STUDIES RELATED TO THE SYNTHESIS
AND BIOSYNTHESIS OF INDOLE ALKALOIDS

by

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M.Sc., University of British Columbia, 1972

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
(Dept. of Chemistry)

We accept this thesis as conforming
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THE UNIVERSITY OF BRITISH COLUMBIA
March, 1978

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ABSTRACT

In Part I, a modified synthesis of radio-labelled secodine (68) and its incorporation into vindoline (7) is described.

In a model study, for the synthesis of side-chain labelled 3-ethylpyridine (74), [2-$^2$H]-($3'$-pyrydyl)-ethane was achieved from the correspondingly labelled 3-acetylpyridine by desulphurization of the intermediate thioketal (93). In a second study, [1-$^3$H]-($3'$-pyridyl)-ethane was synthesized by treating 3-acetylpyridine with sodium borohydride-$^3$H. The resulting alcohol (95) was acetylated, and hydrogenolysis achieved the desired product.

The ester alcohol (74) was coupled to [1-$^3$H]-($3'$-pyridyl)-ethane and the resulting pyridinum salt (90) was reduced to the corresponding piperdeine ester (80) in a "one-pot" synthesis. The conversion of (80) to [19-$^3$H]-secodine was achieved by a known procedure.

In two experiments, [19-$^3$H, $^{14}$CO$_2$CH$_3$]-secodine (68)($^3$H/$^{14}$C ratios = 3.00 and 1.54) was administered to Catharanthus roseus plants. The vindoline (7) which was isolated was shown to have been biosynthesized from the entire secodine molecule ($^3$H/$^{14}$C = 3.31 and 1.35 respectively).

In Part II, a degradation scheme designed to achieve the isolation of the N-methyl group of uleine (1) is described as well as preliminary results from an investigation into the biosynthesis of uleine (1) and olivacine (4).

Variously radio-labelled forms of tryptophan (15), anthranilic acid and secodine (18) were administered to Aspidosperma pyricollum root segments and whole plants. The uleine (1) which was isolated was found to
be inactive in all experiments.

Variously radio-labelled forms of tryptophan (15), anthranilic acid and secodine (18) as well as $^{14}\text{CH}_3$-methionine (30) was administered to *Aspidosperma australe* plants. Uleine (1) and olivacine (4) was isolated. The only incorporation that could be demonstrated was that of $^{14}\text{CH}_3$-methionine (30) into uleine (1) to the extent of 0.168% and 0.147%. The isolation of the N-methyl group from (1) showed that it contained 97% and 98% of the activity.

In Part III, the attempted synthesis of compounds of the preakuammicine- and stemmadenine-series is described.

A new method for the C-18 deoxygenation of curan derivatives using Birch reduction conditions was achieved. Also, a modification of the Oppenauer oxidation of the curenol (36) to achieve improved yields of the aldehyde (37) and nor-fluorocurarine (39) was developed.

The introduction of a carbomethoxy group into the C-16 position of the curan aldehyde derivatives (44) and (50) using a base and methylchloroformate was unsuccessful. Also, the introduction of cyanide into position C-16 of the indole alcohol (52) or indole acetate (57) via the corresponding chloroindolenines was unsuccessful.

The synthesis of product (60), which is believed to be identical with preakuammicine aldehyde (7), was achieved. This material could not be converted into akuammicine (5) or stemmadenine (4). Only the dehydrated indolenine (72) could be obtained. The ring-opening reaction of the corresponding thioacetal derivative (73) yielded the decarboxylated indole thioacetals (75) and (76).
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ACKNOWLEDGMENTS

The author is grateful to Prof. J. P. Kutney for giving
him the opportunity to do research in several interesting areas
of natural products chemistry and also for his encouragement and
supervision.

Many helpful discussions with many helpful colleagues are
gratefully acknowledged. Special thanks are due to Drs. R. Sood
and J. Beck for introducing the author to techniques in biosynthesis.
I also thank Anna Wong for typing the manuscript.
To Brenda,
who endured.
PART I

Studies Related to the Biosynthesis of Vindoline
INTRODUCTION

Man's wonder and curiosity about his natural world is illustrated in his earliest records. Out of curiosity, and often necessity, his early attempts at understanding and using the materials provided by nature was, in fact, the study of organic compounds. From these beginnings the modern field of organic chemistry evolved.

Up until the 19th century, the study of organic chemistry was exclusively the study of natural products. In most cases, organic extracts had been used as folk medicines, perfumes or colouring materials that were obtained from plants or micro-organisms. The classical systematic study of purified natural compounds began with the identification of functional groups, delineation of the carbon skeleton and then attempted synthesis of the compound to confirm the proposed molecular structure.

A bewildering array of molecular structures were discovered. Terpenes, steroids, amino acids, peptides, nucleotides, sugars and alkaloids were some classes of compounds which emerged and soon demanded their own specialties. These class divisions were often arbitrary and unsatisfying. Chemists and botanists began to wonder about the inter-relationships between these classes and the origins of the move complex molecules. Were there any "building blocks" which nature used to construct the molecules of each class, or were there "building blocks" common to several classes of natural products?
Perhaps some of the most elegant early speculations on the biogenesis of natural products were those of Sir Robert Robinson, who is now regarded as the father of this relatively large area of study in organic chemistry. The study of the biogenesis of natural compounds has made possible a re-classification of some classes into families of structures according to their biogenetic origin. For example, we now know that terpenes ranging from the ten carbon (C₁₀) monoterpene through to the thirty carbon (C₃₀) triterpenes as well as the steroids, have all been universally built up from the five carbon (C₅) isoprene unit. The delineation of biosynthetic pathways have often shown the way to simpler and more direct laboratory synthesis which have proven more efficient than approaches that have not taken natural routes into consideration.

One family of natural products which have presented many of the more challenging and subtle chemical problems in structural elucidation, and in synthesis, has been the indole alkaloids. These are compounds whose main structural features are the indole (1) or indoline (2) nucleus plus one basic nitrogen atom elsewhere in the molecule.

(1)  \[ \text{Indole} \]

(2)  \[ \text{Indoline} \]
Indole alkaloids have been found in a large number of tropical and sub-tropical plant families such as Apocynaceae, Loganaceae and Rubiaceae. The Apocynaceae plant family has been particularly productive in providing alkaloids of medicinal importance. Some examples are: (a) reserpine (3) from the genus *Rauwolfia* which is widely used in the treatment of mental disorders; (b) strychnine (4) from various strychnos species is a cardiac and central nervous system stimulant; and (c) vinblastine (5), a bis-indole alkaloid from the genus *Catharanthus* which is now extremely important in cancer chemotherapy.
These alkaloids are representative of the historical classification of indole alkaloids into the four major groups which have been most ardently studied. The carbon skeleton illustrated by resperpine (3) is referred to as being of the Corynanthe family. Strychnine (4) is illustrative of the Strychnos alkaloids. The bis-indole (5) has as its "upper" half an Iboga skeleton a derivative of catharanthine (6) while the "lower" part is a naturally occurring monomeric alkaloid called vindoline (7). The latter is representative of the Aspidosperma family of alkaloids.

The first biogenetic speculations which attempted to establish some order in the great variety of structures found in the indole alkaloid area were offered by Pictet, in 1906. Since all the indolic structures established at that time had a β-aminoethyl group attached to the 3-position of the indole portion, it was logical to speculate that the amino acid tryptophan (8, Figure 1) was decarboxylated to become tryptamine (9), and the latter was then elaborated into the C_{19} and C_{20} indole alkaloids. One half century passed before this speculation was given experimental support. In 1960, Leete showed that tryptaphan, which had been specifically labelled with radioactive carbon 14 in the side chain, was incorporated into three Rauwolfia alkaloids: ajmaline, reserpine and serpentine\(^3,4\). That labelled carbon atom was isolated from these alkaloids, thus leaving no doubt about specific incorporation of the precursor. These very important results initiated a deluge of work in the area of indole alkaloid biosynthesis.

A large number of reviews of indole alkaloid biosynthesis have
appeared over the years, the more recent being particularly informative\textsuperscript{5-10}. Two of these reviews\textsuperscript{8,10} have done an outstanding job of putting order to the total history of work done in this area. Another review at this time cannot be justified. However, it will be instructive in the present work to summarize briefly the highlights of this exciting story if only to put the work to be described later into some perspective.

With the establishment of tryptophan (8) as a fundamental biosynthetic precursor, the origin of the other nine or ten carbon atoms of the complex alkaloids remained a great puzzle. In 1933, Barger and Scholz\textsuperscript{11} thought that a tyrosine derived aldehyde (10, Figure 1) condensed with tryptamine (9) and the product was then elaborated into yohimbine (13) a representative of the then well-known Corynanthe-type. Modifications of this postulate by Hahn\textsuperscript{12} and later by Woodward\textsuperscript{13,14} and Robinson\textsuperscript{1} allowed the hypothetical biogenesis of the Strychnos family of indole alkaloids from 3,4-dihydroxy derivative (14) plus a single acetate unit to give an indolenine (15). Inherent in this scheme was the daring speculation of the oxidation and subsequent cleavage of the phenolic system via intermediate (16) to achieve the Strychnos carbon skeleton (4). The "Barger-Hahn-Robinson-Woodward" hypothesis, illustrated in Figure 1, remained unchallenged for some time.

In 1959, Wenkert and Bringi\textsuperscript{15,16} pointed out three major deficiencies in this hypothesis. Firstly, all the yohimbine-like alkaloids, characterized at that time, possessed the same absolute
Figure 1. The Barger-Hahn-Robinson-Woodward hypothesis for indole alkaloid biosynthesis.
stereo-chemistry at C-15, a fact which seemed unlikely if ring E was indeed aromatic at that level of biosynthesis. Secondly, only one compound of this type possessed an aromatic ring E. And thirdly, the introduction of a carbomethoxy group at C-16 was mechanistically unlikely. They proposed instead that the progenitor of the C\textsubscript{9}-C\textsubscript{10} unit could be prephenic acid (17, Figure 2), a known product of carbohydrate metabolism. The stereospecific rearrangement of the prephenate moiety to the seco-prephenate-formaldehyde unit (SPF,20) fixes the stereochemistry at C-15 in the Corynanthe alkaloids and allows the C-16 carbomethoxy group to be an innate part of the progenitor. This scheme is summarized in Figure 2.

Not only did the SPF unit account well for the non-tryptamine part of the yohimbine-like alkaloids, but could also be seen as part of the Strychnos alkaloids, and by a more complex mechanism it could also account for the C\textsubscript{9}-C\textsubscript{10} part of the Iboga and Aspidosperma alkaloids. Moreover, this structure was seen in a number of monoterpenes, some of which had been isolated from *Strychnos* species. The latter feature led Thomas\textsuperscript{17} to propose that the C\textsubscript{9}-C\textsubscript{10} unit was in fact derived from monoterpenes and had its origin in the condensation of two mevalonic acid moieties (21) to give a cyclic structure (22). This structure could then be elaborated into the cyclopentanoid monoterpenes and the C\textsubscript{9}-C\textsubscript{10} progenitor.

About this same time two laboratories began to consider an acetate derived C\textsubscript{9}-C\textsubscript{10} unit. Schlittler\textsuperscript{18} and Leete\textsuperscript{19-21} suggested that three acetate units condensed with mavalonic acid (18) plus a one-carbon
Figure 2. The Wenkert prephenic acid hypothesis.\textsuperscript{15,16}
unit to give a $C_{10}$ structure with the same carbon skeleton as that of the SPF unit (20). This proposal soon led to some important investigations.

In the early 1960's, the results from many radioactive tracer experiments began to cast doubts on all the hypothesis concerning the origin of the ubiquitous $C_9-C_{10}$ unit. Leete $^{20,21}$, working with Rauwolfia Serpentina from the Apocynaceae family, fed $[2-^{14}C]$-tyrosine, $[2-^{14}C]$-alanine, $[2-^{14}C]$-mevalonic acid and sodium $[1-^{14}C]$-acetate, and isolated ajmaline (26) and reserpine (3), both representing the Corynanthe family. All of these feedings resulted in a negative incorporation except for the sodium acetate experiment. One quarter of the activity

\[
3 \text{CH}_3\text{CO}_2\text{H} \rightarrow \text{HO}_2\text{C} \rightarrow \text{HO}_2\text{C} \rightarrow \text{HO}_2\text{C}
\]

Figure 3. The Thomas-Wenkert monoterpane hypothesis.
isolated was found at positions C-3 and C-19 in ajmaline (26). The Thomas-Wenkert hypothesis required activity at C-14, C-19, C-21 and C-16. Leete then modified the acetate hypothesis to include the condensation of three acetyl-coenzyme-A units (23), one malonyl-coenzyme-A unit (24) and one C₁-unit to give structure (25), which is similar to Wenkert's SPF unit (20) but better explains the experimental results (Figure 4).

![Chemical structure](image)

Figure 4. Leete's modified acetate hypothesis.

Then in 1965, Scott and his collaborators²²,²³ reported the successful incorporation of mevalonate (21), the well-known "building block" in terpene biosynthesis, into the Aspidosperma alkaloid, vindoline (7) biosynthesized in C. roseus. Soon the results of tracer experiments from several laboratories²⁴-²⁸ established mevalonate (21) as a true progenitor in indole alkaloid biosynthesis. Equally exciting was the quick establishment of geraniol (27), the immediate precursor to the monoterpenes, as a precursor in several families of these alkaloids.²⁶,²⁹,³₀
Figure 5 summarizes this new hypothesis as supported by experimental evidence. The monoterpene hypothesis was now on firm ground.

