
				0.051% (−)-Grisabine	368
**A. imene*	Brazil: Amazonas	sb[i]	some	Both the total alkaloids and the tertiary bases (3.5%) are active	136
		st		Imerubrine, **imenine**, homomoschatoline, imeluteine, rufescine, norrufescine	369–371
**A. pahni*	Bolivia: Alto Beni	st		(−)-2′-*N*-Nordaurisoline, (−)-2-*N*-methyllindoldhamine, (−)-2′-*N*-methyllindoldhamine, (+)-coclaurine, (−)-daurisoline, (−)-lindoldhamine, dimethyllindoldhamine, (+)-stepharine, thalifoline	375
A. panurensis	Brazil: Amazonas	st		0.20% (−)-Panurensine, 0.13% (−)-norpanurensine	372
**A. rufescens* (*A. splendida*)	Peru: San Martín	st		0.15% (+)-Aromoline, 0.002% (+)-homo-aromoline, 0.095% (−)-krukovine, 0.002+% splendidine, imenine, lysicamine, 0.02% homomoschatoline	373, 374
	Brazil: Amazonas	st[j]		0.0006% lmerubrine, 0.0058% imenine, 0.0063% homomoschatoline, 0.0008% imeluteine, 0.0006% rufescine, 0.0052% norrufescine	370

(*cont.*)

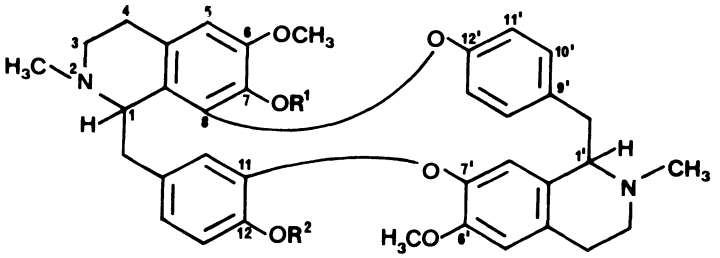
Anomospermum					
**A. grandifolium*	Peru: Loreto	st	some	Tertiary and quaternary bases present	355
Telitoxicum					
T. glaziovii	Brazil: Pará	st		0.04% Non-phenolic bases: 0.0009% lysicamine, 0.0003% telazoline, 0.0002% teladiazoline, 0.0001% *O*-methylmoschatoline; 0.019% phenolic bases	377
**T. minutiflorum*	Brazil: Amazonas	sb	weak; slightly toxic	0.05% Tertiary bases	136, 281
**T. peruvianum*	Peru: San Martín	st		0.13% Tertiary bases: 0.0025% subsessiline, 0.00005% norrufescine, 0.00005% telitoxine, 0.00005% peruvianine, 0.000076% lysicamine, 0.00015% telazoline	376
Cissampelos					
[*C. mucronata*	Angola			**(+)-Isochondrodendrine** and 4 other (minor) bases, possibly incl. cycleanine]	378
**C. ovalifolia*	Guyana	r	active	Ethanolic extract equivalent to 0.3% (+)-tubocurarine	379
				Warifteine, methylwarifteine, *O*-methylcissampareine, and corresponding dihydro derivatives	380, 381
**C. pareira*	Peru: Huánuco	wp		2.5% Crude alkaloids: 1.2% (−)-cissampareine	382
	Jamaica	r/st		0.3% (−)-curine, 0.014% 12-*O*-methyl-(+)-curine	383
	Ghana	r		0.012% Dehydrodicentrine, 0.018% (+)-dicentrine, 0.0033% (−)-cycleanine, 0.002% (+)-insularine, 0.0035% (+)-isochondrodendrine	384
	Madagascar	r		1.6% Total alkaloids: 0.06% (−)-curine, 0.34% **(+)-isochondrodendrine**, 0.22% crude hayatine	385
	India: Kashmir	r		0.65% **Hayatine**, 0.013% hayatinine, hayatidine	386–389
	India: Uttar Pradesh	r		0.15% Hayatine, 0.33% **(−)-curine**	386
	India: Lucknow	rb		0.03% (−)-Curine, 0.074% menismine (as picrate), 0.32% **cissamine**,[k] 0.17% pareirine (as picrate)	390, 391

(*cont.*)

TABLE 1.6 (*cont.*)

Genus and Species[a]	Locality	Plant part[b]	Curarizing activity[c]	Alkaloids present	References
	India: Madras	r + st		1.47% **(−)-Curine**, 0.20% (+)-isochondrodendrine, 0.022% hayatine[l]	170
	India:	l		Cycleanine, (−)-curine, hayatidine, hayatinine, hayatine	392
	China			(±)-Curine, cycleanine[m]	393

[a] The asterisk indicates a species reported to be an ingredient in curares.
[b] The abbreviations for the plant parts are listed in footnote b to Table 1.4.
[c] About the reported curarizing activities, see footnote c to Table 1.4.
[d] The work of Wintersteiner and Dutcher [350] and Dutcher [351, 352] was carried out on a sample of curare known to have been prepared from the single species *C. tomentosum*, the identification of which was documented by herbarium material. Cf. Krukoff and Barneby [135] and Dutcher [351].
[e] King [355] also investigated a commercial product from the Cuzco region of Peru, the identity of the plant source again being assured from accompanying leaf material. (+)-Tubocurarine and (−)-curine were the principal alkaloids present.
[f] Cava et al. [358] retain this name for a readily isolated 1:1 molecular complex of (−)-curine and (−)-tubocurine that behaves as a single substance.
[g] De Lacerda [394] received bark from the same locality where Schwacke had seen the Tikuna preparing curare. He accepted Schwacke's identification of the plant as *Anomospermum grandifolium*. In experiments on various animals, extracts of the material were shown to have the typical paralysing activity of curare. As explained by Krukoff and Moldenke [139: p. 73], the plant concerned was probably *Chondrodendron limaciifolium*, i.e. *Curarea tecunarum*.
[h] Da Rocha et al. [366] reported the isolation of palmatine from *A. grandifolia*, but the identity of their plant material cannot be verified.
[i] In Folkers and Unna [136] as *Elissarrhena grandifolia* (Eichler) Diels. The identification of the documenting collection, *Krukoff 8605*, was later changed to the one indicated [135].
[j] The identity of the material cannot be verified [374].
[k] Identical with the protoberberine alkaloid cyclanoline (**34g**) [391].
[l] Kupchan et al. [170] discuss the origin of the old drug *radix pareirae bravae*, and in view of the similarity in alkaloid composition of some forms of *C. pareira* to that of *Chondrodendron microphyllum* and *Chondrodendron platiphyllum* (q.v.) they suggest that *C. pareira* may have been a further source of the drug. However, the particular combination of alkaloids they encountered has not yet been found in material from Brazil.
[m] Alkaloid B from *Cissampelos pareira* [395].



18a (–)-Curine [(–)-Bebeerine]

$R^1 = R^2 = H$, ···H(1), ···H(1') (*R,R'*)

18b Chondrocurine [(+)-Tubocurine]

$R^1 = R^2 = H$, ···H(1), ◂H(1') (*R,S'*)

18c (–)-Tubocurine

$R^1 = R^2 = H$, ◂H(1), ···H(1') (*S,R'*)

18d (+)-Curine [(+)-Bebeerine]

$R^1 = R^2 = H$, ◂H(1), ◂H(1') (*S,S'*)

18e Chondrofoline

$R^1 = Me$, $R^2 = H$, ◂H(1), ◂H(1') (*S,S'*)

18f Hayatidine

$R^1 = H$, $R^2 = Me$, ◂H(1), ···H(1') (*S,R'*)

18g 12-*O*-Methyl-(+)-curine

$R^1 = H$, $R^2 = Me$, ◂H(1), ◂H(1') (*S,S'*)

18h *O,O*-Dimethylcurine

$R^1 = R^2 = Me$, ···H(1), ···H(1') (*R,R'*)

18i Hayatinine

$R^1 = R^2 = Me$

18j (+)-Tubocurarine

$R^1 = R^2 = H$, $\overset{\curvearrowright}{N}Me_2.X^-$, $\overset{\curvearrowright}{N}'MeH.X^-$, ···H(1), ◂H(1') (*R,S'*)

18k Isotubocurarine

$R^1 = R^2 = H$, $\overset{\curvearrowright}{N}MeH.X^-$, $\overset{\curvearrowright}{N}'Me_2.X^-$, ···H(1), ◂H(1') (*R,S'*)

18l (–)-Tubocurarine

$R^1 = R^2 = H$, $\overset{\curvearrowright}{N}Me_2.X^-$, $\overset{\curvearrowright}{N}'MeH.X^-$, ◂H(1), ···H(1') (*S,R'*)

18m *O,O'*-Dimethyl-(+)-tubocurarine

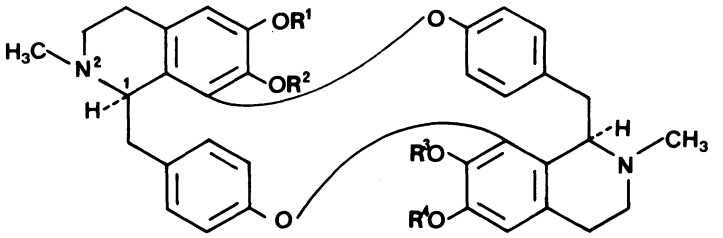
$R^1 = R^2 = Me$, $\overset{\curvearrowright}{N}Me_2.X^-$, $\overset{\curvearrowright}{N}'MeH.X^-$, ···H(1), ◂H(1') (*R,S'*)

18n Chondrocurarine

$R^1 = R^2 = H$, $\overset{\curvearrowright}{N}Me_2.X^-$, $\overset{\curvearrowright}{N}'Me_2.X^-$, ···H(1), ◂H(1') (*R,S'*)

18o *N,O,O'*-Trimethyl-(+)-tubocurarine [Metocurine]

$R^1 = R^2 = Me$, $\overset{\curvearrowright}{N}Me_2.X^-$, $\overset{\curvearrowright}{N}'Me_2.X^-$, ···H(1), ◂H(1') (*R,S'*)



19a (+)-Isochondrodendrine

$R^1 = R^4 = Me$, $R^2 = R^3 = H$ (*R,R'*)

19b Protocuridine

$R^1 = R^3 = H$, $R^2 = R^4 = Me$, stereochemistry unknown

19c Neoprotocuridine

$R^1 = R^4 = Me$, $R^2 = R^3 = H$ or vice versa, stereochemistry unknown

19d Sciadoline

$R^1 = R^3 = R^4 = Me$, $R^2 = H$, $\Delta^{1(2),3}$ (*R'*)

19e Sciadoferine

$R^1 = R^3 = R^4 = Me$, $R^2 = H$, $\Delta^{1(2)}$ (*R'*)

19f (-)-Nor-cycleanine

$R^1 = R^2 = R^4 = Me$, $R^3 = H$ (*R,R'*)

19g Sciadenine

$R^1 = R^3 = R^4 = Me$, $R^2 = H$, ◂H(1) (*S,R'*)

19h (-)-Cycleanine (*O,O*-Dimethylisochondrodendrine)

$R^1 = R^2 = R^3 = R^4 = Me$ (*R,R'*)

(−)-curine (**18a**), respectively (Table 1.6), King thought that the various samples he had worked up had perhaps come from two difficult-to-distinguish species. Possibly relevant to this point is the note on a herbarium specimen of *C. tomentosum*:

T. Plowman, J. Schunke V., and P.M. Perry 11408, coll. 14/12/81,... right bank Río Huallaga, opposite Tocache Nueva, San Martín, Peru. Said to be the most active curare, distinguished from inactive form by having a yellow rather than rose-colored pith when stem is cut; *ampihuasca* (INPA, K; abbreviations, Table 1.1).

However, as already seen (Section 1.2.5.2), according to Ramón Ferreyra (209) in the Huallaga Valley both are used in the large-scale preparation of curare. Thus, in spite of the morphological identity of the leaves, it seems that two forms of *C. tomentosum* can be recognized—one with a whitish wood and one with a reddish wood. Whether King's (−)-tubocurarine, which has only once been isolated, might have come from one or other of these two forms or whether, as Wintersteiner (397) has suggested, the length of time between harvesting and extracting the dried plant material may have been a determin-

ing factor is not known.[13] Similar considerations apply with regard to the origin of the unknown laevorotatory base that has been isolated several times from samples of curare (see below and also Section 1.4.7.7). Be that as it may, in view of the great difference in muscle-relaxant activity of the two tubocurarine isomers (Section 1.5), and the commercial implications that this has, both points merit further study.

King (355) isolated a phenolic tertiary base that he called (+)-tomentocurine. It was subsequently reisolated by Bick and Clezy (356) who suggested that it might belong to the chondrocurine series (cf. **18b**), but with a different arrangement of the hydroxyl and methoxyl functions, rather than to the isochondrodendrine series (cf. **19a**) as King had speculated. There is an added complication in that Dutcher (353) reported on a new quaternary base to which he gave the name (−)-tomentocurarine,[14] $C_{38}H_{44}N_2O_6^{2+}$, chloride: plates (dil. HCl), m.p. 270–275°C (dec.), $[\alpha]_D$ −157° (water), curarizing activity less than 20% of that of (+)-tubocurarine. Dutcher thought that it might be a hydroxyl and methoxyl isomer in the (+)-tubocurarine/(+)-chondrocurarine series. Was the alkaloid perhaps an impure preparation of the unknown laevorotatory base that has been encountered in certain samples of curare? Cf. Section 1.4.7.7.

Especially noteworthy is the proof, well established by NMR (398, 399) and X-ray crystallographic (400, 401) studies, that (+)-tubocurarine (**18j**) is a **mono-** rather than bis-quaternary alkaloid; this structural revision has necessitated some adjustment to the theoretical understanding of the mechanism of its muscle-relaxant action (Section 1.5). X-ray crystallographic studies have shown that the conformations adopted by the dichloride (400) and dibromide (401) salts of (+)-tubocurarine are somewhat different, with the inter-onium distance being fixed at 1.07 nm and 0.90 nm, respectively. The aromatic rings of the two benzyl moieties are oriented perpendicularly to the tetrahydroisoquinoline rings. NMR work on various curine derivatives, including (+)-tubocurarine dichloride, indicates that in solution the disubstituted benzene ring has some degree of rotational freedom, unlike the trisubstituted ring which is fixed because of its *meta*-attachment. The solution conformation of the dichloride is like the crystal conformation that has been determined for the dibromide and the *N,O,O'*-trimethylated derivative (**18o**). This has an

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[13] Wintersteiner noted that extracts of air-dried stems which had been stored for several years had very low curarizing activity; moreover, they had only small amounts of quaternary alkaloids from which no (+)-tubocurarine could be obtained. This is in sharp contrast to the keeping properties, under normal conditions of storage, of extracts of the plant in the form of curares (Section 1.5.1). Perhaps other plant components of the poisons contain natural anti-oxidants which help to retard deterioration of the active principles.

[14] Dutcher pointed out that the two names implied that (−)-tomentocurarine was the quaternary base derived from the enantiomorph of (+)-tomentocurine. Cf. the relationships between the alkaloids with names ending in "-curarine" (**18i–l**) and "-curine" (**18a–d**). However, no such connection has been established.

almost entirely hydrophobic concave surface and a hydrophilic convex surface with the six ether oxygens lying along a fold which divides the molecule into two halves (502).

Acceptable analytical criteria for (+)-tubocurarine have been established (402, 450). Information on TLC and HPLC systems that will allow both the detection and quantitation of the alkaloid and its congeners in curare and biological materials, as well as in pharmaceutical preparations, has been compiled (263, 403–405, 450, 560).[15]

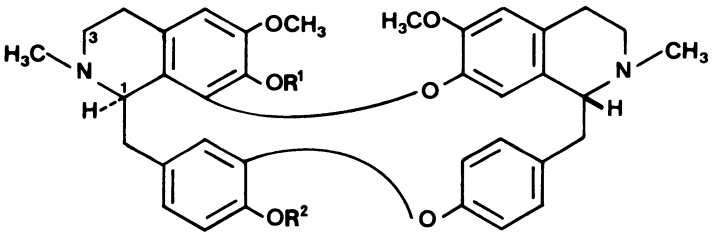
X-ray crystallographic analysis of cycleanine (*O,O*-dimethylisochondrodendrine) (**19h**) shows that its conformation is not dissimilar to that of tubocurarine, with a hydrophobic and a hydrophilic surface and the nitrogens protruding from the body of the molecule on either side (395). Only minor changes in the conformation are to be expected on quaternization.

1.4.6.2. *Curarea*

As evident from Table 1.6, *Curarea* species likewise contain alkaloids based on curine (cf. **18a,d**) and isochondrodendrine (cf. **19a**). In *C. candicans*, the species which has been examined in most detail (359), both these compounds occur in the stems, while the chief dimeric alkaloid of the roots is the bistertiary (−)-limacine (**20d**) in which the two halves are joined head-to-head and tail-to-tail between 8–7′ and 11–12′. Among the accompanying alkaloids, there were small amounts of three *N*-oxides, which, on reduction with zinc and hydrochloric acid, yielded (−)-limacine. Weak peaks at *m/z* 624 in their mass spectra indicated the presence of one extra oxygen atom as compared with the parent base. In the NMR spectra of two of the compounds the location of the signal for the 2-*N*-methyl group was almost unchanged, while that for the 2′-*N*-methyl had moved downfield, from δ 2.64 to 2.97 and 3.45, respectively. To differentiate the two *N*-oxides, it was noted that the H(1′) signals were at δ 4.70 and 4.52. It follows that in the first *N*-oxide the two groups are *anti* and in the second one *syn*, so that the two dimers are, respectively, (−)-limacine 2′β-*N*-oxide (**20e**) and 2′α-*N*-oxide (**20f**). NOE difference spectroscopy confirmed the assignment of the structures.

The NMR data for the third *N*-oxide—the 2-*N*-methyl signal at δ 2.98 and

[15] Tubocurarine figured in one of the longest criminal trials ever held in the United States—the Dr. X or Jascalevich murder trial—in which the defendant was accused of having murdered several patients with curare, i.e. with tubocurarine. The problem resolved itself into whether tubocurarine could be detected in buried cadavers after a lapse of 10 or more years. Both sides in the trial made use of sophisticated methods of detection, among them TLC, HPLC, RIA, and selected-ion direct-inlet MS. The defence was able to show that the embalming fluids and the tissues destroyed tubocurarine at a rate such that no reasonably administered amount could be expected to survive the period during which the cadavers had been buried before being re-examined. At the end of an 8-months trial, the jury brought in a verdict of not guilty after deliberating for less than 2 hours. For the background to this unusual case and the scientific evidence involved, see: Hall and Hirsch (406) and Siegel et al. (407).



20a (–)-2-Nor-limacine
R^1 = H, R^2 = Me, N(2)-H (*R,R'*)

20b (–)-Krukovine
$R^1 = R^2$ = H (*R,R'*)

20c (–)-Caryolivine
R^1 = H, R^2 = Me $\Delta^{1(2),3}$ (*R,R'*)

20d (–)-Limacine
R^1 = H, R^2 = Me (*R,R'*)

20e (–)-Limacine 2'β-*N*-oxide
R^1 = H, R^2 = Me, $\overset{+}{N}$'◂O^- (*R,R'*)

20f (–)-Limacine 2'α-*N*-oxide
R^1 = H, R^2 = Me, $\overset{+}{N}$'···O^- (*R,R'*)

20g (–)-Limacine 2β-*N*-oxide
R^1 = H, R^2 = Me, $\overset{+}{N}$◂O^- (*R,R'*)

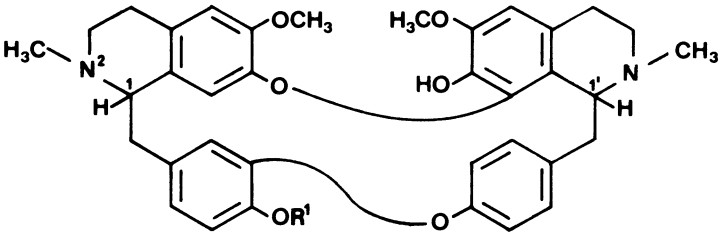
20h Phaeanthine
$R^1 = R^2$ = Me (*R,R'*)

20i *N,N'*-Dimethylphaeanthine
$R^1 = R^2$ = Me, 2 × $\overset{+}{N}Me_2.X^-$ (*R,R'*)

the H(1) signal at δ 4.90—indicated that the extra oxygen atom was in the left-hand part of the molecule with the two functions in an *anti* relationship; again, NOE difference spectroscopy confirmed the proposed structure, (–)-limacine 2β-*N*-oxide (**20g**). The authors suggest that as none of the other dimers present in the plant yielded *N*-oxides the three compounds isolated were the products of enzyme-catalysed reactions rather than artefacts. So far, only a few naturally occurring bis-benzyltetrahydroisoquinoline *N*-oxides have been encountered.

(+)-Limacusine (**21f**), in which the two halves are linked by 7–8' and 11–12' bridges, and candicusine, its 12-*O*-demethyl derivative (**21c**), are among the minor alkaloids present in *C. candicans.*

During their examination of *C. tecunarum* (*Chondrodendron limaciifolium*) wood, along with the known base isochondrodendrine (**19a**), Barltrop and Jeffreys (360) obtained two new alkaloidal components, bases A and B. Bick and Clezy (356) suggest that the former, evidently isomeric with isochon-



21a (–)-1,2-Dehydro-2-nor-limacusine

R^1 = Me, $\Delta^{1(2)}$, no N(2)-Me, ◂H(1') (*R*')

21b (+)-2-Nor-limacusine

R^1 = Me, N(2)-H, ···H(1), ◂H(1') (*R,R*')

21c Candicusine

R^1 = H, ···H(1), ◂H(1') (*R,R*')

21d (+)-Aromoline

R^1 = H, ···H(1), ···H(1') (*R,S*')

21e Macolidine

R^1 = H, ◂H(1), ◂H(1') (*S,R*')

21f Limacusine

R^1 = Me, ···H(1), ◂H(1') (*R,R*')

21g (+)-Homoaromoline

R^1 = Me, ···H(1), ···H(1') (*R,S*')

21h Macoline

R^1 = H, $\overset{+}{N}{}'Me_2.X^-$, ◂H(1), ◂H(1') (*S,R*')

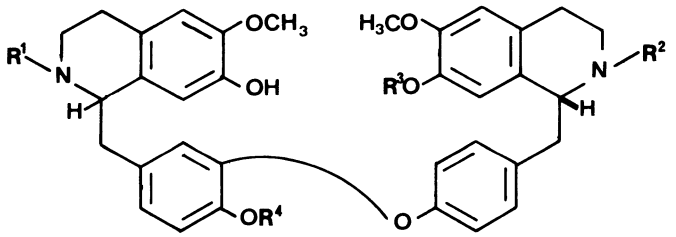
drodendrine, could be a diastereoisomer of protocuridine (**19b**) and that the latter may be an enantiomer of norcycleanine (**19f**).

No quaternary alkaloids have so far been isolated from any member of the genus.

1.4.6.3. *Sciadotenia*

The stems of *S. toxifera* have furnished several bis-tertiary dimeric alkaloids with the isochondrodendrine skeleton. These include sciadoline (**19d**) and sciadoferine (**19e**), with an imino grouping, as well as bases of the *R,R'* series, like isochondrodendrine (**19a**) itself, and of the *S,R'* series, like sciadenine (**19g**). The wood, on the other hand, is reported to contain both curine- and isochondrodendrine-derived dimers. See further Table 1.6.

Along with dimers having one (11–12') and two (7–8' and 11–12') links, (–)-grisabine (**22h**) and 2-norlimacusine (**21b**), *S. eichleriana* has yielded the benzyltetrahydroisoquinoline (+)-coclaurine (**26a**) and also the proaporphine (+)-stepharine (**27a**). *S. cayennensis* has two aporphines, (+)-launobine (**28a**) and (+)-actinodaphnine (**28b**), but no dimeric bases.



22a (–)-Lindoldhamine

$R^1 = R^2 = R^3 = R^4 = H$, ···H(1) (*R,R'*)

22b 2-*N*-Methyllindoldhamine

$R^1 = Me$, $R^2 = R^3 = R^4 = H$, ···H(1) (*R,R'*)

22c 2'-*N*-Methyllindoldhamine

$R^1 = R^3 = R^4 = H$, $R^2 = Me$, ···H(1) (*R,R'*)

22d (–)-*N,N*-Dimethyllindoldhamine (Guattegaumerine)

$R^1 = R^2 = Me$, $R^3 = R^4 = H$, ···H(1) (*R,R'*)

22e 2'-*N*-Nor-daurisoline

$R^1 = R^3 = Me$, $R^2 = R^4 = H$, ···H(1) (*R,R'*)

22f Grisabutine (Magnoline)

$R^1 = R^2 = Me$, $R^3 = R^4 = H$, ◂H(1) (*S,R'*)

22g (–)-Daurisoline

$R^1 = R^2 = R^3 = Me$, $R^4 = H$, ···H(1) (*R,R'*)

22h Grisabine

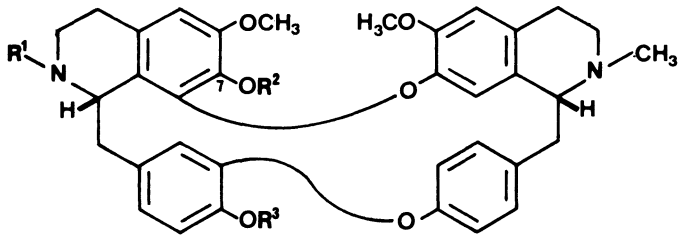
$R^1 = R^2 = R^4 = Me$, $R^3 = H$, ◂H(1) (*S,R'*)

The leaves of several other *Sciadotenia* species have given positive alkaloid tests (408, 409).

1.4.6.4. *Abuta*

Apart from *A. panurensis*, the species of *Abuta* examined so far (Table 1.6) are all known to have been utilized in preparing curare. With two exceptions, *A. grandifolia* and *A. imene*, they have been shown to contain dimeric alkaloids in which the two halves are linked in several different ways: 11–12' (cf. **22**); 8–7' and 11–12', of both the *R,R'* (cf. **20**) and *S,R'* (cf. **23**) series; 7–8' and 11–12' (cf. **21**); and 5–7' and 11–12' (cf. **24**).

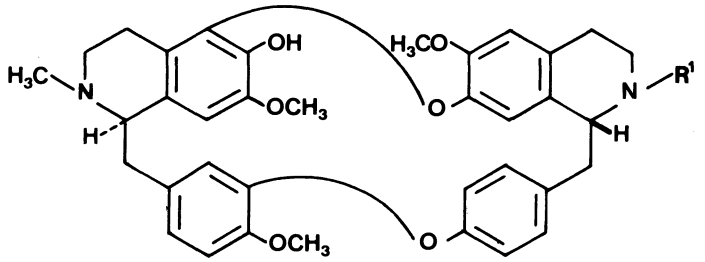
Perhaps the most significant discovery is the isolation by Galeffi and Marini-Bettolo (192) of the principal base present in *A. grisebachii* stem wood, the monoquaternary macoline (**21h**). It is accompanied by some of the corresponding bis-tertiary alkaloid macolidine (**21e**), but the main bis-tertiary bases are peinamine (**23b**) and its 7-*O*-demethyl and *N*-methyl-7-*O*-demethyl derivatives (**23a,c**). Since peinamine has been obtained from a sample of curare as well, it is thought likely that the curare was made with material



23a (–)-7-*O*-Demethylpeinamine
$R^1 = R^2 = R^3 = H$ (*S,R'*)

23b Peinamine
$R^1 = R^2 = H$, $R^3 = Me$ (*S,R'*)

23c (–)-*N*-Methyl-7-*O*-demethylpeinamine
$R^1 = Me$, $R^2 = R^3 = H$ (*S,R'*)

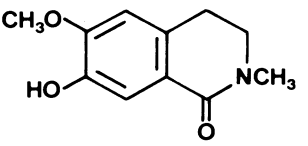


24a (–)-Nor-panurensine
$R^1 = H$ (*R,R'*)

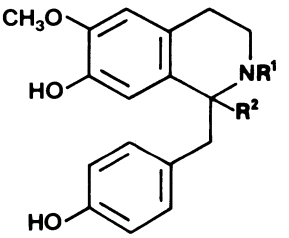
24b (–)-Panurensine
$R^1 = Me$ (*R,R'*)

of this plant (193, 367). The point requires elucidation. Be that as it may, macoline is the only alkaloid so far isolated from any *Abuta* species that is likely to be endowed with curarizing activity comparable to that of the (+)-tubocurarine (**18j**) found in *Chondrodendron tomentosum* (Section 1.4.6.1).

Quite recently, Duté et al. (375) have studied the tertiary alkaloids from the stems of *A. pahni*. Besides a range of known bases, three new dimers with the two halves joined tail-to-tail at 11–12′ were isolated. One is assigned the structure 2′-*N*-nordaurisoline (**22e**) on the basis of NMR and mass spectral evidence. The other two are secondary amines and with formaldehyde/sodium borohydride gave (–)-*N,N*-dimethyllindoldhamine (guattegaumerine) (**22d**). NOE experiments showed that the compounds are 2-*N*- and 2′-*N*-methyl-lindoldhamine (**22b,c**). The CD curves demonstrate that, like (–)-daurisoline (**22g**) and (–)-lindoldhamine (**22a**), all three compounds have the *R,R′* configuration.



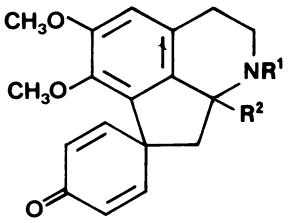
25 Thalifoline



26a (+)-Coclaurine
R^1 = H, R^2 = ···H

26b (–)-*N*-Methylcoclaurine
R^1 = Me, R^2 = ···H

26c (+)-*N*-Methylcoclaurine
R^1 = Me, R^2 = ◂H

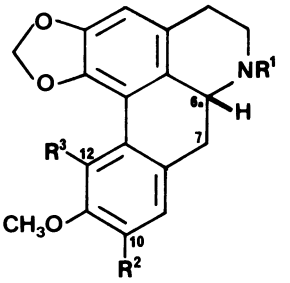


27a (+)-Stepharine
R^1 = H, R^2 = ···H

27b (–)-Stepharine
R^1 = H, R^2 = ◂H

27c (–)-*N*-Formylstepharine
R^1 = CHO, R^2 = ◂H

27d (–)-Pronuciferine
R^1 = Me, R^2 = ◂H



28a Launobine
R^1 = R^2 = H, R^3 = OH

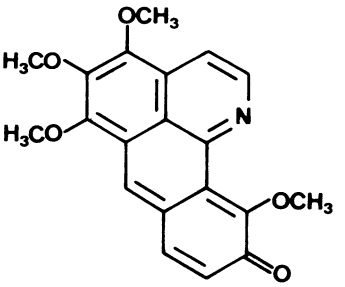
28b Actinodaphnine
R^1 = R^3 = H, R^2 = OH

28c Dehydrodicentrine
R^1 = Me, R^2 = OMe, R^3 = H, $\Delta^{6a(7)}$

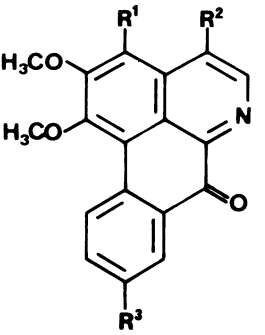
28d Dicentrine
R^1 = Me, R^2 = OMe, R^3 = H

It was not indicated whether the *A. pahni* plant material contained any quaternary bases as well.

Abuta species have also been shown to contain a great variety of other alkaloids, including the isoquinolone thalifoline **(25)**, the benzyltetrahydroisoquinoline coclaurine **(26a)**, aporphinoids of various kinds: the proaporphine stepharine **(27a)**, oxoaporphines (369–371, 374) such as imerubrine **(29)** and imenine **(30f)**, and azafluoranthenes (369, 370), such as rufescine **(31c)**. The tropoloisoquinoline grandirubrine **(32)** has been obtained from the spe-



29 Imerubrine



30a Lysicamine

$R^1 = R^2 = R^3 = H$

30b Peruvianine

$R^1 = R^2 = H$, $R^3 = OH$

30c Homomoschatoline (*O*-Methylmoschatoline)

$R^1 = OMe$, $R^2 = R^3 = H$

30d Splendidine

$R^1 = R^3 = H$, $R^2 = OMe$

30e Subsessiline

$R^1 = OMe$, $R^2 = H$, $R^3 = OH$

30f Imenine

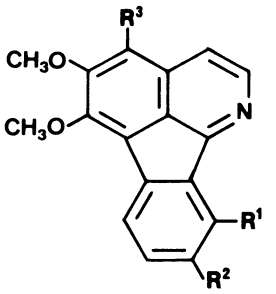
$R^1 = R^2 = OMe$, $R^3 = H$

cies *A. grandifolia* (365) and an isohomoprotoberberine saülatine **(33)** has been isolated from the roots of *A. bullata* Mold. (410).

While the stem wood of *A. fluminum* Krukoff et Barneby, *A. pahni*, *A. rufescens* (*splendida*), *A. solimoesensis* Krukoff et Barneby, and *A. velutina* Gleason is reported to be virtually free of alkaloids (411), the leaves of *A. grisebachii* and *A. panurensis* and the bark of *A. velutina* have furnished positive alkaloid tests (409).

1.4.6.5. *Caryomene*

The above-ground parts of *C. olivascens* Barneby et Krukoff have yielded the bis-benzyltetrahydroisoquinolines (−)-2-norlimacine **(20a)**, a new base (−)-caryolivine **(20c)**, and 1,2-dehydro-2-norlimacusine **(21a)** as the principal components of the alkaloid mixture (412). On the other hand, the fruits have furnished eight non-quaternary bases, including (+)-coclaurine **(26a)**, (−)-stepharine **(27b)**, the protoberberine (+)-pseudopalmatine **(34f)**, and a series of 2,3,10,11-tetra-substituted tetrahydroprotoberberines **(34a–e)**. Bis-benzyltetrahydroisoquinoline alkaloids appear to be absent from the fruit (413).



31a Telitoxine

$R^1 = R^2 = R^3 = H$

31b Nor-rufescine

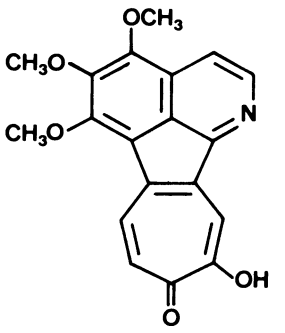
$R^1 = R^2 = H$, $R^3 = OMe$

31c Rufescine

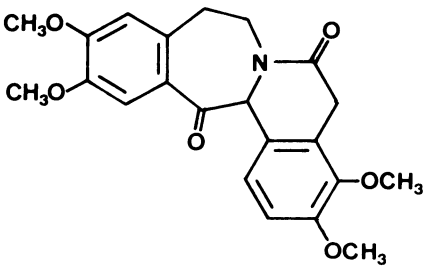
$R^1 = H$, $R^2 = R^3 = OMe$

31d Imeluteine

$R^1 = R^2 = R^3 = OMe$



32 Grandirubrine



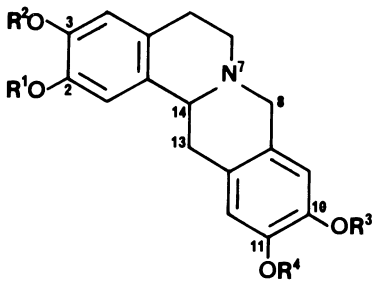
33 Saülatine

1.4.6.6. *Anomospermum*

The assertion by Guha et al. (343) that (+)-tubocurarine is present in the stems of *A. grandifolium* derives from a misreading of the paper by King (355) in which he reported on the curarizing activity of the tertiary- and quaternary-alkaloid fractions calculated in terms of (+)-tubocurarine. No alkaloids have yet been identified. The bark of the species has given positive alkaloid tests (409).

1.4.6.7. *Orthomene*

Up until now, species of *Orthomene* have yielded only negative tests for alkaloids (409).



34a (–)-10-Demethyldiscretine
$R^1 = R^3 = H$, $R^2 = R^4 = OMe$

34b (–)-Govadine
$R^1 = R^4 = OMe$, $R^2 = R^3 = H$

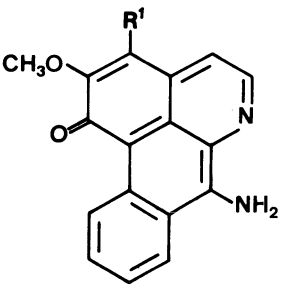
34c (–)-Discretine
$R^1 = H$, $R^2 = R^3 = R^4 = OMe$

34d (–)-Coreximine
$R^1 = R^3 = R^4 = OMe$, $R^2 = H$

34e (–)-Xylopinine
$R^1 = R^2 = R^3 = R^4 = OMe$

34f Pseudopalmatine
$R^1 = R^2 = R^3 = R^4 = OMe$, $\Delta^{7(8),13}$

34g Cissamine (cyclanoline)
$R^1 = R^2 = R^4 = OMe$, $R^3 = H$, $\dot{N}(7)$-Me



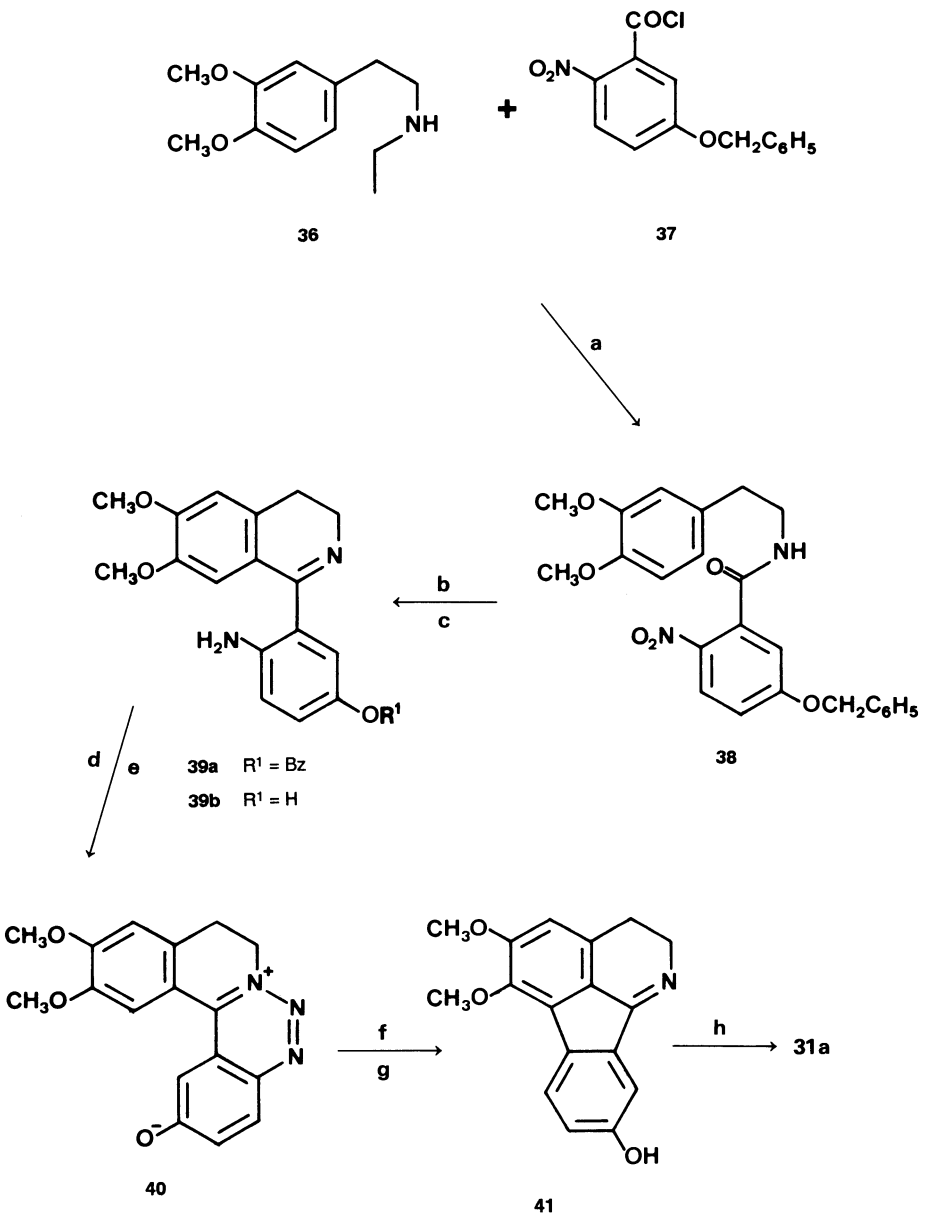
35a Telazoline
$R^1 = H$

35b Teladiazoline
$R^1 = OMe$

1.4.6.8. *Telitoxicum*

No dimeric isoquinoline bases have so far been isolated from the genus, but oxoaporphines (cf. **30**) and azafluoranthenes (cf. **31**) are present in *T. peruvianum* and are the same as or similar to those occurring in *Abuta* species (Table 1.6). Telazoline **(35a)**, one of the so far rare bases containing an oxidized aporphine ring system, is also present (376). The nonphenolic bases from the woody stems of *T. glaziovii* contain very small amounts of oxoaporphines as well as telazoline and the reddish orange teladiazoline. This latter alkaloid appears to be a second representative of the telazoline type, and the structure proposed **(35b)** is derived primarily from a study of the NMR data and decoupling experiments (377).

The structure of the azafluoranthene telitoxine (**31a**) has now been confirmed by synthesis (Scheme 1.2). Condensation of 3,4-dimethoxyphenethylamine (**36**) with 2-nitro-5-benzoyloxybenzoyl chloride (**37**) gave an almost quantitative yield of an amide (**38**) which on Bischler-Napieralski cyclization afforded an excellent yield of the dihydroisoquinoline (**39a**). Catalytic reduction in ethanolic hydrochloric acid furnished the iminoaminophenol (**39b**).



SCHEME 1.2. Synthesis of telitoxine [412]. Reagents: a. $CHCl_3$, K_2CO_3; b. $POCl_3$, MeCN; c. Pd/C, EtOH; d. HNO_2; e. OH^-; f. HCl; g. CH_5Cl, Δ; h. Pd/C, *p*-cymene, Δ.

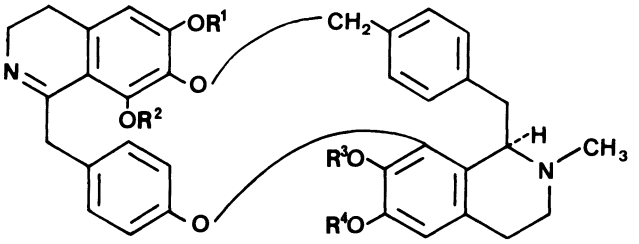
Diazotization, followed by extraction at pH 8, gave a good yield of a triazinium dipole (**40**), the chloride of which on thermolysis produced a 28% yield of dihydrotelitoxine (**41**), along with a small amount of telitoxine (**31a**). Dehydrogenation of the dihydro compound finally gave a 45% yield of telitoxine (412).

The leaves of *T. rodriguesii* Krukoff have given strongly positive tests for alkaloids (409).

1.4.6.9. *Cissampelos*

So far, in material of *C. ovalifolia* only bis-tertiary bis-benzyltetrahydroisoquinolines with the two halves arranged head-to-tail and joined through 7–12′ and 12-8′ ether bridges (cf. **42**) have been found. The 7–12′ bridge is a phenyl benzyl ether linkage instead of the more usual biphenyl ether (380). On the other hand, most of the bases present in *C. pareira* are curine derivatives, among them 0.3% (−)-curine (**18a**) and a little 12-*O*-methyl-(+)-curine (**18g**) (383, cf. 343). The alkaloids of African *C. pareira* include insularine (**43**), in which the two halves are linked head-to-tail by 8–12′, 11–7′, and 12–8′ ether bridges (384); the 11–7′ bridge is a benzyl phenyl ether linkage. See further, Table 1.5.

Hayatine, which has been isolated several times from material of *C. pareira* from Madagascar and India (Table 1.6), has usually been thought to be racemic curine (**18a,d**) (415–417). Recently, however, Verpoorte (418) has critically reconsidered the data and has suggested a number of reasons why the two are unlikely to be identical:



42a Warifteine

R^1 = Me, R^2 = H; R^3 = H, R^4 = Me, or vice versa (*R′*)

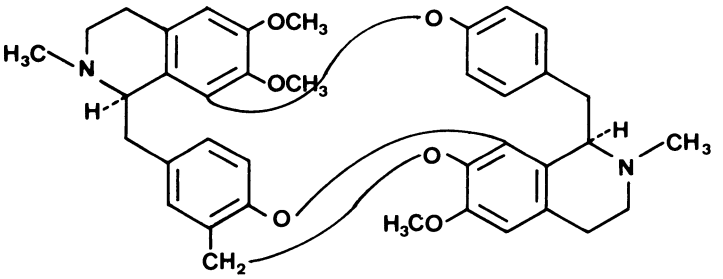
42b Cissampareine (*O*-Methylwarifteine)

$R^1 = R^3 = R^4$ = Me, R^2 = H (*R′*)

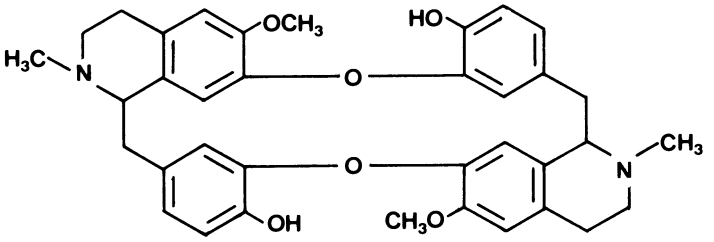
42c *O*-Methylcissampareine (*O,O*-Dimethylwarifteine)

$R^1 = R^2 = R^3 = R^4$ = Me (*R′*)

(The corresponding 1,2-dihydro derivatives of these three alkaloids are also present.)



43 Insularine



44 Hayatine (?) [418, 420]

Isolated from the same plant as (−)-curine (**18a**)
hR_f value different from that of (−)-curine
Separated by means of column chromatography
Different solubilities
Different intensities of coloration with Millon's reagent
Different melting points.

Pharmacology cannot help here, for the head-drop potency of the methiodides of the stereoisomers, (+)- and (−)-curine, is almost the same (419).

Verpoorte speculates that the original symmetrical structure put forward by Agarwal et al. (420) with the two halves joined head-to-tail between 7–11′ and 11–7′ (**44**), may after all be the correct one. Ohiri et al. (in: 418) isolated a small amount of an alkaloid from the African *Synclisia scabrida* Miers (Menispermaceae), whose physical properties are similar to those first reported for hayatine and which on the limited mass and NMR spectral evidence obtained so far has the same structure as that proposed by Agarwal et al. for hayatine. But confirmation, or otherwise, can only come from reisolation of the alkaloid, as the authentic alkaloid is no longer available. However, before undertaking more detailed studies with modern spectroscopic techniques, any new material should be isolated by the method originally used, in

order to ensure that it has exactly the same solubility and other properties as the hayatine originally obtained by Bhattacharji et al.[16]

Chen (393) has reported that the di-ethiodide of the main alkaloid present in Chinese *C. pareira* when subjected to exhaustive Hofmann degradation affords the same nitrogen-free derivative as does (+)-tubocurarine when treated similarly. It is stated that the fully *N,O*-methylated derivative of the alkaloid is identical with a mixture of equal amounts of the *N,O*-methylated (+)- and (−)-curine derivatives; in other words, the original alkaloid should simply be racemic curine, i.e. hayatine as at present commonly understood. The mystery continues!

The bark and leaves of *C. fasciculata* have given positive tests for alkaloids and the leaves of *C. andromorpha* negative tests (408, 409).

1.4.7. Curare

Table 1.5 lists the alkaloids that have been isolated during the chemical investigation of various samples of curare. From some curares only monoterpenoid indole alkaloids have been obtained and from others only bisbenzyltetrahydroisoquinoline bases. Mixed curares have been less thoroughly examined, but alkaloids of both the Loganiaceae and Menispermaceae have been extracted from them. In general, the results of these detailed researches are in line with the classification elaborated by Bauer on the basis of extensive PC studies of museum samples of curare (Section 1.2.6). Its advantage is that it allows a more precise description of the poisons, but it also has the limitation that, because of the current fragmentary knowledge of the chemistry of the plants that furnish the active principles (Sections 1.2.3, 1.2.3.1, 1.4.1, 1.4.6.1), it is not usually possible to determine with any degree of confidence their botanical origin(s) below generic level. All the same, the differences in the main alkaloids present do point to the use of different plants.

The following sections deal with a number of curare alkaloids whose structures have recently been revised on the basis of more modern spectroscopic techniques, and with some newly isolated constituents of curares. There are also several alkaloids with structures that are still not fully established, which could probably be reisolated without too much difficulty.

1.4.7.1. C-Alkaloid O

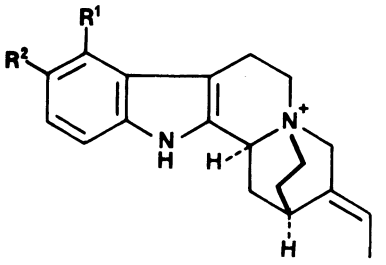
Structure **45a** has now been proposed largely on the basis of NMR and MS studies (435) for C-alkaloid O, a monoquaternary base first isolated from curare by Giesbrecht et al. (304). Supporting evidence comes from a

[16] Sharma (421, cf. 422) has reported on the biosynthesis of supposed hayatine and has shown that (+)- and (−)-*N*-methylcoclaurine (**26b,c**) are both efficient precursors. He interprets the findings in terms of the racemic curine structure. There are no data on the "hayatine" that he added during work-up to dilute the labeled material isolated from the plants fed with radioactive precursors.

comparison with the isomeric 10-substituted cyclized quaternary amine (**45b**), synthesized from 10-methoxygeissoschizol by treatment with tosyl chloride and pyridine at 5°C for 48 hours. Like other compounds obtained from curares, C-alkaloid O may be an artefact. Its 9-substituent is unusual—few such alkaloids are found in *Strychnos* species, presumably the source of the monoterpenoid indole alkaloids found in the curare originally examined by Giesbrecht et al.

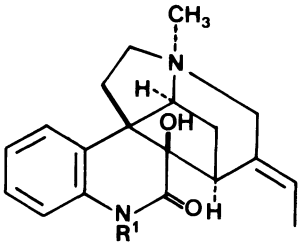
1.4.7.2. Calebassinine 1

Renewed examination by TLC and electrophoresis of calebassinine, another curare alkaloid, but also known to be present in *S. solimoesana* (Table 1.4), has now revealed that it consists of two components, the major one calebassinine 1 together with a small proportion of calebassinine 2. X-ray crystal analysis of calebassinine 1 (436) has shown it to possess the quinoline structure **46a**. The suggestion has been made that it could arise through a number of oxidative transformations from 2,16-dihydroakuammicine (**47**); a variant on the route originally proposed has been outlined (437). Isolation of the alkaloid from *S. solimoesana* (325) would thus imply the occurrence of an akuammicine derivative in the plant, which has not yet been demonstrated.



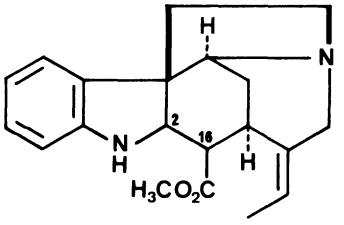
45a C-Alkaloid O
R^1 = OMe, R^2 = H

45b Amine
R^1 = H, R^2 = OMe



46a Calebassinine 1
R^1 = H

46b N_a-Methylcalebassinine 1
R^1 = Me



47 2,16-Dihydroakuammicine

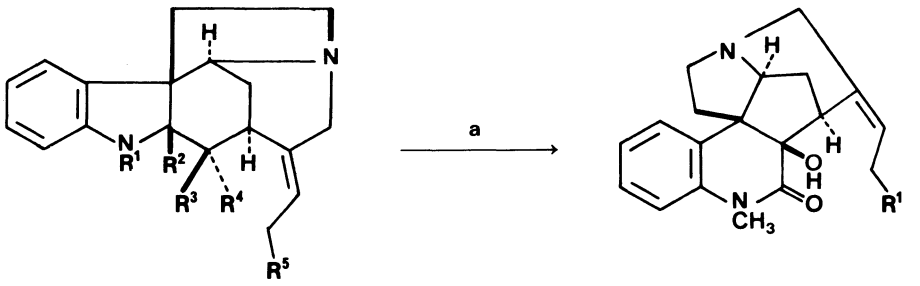
Perhaps a more plausible view is that the alkaloid comes from norfluorocurarine (**2c**), or fluorocurarine (**2f**), the latter being among the alkaloids that have been detected in the plant, via a similar set of reactions; in that case, however, it might be expected to turn up in extracts from several other *Strychnos* species. However that may be, the original isolation of the alkaloid by Schmid and Karrer (434) was from curare and Guggisberg et al. presume that it is an artefact that arose during the preparation of the poison. The experimental method used to isolate it from *S. solimoesana* root bark was a normal acid extraction, followed by separation of the tertiary and quaternary bases, and chromatography of the latter on cellulose—with the various changes in pH and the possibility of aerial oxidation, there is opportunity enough for the formation of artefacts (Section 1.4.3).

An elegant synthesis of N_a-methylcalebassinine 1 methiodide (**46b**) has been achieved by Palmisano et al. (438), utilizing synthetic concepts and strategies, as well as reagents, unimagined at the time the substance was originally isolated. The key step is a base-catalysed α-hydroxyketone rearrangement. The starting point is the Wieland-Gumlich aldehyde (**48a**), which on N_a-methylation (Na, BH_3CN, pH 3.7) and treatment with hydroxylamine/sulphuric acid in water at room temperature yielded the 16β-nitrile (**48b**). Protection of the 18-hydroxy group ($Bu^t(CH_3)_2SiCl$ in DMF) and subsequent oxidative decyanation (Li di-isopropylamide in THF at −78°C, then O_2) and reduction with $SnCl_2$ in HCl at 0°C afforded the ketone (**48c**). Reaction with *m*-chloroperbenzoic acid in chloroform at room temperature was regio- and stereospecific and gave a near quantitative yield of the *N*-oxide (**48d**) which with potassium hydridc in dimethoxyethane in the presence of 18-crown-6 at room temperature furnished a c. 90% yield of the rearrangement product (**49a**). Removal of the *N*-oxide function with H_2/Pd-C/EtOAc gave (**49b**), which when treated with $LiEt_3BH$ in the presence of PPh_3 and $Pd(Ph_3)_4$ in refluxing THF yielded a 6:4-mixture of (**49c**) and (**50**). Treatment of (**49c**) with methyl iodide in acetone gave the N_b-methiodide (**49d**), identical with the compound derived from authentic calebassinine 1 (**46b**) (Scheme 1.3).

Nothing is known about the structure of calebassinine 2.

1.4.7.3. Panarine and Venecurine

Separation of the alkaloids present in two samples of curare with the help of high-speed countercurrent chromatography has led to the isolation of two new monoquaternary bases with a sarpagan skeleton. Panarine, $C_{20}H_{22}N_2O_2$ (**51b**), obtained along with macusine B (**51a**), had similar UV and mass spectra, while the main differences in the ^{13}C NMR spectra of the two alkaloids were the appearance in the panarine spectra of a signal at δ 179.8 and the disappearance of a signal at δ 64.5. X-ray crystallographic analysis of the dihydrate completed the elucidation of the structure and also established the relative stereochemistry. It revealed the presence of a carboxylate function at C(17), which was confirmed by the occurrence of bands



48a Wieland-Gumlich aldehyde $R^1 = R^2 = R^4 = H$, $R^3 = CHO$, $R^5 = OH$

48b $R^1 = Me$, $R^2 = R^4 = H$, $R^3 = CN$, $R^5 = OH$

48c $R^1 = Me$, $R^2 = H$, $R^3,R^4 = O$, $R^5 = OSiBu^tMe_2$

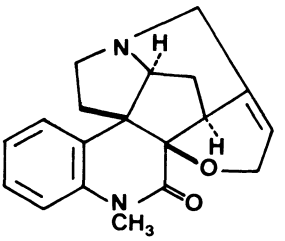
48d $R^1 = Me$, $R^2 = OH$, $R^3,R^4 = O$, $R^5 = OSiBu^tMe_2$, $N_b^+-O^-$

49a $R^1 = OSiBu^tMe_2$, $N_b^+-O^-$

49b $R^1 = OSiBu^tMe_2$

49c $R^1 = H$

49d $R^1 = H$, N_b^+-Me

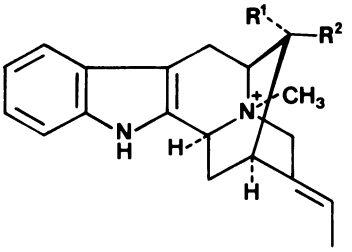


50

SCHEME 1.3. Synthesis of N_a-methylcalebassinine 1 (cf. **46b**). Reagents: a. KH, $C_2H_4(OMe)_2$, 18-crown-6.

at 1597 and 1380 cm^{-1}, and the absence of carbonyl bands, in the IR spectrum. The CD curve, positive at 270 nm and negative at 290 nm, points to a 3*S cis*-configuration; the rigidity of the ring system requires both 5*S* and 15*S*; the chemical shifts of C(16), C(14), C(19), and C(20) indicate 16*R* as in macusine B; and the shifts of C(15) and C(21) are in agreement with an *E* configuration for the ethylidene side-chain, as in most sarpagine-type alkaloids (425). The zwitterion structure, especially with the positive and negative charges γ to each other as in panarine, is rather unusual among naturally occurring alkaloids, the small group of anhydronium bases being the best known example. The interatomic distances are generally close to those established for macusine A (**51c**). However, the geometric parameters for the C(17) COO^- function are intermediate between those for a pure COO^- group and for a normal COOH group, owing evidently to hydrogen bonds involving both oxygen atoms (439).

Venecurine $C_{20}H_{24}N_2O_2$ (**52**), obtained from a different curare sample, was shown on the basis of its spectral data to be a sarpagan derivative related



51a Macusine B

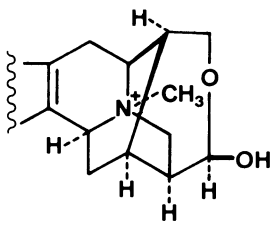
$R^1 = H$, $R^2 = CH_2OH$

51b Panarine

$R^1 = H$, $R^2 = COO^-$

51c Macusine A

$R^1 = CH_2OH$, $R^2 = CO_2Me$



52 Venecurine

to macusine B. The absence in the mass spectrum of a peak corresponding to the loss of a carbomethoxyl function and in the IR spectrum of a carbonyl band suggested the oxygen atoms might be present as a hydroxyl function or in an ether linkage. A signal in the ^1H NMR spectrum at δ 3.07 revealed the presence of a $\equiv\overset{+}{N}—CH_3$ group. Striking differences compared with the spectrum of macusine B (**51a**) were the presence of a 3-proton singlet at δ 1.48, indicative of a methyl group attached to a quaternary carbon atom, and the absence of an ethylidene side-chain. A quaternary carbon with a chemical shift of δ 98.5 pointed to the presence of a hemiketal function as in diaboline (**12a**) and, in view of the unsaturation number 10, plausibly involved in the sixth ring of the molecule. With pyridine/acetic anhydride at 40°C for 8 h, venecurine gave the *N,O*-diacetyl derivative, the difficulty in effecting the acetylation being in line with the presence of a tertiary hydroxyl function. Detailed analysis of the ^1H and ^{13}C, as well as 2D COSY and 2D COSY RCT, NMR spectra and NOESY experiments fully support the structure proposed. The stereochemistry is similar to that of panarine—3*S cis*, 5*S*, 15*S*, 16*R*, 20*R*. In the NOE experiments, the observation of connectivities between CH_3(18) and H(20), H(21a), and H(21b), leads to the conclusion that the base has the 19*R* configuration (426).

Trinervine, the tertiary base corresponding to venecurine, has recently been isolated from material of *S. trinervis* (477).

1.4.7.4. (*R*, *S'*)-2'-Norchondrocurine

Among the alkaloids present in a sample of curare from Peru is the previously unknown base (*R*, *S'*)-2'-norchondrocurine, the structure of which was derived mainly from NMR and mass spectral studies; *N*-methylation with formaldehyde and formic acid to yield (*R*, *S'*)-chondrocurine (**18b**) confirmed the structure proposed (431).

1.4.7.5. Neoprotocuridine and Protocuridine

In analysing a sample of curare, probably from the Amazon, King (306) found that the "non-quaternary" alkaloids made up 38% of the original curare and contained numerous phenolic bases. The two that he isolated and characterized were isomers of the isochondrodendrine series: the pharmacologically inactive protocuridine (**19b**), also obtained by Boehm (189) from curare, and the optically inactive neoprotocuridine (**19c**) which had weak curarizing activity; their stereochemistry has not yet been determined. Cf. also Section 1.4.6.2.

The "quaternary" fraction comprised a further 12% of the curare and from it King was able to prepare a phenolic iodide that had an activity about one-third that of (+)-tubocurarine; it is not certain that the substance was a quaternary base. From the rest of the "quaternary" fraction, after exhaustive methylation King got small amounts of three further bases, one of which was possibly identical with fully methylated protocuridine.

1.4.7.6. Protochondrocurarine and Related Bases

During an investigation of a curare sample, Bodendorf and Scheibe (432) isolated a series of bis-benzyltetrahydroisoquinoline alkaloids. Ether extraction of the basified material afforded the almost inactive tertiary phenolic bases, which, after treatment with methyl iodide, yielded the strongly active and known chondrocurarine (**18n**), presumed to have come from chondrocurine (**18b**). (+)-Protochondrocurarine, an alkaloid isolated from the quaternary fraction, also gave chondrocurarine after *N*-methylation and was evidently a monoquaternary base. It is considered to be identical with chondrocurine metho salt (**18k**) (266: pp. 254–256; 432), and hence isomeric with (+)-tubocurarine (**18j**), which would make it identical with (+)-isotubocurarine (**18k**), a compound that was later synthesized by Soine and Naghaway (480). The enhanced curarizing activity as compared with (+)-tubocurarine is in line with this supposition.

Direct *N*-methylation of the total alkaloids from the sample of curare afforded mainly chondrocurarine and two minor components, (+)-neochondrocurarine and (−)-isochondrocurarine (see below, Section 1.4.7.7), which appear to be monomethyl ethers. The first of these gave the same dimethyl ether as did (+)-tubocurarine and chondrocurarine, while the second one furnished a different product and little can be said about its possible structure.

1.4.7.7. Unknown Laevorotatory Quaternary Dimeric Base

Foster and Turner (440) reported that one of the commercial curare samples analysed by them yielded, instead of the usual (+)-tubocurarine, 1.3% of a laevorotatory quaternary base with $[\alpha]_D$ −184° (water) and curarizing activity about 1/20th of that of (+)-tubocurarine. Because of unsatisfactory analytical figures, Foster and Turner were unwilling to admit that the substance might be identical with the (−)-tubocurarine of King (354). Later researchers

(404, 441) have found what is probably the same monoquaternary base, $C_{37}H_{40}N_2O_6Cl_2$ (?), m.p. 275°C (dec.), $[\alpha]_D$ −199° (methanol), again in commercial samples of curare and also as an impurity in samples of (+)-tubocurarine. The structure of the compound has yet to be established, but its properties show some similarity with those of another weakly active curare alkaloid, likewise of uncertain structure, (−)-isochondrocurarine, $C_{38}H_{42}N_2O_6.2HCl.\frac{1}{2}H_2O$(?), m.p. 278°C (dec.), $[\alpha]_D$ −150° (water), isolated by Bodendorf and Scheibe (432). The relationship of these laevorotatory products to the (−)-tubocurarine of King (354), $C_{38}H_{44}N_2O_6Cl_2.5H_2O$, m.p. 275°C (dec.), $[\alpha]_D$ −258° (water ?), needs to be clarified. See further Sections 1.4.6.1 and 1.4.7.6.

1.4.7.8. Chemical Changes During the Preparation of Curare (see Sections 1.4.2. and 1.4.3)

Methods for preparing curare (Section 1.2.4) vary from simply making and concentrating an extract of the appropriate plant before applying it to the blowgun dart (Waorani) to long and complex procedures utilizing a variety of different plants and lasting several days (e.g. Tikuna).

To establish the active ingredient(s) that were essential to the activity of curare, several of the early explorers attempted to make their own poison: Robert Schomburgk (151) prepared what turned out to be a weak curare from *Strychnos toxifera*; Jobert (442) experimented with both Loganiaceae and Menispermaceae; de Lacerda (443) discussed the tests that Couty and he (444, 445) had previously carried out with extracts from *S. trinervis* and *S. gardneri*; Schwacke (127) noted that extracts of *S. castelnaeana* (*castelnei*) and *S. hirsuta* alone had the same effects as curare; while de Lacerda (394) studied a product made with a species of Menispermaceae, possibly *Curarea tecunarum* (see Table 1.6, footnote g).

Biocca (195) describes how the Makú of the Rio Uaupés and its tributaries in Brazil rather hurriedly prepared a curare, *nam*, in front of him. It comprised several components: the more important ones a *Strychnos* species, *Abuta grisebachii* (?), and an *Anomospermum* (?) species; the less important ones three *Strychnos* species, an unidentified Malpighiacea, and a *Palicourea* or *Psychotria* species (Rubiaceae); but see footnote 4. On comparing the activity with that of other samples of Makú curare he found not only that its potency was much inferior but also that on intramuscular injection in mice the first symptoms, which appeared after some time, were diffuse tremors with the animal resting its head on the ground; death ensued after a prolonged period with the limbs of the animal extended, unable to move, and accompanied by convulsive movements especially of the hind limbs. None of these symptoms were seen with the other samples of Makú curare—they behaved like normal curares. Biocca called his product an "imperfect curare."

Biocca and Ippolito (446) examined the various plant materials chemically and showed that only the *Abuta* species contained diethyl ether- and

chloroform-insoluble bases, presumed to be quaternary bases. In mice, this fraction had only about 3% of the activity of the corresponding fraction from a normal Makú curare; after injection there was a long period of agony accompanied by diffuse tremors. It seemed therefore that in this case the preparation of curare was not just a simple extraction but that chemical changes were also brought about that led to greatly increased potency and to normal curarizing properties. Subsequently, Biocca and Ippolito (447) found that after treating the *Abuta* alkaloid fraction with methyl iodide/sodium methylate the activity had risen to about 75% of that of the control sample of curare and that the symptoms caused were indistinguishable from those of a normal curare. Attempted methylation of the corresponding fraction from the normal Makú curare did not lead to any increase in activity.

Lazzarini Peckolt (448) divided the plants incorporated into curare into three groups: those that yielded alkaloids capable of being *N*-alkylated; those giving rise to an alkaline pH during the concentration of the percolates, thereby allowing the free bases to be liberated from their naturally-occurring forms; and plants containing methyl and other esters capable of furnishing the alkyl groups necessary to form the active quaternary ammonium compounds. His assertions are unconvincing and the experimental evidence he provided in support of them is inadequate, as there were no control experiments.

Later work by Marini-Bettòlo et al. (449) has shed further light on the matter and the results from a series of comparative experiments have provided some support for the conclusions drawn by Biocca and Ippolito. Table 1.7 summarizes their findings. Alkaloids kept for 20 years after extraction from the fresh wood of *Chondrodendron bioccai* (*Curarea toxicofera* ?) greatly increased their potency on being refluxed with excess methyl iodide in methanol for 2 hours. On the other hand, extraction of another portion of the same plant material which had been stored for 20 years showed that it had lost much of its alkylatable alkaloids and attempted methylation did not

TABLE 1.7. Effect of iodomethylation on the toxicity to white mice of certain curares and curare raw materials (from: Marini-Bettòlo et al. [449]).

Material	Toxic dose (mg/kg) i.v.	
	Quaternary bases	Iodomethylated quaternary bases
Makú curare	0.3	0.3
Curarea toxicofera (?)[a]	25.0	1.25
Curarea toxicofera (?)[b]	30.0	30.0
Yanonama (Yanomamo) curare	1.2	2.5
Strychnos sp.	0.3	1.25

[a] Alkaloids extracted 20 years previously; toxicity determined during the course of the research cited.

[b] Alkaloids extracted during the research cited from fragments of wood (from the same source as under a) which had been kept in ambient conditions for 20 years.

alter the potency; see also footnote 13. Similar treatment of *Strychnos*-based Yanomamo curare and of material from a *Strychnos* species caused a loss rather than gain in potency, perhaps as a result of some cleavage of the dimers back to monomeric bases.

These results go some way towards explaining the activity of curares. Table 1.6 shows that in the alkaloid mixtures occurring in the Menispermaceae bis-tertiary bases are in the majority. In the presence of excess methyl iodide these should (bis-)quaternize and thus lead to a big increase in curarizing activity (Section 1.5.4). However, as is evident from Table 1.5, from the few analyses that have been carried out on curares derived partly or entirely from Menispermaceae, monoquaternary or bis-tertiary alkaloids are normally the principal derivatives present, so that any *N*-(and *O*-)methylation taking place during the preparation of the poisons is far from complete. However that may be, the fact that the Waorani can make a curare, though not a very strong one, from the single plant *Curarea tecunarum* (Section 1.2.4), argues for the presence in this species at least of sufficient mono- or bis-quaternized bases to bring about paralysis in not too large animals within a reasonable period of time. Unfortunately, little or nothing is known about the quaternary alkaloids occurring in *C. tecunarum*, and this is also the case for the other species of *Curarea*, as well as the species of *Abuta* that are major ingredients in curares. Nevertheless, it does seem likely that the more elaborate methods of preparation outlined in Section 1.2.4 will enhance the activity of the final product.

Only bis-tertiary and -quaternary dimeric indole alkaloids have so far been isolated from *Strychnos* materials, but the former should, just like the bis-benzyltetrahydroisoquinoline bases already discussed, quaternize on treatment with methyl iodide. The fact that there was no increased toxicity in the experiments by Marini-Bettòlo et al. suggests that dimeric tertiary alkaloids were also absent from their *Strychnos* material, so that there was no opportunity for *N*-methylation and hence for an increase in curarizing activity. But here again, the point needs more detailed study.

1.4.8. Other Plant Constituents

Table 1.3 (Section 1.2.3.2) shows a selection of plant families and genera which provide additives for curare. While representatives of some genera, e.g. *Guatteria* and *Xylopia* (Annonaceae), *Tabernaemontana* (Apocynaceae), or *Ficus* (Moraceae) may yield mucilage or latex which can act as an adhesive, and others, e.g. *Capsicum* (Solanaceae) or *Piper* (Piperaceae), may promote absorption of the poison in the body, i.e. increase the bio-availability of the active principles, there are also some, like *Hippomane* or *Hura* (Euphorbiaceae), which may fulfill both functions. But for most of the additives indicated, it is not clear what contribution they could make to the final poison. For example, *Dieffenbachia* (Araceae), dumbcane, although known to be a dangerous plant in the fresh state, is unlikely to augment the toxicity of the

final product after going through the processes by which the curare is prepared. It is also to be remembered that quantitative information regarding the proportions of the various ingredients in curares is rare. Moreover, possibilities of synergism have not so far been considered.

1.4.9. Alkaloid-Containing Curare Additives

Several families listed in Table 1.3, e.g. Annonaceae, Apocynaceae, Aristolochiaceae, Caesalpiniaceae, Lauraceae, Myristicaceae, and Rubiaceae, have genera recognized as containing alkaloids, many of which are monoterpenoid indole or bis-benzyltetrahydroisoquinoline derivatives analogous to those present in the Loganiaceae and Menispermaceae that furnish the principal active constituents of curares. Other groups of isoquinoline alkaloids are also often present; reviews of relevant groups of alkaloids are as follows: protoberberines (451), aporphines (452), oxoaporphines (453). Generally, not much is known about the pharmacological activity of these substances, and although some of them are toxic in their own right, in most cases there is probably not enough present in curares to have any substantial effect on the overall toxicity. Nevertheless, their presence needs to be borne in mind when assessing the possible contribution they could make to the activity of the poisons into which they are incorporated. The topic requires more space than can be devoted to it here.

The phytochemistry of the Annonaceae has been reviewed by Leboeuf et al. (454) and the alkaloids present in the family have been discussed by Cavé (455) and Cavé et al. (456) The genera listed in the table are known to contain a broad range of such compounds. Thus *Annona* L. and *Xylopia* L. have berberines and tetrahydroberberines, along with benzyltetrahydroisoquinolines and several groups of aporphinoids. The shrubby *A. ambotay* Aublet, in addition to producing flavonoids and an essential oil with a dominant sesquiterpene content, has oxoaporphines, including *O*-methylmoschatoline (**30c**), and trace amounts of geovanine, one of the few known azaanthracene bases (457).

Several species of *Duguetia* A. St. Hil. have been investigated, with most of the attention going to *D. spixiana* C. Martius. The trunk bark (0.3% total alkaloid) of Colombian material afforded a benzyltetrahydroisoquinoline *N*-oxide, a tetrahydroprotoberberine, several phenanthrene derivatives, and many aporphines (including oxoaporphines and 7-hydroxy- and 7-methylaporphines) as the principal bases present (458–460). Trunk bark of the same species from Bolivia (0.2% total alkaloid) contained chiefly 7-hydroxyaporphines and also novel azahomoaporphines; but of the many alkaloids present in the two materials, only six were common to both (461).

Cavé et al. (462) have reviewed the alkaloids occurring in the genus *Guatteria* Ruiz et Pavón. They include an array of tetrahydroprotoberberines and aporphinoids similar to those present in *Duguetia* species. Other types of isoquinoline bases are present as well, but of particular interest is the fact that

these plants also have various bis-benzyltetrahydroisoquinolines. Thus, the stem bark of *G. megalophylla* Diels from Brazil has been found to contain 5% of alkaloids that are related to those present in *Chondrodendron* and *Curarea*: (+)-isochondrodendrine (**19a**), 12′-*O*-methyl-(−)-curine (enantiomer of **18g**), and *O,O*-dimethylcurine (**18h**) (463); a bark extract of *G. gaumeri* Greenman has yielded *N,N*′-dimethyllindoldhamine (guattegaumerine) (**22d**) (464, cf. 465). *G. guianensis* (Aublet) R.E. Fries produces a more complex range of dimers: a group with an 8–7′ ether bridge and an 11–11′ diphenyl bridge (cf. *Ocotea*, below); representatives with 7–8′ and 11–12′ ether bridges; and several with 6–7′, 7–8′, and 11–12′ ether bridges (466, 467). So far, no mono- or bis-quaternary bases have been detected in the genus.

The alkaloids from the trunk bark of Peruvian *Unonopsis spectabilis* Diels have been examined; along with 7,7′-bis-dehydronoraporphines, the main ones present, there are known oxoaporphines and several azafluorenones (468). Stem bark of the Colombian *U. pacifica* R.E. Fries contains a 4,5-dioxo-, a 7-oxo-, and a 7-hydroxyaporphine, along with two 7,7′-bis-dehydronoraporphines (470).

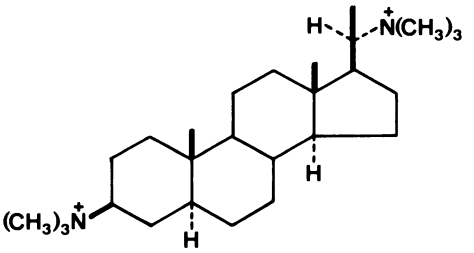
Many genera of the Apocynaceae are well-known for their monoterpenoid indole alkaloids. *Tabernaemontana* L., with over 100 species, has been rather intensively studied and a vast range of such alkaloids, some of which are highly active pharmacologically, has been isolated. Detailed reviews have recently appeared (471, 472).

Both South American and African species of *Malouetia* A. DC., also Apocynaceae and the genus to which *guachamacá* belongs (Section 1.2.1.2), have been investigated chemically and found to contain a range of steroidal alkaloids based on the pregnane skeleton (for a review, see: 77). The bis-quaternary base malouetine (**53**), extracted from bark of the African *M. bequaertiana* Woodson, is undoubtedly the most interesting of these (76, and references cited therein). South American species have been less well studied and only in *M. arborea* (Vell.) Miers have traces of quaternary bases been detected. See further Section 1.6.4.1.

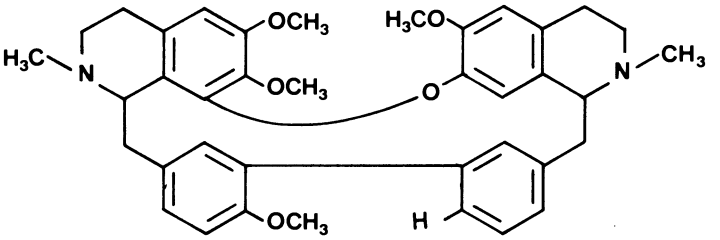
The Aristolochiaceae contain a mixture of alkaloids comprising aristolochic acids, aristolactams, etc. (473), as well as phenanthrenes (561), aporphinoids, and dioxoaporphines.

Folkers (281) and Folkers and Unna (136) demonstrated that alkaloid-containing extracts from stem-bark material of *Capparis sola* J.F. MacBr. (Capparidaceae) collected by Krukoff had distinct paralyzing activity in the frog. The nature of the substances responsible for the activity is not known. It is not likely to derive from the simple quaternary ammonium bases, such as L-stachydrine and L-3-hydroxystachydrine, that have been isolated from or detected in several *Capparis* species (469). A reinvestigation would be of interest.

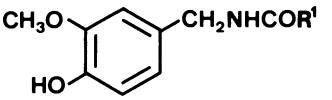
Ocotea Aublet is one of many genera of Lauraceae that are rich sources of various groups of isoquinoline alkaloids; and the fruits of one species, *O. venenosa* Kosterm. et Pinkley, provide an ingredient for one of the arrow



53 Malouetine



54 Rodiasine



55 *N*-(4-Hydroxy-3-methoxybenzyl)alkylamides (capsaicinoids)

R^1 = Up to tridecanoic

Capsaicin

R^1 = 8-Methylnon-*trans*-6-enoic [$Me_2CHCH{=}CH(CH_2)_4$-]

poisons prepared by the Kofán Indians of eastern Ecuador and Colombia. The seeds and bark of this species contain at least eight alkaloids. These include rodiasine (**54**) and a demethyl derivative, which have an 8–7′ ether bridge and an 11–11′ biphenyl bridge joining the two halves (175, 474). Other *Ocotea* species contain benzyltetrahydroisoquinolines, morphinans, and various aporphinoids.

Species of red peppers, or *Capsicum* (Solanaceae), are well-known as spicy ingredients in food, especially in tropical countries. The fruits contain a variable amount of highly pungent principles, the so-called capsaicinoids, which have the general structure *N*-(4-hydroxy-3-methoxybenzyl)alkylamides (**55**). Suzuki and Iwai (186) have provided a recent introductory review. These compounds also have potent pharmacological properties.

1.5. Pharmacology

There is a very extensive literature on the pharmacology of curares and their active principles. The following references will provide an entry into the relevant literature: *Strychnos*-based curare and associated quaternary *Strychnos* alkaloids (41, 252, 481–484); Menispermaceae-based curare and associated alkaloids (252, 344, 345, 347, 353, 419). It is not possible within the limits of this present Section to do more than briefly consider the toxicity and muscle-relaxant activity of curares and their active principles, as a prelude to Section 1.6 which outlines the development of some modern muscle relaxants.

1.5.1. Toxicity of Curares

Bauer and Fondi (190) and Bauer (32, 33, 191, 212–215) determined the head-drop and lethal doses in white mice of more than 70 museum samples of curare, covering most of the region where the poison has been made, and Kebrle et al. (323) established the head-drop doses in white mice of eight different curares. Biocca et al. (55) determined both parameters in rabbits for two samples of curare. Table 1.8 summarizes the findings. Some of the oldest curare samples examined still rank with the most toxic. In this connection, it is of interest to note that some of Vellard's informants emphasized the poor quality of curare prepared during the rainy season (14: p. 89). Doubtless, this is due partly to seasonal and other changes in the alkaloid composition of the essential ingredients, but the question has not been investigated.

Toxiferine-based curares tend to be the most active. Bauer and Fondi (190) assayed two such poisons from the western Brazilian Amazon region, one a Yurí product collected by von Martius in 1820 and the other a Mayoruna preparation obtained by Natterer in 1830. They found LD_{100} 1 and 0.8 mg/kg, respectively.

Products whose main alkaloids are curarine and calebassine are usually somewhat less active. Thus, a curare from southern Venezuela, of Mainatari origin, also collected by Natterer in 1830, had LD_{100} 4 mg/kg. Waser (484) reported that head-drop assay in the mouse of alkaloid fractions isolated by

TABLE 1.8. Head-drop and lethal doses of curares in the white mouse, mostly from Bauer and Fondi [190] and Bauer [32, 33, 191, 212–215].

Main alkaloid(s) present	Head-drop dose (mg/kg)	Lethal dose (mg/kg)
Curarine	1.0, 1.2[a]	2.75, 5
Curarine/Calebassine	0.2–15[b]	>2–20
Toxiferine	0.5–4	0.8–6
Toxiferine/(+)-Tubocurarine	0.5–4	1–6
(+)-Tubocurarine	1–15	2–25

[a] In the rabbit. Data (2 samples) from Biocca et al. [55].
[b] Data from Kebrle et al. [323] fall within this range.

partition chromatography on cellulose from a *Strychnos*-based curare demonstrated that as much as 99.6% of the original activity of the curare had been recovered: 38% was due to curarine, 20% to C-alkaloid G, 12% to calebassine, and about 5% to toxiferine. In the assays of curares by Kebrle et al. (323), the data for the head-drop doses suggest somewhat greater paralysing activity than found in the assays of museum samples by Bauer. The samples were presumably more recent than many of those obtained from museum collections. With one exception, the dominant alkaloids were accompanied by modest amounts of fluorocurarine; the curares all contained little or no toxiferine. The two samples of curare assayed by Biocca et al. (55), both of which had curarine as their main active alkaloid, were found to be quite strong, with LD_{100} 2.75 and 5 mg/kg.[17]

(+)-Tubocurarine-based curares also tend to be less potent. However, the sample of *ticunas*, the notorious curare of the Tikuna Indians, acquired by de Castelnau in 1846 and examined by Bauer (215) and Delle Monache et al. (314), proved to be highly toxic, with LD_{100} 1 mg/kg (214). It contained chiefly *Chondrodendron* alkaloids, including (+)-tubocurarine, but *Strychnos* bases, among them some toxiferine, were also present.

The Indians often assert that curare must be stored under dry conditions if it is to keep its activity; and they believe that curare filled into pots, allowed to dry, and then well sealed does not lose its activity as readily as do the softer and more paste-like or syrupy products that are poured into calabashes or bamboo tubes (14: pp. 138-140). The findings of Bauer and Fondi (190) cited above confirm that curares kept under dry conditions are very stable and lose little of their activity.

1.5.2. Muscle-Relaxant Activity of the *Strychnos* Species Associated with Curare and of Their Alkaloids

Except for *S. solimoesana* and *S. toxifera*, the data presented in Table 1.4 on the *Strychnos* species that are stated to be principal ingredients in curares

[17] One of the more unusual applications of curare has been in whaling. The French whaler Thiercelin (486, 487) experimented with dogs, rabbits, etc., and calculated that 0.5 mg/kg of a 95:5-mixture of a soluble strychnine salt and curare would bring about death within a reasonable time. He had a number of cartridges made up containing 30–40 g of the poison mixed with the explosive which were to be used with the harpoon gun. Of the ten whales struck during the voyage in which the invention was tested, six were recovered, having died in a shorter time than usual, and four were lost. Later, in an old Norwegian pharmacy, Poulsson (488) came across a tin box labelled "strong poison for killing whales." In it were glass tubes containing 14–23 g curare which still had LD_{100} 3–4 mg/kg in rabbits. He concluded that the amounts of poison in the glass tubes were too small to facilitate the capture of whales through the paralysing effect. In this connection, it is of interest to note that as late as 1956 in Sweden (and Norway) mixtures of salts of choline esters and anticholinesterases (organic phosphorus compounds, etc.) were patented as muscle-relaxant poisons to be used in harpoon projectiles for whale hunting (489).

provide little evidence to support their reputation among the Indians. *S. castelnaeana*, especially, has been disappointing. Though reported to have muscle-relaxant activity, C-alkaloid D (Scheme 1.1), the one bis-quaternary alkaloid so far known to be present in it, is relatively weak (head-drop dose >1 mg/kg in the mouse). Nothing is known about the activity of *S. brachiata* and only tertiary bases have been isolated from it. The activity of *S. cogens* is at best weak and the chemistry of the species is unknown. *S. guianensis* varies both in its muscle-relaxant potency and in its alkaloid composition; curarine is the only active bis-quaternary base recognized up till now. The muscle-relaxant power of *S. peckii* varies from none to strong—but again, nothing is known of the alkaloid composition. *S. tomentosa* and *S. mitscherlichii* var. *amapensis*, on the other hand, both have highly active bis-quaternary bases that could account for their use as constituents of curare. There are several species with strong muscle-relaxant activity that the Indians have not utilized in making poisons, among them *S. trinervis*, *S. froesii*, *S. gardneri*, and *S. parvifolia*.

Information about the pharmacology (and/or the chemistry) of most of the remaining species employed is inadequate. In the case of *S. erichsonii*, one of the more frequently exploited species, the activity appears to be due primarily to diaboline and its derivatives; erichsonine (**13**) (Section 1.4.5.2), the only other alkaloid isolated from it, is without pharmacological effects. *S. jobertiana*, a species reported to have only weak activity, may similarly rely on diaboline and related compounds (Section 1.5.3).

The most complete and the most often quoted set of data on the muscle-relaxant activity of the bis-quaternary dimeric indole alkaloids found in *Strychnos* species and curares based on them is due to Waser (252, 483, 484) and the figures given in Table 1.9 are taken from this work. There are, however, several other publications in which similar data are reported (311, 481, 482). The head-drop dose, as determined by i.v. administration in the mouse, ranges from <1 μg/kg for C-alkaloids G and E to 22 mg/kg for calebassinine. As a means of high-lighting certain structure-activity relationships, in several publications the more important bis-quaternary alkaloids are grouped in three triads based on dihydrotoxiferine, curarine, and calebassine (Table 1.9 and Scheme 1.1). In the dihydrotoxiferine triad (**4d–f**), introduction of a hydroxyl group at C(18) and a second one at C(18′) leads in each case to an increase in activity. This is also observed with the members of the curarine triad, though the increase in potency is more striking with its other two members, C-alkaloids G and E; the presence of the 2,2′-oxygen bridge evidently confers greater additional activity. The three alkaloids of the calebassine triad have a much weaker activity than do the alkaloids of the other two triads, which presumably arises from the deformation of the central ring due to the presence of the C(17)–C(17′) bond; see below. The duration of muscle relaxation becomes longer as the polarity of the molecules is increased by the presence of the C(18) and C(18′) hydroxyl groups. The LD_{100} for these various alkaloids is generally about twice the head-drop dose (252, 267, 481, 483, 484).

TABLE 1.9. Head-drop dose, mean lethal doses, and duration of paralysis of some dimeric and other *Strychnos* alkaloids in the mouse i.v. [from Waser: 252, 484].

Alkaloid[a]	Head-drop dose (μg/kg)	Lethal dose (μg/kg)	Duration of paralysis (min.)[b]
Dihydrotoxiferine triad			
Dihydrotoxiferine	30	60	5.5
C-Alkaloid H	16	24	3.7
Toxiferine	9	23	12
Curarine triad			
Curarine	30	50	4
C-Alkaloid G	5	12	7
C-Alkaloid E	4	8	18
Calebassine triad			
Calebassine	240	320	3
C-Alkaloid F	75	120	1.3
C-Alkaloid A	70	150	2
Other dimeric bases			
Caracurine V metho salt		750	
C-Alkaloid D		2000	
C-Alkaloid C		380	
Caracurine II metho salt		350	
Tubocurarine	125		
Monomeric bases			
Fluorocurine	4400	8500	
Calebassinine[c]	22000	44000	

[a] All the alkaloids were chlorides, except fluorocurine which was an iodide.

[b] Dose in the middle between the head-drop and lethal doses

[c] Although the sample must have been impure (see Section 1.4.7.2), the data for the pure alkaloid are not likely to be very different.

The alkaloids of the dihydrotoxiferine triad have a rather rigid box-like structure in which the N_b–$N_{b'}$ (inter-onium) distance is about 1.4 nm. In caracurine II dimetho salt and calebassine, deformation of the central ring because of the extra C(16)–C(16′) or C(17)–C(17′) bridge present (Scheme 1.1) reduces the inter-onium distance to less than 0.9 nm (252). It is to the consequent alteration in configuration (making the molecule bulkier and less flat) that the weak activity of these two alkaloids has been attributed (252), but this view may be questioned, since curarine, in which the presence of the C(2)–C(2′) oxygen bridge again reduces the inter-onium distance to less than 0.9 nm (Section 1.4.4.1), is one of the most potent of the calabash curare alkaloids.

One point of interest is that the activity of C-alkaloids G and E, two of the most potent substances as determined in mice, rapidly decreased and did not become constant until after 1–2 months at about 10% of the original potency. The reason for this curious behaviour is not known, and a later attempt to reproduce the results with new material was unsuccessful. It has been suggested that there may have been some change in configuration (252, 484). The fact that curarine did not behave in the same way perhaps indicates that there

was a change in structure, and not simply in configuration, following reaction between the C(18)- and C(18′)-hydroxyl groups and the central diazacyclooctadiene ring (see Scheme 1.1), thereby leading to a less satisfactory receptor fit. Further study of the phenomenon is desirable.

1.5.3. Other Activities of the *Strychnos* Species Associated with Curare and of Their Alkaloids

In some species, pharmacological effects other than muscle-relaxation may dominate. Thus, as already seen, the activity of *S. erichsonii* extracts appears to be due primarily to the presence of diaboline derivatives. Leaf extracts have analgesic properties, while stem-bark extracts have spasmolytic properties and augment the activity of the central nervous system (50). The bark alkaloids of *S. glabra* are reported to have central rather than peripheral effects (Table 1.4, footnote j). Sublethal doses of aqueous extracts from *S. castelnaeana* cause hypertension, tachycardia, and slight respiratory stimulation; enhancement of the hypertension by atropine and its reduction by hexamethonium (mecamylamine) show that the extract has nicotinic activity. Evidently, the toxicity of the plant must be partly due to the tertiary bases it contains (314).

Akagerine (**14**) is reported to have some cytotoxic activity against cultured B16, Flow 2002, L 1210, and HeLa cells (491).

In addition to its weak muscle-relaxant action (251), bisnordihydrotoxiferine (**4c**) has sedative properties, brought about by a marked depressant effect on the CNS (490). The alkaloid is antidiarrhoeic (476, 562). It exhibits some bacteriostatic activity against both gram-positive and gram-negative bacteria, more especially against *Streptococcus* species associated with dental caries. The related C-alkaloid H is also active (**4e**) (492, 493).

In mice, the bis-tertiary alkaloid caracurine V (3) exhibits weak muscle-relaxant activity at 10 mg/kg (screen-grip test), which was stronger but also lethal at 13 mg/kg. The effect was not opposed by anticholinesterases (251, 492). Like the foregoing bis-tertiary bases, the compound also has some antimicrobial activity (493, 494).

Diaboline (**9a**) is moderately toxic to mice, with LD_{50} i.v. 29 mg/kg, i.p. 150 mg/kg, s.c. 486 mg/kg. It causes diffuse tremors, paralysis of the hind limbs, and death usually from respiratory paralysis (253). Up to 250 mg/kg s.c. there are no convulsions (495). In the rabbit, 40 mg/kg i.v. brings about relaxation of the front and hind limbs and also of the neck muscles, which results in head drop. 70 mg/kg leads to death with distinct signs of respiratory paralysis. Smaller doses, 10–20 mg/kg, in the chloralosed dog lead to slight hypotension, but they also diminish the amplitude of respiratory movements as well as contractions of the gastrocnemius muscle. Up to 40 mg/kg there is no curarizing effect on the mesenteric preparation nor is there any activity on isolated organs (rat diaphragm, rabbit intestine). The alkaloid does not act as an antagonist towards acetylcholine (253).

In urethan-anaesthetized rats, the alkaloid has hypotensive effects which are attributed to a central action. Among the substances potentiating the depressant effect of diaboline on the isolated heart are its acetyl derivatives (496).

Both fluorocurarine (**2f**) and fluorocurine (**7**) have very weak curarizing activity (252). Cf. Table 1.9.

Longicaudatine (**8**) is without muscle-relaxant activity, but it functions as a non-specific spasmolytic, which may arise through action on intracellular Ca^{2+} stores rather than on depolarization-dependent or receptor-operated Ca^{2+} channels (540). The previously reported strong reserpine-like activity (479) has not been confirmed (540).

Macusine B (**51a**) stimulates the CNS. It has hypotensive properties, resulting from peripheral vasodilation, as well as β-adrenergic and α-adrenolytic properties; it also affects tryptamine receptors. In high doses, the alkaloid brings about clonic convulsant effects (497, 498, 491).

1.5.4. Muscle-Relaxant Activity of the Menispermaceae Associated with Curare and of Their Alkaloids

As with the Loganiaceae just discussed, few species have been tested for muscle-relaxant activity (Table 1.6), though it is certainly more widespread than present data indicate. While active quaternary alkaloids have been extracted from *Chondrodendron tomentosum*, nothing is known about the quaternary bases, if any, present in other *Chondrodendron* specics or, indeed, in any of the related *Curarea* species. Rather surprisingly, available information on the widely used *Curarea tecunarum* suggests that it has toxic properties and at best weak paralyzing activity. Extracts from *Cissampelos ovalifolia* roots have muscle-relaxant activity which seems to be on a par with that of *Chondrodendron tomentosum*. The only species other than *C. tomentosum* from which a quaternary base has been isolated is *Abuta grisebachii*, but the pharmacology of the compound concerned, macoline (**21h**), has not been examined.

Both structural and stereochemical factors play a crucial rôle in determining the curarizing potency of the bis-benzyltetrahydroisoquinoline alkaloids. The bis-tertiary alkaloids usually show little or no activity, and it is not until at least one of the nitrogens is quaternized that muscle-relaxant properties become manifest (419). However, there appear to be a number of exceptions (see below). The relative potencies determined depend very much on the test preparation used to assay the compounds, as shown, for example, by the data set out in Table 1.10. This is seen in more practical terms in the differing sensitivities of the muscles in various parts of the body to neuromuscular-blocking agents. With (+)-tubocurarine, for example, the first muscles to be affected are those of the neck and face, then the muscles of the body and limbs are relaxed, and finally the muscles (intercostal and diaphragm) controlling respiration (499).

TABLE 1.10. Molar potencies relative to (+)-tubocurarine (= 1) (from: Marshall et al. [503]).

Compound	Rat diaphragm	Cat tibialis	Cat superior cervical ganglion
(+)-Tubocurarine (**18j**)	1	1	1
N,N'-Dimethyl-(+)-chondrocurine (**18n**)	1.47	1.28	0.71
N,N'-Dimethyl-(−)-curine (cf. **18a**)	0.32	0.91	0.21

It is (+)-tubocurarine and its associated alkaloids that have been at the centre of interest. The monoquaternary (+)-tubocurarine (**18j**) is rather less active than the parent bis-quaternary indole bases. The head-drop dose in the mouse is about 125 μg/kg and in man about 150 μg/kg (252). In contrast, the enantiomeric (−)-tubocurarine (**18l**) is 30–60 times weaker (354). (+)-Chondrocurarine (**18n**), the fully *N*-methylated alkaloid and the base with the bis-quaternary structure originally assigned to (+)-tubocurarine, has almost four times the potency of that alkaloid. It is responsible for a considerable part of the activity of *Chondrodendron tomentosum* extracts (353). Data supposedly for *O,O'*-dimethyl-(+)-tubocurarine (**18m**) are probably for the completely methylated *N,O,O'*-trimethyl derivative (**18o**), since the method used for its preparation also quaternizes the tertiary nitrogen (500). The compound is two to three times more potent than (+)-tubocurarine; it is also known as metocurine and has acquired limited use in surgery as a muscle relaxant (501). The muscle-relaxant properties of quaternized curine derivatives continue to be investigated (see: 345 and references cited therein).

(+)-Tubocurarine (**18j**) in acid solution, and in the body, is protonated. It is therefore able to function as a doubly-charged cation and is the reason why its neuromuscular blocking potency is pH-dependent. The molecular disposition of these bis-benzyl tetrahydro isoquinolines *in vivo* is not known; and while it is probably the hydrophilic side (Section 1.4.6.1) that will become fixed to the protein of the receptor site, simultaneous attachment of the two charged nitrogen atoms appears unlikely, since they are on opposites sides of the molecule (503). The alternative suggestion has been made that the protonated nitrogen could exert an electrostatic repulsion on acetylcholine (cf. 401).

The activity of synthetic (+)-isotubocurarine (**18k**), in which the quaternary and tertiary centres are reversed, i.e. in which N(4'), rather than N(4) is quaternized, is more than twice that of (+)-tubocurarine, as determined using the cat tongue–hypoglossal nerve preparation (480). In this compound, the quaternary nitrogen is adjacent to the *S*-center, rather than to the *R*-center as in the naturally occurring compound, and thus shows that stereochemical factors do play a part in determining the activity; this point also emerges clearly from the data relating to atracurium stereoisomers (Section 1.6.4.2).

(+)-Isochondrodendrine (**19a**) and its bis-quaternized derivative both show modest activity, and again the fully *N,O*-methylated derivative is somewhat more active (395, 397). With an equipotent dose, the bis-tertiary alkaloid warifteine (**42a**) produces nondepolarizing block of about the same duration as (+)-tubocurarine; the head-drop dose in the rabbit is 1.6 mg/kg. In the isolated rat-diaphragm preparation, on the other hand, the compound has only about one-fifteenth of the activity on a molar basis (381). Rodiasine (**54**), likewise a bis-tertiary alkaloid, is stated to exhibit neuromuscular effects similar to those of (+)-tubocurarine (474).

1.5.5. Other Activities of the Menispermaceae Associated with Curare and of Their Alkaloids

References to other pharmacological effects of bis-benzyltetrahydroisoquinoline alkaloids, such as their hypotensive activity and antitumor properties, are to be found in (344, 345). Protoberberine bases may give rise to hypotension and may also have neuroleptic, tranquillizing, or analgesic effects (451); if a sufficient concentration of alkaloids of this type was present in a curare, such effects could contribute to the efficacy of the poison. The pharmacology of some types of aporphines is dealt with briefly in (452). Little or nothing is known about the activities of many of the other groups of alkaloids occurring in Menispermaceae associated with curare.

1.6. Development of Modern Muscle Relaxants

As an appropriate finale to the curare story, the rôle of the poison and its alkaloids in the development of modern muscle-relaxants is outlined.

Sporadic attempts were made during the nineteenth and early twentieth centuries to use curare for the treatment of certain conditions, principally rabies, tetanus, epilepsy and spastic paralysis, and chorea, where it seemed that the muscular relaxation brought about by the drug would have beneficial effects. But the rarity of the substance, the tremendous variation in its activity, and the inability to standardize the product prevented any widespread use (22, 27). However that may be, as early as 1912 Läwen used a sample of Boehm's curarine as an adjunct to anaesthesia, thus anticipating the work of Griffith and Johnson (below) by some 30 years. It is unfortunate that this pioneering effort never received due recognition (22: p. 110, 485: pp. 135–136).

With the large amount of curare brought back by the Gill-Merrill expedition in 1938 (548) and the development of the rabbit head-drop assay by Holaday, the situation began to change. Squibb was able to make a standardized product, "Unauthenticated Extract of Curare," Intocostrin, one unit of which was equivalent to 0.15 mg tubocurarine. This preparation was made available to medical researchers in the United States. In 1938/39 Bennett and McIntyre introduced the use of curare in electro-shock therapy, to prevent the injuries which up till then had been a common occurrence during the

treatment. It was not long before the wider applications of curare came to be investigated and, following the report of Griffith and Johnson in 1942, routine use of the drug in anaesthesia quickly became established. Owing to the circumstances of World War II, little Intocostrin was to be had in Europe. Tubocurarine (Tubarine®), however, became available in Great Britain from Burroughs Wellcome, so that in 1946 Gray and Halton were able to discuss its use in anaesthesia. This exciting period of curare research is dealt with in the books by McIntyre (27), Bryn Thomas (22), and Burnap and Little (42). Muscle relaxants have now been in routine use for almost half a century and several of the early pioneers have published personal accounts of the developments that in due course led to their clinical application: Bennett (506), Betcher (16), McIntyre (27), West (290, 396); see also: Blubaugh and Linegar (507) and Bowman (485).

Somewhat later than (+)-tubocurarine and following the studies of *Strychnos* curare alkaloids, toxiferine underwent clinical trials and was found to be rather more potent and fairly free from side effects, but its very long duration of action and its instability in solution precluded clinical exploitation of the alkaloid (485). However, modification of the toxiferine molecule by replacing the two N_b-methyl groups with N_b-allyl functions produced the semisynthetic compound alcuronium (**4g**), or Alloferin® (267, 508), which is still in use as a short-lasting muscle-relaxant in minor surgery. It is obtained by semisynthesis from strychnine via Wieland-Gumlich aldehyde and subsequent quaternization with allyl bromide. See further, Bowman (485: pp. 139–140).

Also curarine has been tested clinically. It is about equipotent with tubocurarine and its duration of action is similar. Again it has fewer side effects, but its instability and the difficulty of obtaining it has prevented its use in the clinic (485).

Some data on naturally occurring and synthetic muscle relaxants are set out in Table 1.11.

1.6.1. Synthetic Muscle Relaxants

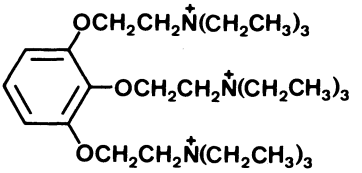
The difficulties encountered in ensuring adequate supplies of curare from which to obtain tubocurarine soon induced medicinal chemists to search for synthetic analogs. It had been realized ever since the work of Crum Brown in the 1860s that quaternization of the basic nitrogen in strychnine and other alkaloids, i.e. the formation of quaternary ammonium compounds, gave rise to muscle-relaxant properties. To begin with, the models chosen were based on King's incorrect structure for tubocurarine and so attention was focussed on molecules having two quaternary nitrogen functions. This early development work has been reviewed by Bovet and Bovet-Nitti (505, 509, 510).

Two lines of research were followed: one was simplification of the ring system and the other was variation of the inter-onium distance. In following out the former approach, among the series of compounds synthesized and

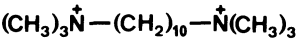
TABLE 1.11. Neuromuscular-blocking potencies of some naturally occurring and synthetic muscle relaxants [512: p. 236, cf. pp. 194–195].

Compound	Neuromuscular block (cat,[a] ED_{95} μg/kg i.v.)	Head-drop dose (rabbit, μg/kg i.v.)	Initial dose (man, μg/kg)[b]	Onset time (mins.)[b]	Duration of relaxation (mins.)[b]
(+)-Tubocurarine	180–230	120–150	10–15 mg[c]	1	30–40
Metocurine	25–30		100–300		25–90
Atracurium	130[d]		300–600	2	15–35
Toxiferine	5.4	4			
Alcuronium	50[d]		300	2	40
Pancuronium	24		40–100	1–3	45
"	8[d,e]				
Dacuronium	600[d,e]				
Vecuronium (Org-NC-45)	38		80–100	2–3	45
Pipecuronium	2[d,e]		70–85	2.5–3	45[f]
Chandonium (HS-316)	70[d,e]		250[g]	1[g]	18[g]
Gallamine	850–900	200–250	80–120	1–2	20–30
Suxamethonium (Succinylcholine)	60–80	120	20–80 mg[c]	0.5	4–6
Decamethonium	30	78	2–2.5 mg[c]	>0.5	15–20

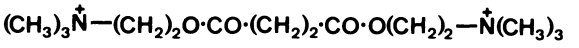
[a] Gastrocnemius muscle.
[b] Taken from Martindale [501]. For some of the drugs, in the U.S. the initial dose administered is generally lower [cf. 545].
[c] Total dose.
[d] ED_{50}.
[e] Tibialis anterior muscle.
[f] From [545].
[g] From [549].



56 Gallamine



57 Decamethonium



58 Suxamethonium

tested by Bovet and his colleagues were ethers derived from phenols with benzene and quinoline or isoquinoline moieties and amino-alcohols related to choline. This work led to the successful introduction into clinical practice of the synthetic drug gallamine triethiodide (**56**), Flaxedil®, a muscle relaxant of moderate duration—a so-called pachycurare; it is still in use (505, 510, 511).

Simpler molecules with two positively charged centres joined by a chain of varying length, like decamethonium (**57**) and esters of the suxamethonium (or disuccinylcholine) (**58**) type and their analogues, were likewise shown to be muscle relaxants, but with a rather short period of activity—leptocurares (510, 511). Both these substances were introduced into clinical practice, but only suxamethonium has kept its position because its brief duration of action, about 5 minutes only, has made it of use in minor surgical procedures such as intubation (501).

Combination of the two approaches—exploiting the potent receptor-affinity of decamethonium and the non-depolarizing action of tubocurarine —led to the introduction of laudexium (**63a**), but it proved to have a number of disadvantages, among them a certain degree of cumulation and a duration of action longer than that of tubocurarine, and the drug was later withdrawn (485).

Over the years, this development work has continued (543) and, though hundreds of synthetic compounds have been produced, most of them have

failed to reach the stage of clinical testing (485). Nevertheless, the fund of knowledge acquired—the data on structure-activity relationships and mechanisms of action—has formed the basis on which present-day muscle relaxants are designed. The many chapters in the volume on *New neuromuscular blocking agents* in the "Handbook of Pharmacology" series are eloquent testimony to the continuing interest in this area (512).

1.6.2. Mechanisms of Action

How peripherally acting muscle relaxants act has been the subject of very extensive investigation, dating back to the classical experiments of Rudolf von Koelliker and Claude Bernard in the middle of the nineteenth century (15). With the development of synthetic muscle relaxants, it soon became apparent that they do not all operate in the same way. They can be divided into several categories, but those that interfere with neuromuscular transmission by interacting with acetylcholine receptors on the end-plates of motor nerves (postjunctional cholinoceptors) are usually divided into two groups: (1) Nondepolarizing acetylcholine antagonists and (2) Depolarizing blocking drugs.

The distinction, although convenient, is not clear-cut and in a series of related compounds there may be some overlap and gradual transition from one mechanism to the other, giving rise to the so-called dual block (519, 539). Among the reviews covering present understanding are those by Bowman (152, 485, 550).

The nicotinic acetylcholine receptor (nAChR) is "the first signal transducer of the nervous system to be thoroughly understood on the molecular level." (513–515) Though much is known about its structure, a comprehensive model that accounts fully for its structure-function relationships is still some distance away. The receptor is a membrane-bound allosteric protein comprising four different polypeptide subunits assembled into a pentamer $\alpha_2\beta\gamma\delta$ arranged in a rosette- or funnel-like structure leading to a narrow central channel. The binding usually of two acetylcholine molecules to the so-called recognition sites on the receptor causes the channel to open, and the consequent influx of sodium ions leads to changes in the electrical condition of the membrane with the resultant onward propagation of an action potential.

Computer modelling involving (+)-tubocurarine, as well as pancuronium and chandonium (Section 1.6.4.1) and other synthetic neuromuscular blocking agents, suggests a receptor with a primary inner site allowing coulombic interaction with the quaternary nitrogen and a secondary site about 0.5–0.6 nm from the primary one and nearer the entrance to the receptor site. The model proposed also accommodates the fact that high blocking activity is associated with molecules exhibiting roughly twofold symmetry, but it does not impute significance to a particular N–N' distance (516).

Curare and its active principles belong to the first of the two groups indicated above. Thus, toxiferine, (+)-tubocurarine, and related compounds are competitive or nondepolarizing muscle relaxants, i.e. they compete with acetylcholine for the recognition sites on the receptor channels. These sites

are relatively nonspecific, since both agonists and antagonists can bind to them. However, unlike acetylcholine, antagonists when they become attached to the recognition site do not bring about opening of the ion channel. Moreover, they are also able to obstruct open ion channels mechanically (517).

The mechanism by which the depolarizing neuromuscular blocking agents, decamethonium, suxamethonium, and similar compounds, operate is less clearly understood. It is thought that they act by mimicking the effects of acetylcholine itself, which when present for long enough in high concentration causes blockade by preventing action potentials being propagated away from the zone surrounding the motor end-plate. Gradual access of the drug to the end-plate allows slow development of depolarization and the local electric currents induced enable the opening of potassium and sodium channels to keep pace with each other, so that no action potential is propagated and a surrounding zone of inexcitability is built up. In the case of acetylcholine, hydrolysis by cholinesterases soon puts an end to its effects, but decamethonium and suxamethonium bind to the receptor more tenaciously and are more resistant to hydrolysis (152, 485).

Muscle relaxants with a depolarizing type of action suffer from several disadvantages, for, as with acetylcholine, their initial response before paralysis sets in may be to stimulate muscle contraction, and this can cause severe postoperative muscle-pain and cramp. It also means that there is a prolonged period during which the muscle is unable to respond to stimulation. Intraocular pressure may be raised and in burned patients they may cause hyperkalaemia. In addition, the neuromuscular-blocking effects of such drugs cannot be readily reversed by anticholinesterases such as neostigmine (152, 485, 501).

1.6.3. Muscle Relaxants—Criteria and Design Requirements

As experience was gained in the practical use of muscle relaxants, it became possible to determine the range of properties desirable in such substances. The following are among those considered to be important (518):

(a) High potency.
(b) Capable of being displaced by large doses of the natural neurotransmitter acetylcholine, thus, in an emergency or at the end of an operation, allowing the anesthetist full control in reversing the blockade and hence the muscle paralysis.
(c) Selective blocking action on the acetylcholine receptors of the skeletal muscles, i.e. high specificity for the neuromuscular junction and freedom from cardiovascular effects.
(d) Rapid onset of action, for use in emergencies.
(e) Short duration of action on administration of a single dose—not longer than 10–15 minutes—and no potentiation or cumulation on repeated administration.
(f) Consistency of response, in combination with a short duration of action,

and rapid recovery which should not be affected by the clinical status of the patient.
(g) Low toxicity.
(h) Formation of inactive metabolites that are rapidly eliminated.
(i) Availability of effective antagonists.
(j) Stability on storage.

These criteria, especially (b), indicate a clear preference for neuromuscular-blocking agents with a nondepolarizing action. At the same time, it is evident that such a formidable array of constraints represents a considerable challenge to the ingenuity of medicinal chemists. Increased knowledge of the structural requirements for nondepolarizing neuromuscular-blocking drugs has, however, permitted a less empirical and more positive approach to their design. Thus, in developing the newer generations of muscle relaxants, features that have been taken into consideration in attempting to meet the above criteria include (485, 519, cf. 520):

(1) Ammonium, i.e. quaternary nitrogen, preferred to other onium functions because of greater blocking power.
(2) Bis- rather than monoquaternary structures for greater potency (but cf. Section 1.6.4.1).
(3) Distance between the two quaternary nitrogens about 1.1 nm, corresponding to about 10 carbon atoms, for maximum neuromuscular- rather than ganglion-blocking potency (but again, cf. Section 1.6.4.2).
(4) Bulky molecule and bulky substituents on the nitrogen atoms to favor a nondepolarizing mode of action.
(5) Inclusion of acetylcholine-like moieties to allow hydrolysis and to increase affinity for the appropriate receptors.

1.6.4. More Recent Developments

The volume in the "Handbook of Pharmacology" series entitled *New neuromuscular blocking agents* (512), already mentioned, presents an account which combines reviews of both basic and applied aspects of research into the structure and function of the neuromuscular junction as well as of progress in the design of improved muscle relaxants. It is a fitting testimony to the tremendous interest in the field. To bring the present story to a close, the following two sections show how the criteria and structural requirements detailed in Section 1.6.3 have shaped the design and development of the newer generations of muscle relaxants. But reference to the book just mentioned will show that many more avenues than can be discussed here are currently being explored.

1.6.4.1. Quaternary Steroid Derivatives

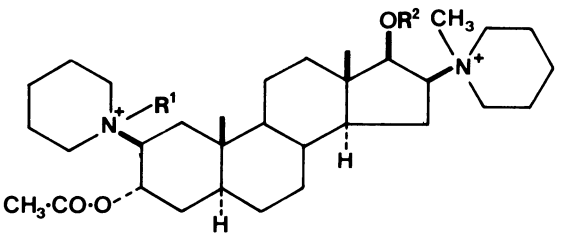
Work in the 1880s on the Venezuelan arrow poison *guachamacá*, known to be derived from species of *Malouetia* (Sections 1.2.1.2 and 1.4.9), led to the

isolation of an alkaloidal substance with curare-like properties (see: 73, 74). Then, in 1960, a bis-quaternary steroidal base, malouetine (**53**), was extracted from bark of the Central African *M. bequaertiana*. In the rabbit it elicited head-drop at ED_{50} 0.15 mg/kg—a dose level similar to that of tubocurarine. Interestingly enough, the compounds epimeric at C(3) and/or C(20) are active at very similar dose levels (521). Though South American species of *Malouetia* also exhibit muscle-relaxant activity, none of the compounds responsible has yet been isolated.

The highly potent toxiferine, with its fixed inter-onium distance, suggested that rigid or semirigid molecules of appropriate structure might be a fruitful source of strong muscle relaxants. And indeed the steroid nucleus has provided, and continues to provide, the frame for many such molecules (522). Be that as it may, during the late 1960s and early 1970s, when testing some monoquaternary steroid derivatives, one of them bearing 3α-acetoxy and 2β-*N*-methylpiperidinium substituents in ring A of androstane, simulating acetylcholine, was found to have about 6% of the muscle-relaxant activity of tubocurarine. Comparison of this derivative with the structures of tubocurarine (**18j**) and malouetine (**53**) suggested to Buckett et al. (523) that addition of a second simulated acetylcholine moiety at the other end of the molecule, in ring D, would give rise to improved activity. The development of pancuronium (**59c**), Pavulon®, the first of the biodegradable muscle relaxants, was the outcome. The required neuromuscular-blocking dose is about one-fifth of that of tubocurarine (Table 1.11).

In accordance with the design requirements listed in Section 1.6.3, in pancuronium the bulk is supplied by the steroid skeleton, which also positions the nitrogen functions at about the correct distance from each other, and bulky substituents and acetylcholine-like moieties are provided by the *N*-methylpiperidinium groups and nearby functional groups. However, the compound still suffered from a number of disadvantages in that it had a slow onset time and a fairly long duration of action (though significantly shorter than that of tubocurarine), as well as having some cardiovascular effects. The drug is metabolized mainly by deacetylation, which leaves the steroid nucleus and its substituents intact, with the consequence that the metabolites formed, the 3-*O*-desacetyl, 17-*O*-desacetyl (dacuronium, **59b**), and 3,17-*O*-didesacetyl derivatives, still retain some muscle-relaxant activity. However, in the case of the first and third of these metabolites it is only about 1/50th of that of pancuronium. The duration of the blockade is also greatly extended in patients with renal or liver dysfunction (524).

The demonstration that tubocurarine was a mono- rather than bis-quaternary compound rekindled interest in the monoquaternary base (**59a**) corresponding to pancuronium, and detailed studies revealed that it actually had a better balance of properties than the bis-quaternary compound. It was introduced into clinical practice in 1983 as vecuronium (ORG-NC-45; Norcuron®). In man, the onset time is 2–3 mins. with doses up to 0.2 mg/kg; the 25% and 90% twitch-strength recovery times are 20 and 40–50 mins.,



59a Vecuronium

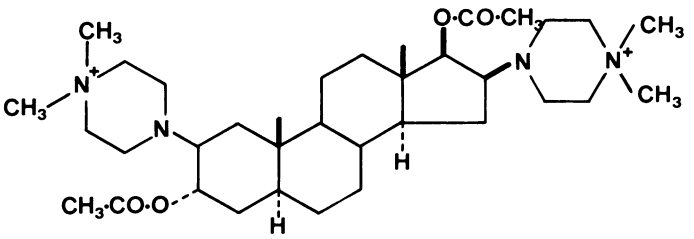
R^1 = H, R^2 = Ac

59b Dacuronium

R^1 = Me, R^2 = H

59c Pancuronium

R^1 = Me, R^2 = Ac



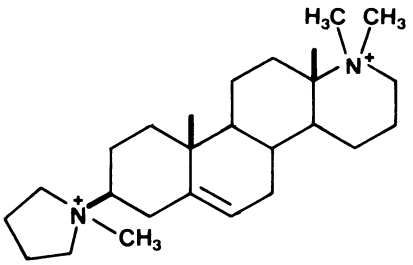
60 Pipecuronium

respectively, for a dose of 0.1 mg/kg. The drug is manufactured as a lyophilized product which also contains a citrate-phosphate buffer and mannitol; it has a shelf-life of two years, but, after being reconstituted, the aqueous solution is stable for only 24 hours. The drug exhibits little cumulation of activity and it has little effect on the cardiovascular system. It is looked upon as fulfilling the requirements for a nondepolarizing neuromuscular-blocking agent with an intermediate duration of action.

The properties and clinical application, etc. of vecuronium have been reviewed (501, 525–527).

Pipecuronium (**60**) (Arduan®) is a further variation on the steroid theme that has undergone clinical trials in Hungary (485, 528). It is currently available in the United States as the bromide salt. It is described as a long-acting, neuromuscular blocker which has no vagolytic activity, but which may require a reduction in dosage for patients with impairment of the renal function (545). Cf Section 1.6.4.2.

Harkishan Singh and coworkers have modified the steroid framework to give a 6-membered D-ring by introduction of a 17a-quaternary nitrogen, which, together with a 3β-*N*-methylpyrrolidino substituent, has yielded a



61 Chandonium

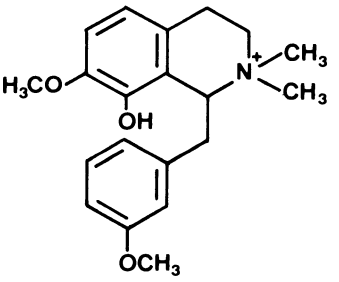
potent, rapidly acting drug of intermediate duration, chandonium (HS-310) (**61**). It has undergone clinical trials, but among its side effects are mild tachycardia and hypertension (529, cf. 530, 549). CNDO molecular-orbital calculations of the charge densities show that there is considerable delocalization of the positive charge on the nitrogen of the C(3) substituent; the nitrogen retains only about 3% of the charge, with most of the rest being located in the pyrrolidine ring itself and its *N*-methyl group and in the adjacent C(3) of the steroid A-ring (531). Doubtless, this delocalization applies to the quaternary functions in other muscle-relaxant molecules.

1.6.4.2. Atracurium

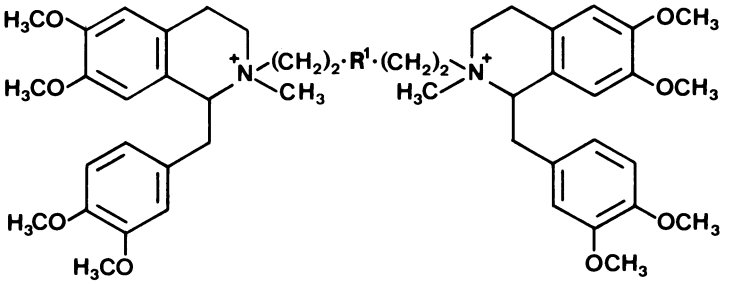
The reasoning that led to the development of Atracurium (Tracrium®) has been discussed in several publications (533–536) and need not be repeated in detail here. Suffice it to note that deficiencies were recognized in the drugs developed in the 1960/70s: slower onset and longer duration of action than suxamethonium, lack of specificity, i.e. freedom from cardiovascular effects, and undue prolongation of muscle relaxation in patients with renal or hepatic impairment.

The fact that a naturally occurring quaternary isoquinoline alkaloid, the papaverinium base petaline (**62**), undergoes spontaneous Hofmann degradation under unusually mild conditions suggested a possible solution to the problem of developing a more advanced (bio)-degradable drug. The project aimed to synthesize a compound which would be stable enough to exert its action for a moderate length of time and to allow storage without deterioration until required, but which would also be labile enough to degrade under the physiological conditions of pH 7.4 and 37°C to essentially inactive materials. Four series of compounds were synthesized and when tested proved that the idea was indeed feasible.

However, it was only with evaluation of the fourth series (**63b**) that the sought-for desirable features were largely realized (532, 533). The structure allowed for changes in the end groups and in the central chain linking them. Evaluation showed that there was good separation between the vagal blocking and neuromuscular paralysing doses, i.e. good specificity, especially for the analogues with tetrahydropapaverinium residues as end groups and n = 5



62 Petaline



63a Laudexium

$R^1 = (CH_2)_6$

63b Polyalkylene diesters

$R^1 = CO\text{-}O\text{-}(CH_2)_n\text{-}O\text{-}CO$

63c Atracurium

$R^1 = CO\text{-}O\text{-}(CH_2)_5\text{-}O\text{-}CO$

or 6 in the central chain. The analogue with n = 5 (BW33A) was finally chosen for detailed study and eventually became atracurium (**63c**).

The electron-withdrawing positively-charged nitrogens and ester carbonyl groups facilitate the Hofmann elimination. The former also promote nucleophilic attack under alkaline conditions (OH^-) at the β C—H bond, thereby allowing fission of the α C—N^- bond, and at the ester carbonyl groups, thus also leading to ester hydrolysis. The rate of degradation of the molecule is dependent on the pH and temperature of the blood and increases when the pH is raised. In man, decomposition takes place via two pathways: the principal one is the Hofmann elimination, which leads to the formation of laudanosine and a quaternary monoacrylate, while a subsidiary one is hydrolysis brought about by nonspecific plasma esterases, which produces a quaternary acid and quaternary alcohol. None of the metabolites formed has significant physiological activity at therapeutic dose levels of atracurium. Inactivation of the drug is thus achieved by both nonenzymic and enzymic mechanisms and is largely independent of the hepatic and renal functions

Cis-isomer *Trans*-isomer

64 Atracurium: *cis-trans*-isomerism

(524, 527, 532–534, 536). Introduced in the early 1980s, like vecuronium, the drug fulfils the requirements for a neuromuscular-blocking agent with an intermediate duration of action.

Atracurium is produced in a three-stage synthesis, by addition of the end-group amine papaverine to the appropriate bis-acrylate in a Michael reaction, and is a mixture of stereoisomers of constant composition. It has four chiral centres: C(1), N(2), C(1′), and N(2′), but the 16 theoretically possible isomers are reduced to 10—four racemates and two *meso*-compounds—because of the symmetry of the compound. There is also *cis-trans*-isomerism around the C(1)–N(2) bond and, relative to the *R* or *S* configuration of the tetrahydroisoquinoline units, *cis* is defined arbitrarily as the arrangement in which the two bulky groups are *cis* to each other (**64a, b**). Quaternary *R*, *R*, *R*, *S*/*meso*, and *S*, *S* stereoisomers have been made, and in their NMR spectra it is observed that the H(8)/H(8′) signal is split, owing to shielding differences arising from the *cis-trans*-isomerism. The major component, at δ 5.9, is assigned to the isomer with the *cis*-arrangement and the minor one, at δ 5.65, to the isomer with the *trans*-arrangement. Analysis by NMR spectrometry or, better, by HPLC, which separates the *cis-cis*-, *cis-trans*-, and *trans-trans*-isomers, shows that in each of the synthesized *R*, *R*, *R*, *S*/*meso*, and *S*, *S* products the three isomers are present in the approximate ratios 10.5 : 6.5 : 1. The molar neuromuscular-blocking potencies of the three products relative to (+)-tubocurarine (= 100) determined using the gastrocnemius muscle—sciatic nerve preparation in anaesthetized cats are: *R*, *R* 231, *R*, *S*/*meso* 133, and *S*, *S* 89, again showing the considerable influence that stereochemistry has on the biological activity (504).

Full neuromuscular block with atracurium is achieved in man with doses in the range 0.26–0.30 mg/kg; the onset time is 1.7–3.0 mins., while the 90% twitch-strength recovery time is about 40 mins. for a 0.3 mg/kg dose. To permit the preparation of small volume injections for clinical use, the alkylating agent finally chosen for the quaternization was methyl benzenesulphonate—the drug is put up in 2.5- or 5-ml ampoules containing 10 mg/ml and the solution is stable for at least two years at the optimal pH of 3.5 (which inhibits possible occurrence of the Hofmann elimination) and storage at 5°C (504, 527, 534, 536).

The properties and clinical use, etc., of atracurium have been reviewed (501, 527, 537, 538).

1.7. Conclusion

In the foregoing pages we have followed the story of curare from the early encounters by the Spanish Conquistadores, through its use by the forest tribes in hunting and occasionally in warfare, through the discovery of its plant sources and the isolation and chemistry of its purified active principles, to the introduction of some of them as valuable muscle relaxants in surgery and to their subsequent role as templates leading to the development of improved products. Curare is thus an excellent example of a material that has made the successful transition from an ethnic product to a purified substance essential to the practice of modern medicine.

Before even the story started, there was a problem—a simple but important one—namely, What do we mean by the term **curare**? While this depends, to some extent at least, on the point of view of the person asking the question, the definition accepted here has been a pharmacological one—that is, a group of dart and/or arrow poisons varying in composition and featuring muscle relaxation as their basic pharmacological action. Poisons with similar properties have been reported from Central Africa and Western Malaysia, so that, in keeping with the foregoing definition, it may be argued that curare is no longer to be thought of as a purely South American product.

In spite of the vast amount of research that has been carried out, especially over the last century and a half, the present account demonstrates that much remains to be done. There is still much to learn about the botany of many of the plants, both Loganiaceae (*Strychnos*) and Menispermaceae (*Curarea*, *Abuta*, etc.), that are presumed to be sources of the active alkaloids. With only a few exceptions, present knowledge of the chemistry of almost half the *Strychnos* species used in curares is very fragmentary or nonexistent. The well-recognized sensitivity of many *Strychnos* alkaloids to the effects of pH, temperature, oxygen, and heat, with the concomitant formation of numerous artefacts, has been, and still is, a major stumbling block to their investigation in plant materials and curares—a multitude of incompletely characterized alkaloids from a host of *Strychnos* species still awaits clarification. Even the plant from which commercial supplies of curare are derived, *Chondrodendron tomentosum*, has not been fully studied: Are the two varieties that can supposedly be recognized in the field chemically identical (Section 1.4.6.1)? What is (are) the source(s) of the unknown weakly active laevorotatory quaternary alkaloid found in some commercial curares (Section 1.4.7.7)? Where did King's weakly active (−)-tubocurarine (Section 1.4.6.1) come from? Is it in fact related to the previous alkaloid? About the distribution of quaternary bases in the Menispermaceae associated with curare nothing is known except for their occurrence in just one *Chondrodendron* and one *Abuta* species; yet, members of other genera, e.g. *Curarea*, *Cissampelos*, etc., are reported to be essential components of curares and are known to deploy muscle-relaxant activity.

The function (if any) of the majority of plants incorporated into curares as

additives, and sometimes as main ingredients, is obscure. Many contain bis-benzyltetrahydroisoquinoline bases, but what pharmacological, or perhaps synergistic, activity these may have is still not known. But this merely serves to emphasize a widely-recognized general point—that study of the pharmacology of natural products has not kept up with their isolation and determination of structure.

It is one of the ironies of the curare story that a large part of the work leading to the development of synthetic analogues with improved muscle-relaxant properties has been based on two myths: (a) the belief that (+)-tubocurarine was a bis-quaternary alkaloid, and (b) the belief that an interonium distance, i.e. the distance between the two hetero-atoms (nitrogen), of about 10 carbon atoms was optimal for muscle-relaxant activity. More recent developments have shown that neither condition is essential: vecuronium is a monoquaternary compound and atracurium has 13 atoms between its two quaternary nitrogen atoms (536). But, however that may be, it is the active principles from curares that have provided both insight and some of the models which lie at the basis of these newer and improved muscle relaxants.

2, 2′-[1,5-Pentanediylbis[oxy(3-oxo-3, 1-propanediyl)]]bis[1-[(3, 4-dimethoxyphenyl)methyl]-1, 2, 3, 4-tetrahydro-6, 7-dimethoxy-2-methylisoquinolinium] dibenzenesulphonate, more conveniently known as atracurium besylate, is indeed a far cry from the raw dart poison of the South American forests. And with its combination of nonenzymic and enzymic degradation pathways, does represent a significant advance as a muscle relaxant, among other things, in that its function is largely independent of the hepatic and renal status of the patient. But, that the development of neuromuscular-blocking agents has not yet come to an end is clear from the desiderata adumbrated by Kharkevich (518): A nondepolarizing, short-acting drug which could be used for controlled neuromuscular block in surgery is still required, and such a substance could become a quasi-universal drug. Other needs are for long-acting neuromuscular blocking agents which will have little effect on haemodynamics and spontaneous respiration or which can be administered orally without causing undue suppression of respiration and hence can also be used in the treatment of neurological conditions characterized by increased muscular tonus. In the development of future generations of muscle relaxants, curare and its alkaloids will surely continue to be a source of inspiration.

1.8. Note added in Proof

In the Conclusion (Section 1.7), certain desiderata—as yet unfulfilled by currently used muscle relaxants—were set out. While these desiderata are indeed still valid, it also has to be realized that surgery itself has been changing. In recent years, outpatient (ambulatory) or day-case surgery has expanded enormously on both sides of the Atlantic and elsewhere, and in the United States 40% of all operations in a hospital setting are now outpatient.

TABLE 1.12. Fragen-Shanks classification of muscle relaxants [from: 551].

Group	Onset time (mins.)	25% Twitch height recovery time (mins.)	95% Twitch height recovery time (mins.)[a]	Examples
A. Ultra-short	1	5–8	15	Suxamethonium
B. Short	1–2	15	30	Mivacurium
C. Intermediate	2–3	20–30	40–60	Vecuronium, atracurium
D. Long	4–6	45–60	90–180	Gallamine, (+)-tubocurarine, metocurine, pancuronium, pipecuronium, doxacurium

[a] The 95% twitch height recovery time corresponds to the time required for clinical recovery.

This has led to changes in anaesthesiological practice and in the use of the associated muscle relaxants, which ideally should have rapid onset and recovery times. Fragen and Shanks (551) have put forward a classification of muscle relaxants in four groups, each with a different time course (Table 1.12).

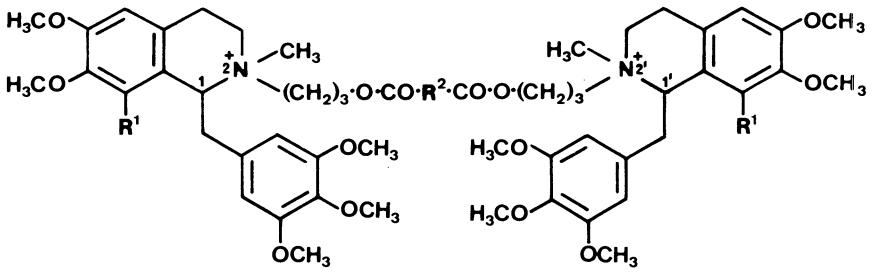
The depolarizing suxamethonium (disuccinylcholine) is the only available ultra-short muscle relaxant, but nondepolarizing muscle relaxants in this category are now being studied. Representatives of the intermediate group, such as vecuronium and atracurium, are suitable for day-case use, while gallamine, metocurine, (+)-tubocurarine, pancuronium, and pipecuronium, etc., are all too long-acting for most day-case procedures.

Two new nondepolarizing bis-benzyl tetrahydro isoquinoline muscle relaxants with negligible cardiovascular effects are now undergoing clinical trials. Mivacurium (BW B1090U) (**65a**) has a short-lasting action, with an ED_{95} of 0.1 mg/kg. The substance is rapidly hydrolysed by plasma cholinesterase—at nearly 90% of the rate of suxamethonium—and some hydrolysis by liver esterases may also take place as well. The recovery time is about half that of vecuronium and atracurium. Minor cutaneous side effects occur sometimes (551–553). Mivacurium has been approved for general use (1992).

Doxacurium (BW A938U) (**65b**) has a relatively long period of action and its ED_{95} is 0.025–0.03 mg/kg. The substance is probably excreted largely unchanged via the kidneys and in cases of renal failure the period of action is almost doubled (553–556).

The development of these two new muscle relaxants highlights the improved understanding of structure-activity relationships in bis-benzyl tetrahydro isoquinolinium derivatives. This has allowed their chemistry and metabolism to be manipulated so as to yield drugs with a short (mivacurium), intermediate (atracurium), or long (doxacurium) duration of action (553).

Like atracurium (Section 1.6.4.2), mivacurium and doxacurium have four chiral centres, at C(1), C(1′), N(2), and (2′). Thus, mivacurium is presented as the dichloride of a mixture of *trans-trans* (1*R*, 1′*R*, 2*S*, 2′*S*), *trans-cis* (1*R*, 1′*R*, 2*S*, 2′*R*), and *cis-cis* (1*R*, 1′*R*, 2*R*, 2′*R*) stereoisomers, all with the central



65a Mivacurium

$R^1 = H$, $R^2 = (CH_2)_2{\cdot}\overset{H}{C}{=}\overset{H}{C}{\cdot}(CH_2)_2$

65b Doxacurium

$R^1 = OMe$, $R^2 = (CH_2)_2$

double bond in the *E*-configuration; their individual muscle-relaxant activities exhibit considerable differences (557, 558). Doxacurium likewise consists of a mixture of the dichlorides of three stereoisomers—a *meso* form (1*R*, 1′*S*, 2*S*, 2′*R*) and two enantiomers (1*R*, 1′*R*, 2*S*, 2′*S*) and (1*S*, 1′*S*, 2*R*, 2′*R*) (558).

A useful reverse-phase HPLC method of assay in biological fluids which is applicable to doxacurium, mivacurium, and many of the currently used muscle relaxants is now available (559).

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Appendix: Chemical Synonymy

Alkaloid 8 from *Strychnos toxifera* = Caracurine metho salt
Alkaloid B from *Cissampelos pareira* = Cycleanine
C-Alkaloid K = Dihydrotoxiferine
C-Alkaloid Q = Macusine B (Hesse [257])
C-Alkaloid Y = Profluorocurine
Caracurine VII = Wieland-Gumlich aldehyde
Cissamine = Cyclanoline
C-Curarine I (Wieland and Pistor [244]) = Toxiferine (Wieland et al. [245]) = Curaine
C-Curarine III = Fluorocurarine
Desacetyldiaboline = Wieland-Gumlich aldehyde
18-Desoxy-Wieland-Gumlich aldehyde = Nor-dihydrofluororcurarine = Nor-hemi-dihydrotoxiferine
18-Desoxy-Wieland-Gumlich aldehyde metho salt = Dihydrofluorocurarine
2,16-Didehydro-18-desoxy-Wieland-Gumlich aldehyde metho salt = Fluorocurarine
7,7′-*O*,*O*-Dimethylisochondrodendrine = Cycleanine
Dihydrofluorocurarine = 18-Desoxy-Wieland-Gumlich aldehyde metho salt
O,*O*-Dimethylwarifteine = *O*-Methylcissampareine
Fluorocurarine = 2,16-Didehydro-18-desoxy-Wieland-Gumlich aldehyde metho salt
Hemi-dihydrotoxiferine = 18-Desoxy-Wieland-Gumlich aldehyde metho salt
Hemitoxiferine [I] = Wieland-Gumlich aldehyde metho salt
Homomoschatoline = *O*-Methylmoschatoline
O-Methylcissampareine = Dimethylwarifteine
Nor-dihydrofluorocurarine = 18-Desoxy-Wieland-Gumlich aldehyde
Nor-hemi-dihydrotoxiferine = 18-Desoxy-Wieland-Gumlich aldehyde
Profluorocurine = C-Alkaloid Y
C-Strychnotoxine [I] (Wieland and Merz [423]) = Calebassine
Toxiferine (Wieland et al. [245]) = C-Curarine I (Wieland and Pistor [244]) = Curarine
C-Toxiferine II (Wieland et al. [247]) = Calebassine
Toxiferine II (King [243]) = Unknown alkaloid ≠ C-Toxiferine II (Wieland et al. [247])
Toxiferine IV (King [243]) = C-Alkaloid A
Toxiferine V (King [243]) = C-Toxiferine I
Toxiferine IX (King [243]) = Caracurine II metho salt
Toxiferine XI (King [243]) = C-Toxiferine I
C-Toxiferine II (Wieland et al. [247]) = Calebassine (Battersby et al. [265])
Wieland-Gumlich aldehyde = Desacetyldiaboline = Caracurine VII

2
Alkaloid Chemistry and Feeding Specificity of Insect Herbivores

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2.1. Introduction

The role of plant secondary natural products and their interactions with insect herbivores have been focal points for research by chemists, botanists, and entomologists for several years and is the topic of several excellent reviews (1, 2, 3, 4). For example, Hedin et al. (5) have an early review of modifications in insect behavior due to host plant secondary natural products. Included among the classes of compounds listed as feeding stimulants, repellents, or deterrents are: flavonoids, terpenoids, phospholipids, glycosides, acids, esters, phenolics, and alkaloids. In general, alkaloids and their glycosides seem to be most often involved as feeding repellents or deterrents, although there are examples when alkaloids are specific feeding cues. Barbosa et al. (6) have linked the presence of alkaloids in deciduous trees with feeding

avoidance by the polyphagous feeding gypsy moth *Lymantria dispar*. They have shown that when the major groups of plant secondary natural products are examined, alkaloids rank the lowest in feeding preference (7). In this paper we will examine potential relationships between the chemistry of alkaloids in the host plants and effects that these compounds may have on insect herbivores. In many cases the specific alkaloid that is produced by a particular plant has different effects on different insects. When the data are available, specific associations between the plant alkaloids and the feeding behavior of the insect herbivore will be made. Examples will be cited throughout the manuscript to indicate that generalist feeding insects often react quite differently to potentially toxic alkaloids than do specialist insect herbivores. Our intent was to describe how minor chemical modifications in alkaloid chemistry modifies the effectiveness of the plants chemical defensive arsenal.

Alkaloids represent a chemically heterogeneous group of compounds not sharing a common biosynthetic pathway, but generally sharing some similar chemical characteristics. Pelletier (8) defines an alkaloid as a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms. Usually the basic nature of alkaloids is attributable to the presence of nitrogen in the form of a heterocyclic ring. This basic nature gives alkaloids a propensity to form salts of endogenous organic acids, and it also gives the plant biochemist a mechanism to extract and isolate them from biological fluids.

Alkaloids are fairly widespread among the dicots. In North America, they occur in 30% of the annuals, and approximately 20% of the perennials (9), and several taxa of fungi also produce alkaloids. For taxonomic purposes, grouping according to biogenetic origins is most useful. Many genus- and family-characteristic patterns are known (10, 11). Gomes and Gottlieb (12) have produced an evolutionary scheme for angiosperms based on alkaloid distribution and biosynthetic pathways. The function of alkaloids is a subject that has generated debate for a number of years. The concept that alkaloids are simple by-products of metabolism which serve only as waste products is no longer a popular one. It has been challenged by an ever growing body of evidence for ecological function as well as by the fact that they turnover rapidly and create considerable metabolic demand for their continual synthesis. In one example using tobacco Robinson has calculated that the plant utilizes more than 17% of its net fixed CO_2 for the production of nicotine in a single day (13).

Within the plant life cycle, alkaloids fluctuate in their concentrations, and these fluctuations are not necessarily correlated with the senescence of the plant. For example, the opium poppy is traditionally harvested after flowering, when the developing ovules are forming, to maximize the recovery of the alkaloid opium. In periwinkle (*Catharanthus roseus*), several indole alkaloids which are rapidly synthesized during germination of the seedlings eventually decline and concentrations increase again as the plant matures (14). Perhaps the single most widely accepted and important function of alkaloids, and the

subject of this chapter, is their physiological or pharmacological effect on herbivores exposed to endogenous levels of alkaloids during feeding. Evidence for a role of alkaloids in providing protection for plants from potential herbivores, including insects, comes from a variety of sources of both a direct and indirect nature.

2.2. Insect Feeding Preferences: Specialists vs Generalists

Levin has hypothesized that a definitive correlation exists between the presence of alkaloid containing species and the latitude at which these species occur. He suggests that herbivore pressure is the driving factor behind this biogeographic distribution with increased numbers of alkaloid containing species residing in the tropical regions where increased pest pressure is exerted. Finding that annual plants have nearly twice the incidence of alkaloids as perennials he suggests that the alkaloid chemical defense strategies should he most effective against the generalist insect herbivores because they lack the mechanisms to detoxify the defensive allelochemicals of more than a few species in an environment with a proliferation of potential host plants (15).

Evidence that chemical defenses which are effective against generalist insect herbivores may be circumvented by specialists for use as either sequestered defensive compounds of their own or as cues for feeding or mating behavior is abundant. For example, the aphid *Acrythosiphon spartii* uses the alkaloid sparteine[1] as a feeding cue (16). Beck (17) points out that the plant hosts to which the insects are adapted contain a wide variety of secondary natural products including alkaloids, phenolics, and various glycosides that are basically deleterious to the insect. It is the ability of the insect to expend considerable metabolic energy to cope with the potential plant toxins that enable it to effectively utilize the plant as a host. Because different plant species or even populations differ qualitatively or quantitatively in their defensive chemistry, an insect adapted to one plant often may not be able to meet the metabolic demands posed by another plant, thereby selecting for a monophagous existence. This concept seems to be reinforced by the examples in this manuscript. The specialization of feeding preference of an insect is often directly proportional to the ability of the insect to cope with extensive alkaloid chemical defensives of the plant host. The polyphagous insect feeders tend to feed on plant hosts with less toxic chemicals or digestibility reducing defensive mechanisms.

This is not to say that the direction of ecological selection is necessarily from a primitive characteristic of polyphagous feeding to monophagy. Indeed, polyphagous feeders are exposed to a wide variety of potentially toxic compounds and must develop mechanisms to handle this diversity of plant chemical defenses. However, the specialization of a monophagous feeding

[1] For the readers convenience Table 2.3 contains the chemical structures of the alkaloids discussed in this manuscript arranged in alphabetical order.

insect is so intimately linked to the host plant that the herbivore is often dependent upon toxic alkaloids in the diet for its survival. One such example is seen in the tetrahydroisoquinoline alkaloids lophocereine and pilocereine (the trimer of lophocereine) which are present in the senita cactus *Lophocereus schotti*. These alkaloids were tested against eight species of *Drosophila* present in the same ecological range. The trimer pilocereine was more toxic than the monomer, possibly due to the stability of the larger molecule. Only *Drosophila pachea*, the normal species which lives on decaying senita cactus stems, was able to tolerate 1% levels of these alkaloids in artificial diets. Not only is *D. pachea* tolerant to lophocereine and pilocereine, but the insect is not able to be grown on artificial media without supplementing the media with extracts of the cactus containing these alkaloids (18).

It should also be remembered that the defensive role of alkaloids in host protection is probably not as simple as the presence or absence of a single functional group on an alkaloid or even whether the alkaloid is in the host plant at all. In many cases, there may be additive effects of feeding deterrent compounds based on the entire chemical composition of the host plant. For example, when the alkaloids nicotine and tomatine, each of which have been shown to exhibit feeding deterrences of 25%, were fed in artificial diets to *Locusta migratoria* the overall feeding deterrence was approximately 50% (19). In this case the alkaloids acted in an additive fashion to produce a more effective chemical defensive mechanism. Adams and Bernays (19) went on to show that this additive characteristic of feeding deterrence was not restricted to alkaloids but was equally effective with alkaloids and various combinations of phenolics, terpenoids, and other miscellaneous secondary plant compounds.

2.3. Simple Pyrrolidine and Piperidine Alkaloids

One of the explanations for the toxic properties of some nonprotein amino acids is their ability to form anomalous proteins. When the structure of a particular compound is structurally analogous to its protein constituent, the nonprotein amino acid may be incorporated. The well-known case of canavanine substitution for arginine in insect proteins is due to the inability of most insects' arginyl-tRNA synthetases to discriminate the two compounds (20). When canavanine containing food is consumed, proteins with disrupted tertiary structure are built and the insects may not survive.

The simple structures of many alkaloids in the pyrrolidine/piperidine group enables them also to compete with protein amino acids. Molecular size, as well as similarity in structure, is important in whether or not substitution in a protein will occur. For example, 3,4-dehydroproline, a toxicant known to compete with proline for prolyl-tRNA synthetase, will incorporate into protein. The nontoxic baikiain (4,5-dehydropipecolic acid), the higher piperidine homologue, however, does not. The size of the molecules offers the

best explanation. In the former, carbon-carbon bond sizes are comparable to those of proline, but baikiain is too small to compete effectively (21).

Similarly, azetidine-2-carboxylic acid and pipecolic acid, the lower and higher homologues of proline, differ significantly in their toxicity to insects. Azetidine-2-carboxylic acid (AZC) is incorporated into proteins, being severely toxic in most cases, since it rotates the α-helix of the polypeptide chain through an angle 15° less than the pyrrolidine ring of proline. It affects tertiary structure in a wide range of organisms including insects, humans and plants (22; 23; 24). Among invertebrates, the growth and development of larvae of the nematode *Ascaris suum* are affected by this compound when disruption of normal cuticle synthesis occurs. The effects are antagonized by proline (25). Toxic effects are observed also in tobacco hornworm, *Manduca sexta*, (26) and *Spodoptera littoralis* (27), but the mechanism has not been determined in these. In contrast to AZC, pipecolic acid is not incorporated into protein and it is only moderately toxic. This piperidine is too big to compete with proline for prolyl-tRNA activation (21).

An alkaloid incorporated into a protein will not automatically be lethal. If the active site and the tertiary structure of the protein are not affected, the effects of incorporation of the alkaloid homolog may go unnoticed. Additionally, even if an anomalous protein is formed, it may not persist long enough to cause deleterious effects. For example, canavanine containing proteins are selectively degraded by a number of eukaryotic organisms. There is apparently little problem for the plants which produce AZC. They are spared protein disruption in their own cells because they have adapted by evolving prolyl-tRNA synthetases with larger active sites unable to recognize the alkaloid, thereby enabling them to produce and store it with impunity (28, 29). One would expect herbivore specialists to evolve similar adaptive measures just as certain bruchid beetles have done for canavanine (30).

2.4. Polyhydroxy Alkaloids

Recently there has been much interest in a group of polyhydroxylated alkaloids which are derivatives of piperidine, pyrrolidine, pyrroline, octahydroindolizine, and pyrrolizidine (31). The piperidine compounds are analogs of 1-deoxypyranose sugar with the ring oxygen replaced by nitrogen. The pyrrolidines are similar analogs of furanose. Thus, for example, deoxymannojirimycin (DMJ) and deoxynojirimycin (DNJ) are analogs of mannose and glucose respectively. Dihydroxymethyldihydroxypyrrolidine (DMDP) is the analog of fructose. Two bicyclic octahydroindolizidines, castanospermine and swainsonine, and the trihydroxypyrrolizidine, alexine, although not structurally resembling monosaccharides, have configurations of hydroxy substitutions which can be compared with sugars.

These polyhydroxy alkaloids are known from a wide range of microorganisms and plant families (32). Legumes are a particularly rich source in which

they may accumulate up to 2% of plant dry weight (33). The common thread among them is their ability to inhibit various glycosidases in insects, mammals, and microorganisms. They are often quite specific in their activity. DNJ, for example, is a potent inhibitor of mammalian α-glucosidase, but has little effect on insect α-glucosidase activity. In contrast DMDP is a potent inhibitor of α-glucosidases of bruchid beetles and insect trehelase (34, 35), but in mammals it preferentially inhibits β-glucosidase activity (32). Loss of the hydroxymethyl group on C (5) of DMDP changes the molecule from a beta to an alpha-glucosidase inhibitor of the glucosidases of mouse digestive mucosa.

Elbein and Molyneux (36) in an earlier review emphasized that three structural features are common in all alkaloid glycosidase inhibitors (AGI) which are toxic to mammals: they have a secondary or tertiary nitrogen atom in a pyrrolidine, piperidine or indolizidine ring; they have at least three hydroxyl groups in a β-position relative to the nitrogen; and they have fixed stereochemical relationships between the hydroxyl groups, which likely accounts for the specificity of the enzyme inhibition observed. Data from insect studies suggest that these same structural constraints may apply. Clearly there is much yet to be learned empirically about specificity of these compounds.

Polyhydroxy alkaloids may also be insect antifeedants. DMDP is a strong feeding deterrent to a number of crop pests including the acridids *Schistocerca gregaria* and *Locusta migratoria* as well as the armyworms *Spodoptera littoralis* and *S. exempta.* Levels of 0.001% reduced feeding in the acridids, higher levels in the armyworms. Concentrations above 1% produced high mortality (37). Electrophysiological studies of *Spodoptera* taste sensilla suggest that DMDP may block both the glucose and fructose receptor sites making insects unable to respond to sugar phagostimulants (31).

Interestingly, at least some insects have adapted to AGIs. For example, the beetle *Ctenocolum tuberculatum,* a specialist feeder on DMDP rich *Lonchocarpus costaricensis,* has an α-glucosidase 100 times less sensitive to this compound than that of the bruchid *Callosobruchus maculatus* (32). All beetles of the *Ctenocolum* genus have a restricted host range which always includes species of *Lonchocarpus* rich in DMDP (32), suggesting that they are using this compound as a feeding cue.

In another remarkable about face, larvae of the moth *Urania fulgens* feed exclusively on species of *Omphalea* (Euphorbiaceae) which contain high levels of 3 different AGIs: DMDP, DMJ, and α-homonojirimycin (HNJ). The larvae, adult moths, and eggs accumulate DMDP and HNJ, presumably from the host plants (38). This is the first report of AGIs in animals. Perhaps noteworthy, HNJ has been found to be one of the most potent inhibitors of avian α-glucosidase (39). The colorful day-flying *Urania* moths seem to be protected as they are reportedly ignored by insect-catching birds (40). Unlike HNJ, the second accumulated AGI, DMDP, is only a weak inhibitor of avian α-glucosidase. Its complementary potent activity against insect glycosidases, however, may serve a protective function in the eggs and larval stages of *Urania* which are vulnerable to insect predation. Pyrrolizidine alkaloids have

been shown to serve this function in eggs of *Utetheisa ornatrix* (41). The third AGI found in the moth's food plant, DMJ, has no significant affect on insect or avian glycosidase activity. Curiously it is not accumulated by the moth.

Among other AGIs, castanospermine, best known for its toxic effects on mammals, is a potent inhibitor of both α- and β-glucosidases of mammalian gut (42). In insects, *Spodoptera* trehalase and lactase are inhibited by castanospermine as are *Callosobruchus maculatus* α- and β-glucosidases (35). Feeding of pea aphids is deterred at 0.0001% artificial diet (43). The polyphagous *Spodoptera littoralis*, oligophagous *S. exempta*, and *Heliothis virescens* are also strongly deterred (31).

Swainsonine, a potent α-mannosidase inhibitor, is the cause of *Swainsona* poisoning or mannosidosis in cattle. The symptoms resemble those of the genetic condition in humans in which α-mannosidase activity is lost and mannose containing oligosaccharides accumulate causing neurological damage. Although toxic affects on insects are not known, swainsonine deters feeding in *Spodoptera littoralis* (32).

In addition to acting as sugar mimics, another proven mode of action for AGIs is their effect on glycoprotein processing. Glycoproteins with their attached oligosaccharides play important roles in a number of biochemical interactions ranging from cell-cell recognition to the infectivity of viruses. Some polyhydroxy alkaloids, including castanospermine, swainsonine, DNJ and DMJ, inhibit various enzymes which elaborate the oligosaccharide side chains (31). The use of these alkaloids as tools for probing structure/activity relationships of glycoproteins is becoming increasingly important. Castanospermine, for example, can reduce the infectivity of HIV by disrupting glycoprotein processing at concentrations not toxic to lymphocytes (44; 45; 46). It would seem only a matter of time before this aspect of AGI activity is explored in plant/insect or plant/viral relationships.

In comparing AGIs with the simple piperidines discussed earlier, some interesting contrasts become apparent. The piperidine alkaloid, pipecolic acid, is only moderately toxic to insects (19, 47, 48). Similarly, its mono- and dihydroxylated derivatives, also show only mild toxic or deterrent effects on insects (49). As already mentioned, these molecules are too big to compete with proline and form anomalous proteins. Additionally, in tests of glycosidase activity neither pipecolic acid nor any of its mono or dihydroxylated derivatives showed any activity towards a broad spectrum of glycosidases (Nash and Fellows, unpublished data). Nonetheless, combinations of mono- and dihydroxylated pipecolic acids do produce both significant antifeeding activity and toxicity in aphids (50, 51). Feeding decreased 38% relative to controls during an initial 2-hour period with a mixture of pipecolic acid, *cis*-5-hydroxypipecolic acid, *cis-trans* 4,5-dihydroxypipecolic acid and the nonprotein amino acid S-(β-carboxyethyl)-cysteine. After 5 days of feeding, considerable toxicity was evident. Survival of treated individuals decreased to 31% of controls. Moderate insecticidal activity was also observed against *Spodoptera frugiperda* (49). While the mechanism is not clear, some of the

compounds act like toxins in *Spodoptera frugiperda* in that they interfere with nutritional physiology by affecting efficiency of conversion of digested food (51a). Although *Spodoptera* can apparently detoxify these alkaloids to some extent, they pay the price in diminished growth.

A single trihydroxylated pipecolic acid, 2(S)-carboxy-3(R),4(R),5(S)-trihydroxypiperidine, an analog of glucuronic acid, is known from plants. This compound, more closely resembling the simple AGIs with its additional hydroxyl group, has significant glycosidase inhibitory activity. Both human liver α-*L*-iduronidase and β-*D*-glucuronidase activity are inhibited, and both α- and β-glucosidase and mannosidase are unaffected (52). The trihydroxy compound does not show potent antifeedant activity against *Spodoptera* or *Heliothis* species (31).

This group of relatively simple and similar compounds shows a gamut of physiological mechanisms which account for their anti-insect activity. Azetidine-2-carboxylic acid forms anomalous proteins; the pipecolic acids increase nutritional costs; the polyhydroxy alkaloids inhibit sugar metabolism, block taste perception, and inhibit glycoprotein processing. While structure examination often results in a correct hypothesis for a given alkaloid's toxicity, clearly only additional empirical evidence will lead to the generalizations we seek.

2.5. Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids represent a group of ester-alkaloids with a bicyclic pyrrolizine backbone and at least one alcoholic function. Originally described in the Compositae, Leguminosae, and Boraginaceae, they have now been found in at least 13 plant families (53). This group of potentially toxic alkaloids is effectively utilized by the host plants to deter herbivory by both insect and mammalian grazers (54). One of the most interesting aspects of this group of plant compounds, however, is the secondary utilization of pyrrolizidine alkaloids by specific insects for their own chemical defense. In the case of *Zonocerus elegans* (elegant grasshopper) the insects are capable of using the pyrrolizidine alkaloids heliotrine, senecionine, and fuchsisenecionine as feeding cues and can detect these compounds as far as 3 meters away (55). Bogner and Boppre (56) have shown that the active agent, which may be volatilized for airborne detection of the pyrrolizidine alkaloids, is the hydroxydanaidal moiety of the parent compound at least in the case of retronecine and heliotridine. Rothschild and Aplin (57) suggest that aposematic insects use cues which attract them to plants containing various toxic substances such as pyrrolizidine alkaloids in order to find a host system that will provide them with the basic nutritional requirements for growth and development as well as an endogenous chemical defense mechanism against predators. As examples they cite the case of *Utethesia pulchelloides* which feeds on *Heliotropium* and stores pyrrolizidine alkaloids produced by the

plant in its body tissue for protection against predators. Some species of insects such as *Arctia caja*, which stores pyrrolizidine alkaloids in its eggs, apparently chemically modify the alkaloids they ingest for their own protection. In contrast, the eggs of *Tyria jacobaeae* contained no alkaloids even though adults were fed on the same diet as *Arctia* (57). The pyrrolizidine alkaloids senecionine and integerrimine and *N*-oxides of these bases are present in the composite *Senecio spathulatus* which is the host plant for the larvae of *Nyctemera annulata*. These alkaloids were found in the frass, larva, adult moth, and eggs of the insect and were correlated with a defensive role in protecting the insect from birds. When a parasitic wasp (*Microplitis*) was raised on the *N. annulata* larvae, the alkaloids were also present in the pupae of the parasite. This is said to be the first report of the transfer of an alkaloid to a carnivorous parasite from a plant (58).

In another example, it was shown that the cinnabar moth (*Callimorpha jacobaeae* L. or *Tyria jacobaeae*) sequesters pyrrolizidine alkaloids from the leaves of ragwort (*Senecio jacobaea*) for it own chemical defense. The alkaloids are found in the plant, larvae, and adult moth (59). The proportions of these pyrrolizidine alkaloids differ quantitatively between the plant and the insect body tissues. The compounds are localized in the imago of the pupa and the integument of the larvae which suggests they may play an active role in the defense of the insect during development (60). Of the six main alkaloids found in the plant, the three dominants are jacobine, jacozine, and jacoline. In the insect a different three alkaloids dominate, senecionine, seneciphylline, and integerrimine. In the frass of the moth larvae the quantitative distribution of the alkaloids mimics that of the plant (59). An examination of the structures of these 6 compounds reveals that the three alkaloids present in high concentrations in the plant and insect frass (jacobine, jacozine, and jacoline) are either epoxides or hydroxylated derivatives of senecionine, seneciphylline and integerrimine. This suggests that the cinnabar moth larvae actively modify the chemistry of the alkaloids and/or have the capability to differentially sequester moderate amounts of specific alkaloids. The formation of the epoxide seems to be a key point in the potential deterrence of these alkaloids as a protective agent for the plant to decrease herbivory. The insect in this case can use the less toxic compounds as a secondary protective agent and must eliminate the more toxic epoxides or modify them chemically.

The importance of the 15,16 double bond of esterified pyrrolizidines was also borne out in a study using the spruce budworm larvae (*Choristoneura fumiferana*) as a model test system (see Table 2.1). Anti-feeding behavior was recorded for a variety of alkaloids and plant extracts containing alkaloids (1). Of the 14 pyrrolizidine alkaloids examined, only two, senkirkine and lasiocarpine, were highly deterrent ($> 75\%$). When the stereochemistry of the 15,16 double bond of these compounds was modified, as in the case of neosenkirkine, or the double bond was epoxidized, as in otosenine, the deterrent activity of the alkaloid was decreased dramatically. This is in sharp contrast to the increase of toxicity associated with epoxide formation in the case of jacobine

TABLE 2.1. Alkaloids tested against the spruce budworm for feeding deterrency at 0.5 mg/ml

Pyrrolizidines		Quinolizidine Alkaloids	
Senkirkine	HD	lupinine	I
lasiocarpine	HD	epilupinine	I
monocrotaline	I	angustifoline	I
echinatine	I	hydroxylupanine	I
retronecine	I	sparteine	D
europine-*N*-oxide	I	α-isolupanine	I
neosenkirkine	I	tetrahydrorhombifoline	I
7-hydroxyheliotridane	I	cytisine	I
heliotrine	MD	lupanine	I
crispatine	D	13-lupanylbenzoate	HD
otosenine	I	13-lupanyltiglate	HD
echinatine-N-oxide	I	13-lupanylcinnamate	HD
senecionine	MD	lupinyl trans-cinnamate	I
europine	I		
Solanum Alkaloids			
tomatidine	0.4 mg/ml	D	
tomatine	1 mg/ml	HD	
solanidine	0.4 mg/ml	D	
α-solanine	0.9 mg/ml	D	
α-chaconine	0.9 mg/ml	HD	

I < 25%, MD > 25%, D > 50%, and HD > 75% deterrence

described earlier, but it does indicate the importance of this functional group in determining toxicity of the pyrrolizidines. Although only these two alkaloids showed highly deterrent activity, only plant extracts which were known to contain alkaloids showed the highest deterrence against the spruce budworm. In this example, an enhanced protective response for the plant against herbivory was obtained in an additive fashion, as mentioned previously, by the combined effect of slightly deterrent alkaloids.

Bentley et al. (1) also reported that two other groups of alkaloids had deterrent activity against the spruce budworm. The quinolizidine alkaloids found in lupines showed a highly specific nature related to deterrent activity. Of thirteen compounds assayed, only three esters of 13-hydroxylupanine were highly active; however, the parent compound was inactive. Thus esterification appeared to represent a mechanism to activate an otherwise innoxious compound. Of the five *Solanum* alkaloids which Bentley et al. tested, all were deterrent or highly deterrent even though they differed considerably in structure. Of the 109 plants tested in this study, only six displayed high feeding deterrence and all of these contained alkaloids. For example, tomato, potato, and nightshade foliage contain various solanum alkaloids, lupine contains quinolizidine alkaloids, coltsfoot contains senkirkine(pyrrolizidine) and valerian contains pyridine alkaloids (1).

There are other examples of the exploitation of the pyrrolizidine alkaloids

by specialist insect herbivores. For example, Danaid butterflies which have been cited for their abilities to store cardenolides as a chemical defense mechanism against predators (61) also sequester pyrrolizidine alkaloids in a similar manner. In particular, male butterflies of both *Danaus plexippus* and *Danaus chrysippus* contained the pyrrolizidine alkaloids seneciphylline, senecionine, and rosmarinine. These alkaloids are present in the host plants fed on by the insects during their larval development and are known to be unpalatable to the insects' predators. The insects were shown to be capable of sequestering pyrrolizidine alkaloids fed in an artificial diet for at least seven days. In a control study in which *D. plexippus* was reared in the wild in the absence of pyrrolizidine alkaloid containing plants, no pyrrolizidine alkaloids were recovered in the insect tissue. Although female butterflies were not as adept as the males in the storage of the toxic alkaloids, it was suggested that they obtained protection through the mimicry of the males. It was further suggested that pyrrolizidine alkaloids replaced the role of the cardenolides in those insects that were not able to feed on cardenolide containing plants or those insects that were not capable of sequestering the cardenolides (62). The utilization of pyrrolizidines for defense is only one use of these plant compounds by insect specialists. In another example of a specialist feeder adaptation the pyrrolizidine ester alkaloid, lycopsamine, was found in hairpencil extracts of two danaid butterflies raised in the field. The males of the species *Danaus hamatus hamatus* and *Euploea tulliolus tulliolus* use the compounds in their hairpencils as an important courtship pheromone. In this case the insect is utilizing a potentially toxic compound produced in the host plant for its own reproductive advantage (63).

It is apparently a general phenomenon that danaidone and closely related pyrrolizidine alkaloids are present in the hairpencils of male *Danaus* butterflies. They evert the hairpencils prior to copulation, and females seek out the males with the most alkaloid. The males visit pyrrolizidine-containing plants including species of *Heliotropium* (Boraginaceae) and feed on the alkaloids necessary to produce the pheromone (64).

In the moth *Uthetheisa ornatrix* the larvae sequester two alkaloids, monocrotaline and usaramine, which they obtain from their food plants, *Crotalaria* sp. This protects them from predatory spiders. Adult females receive additional amounts of alkaloids from males at mating by seminal infusion. They transmit these with alkaloids of their own to their eggs which are also thereby protected (41). The adult males use hydroxydanaidal derived from *Crotalaria* alkaloids to induce mating in females. The pheromone is an indicator of the protective alkaloid load being carried.