Figure 5. The monoterpene origin of the C₉-C₁₀ unit.
The next step in the biosynthesis of the C$_9$-C$_{10}$ unit had to be the identification of the hypothetical cyclopentanoid intermediate (28). As mentioned earlier, there were a number of cyclopentanoid (or iridoid) mono terpenes known which had the correct stereochemistry and, in some cases, co-existed with indole alkaloids in several plant species. Battersby and his co-workers$^{32-34}$ fed radio-labelled venbanalin (31), genipin (32) and loganin (33) to C. roseus and found that only $[^{14}C]CH_3$-loganin (33) was incorporated into three major families of alkaloids. This finding was quickly confirmed by a Swiss group$^{35}$ who were able to incorporate $[^{3}H]_{3}$-loganin into the same three alkaloids in the same plant species. The high levels of incorporation achieved in these studies left no doubt as to the importance of this progenitor. Subsequent work in this area demonstrated the occurrence of loganin (33) in several alkaloid containing plant species and its biosynthesis from mevalonate (21) and geraniol (27) was confirmed.$^{36-38}$

The biosynthetic investigation of ipecoside (34), the first tetra-
hydroisoquinoline monoterpenes to be discovered\textsuperscript{39}, provided a clue to
the next step in indole alkaloid biosynthesis. Battersby speculated

\[
\text{(34)}
\]

that if hydroxyloganin eliminated the elements of $\text{H}_2\text{O}$ with cleavage of
the cyclopentane ring, then one would have a moiety which could
condense directly with tryptamine (9) to give ipecoside (34).\textsuperscript{40} Such
a structure, called secologanin (35), would closely correspond to the
crucial intermediate (29) in the Thomas-Wenkert hypothesis (Figure 5).
The isolation and structural elucidation of three new glucosides lent
great credibility to this idea.\textsuperscript{41,42} Menthiafolin (36a), foliamenthin

\[
\text{(36a)}
\]

\[
\text{(36b)}
\]
and its dihydro-derivative contained the skeleton of secologanin (35) in a masked lactol form.

Battersby and his co-workers were successful in cleaving loganin (33) to secologanin (35) and feeding the latter as a doubly-labelled precursor to *C. roseus* plants. A high level of incorporation into serpentine, ajmalicine (30), catharanthine (6) and perivine was found. In addition, a large scale extract of this plant species revealed the presence of secologanin (35) as a natural product. Thus all the requirements for this compound to be a true precursor to the three families of indole alkaloids were met.  

The next step in delineating this biosynthetic pathway was to look for the product resulting from the condensation of secologanin (35) and the nitrogenous moiety tryptamine (9). An *in vitro* experiment by the Battersby group showed that, at pH 4.5, tryptamine (9) condensed with secologanin (35) to give as a major product vincoside (37) and isovincoside (38) as the minor product. In an elegant series of experiments, reported by these workers, both of these compounds were radio-labelled and fed them to *C. roseus* plants. They found that

![Diagrams of vincoside (37) and isovincoside (38)](attachment:diagrams.png)
only the 3β(R) isomer, vincoside (37), could be incorporated into the three indole alkaloid families. They were also able to demonstrate that both isomers existed as natural products in C. roseus. The problem, however, was that vincoside (37) having the 3β(R) configuration was of opposite stereochemistry possessed by the Corynanthe alkaloids, as represented by ajmalicine (30). It was suggested that C-3 underwent inversion with retention of the hydrogen. This point has been the subject of much controversy. A laboratory investigation showed, in fact, that C-3 of vincoside derivatives was easily epimerized by an oxidative-reductive mechanism. This finding lent great credence to Battersby's original proposal. More recently, a carbon-13 magnetic resonance (CMR) study of various secologanin derivatives and an X-ray diffraction study on vincoside (37) confirmed the earlier assigned stereochemistry of these compounds.

An intriguing result made public in late 1977 by a West German group has added more fuel to this controversy. They reported that in a cell-free enzyme extract from C. roseus cell cultures, only the 3α(S) isovincoside (38) could be incorporated into the three indole alkaloid families found in that species. They also reported that their cell-free preparations catalyzed the formation of only the 3α(S) isomer and the formation of the 3β(R) vincoside (37) was never observed in the five Apocynaceae species Amsonia tabernaemontianum, Rhazia orientalis, Rhazia stricta, Vinca minor and C. roseus. This is very convincing evidence for isovincoside (38) being the obligate precursor. This compound is now more commonly called strictosidine, a name suggested by Smith who first isolated it from R. stricta plants in 1968.
The story of indole alkaloid biosynthesis can at this point be divided between the early stages which form the condensation product, strictosidine \((38)\), arising from monoterpane biogenisis and amino acid biogenisis, and the later stages in which this compound is functionalized and rearranged into the various alkaloid families. Figure 6 summarizes the early stages as proven by experiment.

\[ \text{(21)} \rightarrow \text{(27)} \rightarrow \text{(33)} \rightarrow \text{(35)} \rightarrow \text{(38)} \]

\(\text{(9)}\)

Figure 6. Summary scheme of the early stages of alkaloid biosynthesis as proven by experiment.
A number of investigators soon began unravelling the pathways by which strictosidine (38) was converted into the various alkaloid families. Its conversion to the Corynanthe skeleton could simply be achieved by a loss of a one carbon unit and ring closure to form ring D. Subsequent elaboration of this structure could result in the other members of the Corynanthe family. Figure 7 summarizes some of these proposals.

The natural product cathenamine (39)\textsuperscript{60} appeared to be the most immediate rearrangement product of strictosidine (38) as it was found to accumulate in cell-free enzyme preparations of \textit{C. roseus} that were deprived of NADPH (the widespread natural reduction-oxidation system).\textsuperscript{57,61} Hydrolysis and cleavage of the ethylidene group with loss of formaldehyde from the latter would allow condensation between the now-released aldehyde group and the basic nitrogen. Subsequent dehydration of the intermediate carbonolamine would give cathenamine (39). Reduction of ring C gives ajmalicine (30) and a trivial elaboration of ring E would give geissochizine (40) or corynantheine aldehyde (42a) and corynantheine (42b). Alternatively structure (38) could be elaborated to the immonium species and reduction of the latter would give geissochizine (40) and cyclization of ring E would result in ajmalicine (30).

In 1969, the Battersby group\textsuperscript{62} showed that geissochizine (40) was present in \textit{C. roseus} and was a good precursor not only of the Corynanthe,
Iboga and Aspidosperma alkaloids but also of the Strychnos family represented in that plant by akuamicine (45). Earlier work indicated that corynantheine aldehyde (42a) was not a precursor of ajmalicine (30) or catharanthine (6) in mature C. roseus plants. However, significant incorporation was reported in seedlings of this plant. In fact, subsequent work by Scott’s group suggested that these compounds are all actively involved in biosynthesis in C. roseus seedlings. However, since this group based their conclusions mainly on evidence from thin-layer chromatography the details of biosynthesis in this area must remain an open question.

The elucidation of the biosynthetic events which must take place in the transformation of geissochizine (40) to akuammicine (45) and the other Strychnos alkaloids provided an intriguing challenge. One of the original proposals concerning Strychnos biosynthesis was that of Wenkert (Figure 8). The crucial step in this scheme is the attack at the 3-position of the indole by the activated carbon of the aldehydo-ester (possibly by a radical mechanism) to give a pentacyclic indolenine (41). Subsequent rearrangement could result in the little-known Strychnos alkaloid stemmadenine (43). A transannular cyclization of the indolenine aldehyde and loss of the aldehyde functionality, would give akuammicine (45). The natural products formylstryctamine (41) and its C-16 epimer which were isolated recently from Rhazya stricta add interesting support to the Wenkert hypothesis.
Figure 7. The possible biogenisis of the Corynanthe alkaloids as supported by experiment.
Figure 8. Wenkert's hypothesis for the origin of the Strychnos family of alkaloids.
A second hypothesis was offered by Scott and Quereshi in 1969. They proposed that the hydroxyindolenine (46) could rearrange to an oxindole (47) which in turn could cyclize to preakuamicine aldehyde (44), a structure which possess the Strychnos skeleton. However, it is interesting to note that this type of cyclization is a very difficult one to perform in the laboratory. Compound (44) could then be elaborated to stemmadenine (43) via a ring-opening reaction of a type already known in the laboratory (this reaction will be discussed in detail in Part III of this thesis). A great deal of evidence to support this hypothesis was provided by Scott's laboratory. He reported the isolation of the oxindole (47) and its incorporation into akuammicine (45) in C. roseus seedlings. Also reported was the isolation of the hydroxyindolenine (46) and preakuammicine (48). The co-occurrence of these structures lends great credence to the Scott hypothesis. More recently, Heimberger and Scott were able to incorporate geissoschizine (40) into Wieland-Gumlich aldehyde (49) and strychnine (4).
Figure 9. The Scott hypothesis for the biosynthesis of the Strychos alkaloids.
Wenkert's hypothesis for the biosynthesis of the Aspidosperma and Iboga alkaloids is illustrated in Figure 10. He proposed that the Strychnos skeleton (50), similar to structure (42) (Figure 8) could be the crucial pivotal precursor to the Aspidosperma and Iboga families.

Figure 10. The Wenkert hypothesis for the biosynthesis of the Aspidosperma and Iboga alkaloids.
The important feature in this scheme is the transannular cyclization used to convert structure (52) to (53) and structure (55) to (56). A great deal of effort in our laboratories was made to test this hypothesis in *C. roseus* plants. Despite the success of the corresponding laboratory transformation, the *in vivo* study suggested that this was not a viable route. In the latter study, quebrachamine (57) and cleavamine (58) both labelled with tritium in the aromatic ring, failed to incorporate into catharanthine (6) and vindoline (7).

![Chemical structures](image)

However, out of this work came the surprising result that the Aspidosperma alkaloid tabersonine (59) was incorporated into the more elaborate Aspidosperma alkaloid vindoline (7) and the Iboga alkaloid catharanthine (6). The reverse process, the transformation of catharanthine (6) into tabarsonine (59) could not be demonstrated. This fact strongly suggests the biosynthetic sequence to be Corynanthe -> Strychnos -> Aspidosperma -> Iboga. Sequential feeding experiments by the Scott group using *C. roseus* seedlings provided evidence for this proposal. At this point, one must conclude that the Wenkert hypothesis is incorrect.
Another important observation had been made in our laboratory during the above described study. Administration of [3-\(^{14}\)C]-tryptophan to Vinca minor plants over varying lengths of time and observing the ratios of activity in two "ring-open" alkaloids vincadine (60) and vincaminoreine (61), and two "ring-closed" alkaloids vincadifformine (62) and minovine (63) revealed that the ratios were relatively constant.

\[
\begin{align*}
\text{(60)} & \quad R = H \\
\text{(61)} & \quad R = \text{CH}_3 \\
\text{(62)} & \quad R = H \\
\text{(63)} & \quad R = \text{CH}_3
\end{align*}
\]

This observation appeared to indicate the existence of a pivotal intermediate which was convertible to the "ring-opened" and the "ring-closed" Aspidosperma alkaloids without going through a trans-annular cyclization reaction.

Rationalization of earlier results from Scott's laboratory also demanded a new biosynthetic intermediate. \textit{In vivo} and \textit{in vitro} experiments using the Strychnos alkaloid stemmadenine (43) indicated the latter was converted into both Aspidosperma and Iboga alkaloids. \(80-82\) The structure favoured for this intermediate was the acrylic ester (64), which was closely related to the Wenkert intermediates (51) and (54) but necessarily of a different oxidation level.
The isolation from Rhazya species a new family of dimeric indole alkaloids, called the secamines, related directly to the hypothetical acrylic ester. Later, three related monomeric alkaloids (65), (66) and (67), which were of particular importance, were isolated from the same species.

The Manchester group discovered another group of alkaloids, called presecamines, which underwent acid catalysed rearrangement to the previously characterized secamines. They also found that the presecamines were Diels-Alder type dimers of secodines possessing an α-acrylic ester function. The possible presence of secodine (68) and
its 15,20-dihydroderivative* (67) in the plant strongly supports the hypothesis that dehydroseconidine (64) may be the privotal precursor from which the various families of indole alkaloids is biosynthesized.

This current hypothesis, illustrated in Figure 11, suggests that the Strychnos alkaloid stemmadenine (43) is in biological equilibrium with dehydrosecodine (64) via the intermediate iso-stemmadenine (70). Dehydrosecodine (64) could undergo bond formation between atoms, as shown, to give the carbon skeletons of the Aspidosperma and Iboga types.

The synthesis and radiolabelling of dehydrosecodine derivatives was imperative for the evaluation of this new hypothesis. To date the synthesis of structure (64) has not been possible as it is well known that dihydro-pyridine moieties (71) undergo very facile oxidation to the pyridinium system (78) or rapid rearrangements and polymerization:

* This biogenetic numbering system was suggested by LeMen and Taylor.
Figure 11. Hypothetical biosynthesis of the Aspidosperma and Iboga alkaloids from the Strychnos family via dehydrosecodine.
Therefore, a more practical synthetic target was the tetrahydro-acrylic ester, secodine (68) and its hydrated derivative 16,17-dihydrosecodine-17-ol (69). These two compounds soon became available for biosynthetic investigation.

Battersby's group synthesized the hydroxy ester (69) and determined by isotopic dilution that it was present in Rhazya orientalis plants and possibly present in C. roseus seedlings. Both structures (68) and (69) were synthesized in our laboratories by Drs. R. Sood and J. Beck. In the next section of this thesis, this work and its biosynthetic implications will be described.

Part II of this thesis will discuss investigations into two other families of indole alkaloids for which no biosynthetic evidence has been obtained. These two groups, the ulein-type and the pyridocarbazole-type of indole alkaloids are considered somewhat anomalous in that they do not possess the normal two-carbon bridge between the indole portion and the basic nitrogen atom. In the uleine-type structures this bridge is absent, while in the pyridocarbazole alkaloids a three-carbon bridge is found. Clearly, the evaluation of the biosynthesis of these compounds provides an interesting challenge.

Part III of this thesis describes work directed towards the synthesis of the radio-labelled precursors preakuammicine aldehyde (44), preakuammicine (48), stemmadenine (43) and its iso-derivative (70). As previously described these structures are deeply implicated in the later stages of biosynthesis of all indole alkaloids and the possession of them in suitable radio-labelled form would be extremely desirable.
DISCUSSION

As was revealed in the Introduction, the biosynthesis of both the C$_9$-C$_{10}$ unit from the monoterpene pathway and the tryptamine portion of the structures of the four large indole alkaloid families has been firmly established. The immediate challenge, however, was the testing of the dehydrosecodine hypothesis (Figure 11) put forward independently by Scott$^{80-82}$ and Kutney.$^9$ As mentioned earlier, 16,17-dihydrosecodin-17-ol (69) was synthesized and shown to possibly be present in _C. roseus_ plants by the Battersby group.$^{89}$ The synthesis of (69) and its dehydration product secodine (68) was also accomplished in our laboratories.$^{90,91}$ In this section there will be described a modified synthesis of radio-labelled secodine (68) and its successful incorporation into Aspidosperma alkaloids in _C. roseus_ plants.

Already available in our laboratories$^{90}$ was the 2-carboethoxy indole derivative (73) prepared according to the sequence shown in Figure 8. Using this compound as starting material 16,17-dihydrosecodin-17-ol (69) and secodine (68) were synthesized, by the sequence shown in Figure 13, to obtain sufficient quantities for radio-labelling and plant feeding experiments.

The chloroethylindole (73) was coupled with 3-ethylpyridine (74) in a sealed tube at 120° to give the pyridinium salt (75) in high yield. Sodium borohydride reduction to the tetrahydropyridine derivative (76) followed by lithium aluminum hydride reduction of the ester group gave the alcohol (77), also in good yield.
The next step in this sequence was designed to lengthen the side-chain by one carbon in a manner which would allow the introduction of carbon 14 using an inexpensive and convenient reagent. This was accomplished by displacing the corresponding benzoate (78) with cyanide ion to give compound (79). Subsequent hydrolysis of (79) with methanolic hydrochloric acid gave the corresponding methyl ester (80).

When aromatically labelled secodine (68) was required, tritium was introduced at this stage in the synthesis. The ester (80) was stirred with tritiated trifluoroacetic acid (prepared from trifluoroacetic anhydride and $^3$H$_2$O) for 48 hours. After chromatography and recrystall-
Figure 13. The Kutney Synthesis of secodine (68)²⁰,²¹
zation to constant radioactivity, aromatically labelled (80) was obtainable in 80% yield.

To complete the secodine skeleton, introduction of the hydroxy-methylene group α to the ester group was achieved by treating the anion of (80) with methylformate and carefully reducing the resulting product (81) with sodium borohydride. This reaction had to be performed at -30° and monitored by thin-layer chromatography. Good yields of the ester alcohol (69) were obtained only when precautions against over-reduction to the corresponding diol were exercised.

Dehydration of the alcohol ester (69) using sodium hydride gave the somewhat unstable secodine (68). It was found that it was best to store the alcohol (69) as a crystalline material and prepare the secodine for feeding experiments immediately before use. Thus, we were able to store [ar-3H]-16,17-dihydrosecodin-17-ol and [14CO2CH3]-16,17-dihydrosecodin-17-ol for conversion to the correspondingly labelled secodine precursor (68).

The initial feeding experiments with these new precursors, which were done by Dr. Sood in our laboratories, in Vinca minor revealed that this plant system could not utilize the alcohol (69). Secodine was, however, converted into vincamine (82) and, minovine (83) in a low but definite incorporation. In C. roseus plants a low incorporation of

![Chemical Structures](82) (83)
only secodine (68) into vindoline (7) and catharanthine (6) could be obtained.

Studies with doubly-labelled precursors with a known ratio of radioactivity from tritium in the aromatic ring and carbon-14 in the carbomethoxy group of secodine (68), provided the important information that at least the indolic-ester part of the molecule was being utilized by the plant. Therefore, it would be highly desirable to also have the secodine molecule radio-labelled in the piperideine portion, as well, to confirm utilization of the whole structure. The synthesis of secodine (68) labelled in this way became the next stage in the evaluation of secodine as a precursor common to the biosynthesis of the major families of indole alkaloids.

Clearly, it is of great advantage to introduce radio-labels near the completion of a lengthy synthesis of a desired precursor in consideration of the expense of the experiment and of the safety of the workers involved. If a radio-label is introduced early in the sequence, a higher total activity must be handled during a greater number of manipulations. Thus, if one desired introduction of a radio-label into the piperideine portion of secodine (68) the first synthetic route (Figure 13) would not be particularly favorable. An alternate synthesis of secodine (68) was required.

The basic requirement in a second synthetic route to secodine (68) was that if the piperideine unit carried a radio-label it should be coupled to the tryptamine portion by a simple procedure near the end of the sequence. The alcohol ester (84) was available in our laboratories by the sequence outlined in Figure 14. Due to the initial development of this sequence by
Westcott, bulk quantities of the alcohol ester (84) could be obtained and it was chosen to be the starting material in the new synthesis. The commercially available indole ester (85) was reduced to the corresponding alcohol (86) and converted to the benzoate ester (87). Subsequent introduction of cyanide and methanolysis of the cyano group yielded the homologated ester (89). Treatment of the latter with ethylene oxide in the presence of stannic chloride gave the alcohol (84) in good yield and as a stable material that could be conveniently stored.

![Chemical structure](image)

Figure 14. The Synthesis of ester alcohol (84)

With this alcohol in hand studies were initiated to develop methodology for the coupling with the 3-ethylpyridine unit (74). Our first consideration was to displace the hydroxyl with chloride ion using methanolic hydrogen chloride according to a procedure published by Wenkert. This method met
with only partial success as the product was contaminated with several other components.

Our attention turned to consideration of the corresponding primary tosylate. It is well known that primary tosylates are good leaving groups in nucleophilic displacement reactions. Therefore, if the tosylate derivative could be formed using 3-ethylpyridine as the base, the desired pyridinium salt derivative would be the expected product. This approach was indeed successful. The alcohol (84) was dissolved in methylene chloride and cooled to 0° and freshly recrystallized tosylchloride was added. After two hours this mixture was slowly warmed to reflux until thin layer chromatography (tlc) indicated that only base-line material was present. After removal of the solvent the residue was dissolved in methanol and treated with sodium borohydride to give the tetrahydropyridine structure (80), which was identical with the corresponding material from the first synthesis of secodine shown in Figure 13.

Figure 15. An alternate synthesis of piperdine ester (80).

This sequence allowed us, then, to couple the indolic portion to 3-ethylpyridine and obtain the reduced product (80) in a "one-pot" operation, in 21% yield. Subsequently, this sequence was studied by others
in our group and the yield was raised to 35%.

The next step in our investigations required a method for introducing a radio-label into the piperideine moiety of secodine (68). Tritium was chosen over carbon 14 for this purpose since introduction of the latter would require a more elaborate synthesis of the 3-ethylpyridine skeleton. If tritium were to be used it was desirable, in the first instance, to introduce this isotope into the ethyl side chain rather than into the pyridine ring because the latter undergoes extensive elaboration during the biosynthesis of the various alkaloid skeletons and, therefore, a significant loss of the label would be expected.

Our first consideration for tritium introduction was to exchange the labile protons in the methyl group of 3-acetylpyridine (91) and then remove the oxygen function under conditions which would not allow exchange back to the original inactive system. In a model study using deuterium it was found that, in a two-phase system of tetrahydrofuran and \( \text{H}_2\text{O} \) containing sodium carbonate, more than 95% of the methyl protons could be exchanged as measured by proton nuclear magnetic resonance (p m r). This determination was made by integrating the signal at \( \tau 7.4 \). With this deuterated material in hand we began a study directed at the removal of the oxygen function.

\[
\begin{align*}
\text{(91)} & \quad \text{CH}_3 \\
\text{(92)} & \quad \text{C}_2\text{H}_3
\end{align*}
\]
The conventional procedure for deoxygenation of aldehydes and ketones which would probably not involve a significant degree of enolization concerned the formation of the corresponding ethylene thioketal followed by desulphurization with Raney nickel. Treatment of the deuterated ketone (92) with ethane dithiol and boron trifluoride etherate gave the thioketal (93). Examination of the pmr spectrum of this product revealed multiplet signals at $\tau 6.6$ corresponding to the four methylene protons in the thioketal ring. Integration of the signal at $\tau 7.9$ revealed that only 1.4% loss of deuterium from the methyl group had occurred.

Desulphurization of the thioketal (93) using Raney nickel gave initially a poor recovery of deutero-3-ethylpyridine (94). It was reasoned that perhaps the basic nitrogen of the pyridine ring was coordinating with the Raney nickel in such a way as to retard its liberation. When the reaction mixture was worked up with a 5% solution of aqueous sodium hydroxide a good yield of the product was obtained. When desulphurization of the deuterated thioketal (93) was achieved, the pmr spectrum of the deuterated 3-ethylpyridine (94) showed that approximately 2.1% loss of lable
had occurred on the surface of the Raney nickel. This small loss could be easily tolerated in a subsequent tritiation procedure.

Our second consideration for introducing tritium into the side-chain of 3-ethylpyridine involved labelling the methylene group. Initial studies using sodium borohydride showed that the ketone function of 3-acetylpyridine could be reduced under aprotic conditions. In this manner, sodium borotritiide was efficiently used to introduce tritium into the side-chain. Aprotic conditions minimized loss of label through exchange with proton source.

The next step was the hydrogenolysis of the alcohol group in the product obtained from sodium borohydride reduction of (91). Our plan involved converting the hydroxyl into a better leaving group. We attempted first to make the corresponding tosylate (96), mesylate (97) and benzoate (98) derivatives, but in each case only the starting alcohol could be seen by thin layer chromatography or recovered from the reaction mixture. The
corresponding acetate (99) could, however, be obtained in high yield. The identity of this material was established by its mass spectrum which exhibited a molecular ion peak at m/e 165 and an infrared spectrum which showed the presence of a carbonyl absorption at 1725 cm\(^{-1}\). Furthermore, the pmr spectrum of this product showed a three-proton singlet signal at \(\tau 7.93\) which was expected for the presence of an acetate group.

The subsequent hydrogenolysis of the oxygen functionality was achieved by treating the acetate (99) with an atmosphere of hydrogen gas in the presence of palladium-on-charcoal catalyst in dilute aqueous hydrochloric acid solution. In this way, 3-ethylpyridine (74) was obtained in 65% overall yield.

When this sequence was used to produce radio-labelled material, sodium borohydride-\(^{3}\)H was allowed to react with the carbonyl compound for an extended period of time to ensure a complete transfer of the tritium to the substrate. The reaction was then completed by the addition of inactive sodium borohydride. The overall introduction and retention of the radio-label in this sequence as measured by the specific radioactivity of 3-ethylpyridine (74) was 87%.

Of the two tritium labelled forms of 3-ethylpyridine (74) which were now available, the methylene labelled derivative was chosen for coupling to the indole alcohol (84). The product of this reaction was elaborated, as previously described, to give 16,17-dihydrosecodin-17-ol (69) with a specific radioactivity in the piperidine side-chain of \(2.17 \times 10^{10}\) dpm per millimole.
With side-chain labelled secodine (68) in hand, proof that the entire precursor was being utilized by the plant system to biosynthesize the Aspidosperma alkaloids could be obtained. To further evaluate the biosynthesis of this precursor, doubly-labelled [19-³H, ¹⁴C₂H₃]-secodine having a radioactivity ratio, of tritium over carbon 14, equal to 3.00 was fed to a C. roseus plant using the cotton wick feeding technique. After nine days, the plant was macerated in methanol in a Waring blender and vindoline (7) was isolated in a manner similar to that described by Sood and Beck. This material was recrystallized and reduced with lithium aluminum hydride to vindolinol (100). The latter was then recrystallized to a constant radioactivity with a ratio ³H/¹⁴C equal to 3.31. In view of the results of many feeding experiments in this area, this ratio was considered to be within experimental error. However, to verify this result, a second experiment was carried out.

![Chemical structures](image)

(68) → (7) → (100)

In a second feeding experiment using doubly-labelled secodine (68) a radioactivity ratio of 1.54 was administered to a C. roseus plant. In a manner similar to that described above, the isolated vindoline (7) was
converted to vindolinol (100) and the latter was found to have a constant ratio of radioactivity equal to 1.35. These results are summarized in Table 1.

The important conclusion that can be drawn from the results of these two feeding experiments is that the entire secodine molecule (68) is indeed utilized by the plant system for the biosynthesis of the Aspidosperma alkaloid vindoline (7).

Table 1. The incorporation of [19-3H, 14CO2CH3]-secodine into vindoline (7)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Ratio of Activity Fed (3H/14C)</th>
<th>Ratio of Activity Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.00</td>
<td>3.31</td>
</tr>
<tr>
<td>2</td>
<td>1.54</td>
<td>1.35</td>
</tr>
</tbody>
</table>

The work described in this section forms part of an ongoing program, in our laboratories, designed to evaluate the implications of the dehydrosecodine hypothesis in the biosynthesis of many families of indole alkaloids. Currently work is progressing on the synthesis of a stable chromium complex of dehydrosecodine. Also in the near future these precursors will be evaluated in cell-free enzyme preparations from several plant species.

The next part of this thesis describes some preliminary results from an investigation into the biosynthesis of alkaloids of the uleine- and olivacine-types.
EXPERIMENTAL

Thin layer chromatographic studies were carried out using either Merck silica gel or Woelm neutral alumina as the absorbents. The chromatoplates, 0.3 mm in thickness, were air dried and activated in an oven at 120°C for more than three hours. In preparative scale thin layer chromatography a thicker layer of 0.5 mm was used. In all cases, electronic phosphor (about 2% by weight) was added to the absorbent as fluorescent indicator. The chromatoplates were developed in either chloroform, or a mixture of chloroform plus 2% methanol, and examined under a short and long wavelength ultraviolet scanning lamp and visualized with antimony pentachloride spray or iodine vapors.