Although lepidopterous insects are well known for their cueing responses to numerous secondary plant compounds such as anthocyanins and flavonoids as well as alkaloids, dipterans are not. Recently, a number of flies (Diptera) from four genera of the Chloropidae were found to be attracted to decaying plants containing pyrrolizidine alkaloids as well as pyrrolizidine alkaloid baits containing axillaridine (65). Because both sexes of flies were

observed at the alkaloid baits in approximately equal numbers the behavioral response does not seem to be related to courtship or mating cues. It is possible that the flies, which are normally not thought of as phytophagous feeders, may be storing the alkaloids as a defensive mechanism.

In most cases of secondary protection of insect herbivores by alkaloids the source of the alkaloid is the herbivore diet, i.e. the plant host. There have been several interesting reports on the occurrence of chemical defenses of insects based on alkaloids actively synthesized or at least chemically modified by the insect. One such report showed that deterrence to predation by ants and spiders was afforded to the Mexican bean beetle (*Epilachna varivestis*) by the production of the homotropane alkaloid euphococcinine (66). This compound which actually may be a polyketide, is not present in the body of larvae or newly hatched adults but is accumulated in the beetles blood within one week of emergence. When attacked, the beetle reflex-bleeds emitting droplets of blood containing as much as 5 μg of euphococcinine.

2.6. Quinolizidine Alkaloids

Quinolizidine alkaloids are found in many species of the Fabaceae, Berberidaceae, Leguminosae, and Solanaceae and are derived from lysine (67, 68). Some quinolizidine alkaloids are very toxic insecticides that have the potential of commercial use for the control of insects. For example, the alkaloid ryanodine, which is found in the stems and roots of *Ryania speciosa*, is effective as both an insect contact and stomach insecticide and is particularly effective against Lepidoptera. It has been used to control the codling moth without affecting parasites and predators of the moth (69).

Populations of Colorado perennial lupines show distinct patterns of alkaloids in their inflorescences. These patterns are thought to be correlated with the susceptibility of the host plant lupines used by the larvae of the flower-feeding lycaenid butterfly *Glaucopsyche lygdamus*. In ecological situations where the butterfly is not favored, the lupine populations contain small numbers of alkaloids accumulating in minor concentrations, usually either ammodendrine or epilupinine. In ecological situations where the butterfly is favored, two distinct populations of high alkaloid profiles occur. Those plants with only minor feeding by the butterfly contain three of four mixtures of isomers of lupanine or closely related compounds. Those plants with a higher feeding preference by the insect have mixtures of as many as nine diverse alkaloids including lupanine, hydroxylupanine, and esters of these alkaloids with a great deal of variation among various individuals. This demonstrates that the presence of a variety of alkaloids even in substantial concentrations is not sufficient to deter extensive predation by all insects and that specialists may use them as feeding cues. This diversity of alkaloids and individual variation of alkaloids among a population of plants is interpreted as a mechanism to defend the lupines against specialist herbivores (70), but maximal protection against generalist insects may be best achieved by isometric variation of a few compounds.

Wink and White (71) also discuss the protective role that alkaloids play in lupins by pointing out that the "sweet lupins" (low alkaloid profile) are challenged by a relatively large number of insect pests, while the alkaloid rich "bitter lupins" have only a small number of specialized feeders. The breakthrough of the chemical defenses of the plant by the insect often involves the utilization of the host plant chemicals for the insects' protection. For example, the legume *Petteria ramentacea* contains at least 6 different alkaloids including *N*-methylcytisine, and cytisine. A specialized phloem feeding aphid *Aphis cytisorum* which feeds on the legume has been shown to contain as much as 0.4% or 21 mmol/kg fresh weight of the host alkaloid cytisine with minor amounts of *N*-methylcytisine (71). They estimated that half-maximal inhibitory concentrations of cytisine are in the range of 1–5 mM. Therefore, it is certainly possible that the cytisine obtained from the host phloem sap and stored in the aphid is conferring chemical protection to the aphid against its own predators.

Bentley et al. (72) have tested twelve lupine alkaloids in feeding bioassays at 25 gm/disc against the 6th instar larvae of the spruce budworm, (*Choristoneura fumiferana*). Eight of the alkaloids were present in extracts of *Lupinus polyphyllus* and four others were structurally related (lupinine, epilupinine, α-isolupanine, and lupinyl-*trans*-cinnamate) but not present in the lupines. Of the eight alkaloids present in the lupine, 13-*trans*-cinnamoyloxylupanine and 13-tigloyloxylupanine showed high feeding deterrence of the spruce budworm larvae while the other six alkaloids present in the lupine (lupanine, sparteine, 13-hydroxylupanine, tetrahydrorhombifoline, angustifoline, and 17-hydroxylupanine) as well as the four related alkaloids showed little or no feeding deterrence. In this case it is interesting to note that the 13-hydroxylupanine, the parent compound of 13-*trans*-cinnamoyloxylupanine and 13-tigloyloxylupanine, was completely inactive as a feeding deterrent while the two esters were highly active, demonstrating the highly specific nature of the response correlated with slight chemical changes. These data indicate that the presence or absence of alkaloids may not yield a chemical defensive posture even against insects not adapted to alkaloids. However, subtle changes in the chemical diversity of the alkaloids may provide dramatic defensive tools against specific herbivores (72).

An examination of the effects of alkaloids on insect herbivores may be misleading if distinctions are not made between compounds which display a feeding deterrency and ones that are toxic to the insect. From an ecological standpoint the host plant may derive a very positive advantage from a chemical defensive mechanism composed of alkaloids that simply deter herbivore feeding without necessarily being an insect toxin. On the other hand, a host plant chemical defense which results in the death of a grazing insect herbivore is a powerful selective advantage. In many cases it is difficult to distinguish between feeding deterrency and toxicity without laboratory feeding trials. Miller and Feeny (73) distinguished between toxicity and deterrency using three polyphagous feeding Lepidoptera larvae *Hyphantria cunea*, *Sodoptera eridania*, and *Lymantria dispar*. Six benzylisoquinoline alkaloids including

laudanosine, papaverine, glaucine, berberine, sanguinarine, and aristolochic acid were fed in artificial diets to the test larvae and consumption, growth rates, and toxicity data were recorded. Of the alkaloids tested glaucine had some effect on all three larvae while laudanosine had very little effect except as a slight feeding deterrent in *Spodoptera*. The effects of glaucine are not surprising in view of the fact that the tulip tree, *Liriodendron tulipfera*, contains glaucine and is one of the few tree species that is not devastated by outbreaks of the gypsy moth in the United States (6). Laudanosine on the other hand represents the simplest structure of those tested and the authors speculate that if this compound is correspondingly older evolutionarily than the others, then a selective force has been exerted upon insect herbivores over a longer time to overcome any defensive role that may have been more potent at some distant time (73). The consistent functional group that seems to link the more toxic alkaloids berberine, sanguinarine, and aristolochic acid was a methylenedioxy group on a phenyl ring and not necessarily the parent alkaloid itself. It is possible that this functional group interferes with a mixed function oxidase detoxification system discussed by Brattsten et al. (74), although recent reports indicate that the toxicity of the methylenedioxy group is not related to mixed function oxidases (175). It is interesting to compare the role of this group in modifying the activity of the benzylisoquinoline alkaloids to that of the epoxide functional group in modifying the toxicity of pyrrolizidine alkaloids discussed earlier.

2.7. Maytansinoid Alkaloids

Maytansinoids are a group of ansamacrolide alkaloids found in higher plants and bacteria. Among higher plants they are most frequently encountered in the Celastraceae, Rhamnaceae, and Euphorbiaceae (76). Recently, they have been reported from various moss families (77, 78). In microorganisms a series of maytansinoids are found esterified at the C(3) hydroxyl with simple acyl groups, but lacking nitrogen (ansamitocin P-3). Maytansine was the first to be isolated and characterized, from *Maytenus* species. All ansamacrolides related to maytansine are "maytansinoids". Their unsymmetrical 19–25 membered rings, onto which two six-membered rings are fused, contain a variety of features including single and double carbon bonds, carbonyl groups, lactone or lactam linkages, rings joining nonadjacent positions of aromatic moieties, and alkyl or other substituents at various locations. They may be esterified through the C(3) hydroxyl (maytanacine) or not (maysine). They may have additional fused macrocyclic rings as in treflorine.

Maytansinoids are known for their high level of inhibitory activity in experimental tumor systems (76, 77, 78, 79, 80). A number of analogs have been studied and, although many are effective, none apparently has shown any marked superiority over maytansine (79). A critical feature for activity is the presence of a C(3)- ester group. Considerable variation in the nature of this group has little effect on overall activity (compare maytansine and

maytanacine). The ester linkage may even form part of an additional macrocyclic ring and activity will still be maintained (as in treflorine). Maytansinoids lacking this ester moiety (see maysine), however, are inactive even at higher doses. Etherification of the C(9) hydroxyl, as in maytansine 9 methyl ether, results in marked decrease in antileukemic activity (76).

Maytansinoids inhibit cilia regeneration in *Tetrahymena pyriformis*, and this activity is dependent upon the nature of the C(3)-acyl group (81). Introduction of a hydroxyl into this moiety markedly reduces activity. Introduction of a hydroxyl at C(15) or conversion of the C(14) methyl to a hydroxymethyl group will also lower activity. Loss of the epoxide group at C(4)–C(5) apparently does not affect activity.

Ethanolic extracts of *Trewia nudiflora* seed act as antifeedants for spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) and European corn borer (*Ostrina nubilalis*). Various toxic effects on codling moth (*Laspeyresia pomonella*), redbanded leafroller (*Argyrotaenia velutinana*), plum curculio (*Conotrachelus nenuphar*), striped cucumber beetle (*Acalymma vittatum*), and chicken body louse (*Menacanthus stramineus*) including reduction in progeny, disruption of the normal life cycle, and morphogenic effects were observed (82). Trewiasine and five of its derivatives (dehydrotrewiasine, demethyltrewiasine, treflorine, trenudine, and *N*-methyltrenudone) were responsible for this activity. When incorporated into the diet of *O. nubilalis*, trewiasine showed an LD_{50} of 7.4 ppm. Topical doses at 0.1% caused 70% mortality in *L. pomonella* (83).

Interestingly, all of the compounds known to be active against insects meet the same structural requirements which were shown to be necessary for inhibitory activity in tumor tests and cilia regeneration. The importance of esterification, which has previously been pointed out as necessary for lupine alkaloid toxicity, is seen again here. The mode of action of this bizarre group of compounds remains unknown.

There is a possibility that maytansinoid compounds may be synthesized by bacteria or fungi and transferred to plants. Spjut et al. (84) point out the likelihood of this occurring in mosses. The extreme variation in cytotoxicity from ansamitocin P-3 among different samples led them to look more closely for associated fungi or Cyanobacteria. While mycorrhizal relationships could not be confirmed, the presence of considerable *Nostoc* with the highly active samples suggests the possibility of accumulation in this way. Suwanborirux et al. (78) similarly could not exclude the source of ansamitocin P-3 in *Claopodium crispifolium* from actinomycete contamination.

2.8. Endophyte Produced Alkaloids

While it is unclear about the involvement of maytansinoids in interactions between plants and bacteria or fungi there is ample evidence concerning the interesting phenomenon of fungal endophyte-produced alkaloids. Considerable literature has become available recently demonstrating that associations

between grasses and fungal endophytes which live inside plant tissues represent a major defense of host plants against herbivory. The fungi are principally endophytic but a few epiphytic ones are involved, such as *Atkinsonella hypoxylon* and *Balansia cyperi.* The endophytic fungi are members of the family Clavicipitaceae, tribe Balansia, to which the genus *Acremonium* is closely related. In this unique interaction the fungi themselves are responsible for the synthesis of the alkaloids, as they are produced in pure fungal cultures and are not present in uninfected plants. These fungal infections are associated with changes in host plant growth, reproduction, and toxicity to insect and mammalian herbivores (85, 86).

This plant/fungal defensive interaction was first described in an association between tall fescue (*Festuca arundinacea*) and the endophytic fungus *Acremonium coenophialum* by Bacon et al. (87) and later established as being toxic to ruminant herbivores (88, 89). The toxicosis syndrome of cattle that results from the grazing of infected fescue grass represents a major economic loss to livestock production. Alkaloids suggested to be causally related to toxicity to animals include the pyrrolizidine alkaloids perloline, loline, and the loline derivatives *N*-acetylloline (lolinine) and *N*-formylloline, and the ergopeptine alkaloids represented primarily by ergovaline (90, 91). Perloline, a diazaphenanthrene alkaloid, was originally isolated from perennial ryegrass. Recently at least five ergot alkaloids produced by *A. coenophialum* have been identified in fungal cultures as well as in infected tall fescue grass (91).

Alkaloids, along with specific environmental conditions, have been associated not only with detrimental effects on herbivores but with beneficial effects in endophyte infected tall fescue and perennial ryegrass associations. Described as a mutualistic symbiosis, the endophyte association has been shown to be an effective deterrent to a variety of insect herbivores (92, 93, 94, 95, 96). These reports and others have revealed a positive association between endophyte infection, loline alkaloid production, and resistance to insect pests such as sod webworm, *Crambus* spp. (97), fall armyworm *Sodoptera frugiperda* (98), and the aphids *Rhopalosipum pali* and *Schizaphis graminum* (93). Johnson et al. (93) and Yates et al. (99) demonstrated that *N*-formylloline which is produced in the endophyte/plant association is toxic to the large milkweed bug *Oncopeltus fasciatus.* Several alkaloids from tall fescue were examined for their effects on insect mortality, and in the case of perloline and ergocryptine synergistic effects enhancing the toxicity of each alkaloid were detected.

The polyphagus fall armyworm feeds on numerous host genera, including *Cenchrus, Cyperus, Festuca, Lolium, Paspalum,* and *Stipa.* Reduced herbivory in these genera due to the production of endophytic induced alkaloids has been associated with several fungal genera, including *Balansia, Acremonium, Myriogenospora,* and *Atkinsonella* (92, 100).

In two studies, several species of *Phalaris* (canary grass) were found to contain high levels of tryptamine-type indole alkaloids (101, 102). These alkaloids are apparently produced in endophyte-free tissues, and Corcuera

(103) found that they act as antifeedants and toxins to aphids. Tapper and Rowan (96) found that peramine was the predominant compound deterring feeding by the adult Argentine stem weevil (*Listronotus bonariensis*) on perennial ryegrass (*Lolium perenne*) infected with the endophytic fungus *Acremonium lolii.* They developed an extraction method which also allowed for determination of lolitrem B , a neurotoxin present in endophyte-infected perennial ryegrass which causes the ryegrass staggers disorder in grazing sheep. The alkaloids known as lolitrems have been shown to be directly related to this disorder but have not been associated with insect deterrence. It is likely that the origins of chemical deterrence to insect and animal herbivory are separate (104).

There is ample evidence to indicate that in grasses infected plants are often more vigorous than uninfected plants (105 and 94). In one study, Clay et al (106) found significantly less damage from the leaf spot fungus *Alternaria triticina* on the grass *Panicum agrostoides* when infected with the systemic fungus *Balansia henningsiana.* White and Cole, (107) found that some species of fungi are inhibited *in vitro* when exposed to *Acremonium coenophialum.*

The relative toxicities of endophyte-infected grasses to insect herbivores has been examined only for a few species. Indeed even the distribution and abundance of endophyte-infected grasses is poorly known. The economic benefit of insect deterrence in perennial ryegrass, however, has been commercialized and varieties of endophyte-enhanced turf grasses are produced and available commercially. Many other insects are affected by plants infected with endophytic fungi which produce various alkaloids, but the identity of the specific alkaloid responsible for the deterrent effect is not well known. For example, feeding behavioral avoidance by the fall armyworm to *Paspalum* and *Stipa* occur when these plants are infected with species of the fungal endophytes *Myriogenospora* and *Atkinsonella,* respectively. Clay (104) has found that species of four of the five *Balansiae* genera infecting grasses and sedges caused some degree of insect resistance. The larvae on infected plants typically have lower survival, lower rate of mass gain, increased larval duration, and/or lower pupal mass than larvae on uninfected grasses. In another example, Schmidt (108) found that cutworm (*Agrotis segetum*) larvae exhibited reduced survival and mass gain when feeding on *Dactylis glomerata* infected by *Epichloe typhina.* It is likely that in these examples as well, insect feeding deterrent or toxic alkaloids are produced by the infecting endophytic fungi.

2.9. Pyridine Alkaloids

The pyridine alkaloids arise from a variety of biosynthetic pathways and are among the most widely known and best studied alkaloids in existence. Notable among these is nicotine which is produced and accumulated in relatively high levels by several plants in the Solanaceae. The concentration of alkaloids in plants can be described as existing in a dynamic equilibrium between

synthetic and metabolic processes. Considerable metabolic energy is needed for the maintenance of alkaloids at high levels if they are to be used as defense compounds. It is not surprising that herbivory can effect the concentration of endogenous plant alkaloids. Baldwin (109) has reported that the two main alkaloids, nicotine and nornicotine, of tobacco (*Nicotiana sylvestris*), which are synthesized primarily in the root of the plant and transported to the leaf, are significantly increased by both herbivory by the larvae of *Manduca sexta* and by comparable artificially stimulated herbivory. The two alkaloids have broad spectrum biotic toxicities. Developmental and survival growth factors of the insect are decreased in *Manduca sexta* and *Spodoptera frugiperda* as well as in the parasitic wasps of these species which include *Cotesia congregata* and *Hyposoter annulipes*, respectively, by the ingestion of low concentrations of nicotine in synthetic diets (110). In an earlier study Thurston et al. (111) described the toxicity of nicotine in the poisoning of the green peach aphid (*Myzus persicae*) and the corn leaf aphid (*Rhopalosiphum maidis*) in these terms: "paralysis of the hind and middle legs followed by paralysis of the front legs, a consequent inability of the aphid to move steadily or to keep its balance, a rapid erratic twitching of the legs when severely affected, and rapid desiccation after death". The authors suggested in this and subsequent papers that the toxicity of *Nicotiana* species is due to leaf exudates containing nicotine-related alkaloids, however, they did not empirically determine that nicotine is the causal agent in the leaf exudates (112). In the case of *Nicotiana gossei* it was not necessary for the aphid to ingest the suspected toxic alkaloids, but mere contact with the leaf trichomes was sufficient to kill all test subjects within 18 hrs (113).

To be effective chemical deterrents alkaloids must be detected by potential insect herbivores so that a selective choice can be made. To evaluate the possibility that insect herbivores could detect alkaloids Ishikawa used two sensilla styloconica on the maxilla of the larva of the silkworm, *Bombyx mori*. Various chemicals and extracts from plants fed on by the silkworm were tested to see if they had any effect in stimulating these maxillary hairs. Two alkaloids, nicotine and strychnine nitrate, had the strongest stimulatory effect at the lowest concentration and these results were interpreted as showing that these substances are easily detected by a feeding insect and can act as feeding deterrents (114).

As shown by Baldwin's (109) report, herbivory is not the only mechanical stress that can effect the concentration of alkaloids in plant tissues. In the case of the aporphine alkaloids, injury to the (*Liriodendron tulipifera*) tree stem through mechanical stress caused several alkaloids to be formed in the sapwood of this tree. The alkaloid that was present in normal sapwood was glaucine; however in stressed tissues a series of alkaloids were present including nine aporphine alkaloids: nornuciferine, *N*-acetyltnornuciferine, nuciferine, norushinsunine, dehydroglaucine, norglaucine, glaucine, liriodenine, and *O*-methylatheroline: and eight phenolic aporphine alkaloids: asimilobine,

N-acetylasimilobine, thaliporphine, predicentrine, *N*-methylaurotetanine, lirioferine, liriotuliperine, corunnine. The authors indicate that some of these compounds are probably phytoalexins conferring resistance to the wood from microorganisms. Glaucine in particular has antimicrobial activity (115).

2.10. Indole Alkaloids

The concept of a continual interplay between the evolution of plant defensive alkaloids and the constantly adapting insect herbivores is discussed in relation to alkaloid biochemistry by McKey (116). He points out that toxicity can be enhanced by a single modification of a chemical structure or the convergence of two existing pathways following the introduction of a single enzyme step. For example, indole alkaloids, which are known to be highly toxic, are suggested as being derived from a preexisting pathway for the formation of indole which converged with monoterpenoid metabolism. All known indole alkaloids can be seen as derived from indole coupled with a nine or ten carbon fragment taken from a preexisting monoterpene. Thus a single mutational event could have potentially been responsible for an array of over 600 known indole alkaloids.

Several indole alkaloids are known to directly influence feeding of insect herbivores by acting as direct toxins. For example, gramine (3-*N*,*N*-dimethylaminomethylindole), *N*- methyltryptamine, 5-methoxy-*N*, *N*-dimethyltryptamine, and 5-methoxytryptamine decrease the survival of nymphs of *Rhopalosiphum maidis* with an LD_{50} of 2.9, 3.8, 3.5, 2.3 mM respectively, after 48 hrs of feeding on a synthetic diet. Gramine also decreased survival of *Schizaphis graminum* with an LD_{50} of 0.7 mM after 48 hrs of feeding. These four indole alkaloids also showed feeding deterrent activity on aphids at concentrations as low as 0.5 mM, concentrations which are representative of those found in plants (103).

2.11. Steroidal Alkaloids

Steroidal alkaloids generally possess a four to six ring modified steroidal skeleton with a nitrogen incorporated into the ring or on the side chain of one of the rings. Steroidal alkaloids are prevalent in numerous *Solanum* and *Verarum* species and seem to be active feeding deterrents in several cases. For example, tomatine in a leaf at a concentration of 2 mM/Kg leaf is sufficient to cause a 50% reduction in leaf feeding by Colorado potato beetle larvae (*Leptinotarsa decemlineata*) and 3mM/Kg causes 100% mortality. Imbibition by nymphs of the potato leafhopper (*Empoasca fabae*) is 100% restricted by 0.02 M tomatine, however, the aglycone tomatidine has no effect on the insect. Tomatine also interferes with the growth and development of nymphs of the two-striped grasshopper (*Melanoplus bivittatus*) and the larvae of the mosquito (*Aedes aegypti*) (117; 118).

Bently et al. (119) evaluated the feeding deterrency of steroidal alkaloids on the sixth instar of the spruce budworm, *Choristoneura fumiferana* which were exposed to artificial diets containing five steroidal alkaloids from *Solanum*. These included tomatidine, tomatine, α-solanine, α-chaconine, and solanidine. All showed significant feeding deterrences at 10^{-3} M concentrations and all but tomatidine showed significant feeding deterrence at 10^{-4} M as well. The glycoside tomatine showed the greatest effect. However, the aglycone solanidine showed greater feeding deterrence than either of the other glycosides solanine or chaconine. These authors contend that the diversity of alkaloids rather than generalized chemical components are responsible for the effectiveness of the chemical defense of host plants. In another report the alkaloid tomatine found in *Solanum* was effective as a feeding deterrent alone and in combination with other alkaloids against the leafhopper nymph *Empoasca fabae* (120). In a well designed experiment to evaluate the differences between toxicity and feeding deterrency using the two-striped grasshopper, *Melanoplus bivittatus*, a series of feeding/survival tests were conducted on various secondary compounds including several alkaloids supplied in concentrations up to 1% of the artificial diets (121). In some cases lethal concentrations of alkaloids (solanine, tomatine) had no detectable effects on feeding deterrence and while other alkaloids displayed a marked feeding deterrence (gramine, veratrine) with non-lethal concentrations of the test compounds. Of the alkaloids tested only lupinine and nornicotine effected both the survival and the feeding deterrence of the test insect, although all six alkaloids had a significant role in the plant/herbivore interaction.

In a broad spectrum feeding study, which included some steroidal alkaloids as well as many other secondary plant products, an oligophagous locust, *Locusta migratoria*, was offered more than 100 different plants as food material. Most dicots, which contained higher quantities of alkaloids, were rejected and monocots were by far the preferred food source. Extracts of the rejected dicots inhibited feeding of artificial diets while extracts from the monocots did not affect feeding of the artificial diets (2). Of more than 100 chemicals which were added to artificial diets to test effectiveness as a feeding deterrent, alkaloids and monoterpenoids were consistently the most effective at the lowest concentrations. The alkaloids which were tested in this study were as shown in Table 2.2.

The authors concluded that the higher molecular weight alkaloids were the most deterrent although there was a great deal of variability as to the degree of reaction by the insect. It is interesting to compare the concentration of the alkaloids in the plant to that in the tested media to determine the effectiveness of the alkaloid as a feeding deterrent (2).

Alkaloid toxicity in insects is often reflected in toxicity to other organisms as well. In mammals, for example, sustained tomatine ingestion of a concentration of 40 mg/L in the diet for a period of 200 days did not have any significant anatomical effect on the test animals, however, acute ingestion of 900 mg/kg by rats was fatal (122).

TABLE 2.2. Comparison of alkaloid concentrations in the plant and in artifical diets on the feeding deterrency of *Locusta migratoria.*

Alkaloid tested	concentration	concen. in plant	effect
Aristolochic acid	0.000001%	0.5%	Der
Colchicine	0.001%		Der
Coniin	1%	0.003	Non-der
Jacobine	0.001%	0.25%	Der
Jaconine	0.05%	0.16%	Der
Nicotine	0.002%	7%	Der
Perloline	0.01%	0.05%	Der
Quinine	0.01	0.1	Der
Quinoline	0.5%		Non-der
Senecionine	0.001%	trace	Der
Seneciphylline	0.001%	0.1%	Der
Tomatine	0.15%	0.1%	Der
Gramine	3%	0.5%	Non-der
Halostachine	0.1%	0.3%	Der
Histamine	6%	0.5%	Non-der
Hordenine	10%	0.9%	Non-der

(Der = 50% feeding deterrence)

2.12. Detoxification Mechanisms by Herbivores

Polyphagous insect herbivores have evolved a wide variety of protection mechanisms to deal with plant host chemistry. One mechanism is the activity of mixed-function oxidases in the detoxification of a wide variety of potentially toxic compounds (74). As a consequence of a wide range of substrate utilization and the binding and subsequent reaction with activated oxygen, a wide range of compounds including alkaloids are subjected to oxidative transformations. According to Brattsten the evolution of the mixed-function oxidases in herbivores may have been a response to the exposure of alkaloids, phenolics, and terpenoids. They demonstrated that the mixed function oxidase system in *Spodoptera eridania* is stimulated by the addition of small amounts of secondary plant products such as the alkaloids quinoline and quinazoline. Additionally, the mixed function oxidases are effective in the metabolic degradation of several types of toxic compounds including both nicotine and rotenone through oxidative activity. The authors further speculate that the mixed function oxidase activity is not "fool-proof" as a detoxification mechanism for the polyphagous insect. In the case of the pyrrolizidine alkaloids the aliphatic hydroxylations and epoxidations actually produce compounds with enhanced toxicity. Somehow the plants have counteracted the defensive mixed function oxidase system to use it to their own advantage in the continual chemical defense battle between herbivore and host.

Cavin and Bradley (123) have described another detoxification mechanism in several species of butterflies which feed on *Passiflora* containing

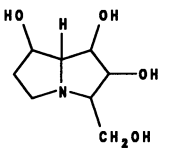
β-carboline alkaloids. Three specialist herbivores of the Heliconiini were shown to have the ability to either undergo a rapid fluid excretion of the β-carbolines or to sequester substantial quantities of harman, harmine and norharman when raised on host species containing these alkaloids. The relative concentrations of each of the alkaloids varied for each of the species tested but in all cases the concentrations of the insects differed from that of the plant host. In *Heliconius ismenius clarescens* which fed on *Passiflora quadrangularis* there was evidence that the insect modified the β-carbolines by the addition of an esterified methoxyl group resulting in even higher levels of the alkaloids in its body. The amount of alkaloids retained by the *Heliconiini* species was greater than that ingested by the generalist feeding insect *Spodoptera exiqua* which excreted virtually all of the alkaloids into the frass without any sequestration of the β-carbolines.

Attention has been paid throughout this manuscript to the differences that some alkaloids exhibit in feeding deterrence and those alkaloids which have toxic or physiological effects on the insect. In some cases the same alkaloid may have more than one effect on different insects, for example the alkaloids vasicine, vasicinol, and vasicinone have severe antifertility effects in *Dysdercus koenigii* but have only mild feeding deterrency in *Aulacophora foveicollis* (124). These differences in activity may represent, among other things, the adaptive nature of both the plant and the insect to an ecological balance between plant host and insect herbivore.

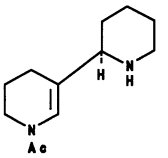
Summary

Empirical data on the involvement of alkaloids in plant insect interactions is appearing more frequently as improvements in analytical techniques permit the separation and identification of the causal agent in plant chemical defenses. Although alkaloids are certainly not the only secondary plant metabolite to be utilized against insect herbivory, they are within the chemical arsenal that is available to many plant species. In looking for generalizations of structure/function relationships the exceptions to the rule seem to be almost as prominent as the generalization itself. One steps on tenuous ground when trying to find them. However, a few arguable points can be made. Often, it is the functional group on the alkaloid which confers the most severe biological activity against the insect. The parent compound may be only moderately active while the highly hydroxylated, epoxide, or methylenedioxy functional groups dramatically change biological activity. Ecologically, each time the plant synthesizes a new or modified alkaloid a potential unique niche is created from which a specialist herbivore feeding insect may derive a specific selective advantage, if it can utilize the plant compound in its chemical defense. A dynamic chemical interplay may develop between the host plant and the insect herbivore. The complexity of such relationships is strikingly seen in tri-trophic level interactions between the plant/insect/and fungal system when the endophytic fungus is responsible for producing the alkaloid utilized by the plant against the insect herbivore.

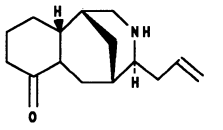
TABLE 2.3. Chemical Structures of Alkaloids.



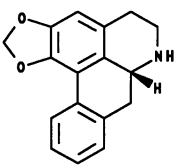
Alexine



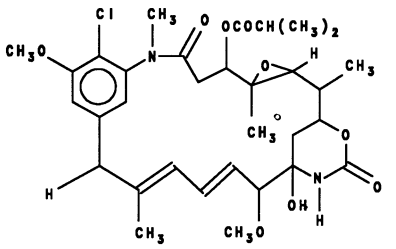
Ammodendrine



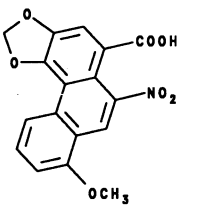
Angustifoline



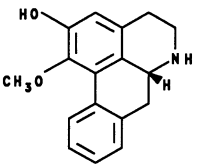
Anonaine



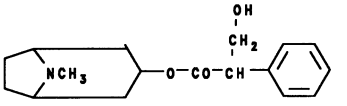
Ansamitocin P-3



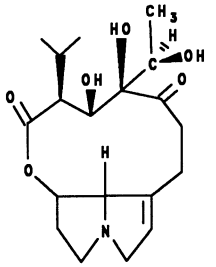
Aristolochic acid



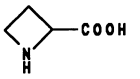
Asimilobine



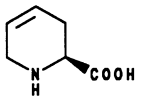
Atropine



Axillaridine



Azetidine-2-carboxylic acid



Baikiain

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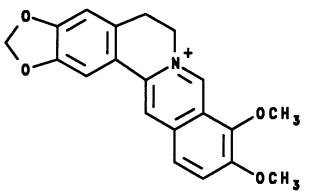
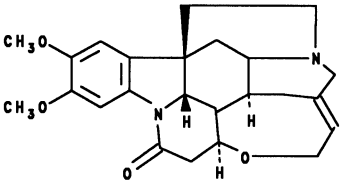
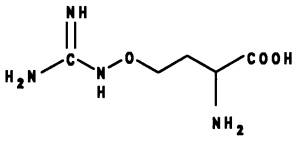


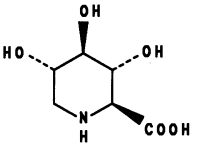
Berberine



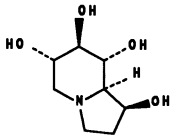
Brucine



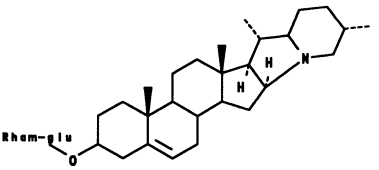
Canavanine



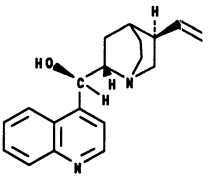
2(S)Carboxy-3(R),4(R),5(S)-Trihydroxypiperidine



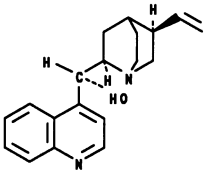
Castanospermine



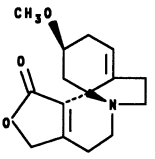
Chaconine



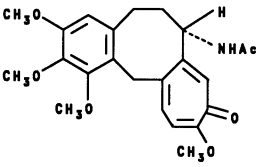
Cinchonidine



Cinchonine

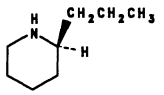


Cocculolidine

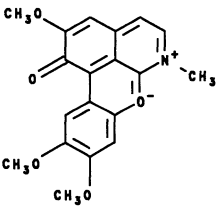


Colchicine

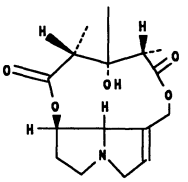
TABLE 2.3 (*cont.*)



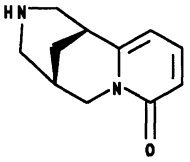
Coniine



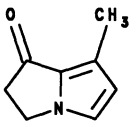
Corunnine



Crispatine



Cytisine



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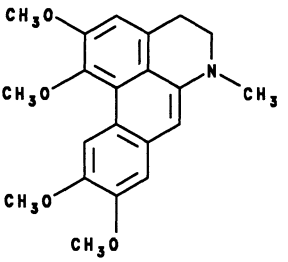
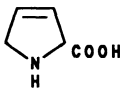
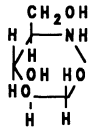


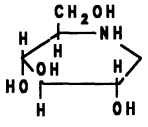
Dehydroglaucine



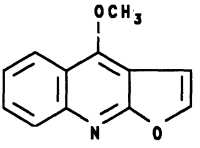
3,4-Dehydroproline



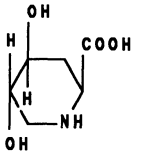
Deoxymannojirimycin (DMJ)



Deoxynojirimycin (DNJ)



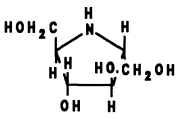
Dictamnine



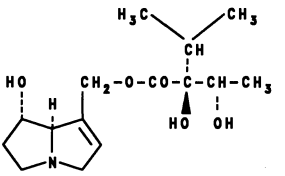
Cis-trans-4, 5-dihydroxypipecolic acid

(*cont.*)

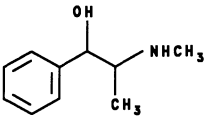
TABLE 2.3 (*cont.*)



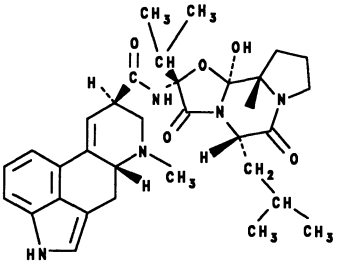
DMDP



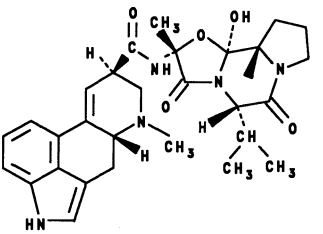
Echinatine



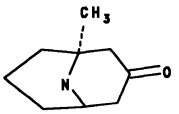
Ephedrine



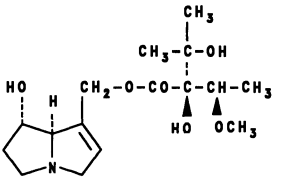
Ergocryptine



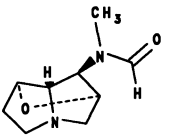
Ergovaline



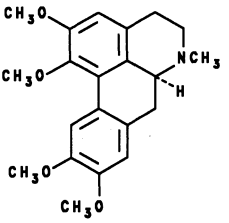
Euphococcinine



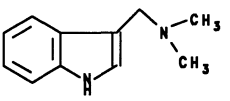
Europine



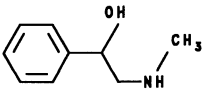
N-Formylloline



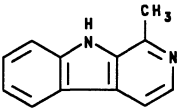
Glaucine



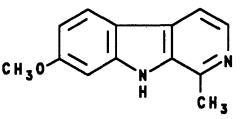
Gramine



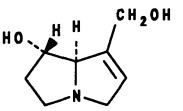
Halostachine



Harman

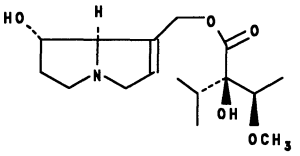


Harmine

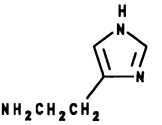


Heliotridine

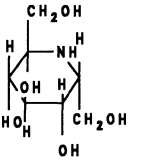
TABLE 2.3 (*cont.*)



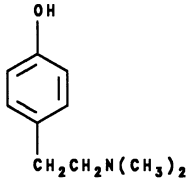
Heliotrine



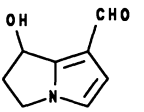
Histamine



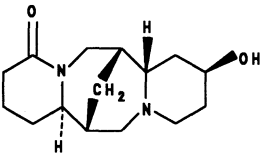
Homonojirimycin
HNJ



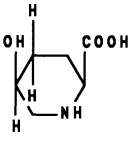
Hordenine



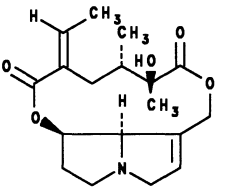
Hydroxydanaidal



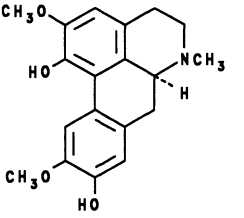
13-Hydroxylupanine



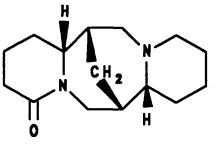
Cis-5-hydroxy-
pipecolic acid



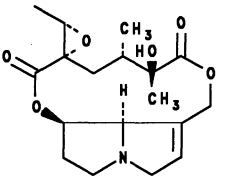
Integerrimine



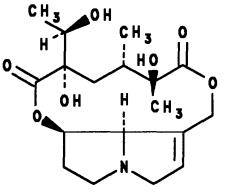
Isoboldine



(+)-Isolupanine



Jacobine



Jacoline

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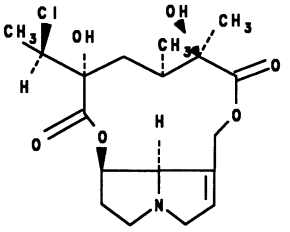
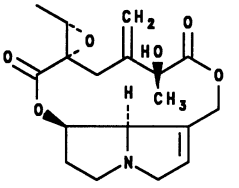
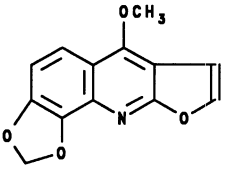


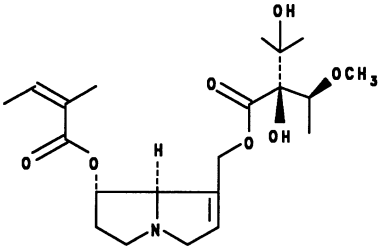
Jaconine



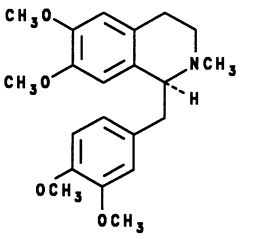
Jacozine



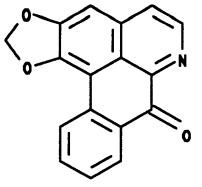
Kokusagine



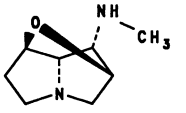
Lasiocarpine



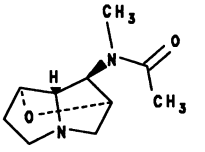
Laudanosine



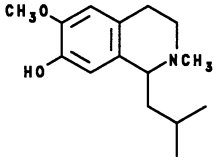
Liriodenine



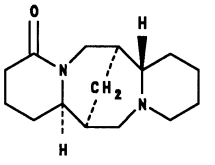
Loline



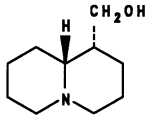
Lolinine



Lophocerine



Lupanine



Lupinine

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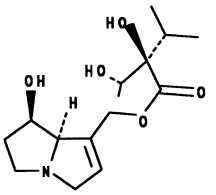
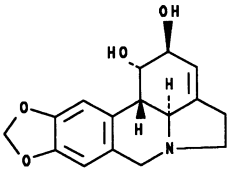
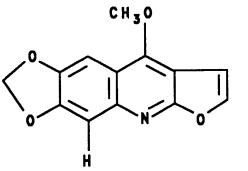


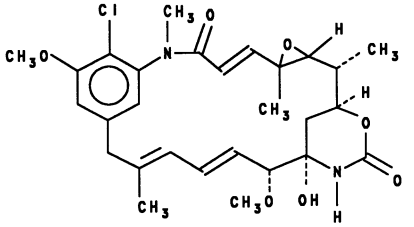
Lycopsamine



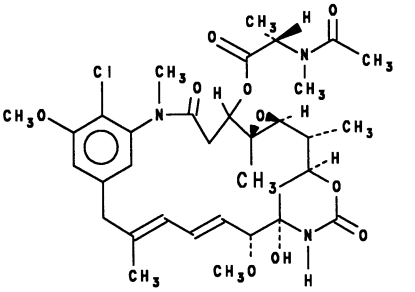
Lycorine



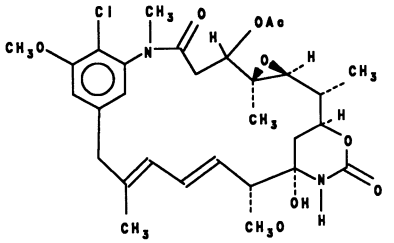
Maculine



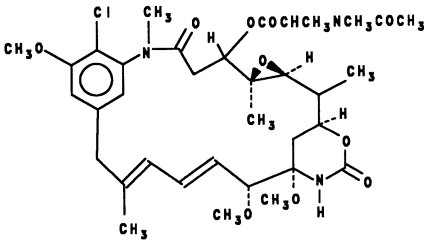
Maysine



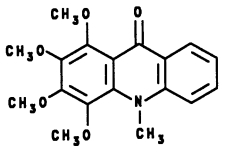
Maytansine



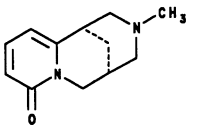
Maytanacine



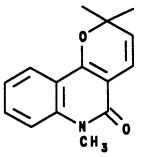
Maytansine 9-methyl ether



Melicopicine



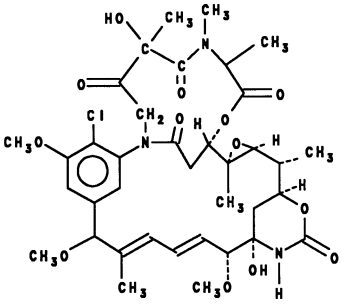
N-Methylcytisine



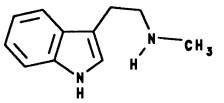
N-methyl flindersine

(*cont.*)

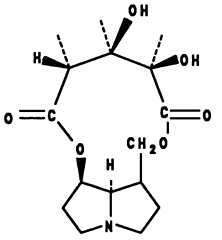
TABLE 2.3 (*cont.*)



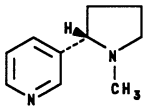
N-Methyltrenudone



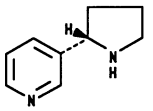
N-Methyltryptamine



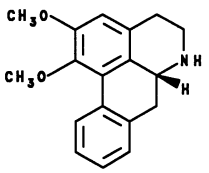
Monocrotaline



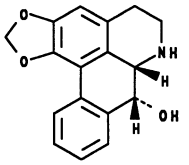
Nicotine



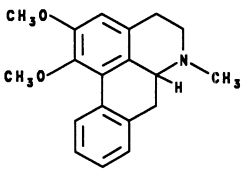
Nornicotine



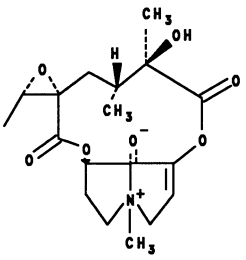
(-) N-Nornuciferine



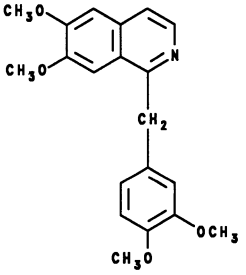
Norushinsunine



Nuciferine



Otosenine



Papaverine

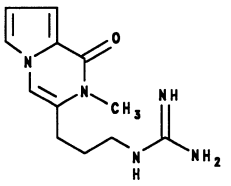


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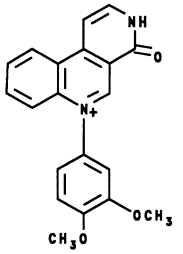
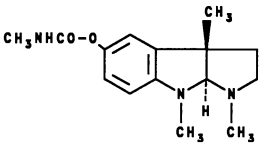
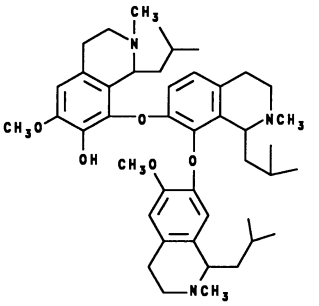
TABLE 2.3 (*cont.*)



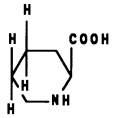
Perloline



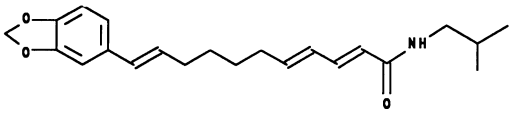
Physostigmine



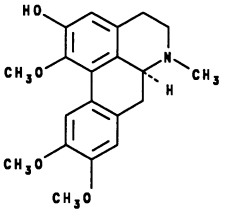
Pilocereine



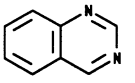
Pipecolic acid



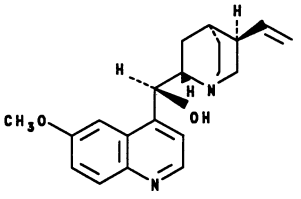
Pipercide



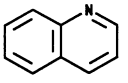
(s)-Predicentrine



Quinazoline



Quinine



Quinoline

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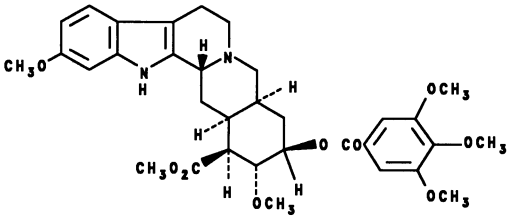
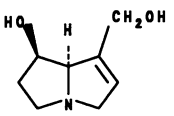
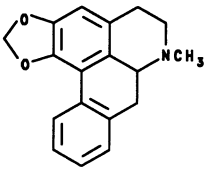


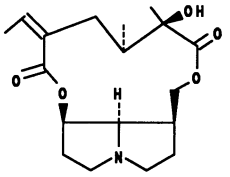
Reserpine



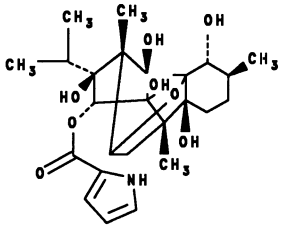
Retronicine



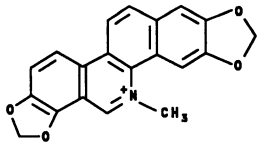
Roemerine



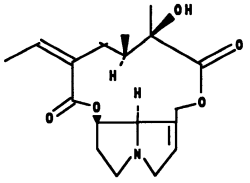
Rosmarinine



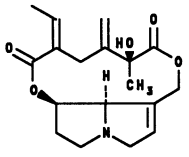
Ryanodine



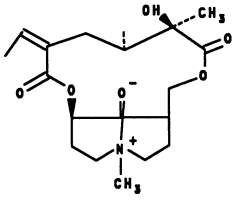
Sanguinarine



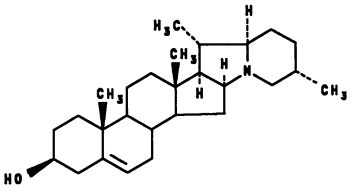
Senecionine



Seneciphylline

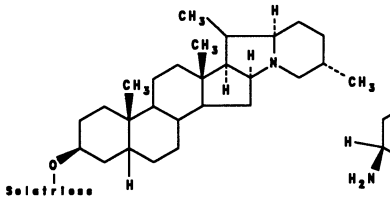


Senkirkine

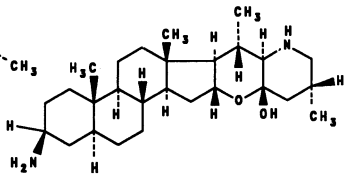


Solanidine

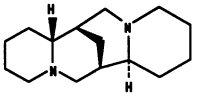
TABLE 2.3 (*cont.*)



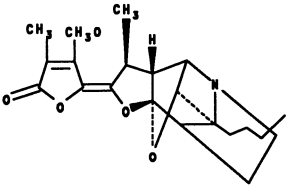
Solanine



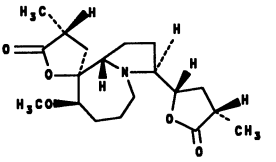
Solanocapsine



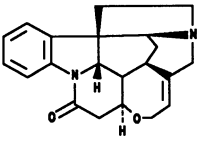
(-) Sparteine



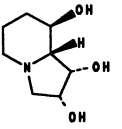
Stemofoline



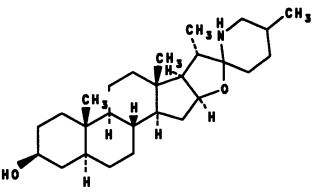
Stemospironine



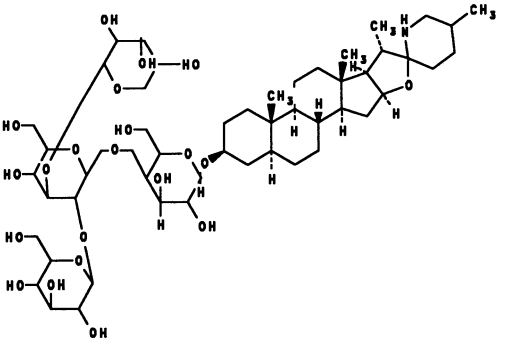
Strychnine



Swainsonine



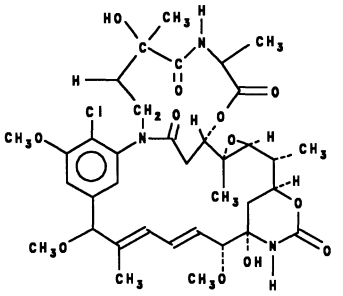
Tomatidine



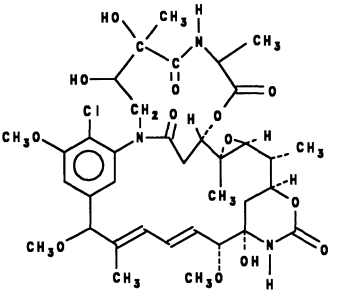
Tomatine

(*cont.*)

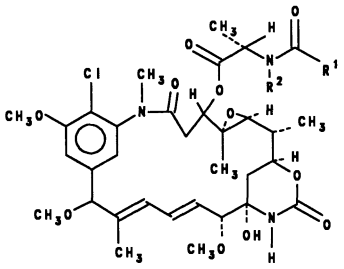
TABLE 2.3 (*cont.*)



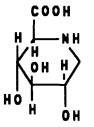
Trefiorine



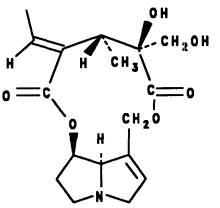
Trenudine



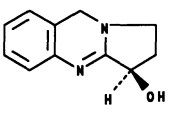
Trewiasine
Dehydrotrewiasine
Demethyltrewiasine



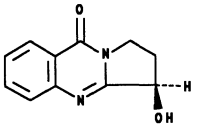
Trans-trans-trans-
Trihydroxypipecolic
acid



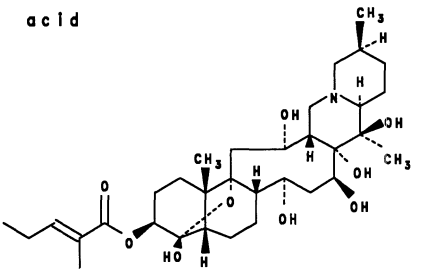
Usaramine



Vasicine



Vasicinone



Veratrine

For convenience we have included the chemical structures of the alkaloids discussed in Table 2.3. Table 2.4 is a representative sample of specific host alkaloids that have been linked to a specific biological activity with a particular insect. Although Table 2.4 is not comprehensive for all alkaloids and all plant/insect interactions, it does serve as a useful guide for further investigations of the evaluation of structure/function relationships in alkaloids.

Even a cursory examination of Table 2.4 reveals that the dominant role for

TABLE 2.4. Biologically active alkaloids affecting insects

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
alexine	*Alexa leiopetala*	*Spodoptera littoralis*	toxin	31
anonaine		*Anona reticulata*	toxin	125
aristolochic acid	Aristolochiaceae	*Lymantria dispar*	toxin, feeding deterrent	73
		Hyphantria cunea	feeding deterrent	73
		Spodoptera eridania	feeding deterrent, toxin	73
		Locusta migratoria	feeding deterrent	2
atropine		*Callosobruchus maculatus*	toxin	47
axillaridine	*Crotalaria scassellatii*	Chloropid flies	insect protection	65
azetidine -2-carboxylate	Liliaceae, Amarylladaceae, Leguminosae	*Spodoptera littoralis*	toxin	27
		Ascaris suum	toxin	25
		Manduca sexta	toxin	26
		Callosobruchus maculatus	toxin	47
berberine	Berberidaceae Papaveraceae	*Lymantria dispar*	toxin, feeding deterrent	73
		Spodoptera eridania	feeding deterrent	73
		Hyphantria cunea	feeding deterrent	73
		Aedes atropalpus	phototoxin	126
brucine	*Cinchona* sp.	*Scitophilus granarius*	mild feeding deterrent	127
		Trogoderma granarium	feeding deterrent	127
		Tribolium confusum	mild feeding deterrent	127
castanospermine	*Castanospermum australe*	*Acyrthosiphon pisum*	feeding deterrent	43 128
		Callosobruchus maculatus	toxin	34
		Tribolium confusum	toxin	34
		Spodoptera littoralis	feeding deterrent, toxin	31 33
		Spodoptera exempta	feeding deterrent	33
		Heliothis virescens	feeding deterrent	31
		Locusta migratoria	feeding deterrent	33

(*cont.*)

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
chaconine	*Solanum*	*Choristoneura fumiferana*	feeding deterrent	1
cinchonidine	*Cinchona* sp.	*Scitophilus granarius*	feeding deterrent	127
		Trogoderma granarium	feeding deterrent	127
cinchonine sulphate	*Cinchona* sp.	*Scitophilus granarius*	feeding deterrent	127
		Trogoderma granarium	mild feeding deterrent	127
cocculolidine	*Cocculus trilobus*	*Prodenia litura*	toxin	129
colchicine		*Callosobruchus maculatus*	toxin	47
		Locusta migatoria	feeding deterrent	2
crispatine		*Choristoneura fumiferana*	medium feeding deterrent	1
cytisine	*Petteria ramentacea*	*Aphis cytisorum*	insect protection	71
danaidone	*Heliotropium*	*Danaus* sp	mating cue	64
(DNJ) deoxyno-jirimycin	*Morus nigra*	*Spodoptera littoralis*	toxin	31
(DMJ) deoxymanno-jirimycin	*Lonchocarpus sericeus*	*Callosobruchus maculatus*	toxin	35
	Omphalea diandra			
dictamine	*Dictamnus*		feeding deterrent	130
cis, trans 4,5-dihydroxy-pipecolic acid	*Calliandra* sp.	*Aphis fabae*	toxin, feeding deterrent	50
(DMDP) dihydroxy methyldihydroxy pyrrolidine	*Omphalea diandra*	*Callosobruchus maculatus*	toxin	35
	Lonchocarpus sp.	*Spodoptera littoralis*	feeding deterrent, toxin	35, 31
	Derris sp.			
	Omphalea diandra	*Spodoptera exempta*	feeding deterrent	33
		Heliothis	feeding deterrent	31
		Schistocera gregaria	feeding deterrent	37
		Locusta migratoria	feeding deterrent	37
		Urania fulgens	insect protection	38
ephedrine		*Callosobruchus maculatus*	toxin	47
euphococcinine		*Epilachna varivestis*	insect protection	66

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
N-formyl loline[1]	Graminae	*Oncopeltus*	toxin	99
	Acremonium sp.	*fasciatus*		93
		Crambus sp.	feeding deterrent	97
		Sodoptera frugiperda	feeding deterrent	98
		Rhopalosipum pali	feeding deterrent	93
		Schizaphis graminum	feeding deterrent	93
glaucine	Papaveraceae	*Hyphantria cunea*	feeding deterrent	73
gramine	Gramineae	*Callosobruchus maculatus*	toxin	47
		Rhopalosiphum maidis	toxin	103
		Schizaphis graminum	toxin	103
		Melanoplus bivittatus	toxin	121
halostachine		*Locusta migratoria*	feeding deterrent	2
harmine	*Passiflora quadrangularis*	*Heliconius ismenius*	insect protection	123
heliotrine		*Choristoneura fumiferana*	mild feeding deterrent	1
α-homonojirimycin (HNJ)	*Omphalea diandra*	*Spodoptera littoralis*	toxin	31
		Urania fulgens	insect protection	38
hydroxydanaidal	*Crotalaria* sp.	*Utethesia ornatrix*	mating cue	41
cis-5-hydroxy-pipecolic acid	*Calliandra* sp.	*Aphis fabae*	toxin, deterrent	50
		Spodoptera littoralis	toxin	51
integerrimine	*Senecio jacobaea*	*Callimorpha jacobaeae*	insect protection	59
	Senecio spathulatus	*Nyctemera annulata*	insect protection	58
isoboldine	*Cocculus trilobus*	*Prodenia litura*	feeding deterrent	129
jacobine	*Senecio jacobaea*	*Callimorpha jacobaeae*	feeding deterrent	59
		Locusta migratoria	feeding deterrent	2
jacobine	*Senecio jacobaea*	*Callimorpha jacobaeae*	feeding deterrent	59
jaconine	*Senecio jacobaea*	*Locusta migratoria*	feeding deterrent	2

(*cont.*)

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
jacozine	*Senecio jacobaea*	*Callimorpha jacobaeae*	feeding deterrent	59
kokusagine	*Orixia japonica*		feeding deterrent	130
lolinine[1]	Graminae	*Crambus* sp.	feeding deterrent	97
	Acremonium sp.	*Sodoptera frugiperda*	feeding deterrent	98
		Rhopalosipum pali	feeding deterrent	93
		Schizaphis graminum	feeding deterrent	93
lasiocarpine		*Choristoneura fumiferana*	feeding deterrent	1
lophocereine	*Lophocereus schotti*	*Drosophila pachea*	feeding stimulant	18
13-lupanyl-benzoate	*Lupinus polyphyllus*	*Choristoneura fumiferana*	feeding deterrent	1
13-lupanyltiglate	*Lupinus polyphyllus*	*Choristoneura fumiferana*	feeding deterrent	1
13-lupanylcinnamate	*Lupinus polyphyllus*	*Choristoneura fumiferana*	feeding deterrent	1
lupinine		*Melanoplus bivittatus*	feeding deterrent, toxin	121
lycopsaminine	*Amsinckia hispida*	*Danaus hamatus*	mating attractant	63
lycorine	Amaryllidoideae Amaryllidaceae	*Schistocerca gregaria*	feeding deterrent	131
maculine	*Flindersia*		feeding deterrent	130
melicopicine	*Teclea trichocarpa*	*Spodoptera exempta*	mild feeding deterrent	132
N-methylflindersine		*Spodoptera* sp.	feeding deterrent	133
monocrotaline	*Crotalaria* sp.	*Utethesia ornatrix*	insect protection	41
nicotine	*Nicotiana* sp.	*Locusta migratoria*	feeding deterrent	19
		Manduca sexta	growth retardent, insect protection	110
		Myzus persicae	toxin	111
		Callosobruchus maculatus	toxin	47
		Bombyx mori	feeding deterrent	114
norharman	*Passiflora guadrangularis*	*Heliconius ismenius*	insect protection	123
nornicotine	*Nicotiana* sp.	*Melanoplus bivittatus*	feeding deterrent, toxin	121
perloline		*Locusta migratoria*	feeding deterrent	19
peramine[1]	*Lolium perenne Acremonium lolii*	*Listronotus bonariensis*	feeding deterrent, reduces ovipositing	96

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
physostigmine	*Physostigma venenosum*		feeding deterrent	134
pilocereine	*Lophocereus schotti*	*Drosophila pachea*	feeding stimulant	18
pipercide	*Piper nigrum*	*Callosobruchus chinensis*	feeding deterrent	135
pipecolic acid	Leguminosae	*Callosobruchus maculatus*	toxin	47
		Aphis fabae	toxin, deterrent	50
		Spodoptera frugiperda	toxin	51
		Locusta migratoria	feeding deterrent	19
		Chortoicetes terminifera	deterrent	48
quinidine	*Cinchona* sp.	*Scitophilus granarius*	feeding deterrent	127
	Cinchona sp.	*Trogoderma granarium*	feeding deterrent	127
quinine	*Cinchona* sp.	*Scitophilus granarius*	feeding deterrent	127
	Cinchona sp.	*Trogoderma granarium*	feeding deterrent	127
		Locusta migratoria	feeding deterrent	19
reserpine		*Callosobruchus maculatus*	toxin	47
roemerine		*Anona reticulata*	toxin	125
rosmarinine	*Senecio pterophorus*	*Danaus plexippus*	male chemical defense	62
ryanodine	*Ryania speciosa*	Lepidopterian moths	toxin	69
sanguinarine	Papaveraceae	*Lymantria dispar*	toxin, feeding deterrent	73
		Hyphantria cunea	feeding deterrent	73
senecionine	*Senecio jacobaea*	*Callimorpha jacobaeae*	insect protection	59
		Choristoneura fumiferana	mild feeding deterrent	1
	Senecio pterophorus	*Danaus plexippus*	male chemical defense	62
	Senecio spathulatus	*Nyctemera annulata*	insect protection	59
		Locusta migratoria	feeding deterrent	2

(*cont.*)

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
seneciphylline	*Senecio jacobaea*	*Callimorpha jacobaeae*	insect protection	59
	Senecio pterophorus	*Danaus plexippus*	male chemical defense	62
		Locusta migratoria	feeding deterrent	2
senkirkine		*Choristoneura fumiferana*	feeding deterrent	1
solanidine	*Solanum*	*Choristoneura fumiferana*	mild feeding deterrent	1
solanine	*Solanum*	*Choristoneura fumiferana*	mild feeding deterrent	1
		Melanoplus bivittatus	feeding deterrent	121
solanocapsine	*Solanum*	*Manduca sexta*	feeding deterrent	136
sparteine	lupines	*Acrythosiphon spartii*	feeding one	16
		Choristoneura fumiferana	mild feeding deterrent	1
		Callosobruchus maculatus	toxin	47
stemofoline	*Stemona japonica*	*Bombyx mori*	feeding deterrent	137
stemospironine	*Stemona japonica*	*Bombyx mori*	toxin	137
strychnine	*Cinchona* sp.	*Trogoderma granarium*	mild feeding deterrent	127
		Callosobruchus maculatus	toxin	47
strychnine nitrate	*Cinchona* sp.	*Trogoderma granarium*	feeding deterrent	127
		Bombyx mori	feeding deterrent	114
swainsonine	*Swainsona* sp. *Astragalus* sp.	*Spodoptera littoralis*	feeding deterrent	32
tecleanthine	*Teclea trichocarpa*	*Spodoptera exempta*	mild feeding deterrent	132
tomatidine	*Solanum*	*Choristoneura fumiferana*	mild feeding deterrent	1
tomatine	Solanaceae	*Locusta migratoria*	feeding deterrent	19
	Solanum	*Choristoneura fumiferana*	feeding deterrent	1
	Solanaceae	*Leptinotarsa decemlineata*	development retardent toxin	118
	Solanaceae	*Empoasca fabae*	feeding deterrent	120
	Solanaceae	*Melanoplus bivittatus*	growth retardent	117
	Solanaceae	*Aedes aegypti*	development retardent	117

TABLE 2.4 (*cont.*)

Alkaloid	Host	Insect	Biological Activity on Herbivore	Reference
trewiasine	*Trewia nudiflora*	*Acalymma vittatum*	feeding deterrent, toxin	83
		Cyclia sp.	feeding deterrent	83
		Menacanthus stramineus	toxin	82
		Conotrachelus nenuphar	toxin	82
		Ostrina nubilalis	toxin, feeding deterrent	82
		Diabrotica howardi	feeding deterrent	82
		Argyrotaenia velutinana	toxin	82
		Laspeyresia pomonella	toxin	82
usaramine	*Crotalaria* sp.	*Utethesia ornatrix*	insect protection	41
vasicine	*Adhatoda vasica*	*Dysdercus koenigii*	severe antifertility effects	124
	Adhatoda vasica	*Aulacophora foveicollis*	feeding deterrent	124
vasicinol	*Adhatoda vasica*	*Dysdercus koenigii*	severe antifertility effects	124
	Adhatoda vasica	*Aulacophora foveicollis*	feeding deterrent	124
vasicinone	*Adhatoda vasica*	*Dysdercus koenigii*	severe antifertility effects	124
	Adhatoda vasica	*Aulacophora foveicollis*	feeding deterrent	124
veratrine (cevadine)	*Veratrum*	*Melanoplus bivittatus*	toxin	121

[1] Alkaloid produced by endophytic fungal/plant association. Host plant and fungal source are listed.

plant-derived alkaloids is the protection of the plant host. Although there are numerous instances in which alkaloids are being utilized by specialists insects for the benefit of the insect, most of the alkaloids in Table 2.4 are functioning either as feeding deterrents or toxins. In many cases it is difficult to distinguish between a feeding deterrent and a toxin, but the semantics used by the original reference has been maintained whenever possible.

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3
Recent Advances in the Synthesis of Yohimbine Alkaloids

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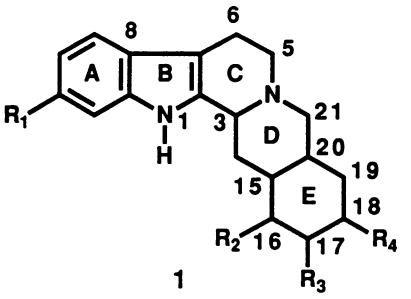
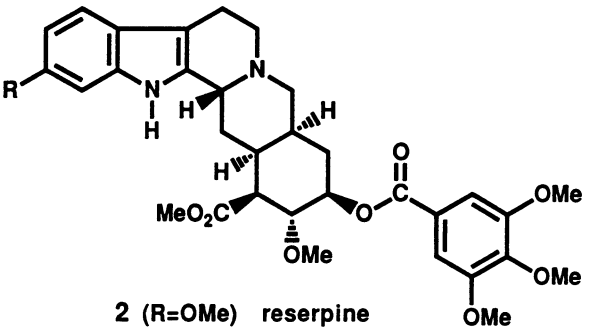
3.1. Introduction

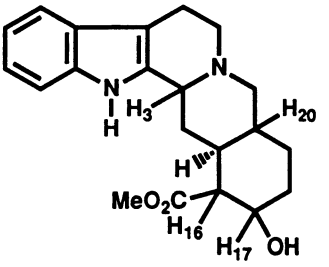
Members of the yohimbine alkaloid family possess a characteristic pentacyclic indole skeleton **1**. Representative compounds in this family include the Rauwolfia alkaloids, such as reserpine (**2**) and deserpidine (**3**), and the yohimbines (**4–9**). These compounds exhibit a wide range of medicinal properties (1, 2). Reserpine, for example, has been used extensively in the treatment





2 (R=OMe) reserpine

3 (R=H) deserpidine

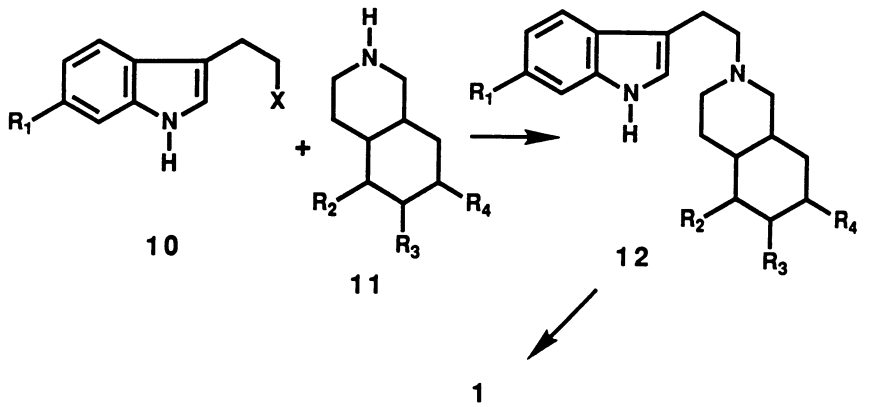


	Yohimbine	H_3	H_{16}	H_{17}	H_{20}
4	**yohimbine**	α	β	β	β
5	**β-yohimbine**	α	β	α	β
6	**pseudoyohimbine**	β	β	β	β
7	**alloyohimbine**	α	β	β	α
8	**α-yohimbine**	α	α	β	α
9	**3-epi-α-yohimbine**	β	α	β	α

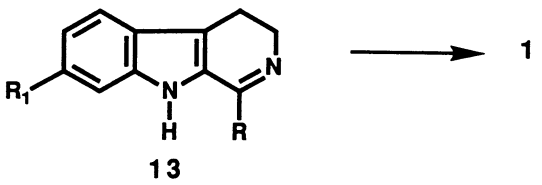
of hypertension and mental disorders. The diverse biological activity of these alkaloids as well as their intriguing structures have piqued the interest of synthetic organic chemists. In particular, reserpine, which possesses six chiral centers, and yohimbine, which has five chiral centers, are particularly challenging synthetic targets. Consequently, a plethora of activity has been directed toward the synthesis of the yohimbine alkaloids.

This chapter will address developments in yohimbine synthesis of the past 10–12 years. A number of reviews have already been published in this area (3–15). Additionally, the spectroscopic properties of these alkaloids have also been investigated (16–18) although a discussion is beyond the scope of this chapter. Unlike other reviews in this field, this report will focus exclusively on the approaches designed to tackle the synthetic challenges presented by the yohimbine alkaloids and will be organized by the methodologies actually used rather than by the specific natural product targets.

Historically, two major synthetic approaches to the yohimbine family have been explored. One is embodied in the first reported synthesis of reserpine (Scheme 3.1) by Woodward (19). Woodward and his co-workers executed a brilliant stereoselective elaboration of the D and E-Ring functionality of the target in their construction of an appropriately substituted cyclohexane aldehyde-acid. Installation of the C-ring following condensation with a tryptophyl unit and epimerization at C(3) completed the reserpine synthesis. Many of the subsequent syntheses of the yohimbines have used similar C-ring closure reactions as key steps to construct the tetrahydro-β-carboline unit comprising the ABC-tricyclic portion of the target natural products. However, unlike the Woodward route, the ensuing approaches utilize very different methodologies for constructing the functionality-rich D and E-rings of the yohimbines. Widely used approaches for DE-ring construction take advantage of cycloaddition reactions, including [2 + 2] cycloadditions (20–23), and Diels-Alder reactions (24–35). Alternatively, sigmatropic rearrangements



SCHEME 3.1



SCHEME 3.2

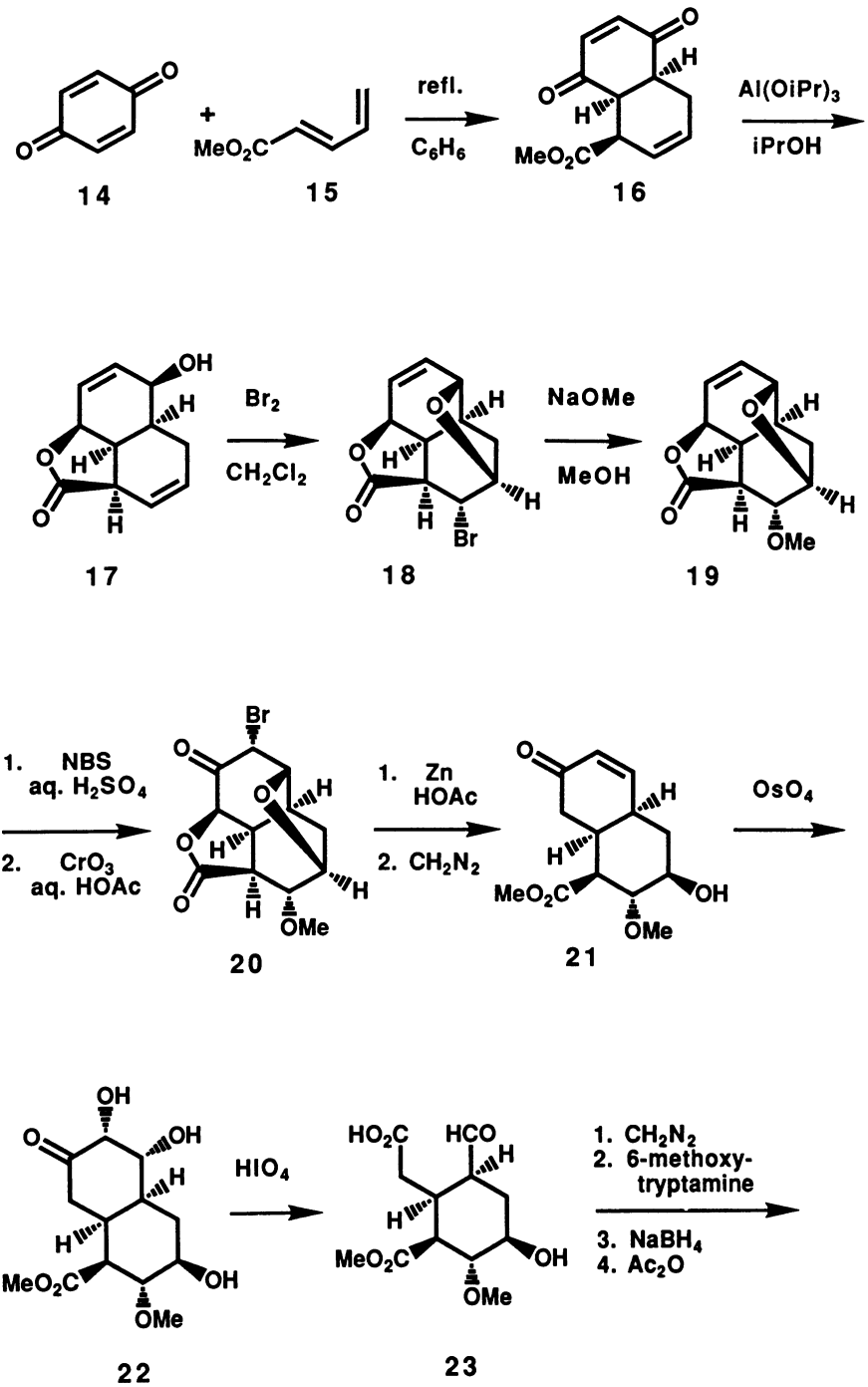
have also been successfully employed for this purpose. These include oxy-Cope (36–38), Cope (39, 40), and zwitterionic amino-Claisen rearrangements (41–44). A third general approach to the D and E-rings has involved Michael addition reactions (45–47).

A second major approach to these alkaloids begins with a β-carboline derivative (ABC-rings) and involves subsequent elaboration of the D and E-rings (Scheme 3.2). This tactic has been executed through the use of Dieckmann cyclizations (3, 11, 48–60) as well as enamide photocyclizations (9, 13, 14, 61–74).

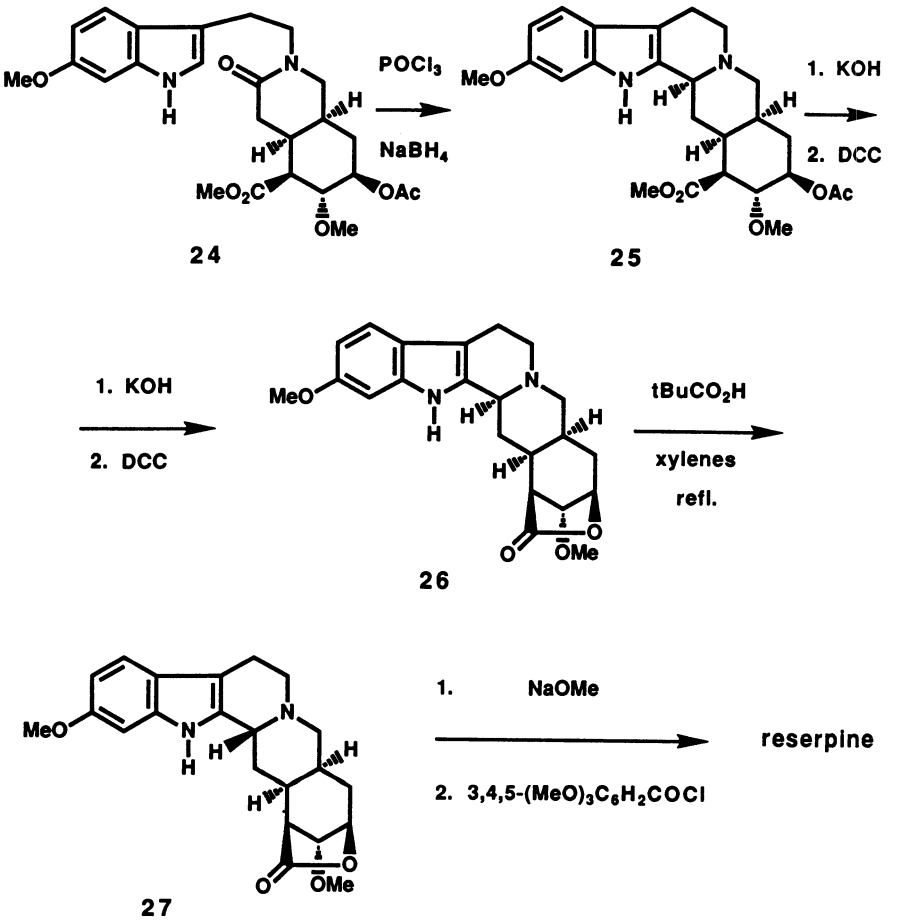
A number of other interesting syntheses which do not employ the synthetic designs discussed above have been developed. A major challenge in all of these syntheses and, in particular those targeted at the Rauwolfia alkaloids, is the generation of the proper stereochemistry at C(3). Several studies (75–80) have been conducted to understand and control this critical issue.

The major focus of this chapter is on the synthetic developments in the yohimbine area in the past dozen years. However, no discussion of this area would be complete without a presentation of the elegant and seminal Woodward (1958) synthesis of reserpine (**2**) (Scheme 3.3). The Woodward route began with a brilliant stereoselective elaboration of the stereochemically- and functionally-rich E-ring and was followed by incorporation of the tryptophyl unit and subsequent C-ring closure. The sequence concluded with a cleverly executed epimerization at C(3) to create the correct 3β-H stereochemistry found in reserpine.

The Woodward synthetic route was initiated with a Diels-Alder reaction between 1,4-benzoquinone (**14**) and diene **15**. The cycloadduct **16** formed in this way underwent Meerwein-Pondorff-Verley reduction to afford tricyclic lactone **17** which was converted to bromoether **18**. Treatment of this substance with methoxide gave the methyl ether **19**. Conversion of **19** to its halohydrin followed by chromium oxidation provided the α-bromo ketone **20** which upon treatment with zinc in glacial acetic acid afforded the bicyclic enone **21**. This substance was transformed to the aldehyde-acid **23** by an osmylation-periodate cleavage sequence. The acid function in **23** was esterified and the aldehyde moiety was condensed with 6-methoxytryptamine. The Schiff base intermediate obtained in this fashion was reduced to give an amine which provided the lactam **24** upon intramolecular acylation. Bischler-Napieralski cyclization of **24** gave the pentacyclic intermediate **25** in which


14
15
refl.
C_6H_6
16
$Al(OiPr)_3$
iPrOH
17
Br_2
CH_2Cl_2
18
NaOMe
MeOH
19
1. NBS
aq. H_2SO_4
2. CrO_3
aq. HOAc
20
1. Zn
HOAc
2. CH_2N_2
21
OsO_4
22
HIO_4
23
1. CH_2N_2
2. 6-methoxy-tryptamine
3. $NaBH_4$
4. Ac_2O

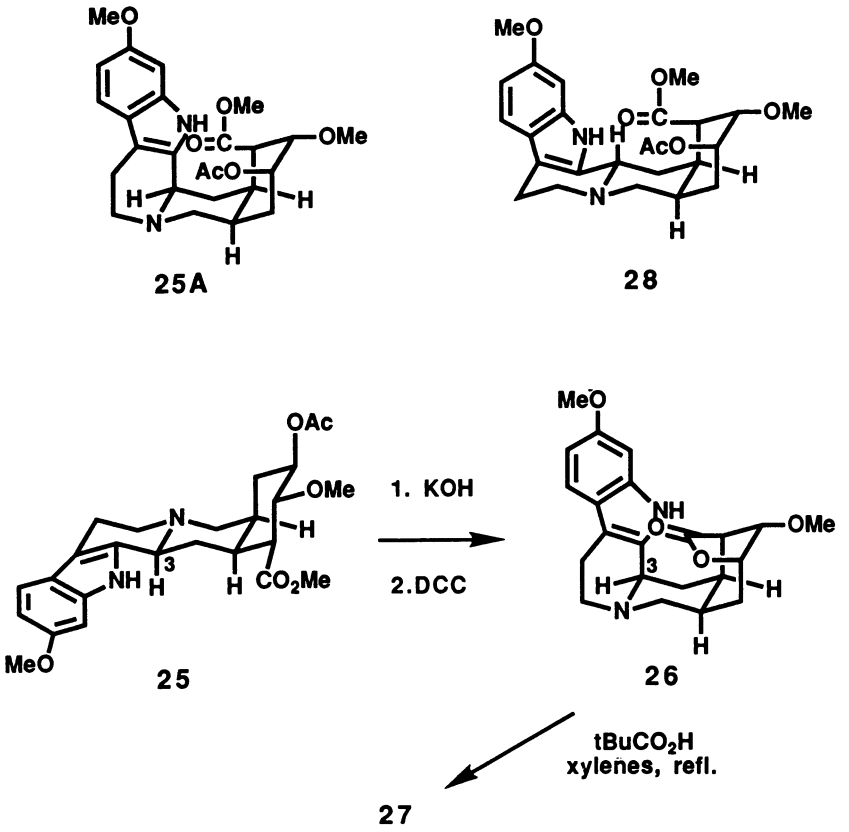
SCHEME 3.3



SCHEME 3.3 (*cont.*)

the H(3) had the opposite (i.e. isoreserpine-like) stereochemistry to that found in reserpine (**2**) (Scheme 3.4). The desired 3β-H isomer **28** exists in a conformation where the E-ring substituents are axial and as a result it is predicted to be higher energy than **25** where the E-ring appendages are equatorial.