Column chromatography was performed using Woelm silica gel or Woelm neutral alumina. The dimensions of the columns were generally maintained at the accepted optimum ratio of diameter to height as 1:10. Throughout this work, the solvents were distilled before use.

Gas liquid chromatography (glc) was performed on a Varian model A-90-P instrument, using helium as a carrier gas at a flow rate of 80-85 ml/min. For routine analysis a column 1/4 inch x 10 feet consisted of 20% carbowax on 60/80 mesh Chromsorb W support packing was used. All work was done using a column temperature of 175-205°.

Ultraviolet spectra were recorded in methanol on a Cary 11 or a Cary 15 recording spectrophotometer and the absorption bands (λ<sub>max</sub>) are recorded in nanometers (nm). Infrared spectra were obtained on a Perkin-
Elmer model 21 double-beam spectrophotometer. Samples were measured in KBr pellets or in chloroform solution. The positions of absorption maxima ($\nu_{\text{max}}$) are quoted in wave numbers (cm$^{-1}$).

Proton magnetic resonance (p.m.r.) spectra were measured in deuterochloroform at room temperature. These were measured at 60 MHz on a Varian T-60 spectrometer. Where additional resolution or double resonance studies were required, the spectra were measured at 100 MHz using a Varian HA-100 instrument. The positions of all p.m.r. absorption signals are given in the Tiers $\tau$ scale with tetramethylsilane as the internal standard at $\tau$ 10.00. For multiplet signals the $\tau$-values given represent the center of the signal.

Mass spectra were measured on an Associated Electrical Industries MS-9 double-focusing mass spectrometer or an Atlas CH-4 mass spectrometer. Fragmentation data is given in mass to charge ratios (m/e) followed by percent relative abundance. High resolution measurements were also determined on the MS-9 instrument using suitable standards of known molecular weight.

Radioactivity was measured using a Nuclear-Chicago Mark I Model 6860 Liquid Scintillation Counter. The measurement in counts per minute (cpm) was converted to disintegrations per minute (dpm) by applying, for each sample, the counting efficiency determined by the external standard technique which uses a barium 133 gamma source. The liquid scintillation solution consisted of 2,5-diphenyloxazole (4 grams) and 1,4-bis[2-(5-phenyloxazoly)]benzene (0.5 grams) dissolved in toluene (1 litre). The background radiation of each counting vial
was determined before it was used. A sample was dissolved in benzene or methanol (1 ml) in the vial and scintillator solution (14 ml) was then added. When doubly labelled samples were counted the instrument was recalibrated and the overlap between the tritium and carbon 14 measurements were obtained for each sample.

The *Catheranthus roseus* plants used in this study were grown in a Department of Horticulture greenhouse, University of British Columbia, under the supervision of Dr. P. Salsbury of the Department of Chemistry.

Melting points were determined on a Kofler block and are uncorrected. Elemental analyses were performed by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia, Vancouver.

**Synthesis of Secodine (68) from 2-Carboethoxy)-3-(8-chloroethyl)-indole (73)**

The synthesis of secodine (68) was previously worked out in our laboratories $^{90,91}$ and has been described in detail. Therefore, only quantities used and yields obtained, as well as some minor changes in procedure will be given for this particular sequence.

**N-[8{3-(2-Carboethoxy)-indolyl}-ethyl]-3'-ethyl-pyridinium chloride (75)**

The carboethoxychloroindole (73) (5g) was dissolved in 3-ethylpyridine (74,16 ml) in thick-walled tube. After sealing, the tube was placed in an oil bath kept at 120° for 24 hours. The tube was then cooled, broken open and the contents were stirred in diethylether for 2 hours and filtered to yield a white solid which was dried in high vacuum (6.3 g).
N-[\beta-\{3-(2-Carboethoxy)-indolyl\}-ethyl]-3'-ethyl-3''-piperideine (76)\textsuperscript{90}

The indole pyridinium chloride (75) (6.3 g) was dissolved in methanol (400 ml) containing triethylamine (6 ml). Sodium borohydride (20 g) was slowly added at room temperature with vigorous stirring. After 2.5 hours most of the solvent was removed in vacuo before water (100 ml) was added. The resulting solution was acidified with 2N hydrochloric acid. After stirring for 30 minutes the solution was basified with sat. sodium bicarbonate solution and extracted with methylene chloride. The combined organic extracts were washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo to yield an amorphous material (76) (6 g).

N-[\beta-\{3-(2-hydroxymethylene)-indolyl\}-ethyl]-3'-ethyl-3''-piperideine (77)\textsuperscript{90}

The piperideine (76) (6 g) from the previous reaction was dissolved in freshly distilled tetrahydrofuran (THF, 70 ml) and was added over 40 minutes to a vigorously stirring suspension of lithium aluminum hydride (8 g) in THF (300 ml) under a nitrogen atmosphere. After the addition was complete, the mixture was slowly warmed to reflux for 2 hours. At the end of this time the reaction was cooled and additions of water, 15% sodium hydroxide solution and water again destroyed the excess reagent. The resulting suspension was filtered, evaporated and dried in high vacuum. Column chromatography on alumina (150 g, act III) permitted the solution of the piperideine alcohol (77) (3.6 g) using a benzene/chloroform gradient.

Benzoylation of the piperideine alcohol (77)\textsuperscript{90}

The material from the above reaction (3.6 g) was dissolved in freshly
dried and distilled pyridine (30 ml) at 0°. Benzoyl chloride (11 ml) was added over 15 minutes and resulting solution was stirred for another 3 hours at 0°. Water (30 ml) was added followed by saturated sodium bicarbonate solution. After the evolution of gas ceased the mixture was extracted with methylene chloride. The organic extract was washed repeatedly with water, dried, evaporated in vacuo and dried in high vacuum overnight to yield a gummy, dark coloured material (78)(5.2g). An alternate benzoylation procedure which avoids using pyridine was later worked out. 89

N-[8-{3-(2-cyanomethylene)-indolyl}-ethyl]-3′-ethyl-3′-piperideine (79) 90

The benzoate piperideine (78)(5.2g) was dissolved in dimethylformamide (135 ml) with potassium cyanide (9.0 g). This mixture was stirred at room temperature for 1 hour before slowly heating to 110°. After about 1 hour the mixture was cooled in an ice-water bath, diluted with water (300 ml) and extracted with methylene chloride. The organic extract was washed repeatedly with water, dried over anhydrous sodium sulphate and evaporated in vacuo to give a thick oil. This oil was dried in high vacuum to give a coloured solid (3.7 g). Subsequent chromatography on alumina (150 g, act III) yielded the nitrile (79)(2.1g).

N-[8-{3-(3-Carbomethoxymethylene)-indolyl}-ethyl]-3′-ethyl-3′-piperideine (80) 90

The indole nitrile (79)(2.1g) was dissolved in freshly dried methanol (about 50 ml, distilled from magnesium). Water (0.5 ml) was added to
constitute 1% of the solution and the mixture was cooled in an ice-water bath. Hydrogen chloride gas was bubbled into the reaction mixture to achieve a saturated solution. This mixture was stirred at room temperature for 2.5 days and then evaporated in vacuo. The residue was partitioned between sat. sodium bicarbonate solution and methylene chloride. The organic extract was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo to give an amorphous material (1.8 g). This product was chromatographed on alumina (100 g, act III) to yield the carbomethoxy indole (80)(1.45 g) which could be recrystallized from methylene chloride, m.p. 85-88°.

\[\text{[Ar-}^{3}\text{H]-indole ester (80)}^{90}\]

The recrystallized and dried indole ester (80)(210 mg) was dissolved in previously prepared trifluoracetic anhydride-[\text{H}] (1.2 g, 0.9 millicuries/m mole) using a vacuum transfer technique. This solution was allowed to stir at room temperature for 2.5 days under a nitrogen atmosphere. After removal of the solvent, the residue was partitioned between a conc. ammonium hydrotide solution and methylene chloride. The organic extract was washed several times with water, dried over anhydrous sodium sulphate and evaporated in vacuo. Subsequent chromatography on alumina yielded the ester (157 mg) which could be recrystallized to a constant specific activity of $4.71 \times 10^7$ dpm/mg.

\[\text{[Ar-}^{3}\text{H]-Secodine aldehyde (81)}^{90}\]

The aromatically labelled indole ester (157 mg) was dissolved in dry
benzene and add dropwise to a suspension of oil-free sodium hydride (160 mg of 55% dispersion in paraffin) in benzene (5 ml) and freshly distilled methyl formate (4 ml) under a nitrogen atmosphere. The reaction temperature was raised to 35° and stirring was continued for 2 hours. The reaction mixture was cooled in an ice-water bath and treated with cold water. Subsequent extract with methylene chloride, drying over anhydrous sodium sulphate and evaporation in vacuo yielded a light yellow foam (180 mg) which was used directly in the next reaction.

\([\text{Ar}^3\text{H}]16\text{-17-Dihyrosecodin-17-ol (69)}\)  

The amorphous material (150 mg) from the above reaction was dissolved in methanol (15 ml) cooled to -30° and treated with sodium borohydride (250 mg) over 1 hour and stirred for an additional 30 minutes until thin-layer chromatography indicated the absence of starting material. This mixture was then treated with 3 drops of 2N hydrochloric acid, diluted with water and reduced in volume in vacuo. Subsequent partitioning between sat. sodium bicarbonate solution and methylene chloride gave an organic extract which was washed with water and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo yielded a slightly coloured foam (180 mg). Column chromatography on alumina (10 g, act III) yielded the secodinol (69)(41 mg) from a benzene/chloroform gradient as a white foam. Recrystallization of this material from dichloromethane afforded a specific activity of 4.54 \times 10^7 \text{dpm/mg}.
Alternative Synthesis of N-\{\beta-[3-(2-Carbomethoxymethyl)-indolyl]-thyl\}-3'-piperdeine (80)

The ester alcohol (84)(190 mg) was dissolved in dichloromethane (15 ml) and 3-ethylpyridine (74)(0.5 ml) at 0° under an inert atmosphere. To the cool reaction mixture p-toluene sulphonyl chloride (600 mg) was added and stirring was continued overnight. This reaction mixture was then warmed to 95° while the dichloromethane was distilled off. Stirring was continued at 95-98° for 8 hours. After cooling, the excess 3-ethylpyridine was removed in high vacuum and the residue was dissolved in methanol at 0°. Sodium borohydride (1g) was slowly added over 1 hour and stirring at 0° was continued for a further 2 hours. Two volumes of water were then added and after stirring for 10 minutes this mixture was extracted repeatedly with dichloromethane (125 ml). The organic extract was dried over anhydrous sodium sulphate and evaporated in vacuo to give a dark coloured residue (340 mg). Chromatography of this material on alumina (15, act.III) using a benzene-dichloromethane solvent gradient yield the desired indole ester (60 mg). This material had physical and spectroscopic properties identical with those of previously prepared material (80).

1-(3'-Pyridyl)-1-thio-ethyleneketal-ethane

3-Acetylpyridine (91)(1g) was dissolved in a mixture of ethane dithiol (5 ml) and boron trifluoride etherate (1 ml) and stirred overnight at room temperature. After removal of the solvent in high vacuum, the residue was partitioned between saturated aqueous sodium bicarbonate
solution and dichloromethane. The organic extract was washed twice with sat. bicarbonate solution and once with water before drying over anhydrous sodium sulphate. Most of the solvent was then evaporated in vacuo and the product was chromatographed on alumina (15 g, act.III) to yield a colourless oil (1.55 g), pmr: 1.0, 1.45, 1.90, 2.78 (multiplets, 4H, aromatic-H), 6.6 (multiplet, 4H, -S-CH₂CH₂-S-), 7.85 (singlet, 3H, -CH₂); mass spectrum: M⁺ 197.

Desulphurization of 1-(3'-pyridyl)-1-thioethylerieketal-ethane

The thioketal (1g) was dissolved in absolute ethanol (50 ml) and refluxed with freshly prepared Raney nickel (20 g) for 16 hours. After cooling this mixture was stirred with 5% aqueous sodium hydroxide for 15 minutes and the inorganic material was filtered off. The residue was washed twice with ethanol and the combined filterates were extracted repeatedly with dichloromethane. After drying over anhydrous sodium sulphate the solvent was removed in vacuo. A glc examination showed the presence of one component which had the same retention time (6 minutes, 175°) as an authentic sample of 3-ethyl pyridine (74). The product was distilled to yield a colourless liquid whose spectral properties were also identical with an authentic sample of (74).

[2'-²H]-3-Acetylpyridine (92)

3-Acetylpyridine (2 g) was stirred with dry tetrahydrofuran (5 ml), deuterium oxide (3 ml) and dried sodium carbonate (0.5 g) overnight in a closed vessel. The reaction mixture was then partitioned and the organic
layer was dried over anhydrous sodium sulphate. After removal of the solvent in high vacuum, the product was examined by pnmr: 7.40 (multiplet, $-\text{CHD}_2$) integrated to show 95% exchange of isotopes.

$[2^-^2\text{H}]-1-(3^-\text{Pyridyl})-1\text{-thioethyleneketal-ethane (93)}$

The deuterated material (1.5 g) from the above reaction was converted to its corresponding thioketal (93)(2.1 g) as previously described and examined by pnmr: 7.9 (multiplet, $-\text{CHD}_2$) integrated to show loss of 1.4% from starting material.

$[2^-^2\text{H}]-3^-\text{Pyridyl})-\text{ethane (94)}$

The above deuterated thioketal (93)(2g) was converted to deuterated 3-ethylpyridine (94)(0.86 g) by the Raney nickel desulphurization procedure previously described. Examination of the product by pnmr: 8.8 (multiplet, $-\text{CHD}_2$) revealed by integration a loss of 3.4% of label from the starting 3-acetylpyridine (92).

$1^-\text{(3^-Pyridyl)-ethanol (95)}$

3-Acetylpyridine (91)(3.5 g) was dissolved in anhydrous dimethoxy-ethane (25 ml) and treated with sodium borohydride (265mg)at 80° for 24 hours. This mixture was then cooled and stirred with 0.1 N aqueous hydrochloric acid for 5 minutes and the resulting mixture was made basic with saturated sodium bicarbonate solution and extracted repeatedly with dichloromethane. The organic extracts were washed with water and dried over anhydrous sodium sulphate. Removal of the solvent in vacuo yielded
a colourless oil which was chromatographed on alumina (25 g, act.III).
This product (95)(3.45 g, b.p. 105-120° at 1.2 mm) had the following
data; uv: similar to 3-ethyl pyridine: $\nu_{\text{max}}$ 3250 (-OH); pmr: 1.56,
2.22, 2.78 (multiplets, 4H, aromatic-H), 4.0 (broad singlet, 1H, -OH),
5.10 (quartet, J=7, 1H, -CH(OH)CH$_3$), 8.55 (doublet, J=7, 3H, -CH(OH)CH$_3$);
mass spectrum: $M^+$ 123.

1-Ethyl-(3'-pyridyl)-acetate (99)

The above alcohol (2.8 g) was dissolved in acetic anhydride and
heated on a steam bath for 45 minutes. The solvent was then removed
in vacuo and the residue was partitioned between 5% aqueous sodium
bicarbonate and dichloromethane. The organic extract was then washed
with water and dried over anhydrous sodium sulphate. After removal
of the solvent the residue (3.2 g) was rapidly chromatographed on
alumina (20 g, act.II) to give a colorless oil (3.0 g): $\nu_{\text{max}}$ 1725
(acetate); pmr: 1.5, 2.30, 2.51 (multiplets, 4H, aromatic-H), 4.05
(quartet, J=7, 1H, -CH(OAc)CH$_3$), 7.93 (singlet, 3H, -O$_2$CCH$_3$), 8.44
(doublet, 3H, -CH(OAc)CH$_3$); mass spectrum: $M^+$ 165.

Catalytic Hydrogenolysis of Acetate (99)

The pyridal acetate (99)(2.0 g) was dissolved in dimethoxyethane
and treated briefly with hydrogen chloride gas. The solvent was removed
in vacuo and the residue was dissolved in water (30 ml). To this solution,
palladium-on-charcoal catalyst (10%, 400 mg) was added and the resulting
mixture was stirred at room temperature in an atmosphere of hydrogen gas.
After one equivalent of hydrogen had been consumed, the reaction mixture was filtered and made basic with sat. aqueous sodium bicarbonate solution and extracted with dichloromethane. The organic extracts were dried over anhydrous sodium sulphate before the solvent was carefully removed by distillation. The residue was then distilled to yield 3-ethyl-pyridine (0.9 g) which was compared with an authentic sample.

\[ \text{[1-}^3\text{H]}-\text{1-(3'-Pyridyl)-ethanol} \]

3-Acetyl pyridine (4.5 g) was dissolved in anhydrous dimethoxy-ethane (30 ml) and treated with dry sodium borohydride (10 mg) at 85° for 2 hours. Sodium borohydride-\(^3\text{H}\) (40 mg, 200 mc) was added and the reaction was continued overnight. Dry sodium borohydride (450 mg) was then added and stirring was continued until examination by tlc on silica gel (chloroform) indicated a complete reaction. This mixture was then worked up as previously described.

\[ \text{[1-}^3\text{H]}-\text{1-Ethyl-(3'-pyridyl)-acetate} \]

The tritiated alcohol from the above reaction was dissolved in acetic anhydride (25 ml) at 100°. This reaction was monitored by tlc and worked up as previously described.

\[ \text{[1-}^3\text{H]}-\text{1-(3'-Pyridyl)-ethane} \]

The tritiated pyridyl acetate from the above reaction was converted to its hydrochloride salt, dissolved in water and treated with palladium-on-charcoal (10%, 80 mg). After hydrogenalysis in
an atmosphere of hydrogen. The reaction mixture was worked up as previously described to yield [1-\(^3\)H]-1-(3'-pyridyl)-ethane (2.6 g, 2.18x10^8 dpm/mg).

\[19-\(^3\)H]-N[β {3-(2-Carbomethoxymethyl)-indolyl}-ethyl]-3’-ethyl-3’-
piperdeine

The ester alcohol (84)(200 mg) was dissolved in dry dichloromethane (15 ml), tritiated ethylpyridine (0.5 ml) with p-toluene sulphonyl chloride (650 mg) at 0°. The reaction was then carried out as previously described to yield the tritiated piperdeine (80) (65 mg).

\[19-\(^3\)H]-Secodine-17-al

The tritiated alcohol (80)(65 g) was dissolved in benzene (10 ml, freshly distilled from lithium aluminium hydride) and methyl formate (4 ml) and treated with sodium hydride (65 mg of a 55% oil suspension). After 2.5 hours the reaction was worked up as previously described.

\[19-\(^3\)H]-16,17-Dihydrosecodin-17-ol

The above product was dissolved in methanol (10 ml) at -35° and treated with sodium borohydride (20 mg) over 2 hours according to the procedure previously described. The product (30 mg) was recrystallized from dichloromethane to give tritiated secodinol (6.56x10^7 dpm/mg).
The Isolation of Vindoline (7) from Feeding Experiments in C. roseus Plants

Vindoline (7) was isolated from whole plants of C. roseus by a procedure similar to that used by others in our laboratories and will only be briefly summarized. The plant was up-rooted and cleaned of soil before it was macerated in methanol in a Waring blender. The resulting pulp was re-macerated twice more with additional quantities of methanol. The combined methanolic extracts were then evaporated and the residue was partitioned between 10% aqueous acetic acid and benzene. This aqueous extract was made basic with saturated sodium bicarbonate solution and extracted with chloroform. The organic extract was then washed with water and dried over anhydrous sodium sulphate before the solvent was removed. Chromatography of the residue on alumina (act.III) yielded vindoline (7), using a benzene-chloroform solvent gradient.

The Administration of \([19-^3\text{H}, ^{14}\text{CO}_2\text{CH}_3]\)-Secodine (68) to C. roseus

In both of the following feeding experiments, the radioactive precursor was dissolved in distilled water (0.5 ml) with 2 drops of IN acetic acid. This solution was administered to the plant via cotton wick (~5 cm) which had been passed through the stem of the potted plant. After the solution had been taken up by the plant distilled water (0.5 ml) with 1 drop of IN acetic acid was added to the container and allowed to be taken up by the plant. After that, the plant was allowed to grow under artificial lights at room temperature for a total of 9 days.
The data relevant to the feeding experiments using C. roseus plants is summarized in Table 2. In both cases the vindoline isolated was converted to vindolinol before recrystallization to constant radioactivity.

Table 2. Additional data associated with Table 1

<table>
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<td>$2.15 \times 10^5$</td>
<td>$6.50 \times 10^4$</td>
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<tr>
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<td>$4.94 \times 10^9$</td>
<td>$3.67 \times 10^5$</td>
<td>$2.72 \times 10^5$</td>
</tr>
</tbody>
</table>

a. $[^{14}\text{CO}_2\text{CH}_3]$-Secodinol was available from the original synthesis by Sood.⁹¹

Vindolinol (100)⁹¹,⁹⁸

Radioactive vindoline (7) was converted to vindolinol by the procedure reported by Beck.⁹¹ Vindoline (7) was dissolved in dry tetrahydrofuran and refluxed with excess lithium aluminum hydride for 2 hours. The excess reducing reagent was destroyed with sat. sodium sulphate solution. Extraction with methylene chloride yielded the desired product, which could be recrystallized to constant radioactivity from diethyl ether.
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PART II

Studies Related to the Biosynthesis of Uleine and Olivacine
INTRODUCTION

In Part I a general introduction to indole alkaloid biosynthesis was presented. Until very recently, all the work in this area had been directed toward the four major families of indole alkaloids, namely the Corynanthe, the Strychnos, the Aspidosperma and the Iboga skeletons. There is, however, an increasing number of indole alkaloids being found in nature in which the tryptamine portion of the skeleton has been altered. Members of the four major families just mentioned possess a two-carbon bridge between the indole nucleus and the basic nitrogen atom ($N_b$). Alkaloids of the uleine-type (1), however, have no such carbon bridge, while members of the apparine (2) and vallesamine (3) family have a one-carbon bridge. On the other hand, a three-carbon bridge is apparent in the pyridocarbazole alkaloids of which the best known examples are olivacine (4), guatambuine (5) and ellipticine (6).
The first question that had to be asked was: are these families of indole alkaloids also derived from tryptophan (15)? Wenkert's early hypothesis was that they were not.\(^1\) He suggested that a precursor of tryptophan, glycosylenanthranilic acid (7) condensed with the seco-prephenate-formaldehyde (SPF) unit (8, see also Figure 2 in Part I) to give an intermediate (10) which with the addition of a methylamine unit, or its biological equivalent, could undergo the appropriate cyclizations to give ellipticine (6), uleine (1) and olivacine (4) as illustrated in Figure 1. Since the publication of this hypothesis the SPF unit (8) has been set aside in favour of the structurally similar seco-loganin moiety (9), the development of which was described in Part I. Since the latter compound could behave in a similar fashion to that of the SPF unit, the Wenkert hypothesis remains intact and worthy of further study.

In 1965, with the structure of apparicine (2) in hand Djerassi and Gilbert\(^2\) suggested that Wenkert's intermediate (13) could also cyclize to provide the one-carbon bridge between the indole and piperidine units to give the apparicine skeleton. The fact that uleine (1) and apparicine (2) co-occur in the same plant species may suggest that these two structures have a common biosynthetic intermediate.\(^3\) Also, it is known in berberine alkaloid biosynthesis that N-methyl groups can be precursors to methylene bridges.
Figure 1. The Wenkert hypothesis for the biosynthesis of ellipticine (6), uleine (1) and olivacine (4).
The first biosynthetic investigations\(^4\) of these alkaloids by Wigfield and Nelson in our laboratories revealed that, in *Aspidosperma pyricollum* plants, tryptophan (15) labelled in the aromatic ring with tritium and in the C-3 position with Carbon 14, was incorporated into apparicine (2) (See Table 1).

However, no activity could ever be detected in uleine (1) which was also isolated in these experiments.

Also of importance was the discovery by our group\(^5\) that stemmadenine (16) was very efficiently incorporated into apparicine (2) to the extent of 0.55%, while the more similar structure vallesamine (17) was incorporated into apparicine in only 0.01%.
These results strongly suggest that a stemmadenine-like precursor is involved in the later stages of biosynthesis with the C-2 carbon atom of the original tryptamine bridge being lost in the later stages of the pathway. Again, no incorporation into uleine (1) could be demonstrated from any of the precursors tried.

With this new insight into the origin of the nitrogenous portion of apparicine, Kim and Erickson\(^6\) modified an earlier mechanistic proposal\(^7\) which allowed the involvement of tryptophan (15) with the extrusion of the two carbon bridge to form the uleine skeletons. Their \textit{in vitro} studies failed to support this proposal. The proposal also suffered from a major deficiency in that it was specific for uleine (1)
and could not take into account the formation of apparicine (2).

Our early results concerning the incorporation of stemmadenine and their own experience with the Polonovski-type fragmentation of N-oxides stimulated a French group\(^3\) to propose a biosynthesis of apparicine (2) which included extrusion of the C-2 carbon from the two-carbon bridge. This idea was supported by in vitro studies on tryptamine derivatives. Further work in our laboratories, by Beck and Fuller, using doubly-labelled secodine (18) revealed that this material was incorporated intact into apparicine (2) in \(A.\) pyricollum plants with retention of the carbomethoxy group.\(^9,^{10}\) The earlier French hypothesis had suggested a concerted loss of this group during the fragmentation reaction. Subsequently, a modified hypothesis was published in 1973 in which loss of the hydroxymethylene group was suggested.\(^{11,12}\) This new hypothesis was a satisfying one in that it had utilized all the experimental evidence to date and had allowed for the biosynthesis of all the "anomalous" alkaloids along very similar pathways. This suggestion is in accord with their co-existence in several plant species.
Figure 3. The Potier-Janot postulate for the biosynthesis of apparicine (2), uleine (1) and the pyridocarbazole alkaloids.
An important point to recognize is that these biosynthetic pathways involve stemmadenine (16), the crucial precursor to the Iboga and Aspidosperma alkaloids discussed in Part I.

A recent synthesis of ellipticine (6) by the Potier group provides an interesting support to their hypothesis. They achieved the cyclization of structure (21) via a Polonovski fragmentation to give (22) in high yield. This reaction is parallel to the conversion (19)\rightarrow(20)\rightarrow(6) in Figure 3. Structure (22) was dehydrogenated to ellipticine (6).

To date all attempts to incorporate any precursor into uleine (1) have failed. It was felt, due to the importance of this problem, that some re-investigations and extension of the earlier studies was necessary. The following section describes further work directed toward the biosynthesis of uleine (1) and olivacine (4).
DISCUSSION

The fact that tryptophan (15), stemmadenine (16) and secodine (18) could all be incorporated into apparicine (2) strongly suggests that uleine (1) could also arise from a similar biosynthetic pathway since they co-occur in several Aspidosperma plant species. As previously mentioned, work in our laboratories showed that the C-3 carbon atom of tryptophan was retained during biosynthesis to form the unusual single-carbon bridge between the indole nucleus and $N_b$ in apparicine (2). The first question that had to be answered with regard to the biosynthesis of uleine (1) was: is this molecule which doesn't possess such a bridge also derived from tryptophan (15) or is it derived from a progenitor of tryptophan as suggested by the Wenkert hypothesis (Figure 1)? Secondly, if tryptophan is involved, what is the fate of carbons-2 and 3? Is the C-3 carbon atom extruded while the C-2 atom is retained to become the N-methyl group of uleine (1) as suggested by the Potier-Janot hypothesis (Figure 3)? And thirdly, what role does the dehydrosecodine hypothesis, as revealed in Part I, play in the biosynthesis of uleine (1).