Woodward designed a clever solution to obtain the desired C(3)-epimer. He reasoned that if **25** could be locked into conformation **25A** in which the E-ring substituents are axially disposed, epimerization at C-3 under equilibrating conditions would furnish the reserpine 3β-H stereochemistry since it would alleviate the strain engendered by the axial bulky indole moiety. In the event, Woodward converted **25** to lactone **26** which enforces the E-ring triaxial conformation. As predicted, when exposed to pivalic acid in refluxing xylenes, **26** cleanly epimerized to produce epimer **27** having the desired ster-



SCHEME 3.4

eochemistry at C(3). The synthesis of reserpine was then completed by a sequence involving methoxide-mediated opening of the lactone followed by trimethoxybenzoylation of the C(18)-hydroxyl group.

The beauty of Woodward's synthesis lies in the elegant and highly stereoselective manner in which the DE-ring system of the target is constructed. Another noteworthy feature is the solution provided to obtain the correct C(3) reserpine stereochemistry. A number of synthetic efforts since this initial achievement have attempted to mimic Woodward's approach in either a specific or general way. In particular, others have tried to effect formal syntheses of reserpine by developing alternative methods to prepare one of the Woodward DE-ring intermediates, such as **21** or **23**. Alternatively, other attempts have been patterned after Woodward's strategy in a more general sense in that the D and E-rings were constructed first followed by appendage of the tryptophyl group. C-ring annulation and C(3)-epimerization have been left as the final issues to be addressed in most, but not all, of the synthetic

routes developed in the intervening years. Although Woodward's brilliant synthesis was reported 20 years before the time period of this review, it will be informative to keep this achievement in mind in evaluating later synthetic efforts in the yohimbine alkaloid field which are reviewed below.

3.2. Approaches Which Address Synthesis of the DE-Ring System Followed by C-Ring Closure

3.2.1. Cycloaddition Approaches

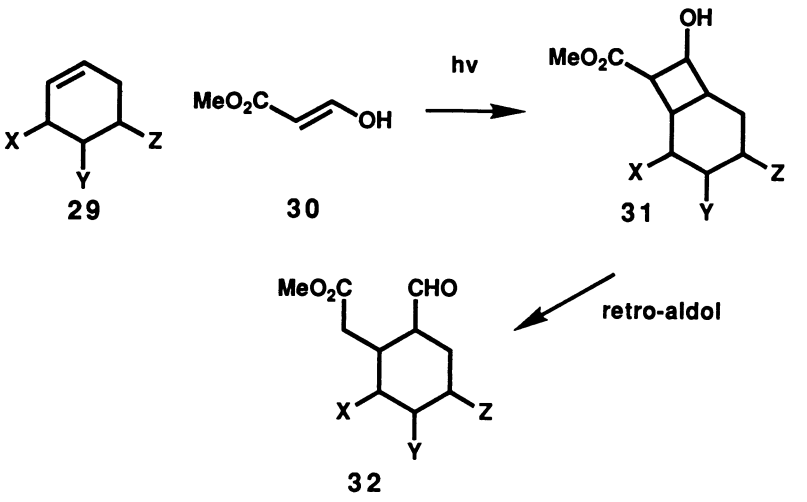
Woodward's strategy for reserpine synthesis involving construction of the stereochemically- and functionality-rich D and E-rings of reserpine first followed by introduction of the tryptophyl moiety and subsequent C-ring closure has been paralleled in many later synthetic endeavors in the yohimbine field. Several general synthetic approaches which have been used for preparation of appropriate DE-ring precursors take advantage of cycloaddition chemistry. In particular, designs based upon ground state and photoinduced [2 + 2] as well as Diels-Alder [4 + 2] cycloaddition reactions have been investigated extensively.

3.2.1.1. [2 + 2] Cycloaddition Approaches

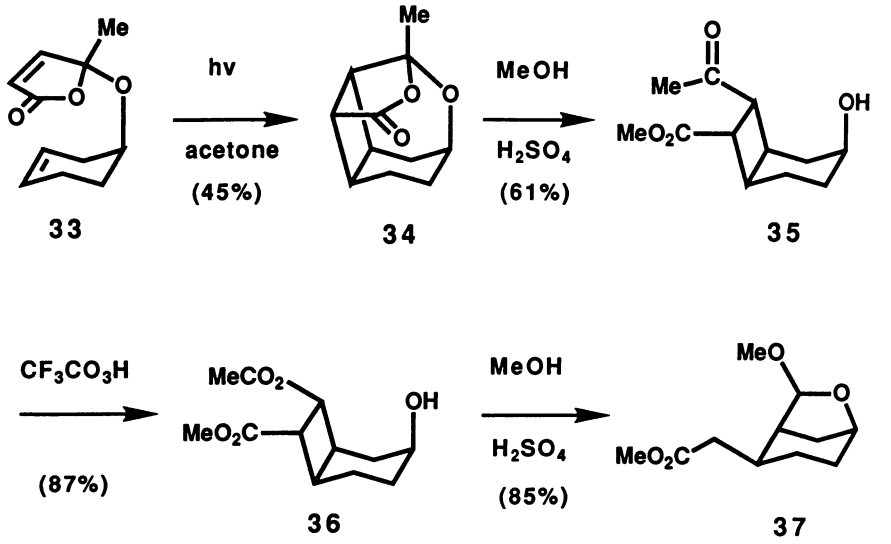
One of the most popular methods for construction of a four membered carbocyclic ring system is [2 + 2] cycloaddition (81–83). Cyclobutane ring formation followed by either ring opening or ring expansion is a strategy which has been applied to the synthesis of the yohimbine alkaloids. Pearlman investigated this approach as a method for elaboration of the DE-ring system of reserpine (**2**) (20, 21). He envisioned that the addition of a formyl acetic acid equivalent **30** to an appropriately substituted cyclohexene **29** would afford cyclobutane **31** which would be capable of undergoing a retroaldol reaction (83) to yield the pentasubstituted cyclohexane **32** (Scheme 3.5). By controlling the nature and stereochemistry of substituents, X, Y and Z, and the newly introduced aldehyde and acetic acid side chains it was proposed that this methodology would be useful for preparation of the key Woodward intermediate **23**.

After examining a number of model systems, Pearlman found that substrates related to the substituted cyclohexenol **33** to be best for this cycloaddition-reteroaldolization sequence (Scheme 3.6) (20). For example, photoinduced intramolecular cycloaddition of the pseudo ester **33** afforded the tetracyclic cyclobutane derivative **34**. Hydrolysis of this substance produced the ketoester **35** which underwent Baeyer-Villager oxidation to generate the retroaldol substrate **36**. Treatment of **36** with acid effected the desired retroaldolization and gave the lactol methyl ether **37** which has the acetic acid, aldehyde and alcohol functionality cis-oriented on the cyclohexane skeleton.

Pearlman utilized this cycloaddition-retroaldol sequence to construct a

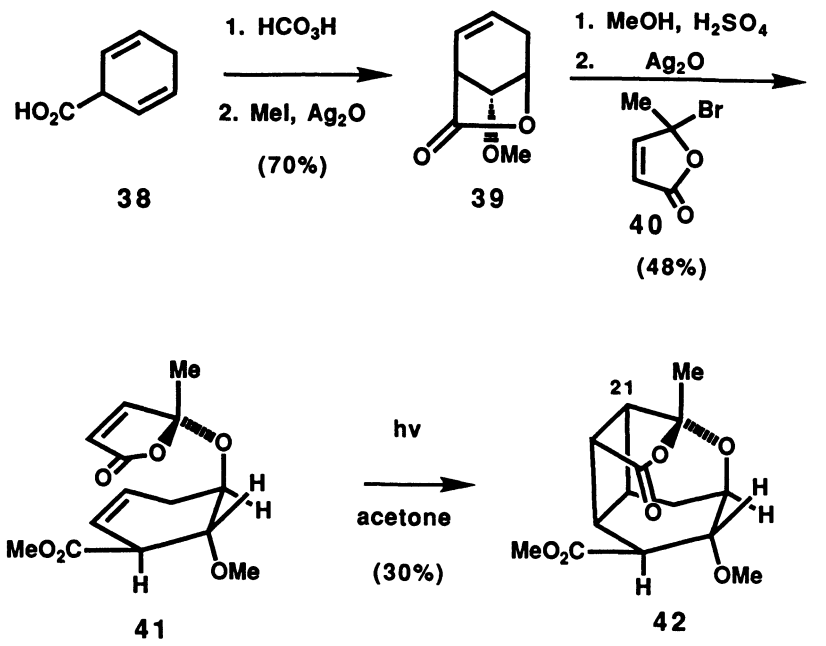


SCHEME 3.5

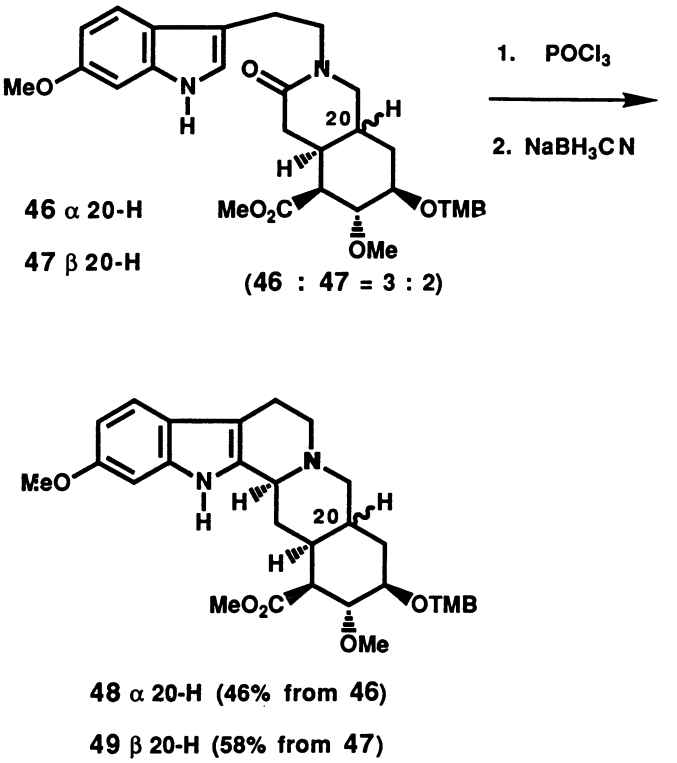


SCHEME 3.6

more highly substituted E-ring precursor of reserpine (Scheme 3.7) (21). The starting material for this effort was 1,4-dihydrobenzoic acid (**38**). Epoxidation with concomitant lactonization and subsequent methylation afforded lactone **39**. Methanolysis of the lactone followed by condensation with the pseudo-acid bromide **40** yielded key intermediate **41** in a 1 : 1 ratio with its inseparable diastereomer. Irradiation of the mixture containing **41** in acetone afforded

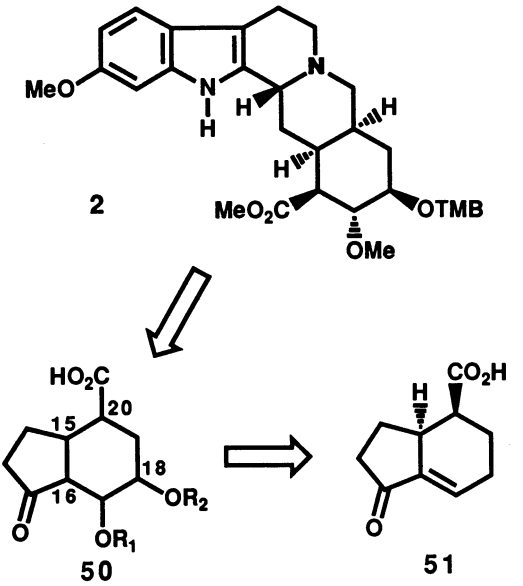

1. HCO3H
2. MeI, Ag2O
(70%)
38
39
1. MeOH, H2SO4
2. Ag2O
40
(48%)
41
hv
acetone
(30%)
42
21
1. MeOH
H2SO4
2. CF3CO3H
(54%)
43
MeOH
H2SO4
44
1. [3,4,5-(MeO)3-
C6H2CO]2O
2. HOAc
45
1. 6-methoxy-
tryptamine
2. NaBH4
(26% from 43)

SCHEME 3.7



SCHEME 3.7 (*cont.*)

cyclobutane **42** in a low (30%) yield. The inefficiency of this process was attributed to the fact that the diastereomer of **41** is unreactive under these photochemical conditions owing, presumably, to its inability to attain a conformation allowing good orbital overlap between the π-centers. Next, as in the model study, the oxidation level of the potential C(21) carbon was adjusted by hydrolysis of the ketal and lactone followed by Baeyer-Villager oxidation to give the β-acetoxycyclobutane ester **43**. This material was heated in acid to effect ester hydrolysis and subsequent retroaldolization to afford the pentasubstituted cyclohexane **44**. This key intermediate in the Pearlman reserpine approach has the five contiguous chiral centers of the DE-ring unit of the target in the correct stereochemical array. Esterification of **44** with subsequent acidic hydrolysis afforded aldehyde **45**, a substance which is closely related to Woodward's reserpine intermediate **23**. At this point, Pearlman has essentially effected a formal reserpine synthesis. However, he elaborated **45** to isoreserpine (**48**) by initial conversion to lactams **46** and **47**. Subsequent Bischler-Napieralski cyclization afforded exclusively products having the isoreserpine stereochemistry at C(3). That no reserpine was formed under this

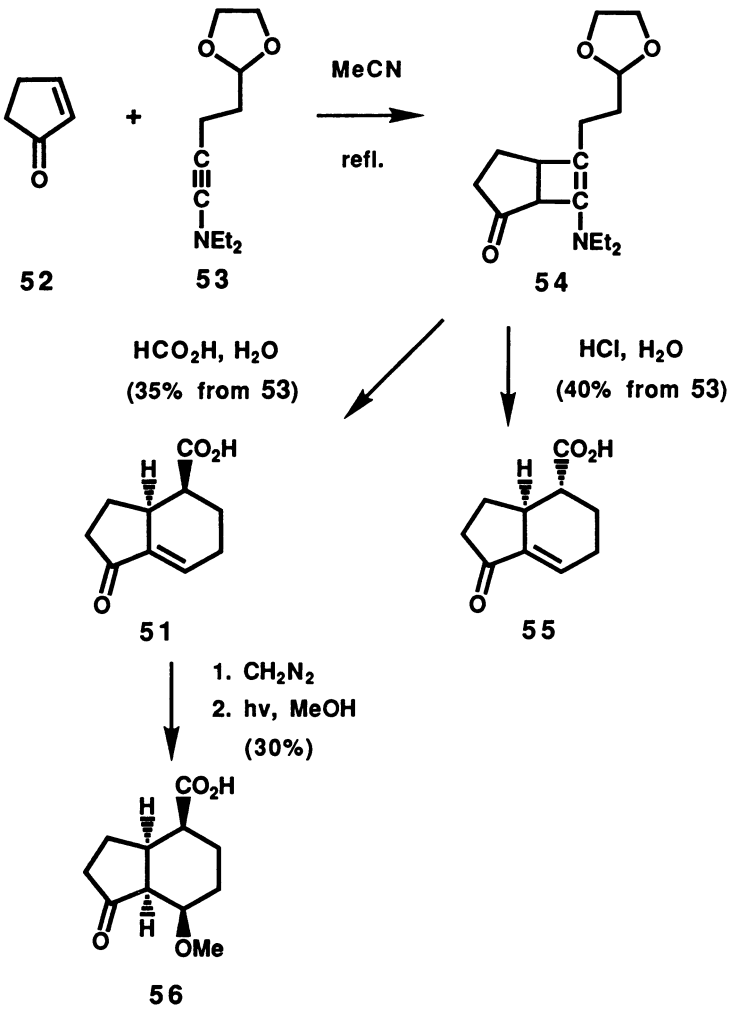


SCHEME 3.8

condition is in accord with Woodward's earlier observations and underscores the need for his clever solution to the problem of generating the correct stereochemistry at C(3) of the target.

A preliminary investigation of the use of a [2 + 2] cycloaddition-ring expansion sequence in the synthesis of reserpine (**2**) was made by Ficini and her coworkers (22). These workers envisioned reserpine as arising from the indanone **50** which in turn would be prepared by appropriate functionalization of the olefinic moiety in enone **51** (Scheme 3.8).

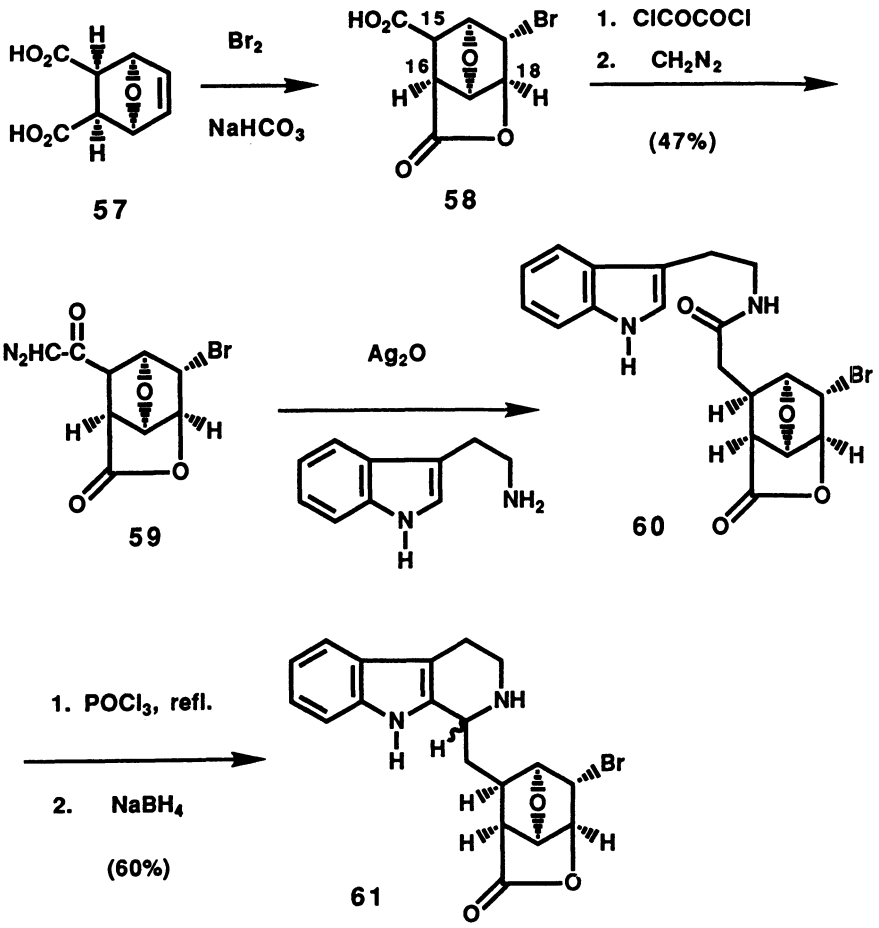
The route employed to prepare indanone **51** involved the cycloaddition-hydrolysis-aldol sequence shown in Scheme 3.9. Accordingly, condensation of cyclopentenone **52** with ynamine **53** (84) afforded the bicyclic enamine **54** which was converted to indanones **51** and **55** by hydrolytic cyclobutane ring opening followed by intramolecular aldol condensation. Interestingly, treatment of **54** with aqueous formic acid yielded indanone **51** which has stereochemistry complementary to that at C(15) and C(20) in reserpine. In contrast, hydrolysis of this substance with aqueous hydrochloric acid afforded the trans-fused indanone **55**. Subsequent to this work, the Ficini group found that esterification of **51** followed by photochemically induced addition of methanol afforded adduct **56** which has four of the reserpine stereocenters in place (23). While no further work on this problem has been reported, these preliminary investigations demonstrate a novel use of [2 + 2] photocycloaddition chemistry in potential approaches to yohimbane alkaloid synthesis.



SCHEME 3.9

3.2.1.2. Diels-Alder Approaches

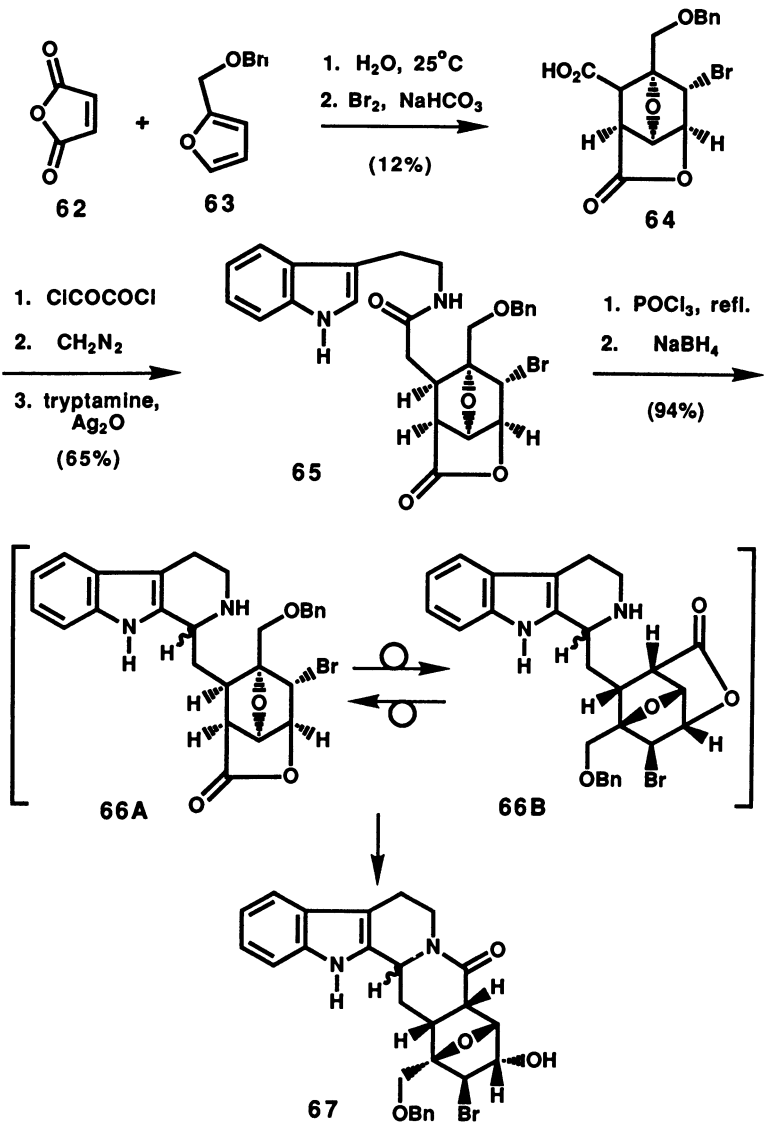
The Diels-Alder reaction is yet another cycloaddition process which has been used extensively to construct the D and E-ring system of the yohimbines. In synthetic organic chemistry, both intermolecular (85–90) and intramolecular (91–93) [4 + 2] cycloadditions have served as powerful tools for the construction of functionalized 6 membered rings. The intermolecular version of this reaction has been used to generate appropriately substituted cyclohexane precursors of reserpine (24–30). Moreover, the intramolecular variant of the



SCHEME 3.10

Diels-Alder reaction has been used to construct the D and E-rings of the yohimbane backbone simultaneously (31–35).

In the initial applications of Diels-Alder chemistry to yohimbine alkaloid synthesis, the Kametani (24–27) and Takano (28) groups have both examined reactions between furan derivatives and maleic anhydride. In the initial investigations of Kametani and his coworkers (24) (Scheme 3.10), the Diels-Alder adduct (**57**) of furan and maleic anhydride underwent bromolactonization to give the tricyclic carboxylic acid **58** (94). This compound has four [C(15), C(16), C(17), and C(18)] of the five contiguous stereocenters of the reserpine E-ring in place. Acid **58** was converted to diazoketone **59** which underwent Wolff rearrangement followed by tryptamine trapping to afford amide **60**. Bischler-Napieralski cyclization of this substance afforded the tetracyclic



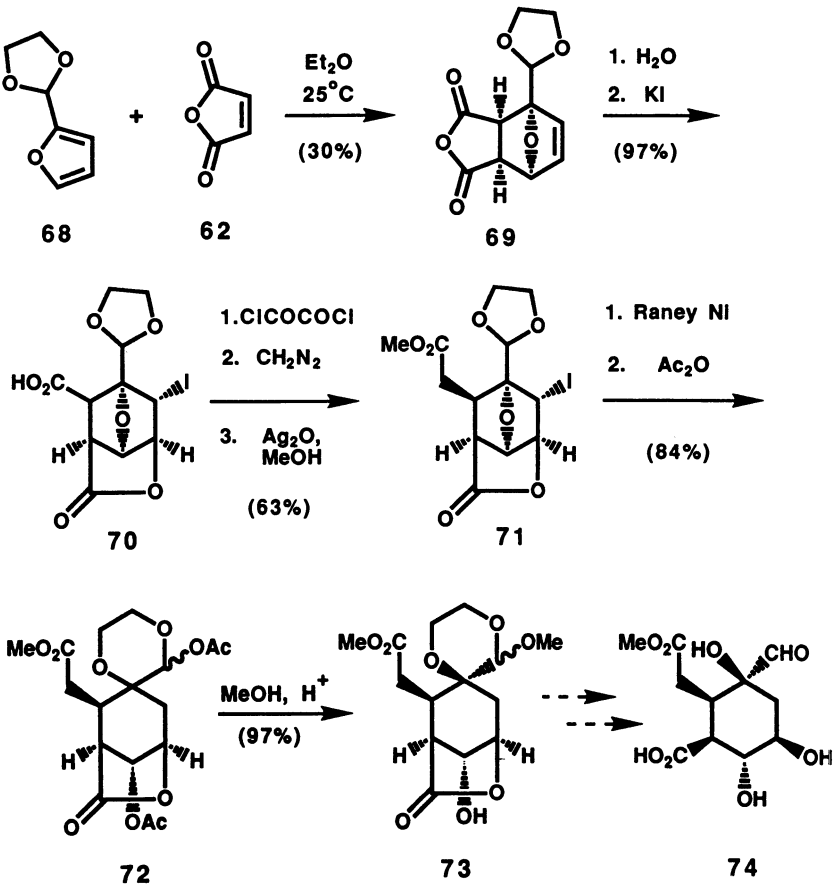
SCHEME 3.11

intermediate **61** which has the reserpine skeleton in place except for the missing C(21) carbon as part of the D-ring.

An alternate route which employed the substituted furan **63** in a Diels-Alder reaction with maleic anhydride (**62**) (25–27) was also explored (Scheme 3.11). The required reserpine C(21) carbon is now present in furan **63** as part of the benzyloxymethyl group. The adduct obtained from reaction of **62** with

63 was treated with bromine to yield the tricyclic lactone carboxylic acid **64** which was then transformed to the amide **65**. Bischler Napieralski reaction afforded the C-ring closed product **66** which was not isolated but rather spontaneously cyclized to form the pentacyclic lactam **67**. Unfortunately, this undesired cyclization involving lactam ring formation, while creating the yohimbane skeleton, placed the E-ring substituents in a wrong positional orientation. Even though this synthetic route was ultimately unsuccessful, it illustrates how the Diels-Alder reaction can be utilized to build rapidly the structurally complex potential E-ring analog of reserpine.

A conceptually similar approach for reserpine E-ring construction via Diels-Alder chemistry was examined by Takano and his coworkers (28) (Scheme 3.12). In this work the cycloadduct **69** obtained by addition of 2-dioxolanyl furan (**68**) to maleic anhydride (**62**) was saponified and iodolactonized to produce the lactone carboxylic acid **70**. Homologation to form the methyl



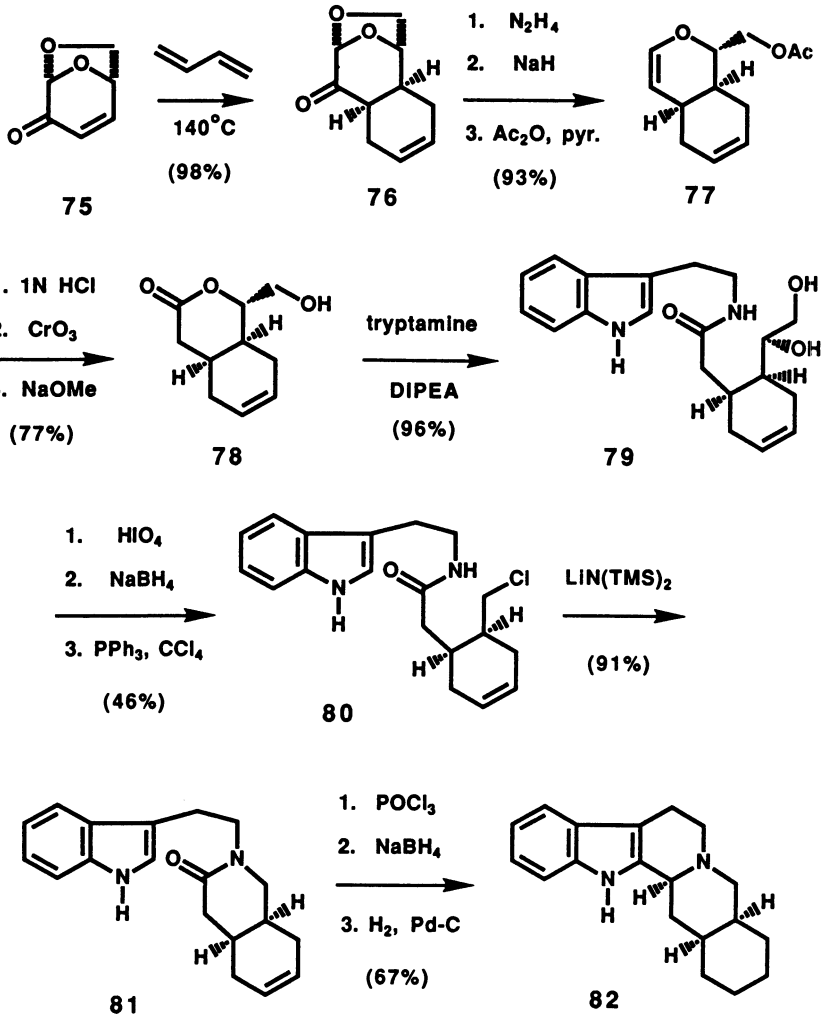
SCHEME 3.12

ester **71** by the Wolff rearrangement was then followed by dehalogenation and subsequent oxo bridge cleavage. Treatment of the deiodinated derivative of **71** with acetic anhydride in sulfuric acid effected an interesting transformation resulting in both cleavage of the bridged ether function as well as rearrangement of the dioxolanyl group to afford dioxanyl lactone **72**. Methanolysis of **72** gave the lactone **73** which in effect is the hydroxylated variant **74** of Woodward's aldehyde acid **23**. While methods to remove the undesired C(20) hydroxyl group in **74** have not been reported, this route illustrates how Diels-Alder reactions of highly functionalized substrates can be used to elaborate the yohimbine E-ring functionality efficiently and with a high degree of stereochemical control.

Isobe and his coworkers (29, 30) utilized Diels-Alder reactions in a quite different fashion in their synthetic approaches to the yohimbine alkaloids. An optically active dienophile was treated with a diene to form the DE ring skeleton which was then elaborated to produce the target yohimbines.

This methodology was initially applied to a synthesis of (−)-alloyohimbane (**82**) (Scheme 3.13) (29). Readily available levoglucosenone (**75**), a product of cellulose pyrolysis, was the starting material. Diels-Alder reaction of **75** with 1,3-butadiene afforded adduct **76** which underwent Wolff-Kishner elimination and subsequent acylation to provide the bicyclic enol ether **77**. Hydrolysis followed by oxidation and saponification afforded hydroxymethyl lactone **78** which was then condensed with tryptamine to yield the amide diol **79**. Periodate cleavage of the diol was followed by conversion to chloride **80** which was cyclized to afford lactam **81**. Subsequent Bischler-Napieralski cyclization followed by hydrogenation of the olefin moiety afforded the target (−)-alloyohimbane (**82**).

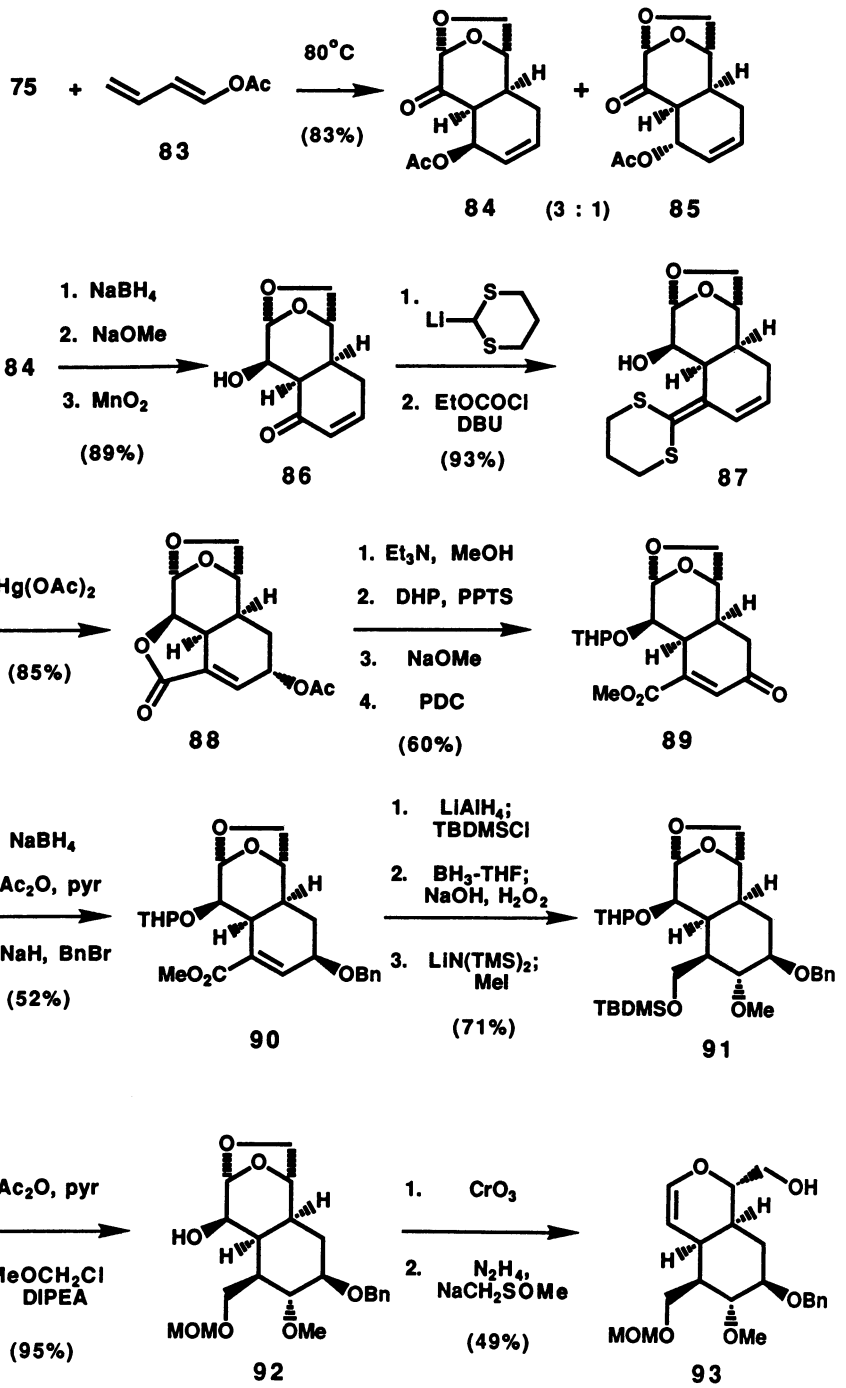
Isobe and his coworkers also utilized the Diels-Alder reaction of levoglucosenone in their synthetic approach to reserpine (**2**) (Scheme 3.14) (30). Cycloaddition of **75** with 1-acetoxy-1,3-butadiene (**83**) afforded adducts **84** and **85** in a 3 : 1 ratio. Reduction of **84** with $NaBH_4$ followed by acetate cleavage and oxidation with MnO_2 afforded enone **86** which was condensed with dithiane anion followed by elimination of water to yield the tricyclic diene **87**. Treatment of **87** with mercuric acetate afforded the tetracyclic lactone **88**. While the mechanism of this transformation is not obvious, Isobe speculated that the process entailed initial mercuration of the endocyclic double bond with subsequent formation of the corresponding thioketene which was trapped by the neighboring hydroxyl group. Elimination of mercury and ethanedithiol then afforded **88**. Lactone **88** was converted by a straightforward sequence to tricyclic enone **89** which was elaborated to the protected allylic alcohol **90**. Next, the desired C(16)-carbomethoxy group had to be protected as a silyl ether prior to introduction of the C(17)-methoxy group by a hydroboration-methylation sequence to yield **91**. This intermediate has all of the E-ring stereocenters of reserpine in place. Finally, a deprotection-protection sequence afforded the selectively deprotected secondary alcohol **92** which was oxidized and then subjected to Wolff-Kishner reduction conditions to give



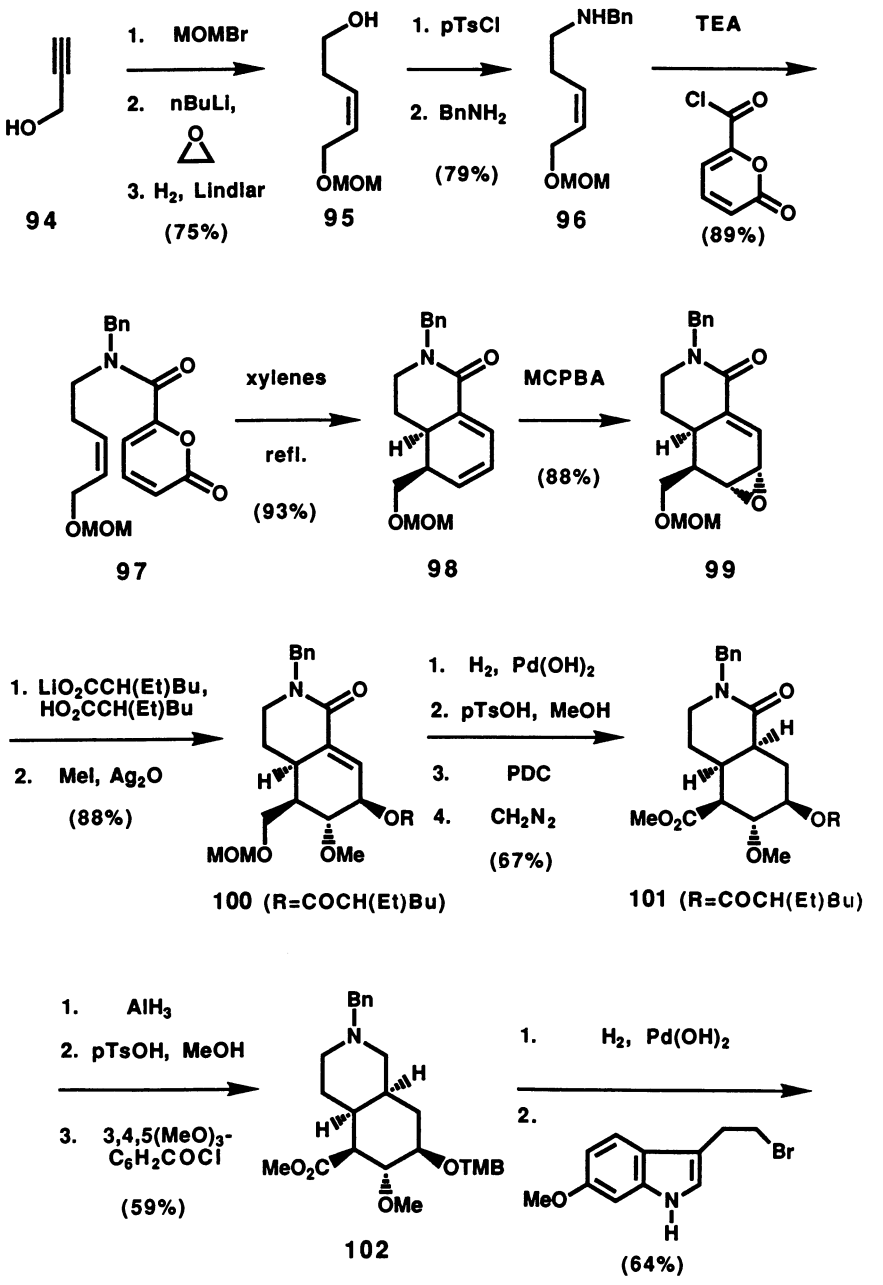
SCHEME 3.13

enol ether **93** which is analogous to an intermediate, **78**, used in the alloyohimbane synthesis. Transformation of **93** to the target, reserpine (**2**), was not reported, but can be easily visualized. While the preparation of **93** required a number of functional and protecting group manipulations, this effort illustrated yet another application of the intermolecular Diels-Alder approach to the synthesis of yohimbine alkaloids.

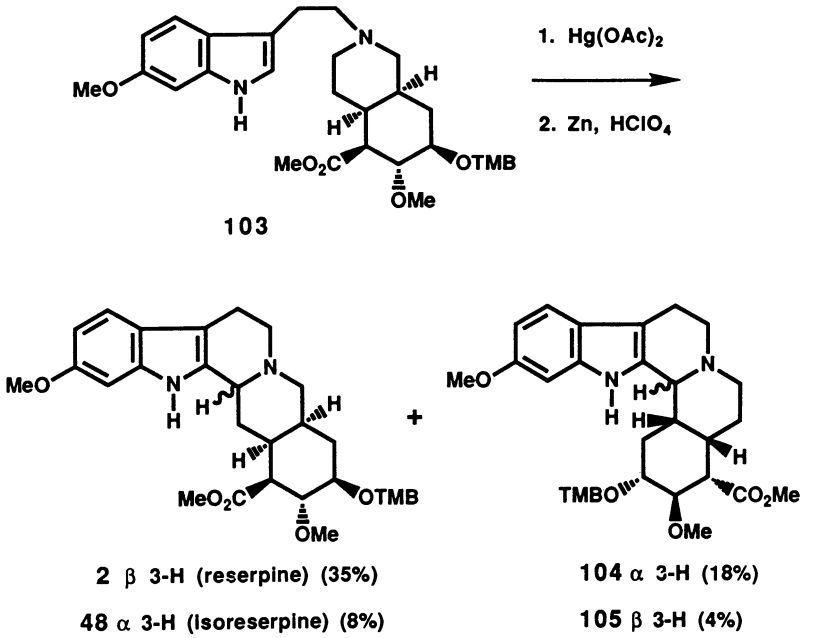
The intramolecular variant of the Diels-Alder reaction has been used extensively by Martin and coworkers (31–35) in the preparation of a number of members of the yohimbine alkaloid family. In this methodology, the D and


75
OAc
83
80°C
(83%)
AcO
84
(3 : 1)
85
1. NaBH4
2. NaOMe
3. MnO2
(89%)
HO
86
1.
Li
S
S
2. EtOCOCl
DBU
(93%)
87
Hg(OAc)2
(85%)
OAc
88
1. Et3N, MeOH
2. DHP, PPTS
3. NaOMe
4. PDC
(60%)
THPO
MeO2C
89
1. NaBH4
2. Ac2O, pyr
3. NaH, BnBr
(52%)
OBn
90
1. LiAlH4;
TBDMSCl
2. BH3-THF;
NaOH, H2O2
3. LiN(TMS)2;
MeI
(71%)
TBDMSO
OMe
91
Ac2O, pyr
MeOCH2Cl
DIPEA
(95%)
MOMO
92
1. CrO3
2. N2H4,
NaCH2SOMe
(49%)
OH
93

SCHEME 3.14


1. MOMBr
2. nBuLi,
3. H2, Lindlar
(75%)
94
95
1. pTsCl
2. BnNH2
(79%)
96
TEA
(89%)
xylenes
refl.
(93%)
97
98
MCPBA
(88%)
99
1. LiO2CCH(Et)Bu,
HO2CCH(Et)Bu
2. MeI, Ag2O
(88%)
100 (R=COCH(Et)Bu)
1. H2, Pd(OH)2
2. pTsOH, MeOH
3. PDC
4. CH2N2
(67%)
101 (R=COCH(Et)Bu)
1. AlH3
2. pTsOH, MeOH
3. 3,4,5(MeO)3-
C6H2COCl
(59%)
102
1. H2, Pd(OH)2
2.
(64%)

SCHEME 3.15



SCHEME 3.15 (*cont.*)

E rings of the target were formed simultaneously. Then, following the approach of Woodward, the D and E rings were elaborated before introduction of the tryptophyl unit and C-ring.

In the synthesis of reserpine (**2**) (Scheme 3.15), Martin and his coworkers (33, 34) used propargyl alcohol (**94**) as the starting material. It was elaborated to homoallylic alcohol **95** which in turn was converted to benzylamine **96**. Condensation of **96** with the pyrone acid chloride afforded triene **97**, the substrate for the key intramolecular Diels-Alder reaction. When **97** was refluxed in xylenes, the desired cycloadduct **98** was formed in excellent yield. This compound was selectively epoxidized at the C(17)–C(18) double bond to afford α-epoxide **99**. After considerable experimentation, this epoxide was opened efficiently with lithium 2-ethylhexanoate and the liberated hydroxyl group was methylated to afford intermediate **100** in which four [C(15), C(16), C(17), C(18)] of the five contiguous chiral centers of reserpine are in place. Hydrogenation of **100** followed by conversion of the MOM protected alcohol to the corresponding methyl ester gave the *cis*-decahydroisoquinoline **101**. After the lactam was reduced, the C(18)-hydroxy was deprotected and subsequently acylated to give **102** with all of the reserpine E-ring functionality in place. Debenzylation followed by condensation with 6-methoxytryptophyl bromide afforded 2,3-seco-reserpine (**103**). Closure of the C-ring was then

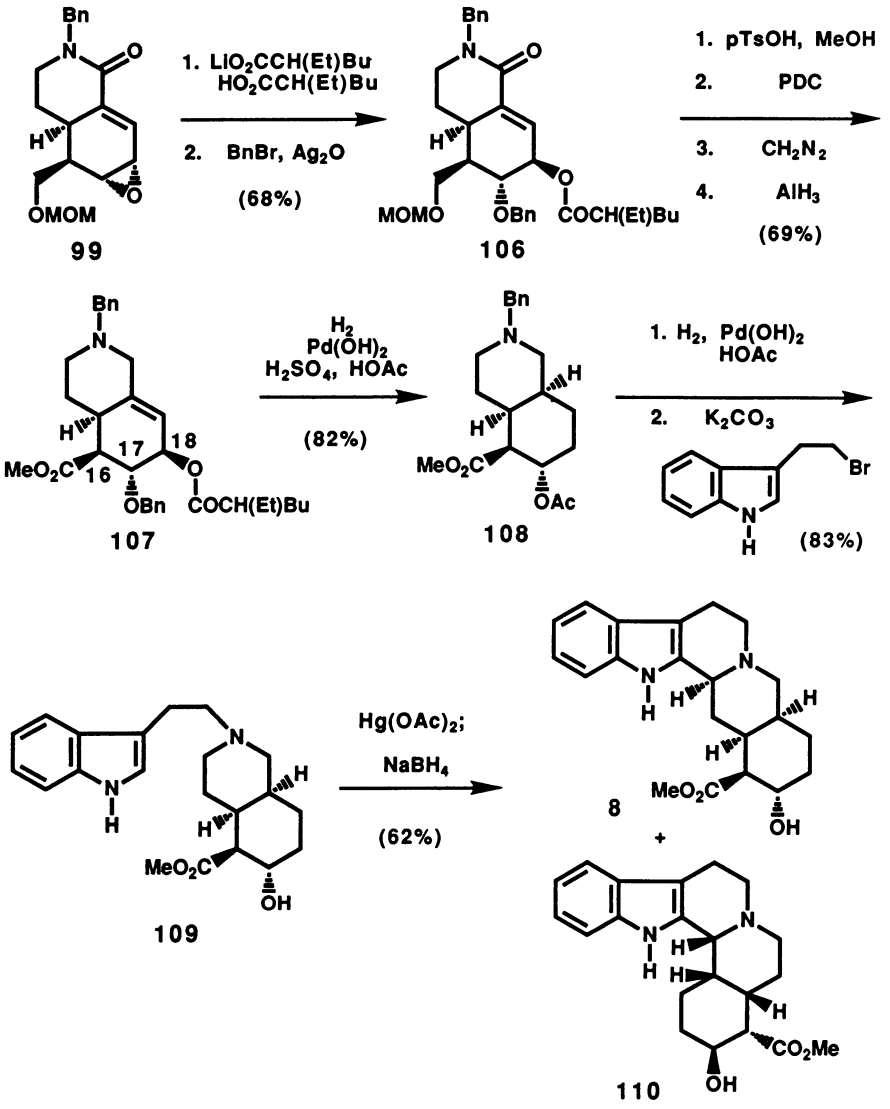
effected by a mercuric acetate-mediated oxidative cyclization with subsequent reduction of the intermediate iminium species by zinc.

Interestingly, the major product of this cyclization was reserpine (**2**); in addition, a modest amount of isoreserpine (**48**) and the inside isomers **104** and **105** were also formed. These results are particularly intriguing in light of the observation that $POCl_3$- $NaBH_4$ mediated Bischler-Napieralski cyclization of Woodward's intermediate **24** afforded only the isoreserpine stereoisomer. While both reactions presumably proceed by generation of an iminium species which is trapped by the indole followed by subsequent reduction, the seemingly subtle differences in the reaction conditions are enough to effect drastic differences in the ratio of the reserpine and isoreserpine stereoisomers which form.

Martin's group has utilized the intramolecular Diels-Alder reaction in the preparation of several other yohimbine alkaloids. For example, epoxide **99** is a key intermediate in the synthesis of α-yohimbine (**8**) (Scheme 3.16). (32, 34) As in the reserpine synthesis, the epoxide was opened with lithium ethylhexanoate. The liberated hydroxyl group was protected as the benzyl ether in **106**. Then, as before, the lactam was reduced with alane followed by conversion of the MOM ether to the methyl ester. Hydrogenolysis of **107** effected double bond reduction, selective O-debenzylation, and deoxygenation at C-18 to afford **108** which has the α-yohimbine E-ring functionality in place. Hydrogenolysis of the benzylamine group in **108** and subsequent tryptophylation afforded 2,3-seco-α-yohimbine (**109**). Treatment of **109** with mercuric acetate followed by reduction with $NaBH_4$ afforded α-yohimbine (**8**) and its inside isomer **110** in a 1 : 1 ratio.

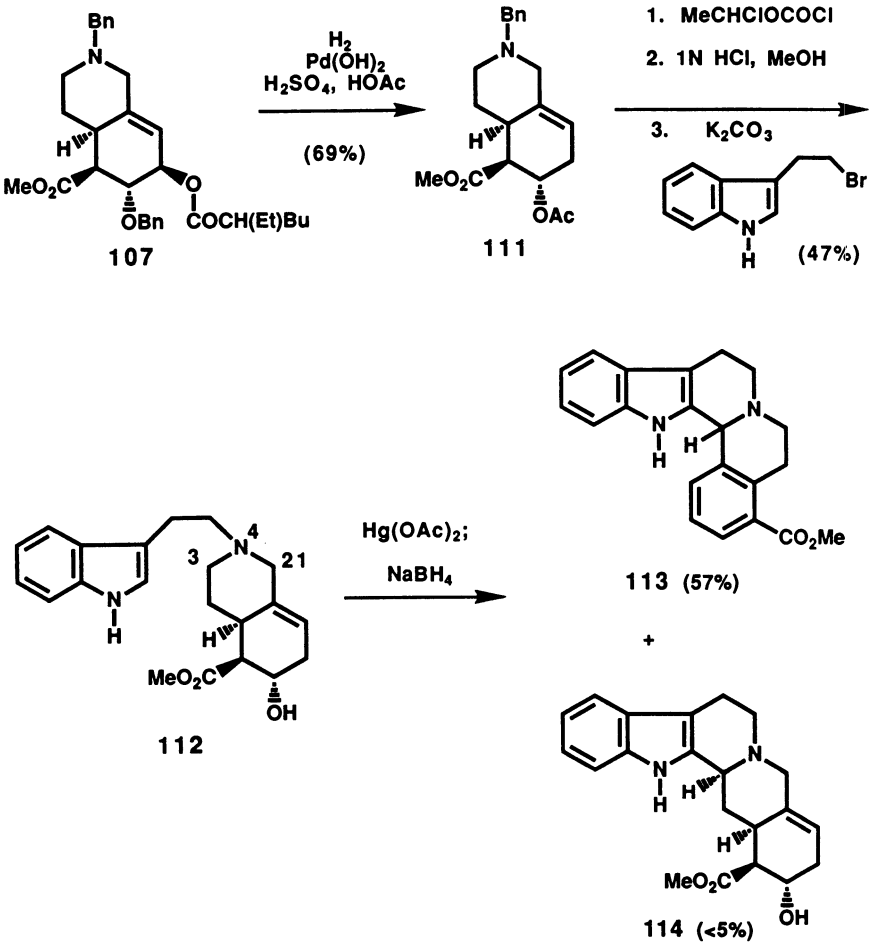
Martin and his coworkers also attempted a synthesis of 19,20-dehydroyohimbine (**114**) using intermediate **107** (Scheme 3.17). Hydrogenolysis conditions were chosen to selectively debenzylate the O-17 hydroxyl and cause deoxygenation at C(18) to yield **111**. Debenzylation of the nitrogen function in **111** under noncatalytic conditions followed by tryptophylation gave **112**, the seco analog of the target. Disappointingly, sequential treatment of **112** with mercuric acetate and $NaBH_4$ yielded predominantly the inside aromatized yohimbine derivative **113** along with only trace amounts of the desired dehydroyohimbine **114**. Apparently, formation of the conjugated C(21)–N(4) iminium salt was more favorable than generation of the C(3)–N(4) iminium salt. Despite the failure of the cyclization step, this route illustrated how the functionality introduced in the intramolecular Diels-Alder reaction could be modified to prepare a new E-ring permutation in the yohimbine family.

In the previous examples, Martin and his coworkers utilized the intramolecular Diels-Alder reaction to construct the DE-ring system of the pentacyclic targets and then, following the strategy of Woodward, introduced the tryptophyl moiety followed by C-ring closure. The Martin group has synthesized other members of the yohimbine family by routes which do not adhere



SCHEME 3.16

to this pattern, but which also employ the intramolecular Diels-Alder reactions as key steps. Two of these targets are yohimbane (**120**) and 16,17-dehydroyohimbane (**121**) (Scheme 3.18) (31). These syntheses commenced with acylation of *N*-allyltryptamine (**115**) with acid chloride **116** to afford triene **117**. In the key step, intramolecular Diels-Alder reaction of **117**, yielded the cis and trans ring fused products **118** and **119** in a ratio of 1.9 : 1. The trans

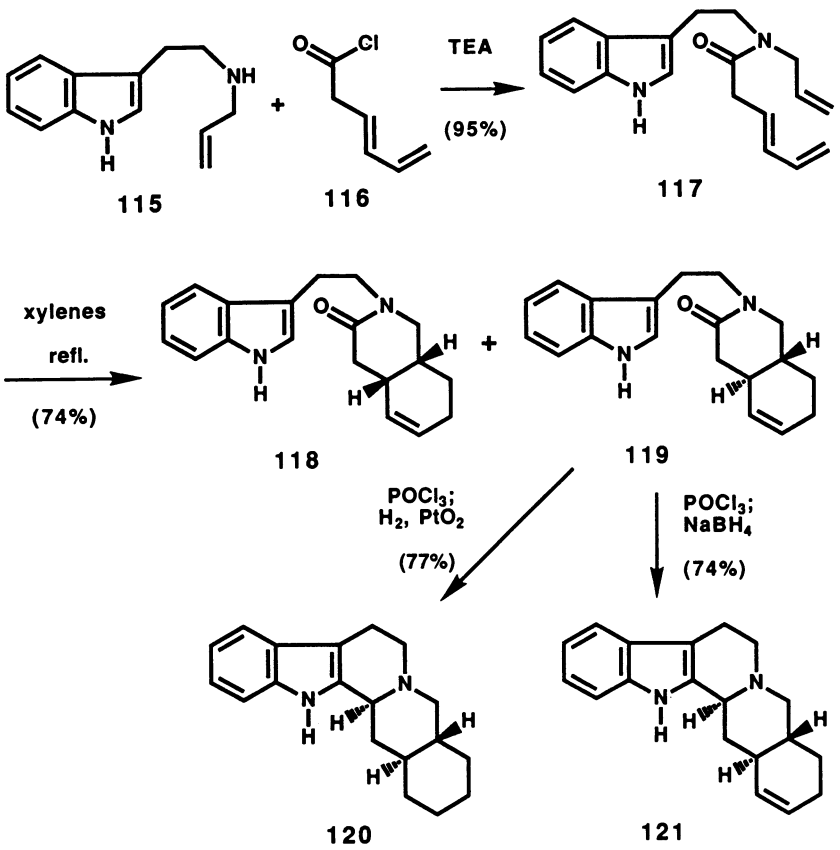


SCHEME 3.17

product **119** was cyclized with $POCl_3$ followed by catalytic hydrogenation to yield yohimbane (**120**) while sequential treatment of **119** with $POCl_3$ and $NaBH_4$ afforded the 16,17-dehydro derivative **121**.

Finally, Martin and Geraci (35) have recently employed an intramolecular Diels-Alder reaction in an elegant synthesis of oxogambirtannine (**127**) (Scheme 3.19). Sequential condensation of β-carboline **122** with the pyrone acid chloride **123** and ketene acetal **124** afforded triene **125**. In refluxing mesitylene, **125** underwent Diels-Alder reaction to produce the pentacyclic intermediate **126** which then aromatized to form the target **127**.

In summary, the efforts of Martin and his group illustrate well the utility of the intramolecular Diels-Alder reaction in the synthesis of yohimbine alkaloids. Through the use of this reaction, partially reduced isoquinoline

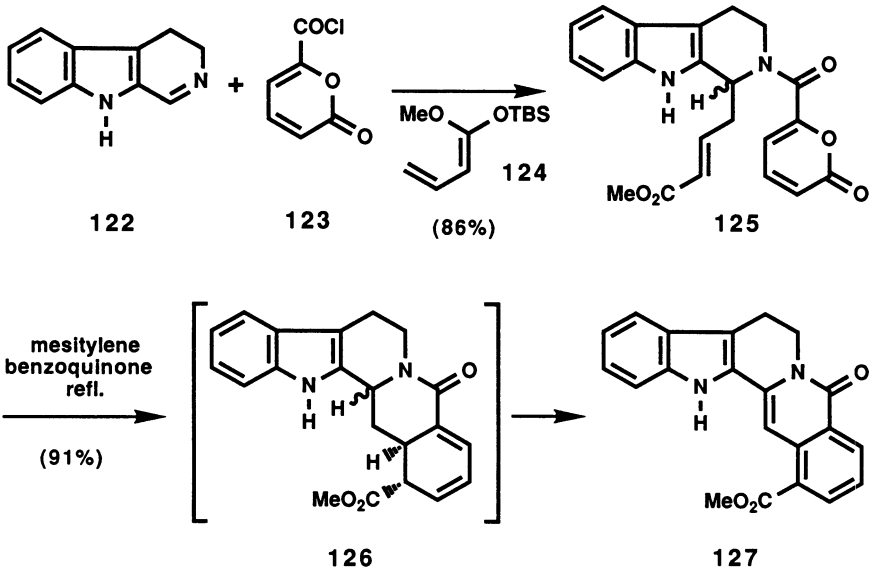


SCHEME 3.18

derivatives were prepared. These DE-ring precursors were then appropriately elaborated with good stereocontrol to afford a wide spectrum of representative yohimbine structures ranging from the relatively simple yohimbane (**120**) to the functionally embellished reserpine (**2**).

3.2.2. Sigmatropic Rearrangements

Sigmatropic rearrangements (95, 96, 97) are another class of reactions which have been employed extensively in the generation of the DE-ring portion of the yohimbine alkaloids. Reactions of this type which have been utilized include oxy-Cope, Cope, and zwitterionic amino-Claisen rearrangements. Generally, these reactions were employed early in the synthetic routes to generate a cis-fused DE-ring precursor and typically olefin elaboration was utilized to introduce additional E-ring functionality. With a DE-ring system in hand in these strategies, the tryptophyl unit was appended and the C-ring closed as in the Woodward reserpine synthesis.

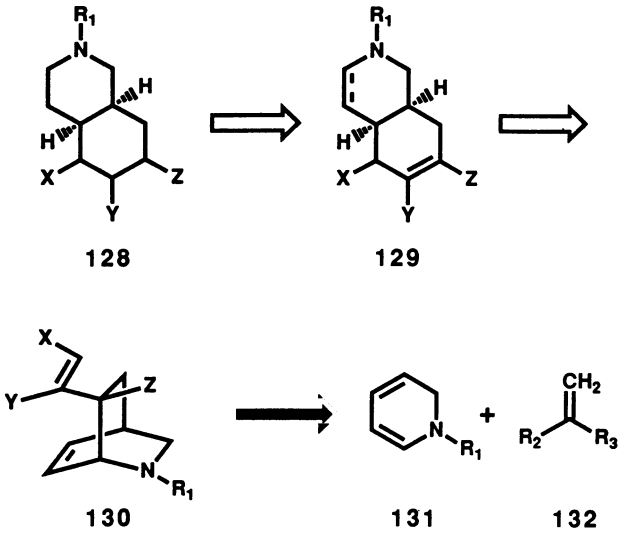


SCHEME 3.19

3.2.2.1. Oxy-Cope Rearrangements

Oxy-Cope rearrangements (95, 98, 99) have been extensively used in natural product synthesis to generate 1-oxo-hexa-1,5-diene systems which are then elaborated to introduce the functionality presented in the selected targets. Wender and his coworkers have utilized this methodology in a key step in the synthesis of reserpine (36, 37). They envisioned that the DE-ring precursor **128** could arise from the hydroisoquinoline **129** which could be generated by oxy-Cope rearrangement of isoquinuclidene **130** (Scheme 3.20). This key intermediate, in turn, could be prepared by Diels-Alder reaction of dihydropyridine **131** with an appropriately substituted dienophile **132**.

In this reserpine synthetic route (Scheme 3.21), Diels-Alder reaction of dihydropyridine **133** (100) and methyl α-acetoxyacrylate **134** afforded isoquinuclidenes **135** and **136** (36). Addition of lithium *t*-butyl acetate to **135** afforded the β-ketoester **137** which was converted to the corresponding enol ester **138**. When **138** was refluxed in xylenes, the cis-fused hexahydroisoquinoline **139**, in which the reserpine C(15), C(16), and C(20) stereocenters are in place, formed in good yield. Hydrogenation of the enamine function in **139** followed by reduction of the enol ester afforded **140** which has the C(17)-methoxy group with the required relative stereochemistry. Acylation of ketone **140** yielded the C(18), C(19) enol ester which was hydrogenated stereoselectively to produce **141** in which the C(18) stereochemistry is set. Deprotection of the nitrogen function followed by tryptophylation afforded **142** which was then cyclized to provide the anticipated isoreserpine diol **143**



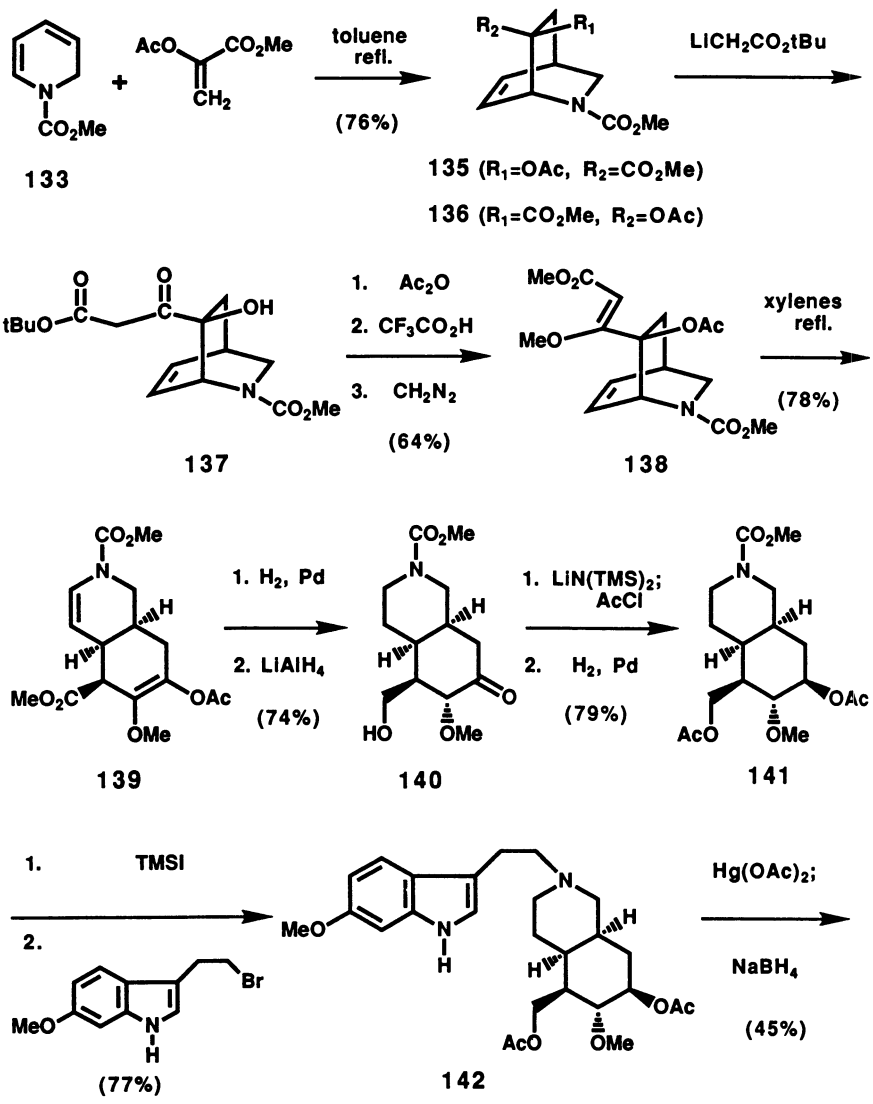
SCHEME 3.20

along with 30% of an isomeric material, assigned as the inside reserpine product. Next, **143** was O-acylated and then through a lengthy sequence of steps, the hydroxymethyl group was converted to the desired carbomethoxy functionality with concomitant oxidation of the indole to afford the hydroxyindolenine **145**. This compound was reduced to form isoreserpine (**48**) which could be epimerized to yield reserpine (**2**) by literature methods (101, 102).

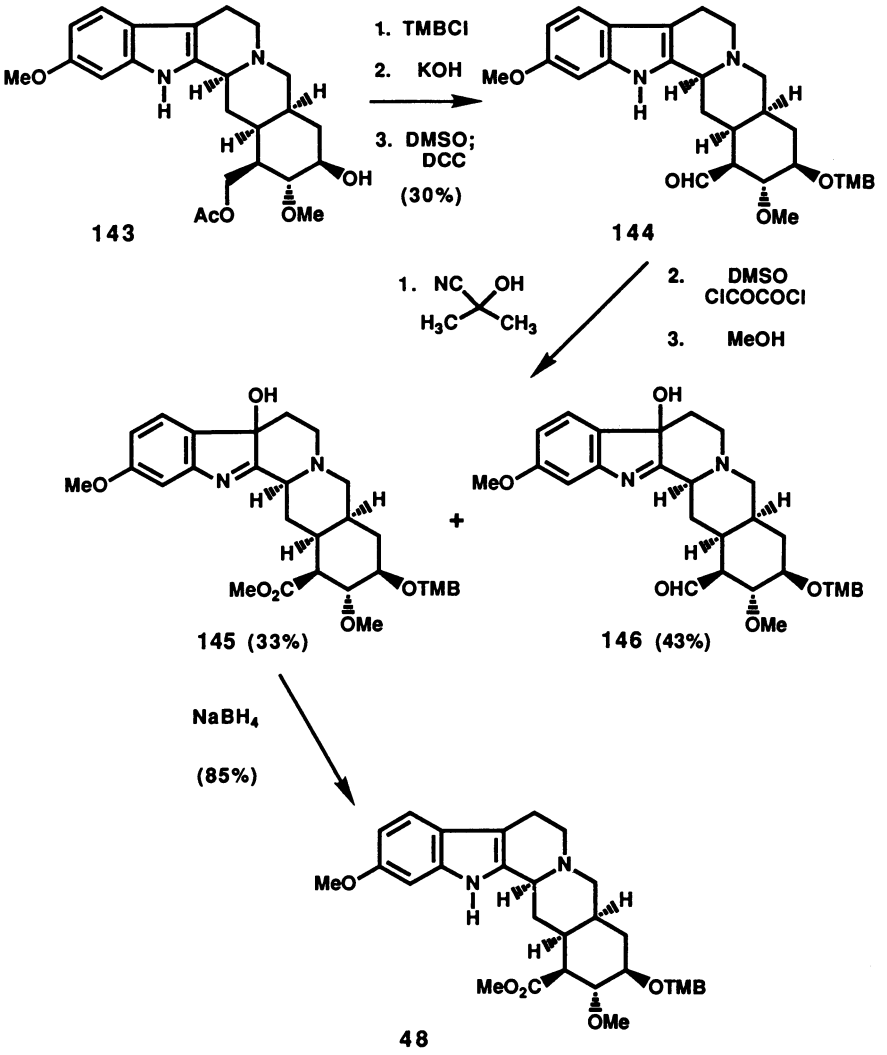
Wender's group was able to streamline the synthesis considerably (Scheme 3.22) (37) by making the latter part of the sequence from intermediate **140** to reserpine more efficient. Accordingly, the intermediate **140** was converted to methyl ester **147**. Reduction of the ketone with cerium-mediated borohydride followed by acylation afforded **148** which has all of the reserpine E-ring functionality installed. Subsequent nitrogen deprotection and tryptophylation afforded seco-reserpine **103**, an intermediate in Martin's (33, 34) approach to reserpine.

Another approach to the DE-ring system of reserpine utilizing an oxy-Cope rearrangement strategy has been reported by Jung and Light (Scheme 3.23) (38). Their targeted DE-ring precursor **149** was planned to arise from the cis-fused hydrindanone **150** which then could be prepared from **151**, the oxy-Cope rearrangement product of bicyclopentene **152**. This substance, in turn, could readily be prepared from norbornenone **153**.

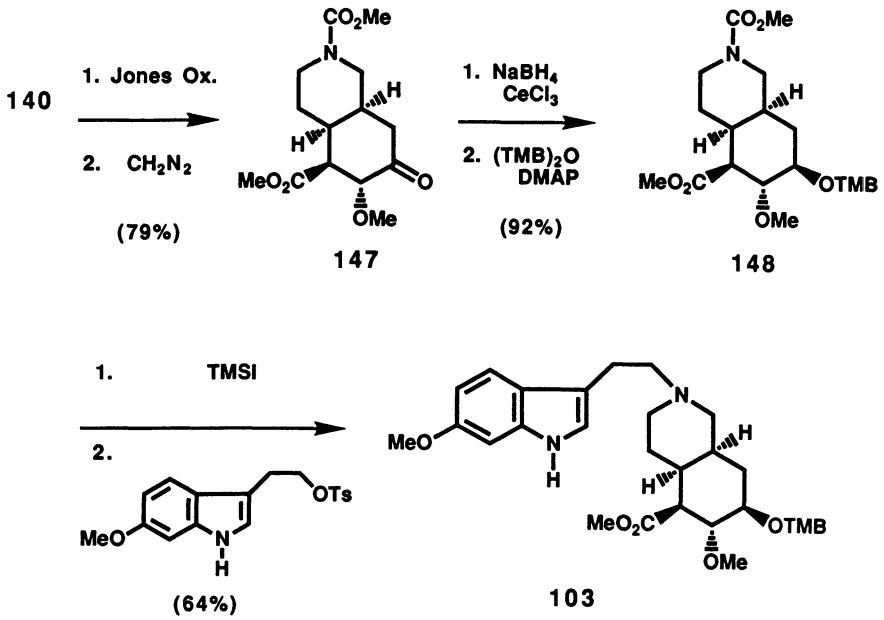
In the synthetic sequence (Scheme 3.24), the readily available ketone **153** was condensed with a propargyl alcohol derivative to yield **154** which was then reduced to form the diene **155**. Treatment of **155** with sodium hydride in refluxing THF effected the crucial anionic oxy-Cope rearrangement to afford


toluene
refl.
(76%)
LiCH2CO2tBu
133
135 (R1=OAc, R2=CO2Me)
136 (R1=CO2Me, R2=OAc)
1. Ac2O
2. CF3CO2H
3. CH2N2
(64%)
xylenes
refl.
(78%)
137
138
1. H2, Pd
2. LiAlH4
(74%)
1. LiN(TMS)2;
AcCl
2. H2, Pd
(79%)
139
140
141
1. TMSI
2.
(77%)
Hg(OAc)2;
NaBH4
(45%)
142

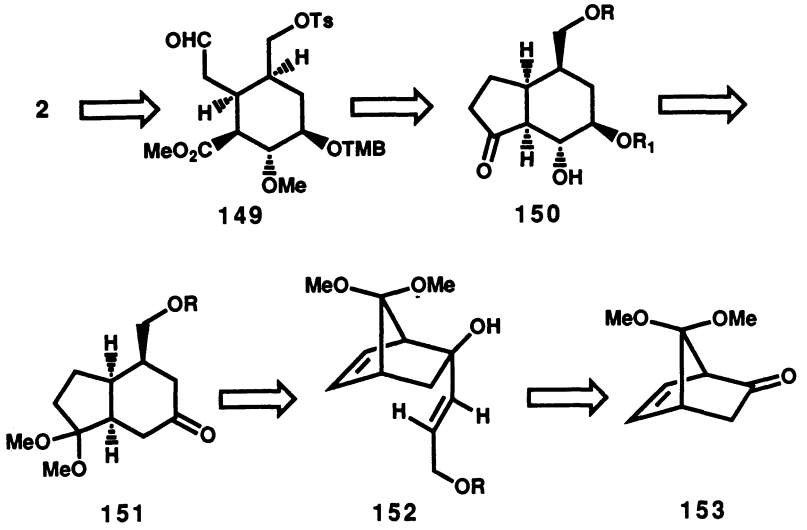
SCHEME 3.21



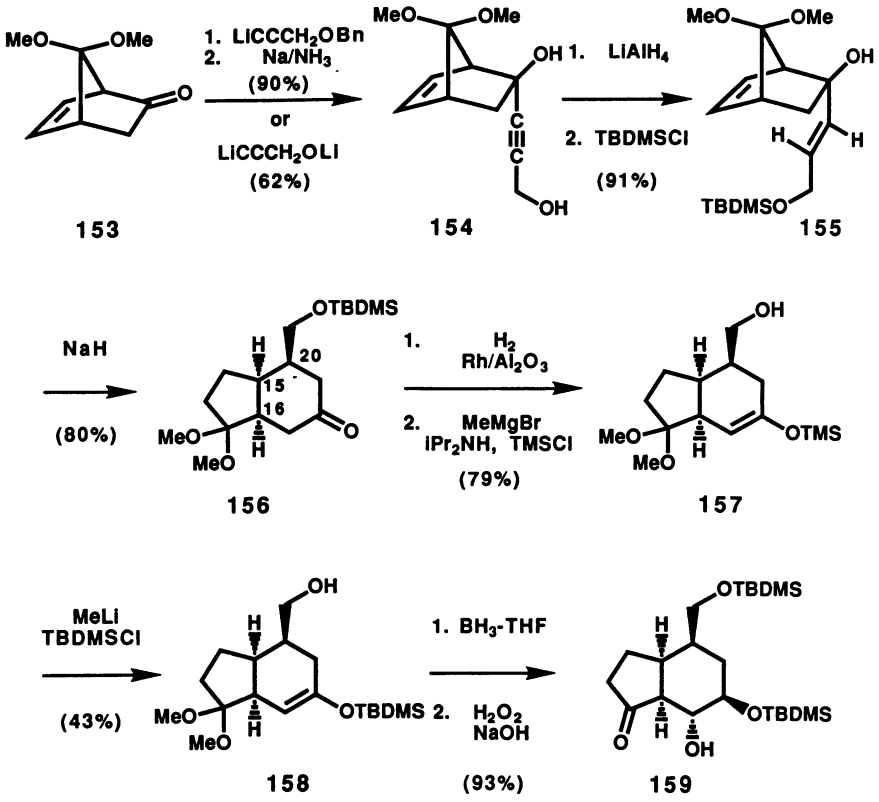
SCHEME 3.21 (*cont.*)



SCHEME 3.22



SCHEME 3.23



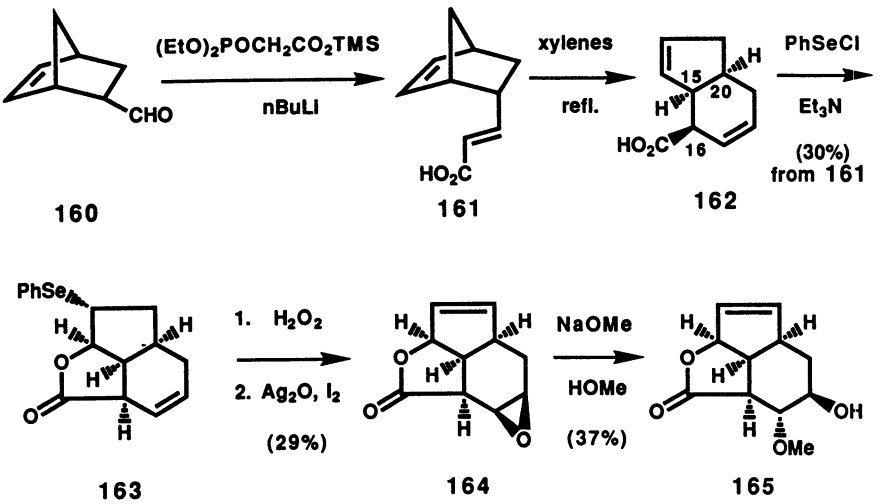
SCHEME 3.24

156 which has three of the reserpine stereocenters, C(15), C(16), and C(20), in place. After hydrogenation of the double bond the next issue addressed in the synthesis was introduction of the C-17 hydroxyl group. The trimethylsilylenol ether for this purpose was formed regioselectively under thermodynamically controlled conditions. Attempts to introduce the C(17) hydroxyl by hydroboration of **157**, however, proceeded in low yield. Consequently, **157** was transilylated to form the more durable TBDMS-enol ether **158**. In contrast, hydroboration of this substance afforded in excellent yield the alcohol **159** in which the C(17)-hydroxyl was introduced stereoselectively from the less hindered, convex face of the molecule. Surprisingly, under the basic conditions of the hydroboration, the ketal was converted to the ketone. Presumably, hydrolysis was assisted by the C(17)-boron atom in the borane intermediate. Although no further chemistry has been reported in this series, this work nicely illustrates the utility of the oxy-Cope rearrangement in stereoselectively generating a reserpine E-ring precursor.