Similar questions may be asked of the biogenetic origin of the pyridocarbozole derivative olivacine (4), a member of the "anomalous" family of alkaloids having a three-carbon bridge between the indole nucleus and $N_b$. The Potier-Janot hypothesis (Figure 3) suggests that both uleine (1) and olivacine (4) are derived from the same intermediate, from which the C-3 carbon atom originally in tryptophan has been extruded. The formation of the fully aromatic structure of olivacine (4) also
requires loss of the C-2 atom which had been implicated as the N-methyl group. Figure 4 summarizes the expected positions of radio-label in uleine (1) and olivacine (4) as would arise from the appropriately labelled precursors tryptophan (15), stemmadenine (16) and secodine (18).

We had available in our laboratories two Aspidosperma plant species, A. pyricollum and A. australis, in which uleine (1), apparicine (2) and olivacine (4) co-occur. This provided us with a unique opportunity to study the biosynthesis of these unusual alkaloids. As mentioned earlier the study of apparicine (2) was begun first, in our laboratories, in A. pyricollum plants. The biosynthetic evidence obtained is summarized in Table 1.

Experiments 1 and 2 in Table 1, reported by Nelson and Wigfield, showed for the first time that tryptophan is indeed a precursor of apparicine (2) and that it is the C-2 carbon atom which is extruded at some point in the biosynthesis. The Strychnos alkaloid stemmadenine (16) was found to be incorporated in the surprisingly high level of 0.55%, while the more structurally similar vallesamine (3) was incorporated in only 0.01%. Although the incorporation of variously labelled secodine (18) precursors was generally low (experiments 5-8) there can be little doubt that the proposed stemmadenine $\rightarrow$ secodine bio-equilibrium is operative in the biosynthesis of this molecule. In none of these experiments could incorporation into uleine (1) be demonstrated.

Clearly, it would be of great interest to expand the biosynthetic investigation of uleine (1) in A. pyricollum plants in an attempt to understand the negative results obtained to date, and then evaluate possible biosynthesis of this molecule in another Aspidosperma plant species.
Figure 4. Expected positions of radio-label in uleine (1) and olivacine (4) from available precursors as suggested by the Potier-Janot hypothesis (Figure 3).
Table 1

Incorporation studies into apparicine (2) in *A. pyricollum* plants

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<th>Entry(ref)</th>
<th>Precursor</th>
<th>Percent incorporation</th>
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<th>Ratio Activity Isolated(^{3}H /^{14}C)</th>
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<td>5(9)</td>
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<td>6(9)</td>
<td>([\text{Ar}^{3}H, 1^{14}C]\text{CO}_2\text{CH}_3])-secodine(18)</td>
<td>0.014, 0.015</td>
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<td>7(9)</td>
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<td>2.05</td>
</tr>
</tbody>
</table>
In *A. australis* plants, similar precursors could be administered to investigate their possible participation in the biosynthesis of uleine (1) and the pyridocarbazole alkaloid olivacine (4). The biosynthesis of the latter class of alkaloids has not been investigated before.

The remaining part of this discussion deals with the current investigation of the biosynthesis of uleine (1) and olivacine (4).

The hypothetically labelled structure of uleine (1) in Figure 4 illustrates that an investigation into the biosynthesis of this molecule would be greatly enhanced if a degradation scheme could be adapted to permit the isolation of some of the carbon atoms which are expected to bear a radio label. With this objective in mind, the following plan was implemented.

Our first objective was to isolate the N-methyl group of uleine (1). Uleine was first isolated by Schmutz et al. from *A. ulei* in 1957. The structure proposed at that time was, however, incorrect and was replaced by the now proven structure proposed by Buchi two years later. The latter group also succeeded in the total synthesis of uleine in 1971. The structural proof of this molecule was based on a classical Hofmann degradation scheme which permitted the removal of the basic nitrogen from the indole portion. This scheme, then, could probably be adapted to permit the isolation of the N-methyl group in a manner which would be useful in a biosynthetic investigation.

To obtain bulk quantities of uleine for our degradation work and subsequent plant feeding experiments we had available ethanolic extracts of *A. australis* plants collected in Brazil. The extracts were dried and treated with a 15% aqueous acetic acid solution, filtered to remove
insoluble material, and the resulting solution was washed with petroleum ether to remove any remaining non-alkaloidal material. This acidic solution was made basic with aqueous sodium hydroxide and extracted with diethyl ether to yield the alkaloid containing fraction. Subsequent chromatography on silica gel yielded quantities of uleine (1), apparicine (2) and olivacine (4) and several other components.\textsuperscript{17,18}

A similar isolation scheme was used to obtain further quantities of uleine (1) and olivacine (4) from \textit{Aspidosperma Olivaceum} plant extracts. Chromatography of the alkaloidal fraction using deactivated alumina as adsorbent provided an easier method for obtaining ulein (1) as pure crystalline material.

Uleine (1, m.p.75-80\(^\circ\)) was identified by its characteristic ultraviolet spectrum (\(\lambda_{\text{max}}\) 210, 307, 316), mass spectrum; and pmr spectrum. The latter exhibited two singlet absorptions of \(\tau 4.64\) and 4.72 which are characteristic of exocyclic methylene protons. Also present was a singlet at \(\tau 7.60\) which was assigned to the N-methyl group. The spectral and physical data were found to be identical with published data\textsuperscript{17} as well as with an authentic sample of uleine (1).\textsuperscript{18}

Olivacine (4, m.p.312-315\(^\circ\)) was similarly identified. Its ultraviolet spectrum (\(\lambda_{\text{max}}\) 224, 338, 277, 288, 315, 329, 375) showed the presence of the fully aromatic pyridocarbazole system. The pmr spectrum exhibited besides the seven aromatic ring protons, a singlet at \(\tau 6.86\) which was assigned to the C-1 methyl group and another singlet at \(\tau 7.21\) was attributed to the C-5 methyl group. The spectral and
physical data on this compound were identical with published data and with an authentic sample of olivacine (4).

The first degradation of uleine (1) that we considered was one involving two classical Hofmann elimination reactions which would remove the basic nitrogen with its methyl group from the rest of the molecule. Uleine (1) was converted to its quaternary salt with methyl iodide to give a very high yield of the methiodide derivative m.p. 205-206°. The mass spectrum of this compound exhibited the parent peak (m/e 281) as the base peak which corresponded to the addition of one methyl group to the uleine molecule. The identity of structure (23) was confirmed by elemental analysis.

Uleine methiodide (23) was refluxed with potassium hydroxide in aqueous ethanol to give a single product which had an ultraviolet spectrum ($\lambda_{max}$ 235(sh), 248, 262, 287, 293, 307) characteristic of a carbazole derivative. The mass spectrum of this product showed a molecular parent peak at m/e 280. The pmr spectrum, apart from two groups of aromatic proton signals, revealed a quartet at $\tau$7.10 and a methyl triplet at $\tau$8.75 indicating an ethyl side-chain, similar to that in uleine (1). Also there appeared a three proton singlet at $\tau$7.50 which was assigned to a methyl group attached to an aromatic ring and a six-proton singlet at $\tau$7.61 which was assigned to two N-methyl groups. These data are consistent with structure (24). Uleine methiodide (23) underwent the expected Hofmann fragmentation reaction to give an intermediate (24), which underwent facile aromatization to give the carbazole system (25) in 82% yield.
The carbazole derivative (25) was reacted with methyl iodide to give its corresponding methiodide salt (22, m.p. 300-302°) which formed in quantitative yield.

The next step in the degradation was to perform a Hofmann elimination reaction on the methiodide (26) to remove the basic nitrogen from the carbazole part of the molecule. Initial attempts, contrary to a literature report, failed to achieve the desired fragmentation when potassium hydroxide dissolved in ethylene glycol-water solution at 200°C, was employed. However, potassium t-butoxide in freshly dried t-butanol achieved the elimination at 80°. The product (27) from this reaction had an ultraviolet spectrum ($\lambda_{\text{max}}$ 228, 248, 265, 292, 306 nm) expected for a carbazole system. Its mass spectrum showed a molecular ion as the parent peak at m/e 235 with a facile loss of two methyl groups to give fragments at m/e 220 and 205. The pmr spectrum revealed a multiplet centered at $\tau$3.54 which was assigned to a vinyl group bonded to an aromatic ring.

In order for this degradation scheme to be useful in a biosynthetic investigation it was necessary to evaluate the sequence starting with 15-20 milligrams (mg) of uleine (1), this being the expected quantity obtainable from plant feeding experiments. It was also essential to be able to trap efficiently the trimethylamine (28) which was liberated in the last reaction of this sequence. This requirement was achieved by a slow passage of dry nitrogen gas through the reaction mixture into a collector containing a methanolic solution of methyl iodide. Crystalline tetramethylammonium iodide (29) was obtained in an overall yield of 70-75%.
Figure 5. Degradation of uleine by means of Hofmann elimination reactions.
It was also desirable to evaluate this degradation scheme using radioactive material before proceeding with the plant feeding experiments in order to discover any further difficulties which might arise. Uleine (1)(20mg) was reacted with $^{14}$C-methyl iodide and the resulting quaternary ammonium salt (19) was recrystallized to a constant specific radioactivity of $7.17 \times 10^5$ disintegrations per minute (dpm) per millimole. Degradation of this material by the procedure described above yielded tetramethyl ammonium iodide (25) (72% yield), which was recrystallized to a constant radioactivity of $6.95 \times 10^5$ dpm per millimole. This recovery of 97% of the specific activity was considered within the limits of experimental error.

A biosynthetic investigation of uleine was now initiated. The first plant species chosen for this study was A. pyricollum. This plant is a slow growing shrub, indigenous to South America, which stores uleine in the roots. We found that by removing some fibrous roots or the tap root for use in feeding experiments the remaining plant could be maintained in a satisfactory growing state for subsequent experiments.

In our initial feeding experiments using either fibrous or tap roots the water solubilized precursor was administered topologically to the system. At the end of the feeding time, the roots were masurated in methanol and the normal alkaloid extraction procedure, as described earlier, was used to obtain a crude extract. In each experiment uleine (1)(20mg) was added to this extract to act as a carrier for small amounts of radioactive uleine which may have been present in quantities insufficient to study directly. This technique of radio-dilution allows one to study
the total incorporation into a compound but not specific incorporation (defined as radioactivity per millimole) since the amount of compound obtained from the plant is never known.

Table 2 summarizes the feeding experiments using A. pyricollum. In experiments 1 and 2, tryptophan labelled in the aromatic ring with tritium and with carbon-14 in the side chain (C-2) was administered hydroponically to tap root and fibrous roots, respectively. After a time period of 6 days, uleine (1) was isolated and recrystallized until an insignificant amount of radioactivity was detected. It is generally accepted in the field of biosynthesis that if a total incorporation of a precursor into a target molecule is below 0.001% the results should be viewed as being negative. In the third experiment in this plant species, the acetate salt of secodine (18) labelled with carbon 14 in the carbomethoxy group was similarly administered to a tap root. Again, incorporation into uleine (1) could not be demonstrated. The preparation of this precursor is described in Part I.

A second series of feedings to A. pyricollum were initiated to test the possibility that the production of uleine (1) in the plant was a slow process and therefore the administration of tryptophan (15) or its precursor anthranilic acid to a growing plant may result in some incorporation into uleine. The resources required to do a complete time study of incorporation were not available at that time. In experiments 4 and 5, tryptophan and anthranilic acid were administered in very dilute acetic acid solutions to growing plants using the wick feeding technique. The results of these long term feeding experiments in whole plants
Table 2. Incorporation studies in *A. pyricollum* plants

<table>
<thead>
<tr>
<th>Exp.No.</th>
<th>Precursor</th>
<th>Activity Fed(dpm)</th>
<th>Percent incorporation into Uleine(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$[\text{Ar-}^3\text{H, 2-}^{14}\text{C}]$-tryptophan (15)$^a$</td>
<td>$^3\text{H} - 1.13\times10^7$</td>
<td>$^{14}\text{C} - 1.14\times10^8$</td>
</tr>
<tr>
<td>2</td>
<td>$[\text{Ar-}^3\text{H, 2-}^{14}\text{C}]$-tryptophan (15)$^a$</td>
<td>$^3\text{H} - 2.34\times10^8$</td>
<td>$^{14}\text{C} - 7.62\times10^9$</td>
</tr>
<tr>
<td>3</td>
<td>$[^{14}\text{C}_2\text{CH}_3]$-secodine (18)$^b$</td>
<td>$3.70\times10^7$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>$[\text{Ar-}^3\text{H, 2-}^{14}\text{C}]$-tryptophan (15)$^a$</td>
<td>$^3\text{H} - 3.89\times10^8$</td>
<td>$^{14}\text{C} - 4.92\times10^7$</td>
</tr>
<tr>
<td>5</td>
<td>$[\text{Ar-}^3\text{H}]$-anthranilic acid$^a$</td>
<td>$2.22\times10^9$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Available from New England Nuclear

$^b$ Available from the original synthesis by Sood in our laboratories.
appeared also to be negative. Additional data concerning the feeding experiments in *A. pyricollum* appear in Table 4.

Another plant species which had become available in our laboratories was *Aspidosperma australe*. Not only did this plant biosynthesize uleine (1) but also it produced several pyridocarbazole alkaloids of which olivacine (4) was of particular interest. The plants used in this work were eighteen to twenty-four months old and twenty to twenty-five centimeters tall. In this series of experiments, the precursor was administered directly to the tap root of the whole plant and the plant was then allowed to grow hydroponically in a test tube during a feeding time of 6 days. As before the target compounds uleine (1) and olivacine (4) were obtained by dilution of the alkaloidal extracts with inactive alkaloids and subsequent isolation.