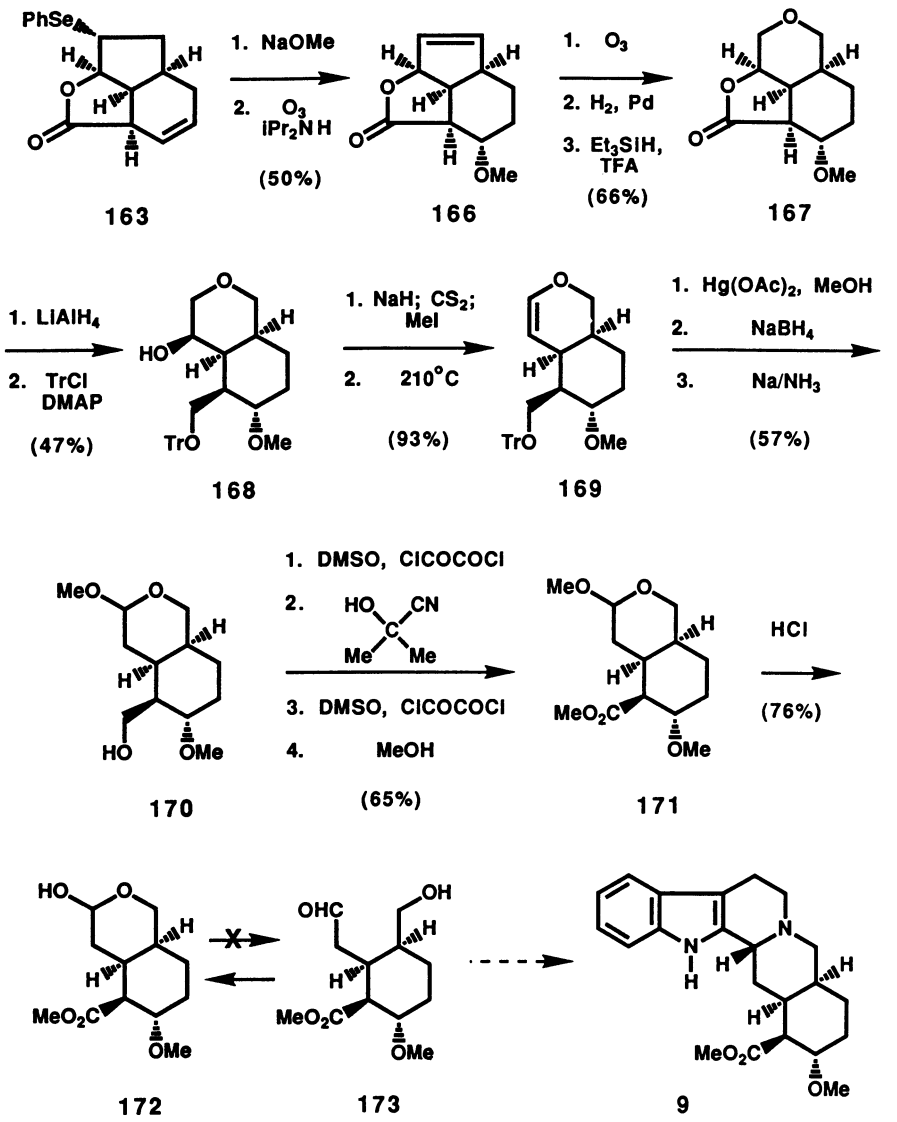
3.2.2.2. Cope Rearrangements

The Cope rearrangement (95, 96, 98) has also been utilized as a key design element in yohimbine alkaloid synthesis. In an approach analogous to that used by Jung and Light (38), Polniaszek and Stevens have employed a Cope rearrangement of a norbornene derivative to prepare a DE-ring precursor of yohimbine and reserpine (39). Norbornene **160**, the Diels-Alder adduct from cyclopentadiene and acrolein, was converted to the acrylic acid derivative **161** (Scheme 3.25). Heating **161** in refluxing xylenes effected Cope rearrangement to produce the hydrindane **162** which has the C(15), C(16), and C(20) stereocenters of the reserpine DE-rings in place. Selenolactonization of **162** to afford the tricyclic intermediate **163** was followed by selenoxide elimination and regioselective epoxidation to give the β-epoxide **164**. Treatment of **164** with methoxide effected regioselective ring opening to yield **165** which contains the five contiguous chiral centers of reserpine with correct relative stereochemistry. No further work elaborating this intermediate to reserpine was reported.

The tricyclic lactone, **163**, was also transformed to a yohimbine precursor by Polniaszek and Stevens (Scheme 3.26). Treatment of **163** with methoxide induced a double bond migration which was followed by Michael addition of methoxide. Subsequent oxidation and thermolysis effected selenoxide elimination to provide **166**. Ozonolysis of the double bond followed by reduction yielded a mixture of bis-hemiacetals which were reduced with triethylsilane in trifluoroacetic acid to provide the tricyclic pyran **167**. Reduction of the lactone and tritylation of the primary alcohol function afforded **168** which was subjected to xanthate ester elimination to give the enol ether **169**. Oxymercuration-reduction and detritylation afforded the methylpyranoside



SCHEME 3.25



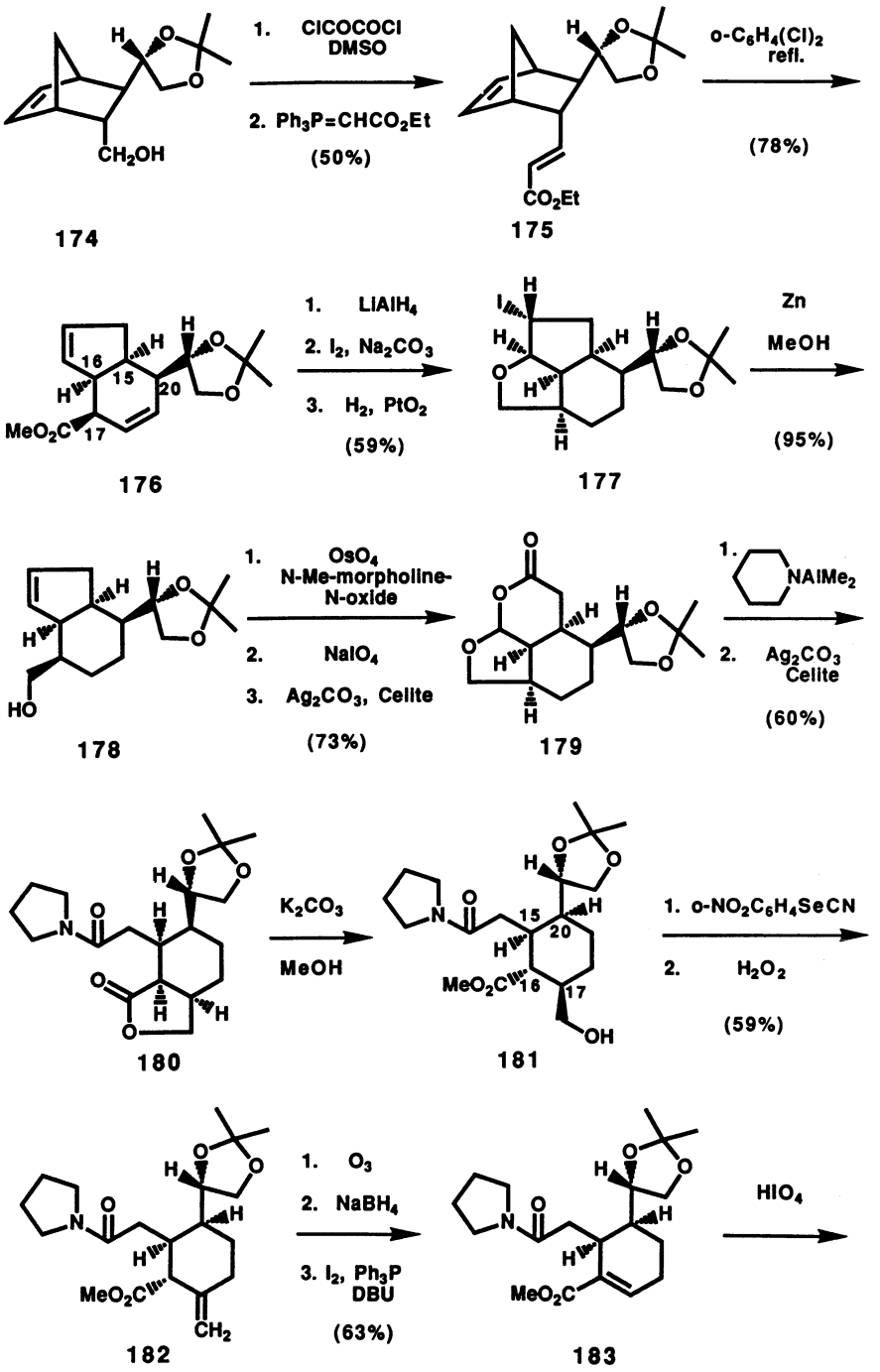
SCHEME 3.26

170. The primary alcohol was converted to methyl ester **171** which was hydrolyzed to hemiacetal **172**, the ring closed form of aldehydo-alcohol **173** and a precursor of 3-epialloyohimbine (**9**). While the syntheses of the target molecules were not completed, the work of Polniaszek and Stevens illustrates how Cope rearrangements can be used effectively to generate cis-fused DE-ring precursors of the yohimbine alkaloids.

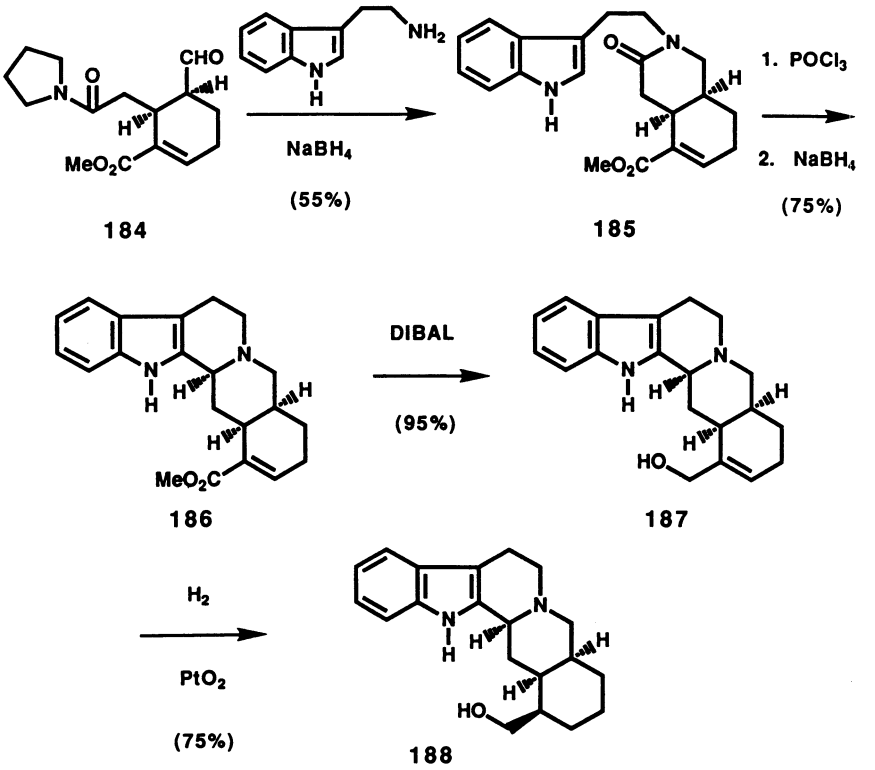
Another application of the Cope rearrangement methodology to the synthesis of members of the yohimbine family is illustrated in the investigations of Takano and his coworkers concerning the chiral synthesis of nitraraine (**187**) and dihydronitraraine (**188**) (Scheme 3.27) (40). In a fashion similar to the Jung (38) and Stevens (39) works, Takano's synthetic route began with a norbornene derivative, **174**. This enantiomerically pure, chiral substance was oxidized to generate the corresponding aldehyde which underwent a Wittig reaction to afford the Cope rearrangement substrate **175**. Heating **175** in refluxing *o*-dichlorobenzene led to the desired rearrangement which efficiently yielded the indene **176** in which the C(15), C(16), and C(20) stereocenters of the targets are in place. This intermediate was then elaborated through a lengthy sequence o the desired targets. Accordingly, reduction of the ester function in **176** followed by iodoetherification and hydrogenation permitted selective reduction of the C(18)–C(19) double bond to yield **177** which was subjected to a dehalogenation-elimination sequence to afford the indene **178**. Hydroxylation of the double bond in **178** followed by periodate cleavage and oxidation of the resulting lactol gave the tricyclic lactone **179**. Amidation and oxidation to the lactone **180** and subsequent methanolysis provided methyl ester **181**. The next issue addressed in the synthesis was the removal of the undesired C(17)-hydroxymethyl group. This aim was achieved by sequential conversion to the selenide, oxidation, and selenoxide elimination to give the exocyclic olefin **182**. Then, ozonolysis of the double bond followed by reduction to the alcohol and its conversion to the corresponding iodide followed by elimination completed the lengthy sequence required for removal of the hydroxymethyl group. Hydrolysis of the isopropylidene group in the resultant α,β-unsaturated ester **183** and periodate cleavage of the formed vicinal diol afforded aldehyde **184**. Condensation with tryptamine and subsequent sodium borohydride reduction afforded the tetracyclic lactam **185** which was cyclized to produce the pentacyclic intermediate **186**. Reduction of the methyl ester function in **186** afforded nitraraine (**187**) which was hydrogenated to yield its dihydro-derivative **188**. The relevance of these syntheses is confounded by the fact that the spectroscopic and physical properties of the synthetic materials were not found to be identical to those of the natural products. In spite of this problem, Takano's efforts illustrate how sigmatropic rearrangements can be utilized to generate cis-fused indene derivatives which possess functionality that can be elaborated stereoselectively to that found in yohimbine targets.

3.2.2.3. Zwitterionic Amino-Claisen Rearrangements

The zwitterionic amino-Claisen rearrangement (41–44), a variant of the aza-Cope reaction (103–105), is another sigmatropic process which has been successfully employed in the synthesis of yohimbines. Mariano and his coworkers have presented designs for the synthesis of reserpine (**2**) and/or deserpidine (**3**) in which the target was envisioned as arising from β-enaminoester **189** by


1. ClCOCOCl DMSO
2. $Ph_3P=CHCO_2Et$ (50%)
$o\text{-}C_6H_4(Cl)_2$ refl. (78%)
CH_2OH
CO_2Et
174
175
1. $LiAlH_4$
2. I_2, Na_2CO_3
3. H_2, PtO_2 (59%)
Zn MeOH (95%)
MeO_2C
176
177
1. OsO_4 N-Me-morpholine-N-oxide
2. $NaIO_4$
3. Ag_2CO_3, Celite (73%)
1. NAlMe2
2. Ag_2CO_3 Celite (60%)
HO
178
179
K_2CO_3 MeOH
1. $o\text{-}NO_2C_6H_4SeCN$
2. H_2O_2 (59%)
180
181
1. O_3
2. $NaBH_4$
3. I_2, Ph_3P DBU (63%)
HIO_4
CH_2
182
183

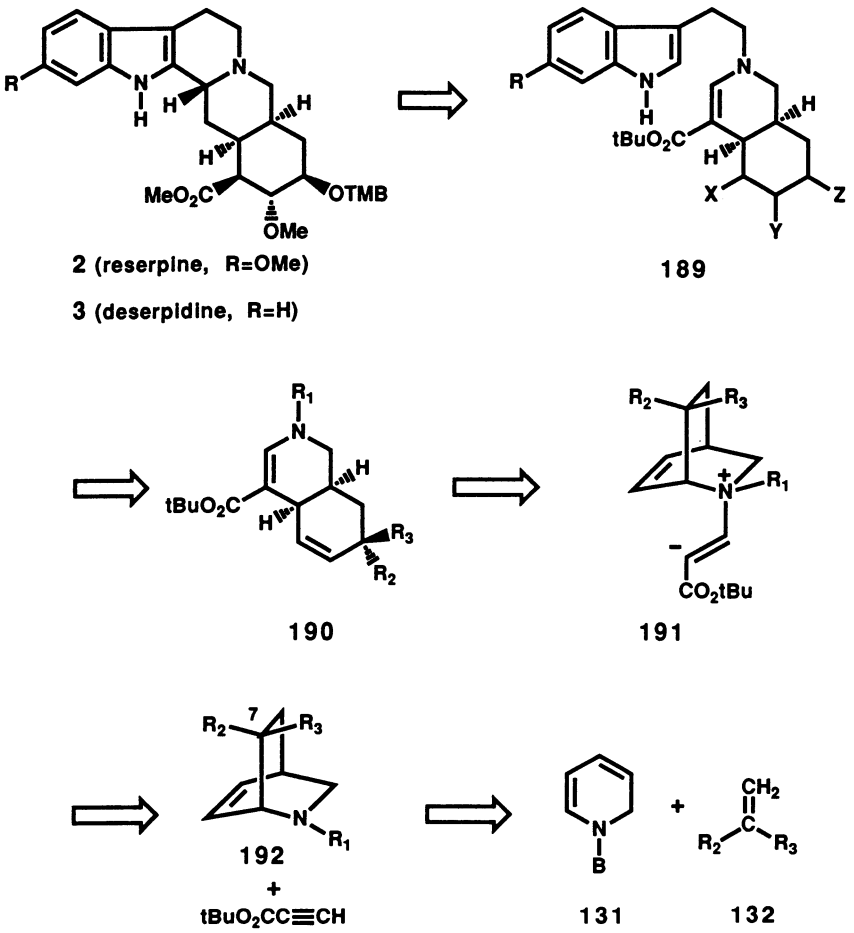
SCHEME 3.27



SCHEME 3.27 (*cont.*)

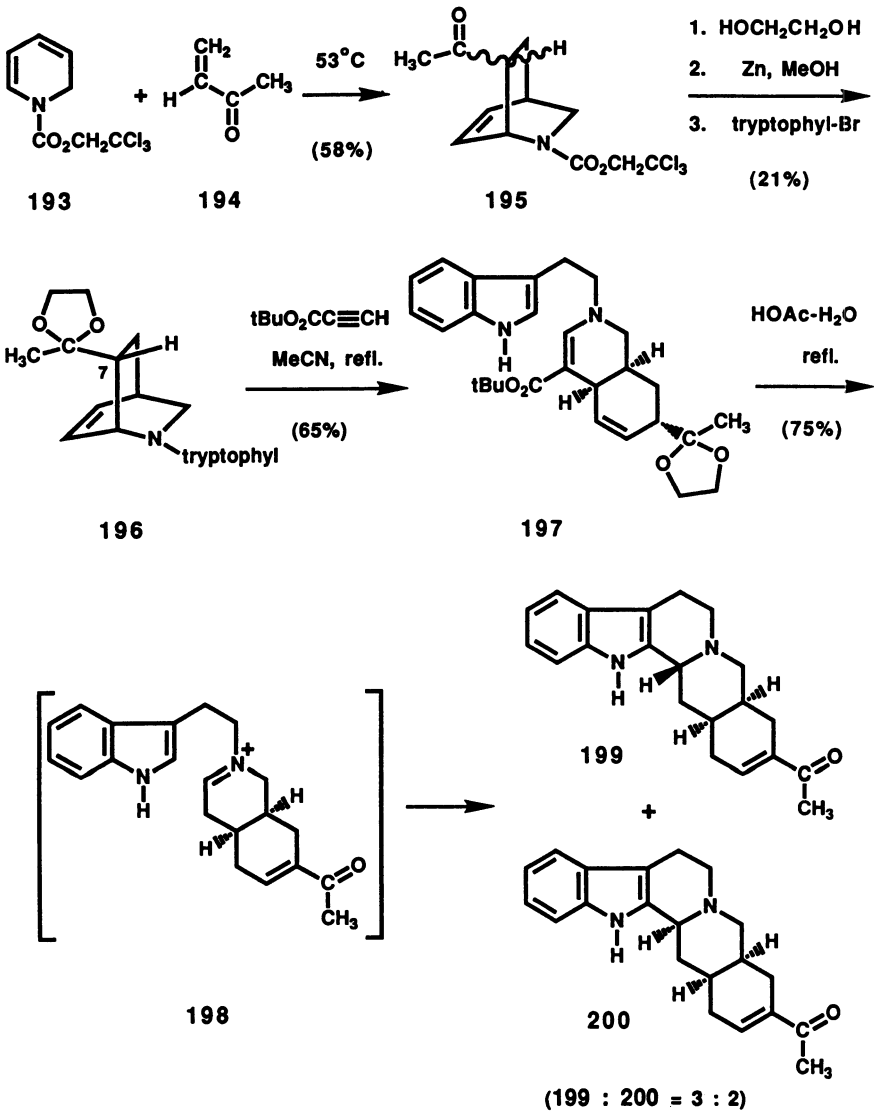
acid-mediated cyclization using conditions developed earlier by Wenkert (106, 107) (Scheme 3.28). The *N*-tryptophyl-hydroisoquinoline **189**, in turn, could be prepared from **190**, an intermediate that could potentially be generated by amino-Claisen rearrangement of the zwitterion **191** resulting from condensation of isoquinuclidene **192** with *t*-butyl propiolate. This key step is predicted to produce a cis-fused hexahydroisoquinoline. As in Wender's approach, the requisite isoquinuclidene could arise by Diels Alder reaction of an appropriate dihydropyridine **131**—dienophile **132** pair.

Model studies have demonstrated that the approach of Mariano and his coworkers does indeed permit rapid construction of the pentacyclic yohimbine skeleton (Scheme 3.29) (41, 42). For example, Diels-Alder reaction of dihydropyridine **193** with methyl vinyl ketone (**194**) afforded isoquinuclidene **195** which was sequentially ketalized, the nitrogen deprotected, and tryptophylated to yield the model substrate **196**. Treatment of **196** with t-butyl propiolate effected the crucial zwitterionic amino-Claisen rearrangement to efficiently provide the hexahydroisoquinoline **197** having the crucial cis-DE-ring fusion in place. Treatment of **197** under the Wenkert cyclization condi-



SCHEME 3.28

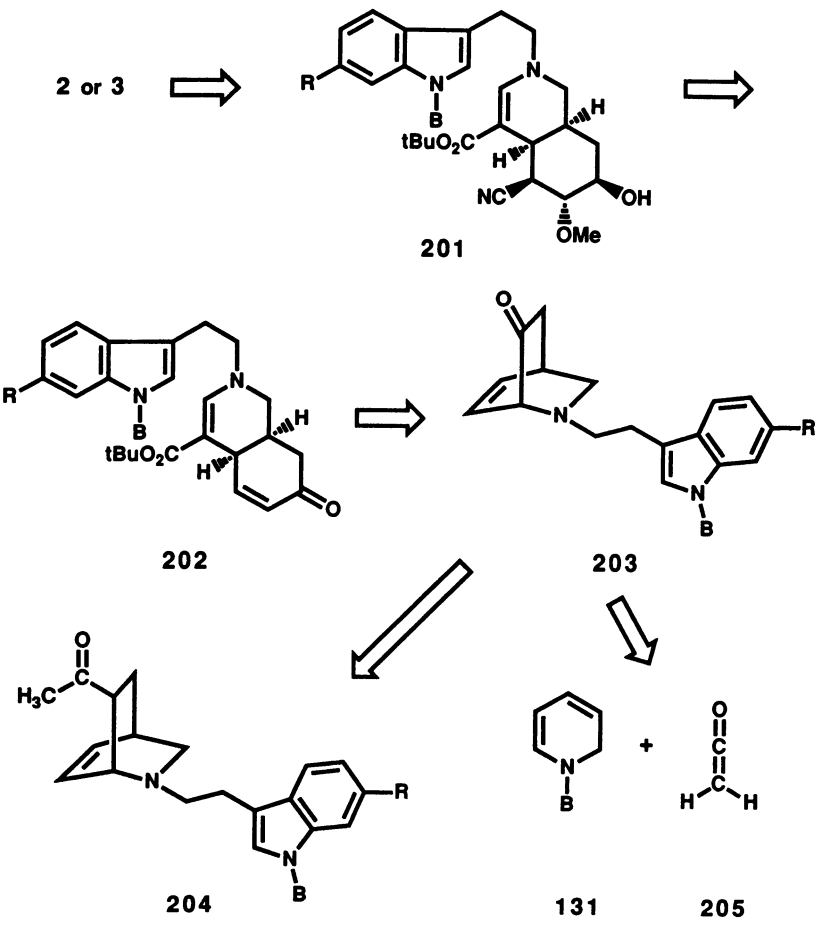
tions (aqueous acetic acid, reflux) resulted in hydrolytic decarboxylation of the *t*-butyl *β*-enaminoester function and yielded the C(2)–C(3) iminium cation **198** which was then captured by intramolecular nucleophilic attack by the indole ring. Concomitant deketalization and olefin migration led to formation of the observed pentacyclic yohimbanes, **199** and **200**, in a 3 : 2 ratio. An intriguing feature of this cyclization process was its lack of stereoselectivity; a slight preference exists for epimer **199** in which the H(3)-proton has the *β* (reserpine-like) stereochemistry. A possible explanation for this observation is that, unlike the iminium ion intermediate in the $Hg(OAc)_2$ oxidation of Martin (33, 34) (see above) which has a highly functionalized E-ring, the iminium species **198** derived from **197** lacks E-ring stereodirecting functionally. As a result, the two transition states for iminium ion cyclization having



SCHEME 3.29

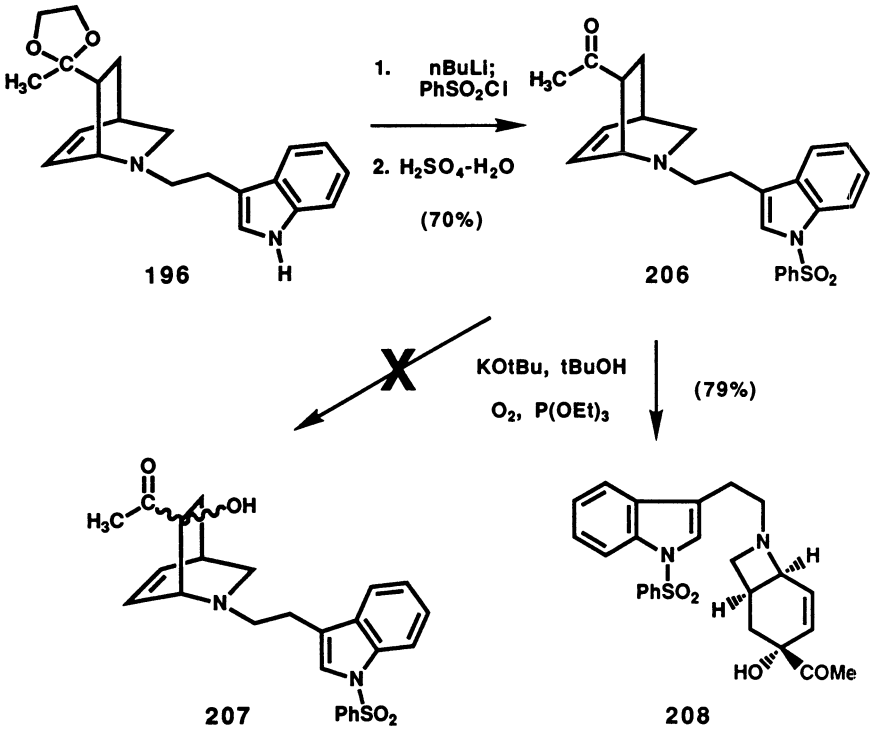
the stereoelectronically preferred axial approach of the indole moiety are of near equal energy.

Clearly, this investigation demonstrated that the combined zwitterionic amino-Claisen rearrangement Wenkert cyclization methodologies constitute a rapid procedure for generating functionalized yohimbines. Moreover, the strategy is flexible and allows for the introduction of appropriate E-ring



SCHEME 3.30

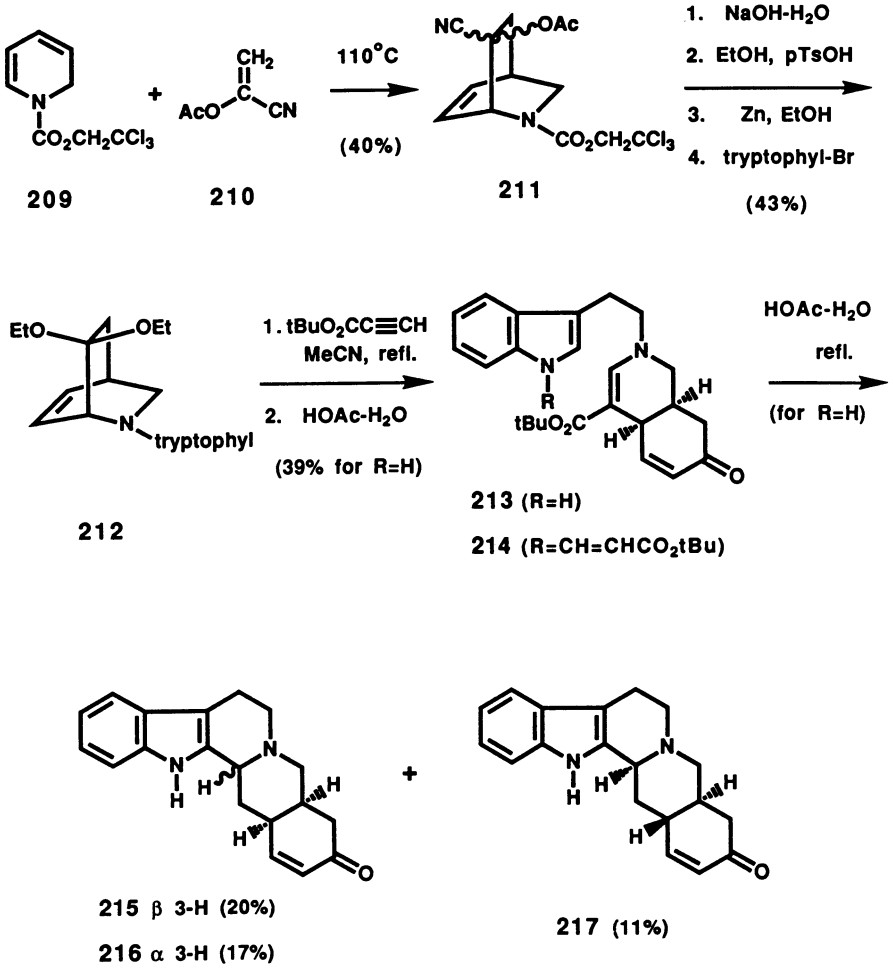
functionality found in interesting yohimbine targets. An example of this is found in the route developed by Mariano and his coworkers for synthesis of deserpidine (**3**) (44). The plan for this sequence focused on the enone **202** as a potentially viable intermediate for the elaboration of E-ring functionality of the target by 1,4-silylcyanation to afford an intermediate β-cyanosilyl enol ether followed by hydroboration-oxidation and methylation to give the properly functionalized *N*-tryptophylisoquinuclidene **201**. It was envisaged that the key enone **202** in this route could arise by zwitterionic amino-Claisen rearrangement of a masked derivative of 7-keto isoquinuclidene **203** which, in principle, could be prepared from **204** by an oxygenation-ketone reduction-periodate cleavage sequence developed earlier by Buchi (108). Alternatively, isoquinuclidene **203** could be generated by Diels-Alder reaction of the dihydropyridine **131** with the ketene equivalent **205**.



SCHEME 3.31

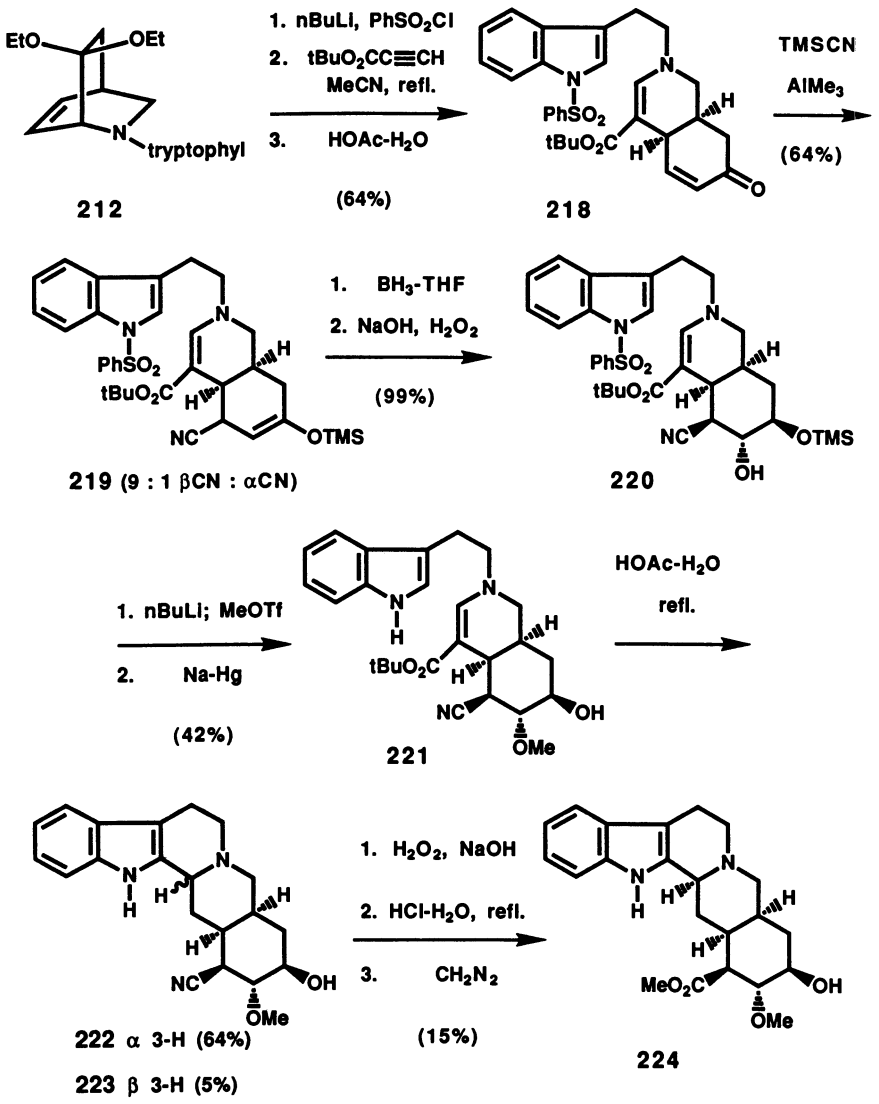
In practice, isoquinuclidene **196** was prepared, protected as the *N*-benzenesulfonyl derivative and hydrolyzed to give the Buchi-oxygenation substrate **206** (Scheme 3.31) (43). Interestingly, oxygenation of **206** under Buchi's conditions did not yield the desired hydroxy ketone **207** but rather gave a substance tentatively characterized as the bicyclic azetidine **208**. Presumably, under the basic reaction conditions, isoquinuclidene **206** forms a dienolate anion by a pathway involving nitrogen elimination and readdition to the intermediate dienone.

A successful method for preparation of an isoquinuclidene related to **203** based on Diels-Alder reaction between dihydropyridine **131** and a ketene equivalent was developed. This approach involving preparation and zwitterionic amino-Claisen rearrangement of the isoquinuclidene **203** to form the enone **202** followed by subsequent E-ring elaboration and Wenkert cyclization ultimately culminated in a formal synthesis of deserpidine (**3**) (Scheme 3.32) (44). Mariano and his coworkers found that Diels-Alder reaction of the *N*-trichloroethoxycarbonyl-1,2-dihydropyridine (**209**) and the masked ketene equivalent, **210**, reacted to afford the isoquinuclidene **211** as an epimeric mixture of 7-cyano-7- acetoxy isomers. The α-cyanoacetate functionality in



SCHEME 3.32

211 was hydrolyzed to give the corresponding ketone which was protected as the diethylketal. Removal of the trichloroethoxy carbonyl group and subsequent tryptophylation afforded the zwitterionic amino-Claisen rearrangement substrate **212**. When this material was treated with *t*-butyl propiolate followed by allylic ketal hydrolysis, the desired enone **213** was formed in modest yield along with the indole *N*-acrylate **214**. Wenkert cyclization of **213** gave both the desired 3β-H epimer **215** and the 3α-H analog **216** in near equal amounts. In addition, a significant amount of the H(15), H(20) *trans* isomer **217** was also produced under these conditions. The poor stereoselectivity in the generation of the H(3)-stereocenters in the products of this reactions is most probably due to a lack of a strong conformational bias in the transition



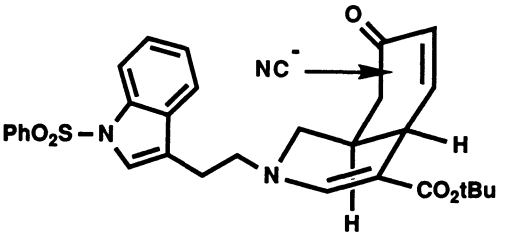
SCHEME 3.33

state for cyclization of the intermediate iminium ion. Furthermore, the trans-DE-ring fusion in **217** is likely caused by epimerization at the γ-enone center [C(15)] under the acidic conditions.

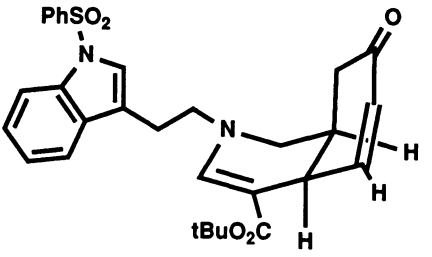
As a result of the low yields for both the zwitterionic amino-Claisen and Wenkert cyclization processes, an alternative route to deserpidine was investigated (Scheme 3.33) (44). The authors reasoned that protection of the indole nitrogen would prevent the competing Michael addition of the indole moiety

to the propiolate ester in the zwitterionic amino-Claisen rearrangement process. Also, *N*-protection would permit functional group manipulation in the enone grouping without concern for competitive reaction of the indole nucleus. With E-ring functionalization complete, the indole would then be deprotected and C-ring closure could be conducted under the Wenkert cyclization conditions. According to this plan, indole **212** was converted to its benzenesulfonyl derivative and then subjected to zwitterionic amino-Claisen rearrangement conditions with subsequent hydrolysis of the allylic ketal. As anticipated, this process afforded the hydroisoquinolin-enone **218** in good yield.

The enone **218** was then transformed to its E-ring functionalized derivative **221** in a highly stereoselective and efficient manner. Key to the success of this design is the fact that enone **218** exists in a single conformation **218A** (Figure 3.1). Presumably **218A** is favored over the alternate conformation **218B** because of an unfavorable $A_{1,2}$ interaction between the *t*-butoxycarbonyl group and H(16) in **218B**. Stereoelectronic (axial) control of cyanide conjugate addition to the enone function in **218** should favor formation of the β-cyano product **219**. Indeed, when **218** was treated with trimethylsilylcyanide under Lewis acid catalyzed conditions, the β-epimer is produced predominantly (9 : 1 $\beta : \alpha$ ratio). Hydroboration of the silylenol ether function in **219** dis-



218A

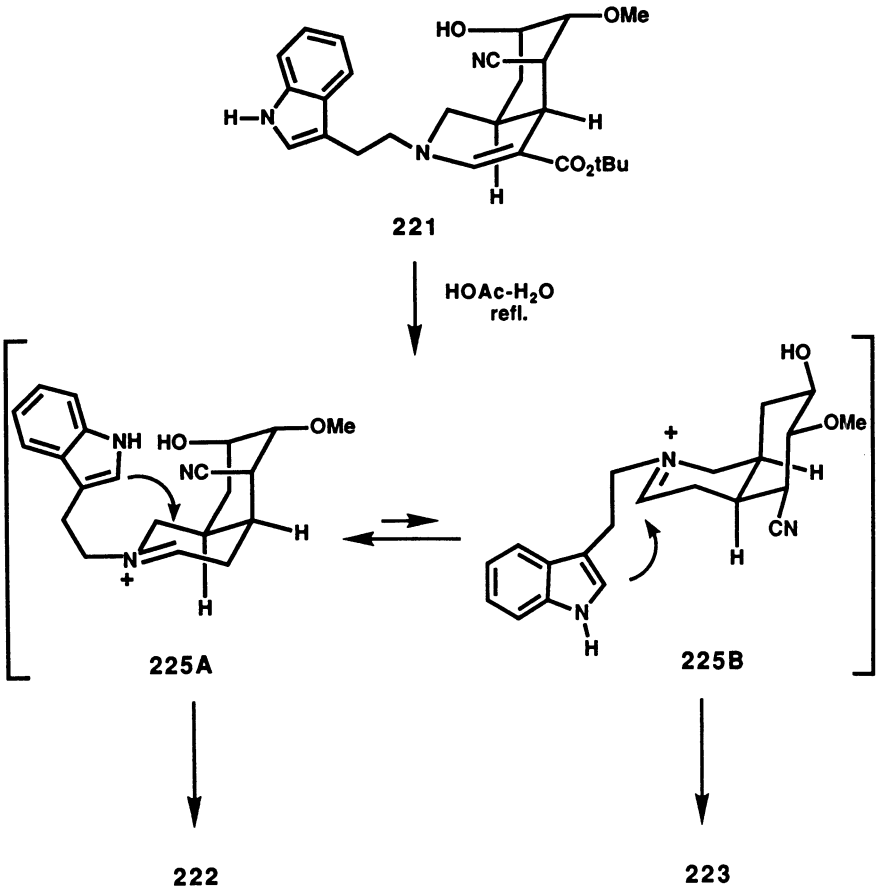


218B

FIGURE 3.1. Conformer preferences for the hydroisoquinolin-enone **218**.

played a convex face preference and yielded differentially protected trans-diol **220**, a substance which has the five contiguous centers of deserpidine E-ring installed. This sequence illustrates how the conformational preference in **218** can be used for the stereoselective introduction of yohimbine functionality.

The remaining steps in this route to deserpidine involved straight forward chemistry. Methylation of the O(17)-hydroxyl group followed by removal of the indole benzenesulfonyl protecting group and concomitant cleavage of the TMS-ether afforded **221**, a substance properly constituted for the key Wenkert cyclization step. When this material was heated in aqueous acetic acid it yielded almost exclusively the yohimbane **222** having the, 3α-H (isodeserpidine-like) stereochemistry along with only a trace amount of the 3β-H epimer (deserpidine-like stereochemistry) **223**. This result is surprising on the basis of kinetic considerations (Scheme 3.34) since the 3β-H (reserpine) epimer is expected to be the major product of cyclization of an intermediate



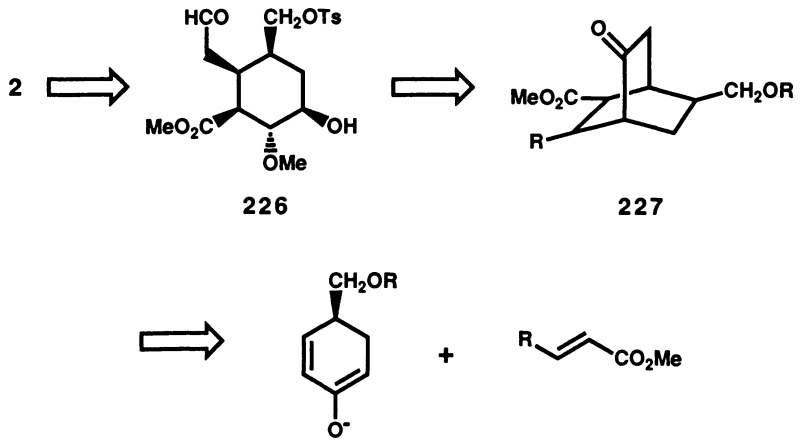
SCHEME 3.34

iminium ion **225**. This intermediate should exist in the all-equatorial conformation **225B**, and indole attack is expected to occur from the axial direction. It was shown that the product ratio does not reflect thermodynamic control since **223** does not epimerize to **222** under the conditions employed for this Wenkert cyclization process. These results point out that the product ratio obtained in these C(2)–C(3) bond forming reactions which presumably occur *via* an iminium cation intermediate are under kinetic control. However, the factors controlling C(3) stereochemistry are poorly understood. Finally, a formal deserpidine synthesis was completed by conversion of **222** to the corresponding methyl ester **224**, a substance which has previously been transformed to deserpidine by Szantay (54).

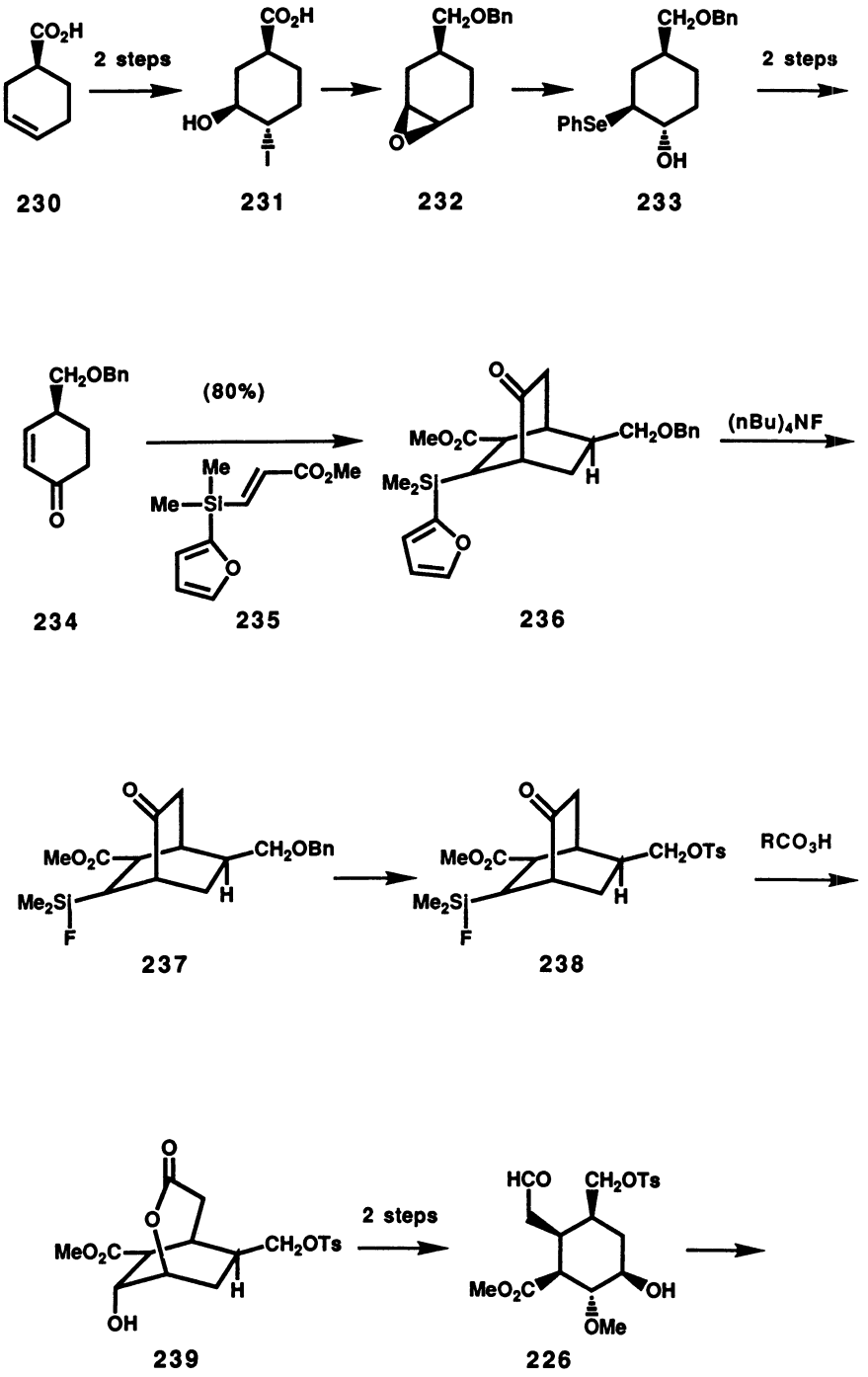
3.2.3. Michael Additions

Another general synthetic approach to the yohimbine alkaloids has involved the use of Michael addition reactions (109, 110). Stork has successfully utilized this chemistry in an elegant, enantiospecific synthesis of optically active reserpine. Stork's design (Scheme 3.35) employed the pentasubstituted cyclohexane **226**, a substance closely analogous to Woodward's intermediate **23** as a key synthon. This intermediate was envisaged as arising by Baeyer-Villager oxidation and subsequent ring opening of a bicyclic ketone precursor **227**. Ketone **227** could be prepared by a double Michael reaction of one enantiomer of an appropriately substituted, cyclohexadienolate anion **228** with a β-substituted acrylate **229**. This strategy resulted in a concise, stereoselective synthesis of reserpine.

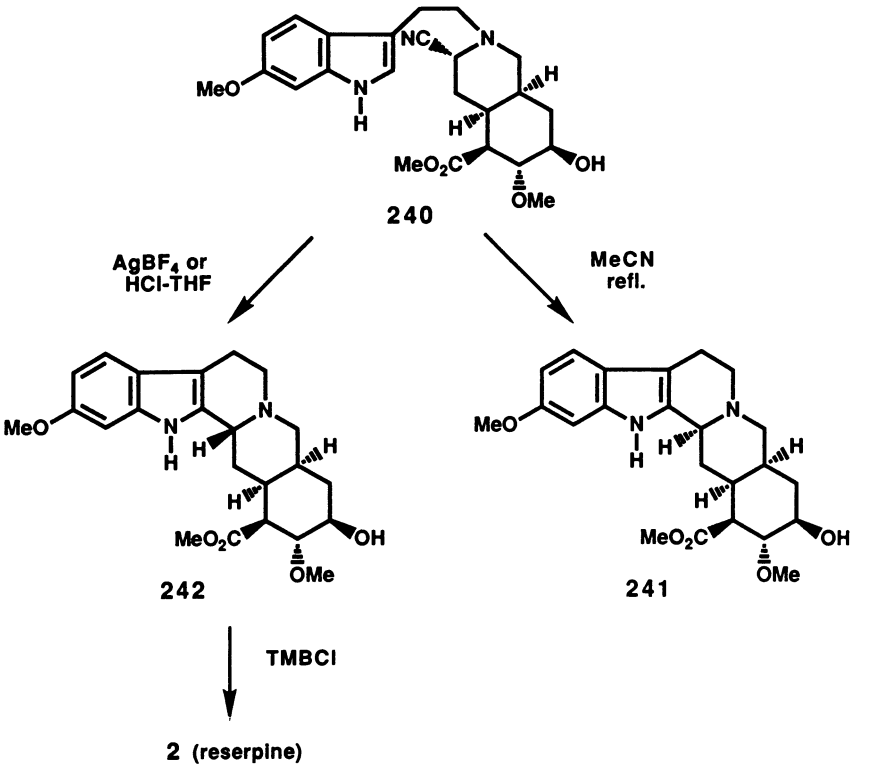
Stork's synthesis (Scheme 3.36) began with the enantiomerically pure cyclohexene carboxylic acid **230** which was converted to the enone **234**, the



SCHEME 3.35


CO2H
2 steps
CO2H
HO
CH2OBn
O
CH2OBn
PhSe
OH
2 steps
230
231
232
233
CH2OBn
O
(80%)
Me
Me—Si
CO2Me
O
MeO2C
Me2Si
O
CH2OBn
H
(nBu)4NF
234
235
236
O
MeO2C
Me2Si
F
CH2OBn
H
O
MeO2C
Me2Si
F
CH2OTs
H
RCO3H
237
238
O
O
MeO2C
CH2OTs
H
OH
2 steps
HCO
CH2OTs
MeO2C
OH
OMe
239
226

SCHEME 3.36



SCHEME 3.36 (*cont.*)

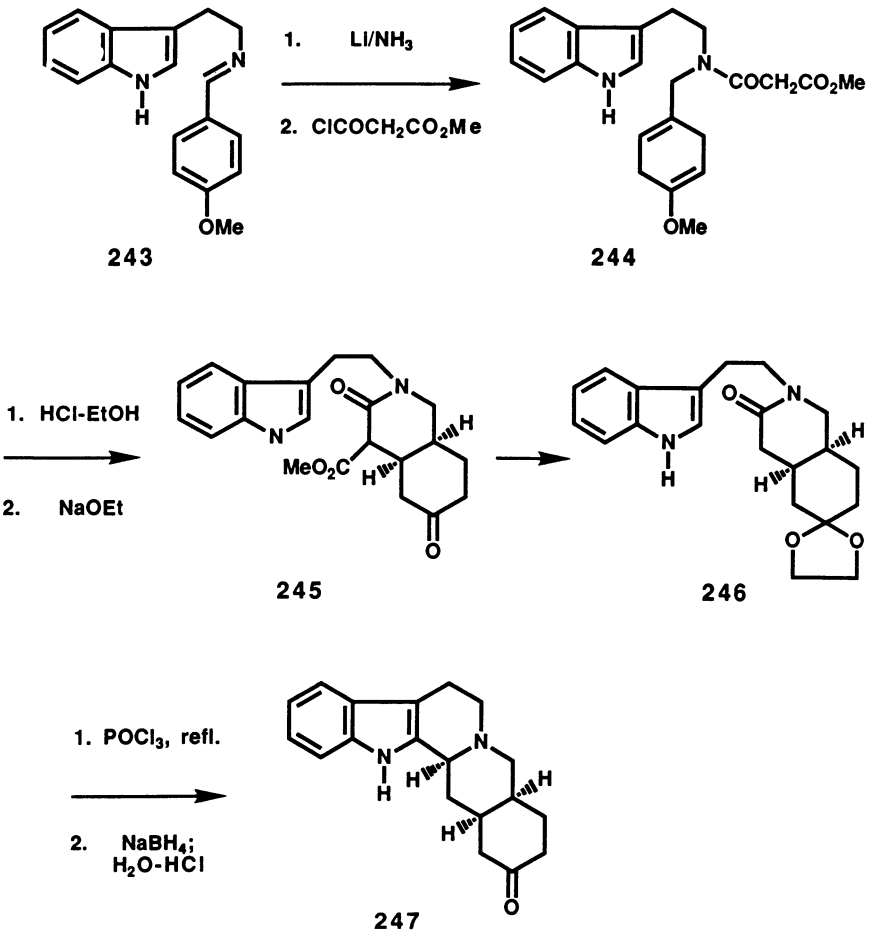
substrate for the crucial double Michael addition step. The sequence for preparation of enone **234** involved transformation of **230** to the iodohydrin **231** which was then converted via epoxide **232** and selenide **233** to **234**. The dienolate generated from **234** was then treated with the β-silyl-acrylate **235** to afford the desired bicyclic ketone **236** in good yield. The silylfuran group of **236** was transformed to the corresponding fluorosilane functionality in **237**. Intermediate **237** was then converted to tosylate **238** which was treated with peracid to effect not only the desired Baeyer Villager oxidation but also oxidative removal of the silicon substituent. Ring opening of the formed bicyclic lactone **239** followed by reduction of the liberated carboxylic acid to the corresponding aldehyde afforded the pentasubstituted cyclohexane **226**, a substance having the five contiguous chiral centers of reserpine in place. Reaction of **226** with 6-methoxytryptamine afforded an intermediate iminium cation which was stereoselectively trapped with cyanide to yield **240**. An extremely interesting aspect of the Stork synthetic route concerns the stereoselectivity of the C-ring forming cyclization of the cyanoamine **240**. When **240** was heated in refluxing acetonitrile, it was converted to the isoreserpine

alcohol **241**. This is an unexpected result since it was anticipated that axial indole attack on the intermediate cyclic iminium cation would result in formation of the corresponding reserpine alcohol **242** with 3β-H stereochemistry. Stork rationalized this result by suggesting that reaction under these conditions proceeds through a tight ion pair in which the axial iminium cation face is blocked by the cyanide leaving group. Thus, the indole nucleophile would have to approach from an equatorial direction to generate the observed isoreserpine stereo-chemistry at C(3). In light of this, the observation that treatment of **240** with either silver tetrafluoroborate or aqueous HCl in tetrahydrofuran gave the reserpine alcohol **242** is instructive. Stork suggested that under these reaction conditions, the iminium-cyanide ion pair is completely dissociated thus allowing the indole ring to attack the iminium species from the axial α-face to yield the reserpine precursor **242**. The observation that the 3β-H (reserpine) stereoisomer **242** formed in cyclization of a "free" iminium salt is rather intriguing in light of the observations made by Mariano (see above) and suggests that the stereoselectivity of the ring closure is subject to very subtle changes in the reaction conditions. Trimethoxybenzoylation of **242** then completed this interesting synthesis of reserpine.

Stork and Livingston have also employed a Michael reaction methodology in a concise synthesis of alloyohimbone **247** (Scheme 3.37). In this sequence, the Schiff base **243**, prepared from tryptamine and 4-methoxybenzaldehyde, underwent sequential Birch reduction and acylation to afford the cyclohexadiene **244**. Hydrolysis provided the corresponding α, β-unsaturated ketone. Under basic conditions, the key Michael addition took place to yield the cis-fused bicyclic lactam **245**. Decarboxylation and ketalization afforded **246** which underwent Bischler-Napieralski cyclization and subsequent deketalization to afford alloyohimbone **247**. All in all, Stork has shown that Michael addition methodologies can be successfully employed to construct the pentacyclic yohimbine alkaloid skeleton in an efficient stereoselective manner.

A conceptually similar approach to alloyohimbone (**247**) was investigated by Godleski and Villhauer (47). These workers utilized a palladium-mediated equivalent to the Michael reaction to construct the D-ring of the target alkaloid (Scheme 3.38). The starting material for this synthesis was cyclohexene **248** which contains the β-acetoxy vinyl sulfide unit as an α,β-unsaturated carbonyl equivalent. Aldehyde **248** was condensed with tryptamine to afford an imine which was reduced. Acylation of the resulting amine gave the α-sulfonyl acetamide **249**. In the key step of this alloyohimbone synthesis, the anion of **249** was treated with palladium to effect the closure on the allylic acetate to yield the cis-fused *N*-tryptophyhydroisoquinoline **250** in which the vinyl sulfide serves as a masked carbonyl group. Reductive removal of the sulfone and subsequent Bischler-Napieralski reaction provided the pentacyclic vinyl sulfide **251** which was hydrolyzed to form alloyohimbone (**247**). Removal of the ketone afforded alloyohimbine (**82**).

Attempts to convert the vinyl sulfide function in **251** to the hydroxy ester

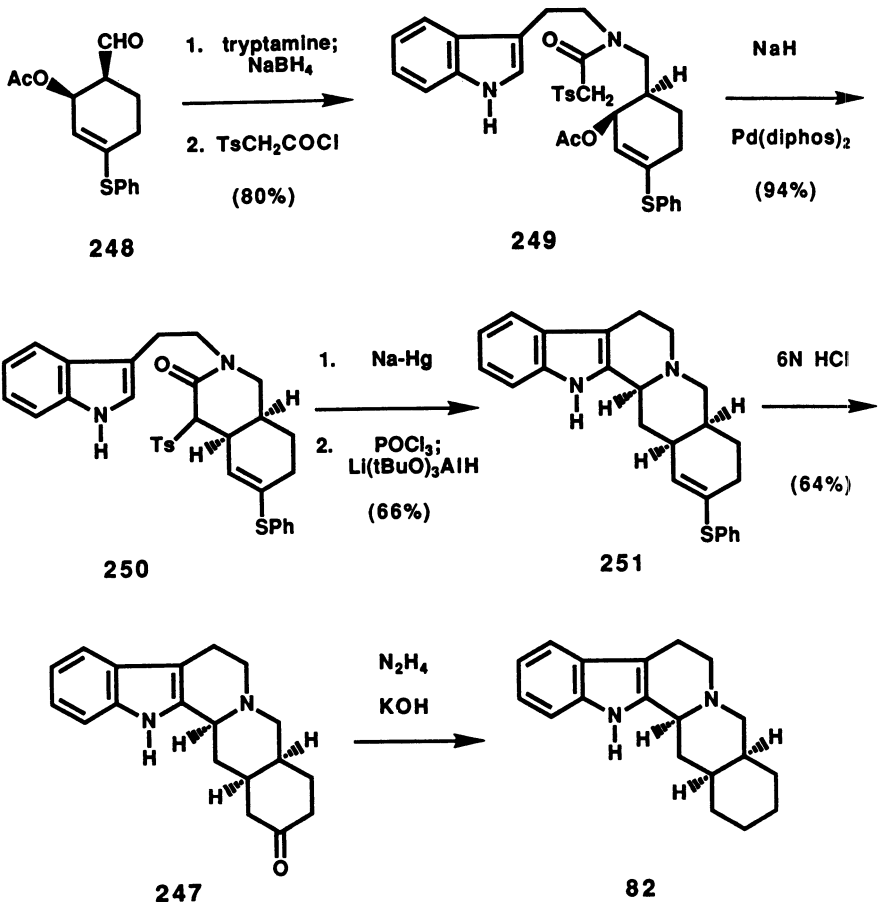


SCHEME 3.37

groups of alloyohimbine (7), however, were unsuccessful (Scheme 3.39). Accordingly, protection of the indole nitrogen followed by allylic cyanation gave **252** which resisted attempts to hydrolytically convert the vinyl sulfide group to the ketone function found in **254**. Despite the difficulty encountered in elaborating E-ring functionality, Godleski's route demonstrates how palladium catalyzed substitutions can be utilized to quickly construct the yohimbine skeleton.

3.3. Alternative Synthetic Approaches to the Yohimbine Alkaloids

In the first part of this survey, approaches to yohimbine alkaloid synthesis which involved initial elaboration of the DE-ring system followed by introduction of the tryptophyl unit and subsequent C-ring closure were addressed.

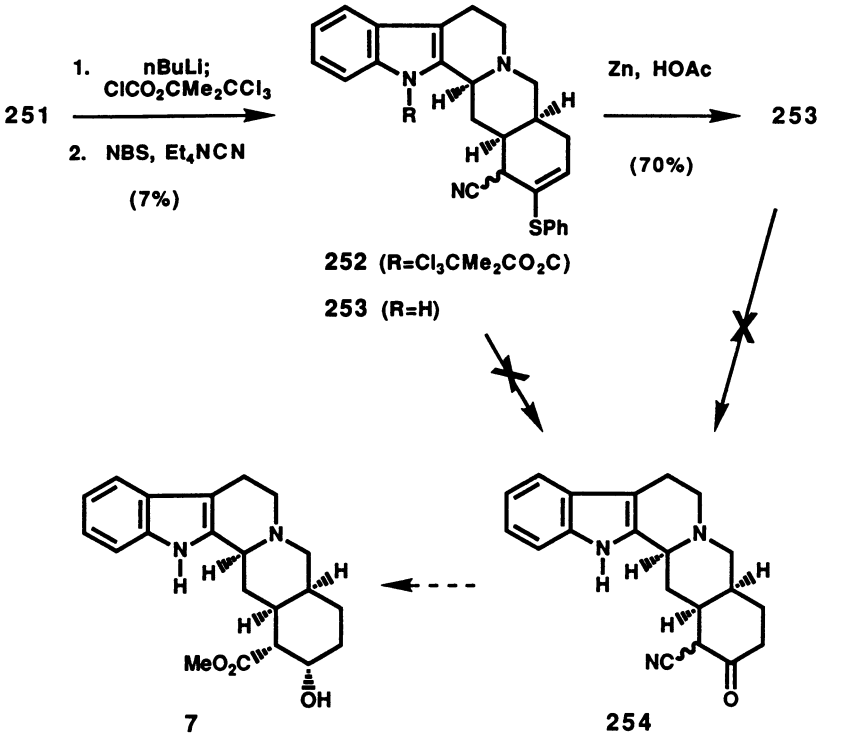


SCHEME 3.38

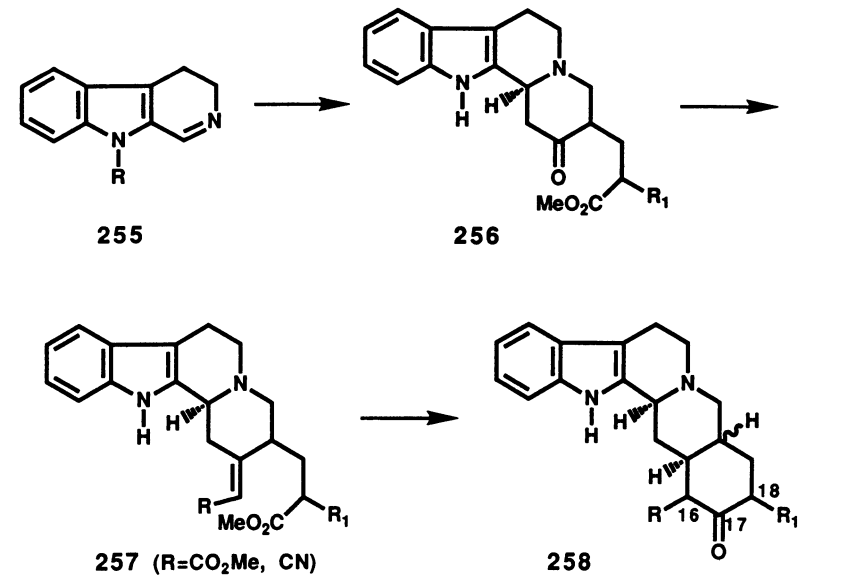
In this section, synthetic strategies which begin with the ABC portion of the yohimbine target and then elaborate the D and E-rings will be addressed. Two synthetic methodologies which have been principally employed in this general approach are Dieckmann cyclizations (3, 11, 48–60) and enamide photocyclizations (9, 13, 14, 61–74). Applications of these methodologies will be addressed first, and then several alternative routes to the yohimbine alkaloids which also employ an ABC-ring precursor as the starting point for the synthesis will be presented.

3.3.1. Dieckmann Cyclizations

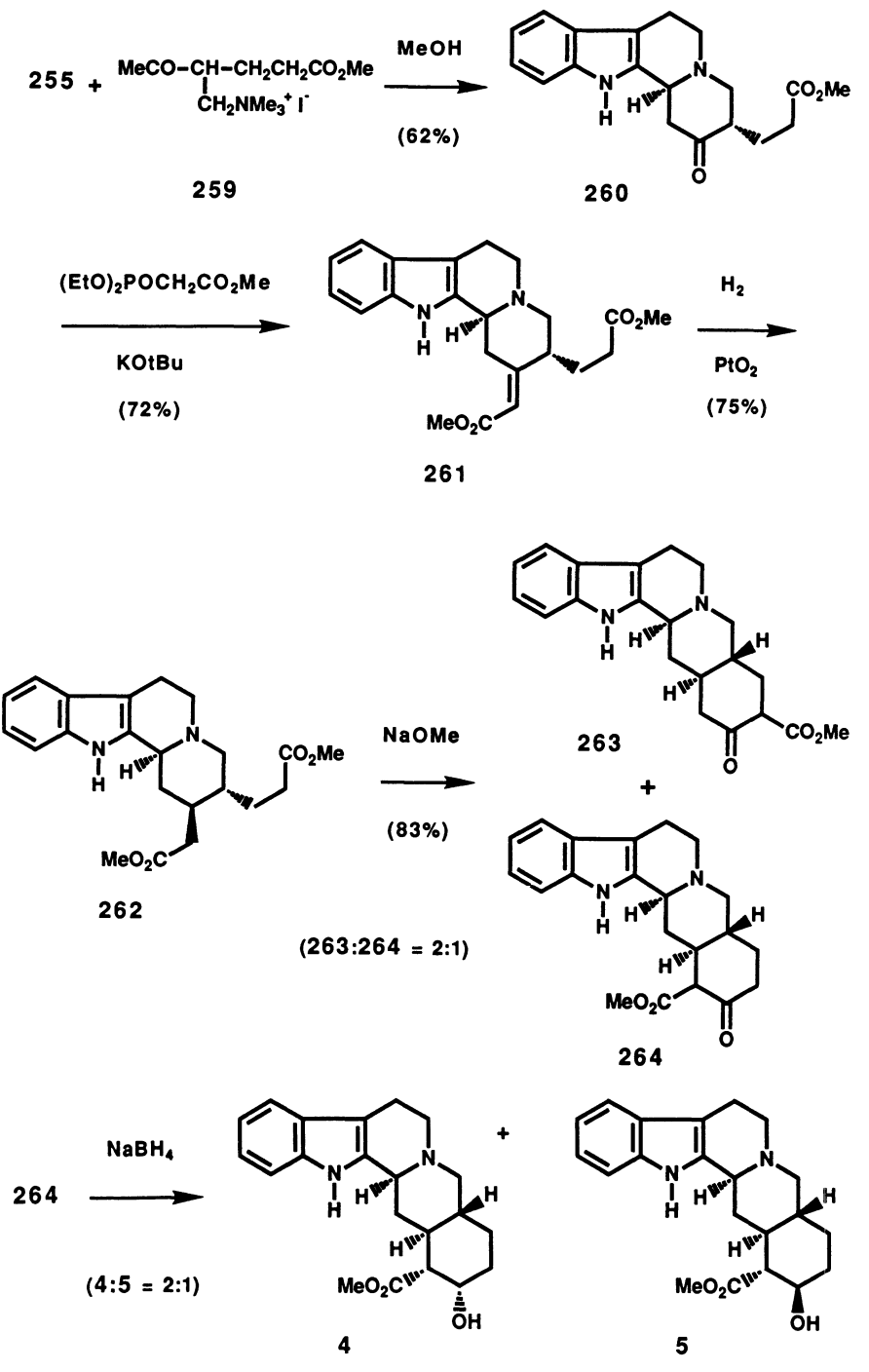
Szantay has utilized Dieckmann cyclization strategies to prepare a variety of representative yohimbines (Scheme 3.40). Typically, 3,4-dihydro-β-carboline **255** was transformed to the tetracyclic ketone **256**. Introduction of a second



SCHEME 3.39



SCHEME 3.40


255 +
MeCO-CH-CH2CH2CO2Me
CH2NMe3+ I-
MeOH
(62%)
259
260
(EtO)2POCH2CO2Me
KOtBu
(72%)
261
H2
PtO2
(75%)
262
NaOMe
(83%)
263
+
264
(263:264 = 2:1)
NaBH4
264
(4:5 = 2:1)
4
5

SCHEME 3.41

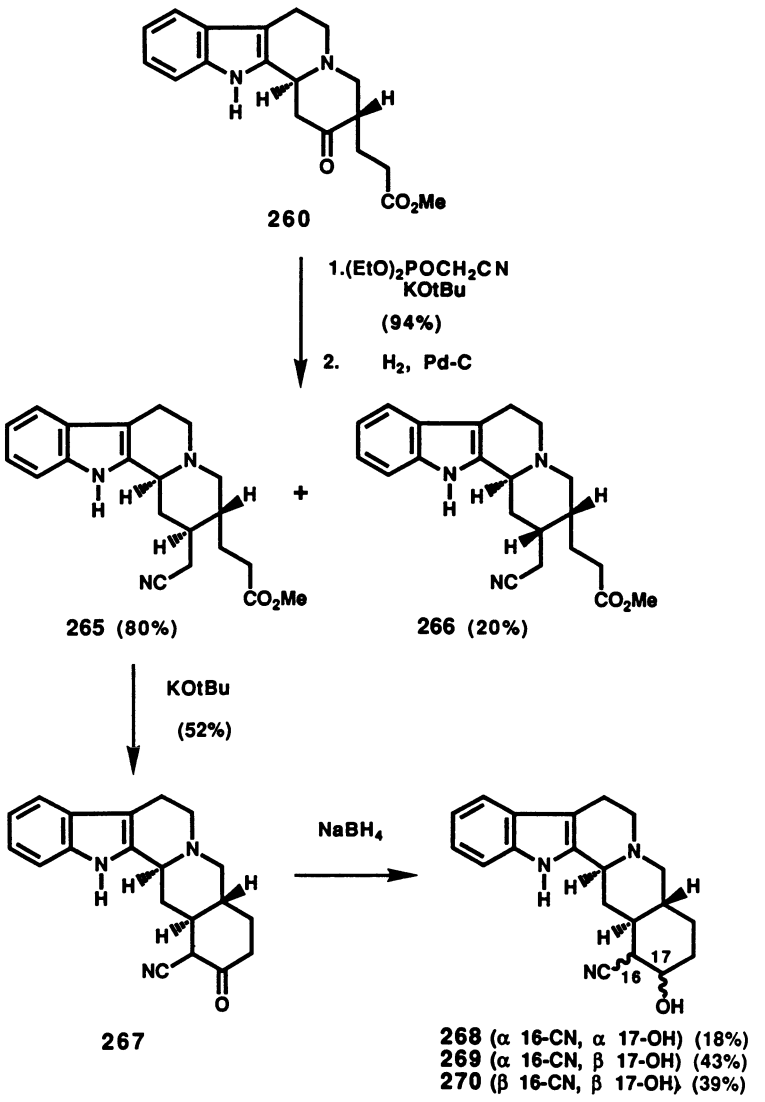
activated methylene unit was achieved by a Wittig olefination-hydrogenation sequence to afford the Dieckmann substrate **257**. Treatment with base effected annulation to produce the pentacyclic product **258** which possesses the yohimbine skeleton. The C(17)-ketone group in **258** serves as appropriate functionality to introduce the yohimbine O(17)-hydroxyl moiety and the R and R′ groups are useful for elaborating the desired C(16) and C(18) functionality of appropriate targets.

Szantay and coworkers initially synthesized yohimbine (**4**) and β-yohimbine (**5**) utilizing this strategy (Scheme 3.41) (48, 49). Condensation of β-carboline **255** with ketoester **259** afforded tetracyclic ketone **260** in good yield. Wadsworth-Emmons olefination followed by hydrogenation afforded the diester **262** which has the requisite yohimbine trans DE-ring fusion stereochemistry in place. Treatment of **262** with sodium methoxide led to formation of the Dieckmann cyclization products **263** and **264** as a 2 : 1 regioisomer mixture. Subsequent reduction of **264** afforded yohimbine (**4**) and β-yohimbine (**5**) in a 2 : 1 ratio. While this synthetic strategy permitted rapid construction of the pentacyclic yohimbine skeleton, the poor regioselectivity of the Dieckmann cyclization step detracts slightly from the usefulness of this approach.

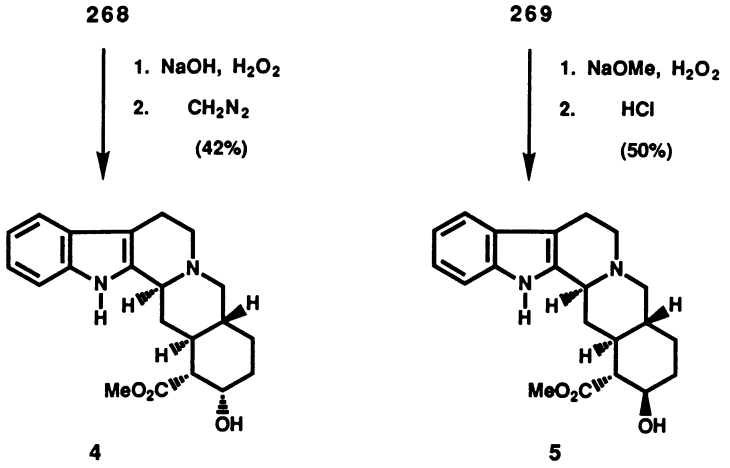
Szantay and coworkers were able to improve the efficiency of the yohimbine synthetic sequence by utilizing the nitrile **265**, derived from the ketone **260** (Scheme 3.42) (48, 49). Dieckmann cyclization of **265** afforded regioselectively the α-cyano ketone **267** which on treatment with sodium borohydride provided the epimeric β-cyano alcohols **268**, **269**, and **270**. Nitriles **268** and **269** were then independently converted to yohimbine (**4**) and β-yohimbine (**5**), respectively.

In subsequent studies, Szantay's group further improved their syntheses of yohimbine (**4**) and β-yohimbine (**5**). In addition, the ketone **260** was resolved, thus effecting a chiral synthesis of these alkaloids (Scheme 3.43) (55). The levorotatory enantiomer of ketone **260** was converted to the α,β-unsaturated ester **(+)-261** which was then cyclized by the Dieckman method to afford **(−)-271**. Treatment of this unsaturated ketone with sodium borohydride in the presence of nickel chloride afforded a 2 : 1 mixture of (−)-β-yohimbine (**(−)-5**) and (+)-yohimbine (**(+)-4**). Furthermore, the (+)-ketone (**(+)-260**) was carried through the aforementioned sequence to yield a mixture of (+)-β-yohimbine (**(+)-5**) and (−)-yohimbine (**(−)-4**).

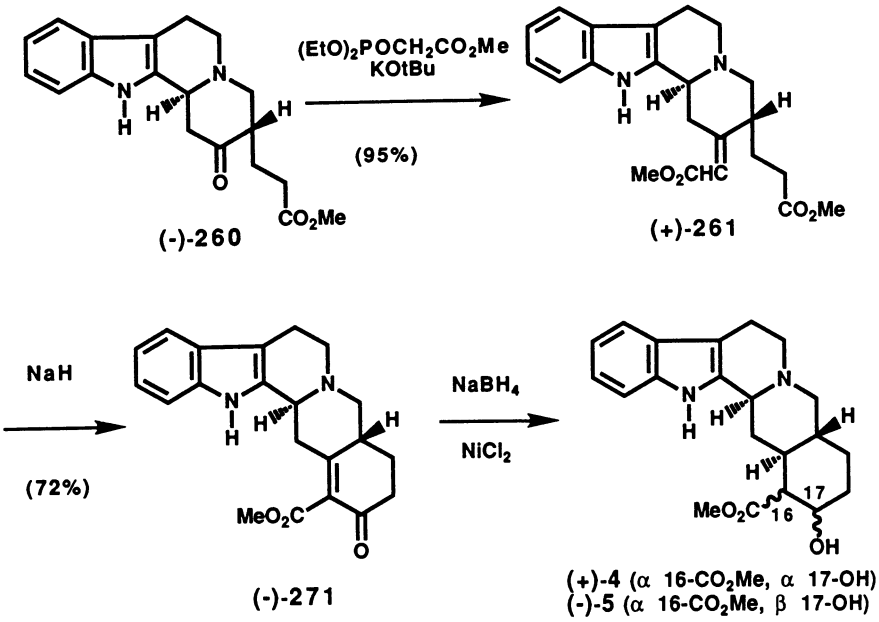
In addition to executing the syntheses of yohimbine (**4**) and β-yohimbine (**5**), Szantay has also developed methods to elaborate the ketone **260** to alloyohimbine (**7**) and α-yohimbine (**8**) by utilization of the Dieckmann strategy (Scheme 3.44) (50). Ketone **260** was condensed with methyl cyanoacetate to afford the α,β-unsaturated ester **272**. Reduction with $NaBH_4$ followed by saponification provided diacid **273** which has the cis-stereochemistry required for the DE-ring fusion in the yohimbine targets. Subsequent decarboxylation of **273** followed by hydrolysis and esterification afforded diester **274** which underwent the key Dieckmann cyclization in modest yield to


260
1.(EtO)2POCH2CN
KOtBu
(94%)
2. H2, Pd-C
265 (80%)
266 (20%)
KOtBu
(52%)
NaBH4
267
268 (α 16-CN, α 17-OH) (18%)
269 (α 16-CN, β 17-OH) (43%)
270 (β 16-CN, β 17-OH) (39%)

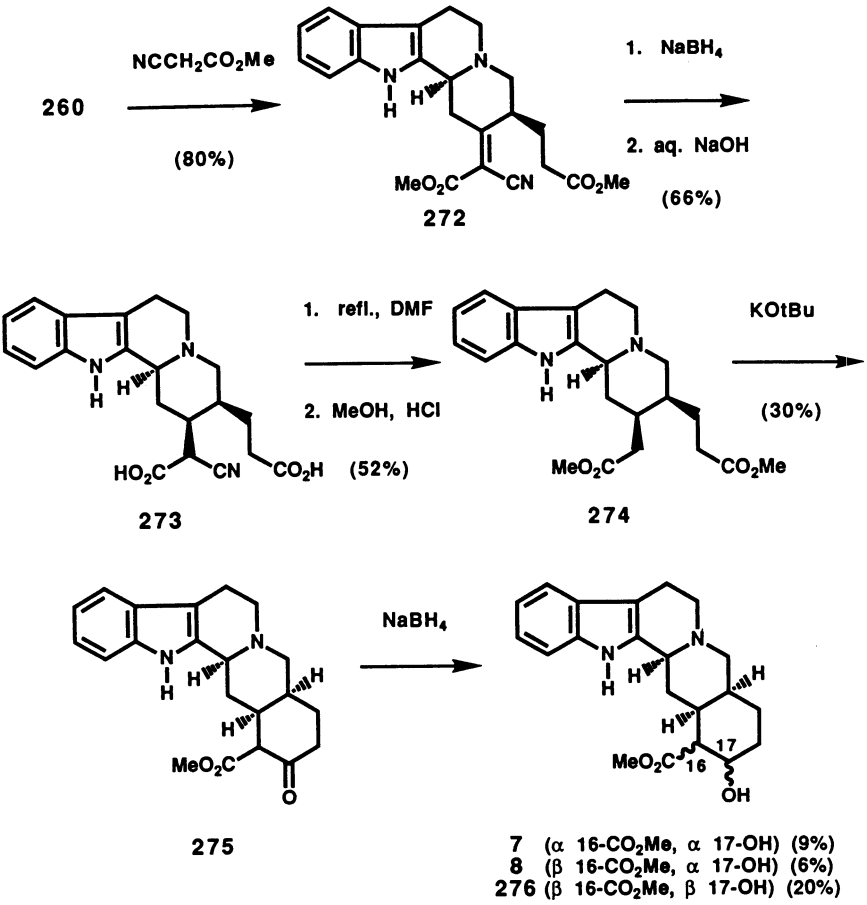
SCHEME 3.42



SCHEME 3.42 (*cont.*)



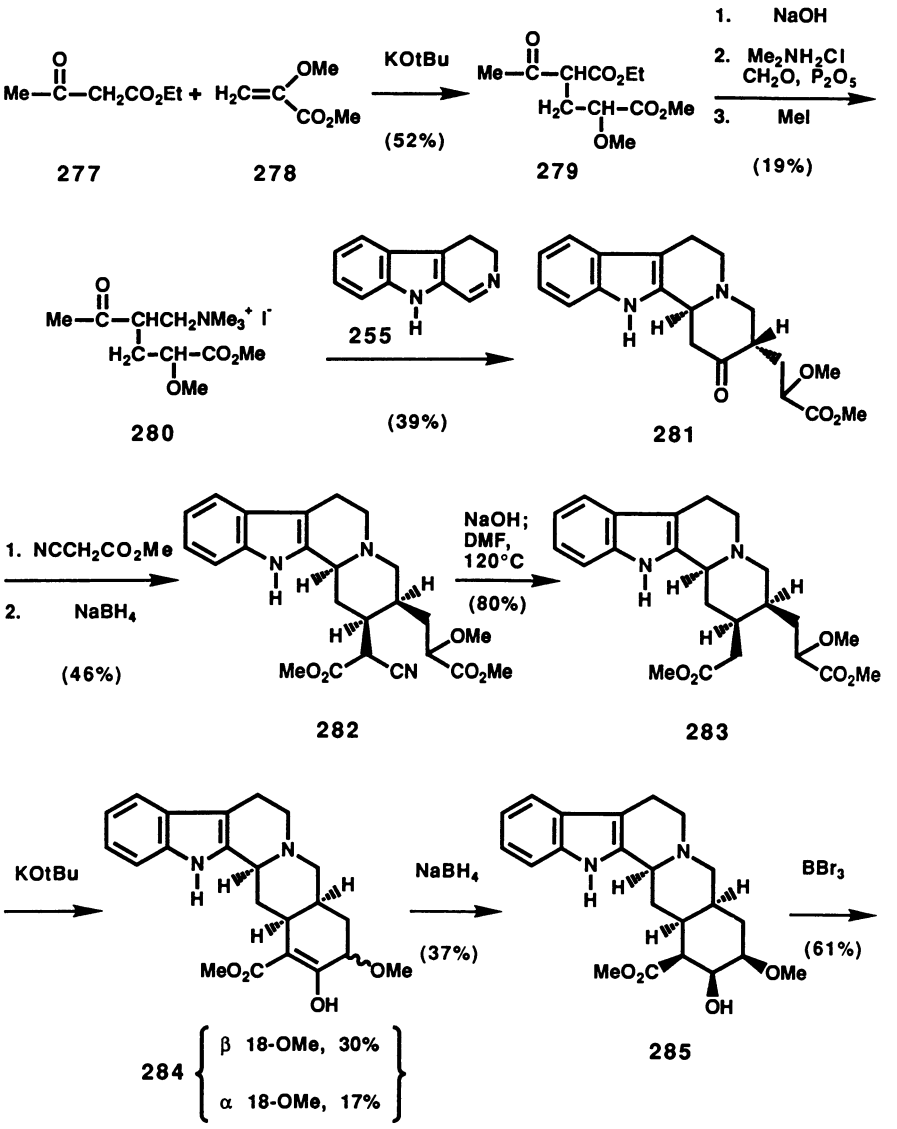
SCHEME 3.43



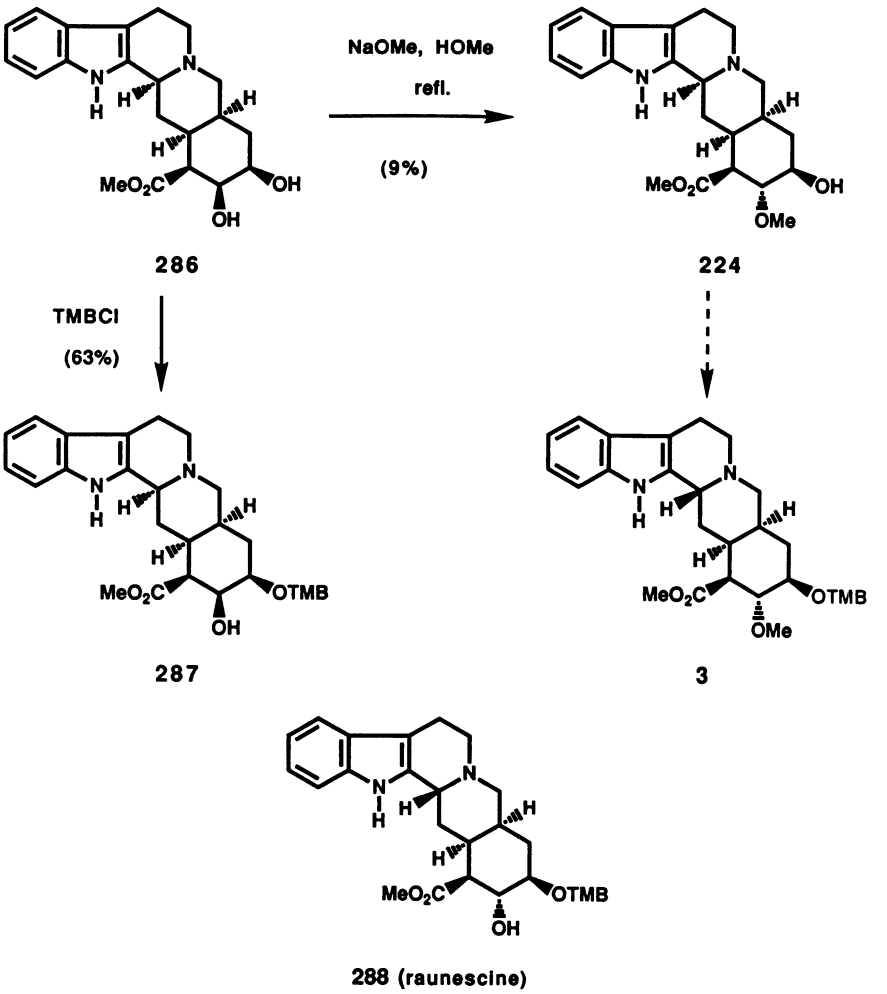
SCHEME 3.44

afford the pentacyclic β-ketoester **275**. Reduction of this substance with $NaBH_4$ gave mainly the undesired stereoisomer **276** in addition to lesser quantities of alloyohimbine (**7**) and α-yohimbine (**8**).