Table 3 summarizes the results of these experiments. Experiments 1 and 2 show that neither anthranilic acid nor tryptophan could be incorporated significantly into uleine (1) or olivacine (4) under these experimental conditions. In the hope that a later stage precursor, being closer to the end of the biosynthetic pathway, might be elaborated into these alkaloids two feeding experiments using doubly-labelled secodine (18) were performed. The procedure for preparing secodine (18) immediately prior to its administration to the plant was the same as that described in Part I. No incorporation of secodine (18) into uleine (1) or olivacine (4) could be demonstrated.

Another biogenesis of the N-methyl group of uleine (1) was considered. It is well known that the main source of methyl groups in biological systems is the amino acid methionine. Methionine (30) in its active form as S-
adenosylmethionine (31) could transfer its S-methyl group to the biological equivalent of des-N-methyluleine (32). The latter (32)

\[
\text{CH}_3\text{-S}\text{-CH}_2\text{CH}_2\text{CH-CO}_2\text{H}
\]

(30)

...(31) could possibly be on the biosynthetic route to uleine (1) or it could be in biological equilibrium with the methylated derivative.
Therefore it was decided to label the "C\textsubscript{1}-pool" in the plant with methionine (30) labelled with carbon 14 in the S-methyl group. As shown by experiments 5 and 6 in Table 3, a high incorporation of methionine into uleine (1) was found. To ascertain the precise position of label in the isolated uleine the previously described degradation scheme permitted the isolation of 97\% and 98\% of the specific radioactivity obtained as the tetramethylammonium iodide salt (29).

The results of the biosynthetic investigations described in this section do not provide any direct information relating to either the Wenkert hypothesis\textsuperscript{1} or the Potier-Janot hypothesis\textsuperscript{11} concerning the biosynthesis the uleine and olivacine type of alkaloids. The incorporation of anthranilic acid is essential to the Wenkert hypothesis as is the incorporation of tryptophan (15) to the Potier-Janot hypothesis. The latter would also require the incorporation of secodine (18) since it has already been demonstrated (as described in Part I) that secodine is an active precursor to the Aspidosperma and Iboga type of alkaloids by way of the dehydrosecodine (18) \rightleftharpoons stemmadenine (16) equilibrium (Figure 4). However, the fact that none of these precursors could be elaborated by \textit{A. pyricollum} or \textit{A. austral} into more complex alkaloids under the conditions described does not mean that these compounds are necessarily on the true biosynthetic pathway. Caution must be exercised in interpreting negative results in biosynthetic investigations due to three problems inherent to the administration of material to plants.

The first problem that must be overcome is one of transportation to the site of biosynthesis within the plant. We know that the plant has
Table 3. Incorporation studies in *A. australe* plants

<table>
<thead>
<tr>
<th>Exp.No.</th>
<th>Precursor</th>
<th>Activity Fed (dpm)</th>
<th>Percent Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[Ar-(^3)H]-anthranilic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.22x10^9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>[Ar-(^3)H, 2-(^14)C]-trypotphan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3</td>
<td>[Ar-(^3)H, (^14)CO(_2)CH(_3)]-secodine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3(^H) - 2.48x10^7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14(^C) - 7.44x10^6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>[19-(^3)H, (^14)CO(_2)CH(_3)]-secodine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3(^H) - 1.19x10^7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14(^C) - 7.50x10^6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>5</td>
<td>[(^14)CH(_3)]-methionine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.22x10^8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.168</td>
</tr>
<tr>
<td>6</td>
<td>[(^14)CH(_3)]-methionine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.22x10^8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.147</td>
</tr>
</tbody>
</table>

<sup>a</sup> Available from New England Nuclear

<sup>b</sup> [\(^14\)CO\(_2\)CH\(_3\)]-secodine was available from the original synthesis by Sood in our laboratories

<sup>c</sup> Synthesis of [Ar-\(^3\)H]-secodine and [19-\(^3\)H]-secodine is described in Part I
absorbed a large percent of the precursor because the residual radioactivity in the vessel is always measured. But we don't know where in the plant the biosynthesis of the alkaloids occurs nor do we know if our precursor is transported to where it may be required.

The second problem is one of permeability of the cell walls behind which biosynthesis takes place. This problem is believed to be more serious with larger molecules such as secodine (18). It may be that under normal conditions the biosynthetic building blocks are attached to carrier protein molecules and unattached precursors may be treated as foreign material by the plant systems.

The third problem arising in a biosynthetic investigation is the question of whether or not the plant is actively biosynthesizing the target compounds during the time of the experiment. It is possible that these compounds are biosynthesized very slowly and stored or they may be biosynthesized only during certain growth periods in the life of the plant and again simply stored during other times. If biosynthesis is occurring very slowly it is likely that the precursor that was fed would undergo biodegradation before a significant amount of it could be used to synthesize the alkaloids under investigation.

In order to overcome some of these problems inherent in the administration of precursors to plant systems a great deal of material and work would be required. One way around these problems is to work with cell-free extracts of the enzyme systems of the plant species. Work in this area will be initiated in our laboratories in the near future.

The fact that uleine (1) was efficiently methylated with carbon 14 labelled methionine (30) casts grave doubt on the Potier-Janot hypothesis.
which required the N-methyl group to come from C-2 carbon of tryptophan (15). It is therefore logical to speculate that des-N-methyluleine (32) is an immediate precursor to uleine (1) and that methylation of the basic nitrogen atom occurs as the last step in the biosynthesis. This possibility is supported by the discovery of the co-existence of the des-N-methyl derivative (32) with uleine (1) in A. dasycarpon A. DC. and A. vargasii A. DC. The study of this methyl transfer reaction by the methyl transferase enzyme may be a good way to initiate an investigation of cell-free enzyme extracts of the Aspidosperma species.

Part III of this thesis describes a synthetic study designed to make available, for biosynthetic evaluation, appropriately labelled derivatives in the preakuammicine- and stemmadenine-series.
EXPERIMENTAL

For a description of general experimental information the reader is referred to the experimental section of Part I.

Radioactivity was measured using a Nuclear-Chicago Mark I or a Mark II Model 6860 Liquid Scintillation Counter. The details of the counting procedure were previously described and are pertinent to this section.

The *Aspidosperma pyricollum* plants and the *A. australis* plants were grown from seeds in a Dept. of Horticulture greenhouse, University of British Columbia, under the supervision of Dr. P. Salsbury of the Dept. of Chemistry. The seeds were collected by Dr. B. Gilbert in the vicinity of Rio de Janeiro, Brazil.

The Isolation of Alkaloids from *Aspidosperma australis* (Mull. Argov.) Extract

The following procedure was adapted from that published by Ondetti and was later used by others in our laboratories.

The methanolic extract of *A. australis* plants collected in the vicinity of Rio de Janeiro, Brazil in 1967, was dried to give a dark coloured amorphous solid (15.8 g). This material was then suspended in a cooled solution of methanol (15 ml) and 15% aqueous acetic acid (100 ml) and stirred in an ice-water bath for 1 hour. The suspension was then filtered and washed with petroleum ether (2x50 ml). The acidic extract was carefully made basic with a cooled aqueous sodium hydroxide solution (50%) while ice was added periodically to keep the temperature down. After raising the pH to 10–12, the resulting suspension was extracted with diethyl ether (3x100 ml) followed by extraction with methylene chloride (3x100 ml). These organic
extracts were combined, washed once with water, and dried over anhydrous sodium sulphate. Removal of the solvent in vacuo yielded a brown solid (1.6 g). An examination of this material by thin-layer chromatography (alumina, 15% methanol in ethylacetate) revealed a larger number of components which separated into a "polar" group and a "non-polar" group. Subsequent column chromatography on alumina (act. III) using an ethylacetate/methanol gradient yielded two fractions of alkaloids which were separately partitioned.

The polar fractions were re-chromatographed on alumina (act. III) using a toluene/chloroform gradient to obtain almost pure uleine (1). This material (120 mg) was recrystallized from methanol to give colourless cubic crystals, m.p. 75-80° (lit. m.p. 76-80°). The identity of this compound was unambiguously established by comparison of its physical and spectroscopic data with an authentic sample of uleine (1) as well as with published data.

The less polar fractions from the first chromatographic separation were dissolved in chloroform containing 5% methanol and several drops of pyridine and re-chromatographed on silica gel (act. III). Elution with a chloroform/ethylacetate gradient yielded olivacine (4) (210 mg) and continued elution using an ethylacetate/methanol gradient yielded guatambuine (190 mg). The fractions containing olivacine (4) were recrystallized from methanol to give light yellow needle-like crystals, m.p. 312-315° (lit. m.p. 314-316). The identity of this compound was unambiguously established by comparison of its physical and spectroscopic data with an authentic sample of olivacine (4) and with published data.
The guatambuine obtained was identified by comparison with a sample obtained by partial synthesis and used in another study in our laboratories by Dr. Grierson.

The Isolation of Alkaloids from Aspidosperma Olivaceum Extract

The methanolic extract of A. Olivaceum plants collected in the vicinity of Rio de Janeiro, Brazil, was dried to give an amorphous solid (26 g). In the way described in the previous extraction procedure, this extract was taken up in 15% aqueous acetic acid-methanol solution, filtered and washed with petroleum ether. The solution was made basic with aqueous sodium hydroxide solution and extracted with methylene chloride to yield an alkaloidal extract (3.0 g). This material was subsequently chromatographed on alumina (150 g, act.III). The appropriate fractions obtained from elution with a toluene/chloroform gradient were pooled to give uleine(1)(240 mg) which could readily be crystallized and identified by comparison with previously obtained material.

Uleine methiodide (23)

Uleine (1)(75 mg) was dissolved in a solution of methanol (3 ml) and methyl iodide (1 ml) and let stand at 5° overnight.

Removal of the solvent from the reaction mixture in vacuo yielded a slightly coloured crystalline material. Recrystallization of this product from ethanol gave colorless, short needle-like crystals (95 mg) which were dried in high vacuum, m.p. 205-206° (lit. m.p. 204-206°)\textsuperscript{14}; $\lambda_{\text{max}}$ 283(sh), 290, 302, 305(sh), 312(sh); mass spectrum: 281(100), 266(10),...
222(22), 207(29), 205(29), 204(23), 194(9), 128(77), 127(44).

Anal. Calculated for C$_{19}$H$_{25}$N$_2$I: C,55.88; H,6.17; N,6.86. Found: C,56.13; H,6.00; N,6.69.

1-Methyl-2-(¿-dimethylaminoethyl)-3-ethylcarbazole (25)

Uleine methiodide (19)(62 mg) was dissolved in a solution of ethanol (3 ml), water (2 ml) and potassium hydrotide (75 mg) and the mixture was refluxed for 1.5 hours. Most of the solvent was then removed in vacuo and the residue was partitioned between water and diethylether. The aqueous layer was extract several times with diethylether (50 ml) and the combined organic extracts were washed once with water and dried over anhydrous sodium sulphate.

Removal of the solvent yielded a light brown glassy material which could be crystallized from diethylether/petroleum ether solution to give a crystalline product, m.p. 114-116° (lit. m.p. 115-116°)$^{14}$; $\lambda_{\max}$ 230, 248, 262, 287(sh), 293, 307; pmr signals: 1.9-2.3 (3H, multiplet, ar-H), 2.6-2.8 (2H, multiplet, ar-H), 7.1 (4H, multiplet, ar-CH$_2$-CH$_2$-N), 7.20 (2H, quartet, ar-CH$_2$-CH$_3$), 7.50(3H, singlet, ar-CH$_3$), 7.61(6H, singlet,-N(CH$_3$)$_2$), 8.75(3H, triplet, -CH$_2$CH$_3$); mass spectrum: 280(86), 235(22), 222(29), 220(29), 206(38), 204(38), 186(66), 171(100).

Anal. Calculated for C$_{19}$H$_{24}$N$_2$: C,81.38; H,8.63; N,9.99. Found: C,81.10; H,8.59; N,10.10.

This material was found to be identical with a degradation product ofquatambuine obtained by Dr. Grierson in our laboratories.$^{21}$

1-Methyl-2-(¿-trimethylaminoethyl)-3-ethylcarbazole iodide (26)

The carbazole amine(21)(40 mg) was dissolved in a solution of
methanol (3 ml) and methyl iodide (1 ml) and let stand at 5° overnight. Removal of the solvent in vacuo gave a slightly coloured crystalline material (61 mg). A portion of this material was recrystallized from ethanol and dried in high vacuum overnight, m.p. 300-302° (lit. m.p. 300-302°).

Anal. Calculated for C_{20}H_{27}N._I: C, 56.87; H, 6.44; N, 6.63. Found: C, 56.60; H, 6.37; N, 6.59.

Hofmann Fragmentation of Compound (26)

The carbazole methiodide (22) (60 mg) was added to freshly distilled t-butanol (10 ml) in which potassium metal (53 mg) had been dissolved. The reaction vessel, a two-necked round bottom flask, was equipped with a source of dry nitrogen gas and a glass tube outlet which extended into a collector charged with methyl iodide-methanol solution. In this way a slow stream of nitrogen gas was passed through the reaction mixture to carry with it any volatile material, formed during the reaction, into the collecting flask. The reaction mixture was stirred in an oil bath set at 80° for 2 hours. The system was continuously purged with nitrogen gas as the reaction mixture was allowed to cool to room temperature.