Szantay and his coworkers have also examined the feasibility of using the Dieckmann cyclization methodology to prepare more highly functionalized yohimbines, in particular deserpidine (**3**) and analogs of raunescine (**288**) (Scheme 3.45) (51–54). Condensation of ketoester **277** and methoxyacrylate **278** afforded the ketodiester **279** which was transformed to the corresponding ammonium salt **280**. Reaction of **280** with tetrahydro-β-carboline **255** gave tetracyclic ketoester **281** which was condensed with methyl cyanoacetate, followed by reduction, hydrolysis and decarboxylation to provide diester **283**. Treatment of this substance with base effected the crucial Dieckmann cyclization to give the highly functionalized yohimbane **284** with a cis DE-ring

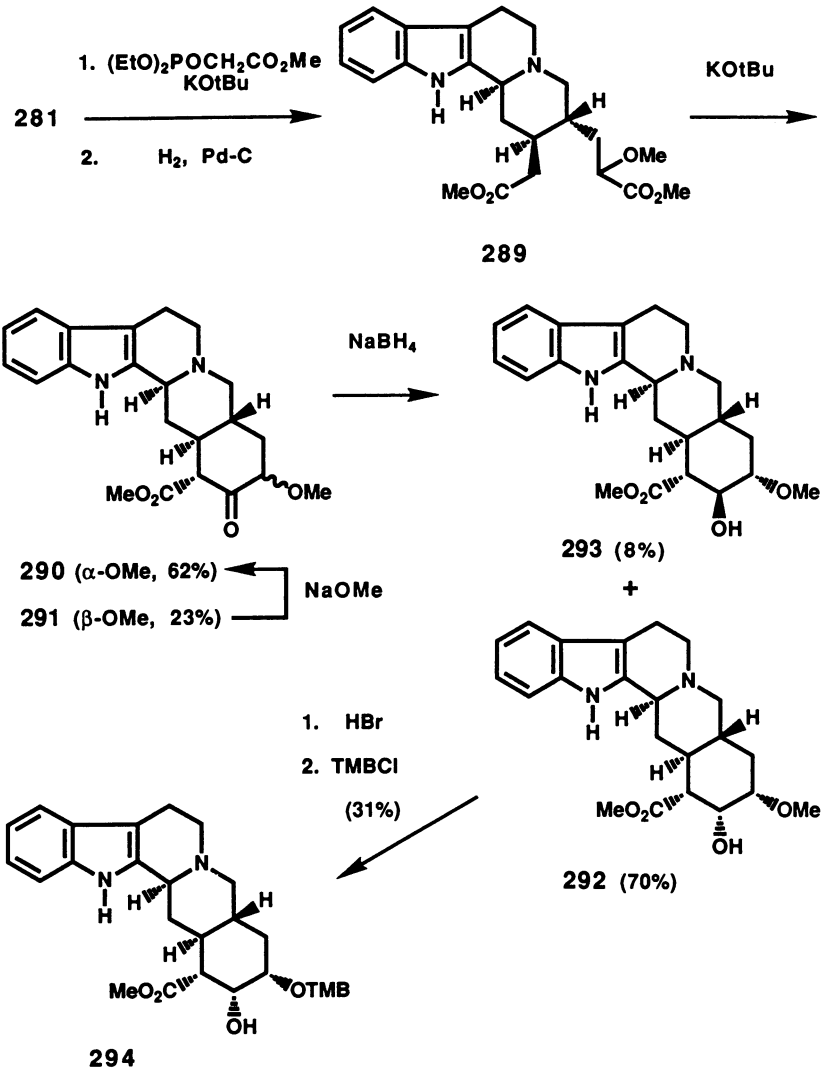

KOtBu
(52%)
1. NaOH
2. Me2NH2Cl
CH2O, P2O5
3. MeI
(19%)
277
278
279
280
255
(39%)
281
1. NCCH2CO2Me
2. NaBH4
(46%)
NaOH;
DMF,
120°C
(80%)
282
283
KOtBu
NaBH4
(37%)
BBr3
(61%)
284
β 18-OMe, 30%
α 18-OMe, 17%
285

SCHEME 3.45



SCHEME 3.45 (*cont.*)

fusion. Reduction of **284** provided **285** which was demethylated to yield the C(17), C(18) diol **286**. Selective trimethoxybenzoylation of the 18-hydroxyl group afforded 3-epi-17-epi-raunescine **287**. Szantay solved the problem of stereoselective installation of an 17α-hydroxy and 17α-methoxy group, required for respective syntheses of raunescine (**288**) and deserpidine (**3**), by first effecting elimination of water across the C(16)–C(17) bond in **286** or its C(18) ester followed by stereo-controlled conjugate addition of water or methoxide. These sequences yielded raunescine (**288**) and **224**, a precursor of deserpidine since acylation and H-3 epimerization had already been reported (112).

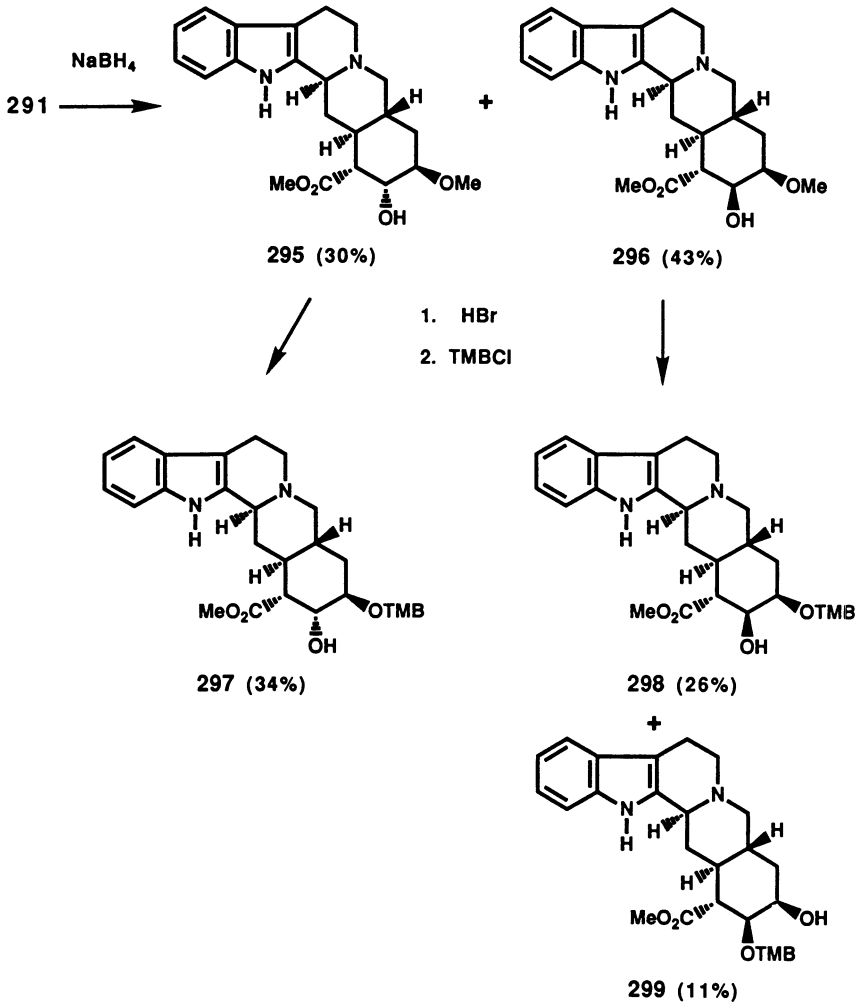


SCHEME 3.46

Szantay also utilized the Dieckmann cyclization strategy to prepare other 18-hydroxy yohimbine analogs (Scheme 3.46) (53). For example, Wadsworth-Emmons condensation of tetracyclic ketone **281** followed by hydrogenation provided diester **289**. Dieckmann cyclization of **289** then afforded the epimeric pentacyclic esters **290** and **291**. Treatment of **291** with sodium methoxide effected epimerization to the desired C(18)α methoxy epimer **290**. Sodium

borohydride reduction of the ketone function in **290** afforded predominantly the alcohol **292** in addition to a small amount of its C(18)-epimer **293**. Demethylation of **292** followed by trimethoxybenzoylation afforded **294**, a yohimbine analog.

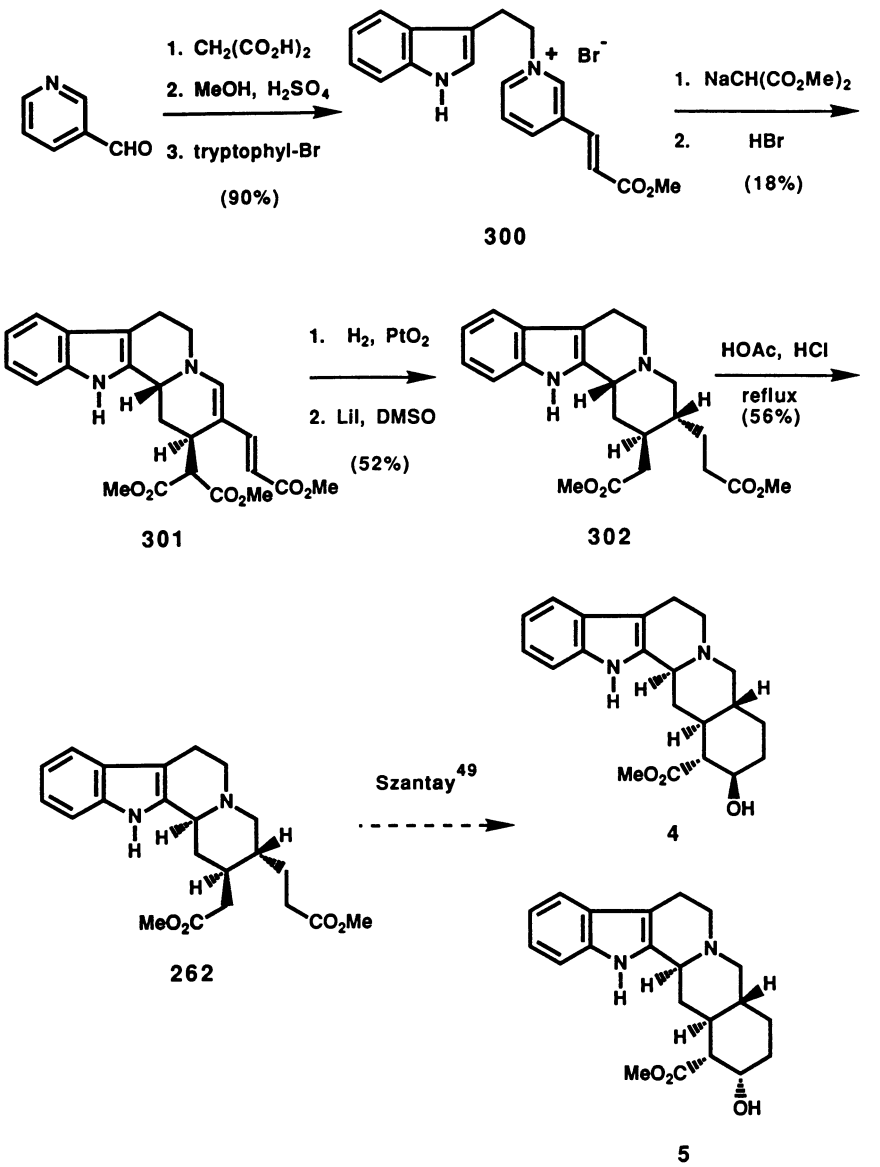
Finally, Szantay and his coworkers utilized the pentacyclic ketone **291** as a key intermediate in the preparation of several analogs of raunescine (Scheme 3.47) (53). Accordingly, reduction of **291** afforded pentacyclic alcohols **295** and **296**. Demethylation and subsequent acylation of **295** afforded 3-epi-16-epi-20-epi raunescine (**297**). When **296** was subjected to the same reaction conditions, the isomeric yohimbines **298** and **299** were formed. In summary,



SCHEME 3.47

the efforts of the Szantay group illustrate how Dieckmann cyclization processes can be used to construct the pentacyclic yohimbine skeleton.

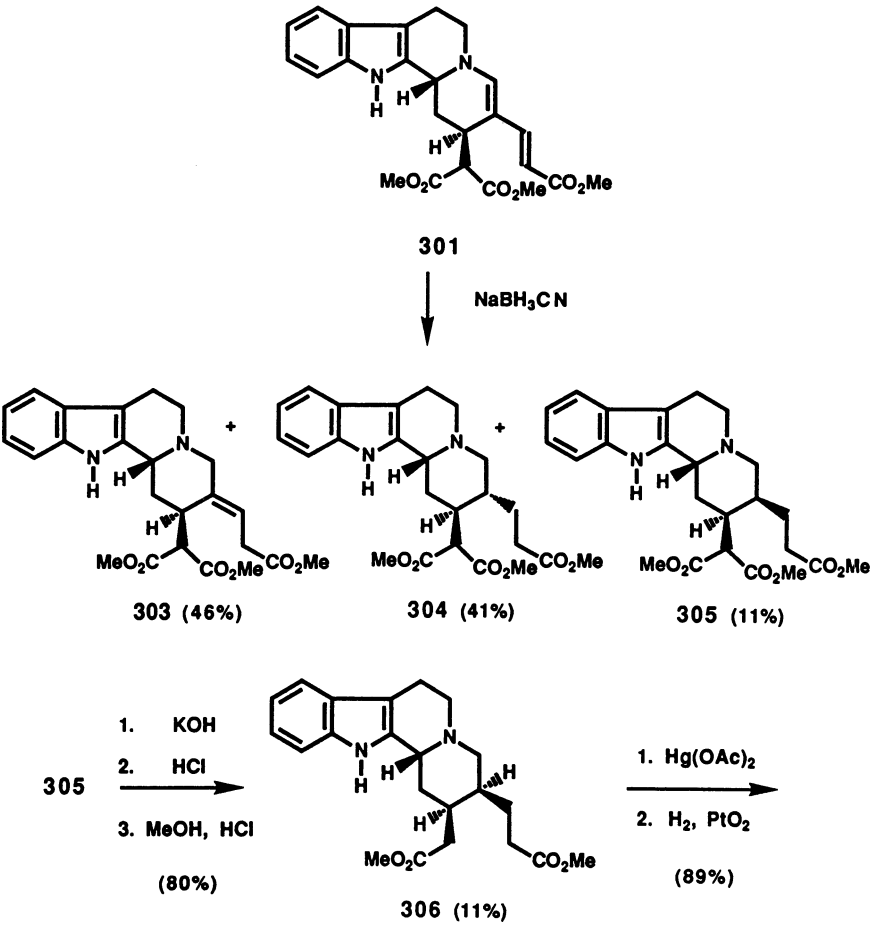
Wenkert has also employed a Dieckmann cyclization strategy to prepare a variety of yohimbine alkaloids (56–58). In formal syntheses of yohimbine (**4**) and β-yohimbine (**5**) (Scheme 3.48) (56), nicotinaldehyde was initially



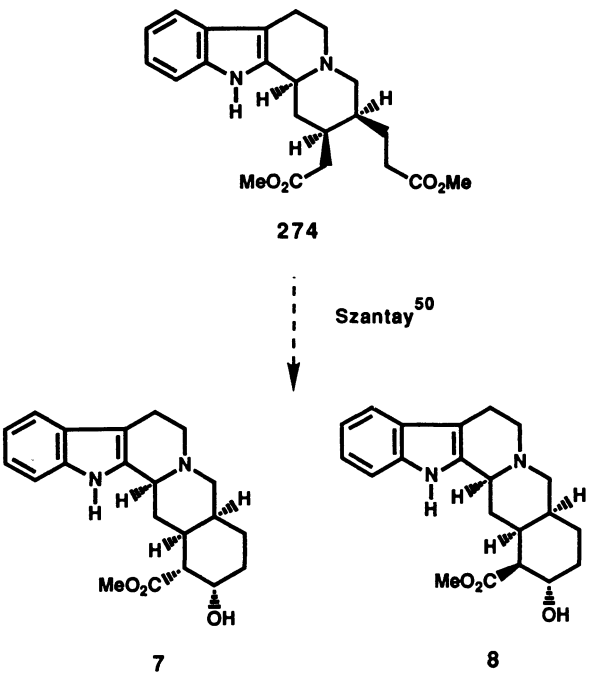
SCHEME 3.48

condensed with malonic acid followed by esterification and alkylation with tryptophyl bromide to provide pyridinium bromide **300**. This material was treated with the sodium salt of malonic acid diethyl ester followed by treatment with HBr to induce cyclization via an intermediate iminium cation to produce tetracyclic diester **301**. Hydrogenation followed by a saponification-decarboxylation sequence yielded diester **302**. Epimerization of 302 was achieved in refluxing acid to yield the α 3(H)-epimer **262** which Szantay had previously converted using the Dieckmann cyclization methodology to yohimbine (**4**) and β-yohimbine (**5**) (49).

Wenkert also utilized the Dieckmann methodology to prepare alloyohimbine (**7**) and α-yohimbine (**8**) (Scheme 3.49) (56). Reduction of **301** afforded a mixture of products including desired triester **305** as a minor component.



SCHEME 3.49

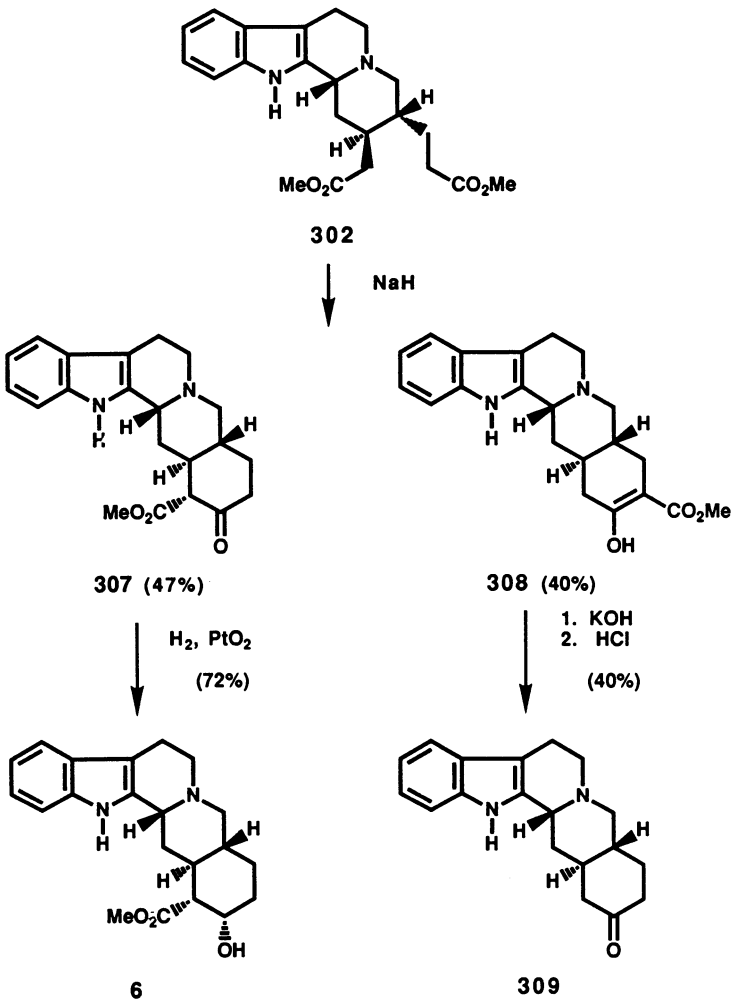


SCHEME 3.49 (*cont.*)

Saponification and decarboxylation of **305** afforded diester **306** which underwent a mercuric acetate mediated epimerization to give its C(3)-epimer **274** which Szantay had previously transformed by use of Dieckmann processes to targets **7** and **8** (50).

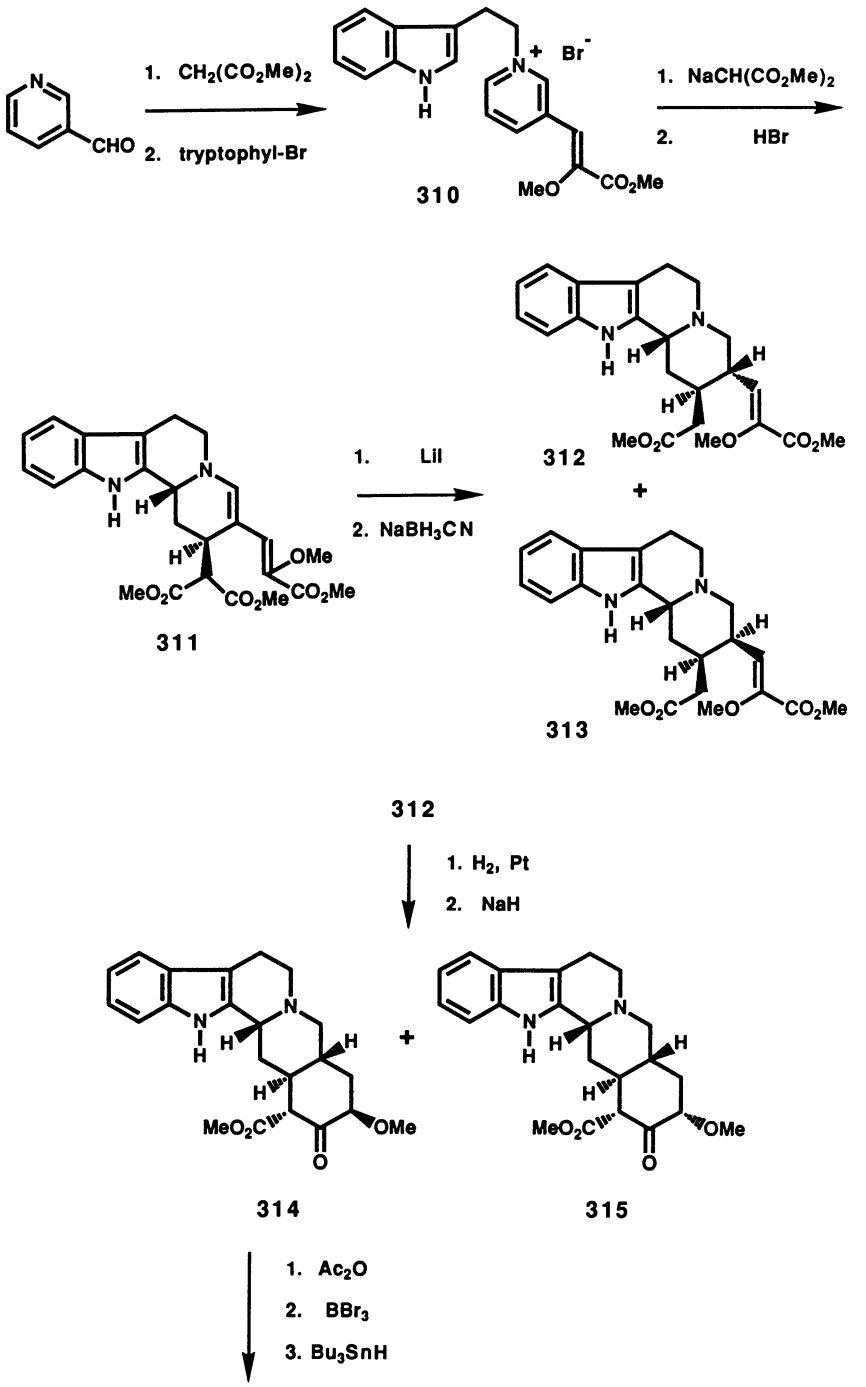
Wenkert also applied the Dieckmann methodology to the preparation of pseudoyohimbine (**6**) and pseudoyohimbone (**309**) (Scheme 3.50) (56). Treatment of **302** with base effected the desired cyclization to provide regioisomers **307** and **308**. Ketoester 307 was hydrogenated to yield pseudoyohimbine (**6**). Additionally, **308** was saponified and decarboxylated to afford pseudoyohimbone (**309**).

In continuing studies in this area, Wenkert and his coworkers have developed a new synthetic route utilizing Dieckmann chemistry to prepare pseudoyohimbine (**6**) (Scheme 3.51) (57). This strategy also permitted the preparation of **313** which serves as a key intermediate in the synthesis of the more highly functionalized yohimbines, deserpidine (**3**) and raunescine (**288**). In the synthesis of pseudoyohimbine (**6**), nicotinaldehyde was condensed with methyl methoxyacetate followed by alkylation with tryptophyl bromide to afford pyridinium salt **310**. This material was treated with the anion of the dimethyl ester of malonic acid followed by acid mediated cyclization to afford

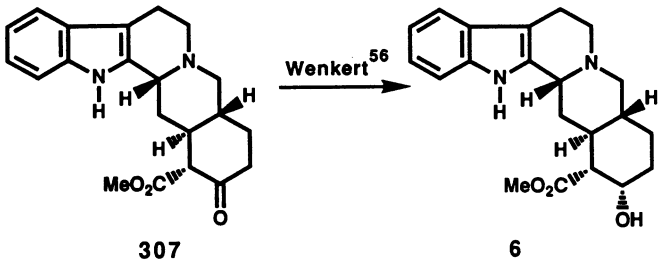


SCHEME 3.50

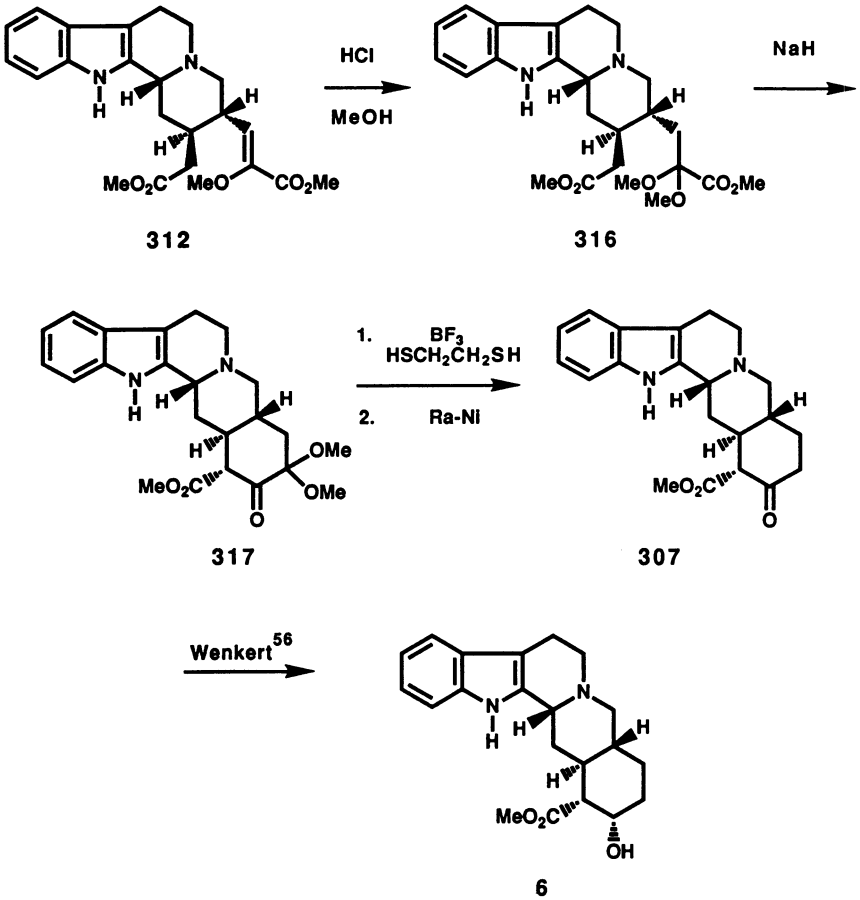
tetracyclic triester **311**. A saponification-decarboxylation-reduction sequence gave a mixture of diesters **312** and **313** in a 1 : 2 ratio. While **312** was converted to pseudoyohimbine (**6**), intermediate **313** was elaborated to more highly oxygenated yohimbines. In the synthesis of pseudoyohimbine, hydrogenation of **312** followed by Dieckmann cyclization afforded ketoesters **314** and **315**. Next, removal of the 18-methoxy group of **314** was addressed. Ketoester **314** was first acylated; conversion of the 18-methoxy group to the corresponding bromide followed by reductive dehalogenation afforded **307**, a substance which Wenkert had previously transformed to pseudoyohimbine (56).


1. $CH_2(CO_2Me)_2$
2. tryptophyl-Br
CHO
Br⁻
310
1. $NaCH(CO_2Me)_2$
2. HBr
311
1. LiI
2. $NaBH_3CN$
312
313
1. H_2, Pt
2. NaH
314
315
1. Ac_2O
2. BBr_3
3. Bu_3SnH

SCHEME 3.51



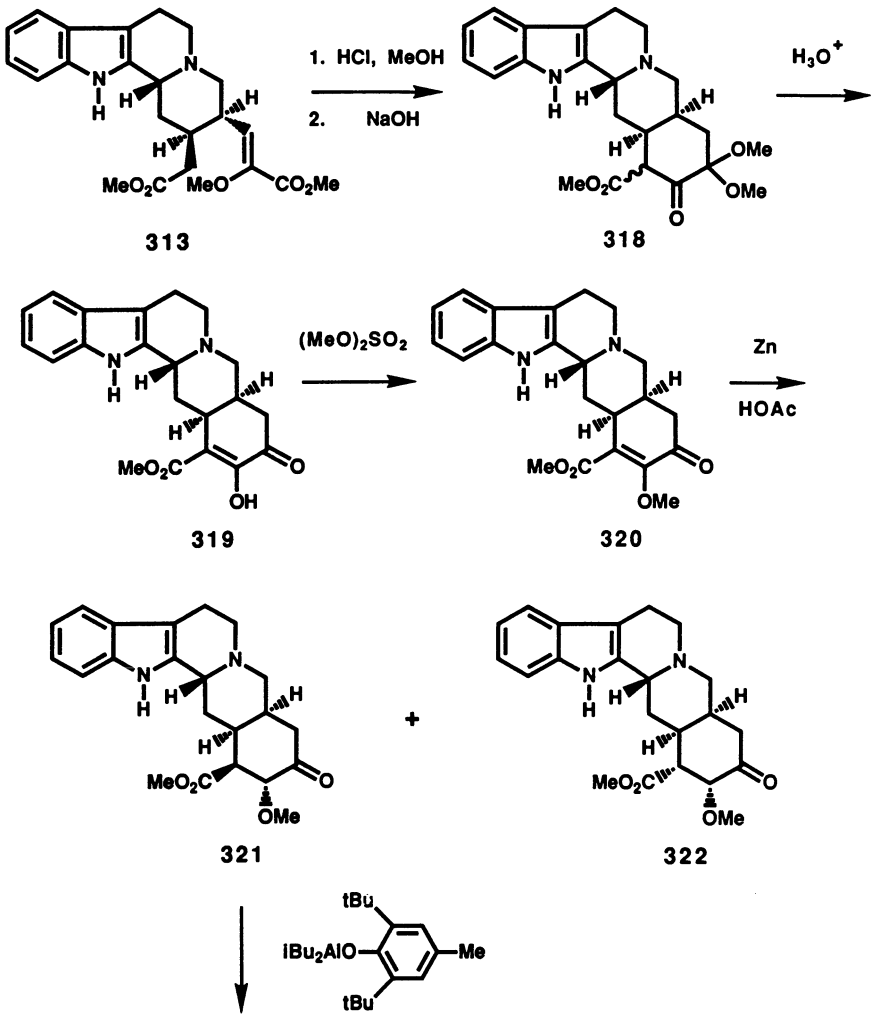
SCHEME 3.51 (*cont.*)



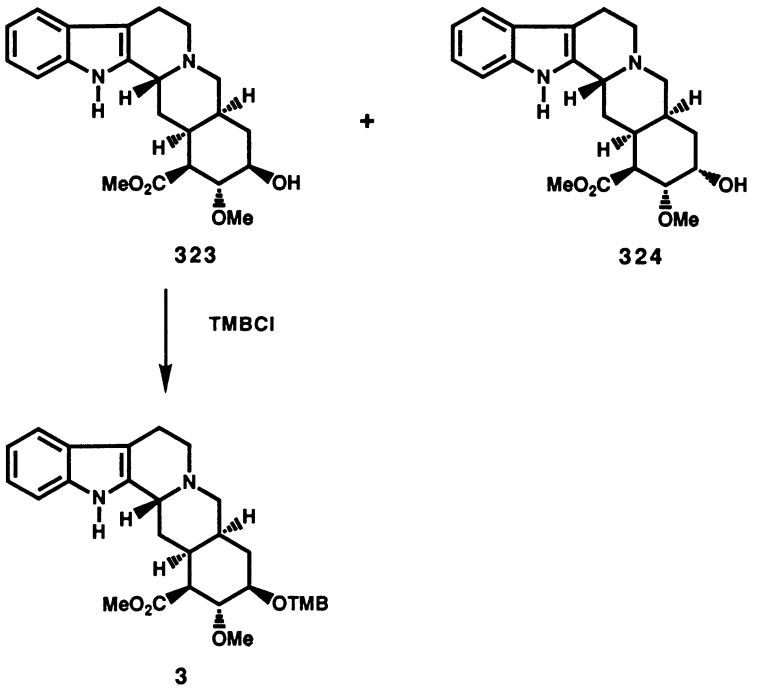
SCHEME 3.52

Wenkert has also reported an alternative preparation of the pseudoyohimbine precursor **307** (Scheme 3.52) (58). Enol ether **312** was converted to ketal **316** which underwent the crucial Dieckmann cyclization to afford ketal **317**. Removal of the oxygen functionality at C(18) was achieved by conversion to the corresponding thioketal which was desulfurized to provide **307**.

Wenkert has applied the Dieckmann methodology to the preparation of the more highly oxygenated yohimbines, deserpidine (**3**) and raunescine (**288**) (57, 58). To prepare deserpidine (Scheme 3.53), enol ether **313** was first converted to its ketal. Dieckmann cyclization then afforded the ketoester ketal



SCHEME 3.53

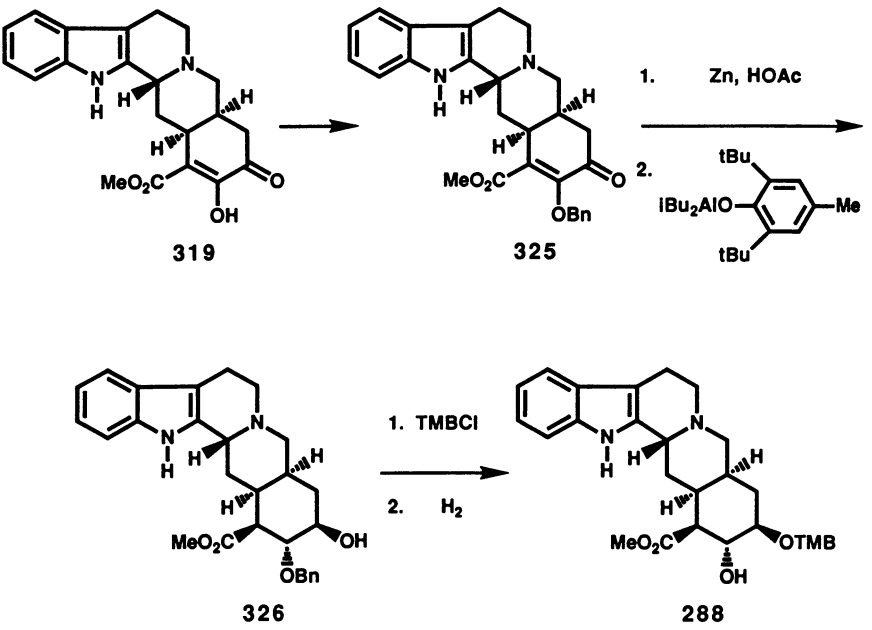


SCHEME 3.53 (*cont.*)

318. Deketalization afforded the diosphenol **319** which was methylated to yield **320** followed by subsequent reduction to afford a 2 : 1 mixture of **321** and **322**. Ketone **321** upon treatment with a hindered reducing agent provided predominantly the C(18)β alcohol **323** which was trimethoxybenzoylated to yield the target deserpidine (**3**).

By using this same Dieckmann cyclization strategy. Wenkert and his coworkers have accomplished a synthesis of raunescine (**288**) (Scheme 3.54) (58). Accordingly, ketoester **319** was benzylated to provide enol ether **325**. Sequential reduction of the double bond and ketone of **325** by the protocol employed in the deserpidine synthetic route provided the C(18) alcohol **326**. Acylation of the hydroxyl group and hydrogenolysis gave raunescine. Similar to the efforts of Szantay, Wenkert's investigations clearly demonstrate how Dieckmann cyclization chemistry can be applied to the synthesis of a variety of yohimbine alkaloids.

A final illustration of how Dieckmann cyclizations have been utilized in yohimbine alkaloid synthesis is found in the work of Winterfeldt and coworkers (59, 60). In these studies, this methodology was employed to construct the pentacyclic alkaloid skeleton found in thioketal **335** (Scheme 3.55). Reaction of β-enaminoester **327**, prepared by condensation of tryptamine and



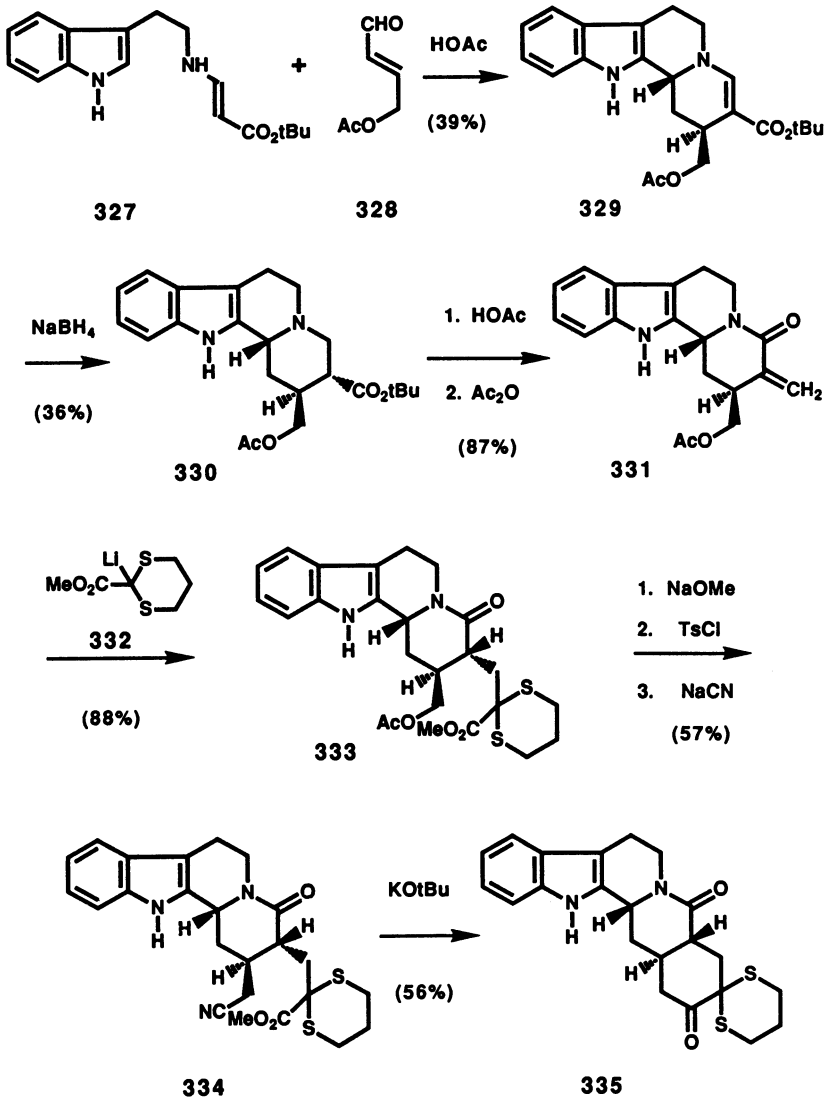
319 325 326 288

SCHEME 3.54

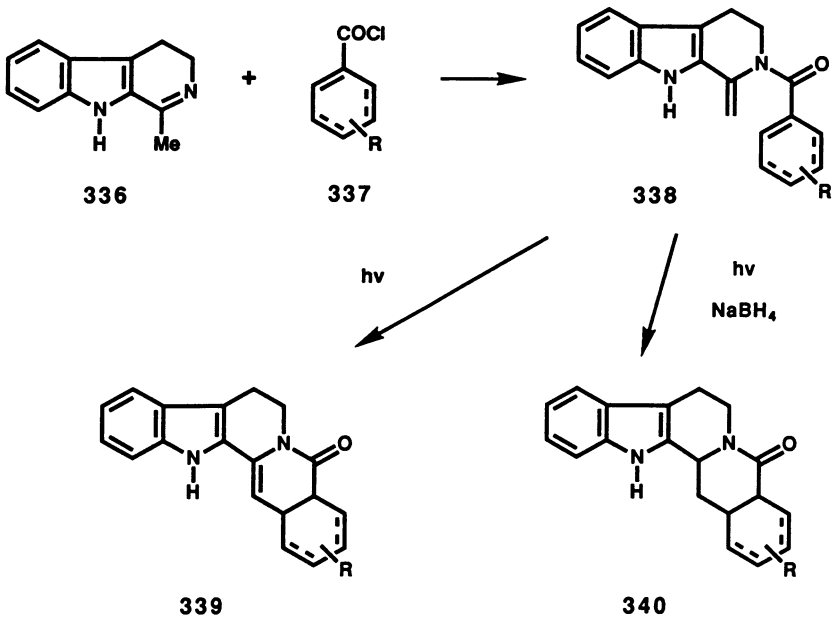
t-butyl propiolate, with the α,β-unsaturated aldehyde **328** afforded the tetracyclic enaminoester **329**. Reduction afforded ester **330** which underwent an interesting transformation to α-methylene lactam **331** when heated in acid. Michael addition of the dithiane anion **332** to **331** afforded acetate **333** which was converted to the corresponding nitrile **334**. Dieckmann cyclization of **334** afforded **335**, a substance which possesses the pentacyclic yohimbine skeleton. While no further transformations of **335** were reported, the functionality in the E-ring could permit elaboration of this yohimbane to a variety of alkaloids.

3.3.2. Enamide and Enamine Photocyclizations

Enamide and enamine photocyclizations (9, 13, 14, 113) are central to yet another strategy which has been successfully employed by Ninomiya (9, 13, 14, 61–71), Kametani (74), and Atta-ur-Rahman (72, 73) in the synthesis of yohimbine alkaloids. These approaches typically begin with harmalane (**336**) which contains the ABC unit of the pentacyclic yohimbine targets (Scheme 3.56). Condensation of harmalane with an appropriately substituted cyclohexene carboxylic acid or benzoyl chloride **337** affords seco D-ring intermediate **338**. To effect D-ring closure, the enamide chromophore in **338** is photocyclized to form pentacyclic enamide **339**. Alternatively, photoreaction


CHO
HOAc
(39%)
CO2tBu
AcO
327
328
329
NaBH4
(36%)
1. HOAc
2. Ac2O
(87%)
CH2
330
331
LiS
MeO2C
332
(88%)
333
1. NaOMe
2. TsCl
3. NaCN
(57%)
NC
KOtBu
(56%)
334
335

SCHEME 3.55

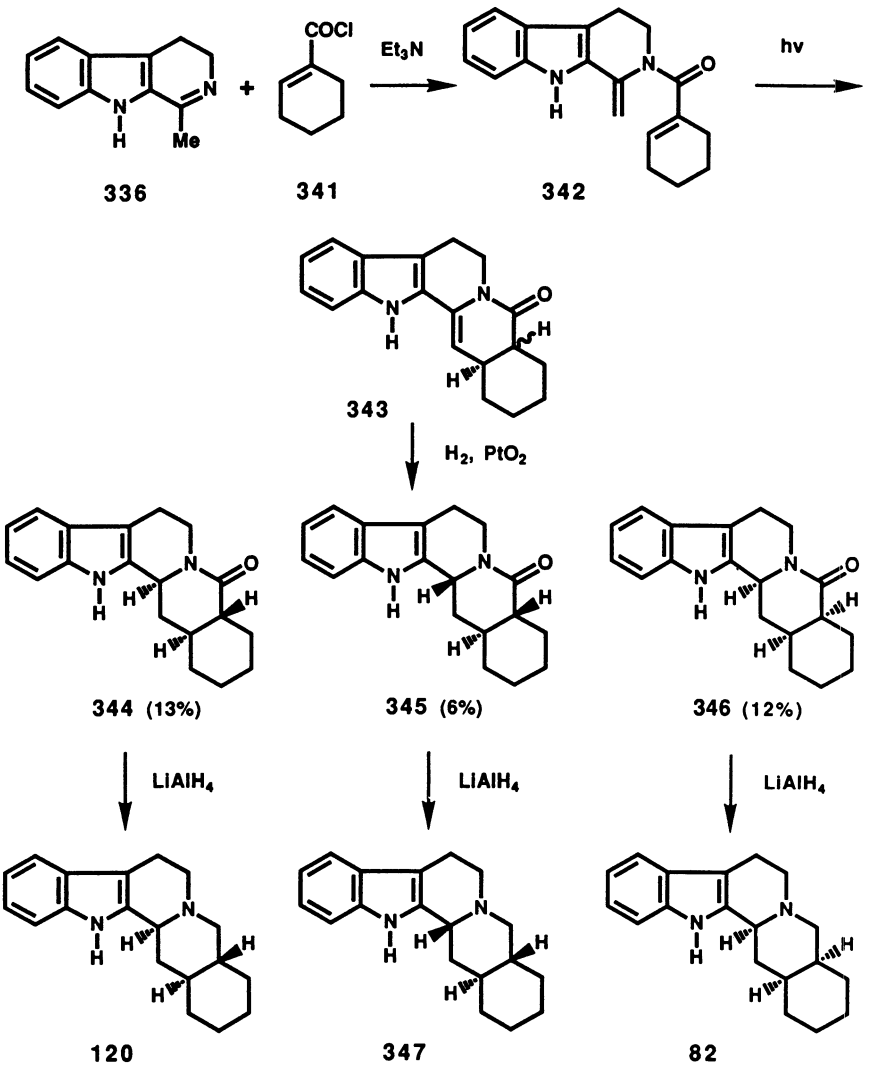


SCHEME 3.56

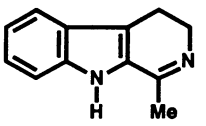
of **338** is carried out in the presence of a reducing agent to provide amide **340**. Appropriate functional group manipulations are conducted to arrive at the yohimbine targets.

An early application of enamide photocyclizations to yohimbine alkaloid synthesis is illustrated by Ninomiya and coworkers' synthesis of yohimbane (**120**), epiyohimbane (**347**) and alloyohimbane (**82**) (Scheme 3.57) (61, 63, 69). These compounds were prepared by a short sequence starting with harmalane (**336**) which was condensed with acid chloride **341** to provide enamide **342**. Irradiation of **342** in benzene followed by reduction afforded a mixture of the stereoisomeric pentacyclic lactams **344**, **345**, and **346** which were reduced to provide yohimbane (**120**), epiyohimbane (**347**), and alloyohimbane (**82**), respectively. While the yield of the photocyclization process was modest, this route demonstrated how enamide photocyclizations can be used to rapidly construct pentacyclic yohimbane targets.

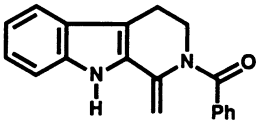
The synthesis of yohimbane (**120**) and alloyohimbane (**82**) was improved by using a modified sequence which featured reductive photocyclization as the key step (Scheme 3.58) (63, 69). Condensation of harmalane (**336**) with benzoyl chloride afforded enamide **348** which upon irradiation in the presence of sodium borohydride afforded a mixture of functionalized yohimbanes **349**, **350**, and **351**. The overall yields and product distribution were found to be dependent on the solvent employed with the maximum yield of **350** (98%) being obtained in a mixture of MeCN and MeOH. Hydrogenation of **349**


COCl
Et3N
hv
Me
336
341
342
343
H2, PtO2
344 (13%)
345 (6%)
346 (12%)
LiAlH4
LiAlH4
LiAlH4
120
347
82

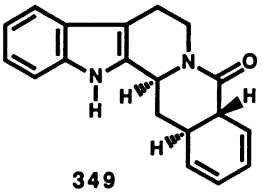
SCHEME 3.57



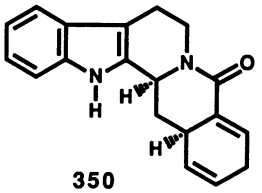
336 + PhCOCl $\xrightarrow{Et_3N}$



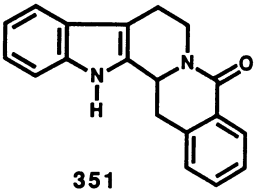
348 $\xrightarrow[NaBH_4]{hv}$



349 +



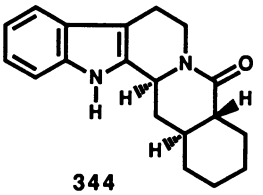
350 +



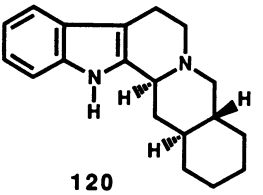
351

Solvent	349	350	351
PhH-MeOH	7%	21%	20%
Et_2O-MeOH	17%	32%	5%
MeCN-MeOH	–	98%	–

349 $\xrightarrow[(88\%)]{H_2, PtO_2}$

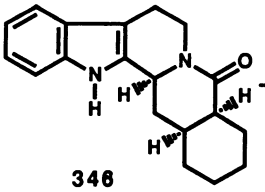


344 $\xrightarrow[(85\%)]{LiAlH_4}$

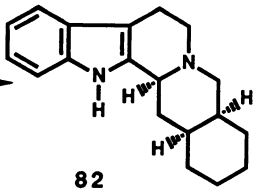


120

350 $\xrightarrow[(46\%)]{H_2, PtO_2}$ 344 +



346 (41%) $\xrightarrow[(85\%)]{LiAlH_4}$

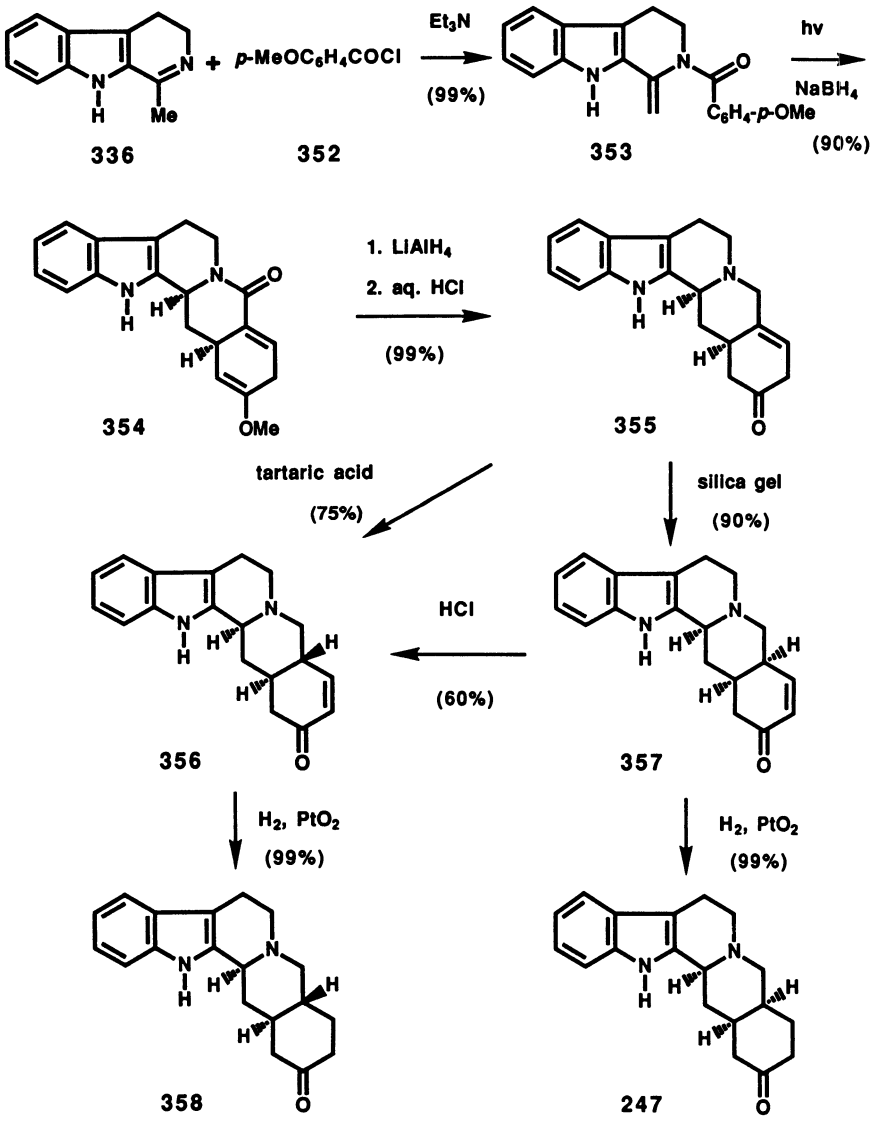


82

SCHEME 3.58

afforded lactam **344** which was converted to yohimbane **120**. Alternatively, **350** was transformed to amides **344** and **346** which served as the respective precursors of yohimbane (**120**) and alloyohimbane (**82**).

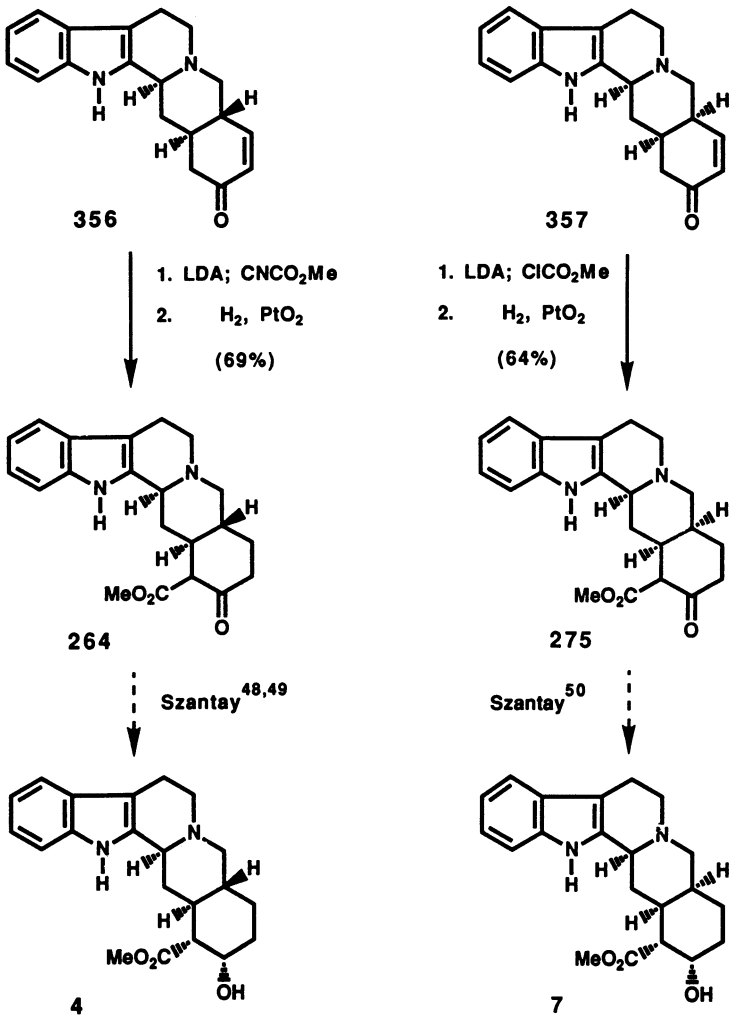
Ninomiya's group has also utilized the enamide photocyclization strategy to prepare alloyohimbone (**247**) and yohimbone (**358**) (Scheme 3.59) (9, 64, 65, 70). In these syntheses, harmalane (**336**) was condensed with anisoyl chloride **352** to provide enamide **353**. Reductive photocyclization of this substance



SCHEME 3.59

afforded in good yield pentacyclic enol ether **354** which was reduced and hydrolyzed to β,γ-unsaturated ketone **355**. Interestingly, heating **355** at reflux with tartaric acid led to formation of the trans-fused enone **356**. Alternatively, when **355** was subjected to silica gel or base treatment, the cis-fused enone **357** was generated. Treatment of **357** with acid provided its C(20)-epimer **356**. Finally, hydrogenation of **356** and **357** afforded yohimbone (**358**) and alloyohimbone (**247**), respectively.

Ninomiya and his coworkers were able to adapt this preparative sequence to a formal syntheses of yohimbine (**4**) and alloyohimbine (**7**) (Scheme 3.60) (65, 70). Accordingly, enones **356** and **357** were acylated at C-16 and then

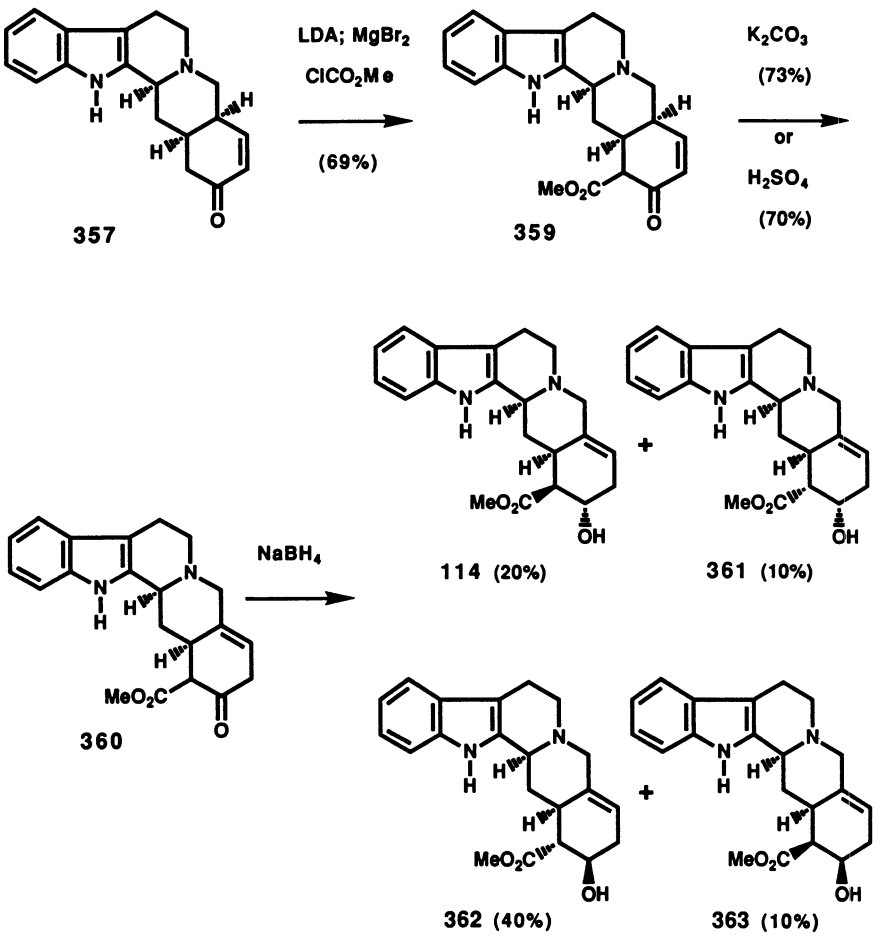


SCHEME 3.60

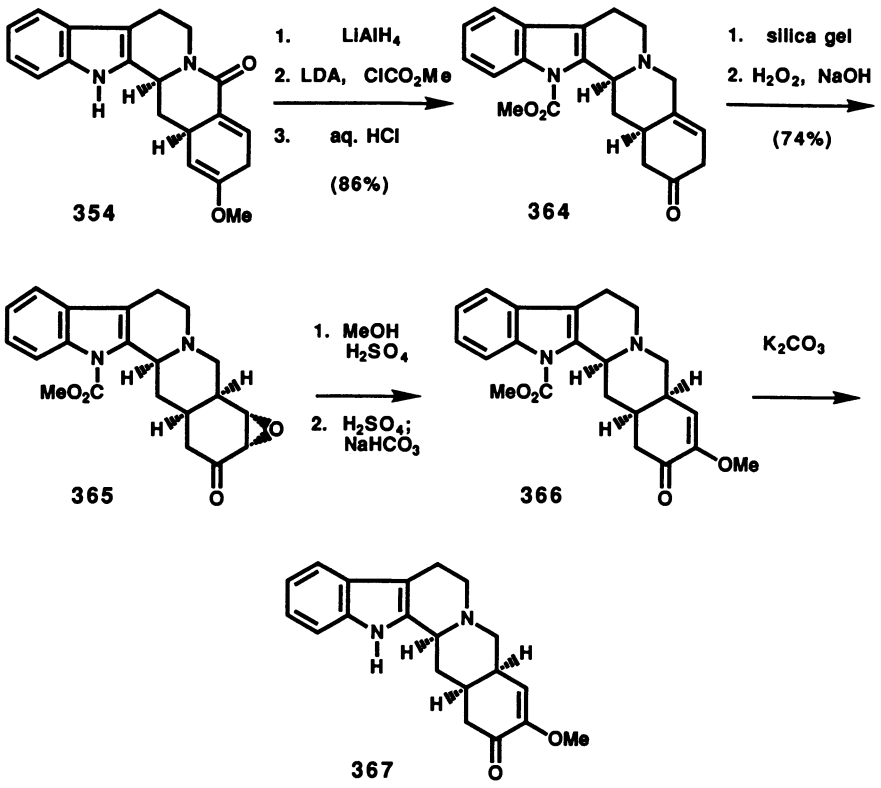
hydrogenated to yield β-ketoesters **264** and **275**, respectively. Ketoester **264** was an intermediate in Szantay's synthesis of yohimbine (**4**) (48, 49) while **275** was a precursor in the Szantay preparation of alloyohimbine (**7**) (50).

Yet another extension of the enamide photocyclization methodology is exemplified by Ninomiya's synthesis of the 19,20-dehydroyohimbines **114**, **361**, **362**, and **363** (Scheme 3.61) (67). In this work, the pentacyclic enone **357** was transformed to the β-ketoester **359** which was readily isomerized to its β,γ-unsaturated derivative **360**. The ketone was reduced to provide the epimeric 19,20-dehydroyohimbines **114**, **361**, **362**, and **363**.

The Ninomiya group was also able to apply the enamide photocyclization strategy to the synthesis of the highly functionalized yohimbine, deserpidine



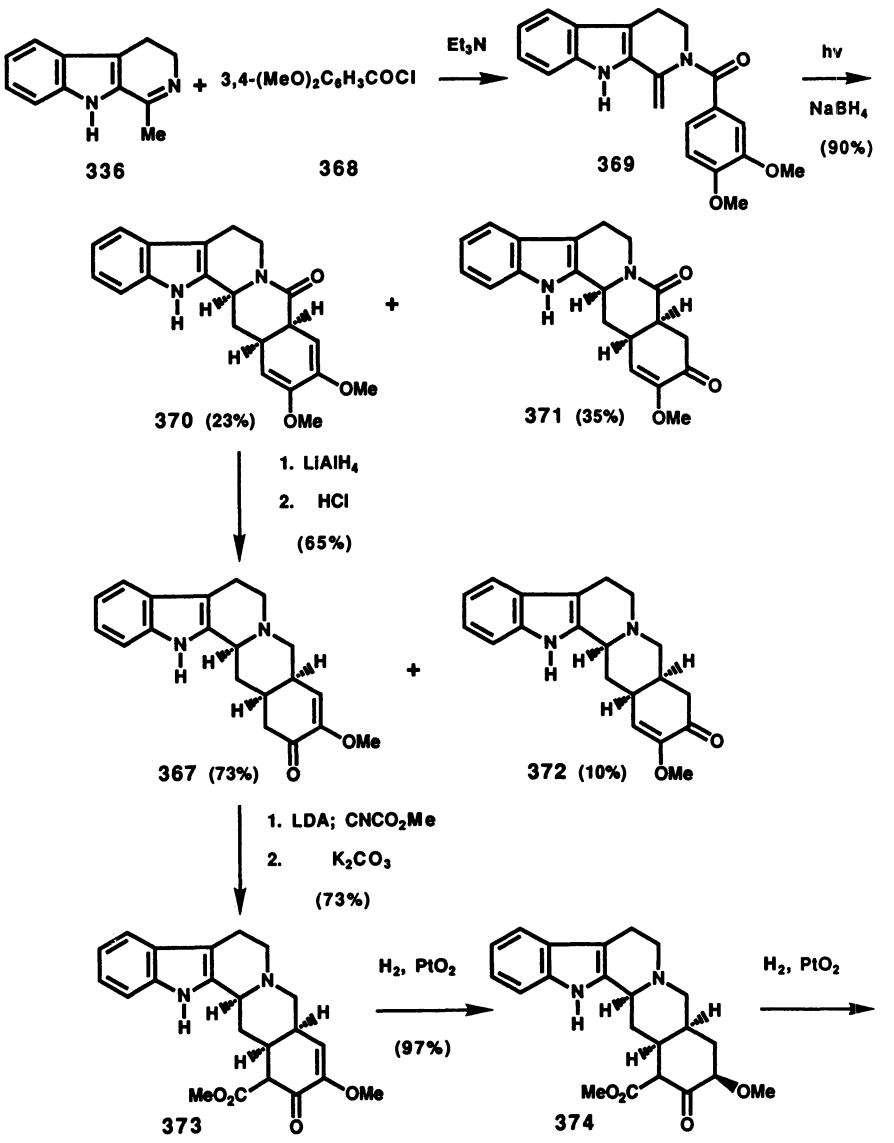
SCHEME 3.61



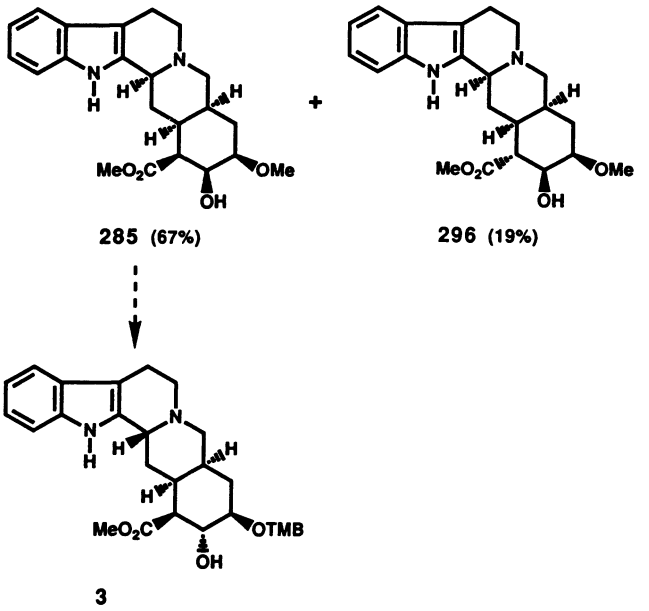
SCHEME 3.62

(3) (Scheme 3.62) (66, 71), Reduction of the amide followed by protection of the indole nitrogen in the tetracyclic lactam **354** with subsequent hydrolysis of the enol ether function afforded ketone **364**. Isomerization to give the corresponding α,β-unsaturated ketone was followed by treatment with basic hydrogen peroxide to provide epoxy ketone **365**. Epoxide opening followed by methanol elimination afforded α-methoxy enone **366** which was deprotected to yield **367**.

A more efficient route to the pentacyclic ketone **367** not requiring indole protection has been developed by the Ninomiya group (Scheme 3.63) (66, 67). Harmalane (**336**) was condensed with 3,4-dimethoxybenzoyl chloride (**368**) to provide enamide **369** which upon irradiation under reducing conditions afforded a mixture of desired bis-enol ether **370** and corresponding ketone **371**. Lactam **370** was reduced, and the C(16), C(17) enol ether was hydrolyzed to afford predominantly **367** in addition to a small amount of the regioisomeric hydrolysis product **372**. Treatment of **367** with methyl cyanoformate


Et3N
hv
NaBH4
(90%)
336
368
369
370 (23%)
371 (35%)
1. LiAlH4
2. HCl
(65%)
367 (73%)
372 (10%)
1. LDA; CNCO2Me
2. K2CO3
(73%)
H2, PtO2
(97%)
H2, PtO2
373
374

SCHEME 3.63



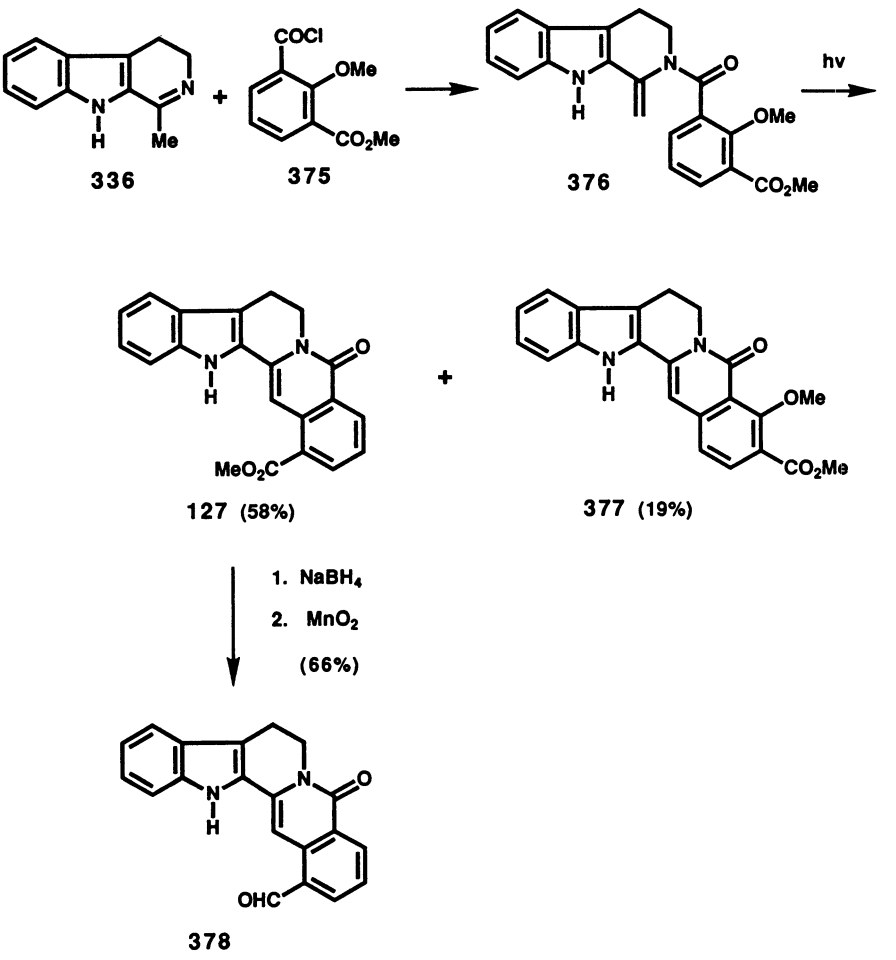
SCHEME 3.63 (*cont.*)

effected acylation at C(16) as well as at the indole nitrogen. Subsequent basic hydrolysis provided **373**. Successive hydrogenation of the enol ether and then of the ketone afforded the yohimbine **285** which Szantay had previously elaborated to deserpidine (**3**) (53, 54).

A further application of the enamide photocyclization in yohimbine synthesis is demonstrated by Ninomiya's preparation of naucleficine (**378**) (Scheme 3.64) (68) Enamide **376**, prepared from **336** and aroyl chloride **375**, was irradiated under non-reducing conditions to form oxygambirtannine (**127**). Finally, a reduction-oxidation sequence afforded the target.

A variation on Ninomiya's enamide photocyclization strategy is illustrated in the synthesis of nauclefine (**382**) (Scheme 3.65) (62). Condensation of harmalane (**336**) and isonicotinyl chloride (**379**) afforded spirocycle **380** which was hydrolyzed to pentacyclic enamide **381**. Irradiation of **381** yielded target **382**.

Other research groups have also investigated the viability of strategies based on enamide photocyclizations to prepare yohimbines. For example, Kametani and his coworkers tested this approach in a formal asymmetric synthesis of yohimbol (**390**) (Scheme 3.66) (74). The optically active harmalane derivative **383**, prepared from L-trytophan, was condensed with anisoyl chloride (**352**) to afford enamide **384**. Irradiation of **384** yielded a mixture of the desired pentacyclic lactam **385** in addition to enamide **386**. The amide carbonyl in **385** was reduced to provide **387** as well as its C(3)-epimer **388**.



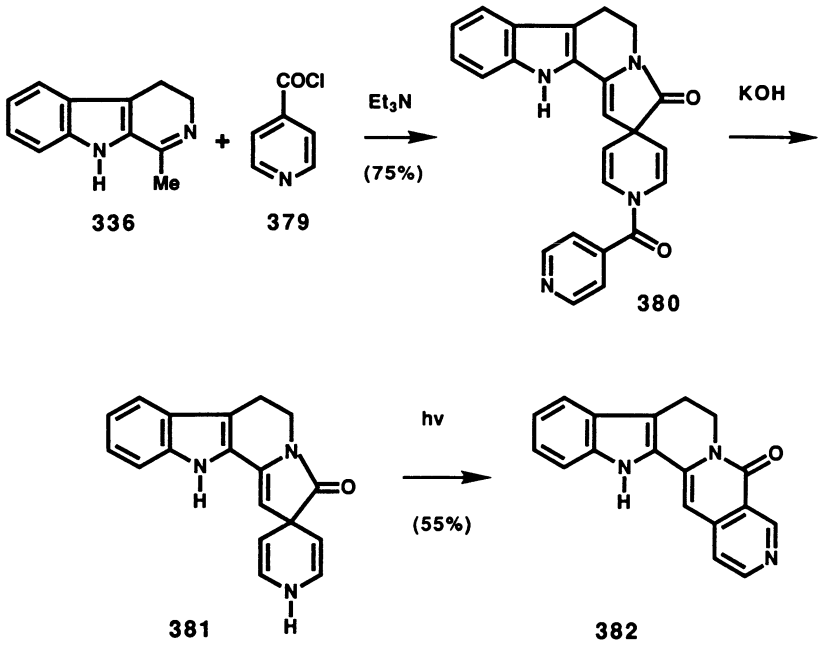
336 375 376 127 (58%) 377 (19%) 378

SCHEME 3.64

The carbomethoxy group of **387** was removed to give in poor yield the yohimbane **389** which had previously been converted to yohimbol (**390**) by Okamura and Yamada (114).

The closely related enamine cyclization process has also been applied to the synthesis of gambirtannine derivatives by Atta-ur-Rahman (Scheme 3.67) (72, 73). Condensation of 6-methoxyharmalane (**391**) with **392** afforded a separable mixture of the enamines **393** and **394**. Irradiation of **393** afforded 11,16-dimethoxy-16-des(methoxycarbonyl) gambirtannine (**395**) in good yield. Under the same conditions, enamine **394** yielded 11,16-dimethoxy-14-(13-methoxybenzyl)-6-des(methoxycarbonyl) gambirtannine (**396**).

In summary, the efforts of a number of research groups have demonstrated

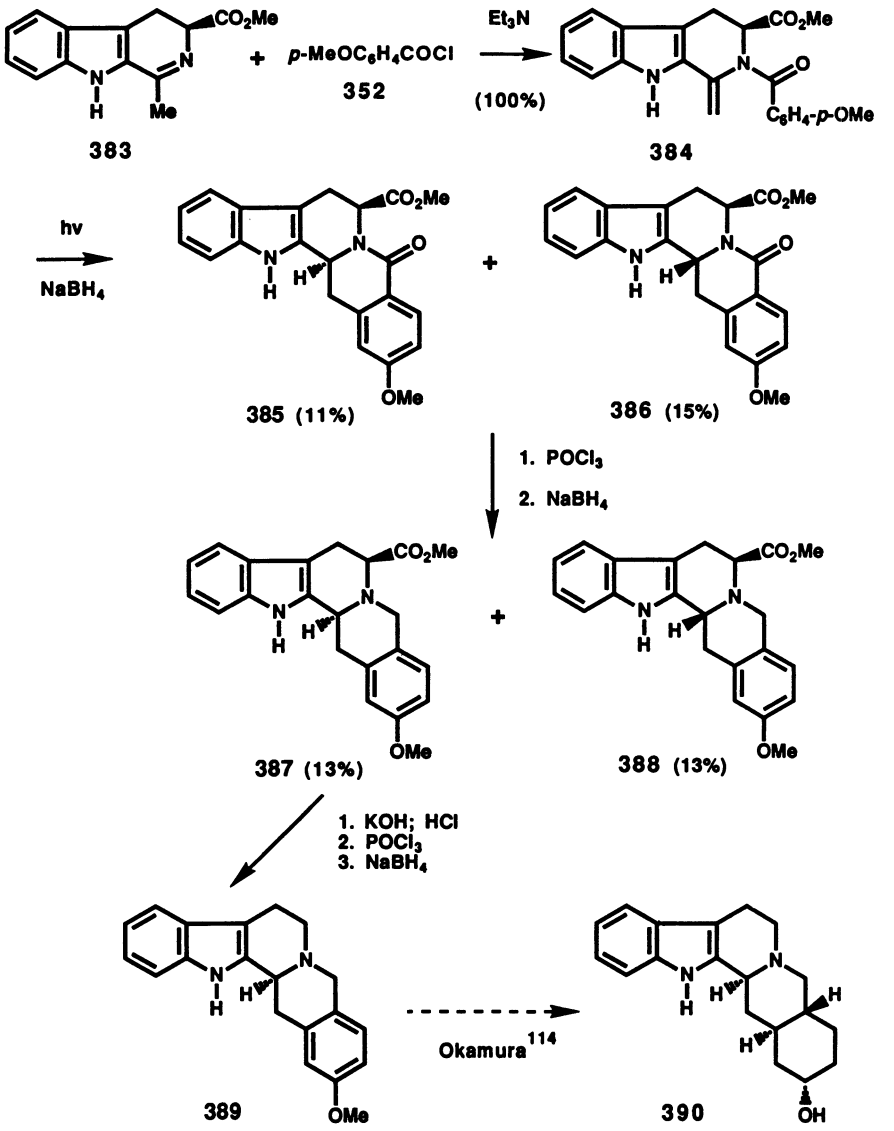


SCHEME 3.65

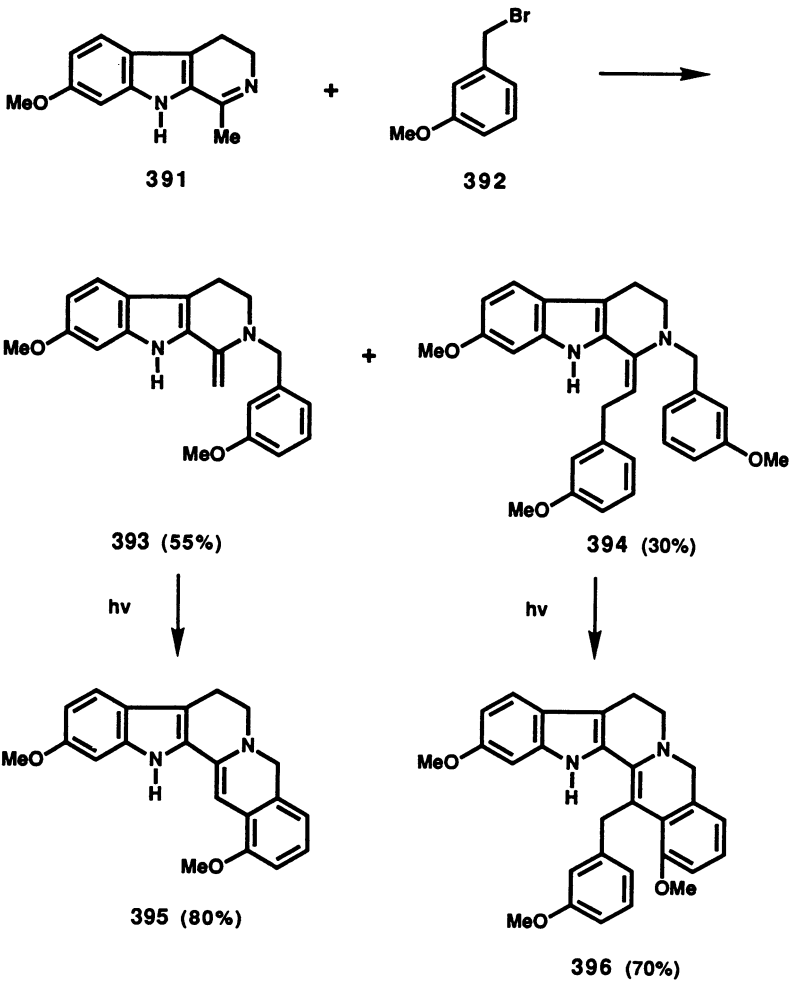
that enamide photocyclizations indeed represent a viable strategy for the synthesis of yohimbine alkaloids. This approach has permitted rapid construction of the pentacyclic targets from simple starting materials. Furthermore, this route is quite versatile in that photocyclization substrates can bear a variety of functional groups needed to elaborate the E-ring functionality arrays in complex targets.

3.3.3. Alternative Approaches Utilizing β-Carboline Derivatives

A number of synthetic efforts directed toward the yohimbine alkaloids have utilized β-carboline derivatives as starting materials. These approaches do not collectively follow a general synthetic strategy and as a result are discussed as a group at this point. An early effort in this context was reported by Winterfeldt and his coworkers who developed a concise method for construction of the yohimbine skeleton from the known (115) β-carboline **397** (Scheme 3.68) (116, 117). Birch reduction of **397** afforded enol ether **398** which was hydrolyzed. The resulting β,γ-unsaturated ketone was treated with paraformaldehyde in acid to yield pentacyclic enone **401** in good yield. This interesting transformation presumably proceeded through iminium species **399** which undergoes sigmatropic rearrangement to **400** followed by ring closure on the iminium salt by the dienol moiety to afford **401**.


CO2Me
N
H
Me
383
+ p-MeOC6H4COCl
352
Et3N
(100%)
CO2Me
N
H
O
C6H4-p-OMe
384
hv
NaBH4
CO2Me
N
H
H
O
OMe
385 (11%)
+
CO2Me
N
H
H
O
OMe
386 (15%)
1. POCl3
2. NaBH4
CO2Me
N
H
H
OMe
387 (13%)
+
CO2Me
N
H
H
OMe
388 (13%)
1. KOH; HCl
2. POCl3
3. NaBH4
N
H
H
OMe
389
Okamura[114]
N
H
H
H
H
OH
390

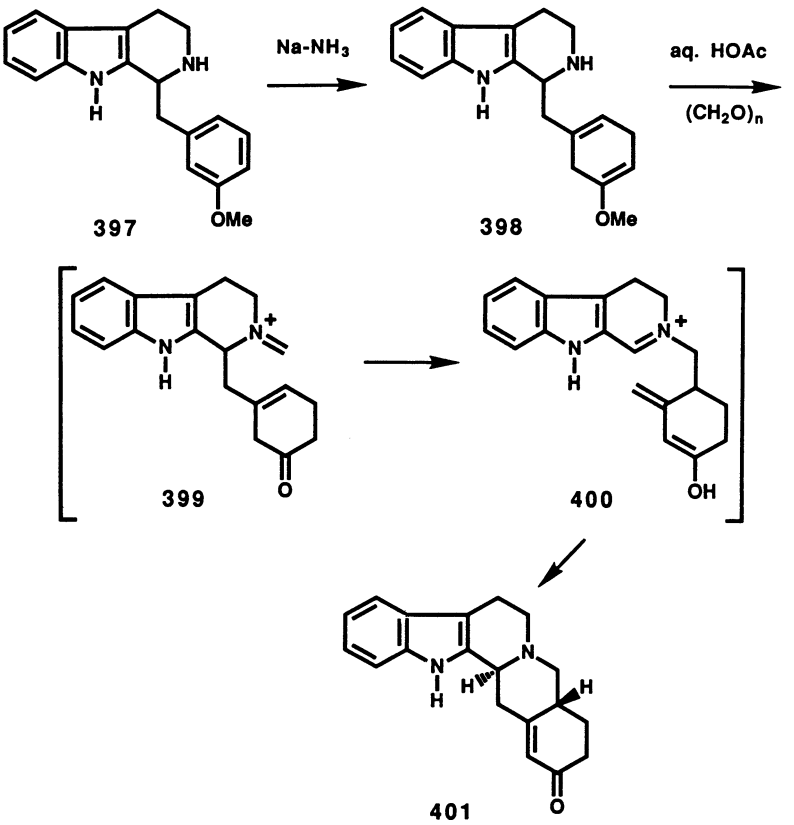
SCHEME 3.66



SCHEME 3.67

Meyers has investigated the conversion of β-carboline derivatives to pentacyclic yohimbines initially in methodology studies and then in target oriented investigations. In their preliminary efforts, Meyers and his coworkers converted tetrahydro-β-carboline **402** to formamidine **403** (Scheme 3.69) (118). Alkylation of the lithio-anion of **403** followed by removal of the formamidine moiety and subsequent acid induced lactamization afforded **406** which possesses the pentacyclic yohimbine skeleton.

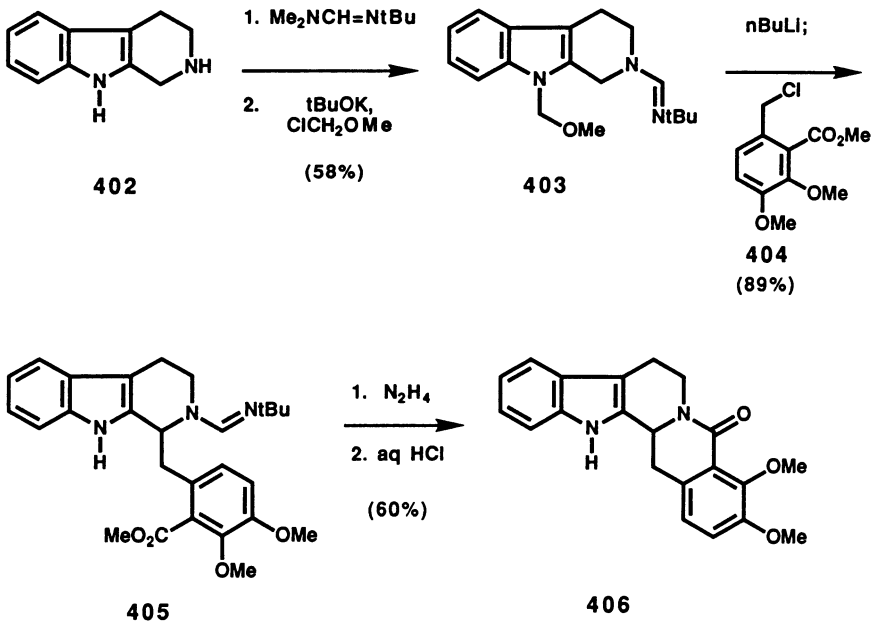
Next, Meyers applied this methodology to the synthesis of yohimbone (**358**) (Scheme 3.70) (119) in an approach very similar to that employed by Winterfeldt (117). Tetrahydrocarboline **402** was converted to formamidine



SCHEME 3.68

407 which was alkylated to provide **408**. Removal of the formamidine group followed by Birch reduction afforded enol ether **398** (117). As in the Winterfeldt approach, the enol ether group was hydrolyzed and the resulting enone was treated with formaldehyde to afford the pentacyclic enone **401** which served as a precursor of yohimbone (**358**).

This methodology has also been used by Meyers in an asymmetric synthesis of (−)-yohimbone ((−)-**358**) (Scheme 3.71) (119). In this sequence, condensation of β-carboline **402** with the enantiomerically pure amidine **409** provided chiral formamidine **410**. Alkylation followed by hydrolytic cleavage of the methoxymethyl group and subsequent removal of the formamidine afforded **397** which upon reduction with dissolving metal gave enol ether **398**. When **398** was treated with acid followed by formaldehyde, cyclization occurred to produce racemic enone **401**. Meyers rationalized this stereochemical observation by stating that the sigmatropic rearrangement step

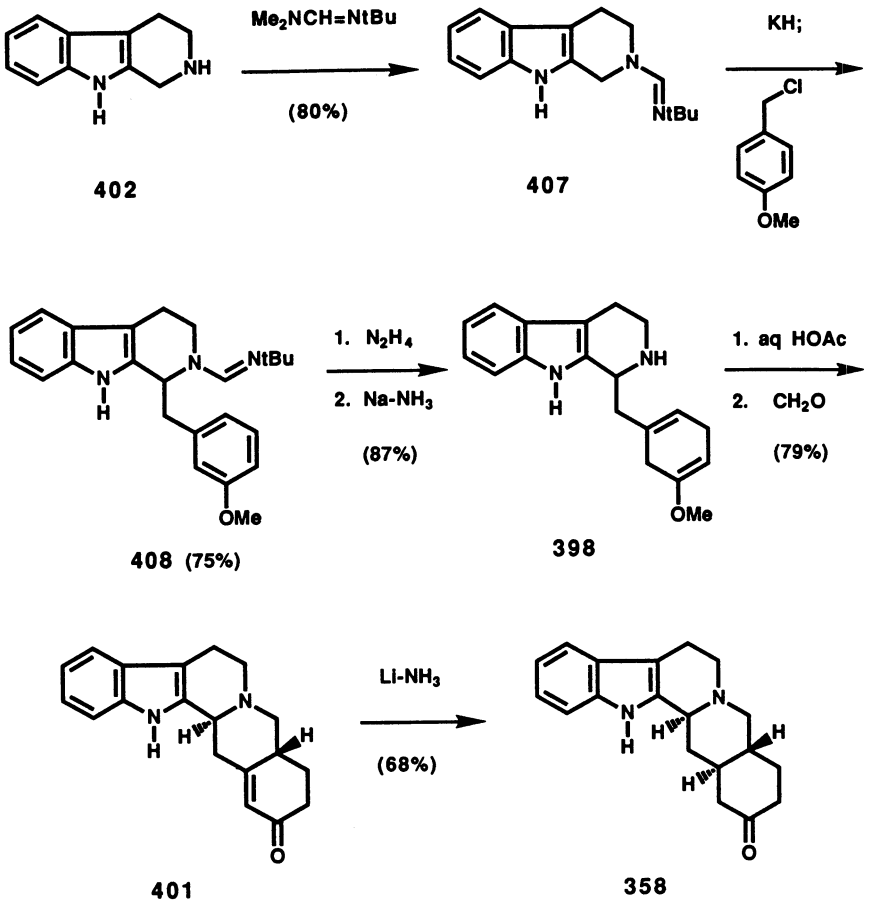


SCHEME 3.69

proceeded with retention of stereochemical integrity, but that the subsequent "ene" process (Scheme 3.68) occurred from both faces of the iminium salt and, as such, resulted in complete loss of chirality. To circumvent this problem, intermediate ketone **412** was reduced to yield alcohol **413**. When this alcohol was treated with formaldehyde and acid, pentacyclic derivative **414** formed. Swern oxidation followed by alumina-mediated methanol elimination afforded **401** which was reduced to yield enantiomerically pure (−)-yohimbone (**(−)-358**).

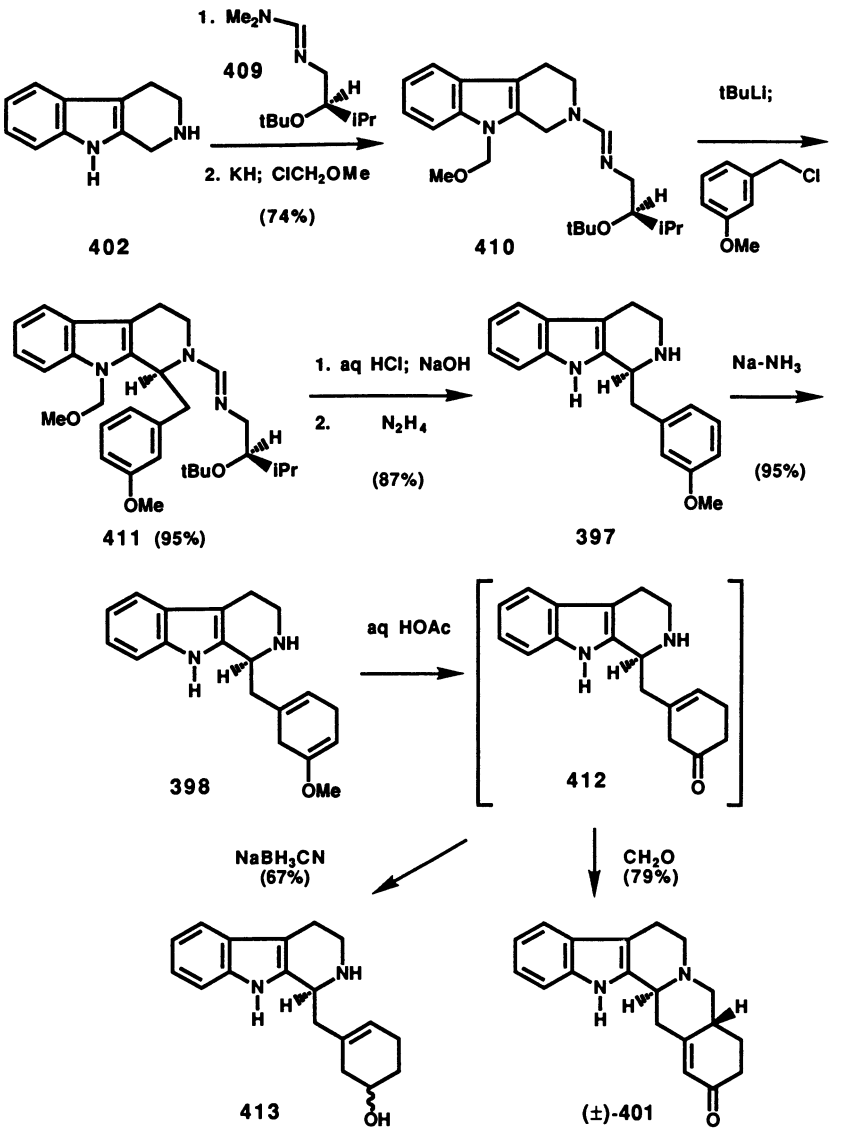
Yet another example of a β-carboline elaboration of the yohimbine skeleton is found in the work of Danishefsky (Scheme 3.72) (120). Diels-Alder reaction of β-carbolines **255** or **415** with the readily prepared diene **416** afforded pentacyclic lactams **417** or **418**, respectively. Amide reduction of **417** followed by enol ether hydrolysis afforded known yohimbane ketone **419** (114, 121). Attempts to isomerize the π-bond in **419** to give the α,β-unsaturated ketone **401** were unsuccessful. Nevertheless, this chemistry demonstrates how Diels-Alder reactions of β-carboline derivatives can be used to construct the pentacyclic skeleton of the yohimbines in an efficient fashion.

In a manner similar to Danishefsky, Hua and his coworkers also utilized a cycloaddition strategy to prepare (−)-alloyohimbane (**(−)-82**) and (+)-epialloyohimbane (**(+)-426**) (Scheme 3.73) (122). Harmalane (**336**) was converted to the corresponding sulfoxide **420**. The D and E rings of the yohim-

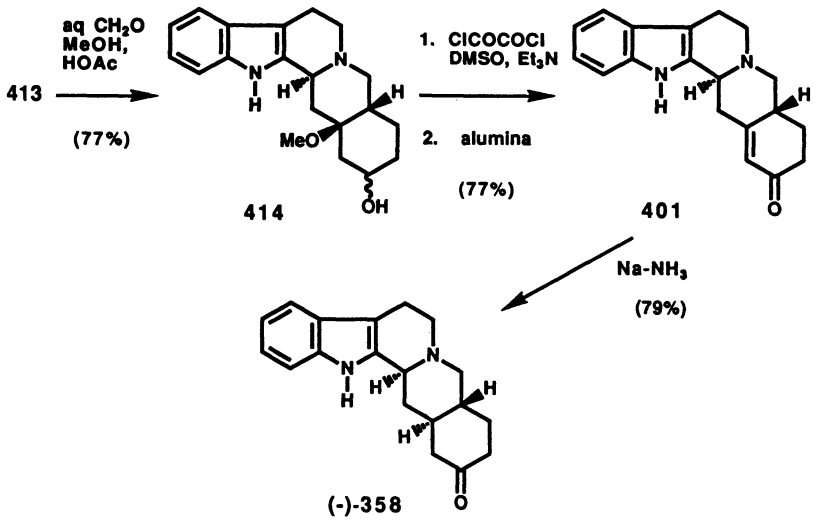


SCHEME 3.70

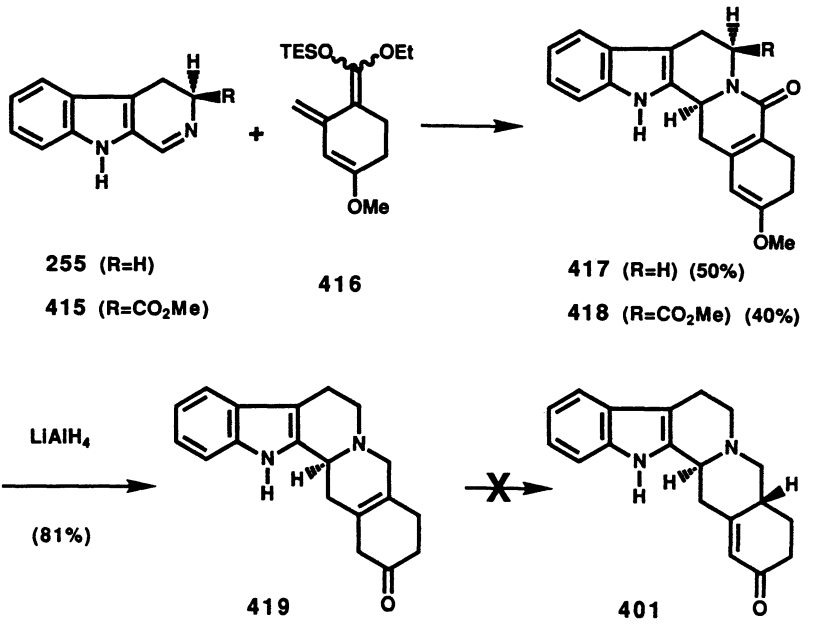
bane were elegantly appended in a single step by condensation of **420** with methyl cyclohexenecarboxylate (**421**) to yield the pentacyclic products **422** and **423**. These intermediates were believed to be separable, slowly interconverting rotamers which arose from restricted rotation about the C(14) sulfur bond. Exact structure assignments, however, could not be made. Each of these compounds was independently converted to the targets. Intermediate **422** was reduced to yield diastereomeric sulfoxides **424** and **425**. Desulfurization followed by amide reduction afforded (−)-alloyohimbane ((−)-**82**) and (+)-epialloyohimbane ((+)-**426**), respectively, in 57% overall yield from **422**. Alternatively, when rotamer **423** was treated with sodium cyanoborohydride, a 1 : 2 mixture of **424** and **425** formed in an 80% yield. The desulfurization-reduction sequence was repeated as before to provide **82** and **426** in 55% yield from **423**.


1. Me2N
409
tBuO
iPr
2. KH; ClCH2OMe
(74%)
402
410
tBuLi;
Cl
OMe
MeO
1. aq HCl; NaOH
2. N2H4
(87%)
411 (95%)
397
Na-NH3
(95%)
398
aq HOAc
412
NaBH3CN
(67%)
CH2O
(79%)
413
(±)-401

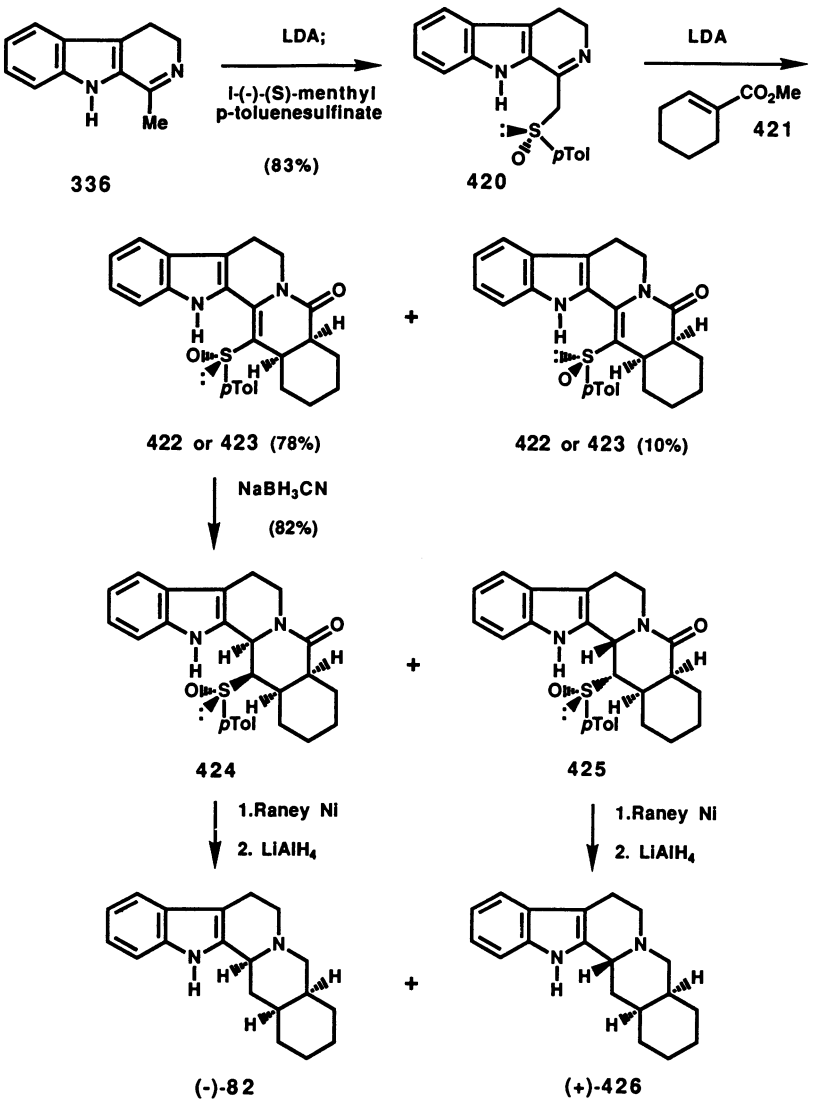
SCHEME 3.71



SCHEME 3.71 (*cont.*)

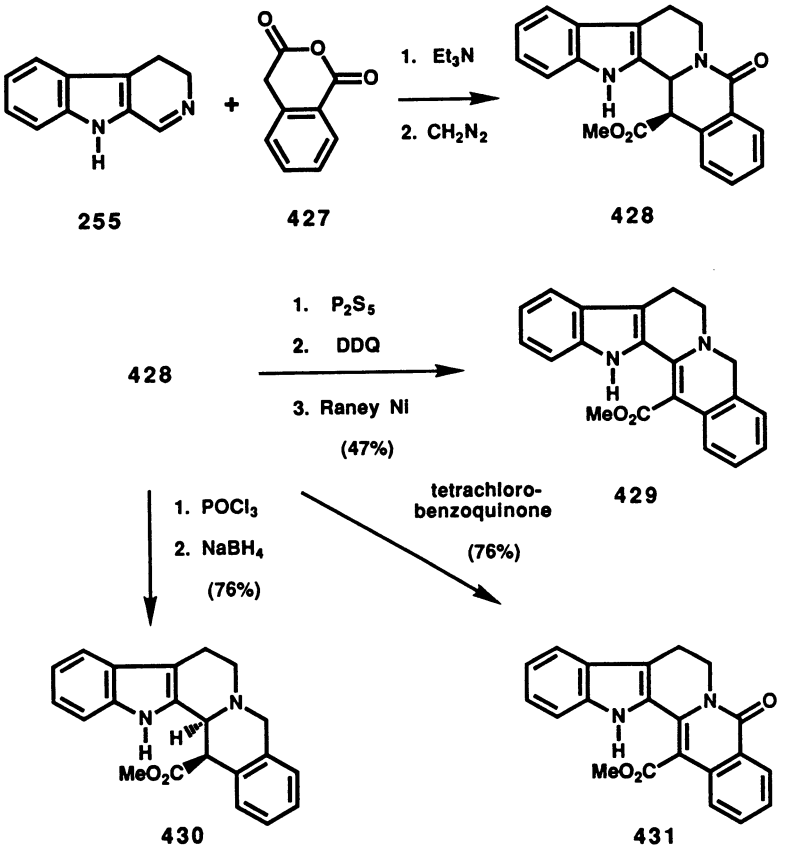


SCHEME 3.72



SCHEME 3.73

Yet another example of yohimbine alkaloid synthesis by use of a single step appendage of the DE rings onto a ABC-ring containing unit is found in Haimova's concise synthesis of several isogambirtannine derivatives (Scheme 3.74) (123, 124). Condensation of carboline **255** with homophthalic anhydride (**427**) gave the pentacyclic intermediate **428**. Conversion of this substance to the corresponding thiolactam followed by oxidation and desulfurization provided isogambirtannine (**429**). Alternatively, direct reduction of **428** afforded

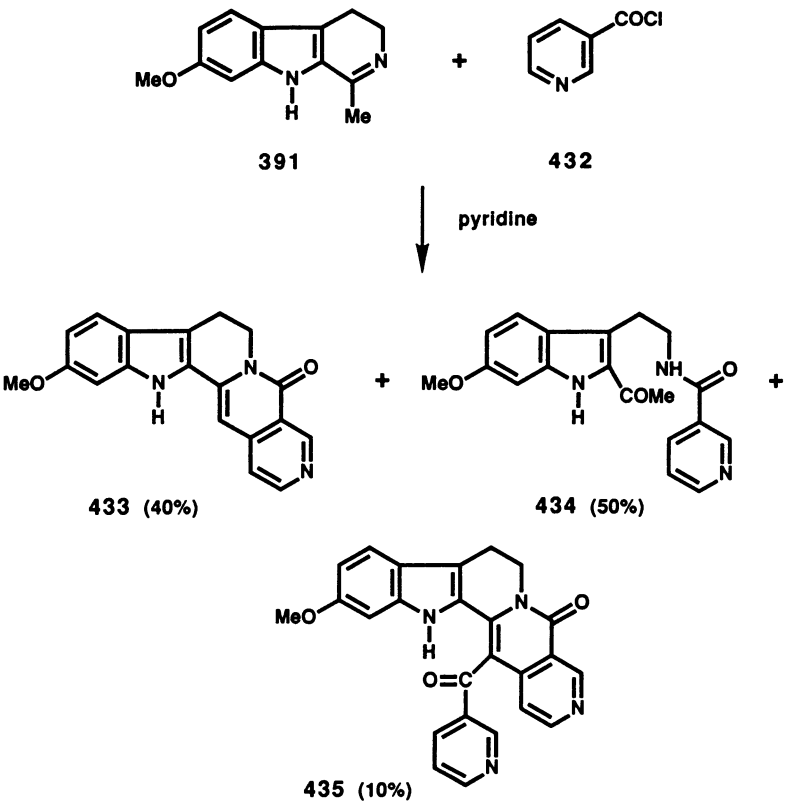


SCHEME 3.74

isodihydrogambirtannine (**430**) while oxidation of this material gave iso-oxogambirtannine (**431**).

In the syntheses of 11-methoxynauclefine (**433**) (Scheme 3.75) and 11-methoxyrutaecarpine (**440**) (Scheme 3.76), Atta-ur-Rahman and Ghazala demonstrated how the pentacyclic yohimbine skeleton could be rapidly constructed from β-carboline derivatives (125). Condensation of **391** with nicotinyl chloride (**432**) afforded the yohimbane **433** in addition to its seco-CD derivative **434** and pentacyclic analog **435**. Thus, a one step synthesis of 11-methoxynauclefine (**433**) was achieved.

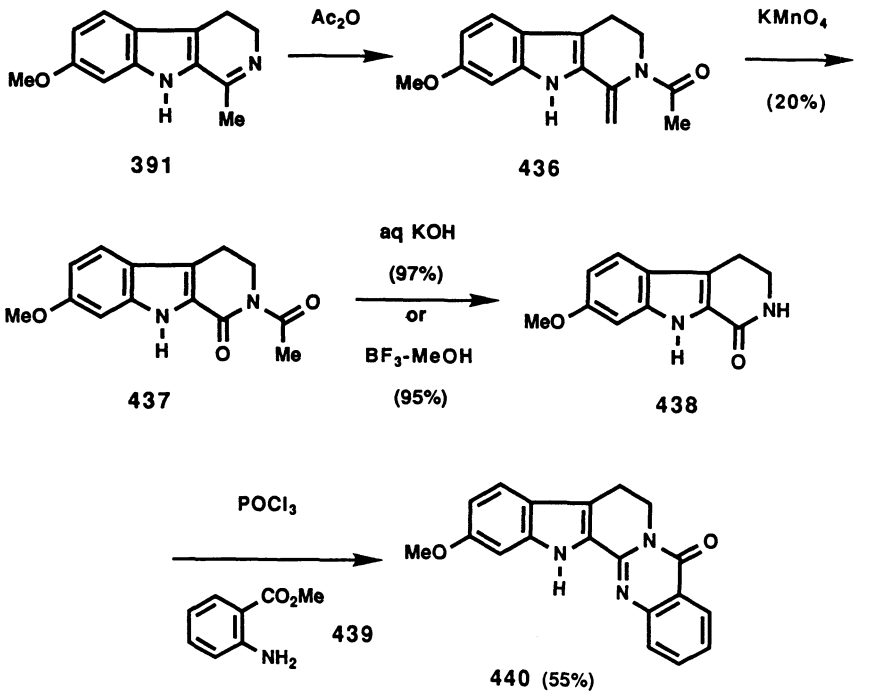
Furthermore, in this work derivatized β-carboline **391** was modified and elaborated to 11-methoxyrutaecarpine (**440**) (Scheme 3.76) (125). Acylation of **391** followed by oxidation afforded imide **437** which was oxidized and deacetylated to provide amide **438**. Condensation of **438** and amino benzoate **439** afforded target azayohimbane **440**.



SCHEME 3.75

Lastly, Atta-ur-Rahman and Firdous were able to generate a 21-carbomethoxy yohimbane derivative by a one-step appendage of the DE-rings to a β-carboline derivative (Scheme 3.77) (126). Methoxy substituted β-carboline **391** was treated with bis-anhydride **441**, prepared by condensation of cyclohexanone and oxalyl chloride, to afford pentacyclic zwitterionic substance **442** or **443**, depending on the alcoholic solvent employed. Reduction of **442** afforded the pentacyclic enamine **444** in addition to **445**, whereas reduction of the corresponding ethyl ester **443** provided **446** and **447**.

In summary, the research groups of Winterfeldt, Meyers, Danishefsky, Hua, Haimova, and Atta-ur-Rahman utilized very different methodologies to arrive at the desired pentacyclic yohimbine targets. The unifying theme is that each synthetic approach demonstrated how the DE-ring portion of the pentacyclic skeleta can be joined to a β-carboline derivative to generate yohimbines with high efficiency.

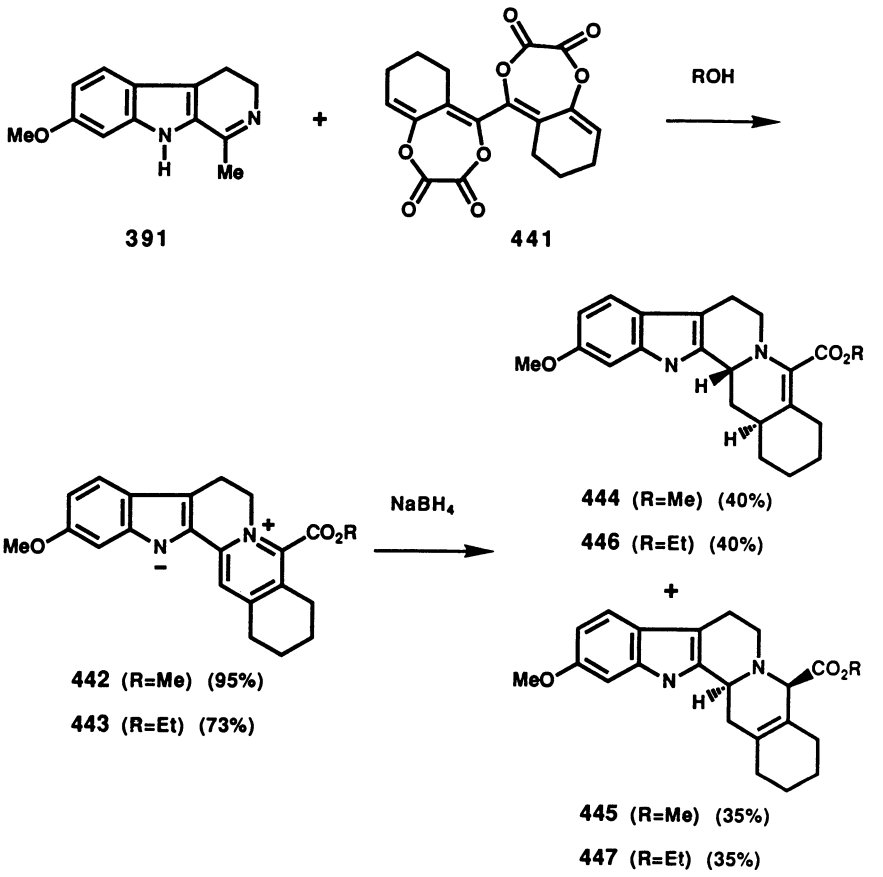


SCHEME 3.76

3.3.4. Additional Syntheses in Which Construction of the Yohimbine Skeleton is Completed by D-Ring Closure

A number of strategies for synthesis of yohimbine alkaloids involve sequences in which construction of the pentacyclic skeleton is completed by D-ring closure. These approaches typically do not utilize a β-carboline starting material and, as such, will be addressed separately in this section. Examples of this are found in syntheses of (−)-yohimbone ((−)-**358**) and (−)-alloyohimbone ((−)-**247**) by Okamura and Yamada (Scheme 3.78) (114). Both syntheses begin with the condensation of L-tryptophan carboxamide (**448**) with epoxide **449** which afforded the diastereomeric seco-D-ring intermediates **450** and **451** in a 1.5 : 1 ratio. Subsequent treatment of the cis isomer **450** with paraformaldehyde provided pentacyclic yohimbane **452**. Removal of the carboxamido group yielded **389** which was converted by dissolving metal reduction and hydrolysis to ketone **419**. Isomerization of the double bond followed by catalytic hydrogenation afforded the optically active yohimbones (−)-**358** and (−)-**247**.

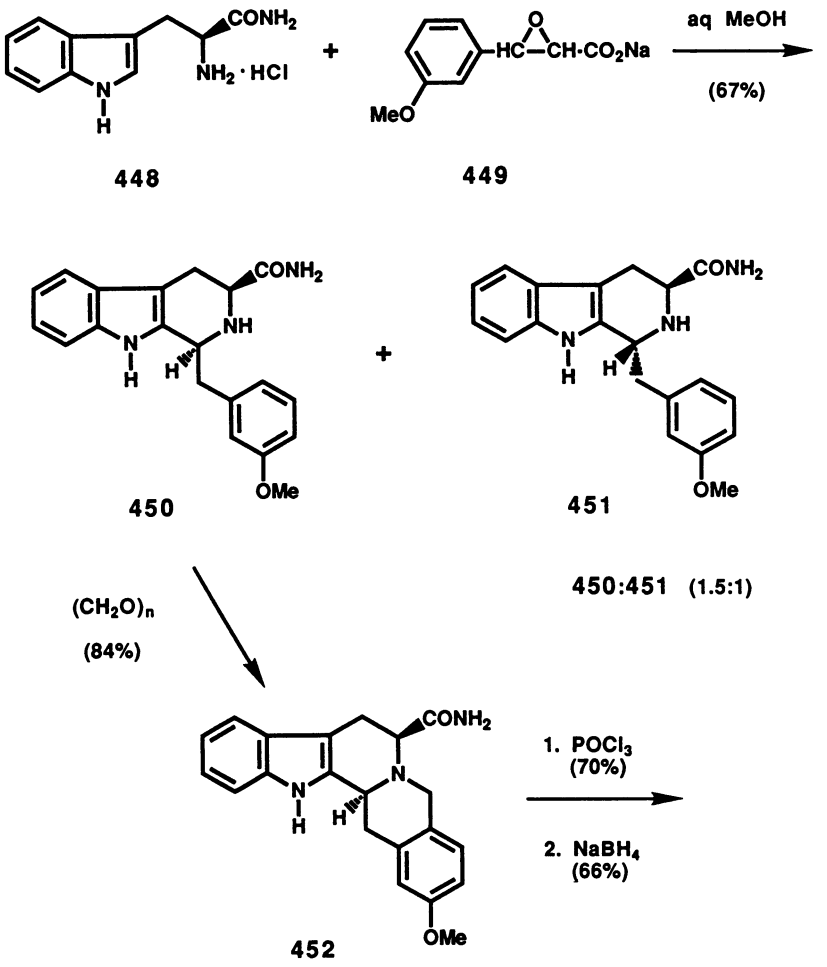
Kametani and his coworkers have synthesized alloyohimbane (**82**) and yohimbane (**120**) by using an approach in which the pentacyclic skeleton of the targets was completed by a D-ring closure process (Scheme 3.79) (127,



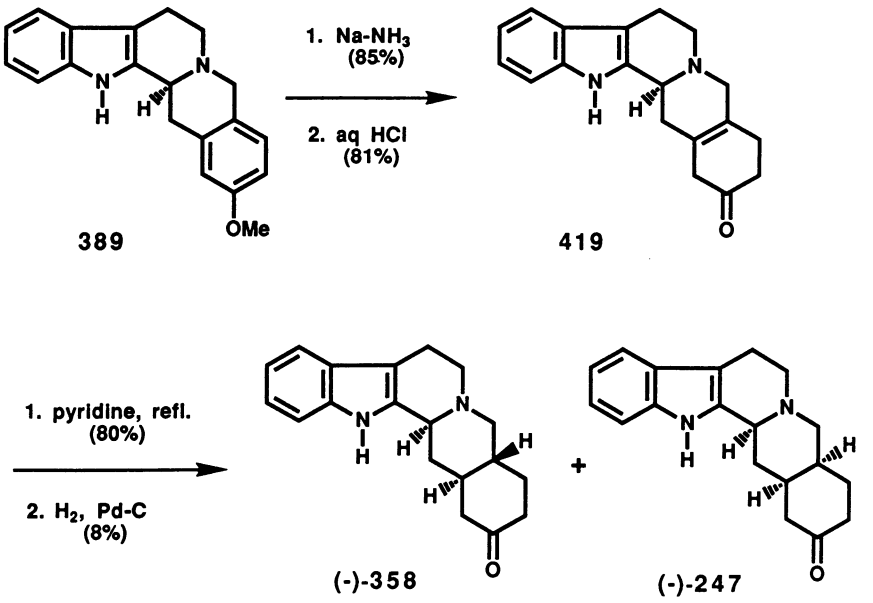
SCHEME 3.77

128). The synthesis began with the silver ion catalyzed reaction between diazoketone **453** and tryptamine which afforded amide **454**. When **454** was subjected to Bischler-Napieralski cyclization conditions, tetracyclic ester **455** formed along with the pentacyclic lactam **456** containing a trans DE-ring fusion. Treatment of **455** with mild base effected cyclization to produce the cis-fused pentacyclic lactam **457** which was reduced to provide alloyohimbane (**82**). Alternatively, lactam **456** was reduced to provide **458**, a substance which Ninomiya had previously converted to yohimbane (**120**) (61).

In a concise synthesis of 15,16,17,18,19,20-hexahydroyohimbane (**461**), Pandey and Tiwari also utilized D-ring closure as the final step in a sequence for generation of the yohimbane skeleton (Scheme 3.80). Cyclization of the amino-aryl bromide **459** was achieved by a palladium-mediated carbon monoxide insertion reaction. The resulting amide **460** was reduced to provide the desired target **461**.


CONH2
NH2 · HCl
N
H
448
+
O
HC—CH·CO2Na
MeO
449
aq MeOH
(67%)
CONH2
NH
N
H
H
OMe
450
+
CONH2
NH
N
H
H
OMe
451
450:451 (1.5:1)
(CH2O)n
(84%)
CONH2
N
N
H
H
OMe
452
1. POCl3
(70%)
2. NaBH4
(66%)

SCHEME 3.78

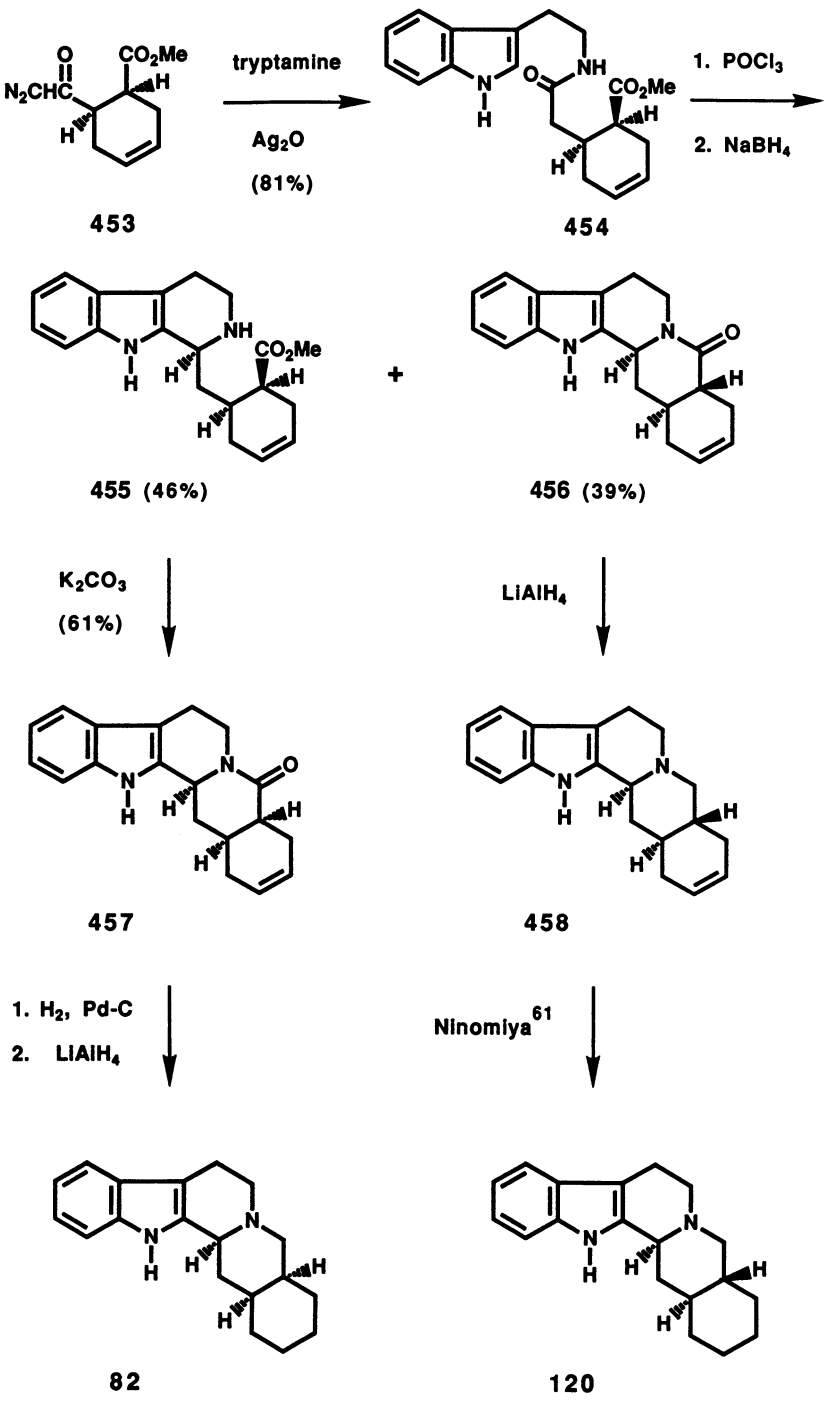


SCHEME 3.78 (*cont.*)

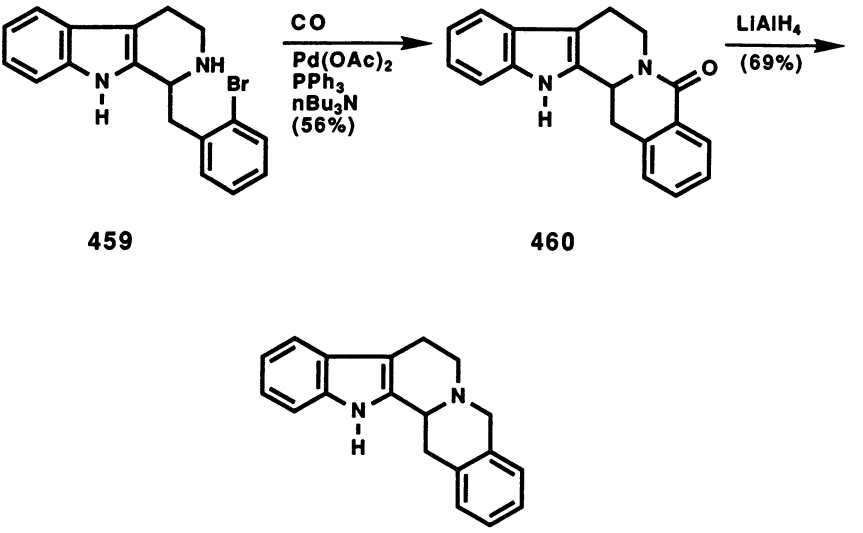
An interesting D-ring closure was used by Pakrashi and his coworkers to synthesize normalindine (**466**) and norisomalindine (**467**) (Scheme 3.81) (130). Imine **462**, prepared by condensation of tryptamine and 3-acetylpyridine, was reduced and the formed amine was acetylated to afford amide **463**. Bischler-Napieralski cyclization of **463** provided tetracyclic iminium bis-perchlorate salt **464** which when treated with triethylamine and pivaloyl chloride underwent a second cyclization to yield the pentacyclic enamine **465**. Reduction of the olefinic group in **465** afforded the desired azayohimbines, **466** and **467**.

3.4. Additional Yohimbine Alkaloid Synthesis Strategies

One strategy which has been successfully utilized in yohimbine alkaloid synthesis involves the use of cyclization reactions of either pyridinium or isoquinolinium cations to install the respective D or DE-ring units. Wenkert and his coworkers have popularized this methodology, as exemplified by their synthesis of hexahydroyohimbine (**473**) (Scheme 3.82) (131). In this sequence, *N*-tryptophylpyridinium salt **468**, prepared by reaction of 3-formylpyridine and tryptophyl bromide, was treated with the enolate anion of methyl acetoacetate to afford isoquinolone **469**. Methylation of this material provided isoquinolinium salt **472** which upon reduction followed by acid mediated cyclization provided yohimbane **473**. This methodology represents a rather


tryptamine
Ag2O
(81%)
1. POCl3
2. NaBH4
453
454
455 (46%)
456 (39%)
K2CO3
(61%)
LiAlH4
457
458
1. H2, Pd-C
2. LiAlH4
Ninomiya[61]
82
120

SCHEME 3.79

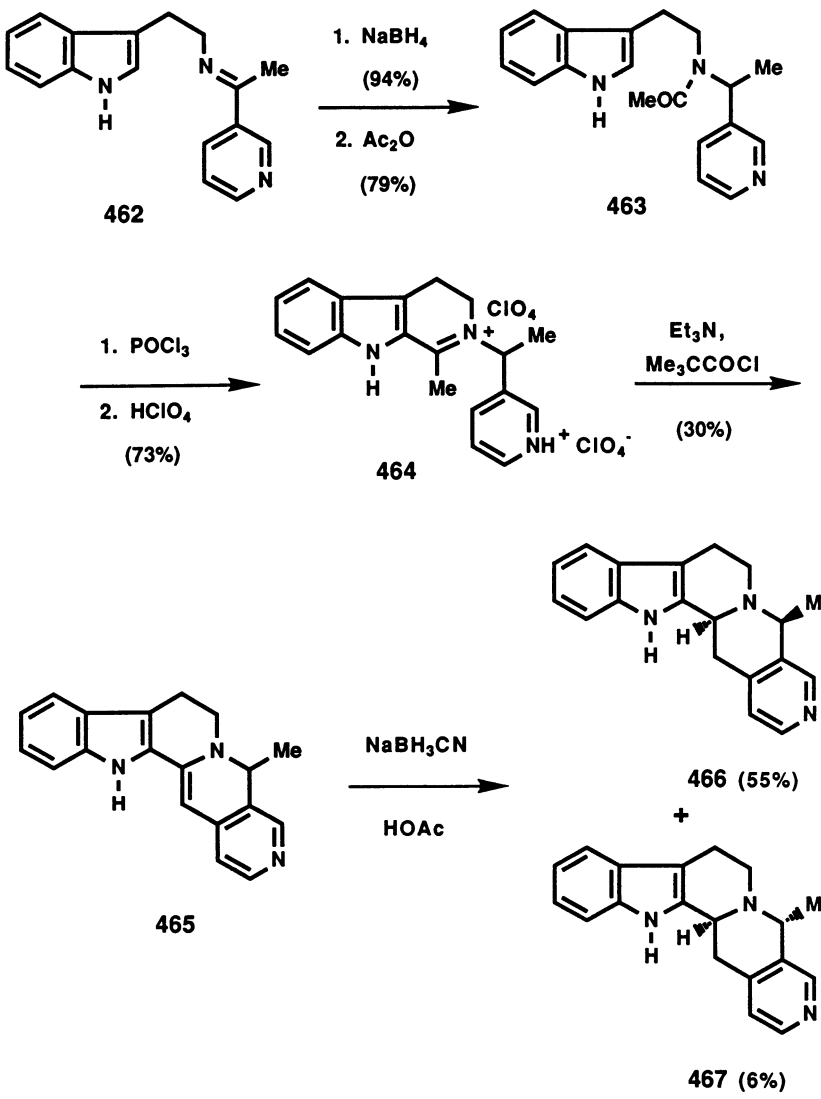


SCHEME 3.80

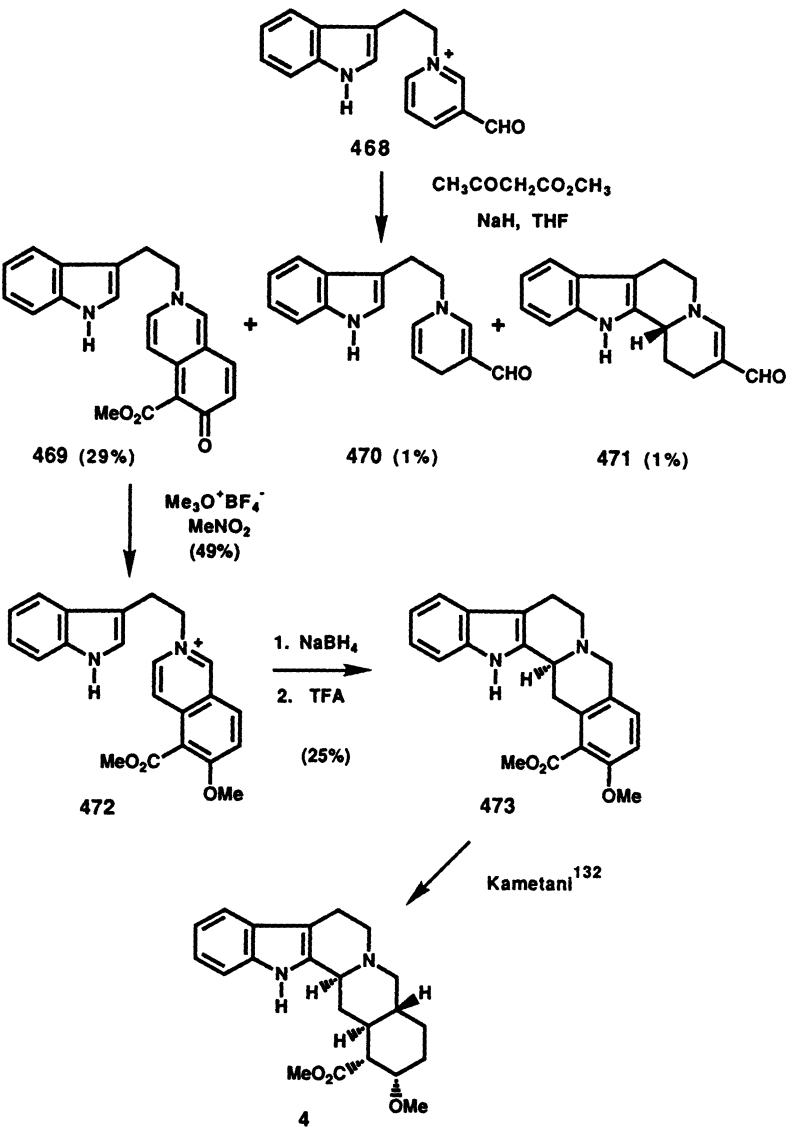
expedient procedure for construction of the yohimbine skeleton. Moreover, the route described constitutes a formal synthesis of yohimbine (**4**) as **473** is an intermediate employed in Kametani's synthesis of this natural product (132).

In a manner quite similar to that of Wenkert, Lounasmaa and his coworkers have utilized isoquinolinium salt cyclization reactions to prepare yohimbanes. For example, isoquinoline **474** was alkylated with tryptophyl bromide to yield *N*-tryptophyl isoquinolinium salt **476** (Scheme 3.83) (133). Reduction of **476** with dithionate directly afforded dihydrogambirtannine (**478**) which could be converted by oxidation to ourouparine (**480**). Further reduction with dithionite provided gambirtannine (**482**). These reactions were also carried out on compounds in the descarbomethoxy series resulting in syntheses of the gambirtannine analogs **479**, **481**, and **483**.

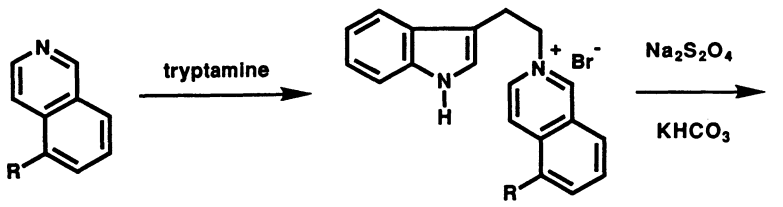
Lounasmaa also used isoquinolinium intermediates to prepare alloyohimbane (**82**) and epialloyohimbane (**426**) in an unusually succinct fashion (Scheme 3.84) (134). Accordingly, tetrahydroisoquinolinium salt **484** was converted to nitrile **485** which was cyclized to form the pentacyclic intermediate **486**. Catalytic hydrogenation gave alloyohimbane (**82**). Alternatively, **486** was converted to its BOC-protected derivative **487** which upon hydrogenation yielded the H(3)-epimer **488** in addition to recovered starting material (30%) and an indole ring 2,3-reduction product. It is interesting that hydrogenation of **487** gives a product with opposite C(3) *vs.* C(15), C(20) stereochemistry to that obtained from reduction of the unprotected derivative **486**. The reason


1. NaBH4
(94%)
2. Ac2O
(79%)
462
463
1. POCl3
2. HClO4
(73%)
464
Et3N,
Me3CCOCl
(30%)
NaBH3CN
HOAc
465
466 (55%)
+
467 (6%)

SCHEME 3.81

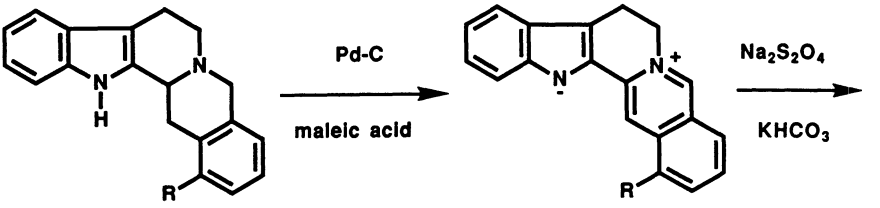

468
CH3COCH2CO2CH3
NaH, THF
469 (29%)
470 (1%)
471 (1%)
Me3O+BF4-
MeNO2
(49%)
1. NaBH4
2. TFA
(25%)
472
473
Kametani[132]
4

SCHEME 3.82



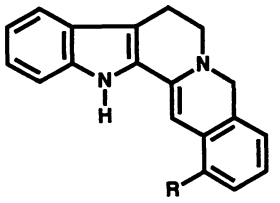
474 (R = CO_2Me)
475 (R = H)

476 (R = CO_2Me) (92%)
477 (R = H) (88%)



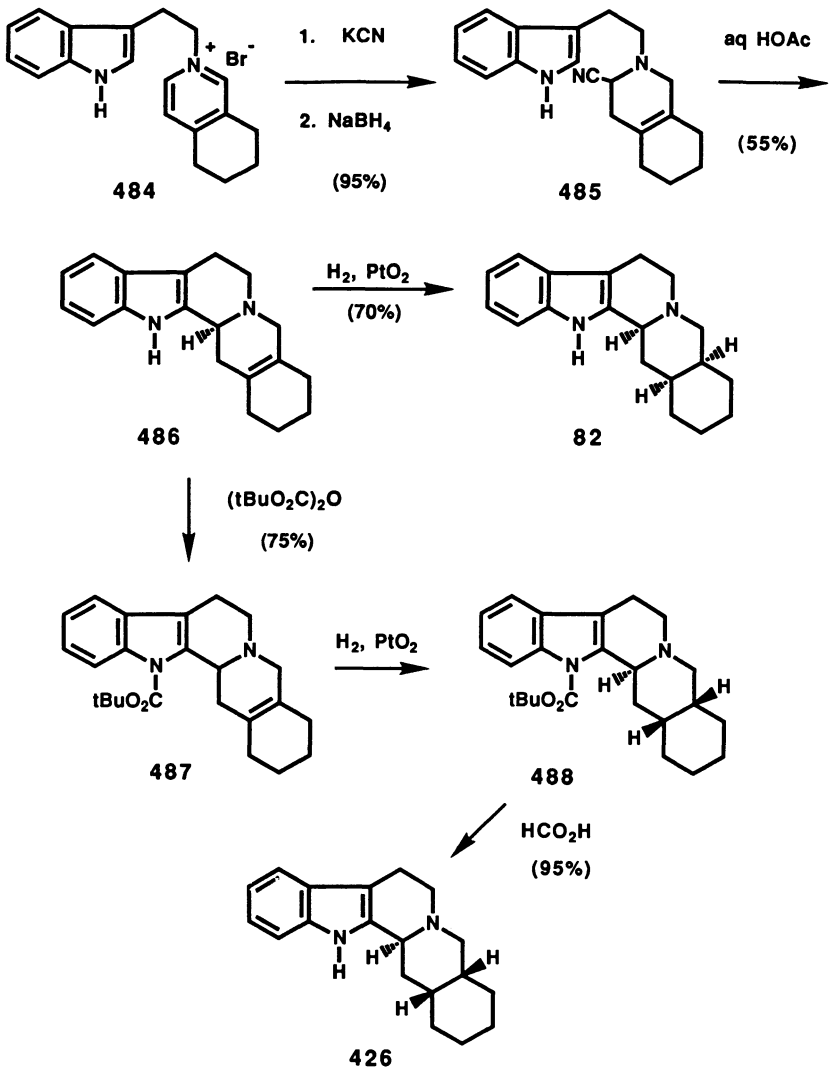
478 (R = CO_2Me) (14%)
479 (R = H) (26%)

480 (R = CO_2Me) (45%)
481 (R = H) (54%)

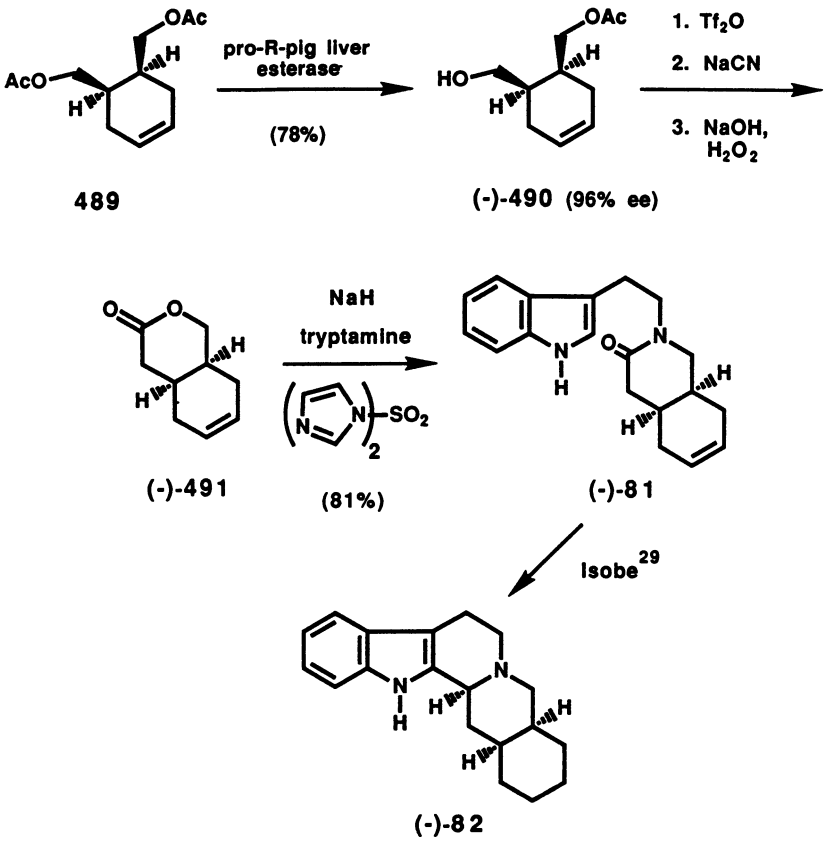


482 (R = CO_2Me) (26%)
483 (R = H) (22%)

SCHEME 3.83


1. KCN
2. NaBH4
(95%)
aq HOAc
(55%)
484
485
H2, PtO2
(70%)
486
82
(tBuO2C)2O
(75%)
H2, PtO2
tBuO2C
487
488
HCO2H
(95%)
426

SCHEME 3.84

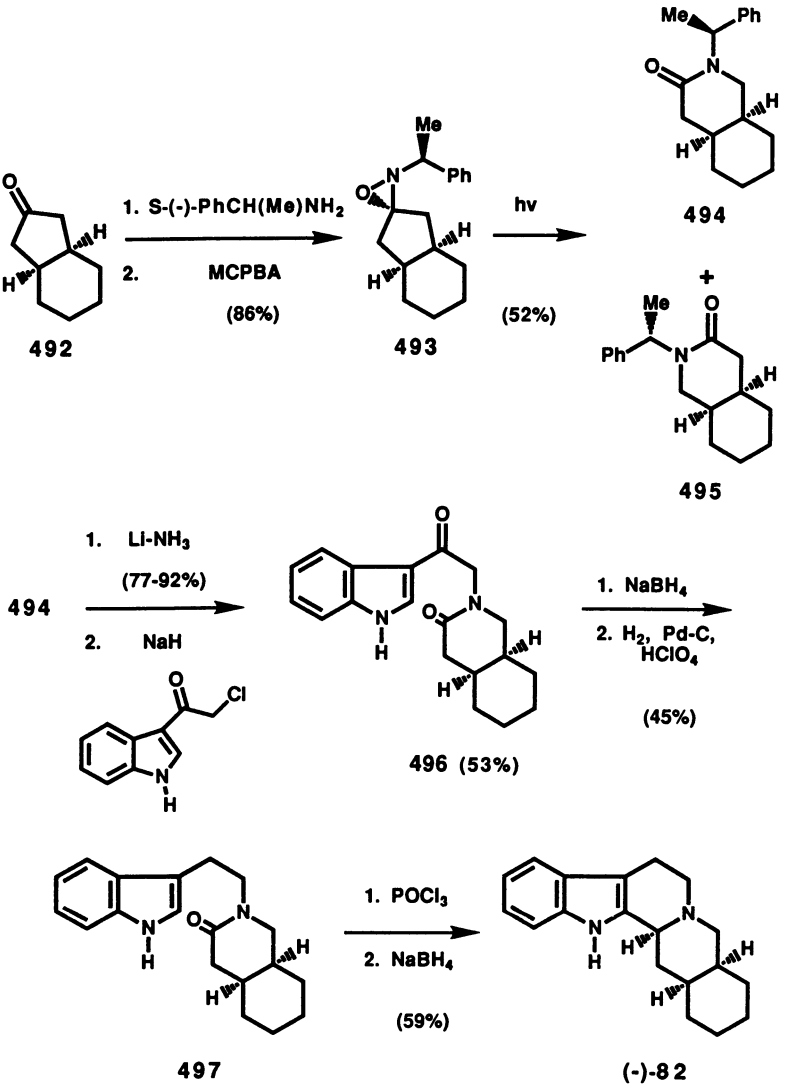


SCHEME 3.85

for this difference is not obvious. Removal of the *N*-protecting group of **488** afforded epialloyohimbane (**426**).

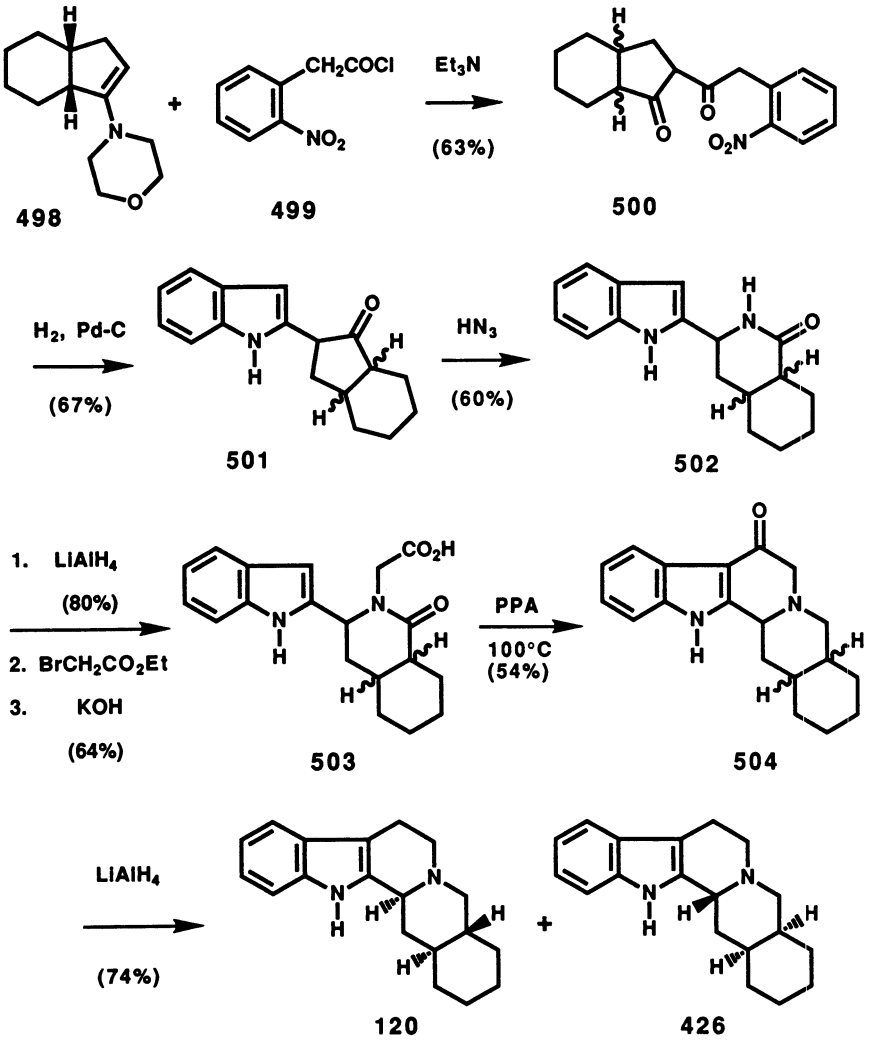
Recently, two enantioselective syntheses of (−)-alloyohimbane (**82**) have been reported. The synthetic route utilized by Riva's group featured an enzymatic hydrolysis as the key step (Scheme 3.85) (135). Prochiral diester **489** was hydrolyzed with pig liver esterase to provide the hydroxyester **(−)-490** in good yield and with a high enantiomeric excess. One carbon homologation of **(−)-490** followed by subsequent lactonization generated the bicyclic lactone **(−)-491**. Condensation of **(−)-491** with tryptamine afforded tetracyclic lactam **(−)-81**, an intermediate in Isobe's synthesis of (−)-alloyohimbane (**(−)-82**).

Aube and his coworkers have also carried out an enantioselective synthesis of (−)-alloyohimbane (**(−)-82**) (Scheme 3.86) (136, 137). Their route highlighted a photochemical nitrogen insertion reaction of the intermediate oxaziridine **493**. Bicyclic ketone **492** was condensed with (*S*)-(−)-α-



SCHEME 3.86

methylbenzylamine to give an imine which was epoxidized to afford a mixture of the four possible diastereomeric oxaziridines in a ratio of 66 : 17 : 10 : 7. The major product in this mixture was the desired **493**. Irradiation of the oxaziridine **493** effected ring expansion via nitrogen insertion to afford lactam **494** in addition to its regioisomer **495**. Lactam **494** was debenzylated and then alkylated to provide **496** which upon reduction of the ketone and Bischler-Napieralski cyclization gave (−)-alloyohimbane ((−)-**82**).

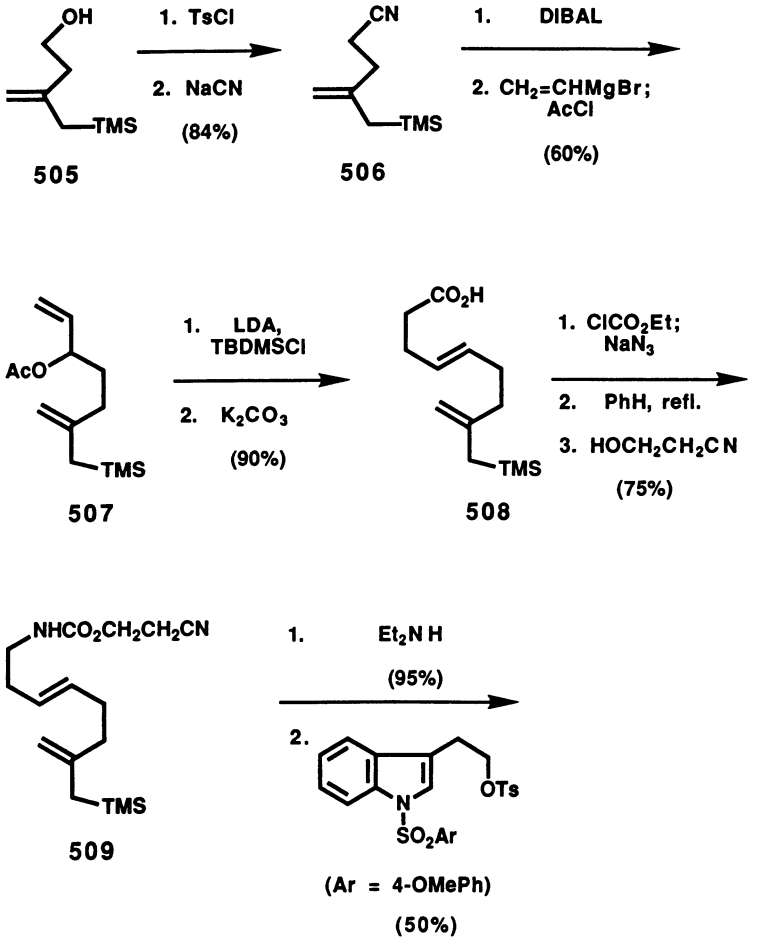


SCHEME 3.87

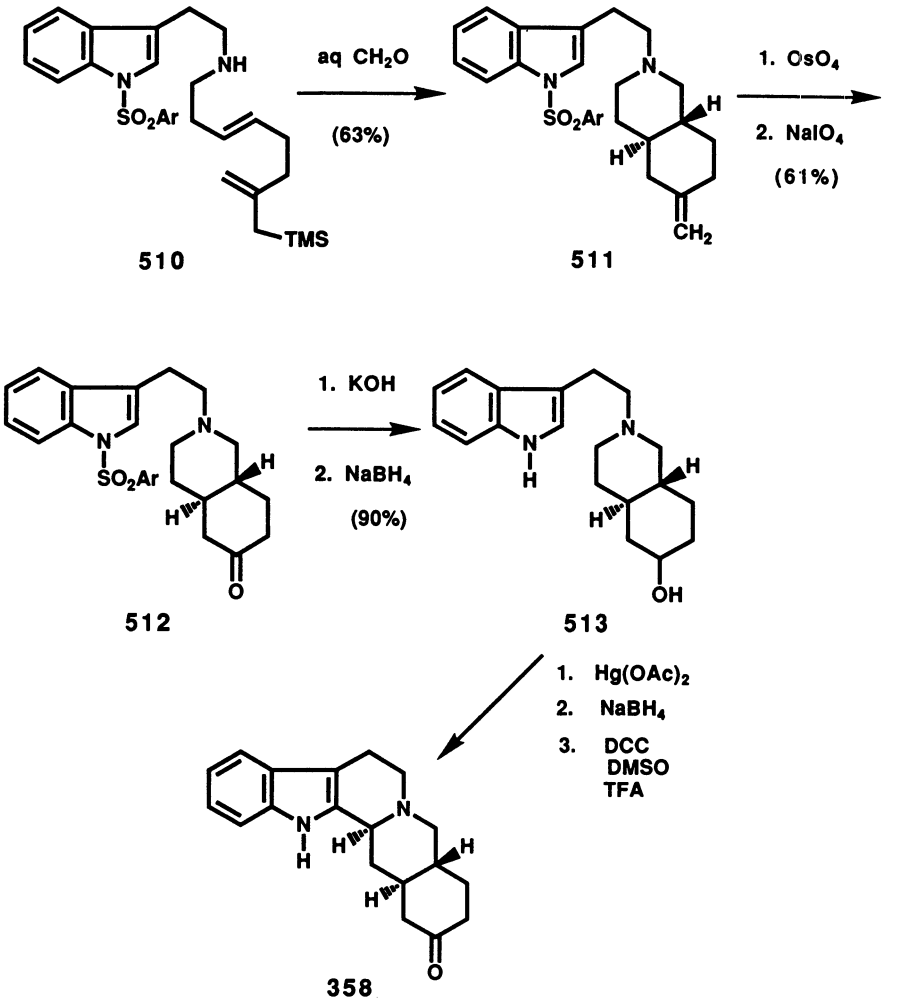
Thus far, we have reviewed several synthetic approaches to the yohimbines that involve C-ring closures by formation of the bond formation between C(2) and C(3). Interestingly, in the synthesis of yohimbane (**120**) and epialloyohimbane (**426**), Rosenmund and his coworkers employed a C(6), C(7) bond forming cyclization as a key step in the routes to these targets (Scheme 3.87) (138). Condensation of bicyclic enamine **498** with acid chloride **499** produced indanone **500**. Catalytic reduction of the nitro group in **500** resulted in cyclization to produce ketoindole **501**. Schmidt rearrangement then afforded tetracyclic lactam **502**. Reduction of the amide followed by alkylation of the

resulting amine with ethyl bromoacetate and subsequent saponification produced carboxylic acid **503**. Treatment of this substance with polyphosphoric acid promoted cyclization to generate the pentacyclic ketone **504** which was reduced to afford a mixture of yohimbane (**120**) and epialloyohimbane (**426**).

In an elegant synthesis of yohimbone (**358**), Grieco and Fobare took advantage of an interesting polyolefin cyclization reaction in which an iminium ion acted as the initiator and an allyl silane as the terminator to simultaneously generate the D and E-rings (Scheme 3.88) (139). In this route, alcohol **505** was converted to nitrile **506** which after reduction to the corresponding aldehyde followed by a two carbon homologation and subsequent acylation afforded allylic acetate **507**. Application of the Ireland ester-enolate Claisen rearrangement protocol gave carboxylic acid **508**. Next, a modified



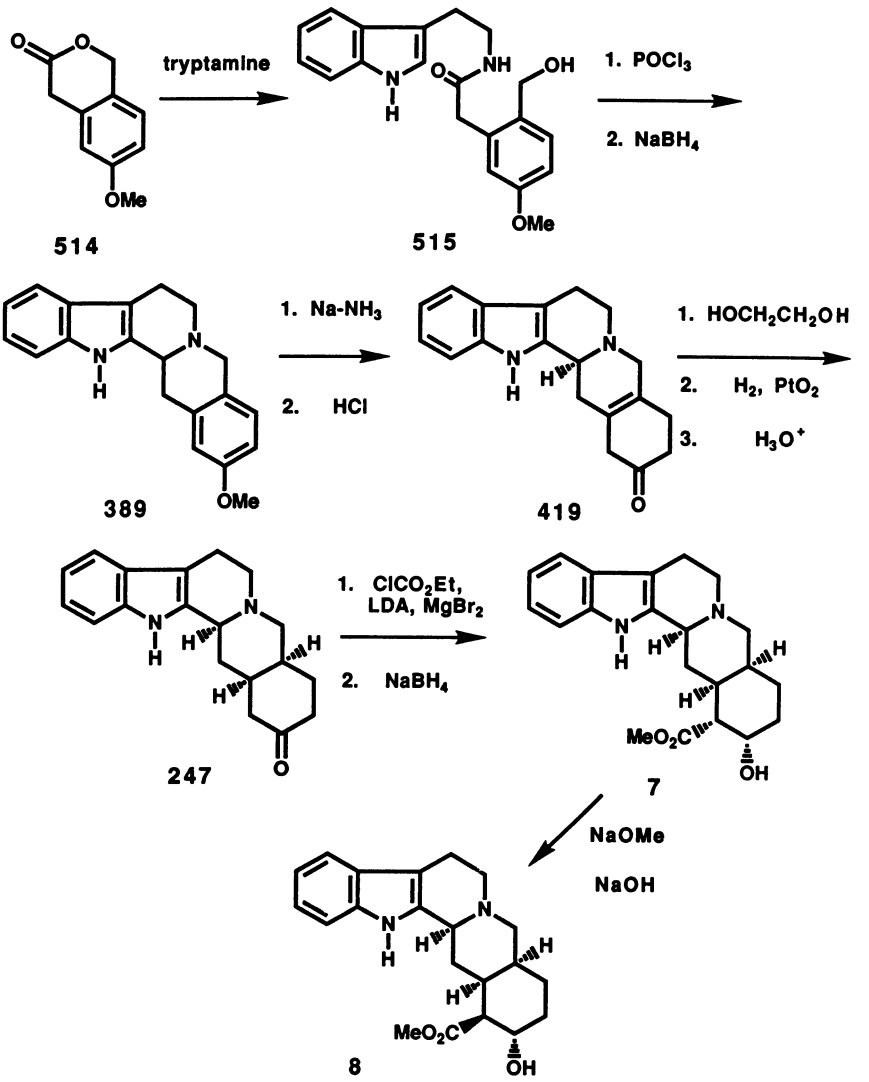
SCHEME 3.88



SCHEME 3.88 (*cont.*)

Curtius rearrangement with capture of the intermediate isocyanate by 3-hydroxypropionitrile yielded carbamate **509**. Deprotection of the nitrogon in **509** followed by alkylation afforded **510**, the polyolefin cyclization substrate. Treatment of this material with formaldehyde effected the desired cyclization yielding the tetracyclic intermediate **511** in high yield. Conversion to ketone **512** by excision of the exocyclic methylene group was followed by removal of the *N*-sulfonyl group and reduction to provide alcohol **513**. C-ring closure and oxidation of the hydroxyl group then afforded yohimbone (**358**).

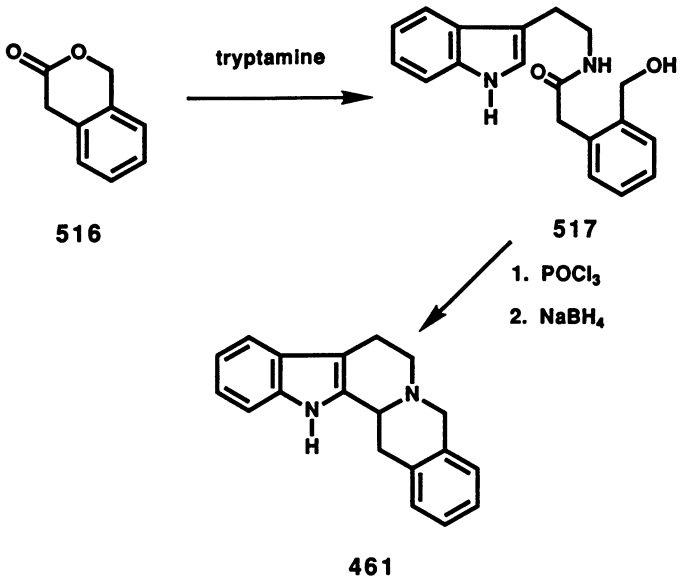
Chatterjee has synthesized a variety of yohimbine alkaloids utilizing 3-isochromanone derivatives as DE-ring precursors (Scheme 3.89) (140). For



SCHEME 3.89

example, condensation of 6-methoxy isochromanone (**514**) and tryptamine gave amide **515** which upon treatment with phosphorus oxychloride and subsequent reduction afforded pentacyclic yohimbane **389**. Birch reduction of this substance followed by acid hydrolysis provided ketone **419** which served as a precursor of both alloyohimbine (**7**) and its C(16)-epimer, rawolscine (α-yohimbine) (**8**).

A similar approach was used by Chatterjee in a synthesis of decarbo-

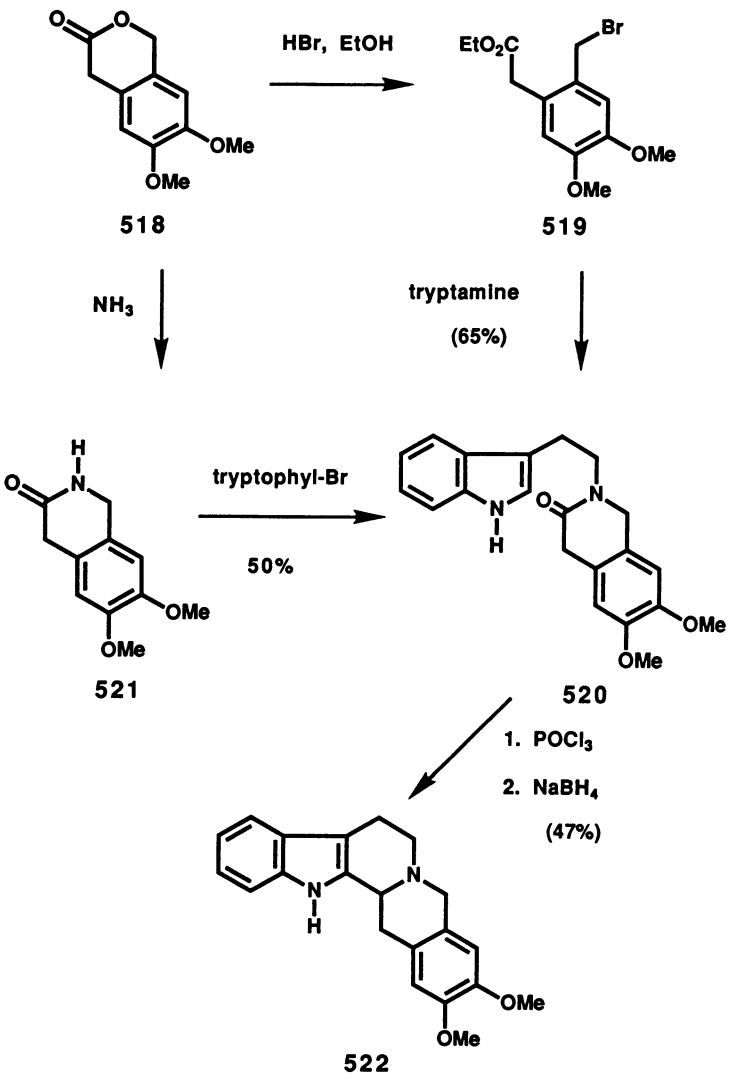


SCHEME 3.90

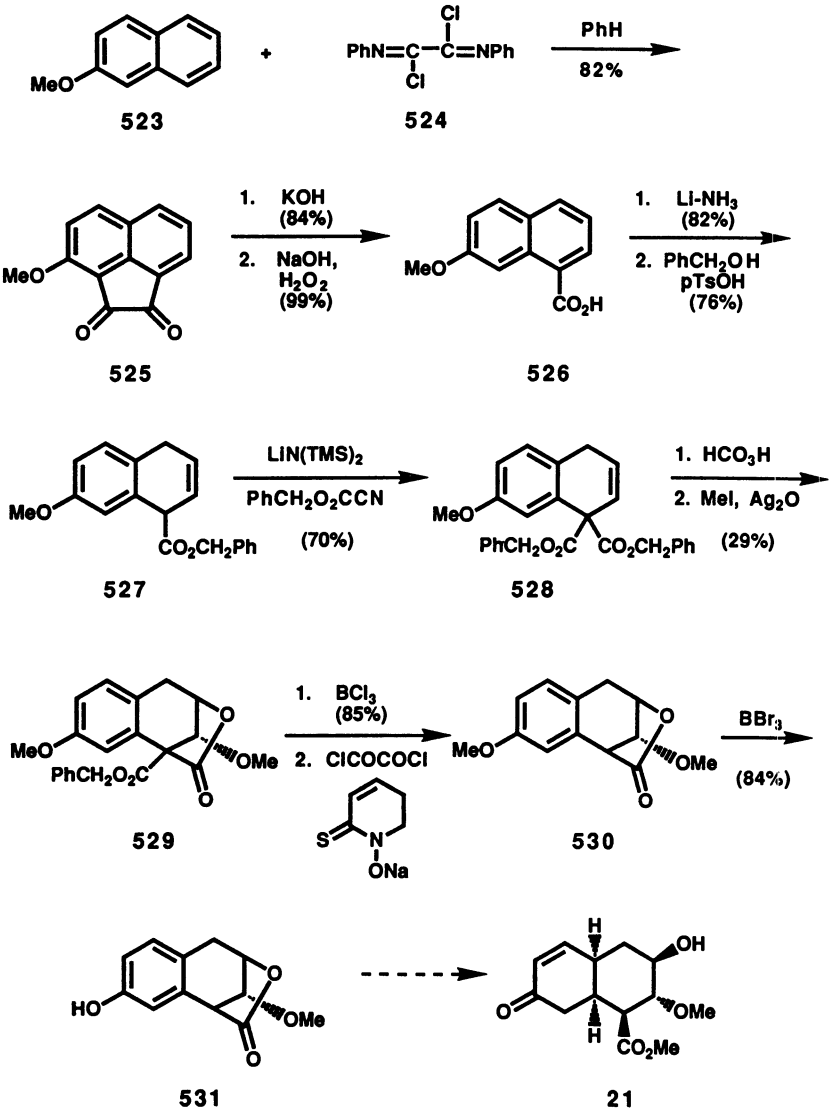
methoxy dihydrogambirtannine (**461**) (Scheme 3.90) (140). Here reaction of 3-isochromanone (**516**) with tryptamine afforded amide **517** which was cyclized to generate **461**.

A related synthetic approach to yohimbine synthesis was utilized by Pandey and his coworkers (Scheme 3.91) (141, 142). Thus, isochromanone **518** was converted to bromoester **519** which was then condensed with tryptamine to afford tetracyclic amide **520**. Alternatively, **520** could be prepared by treatment of **518** with ammonia to provide **521** which was then *N*-tryptophylated. Cyclization of **520** under standard conditions provided the dimethoxyyohimbane derivative **522**.

A final synthetic effort in the yohimbine alkaloid area concerns the studies reported by Loewenthal and his coworkers (Scheme 3.92) (143). The aim was to develop an efficient method to prepare the bicyclic enone **21**, which serves as a key intermediate in the Woodward reserpine synthesis strategy (19). The route for preparation of **21** began with Friedel Crafts reaction of 2-methoxynaphthalene (**523**) with the oxalyl chloride equivalent **524**, a process which afforded the acenaphthenoquinone **525**. Oxidative-decarboxylation of **525** yielded the naphthalene-carboxylic acid **526** which was transformed by Birch reduction and esterification to the dihydro-derivative **527**. Carboxylation then provided geminal diester **528** which was epoxidized. Sequential lactonization and methylation afforded tricyclic lactone **529**. Ester cleavage with subsequent decarboxylation gave lactone **530** which was demethylated to provide **531**. While no further effort was given to the development of this


HBr, EtOH
EtO2C
Br
OMe
OMe
518
519
NH3
tryptamine
(65%)
tryptophyl-Br
50%
521
520
1. POCl3
2. NaBH4
(47%)
522

SCHEME 3.91


MeO
523
PhN=C(Cl)-C(Cl)=NPh
524
PhH
82%
1. KOH (84%)
2. NaOH, H2O2 (99%)
525
526
1. Li-NH3 (82%)
2. PhCH2OH pTsOH (76%)
LiN(TMS)2
PhCH2O2CCN
(70%)
527
528
1. HCO3H
2. MeI, Ag2O
(29%)
PhCH2O2C
CO2CH2Ph
1. BCl3 (85%)
2. ClCOCOCl
529
530
BBr3
(84%)
ONa
HO
531
21
OH
OMe
CO2Me

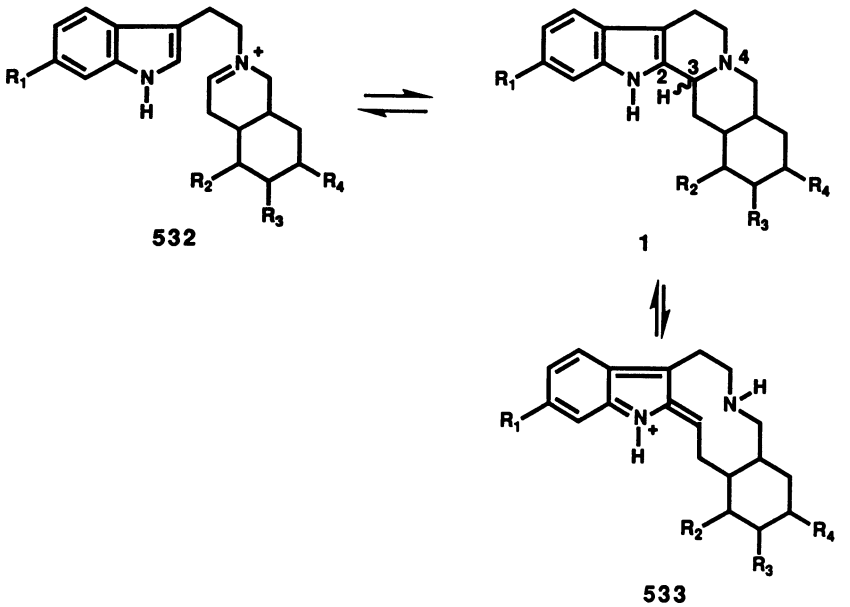
SCHEME 3.92

sequence, it is presumed that a sequence beginning with Birch reduction-hydrolysis of **530** would yield the key Woodward intermediate **21**.

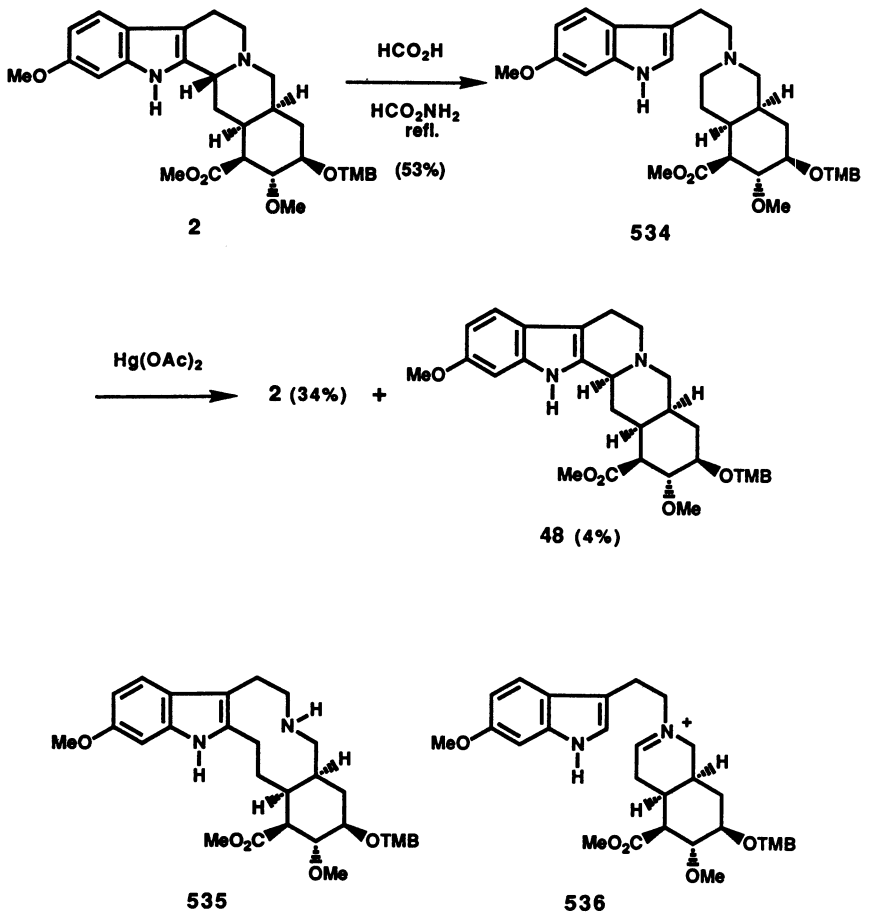
3.5. Investigations of C(3) Epimerization and C(2)–C(3) and C(3)–N(4) Bond Cleavage Processes

As can be seen by reviewing the chemistry outlined above, a variety of cleverly devised strategies have been employed for the synthesis of the structurally complex yohimbine alkaloids. One elusive problem in many of these approaches has been the proper adjustment of the C(3) stereochemistry, particularly in approaches to reserpine (**2**) and deserpidine (**3**). It is well-known that epimerization at C(3) of the yohimbine skeleton **1** can occur under acidic conditions presumably *via* a mechanism involving cleavage of either the C(2)–C(3) or C(3)–N(4) bond to afford the respective iminium cation **532** or α-indolylcarbinyl cation **533**. In this section, we will review investigations which focus on this epimerization process.

An early study in this area was conducted by Sakai and Ogawa (Scheme 3.94) (75, 76). They found that when reserpine (**2**) was heated in refluxing formic acid and formamide, only **534**, the product arising from C(2)–C(3) bond cleavage, was formed. Importantly, the tetracyclic amine **535**, which would have been generated by a C(3)–N(4) bond breakage, was not detected. These results indicate that acid catalyzed yohimbine ring cleavage—and presumably C(3) epimerization—proceeds *via* the iminium species **536**. More-



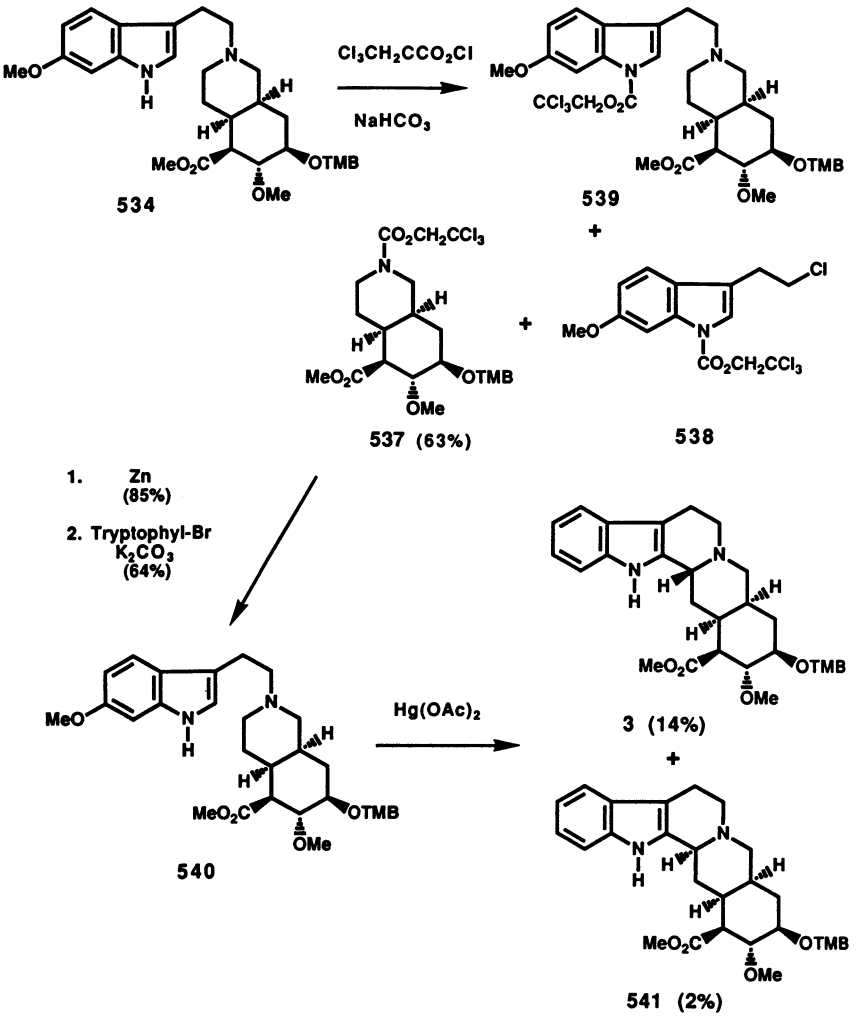
SCHEME 3.93



SCHEME 3.94

over, these workers found that when the seco-reserpine derivative **534** was treated with mercuric acetate, reserpine (**2**) was the major product formed in addition to a small amount of isoreserpine (**48**). Additionally, **534** was converted to deserpidine (**3**) (Scheme 3.95) (75). Accordingly, dealkylation of the tertiary amide afforded carbamate **537** which upon removal of the trichloroethoxycarbonyl group and subsequent tryptophylation provided **540**. Mercuric ion-mediated cyclization yielded deserpidine (**3**) in addition to a small amount of isodeserpidine (**541**). Thus, kinetically controlled cationic cyclization of the iminium ions related to **536** formed in the mercuric acetate induced reactions appears to favor production of the 3β-H stereochemistry.

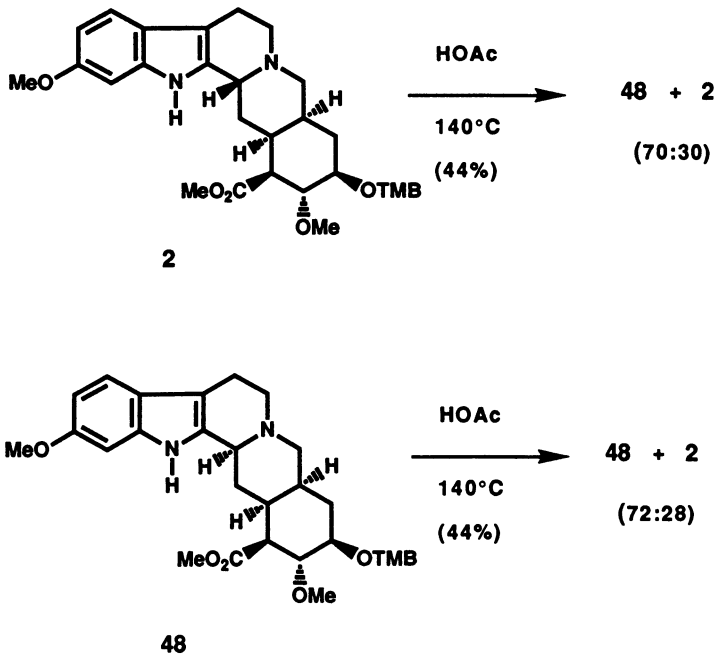
Schiffl and Pindur have examined the C(3) epimerization of reserpine (**2**)



SCHEME 3.95

and isoreserpine (**48**) occurring in refluxing acetic acid over extended time periods (Scheme 3.96) (79). When reserpine (**2**) was subjected to these conditions, a 70 : 30 mixture of isoreserpine (**48**) and reserpine (**2**) resulted. Under the same conditions, isoreserpine (**48**) gave rise to a 72 : 28 mixture of isoreserpine and reserpine. That a very similar product ratio resulted in each case is reflective of the relative thermodynamic stabilities of the two yohimbines.

Cook and coworkers have conducted a more recent investigation of the C-3

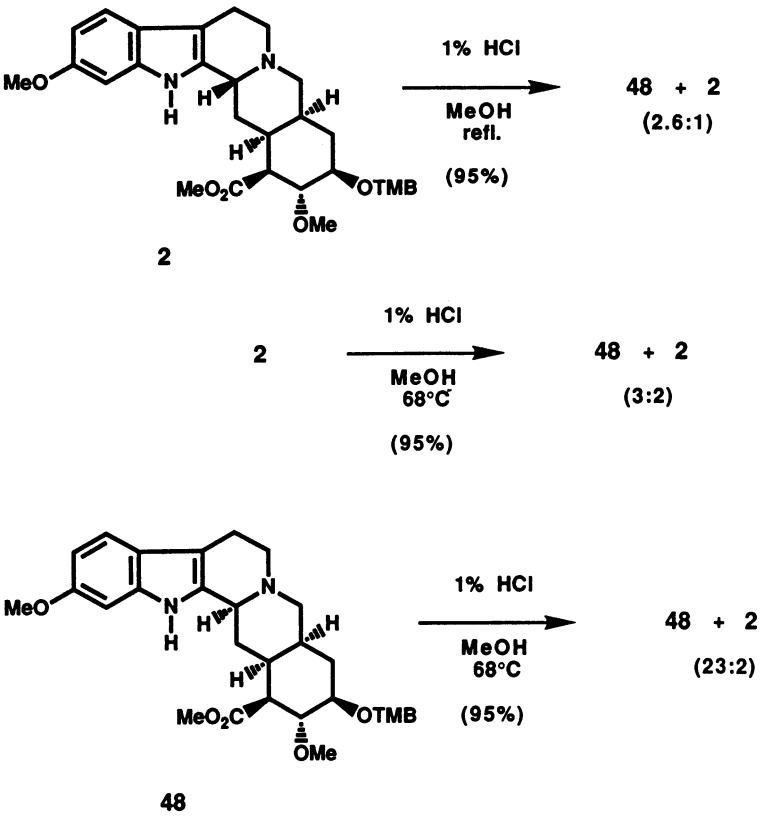


SCHEME 3.96

epimerization chemistry (Scheme 3.97) (80). Their observations were somewhat different from those of the Sakai (75, 76) and Pindur groups (79). For example, Cook found that upon prolonged heating at reflux in anhydrous methanolic-HCl, reserpine (**2**) gave isoreserpine (**48**) and reserpine (**2**) in a 2.6 : 1 ratio. When reserpine was heated in methanolic HCl for a shorter period of time, isoreserpine and reserpine were isolated in a 3 : 2 ratio. Under the same conditions, isoreserpine afforded a 23 : 2 mixture of isoreserpine to reserpine.

Bond cleavage reactions of reserpine and isoreserpine have also been investigated by Cook (Scheme 3.98) (80). When reserpine (**2**) was heated in acetic acid solution containing zinc, isoreserpine (**48**) and recovered reserpine (**2**) were formed along with a small amount of the C(3)–N(4) cleavage product **535** and a trace quantity of the C(2)–C(3) cleavage product, **534**. These results are strikingly different from those observed when reserpine was heated in a formic acid-formamide mixture (75, 76). Moreover, when isoreserpine (**48**) was subjected to these same reaction conditions, a very similar distribution of products was formed.

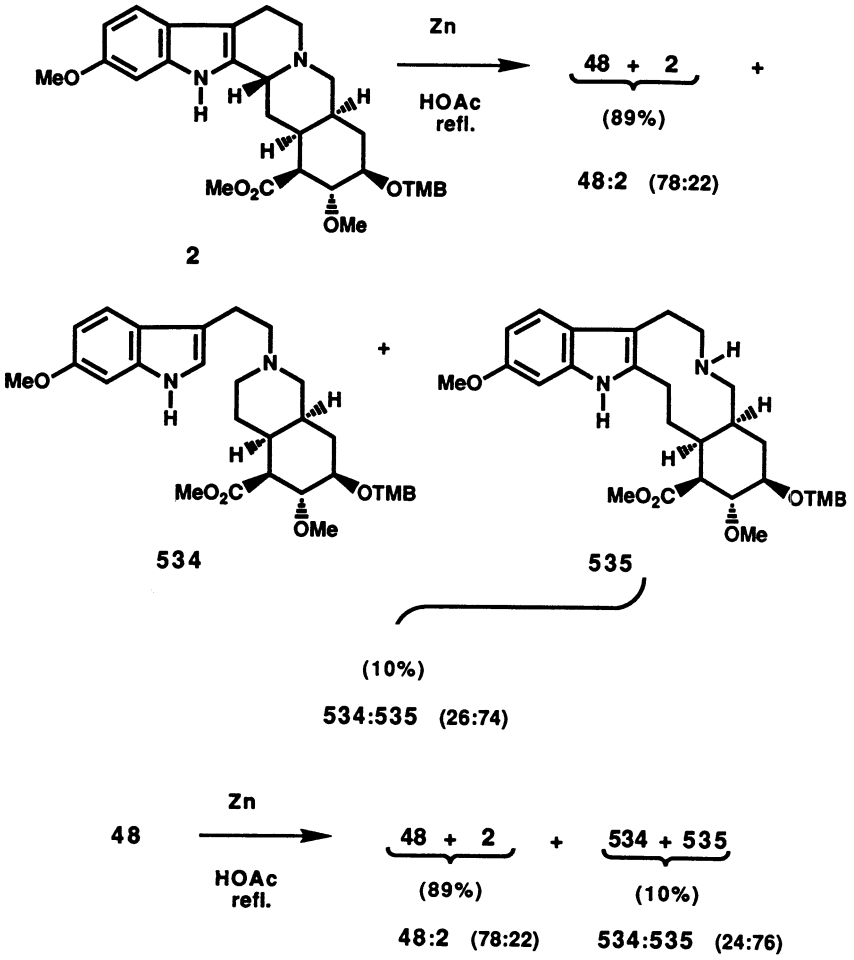
Szantay and his coworkers have also investigated the C-3 epimerization reaction of yohimbine derivatives (Scheme 3.99) (72). For example, when 3-epialloyohimbine (**542**) was subjected to an oxidation-reduction sequence,



SCHEME 3.97

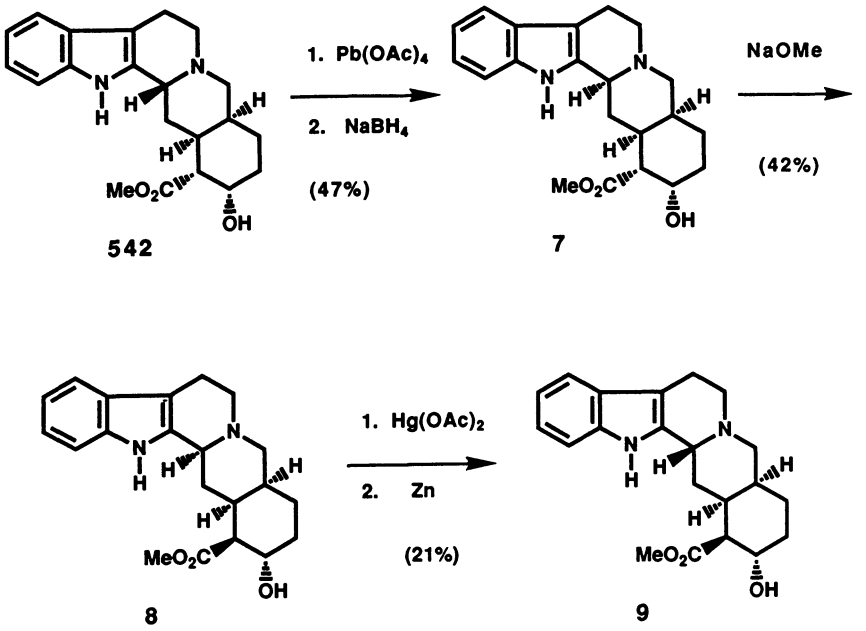
alloyohimbine (**7**) was formed. Subsequent epimerization of **7** at C(16) afforded α-yohimbine (**8**) which was treated with mercuric acetate followed by zinc to provide 3-epi-α-yohimbine (**9**). Similar studies were also conducted on 17 epi-α-yohimbine (**543**) (Scheme 3.100) (77). In this case, successive treatment with mercuric acetate and zinc afforded 3,17-epi-α-yohimbine (**544**) in addition to unreacted starting material (15%). It should be noted that the product yields in these transformations range from poor to moderate.

In a related investigation, Banerji and Siddhanta examined C(3)–C(4) bond cleavage of venenatine (**545**) (Scheme 3.101) (78). Interestingly, when venenatine was treated with cyanogen bromide, C(3)–N(4) bond rupture occurred to yield the ring opened products, **546** and **547**. Furthermore, when **546** was heated in glacial acetic acid, venenatine **545** formed exclusively. Alternatively, when **547** was subjected to the same conditions, the only product observed was alstovenine (**548**). Therefore, it appears that these cyclization reactions do not proceed *via* a common indolylcarbinyl cation.

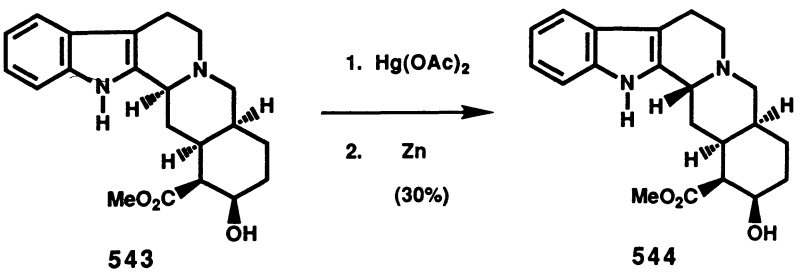

Zn
HOAc
refl.
48 + 2
(89%)
48:2 (78:22)
+
MeO
N
H
MeO2C
OTMB
OMe
2
534
535
(10%)
534:535 (26:74)

48
Zn
HOAc
refl.
48 + 2
(89%)
48:2 (78:22)
+
534 + 535
(10%)
534:535 (24:76)

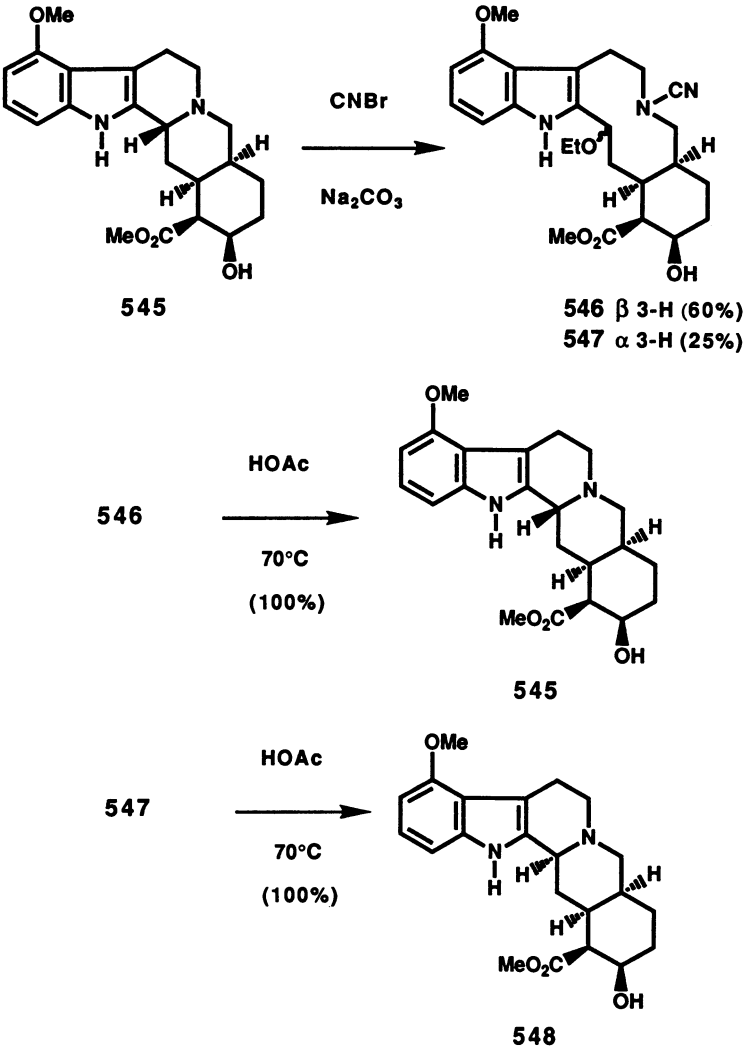
SCHEME 3.98



SCHEME 3.99



SCHEME 3.100


OMe
CNBr
Na_2CO_3
545
546 β 3-H (60%)
547 α 3-H (25%)
546
HOAc
70°C
(100%)
545
547
HOAc
70°C
(100%)
548

SCHEME 3.101

While no sweeping generalizations can be made from the series of experiments outlined above, it is possible to conclude that in the case of reserpine and deserpidine strong biases exist for the C(3) iso-stereoisomers having the α 3(H) configurations. Furthermore, the degree of C(2)–C(3) *vs.* C(3)–N(4) bond cleavage operating in the mechanism for C(3) epimerization appears to depend critically on the reaction conditions employed.

Conclusions

The yohimbine alkaloids represent formidable synthetic challenges not only because of their unique pentacyclic structures and the presence of complex functionality, but also because of the stereochemical issues associated with the large number of chiral centers present in these natural products. In the past dozen years, a considerable effort has been expended in devising solutions to the synthetic problems posed by these highly functionalized pentacyclic alkaloids. While control of C(3) stereochemistry in many of the strategies developed remains an elusive issue, many other problems have been cleverly solved. As a result, a variety of new synthetic methods have been developed to accomplish yohimbine syntheses. The synthesis of targets in this alkaloid family is still an active area of research in natural products chemistry and the future should bring forward a variety of new and interesting strategies to tackle yohimbine syntheses.

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4
The Loline Group of Pyrrolizidine Alkaloids

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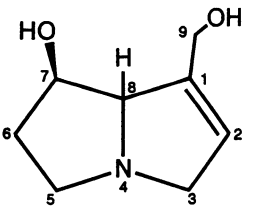
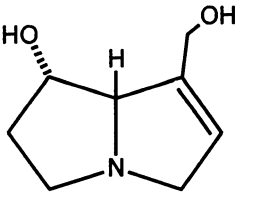
4.1. Introduction

Pyrrolizidine alkaloids are of common occurrence in plants, particularly in genera such as *Senecio*, *Crotalaria*, *Symphytum*, *Echium* and *Heliotropium* (1). Retronecine (**1**) and heliotridine (**2**), and numerous simple and complex esters of these and related alkaloids, are typical pyrrolizidines. Many pyrrolizidine alkaloids are potent hepatotoxins and carcinogens (2); thus, the pyrrolizidines have been widely studied and are considered to be among the most important groups of natural products in terms of their effects on human health and economic activities (3).

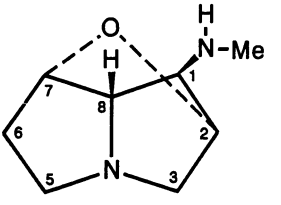
Typical pyrrolizidine alkaloids such as **1** possess CH_2OR functions at C(1), are unsaturated at C(1), C(2) and usually have additional oxygen functions at C(7) and elsewhere. Loline (**3**) and its derivatives differ from typical pyrrolizidine alkaloids in that they all have an additional nitrogen function, NRR' rather than CH_2OR at C(1). In addition, these alkaloids are fully saturated, and possess a remarkably stable ether linkage between C(2) and C(7). Although structural features distinguish these two groups, the loline alkaloids have been considered along with the more widely known pyrrolizidine alkaloids (4). The lolines have only been reported from a few genera of grasses (Poaceae), most often *Lolium* and *Festuca*, and in *Adenocarpus* species (Fabaceae). Their occurrence in grasses is closely associated with the presence of endophytic fungi, particularly *Acremonium coenophialum* (5). This review summarizes available knowledge concerning the loline alkaloids through 1990.



1 Retronecine

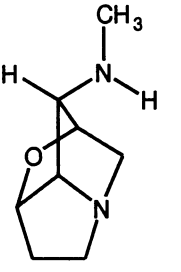


2 Heliotridine



3 Loline

≡



4.2. Naturally Occurring Alkaloids of the Loline Group

4.2.1. Loline Alkaloids from *Lolium* Species

Hofmeister (6) isolated a basic compound "temuline," $C_7H_{12}N_2O$, from *Lolium temulentum* L. in 1898 and Hannig (7) reported that alkaloids were detectable only in caryopses which were invaded by a fungus. However, Katz (8) was unable to find any alkaloids in *L. temulentum*. Reinvestigation of *L. temulentum* by Dannhardt and Steindl in 1985 (9) gave loline (**3**) as the predominant alkaloid. These workers speculated that temuline was actually norloline (**4**) (*N*-demethylloline), which arose as an artifact during the Hofmeister isolation procedure.

The first isolation of loline was by Yunusov and Akramov in 1955 (10) from *L. cuneatum* Nevski. Loline was originally assigned an erroneous structure (**5**), but the structure was revised in 1965 (11). The structure and absolute configuration of the loline group of alkaloids was firmly established in 1972 (12) by X-ray crystallography of loline dihydrochloride. Yunusov et al. continued studies of *L. cuneatum* during the period 1955–1976 and their work resulted in isolation and/or preparation of numerous compounds, including loline (**3**), norloline (**4**), *N*-acetylloline (or lolinine) (**6**) (13), *N*-acetylnorloline (**7**) (14), *N*-methylloline (**8**), *N*-formylloline (**9**) (15), and *N*-formylnorloline (**10**) (16). Additional compounds reported include *N*-acetylloline *N*-oxide (**11**) (16) and lolidine, a chlorine-containing dimeric alkaloid, (**12**) (17).

4.2.2. Loline Alkaloids from *Festuca* Species

Yates and Tookey (18) isolated a compound termed "festucine," $C_8H_{14}N_2O$, from *Festuca arundinacea* Schreb in 1965 as part of a study on cause(s) of fescue toxicity in cattle. Following a detailed study of the chemical and physical properties of festucine, these workers concluded that festucine was either loline (**3**), or a positional isomer of this alkaloid.

Aasen and Culvenor (19) confirmed the identity of loline and festucine by direct comparison of the dihydrochloride salts of loline isolated from *L. cuneatum* and festucine isolated from *F. arundinacea*. The two samples were identical with regards to melting point, mixture melting point, infrared spectra, and proton NMR spectra. Thus, loline became the preferred name and, although the absolute configuration remained unknown, structure **3** was preferred over structure **5**. The absolute configuration of **3**, as mentioned in the previous section, was determined by X-ray crystallography of the dihydrochloride (12).

Literature concerning the chemistry of loline alkaloids was first reviewed in 1972 (20). Robbins et al. (21), using much milder isolation procedures than those of Yates and Tookey (18), also reported the occurrence of *N*-acetylloline (**6**), *N*-acetylnorloline (**7**), and *N*-formylloline (**9**) in *F. arundinacea* seed. Procedures for multiple-gram scale isolation of loline dihydrochloride from *F. arundinacea* seed, and its conversion in high yield to any of the monomeric

loline derivatives known to occur in *Lolium* or *Festuca* species have been described (22).

4.2.3. Norloline and Derivatives from *Adenocarpus* Species

Ribas and Barreiro (23) reported isolation of an alkaloid "decorticasine," $C_7H_{12}N_2O$, from *Adenocarpus decorticans* in 1953. In 1960 it was suggested that the product resulting from hydrolysis of decorticasine was identical with norloline (**4**) (14). Some confusion concerning names appears in the literature, but decorticasine is now considered to be *N*-propionylnorloline (**13**) (24). Depropionyl-*N*-methyldecorticasine was first mentioned in 1959 but was assigned a structure that later proved to be erroneous (25). Depropionyldecorticasine has been demonstrated to be identical with norloline (**4**) and *N*-methyldepropionyldecorticasine (**3**) to be identical with festucine [i.e., loline (**3**)] (26). Also, *N*-butrylnorloline (**14**), *N*-isobutrylnorloline (**15**), and *N*-isovalerylnorloline (**16**) have been reported in *A. decorticans* (24). Recently, *N*-acetylnorloline (**7**), and compounds **13** and **14** were reported from several additional species of *Adenocarpus* (27).

4.2.4. Additional Sources of Loline Alkaloids.

The occurrence of loline alkaloids in grasses has been known, at least since the report of Bacon, et al. (28) to be closely associated with the presence of fungal endophytes; particularly with *Acremonium coenophialum* Morgan-Jones and W. Gams (29). Bush, et al. (30), and Jones, et al. (31) have demonstrated that the endophyte is required for any significant accumulations of loline alkaloids in tall fescue or in *Lolium-Festuca* hybrids. Siegel et al. (5) have shown that only host-endophyte systems that include *Acremonium* species accumulate loline alkaloids. These investigators found lolines to be present in endophyte-infected *F. gigantea* and *Poa autumnalus*. Takeda (32) identified lolines in urine from horses and suggested that an unidentified *Poa* species was the source. Additional studies in our laboratory have resulted in isolation of lolines from an endophyte-infected *Stipa* species (33).

4.3. Isolation and Characterization of Loline Alkaloids

4.3.1. Chromatographic Techniques

4.3.1.1. Adsorption Chromatography

Loline alkaloids have been isolated using paper chromatography (18), chromatography on alumina (9, 21), and thin-layer chromatography (21). Traditional chromatography with solid supports results in pronounced tailing of alkaloids. Extracts of endophyte infected fescue contain in, addition to the lolines, diazaphenanthrene and ergot peptide alkaloids (34). Therefore, isolation and separation of the pure lolines are complicated by the presence of these structurally unrelated alkaloids. Reverse phase chromatography does

not appear to be developed as an analytical tool, or for preparative separation, of lolines.

Chloroform-methanol (9 : 1) has been used with limited success as the developing solvent on silica gel plates while potassium iodoplatinate spray reagent has been used to visualize these compounds (22). Recently we observed that loline alkaloids from tall fescue may be separated from ergot-peptide alkaloids by passing an alkaloidal concentrate through Sephadex LH-20 and eluting with methanol. Under these conditions, ergot-peptide alkaloids are retained by the support while lolines pass rapidly through the column. Ergot alkaloids may then be recovered by exhaustive elution (35).

Conversion of loline (**3**) to norloline (**4**) required a rapid means to distinguish these two compounds (22). Detection of (**3**) and (**4**) on tlc plates was accomplished by spraying with 2-hydroxy-1-naphthaldehyde; this reagent has been used as an amino function protection reagent in peptide synthesis (36) and is reactive to compounds having free primary or secondary amino functions. Primary amines such as **4** are lemon yellow in color, secondary amines such as **3** are orange in color, and tertiary amines such as **8** are unreactive with this spray reagent.

4.3.1.2. Countercurrent Chromatography

Countercurrent chromatography (CCC) offers distinct advantages for isolation of polar compounds, labile substances and materials of biological origin (37). As opposed to silica, alumina, or cellulose, excessive or irreversible retention is eliminated with CCC. Separation of these compounds in a mixture is based on liquid-liquid partition, and the mild operating environment limits sample denaturation and decomposition. A crude fescue alkaloid mixture (1 g) was separated by high speed CCC in less than eight hours using a two-phase solvent system composed of chloroform-methanol-water (5 : 4 : 3, v/v/v) (38). *N*-methylloline (**8**), *N*-acetylloline (**6**), and *N*-formylloline (**9**) were separated and recovery of these alkaloids was quantitative (Fig. 4.1).

4.3.1.3. Gas Chromatography

Gas chromatographic analyses of loline alkaloids have been reported (21, 39) but, until recently, literature values for lolines must only be regarded as semi-quantitative. A total figure has most often been reported for the two predominant alkaloids in tall fescue, **6** and **9**, and the minor loline-type alkaloids generally have not been identified. A quantitative capillary gas chromatographic method for routine analysis of loline-type alkaloids in tall fescue seed and forage has been described (40). Filtered solvent extracts of seed, in CH_2Cl_2/MeOH/NH_4OH (75 : 25 : 0.5), with phenylmorpholine as the internal standard may be analyzed directly; however, forage samples require additional cleanup by ion exchange chromatography to remove interfering substances. Compound identities are easily confirmed by mass spectro-

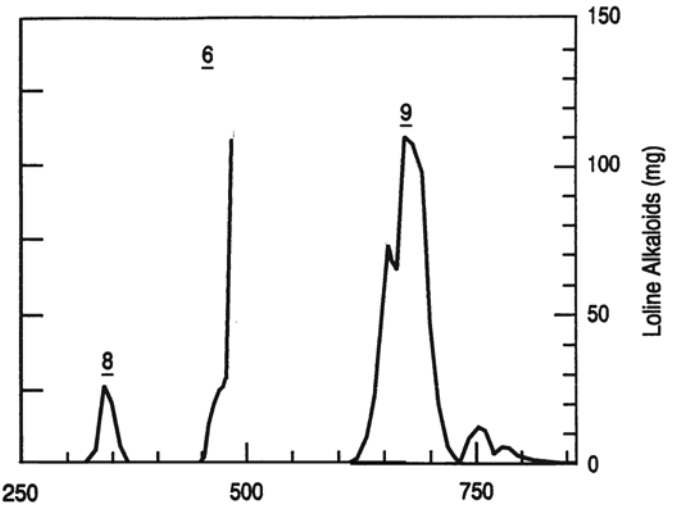


FIGURE 4.1. Countercurrent Chromatogram of Loline Alkaloids; *N*-methylloline (**8**, 61 mg), *N*-acetylloline (**6**, 305 mg), and *N*-formylloline (**9**, 601 mg).

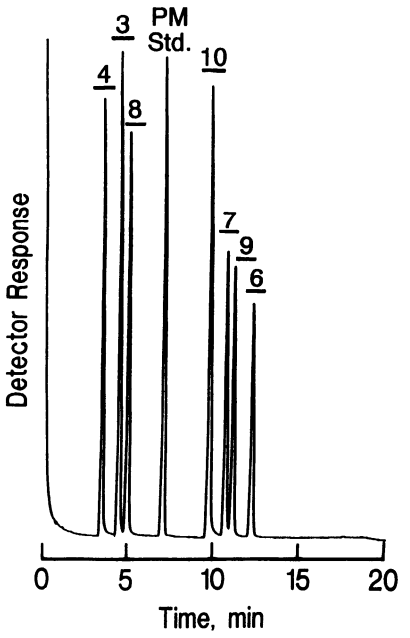


FIGURE 4.2. Capillary Gas Chromatographic Analysis of Standard Loline Alkaloids with Phenylmorpholine (PM) as Internal Standard.

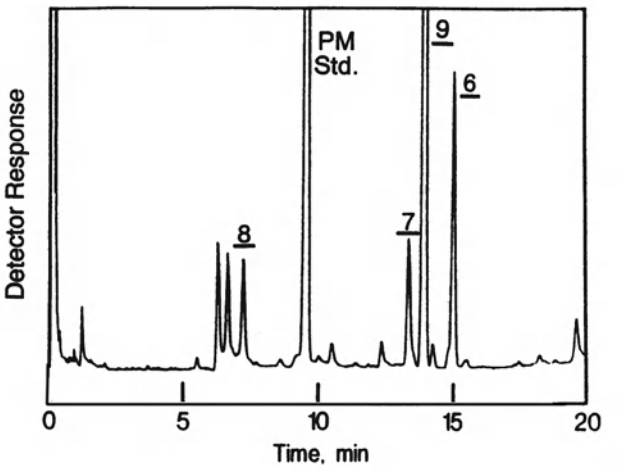


FIGURE 4.3. Typical Capillary Gas Chromatographic Analysis of An Extract of Endophyte-Infected Tall Fescue Seed.

metry and by direct comparison with known standards. Chromatography of a mixture of reference standards revealed that lolines elute in the order shown in Figure 4.2 (40). Modifications of the method that require less time per analysis (shorter columns, faster flow rates, higher operating temperatures) are now being used by others investigators (32).

Capillary GC analysis of a typical fescue seed extract is shown in Figure 4.3. Although small peaks corresponding closely in retention time to **4**, **3**, and **10** are often observed, mass spectral analysis revealed that the fragmentation patterns of these substances did not contain the m/z 82 ion characteristic of loline and its derivatives. The most common lolines found in endophyte-infected tall fescue are **6** and **9** along with lesser amounts of **7** and **8**.

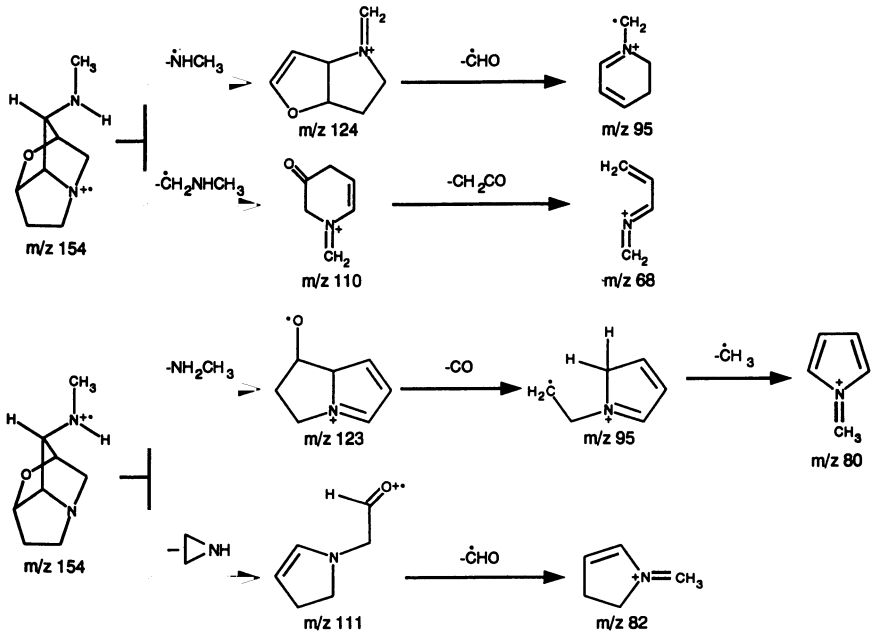
4.3.2. Mass Spectra

Mass spectra of the loline alkaloids have normally been reported during their characterization (9, 21, 22, 32) and a combination of gas chromatography and mass spectrometry is the most rapid method for positive identification of lolines. Mass spectral data for loline (**3**) and some derivatives are given in Table 4.1. A base peak at m/z 82 is characteristic of the lolines and, with the exception of *N*-formyl derivatives, molecular ions are prominent. Both *N*-formylloline (**9**) and *N*-acetylnorloline (**7**) have molecular weight 182 but are readily distinguishable by the mass fragmentation patterns. A diagnostic fragment at m/z 139 is exhibited by **7** but there is no corresponding fragment in the spectrum of **9**. The GC retention times for these two compounds also differ. Alkaloids **8** and **10** also have the same molecular weight, but are separated by GC, and **10** has a distinct fragment ion at m/z 140 (M − 28) vs

TABLE 4.1. Mass Spectra of Loline and Some Derivatives.[a]

Compound	Prominent Ions (m/z, % relative abundance)
Norloline (**4**)	$[M]^+$140 (4.3), 123 (24), 111 (15), 97 (22), 95 (15), 82 (100), 69 (26)
N-Formylnorloline (**10**)	$[M]^+$168 (0.3), 140 (3), 123 (5), 95 (17), 82 (100), 69 (26)
N-Acetylnorloline (**7**)	$[M-29]^+$153 (2.6), 139 (1), 123 (7), 95 (21), 82 (100), 69 (34)
Loline (**3**)	$[M]^+$154 (3.5), 123 (12), 110 (34), 95 (31), 82 (100)
N-Formylloline (**9**)	$[M-28]^+$154 (11), 123 (9), 110 (9), 95 (24), 82 (100)
N-Acetylloline (**6**)	$[M]^+$196 (2.1), 167 (5), 153 (8), 123 (23), 95 (43), 82 (100), 42 (42)
N-Methylloline (**8**)	$[M]^+$168 (1.7), 123 (42), 95 (68), 82 (100)

[a] Petroski, et al., (22), eims, 70 eV.



SCHEME 4.1

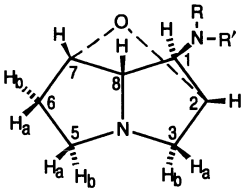
M − 45 for **8** (i.e. m/z 123, 43%). Takeda (32) examined the fragmentation of loline (**3**) using tandem ms methods and his conclusions are summarized in Scheme 4.1.

4.3.3. Nuclear Magnetic Resonance

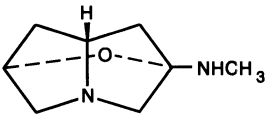
Detailed NMR studies of loline alkaloids have been surprisingly few. Aasen and Culvenor (19) examined the ^1H NMR spectrum of loline dihydrochloride in D_2O and reported that the spectrum was remarkably simple compared

with spectra of other pyrrolizidine derivatives. This was attributed to the near-equality of some chemical shifts and to small values of the vicinal coupling constants involving H(2) and H(7). Using a Dreiding model of loline, the authors reported approximate dihedral angles of 45° for 2,3a and 75° for 2,3b. The coupling of H(2) with H(3b) was too small to be observed. Robbins et al. (21), recorded ^{1}H NMR spectra of *N*-acetylloline (**6**), *N*-formylloline (**9**), and *N*-acetylnorloline (**7**) and noted that *N*-formylloline (**9**) exists in solution as a mixture of two rotational isomers. Petroski et al., confirmed that **9** exists in solution as a mixture of two rotamers, but only one rotational isomer was apparent in the spectrum of *N*-formylnorloline (**10**) (22). LaPlanche and Rogers (41) had earlier observed and offered an explanation for the phenomenon of rotational isomerism in compounds such as *N*-isopropyl-*N*-methylformamide.

Definitive assignments of all ^{1}H and ^{13}C signals in NMR spectra of the lolines were reported in 1989 (22). Two-dimensional homonuclear (COSY) experiments, DEPT experiments, and ^{1}H/^{13}C heteronuclear correlation experiments were conducted on the series of alkaloids (**3**, **4**, **6**–**10**, **13**–**15**) and are summarized in Tables 4.2 and 4.3. Assignments for both rotamers of **9** were relatively straightforward as the minor rotamer peaks (40%) were only 2/3 the size of those for the major rotamer (60%). Roeder (42) has reviewed available ^{13}C NMR data of the typical pyrrolizidine alkaloids (42).



4 R=R′=H
6 R=CH_3, R′=Ac
7 R=H, R′=Ac
8 R=R′=CH_3
9 R=CH_3, R=CHO
10 R=H, R′=CHO
13 R=H, R′=$COCH_2CH_3$
14 R=H, R′=$CO(CH_2)_2CH_3$
15 R=H, R′=$COCH(CH_3)_2$
16 R=H, R′=$COCH_2CH_2(CH_3)_2$



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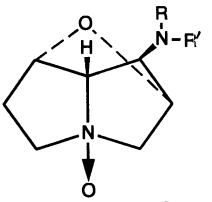

11 R=CH_3, R′=Ac

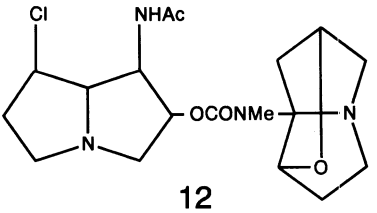

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TABLE 4.2. ^{13}C Nmr Assignments for Loline (**3**), *N*-Formylloline (Rotamers **9** and **9'**), *N*-Acetylloline (**6**), *N*-Methylloline (**8**), Norloline (**4**), *N*-Formylnorloline (**10**), *N*-Acetylnorloline (**7**), Hydroxychlorololine.2HCl (**17A**), and Loline.2HCl (**3A**)[a].

Carbon Assign	Mult.	Compound									
		3	9	9'	6	8	4	10	7	17A	3A
1	d	67.8	65.3	62.4	64.1	74.1	60.5	55.8	57.6	68.2	65.9
2	d	73.4	73.9	73.0	73.4	74.2	76.2	73.6	73.8	73.6	73.9
3	t	60.6	60.4	60.8	61.0	61.2	60.8	60.8	60.9	60.4	64.2
5	t	54.0	54.4	54.4	54.7	54.3	54.5	53.7	54.6	57.6	58.1
6	t	33.5	32.8	32.6	33.0	33.4	34.1	31.7	33.9	36.1	31.6
7	d	81.0	81.8	80.0	80.6	82.0	81.7	80.0	80.9	60.8	83.2
8	d	69.0	67.4	67.8	67.8	69.2	71.9	69.7	69.6	77.5	72.2
NMe	q	34.7	33.4	29.8	33.9	44.4	—	—	—	34.8	36.5
NMe	q	—	—	—	—	44.4	—	—	—	—	—
HC—O	d	—	162.1	163.5	—	—	—	161.8	—	—	—
Me*C*—O	s	—	—	—	171.5	—	—	—	170.2	—	—
*Me*C—O	q	—	—	—	22.3	—	—	—	23.1	—	—

[a] Spectra were recorded at 75.5 MHz. Chemical shifts are reported relative to $CDCl_3$ (δ 77.0) for all compounds except **17A** and **3A**. For compounds **17A** and **3A**, spectra were recorded with D_2O solutions relative to internal acetone at δ 30.5.

4.3.4. X-Ray Crystallography

The structure and relative configuration of loline (3) was established by an X-ray study on its dihydrochloride by McMillan (43) and the absolute configuration of loline dihydrochloride was determined by Bates and Morehead (12). Wilson et al. (44) have reconfirmed the structure and absolute configuration of loline. The absolute configuration of norloline (**4**), derived from *Adenocarpus* species, has not been determined but it would be of interest to know whether or not lolines derived from this source possess the same absolute configuration, or are mirror images, of lolines from the Poaceae.

4.3.5. Reactions and Chemical Interconversions of Loline Alkaloids

Early work on *Lolium* alkaloids (10, 11), on *Adenocarpus* alkaloids (23–26), and on *Festuca* alkaloids (18, 21) relied heavily on chemical reactions and interconversions. By 1960, Yunusov and Akramov (14) had reported demethylation of loline (**3**) to norloline (**4**), methylation of loline to *N*-methylloline (**8**), acetylation to *N*-acetylloline (**6**), and conversion to a hydroxychloro derivative. Yates and Tookey (18) prepared the same compound (cleavage of the C(2)–C(7) ether linkage using HCl) but the complete structure of this hydroxychloro derivative (17) was only recently elucidated (22). Preparation of **17** was achieved from **3** by the action of concentrated HCl in a sealed tube for several hours at 160°. In mild base **17** is relatively unstable and reverts to

TABLE 4.3. ^{1}Nmr Chemical Shifts (δ and Proton Couplings (J, Hz) for Loline (**3**), *N*-Formylloline (Rotamers **9** and **9'**), *N*-Acetylloline (**6**), *N*-Methylloline (**8**), Norloline (**4**), *N*-Formylnorloline (**10**), *N*-Acetylnorloline (**7**), Hydroxychlorololine.2HCl (**17A**), and Loline.2HCl (**3A**)[a].

Proton Assign	Mult.	Compound									
		3	9	9'	6	8	4	10	7	17A	3A
		Chemical Shifts									
1	dd	3.03	3.68	3.82	4.05	2.53	3.48	4.52	4.41	3.77	4.23
2	dd	3.71	4.05	4.56	4.38	3.82	3.72	4.29	4.17	4.66	4.79
3a	dd	3.10	3.09	3.10	3.03	3.36	3.38	3.43	3.29	3.92	4.15
3b	d[b]	2.10	2.33	2.29	2.26	2.18	2.29	2.53	2.42	3.37	3.55
5a	ddd	2.62	2.83	2.83	2.84	2.78	2.80	3.04	2.90	3.52	3.73
5b	ddd	2.77	2.91	2.91	2.93	2.89	2.98	3.17	3.10	3.92	3.73
6a	dddd	1.65	1.80	1.80	1.81	1.75	1.84	2.07	1.98	2.34	2.28
6b	ddd	1.74	1.93	1.94	1.92	1.86	1.94	2.18	2.08	2.74	2.37
7	dd	4.10	4.34	4.27	4.29	4.25	4.29	4.50	4.44	4.80	4.72
8	dd	2.84	3.30	3.34	3.10	3.01	2.92	3.32	3.09	4.48	4.79
NH	bm	—	—	—	—	—	—	7.39	6.21	—	4.76
NMe	s	2.19	2.76	2.94	3.04	2.12	—	—	—	2.79	2.79
NMe	s	—	—	—	—	2.12	—	—	—	—	—
HC—O	s	—	7.25	8.23	—	—	—	8.17	—	—	—
MeC—O	s	—	—	—	1.96	—	—	—	1.97	—	—
		Proton Couplings									
1, 2		1.6	1.5	1.4	2.7	1.2	<2	2.1	2.5	4.9	<2
2, 3a		<2	<2	<2	<2	1.2	1.6	<2	1.0	5.6	1.0
2, 3b		—	—	—	—	—	—	—	—	5.6	—
3a, 3b		11.6	11.9	11.8	11.7	10.9	11.7	12.0	11.8	13.1	13.9
5a, 5b		12.3	13.1	13.1	12.8	12.8	12.8	12.9	12.8	12.1	12.8
5a, 6a		9.8	9.3	9.3	9.1	9.3	9.3	9.3	9.3	7.1	8.2
5a, 6b		7.3	7.1	7.1	7.4	7.2	7.5	7.5	7.4	5.0	7.7
5b, 6a		3.8	4.4	4.4	4.2	4.2	3.8	4.0	3.6	5.3	5.0
5b, 6b		8.3	7.8	7.8	8.4	8.4	8.3	8.6	8.3	6.4	9.6
6a, 6b		14.3	14.3	14.3	14.2	14.0	14.3	14.3	14.4	15.0	14.6
6a, 7		4.3	4.3	4.3	4.3	4.4	4.4	3.9	4.3	4.6	4.8
6b, 7		—	—	—	—	—	—	—	—	4.9	—
7, 8		1.9	1.8	1.8	<2	1.8	1.9	2.2	1.9	3.2	2.2
8, 1		1.8	2.0	2.0	<2	1.3	1.8	<2	1.6	6.1	1.9

[a] Spectra were recorded at 300 MHz. Chemical shifts are reported relative to tetramethylsilane with $CDCl_3$ solutions for all compounds except **17A** and **3A**. For compounds 17A and **3A**, spectra were recorded with D_2O solutions, chemical shifts are relative to internal acetone (δ 2.12).
[b] In the case of **17A**, the C(2) proton is also coupled to proton 3b.

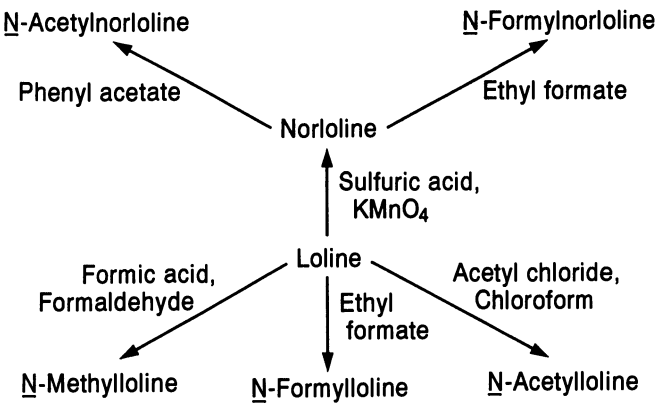


FIGURE 4.4. General Procedures for Preparation of Loline Derivatives.

3 (18). Loline (**3**) and norloline (**4**) have been converted to several additional *N*-acyl derivatives by treatment with the appropriate acid chloride (45). A general scheme for preparation of derivatives from **3** is shown in Figure 4.4.

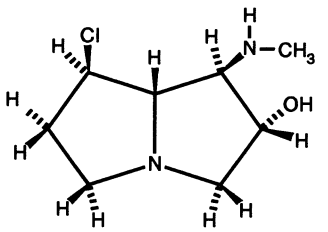
4.4. Synthesis and Biosynthesis of the Loline Alkaloids

4.4.1. Synthetic Approaches

Wilson et al. (44) and Glass et al. (46) attempted syntheses of loline. Synthetic strategy involved bromination of 3-aza-9-oxabicyclo[4.2.1]non-7-ene (**18**) leading directly to **19** and the bridged ring system of the lolium alkaloids. It was thought that bromine in the tricyclic intermediate (**19**) could be displaced by an appropriate nitrogen nucleophile, such as methylamine, leading to members of the loline class. Attempts to carry out this transformation using a variety of nucleophiles and reaction conditions were unsucessful. Structural analysis of **19** by X-ray methodology (46) revealed a small angle (88°) between C(1), C(7), and C(4) which would make substitution very difficult. Electron-electron repulsion between lone-pair electrons on the bridgehead nitrogen and the incoming nucleophile may also make displacement electronically unfavorable.

4.4.2. Total Synthesis

Total synthesis of loline (**3**) and norloline (**4**) was accomplished using a nitrone-based strategy (47). Methyl 2-hydroxy-7,7-dimethoxy-8-pyrrolizidine-1-carboxylate (**20**) was prepared from nitrone (**21**) and methyl 4-hydroxycrotonate (**22**). Epimerization at C(1) was accomplished with sodium methoxide in methanol to yield **23**. Reduction of **23** with $LiAlH_4$ produced **24** which, upon acetylation, resulted in **25**. The ketone **26** was obtained from **25** by treatment with excess TFA at room temperature. The convex face of **26**



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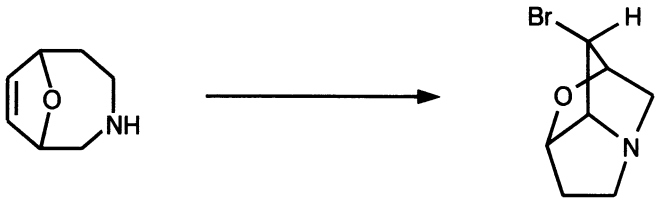
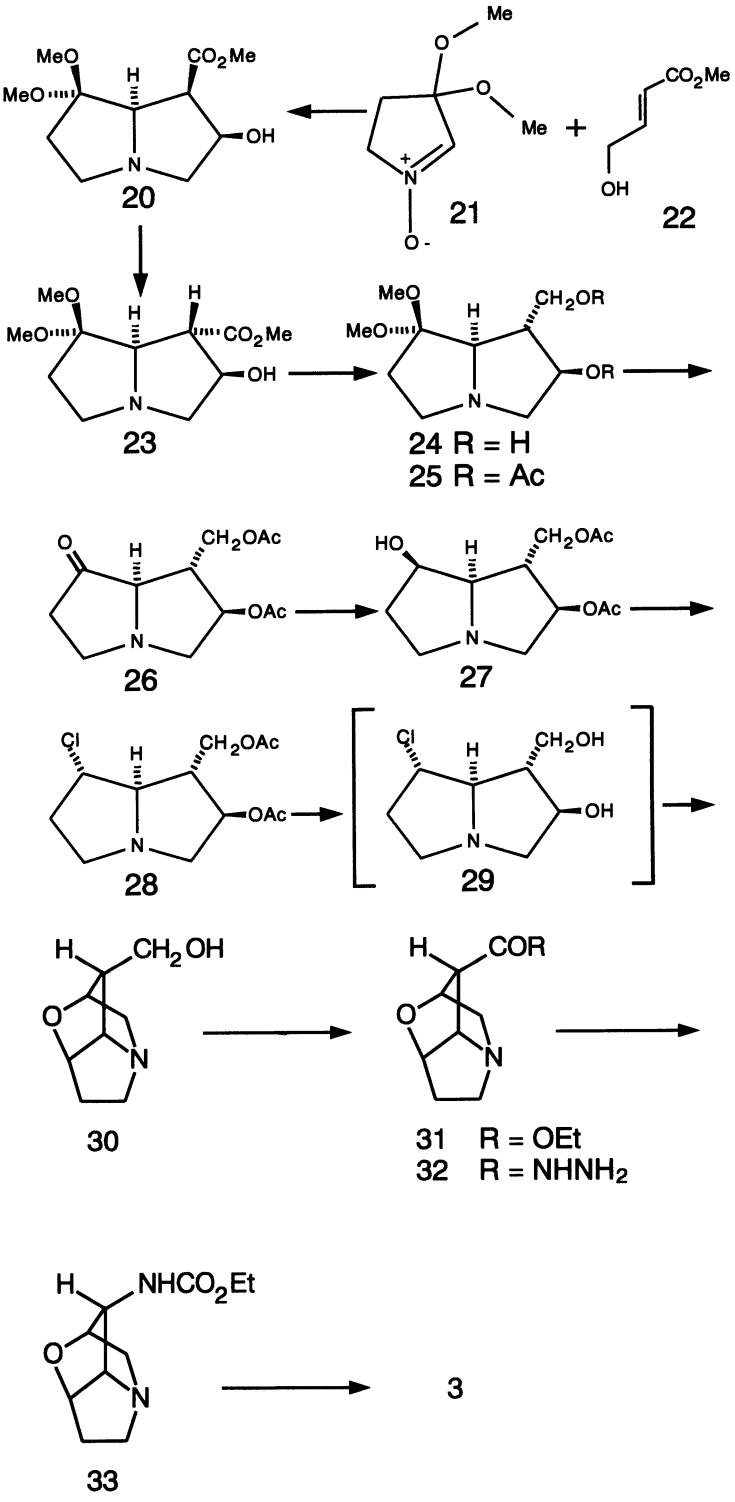


18 19

is less hindered, therefore, hydrogenation with Adams catalyst in glacial acetic acid results in stereospecific reduction to alcohol **27**. Treatment of **27** with Vilsmeier reagent (thionyl chloride in dry DMF) resulted in chlorine displacement of the C(7)-hydroxyl with inversion of configuration (i.e., compound **28**). Treatment of **28** with sodium methoxide in methanol removed both acyl protecting groups, promoted intramolecular cyclization of the postulated diol **29** and resulted in **30** with the correct stereochemistry for the loline alkaloids. Oxidation of alcohol **30** to the carboxylic acid with Jones reagent followed by direct esterification with ethanol and H_2SO_4 in benzene, yielded **31**. Compound **31** was refluxed with hydrazine hydrate to give **32**. Unpurified **32** was then treated with isoamyl nitrite and anhydrous HCl in ethanol to give a product mixture of the desired carbamate (**33**) and ester (**31**). The mixture was separated on an alumina column and the carbamate **33** was reduced with $LiAlH_4$ in refluxing THF to yield loline (**3**). Prolonged reflux of **33** in methanol-water containing 10% NaOH gave norloline (**4**) as the crystalline carbonate.

4.4.3. Biosynthesis

There are no reported studies of loline alkaloid biosynthesis. Loline has been thought to arise via spermidine and retronecine (**1**) (21); however, this pathway would require loss of a carbon, possibly via a Curtlus type rearrangement, to form the norpyrrolizidine system found in loline-type alkaloids. There is no literature precedent for such a rearrangement in alkaloid


MeO
MeO
H
CO2Me
OH
N
20
Me
O
O
Me
+
N
O -
21
CO2Me
OH
22
MeO
MeO
H
H
CO2Me
OH
N
23
MeO
MeO
H
CH2OR
OR
N
24 R = H
25 R = Ac
O
H
CH2OAc
OAc
N
26
HO
H
CH2OAc
OAc
N
27
Cl
H
CH2OAc
OAc
N
28
Cl
H
CH2OH
OH
N
29
H
CH2OH
O
N
30
H
COR
O
N
31 R = OEt
32 R = NHNH2
H
NHCO2Et
O
N
33
3

biosynthesis. Several laboratories are attempting to produce alkaloids associated with endophyte-infected tall fescue by *Acremonium* fermentations and, if loline derivatives are found to be products of such fermentations, the loline biosynthesis problem could be simplified considerably.

4.5. Biological Activities of the Loline Alkaloids

4.5.1. Effects of Loline Derivatives in Animals

Karimov and Kamilov (48) first reported on the pharmacology of loline dihydrochloride in cats and dogs. Loline dihydrochloride in intravenous doses of 1–60 mg/kg gave an immediate and considerable deepening in respiration and a dose-related decrease in blood pressure that lasted 2–15 minutes.

Using pure loline dihydrochloride, Dannhardt and Stendl (9) reported no toxicity in mice (intraperitoneal, 200 mg/kg) and concluded that loline (**3**) has scarcely any toxicity.

Yates and Tookey (18) reported that loline (**3**) was lethal to mice when administered intravenously at 400 mg/kg but that it was without apparent effect when administered orally at 1000 mg/kg.

Loline dihydrochloride does not appear to interfere with; α_1 adrenergic (50), serotoninergic (51), muscarinic (52), nicotinic (53), and/or benzodiazepine receptors (54).

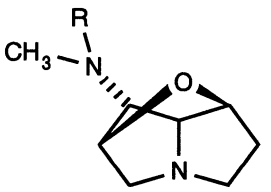
Many investigators have attempted to assess the role of lolines in "fescue toxicity" that is commonly seen in cattle fed high levels of endophyte-infected tall fescue (18, 20, 21, 34). The symptoms of fescue toxicity are similar to classical ergotism and consist of prolactin reduction, increased body temperature, reproductive problems and dry gangrene of the extremeties (hooves, tips of tail and ears) which is characteristic of vasoconstriction. The ergot-peptide alkaloids are also known constituents of endophyte-infected tall fescue (34).

N-Acetylloline (**6**) has been reported to produce vasoconstriction *in vitro* (49); thus, **6** may enhance the effect of ergot alkaloids (34).

Loline normally occurs as *N*-acylated derivatives and there are essentially no reports of the effects of pure *N*-acylloline derivatives in higher animals. Additional studies relating to the pharmacology of loline derivatives are currently in progress.

4.5.2. Phytotoxicity of Loline Derivatives

Several naturally occurring and synthetic *N*-acyl derivatives of loline (**3**) were tested for phytotoxicity against germination and seedling growth of alfalfa (*Medicago sativa* L.) and annual ryegrass (*Lolium multiflorum* L.) (45). Phytotoxicity was tested against both a broad-leaved plant and a grass to detect any general selectivity. Synthetic *N*-acylloline derivatives (**34**–**49**) were prepared for comparison of their phytotoxicity with *N'*-acylnornicotines. Derivatives such as *N'*-hexanoylnornicotine, *N'*-octanoylnornicotine, and *N'*-



34 R = propionyl
35 R = butyryl
36 R = *n*-hexanoyl
37 R = *n*-octanoyl
38 R = *n*-nonanoyl
39 R = *n*-decanoyl
40 R = *n*-dodecanoyl
41 R = *n*-tetradecanoyl
42 R = *n*-hexadecanoyl
43 R = methyl
44 R = isobutyryl
45 R = cyclopropanecarboxoyl
46 R = benzoyl
47 R = cinnamoyl
48 R = *p*-*n*-hexylbenzoyl
49 R = 3-methyl-2-butenoyl

decanoylnornicotine had been reported to exhibit tobacco seed germination and growth inhibiting activity (55).

Loline (**3**), norloline (**4**), and low molecular weight derivatives such as *N*-formylnorloline (**10**), *N*-formylloline (**9**), *N*-acetylloline (**6**) and *N*-methylloline (**8**) exhibited no observed phytotoxicity when tested at doses between 0.1 and 100×10^{-8} mole/seed. These results suggest that naturally occurring loline alkaloids are not general allelopathic agents. However, a structure activity relationship occurred between straight *N*-acyl chainlength and seedling-length inhibition (Fig. 4.5). Maximum phytotoxicity was observed with loline derivatives having *N*-acyl chainlengths between nine and fourteen carbons. *N*-Acyl derivatives with three carbons or less exhibited no phytotoxicity.

Both loline and nornicotine *N*-unacylated derivatives and derivatives having short *N*-acyl chains demonstrated little or no phytotoxicity. Those derivatives having *N*-acyl chainlengths of six to ten carbons (nornicotines) or nine to fourteen carbons (lolines) demonstrated high levels of phytotoxicity. An intermediate-chainlength *N*-acyl function may make such alkaloid derivatives sufficiently lipophilic to move across cell membranes into developing seedlings.

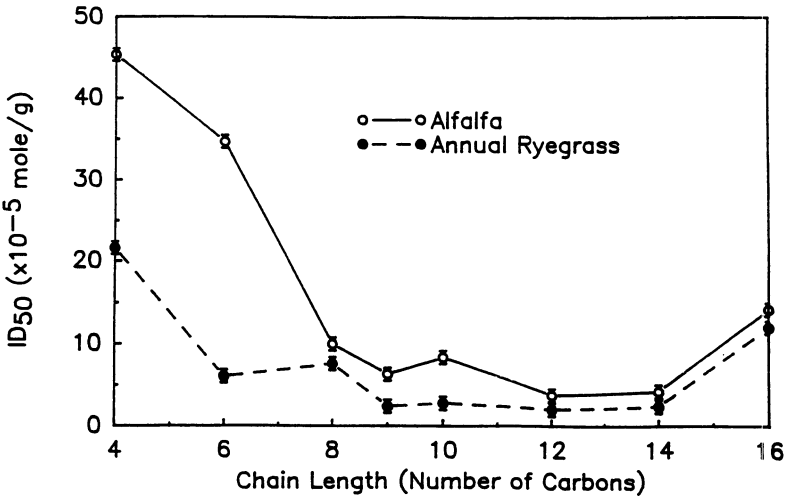


FIGURE 4.5. Loline Derivatives: Structure-Activity Relationship Between Straight *N*-acyl Chain Length and Seedling Length Inhibition.

4.5.3. Insect Deterrency/Toxicity of Lolines

Endophyte-infected perennial ryegrass and tall fescue are both significantly more resistant to certain insects than their uninfected counterparts. Insects reported to be affected by the feeding deterrent/toxic factor(s) in these plants include the aphids *Rhopalosphum padi* and *Shizaphis graminum* (5, 56); the large milkweed bug *Oncopeltus fasciatus* (56, 57); sod webworm, *Crambus* species (58); fall armyworm, *Spodoptera frugiperda* Smith; and European corn borer, *Ostrinia nubilalis* Hubner (59). *N*-formylloline (**9**) and *N*-acetylloline (**6**) appear to be important alkaloids contributing to insect deterrence of grasses infected with *Acremonium* endophytes as these alkaloids are nearly as effective as nicotine sulfate against the greenbug (*Shizaphis graminum*) (59).

Summary

The loline alkaloids are an important group of pyrrolizidine alkaloids found in grass species. Their occurrence is closely associated with infection of the grass with fungal endophytes. The lolines also occur in one genus of the Fabaceae (*Adenocarpus*). Structures have been confirmed by X-ray crystallography, total synthesis, and chemical interconversion. Loline alkaloids are readily available from natural sources and it is not unusual for endophyte-infected tall fescue to contain the equivalent of two grams of loline per kilogram of seed.

Lolines possess a stable ether linkage and procedures are reported for their interconversion to any of the naturally-occurring derivatives. The biosynthe-

sis of the loline alkaloids must differ from that of the more widely studied pyrrolizidine alkaloids; postulations have been presented but proven mechanisms are unknown.

Increased resistance of plants containing loline alkaloids towards certain insect pests is well documented and several synthetic long-chain *N*-acyl loline derivatives inhibit seed germination and seedling growth of alfalfa and annual ryegrass. Information concerning the pharmacology and toxicology of pure loline derivatives is limited. Nevertheless, these alkaloids may contribute to health and performance problems reported in cattle. In contrast to other pyrrolizidine alkaloids, the lolines are not considered to be hepatotoxic or carcinogenic. Further research is necessary to define their effects on animal health and whether food products from animals that consume lolines pose a threat to human health.

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