The reaction mixture was evaporated in vacuo and the residue was partitioned between water and diethylether. The aqueous layer was washed several times with diethylether (60 ml) and the combined organic extract was washed once with water and dried over anhydrous sodium sulphate. Removal of the solvent in vacuo yielded a brown amorphous material (12 mg). A thin-layer chromatography (silica gel, CHCl₃) examination of this product
revealed one major component plus several more polar minor components. Attempted purification of the major product by preparative thin-layer chromatography yielded a slightly yellow glass which crystallized only poorly from diethylether/petroleum ether solution (lit. m.p. 67-68°). Lack of material prevented further attempts to purify this product.

\[ \lambda_{\text{max}} \] 228(sh), 235, 248, 265, 292(sh), 306; pmr signals: \( \tau \)1.9-2.3 (3H, multiplets, ar-H), 2.6-2.8(2H, multiplets, ar-H), 4.4(1H, doublet of doublets, J=17 and 3, -CH=CH\textsubscript{2} \text{cis}), 6.7(1H, doublet of doublets, J=24 and 3, -CH=CH\textsubscript{2} \text{trans}), 7.20(2H, quartet, J=7, ar-CH\textsubscript{2}CH\textsubscript{3}), 7.50 (3H, singlet, ar-CH\textsubscript{3}), 8.75(3H, triplet, J=7, -CH\textsubscript{2}-CH\textsubscript{3}); mass spectrum: 235(100), 220(90), 205(56), 204(39), 117(19), 107(20), 103(21), 102(26); high resolution mass measurement: Calculated for C\textsubscript{17}H\textsubscript{17}N:235.1360. Found: 235.1371.

The solvent in the trap was evaporated \textit{in vacuo} to give a colourless precipitate (19 mg) which was recrystallized from methanol to yield long needle-like crystals which were crushed and dried in high vacuum. This material was readily identified as tetramethylammonium iodide (31) based on the following data: m.p. 230° (lit. m.p. 230°)\textsuperscript{23}; mass spectrum: 142(100), 127(94), 59(33), 57(76).

\textbf{Anal.} Calculated for C\textsubscript{4}H\textsubscript{12}NI: C,23.89; H,6.02; N,6.96. Found: C,23.96; H,6.10; N,7.07.

The Isolation of Uleine (1) from Feeding Experiments in Aspidosperma pyricollum plants

The following procedure was used to obtain uleine (1) from all the feeding experiments using \textit{A. pyricollum} plants. The root segments or whole plants were cut up with scissors before being macerated in a
Waring blender with methanol. The resulting pulp-like material was filtered and re-macerated in methanol (4 times) until the filtrate became colourless. The combined methanolic extracts were then evaporated in vacuo. This residue was then suspended in cool aqueous acetic acid (10%) and filtered. The filtrate was washed twice with diethylether before being basified with cooled aqueous sodium hydroxide solution (50%). The extraction of this suspension with methylene chloride yielded the crude alkaloidal extract. At this point, uleine (20 mg) was added to the extract and re-isolated by column chromatography on alumina (usually 25 g, act.III) using a toluene/chloroform gradient. Further purification was achieved by recrystallization from methanol. Usually two recrystallizations were done before the material was dried in high vacuum overnight in preparation for counting the radioactivity in it. Samples between 0.150 and 0.250 mg in weight were dissolved in benzene (1 ml) and counted as described previously in Part I. At least two recrystallizations were done between measurements of radioactivity. This procedure was similar to that used by others studying the same plant system in our laboratories.

The Administration of Radioactive Precursors to A. pyricollum plants

In all the feeding experiments using root segments (nos. 1-4) the radioactive precursor was dissolved in a solution of ethanol and water (0.5 ml, 1:4) with 1 or 2 drops of 1N acetic acid. Dissolution could be aided by sonication. This solution was applied directly to freshly cut roots which were contained in test tubes. After absorption of the solution by the root bark, the container which had the radioactive solution in it
was washed with two aliquots (0.5 ml) of the solvent mixture described and applied to the roots. Further washings with distilled water were applied to the root throughout the incubation period in order to keep them moist.

At the end of the feeding time this container, the pipette and the test tube in which the root had been were all washed with methanol. This wash solution was then measured for radioactivity in case a correction had to be applied to the radioactivity fed to the plant. In all cases the residual radioactivity was found to be insignificant.

In the two experiments (nos. 5 and 6) in which whole plants were fed, the wick method of feeding was used. This technique was employed in a manner similar to that described in Part I.

The data pertaining to each feeding experiment are given in Tables 2 and 4.

Table 4. Additional data associated with Table 2

<table>
<thead>
<tr>
<th>Exp.No.</th>
<th>Plant System</th>
<th>Plant weight(g.)</th>
<th>Feeding Technique</th>
<th>Weight of precursor(mg)</th>
<th>Feeding Time(days)</th>
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<tbody>
<tr>
<td>1</td>
<td>tap root</td>
<td>37.0</td>
<td>hydroponic</td>
<td>7.01</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>fibrous root</td>
<td>14.1</td>
<td>hydroponic</td>
<td>9.56</td>
<td>6</td>
</tr>
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<td>3</td>
<td>tap root</td>
<td>27.2</td>
<td>hydroponic</td>
<td>2.46</td>
<td>5</td>
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<td>4</td>
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<td>197</td>
<td>wick</td>
<td>8.94</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>whole plant</td>
<td>183</td>
<td>wick</td>
<td>0.03</td>
<td>91</td>
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</table>
The Isolation of Uleine (1) and Olivacine (4) from Feeding Experiments in Aspidosperma australe plants

The procedure used to obtain uleine (1) and olivacine (4) from all the feeding experiments using A. australe plants was very similar to that described above for experiments using A. pyricollum plants. In this study, however, both uleine (20 mg) and olivacine (20 mg) were added to the crude alkaloidal extract, and then isolated by chromatography on alumina (act.III).

The Administration of Radioactive Precursors to A. australe plants

In all the feeding experiments using this plant the following procedure was used. Immediately before each experiment the plants were up-rooted and the soil was carefully brushed from the root system. Each plant was placed in a test tube. The radioactive precursor was dissolved in a solution of ethanol and water (0.5 ml, 1:4) with 1 or 2 drops of IN acetic acid. This solution was applied directly to the root system of each plant. After absorption of the solution by the root bark, the container which had the radioactive solution in it was washed with two aliquots of the solvent mixture which were also applied to the roots. The roots were kept moist during the feeding period by the periodic addition of a little water as the plants were allowed to grow under fluorescent lights. The data which is pertinent to these experiments is summarized in Tables 3 and 5.
Table 5. Additional data associated with Table 3.

<table>
<thead>
<tr>
<th>Exp.No.</th>
<th>Number of plants</th>
<th>Total wt of plants(g)</th>
<th>Weight of precursor(mg)</th>
<th>Feeding Time(days)</th>
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<td>4</td>
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<td>5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>27.9</td>
<td>1.12</td>
<td>5</td>
</tr>
</tbody>
</table>

The Degradation of Uleine (1) Isolated from Feeding Experiments 5 and 6.

The radioactive uleine (1) isolated was degraded as previously described to yield tetramethylammonium iodide (29) which was recrystallized from methanol to constant radioactivity.

Experiment 5:

14 mg of uleine (4.96 x 10^6 dpm/mmmole) yielded 5 mg of tetramethylammonium iodide (4.80 x 10^6 dpm/mmmole).

Experiment 6:

12 mg of ulein (4.47 x 10^6 dpm/mmmole) yielded 4.1 mg of tetramethylammonium iodide (4.38 x 10^6 dpm/mmmole).
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18. We are grateful to Dr. C. Djerassi, Stanford University, Dr. J. Joule, Manchester University, Dr. J. Schmutz, Research Institute, A. Warderé, S. A., Bern, and Dr. B. Gilbert, Laboratorio de Quimica Organica, Faculdade Nacional de Farmacia, Rio de Janeiro for providing samples of these alkaloids.
20. We are grateful to Dr. D. Henry, Stanford Research Institute, Menlo Park, California, for samples of pyridocarbazole alkaloids.
PART III

Studies Related to the Synthesis of
Preakuammicine- and Stemmadenine-
Type Alkaloids
INTRODUCTION

A great deal of elegant work has led to a good understanding of the early stages of indole alkaloid biosynthesis. However, a great deal of work has yet to be done in order to fully understand the relationships between the various families of indole alkaloids in the later stages of biosynthesis. In Parts I and II of this thesis, a number of hypothesis supported by experimental work was described to outline the developments in this area. In this Part, there will be described the attempted synthesis of compounds believed to be important in the later stages of indole alkaloid biosynthesis.

There is much evidence to support the conjecture that the first of the major families of alkaloids to be biosynthesized is the Corynanthe, but the way in which the latter is converted to the Strychnos, Iboga and Aspidosperma skeletons is not clear and has been the subject of much speculation. Figure 1 summarizes two hypothesis for this conversion. Wenkert\(^1\), in 1965, proposed that the Corynanthe alkaloid geissoschizine (1) underwent cyclization (route A) and rearrangement to give the pentacyclic structure (2) which could easily undergo bond fission to give the ring-opened Strychnos skeleton (3). A two-step reduction of the latter would give the little known alkaloid stemmadenine (4), while ring-closure and loss of the aldehyde group would result in akuammicine (5). An alternative proposal by Scott and Quereshi\(^2\) (route B) involved a known rearrangement of the hydroxyindoline derivative of geissoschizine (1) to form the oxindole (6). Cyclization of this structure would give the
indolenine preakuammicine aldehyde (7). A simple reduction of the aldehyde group of the latter would give preakuammicine (8), a compound the Scott group claim to have found as a natural product in Catharanthus roseus seedlings. However, full characterization data on this compound has not been made available. These investigators also claim to have converted preakuammicine (8) into both stemmadenine (4) and akuammicine (5).²

The biosynthesis of other major families of indole alkaloids from the Strychnos family is believed to occur by elaboration of stemmadenine (4). Figure 2 summarizes the biosynthetic schemes which are supported by experimental evidence (described in detail in Parts I and II). The current hypothesis is that stemmadenine (4) is in biological equilibrium with dehydrosecodine (9) via iso-stemmadenine (10). Variously labelled forms of secodine have been shown in our laboratories³ to be incorporated into the Aspidosperma⁴(11), the Iboga⁵(12) and the Huntaria⁶(13) families of alkaloids as well as into the anomalous structure apparicine (14).⁷,⁸

Stemmadenine (4) is also believed to be the precursor to three smaller families of indole alkaloids which have more diverse carbon skeletons.⁹ These are compounds of the ellipticine, olivacine and uleine types.

The biomimetic conversion of stemmadenine (4) to members of the Iboga and Aspidosperma families has been the subject of much controversy. Scott and Quereshi¹⁰ claimed to have rearranged this molecule to tabersonine and catharanthine (12) in hot acetic acid. These results were said to support the proposal that a dehydrosecodine-like molecule must be a pivotal precursor of the Iboga and Aspidosperma families. However, a careful
Figure 1. The proposed biosynthesis of the Strychnos alkaloids from the Corynanthe family.
Figure 2. The implication of the dehydrosecodine \( \rightleftharpoons \) stemmadenine bio-equilibrium in indole alkaloid biosynthesis as supported by experiment.
investigation of this work by the Smith group failed to confirm these results.\textsuperscript{11}

Two other biomimetic conversions of stemmadenine (4) have been claimed. Scott\textsuperscript{12} reported that a ring-contraction reaction which resulted in a 20% yield of the natural product vallesamine (15) had been achieved. This conversion involved a modified Polonovski fragmentation of the N-oxide. However, no experimental details are available. This reaction is very similar to the previously proposed conversion of stemmadenine (4) to alkaloids possessing the more diverse carbon skeletons.\textsuperscript{9}

A second reaction of stemmadenine (4) which may have biological significance was reported by Sandoval et al.\textsuperscript{13} They claimed to have achieved an oxidative ring-closure which yielded the product condylocarpine (16), a member of the Aspidosperma family with which it co-occurs in some plant species.

\textbf{Figure 3. Some biomimetic conversions of stemmadenine (4).}
Stemmadenine (4) was first isolated, in 1958, from the fruit of *Stemmadenia Donnell-Smithii*. Subsequent work revealed the novel ring-opened Strychnos carbon skeleton, but the stereochemistry at C-16 was not determined. The only reported attempt at synthesizing stemmadenine (4) derivatives is that by Sniekus who achieved the model compound (17).

Synthetic work in the Strychnos area was initiated in our laboratories in 1968. The plan was to convert the available Corynanthe alkaloid (18) via its chloroindolenine to structures of the type (19) and then to cyclize the latter to achieve the 19,20-dihydro-preakuammicine series (20). All efforts to achieve this cyclization failed.

Clearly, in order to fully evaluate the central role which stemmadenine (4) appears to play in indole alkaloid biosynthesis various radio-labelled forms of this molecule must be made available. It would also be of great interest to have available derivatives of the preakuammicine type (7) and (8) in order to investigate the chemistry of these compounds and also to evaluate their role in the biosynthesis of indole alkaloids.
DISCUSSION

In order to evaluate effectively the roles played by the preakuammicine and the stemmadenine series in the biosynthesis of indole alkaloids a synthesis of these compounds had to be developed. This synthesis not only had to make available reasonable quantities of these materials but also make possible the introduction of a radio-label in certain positions in the structures. Also, since the stereochemistry at position C-16 in these compounds was unknown it would be desirable to synthesize both epimers of each compound.

There was available in the literature descriptions of a great deal of work bridging the degradation chemistry of strychnine (29)\textsuperscript{17} and the constituents of calabash curare.\textsuperscript{17} Curare is an extract preparation from several species of the plant genus *Strychnos* which was used by the South American natives as arrow head poisons. Due to the high biological activity of these preparations the chemical and botanical interest in them led to the identification of a large number of molecular structures possessing the basic *Strychnos* skeleton (21a). The system of

\begin{center}
\includegraphics[width=0.5\textwidth]{image}
\end{center}

\textsuperscript{(21a)}
nomenclature used here is that proposed by Janot and Le Men following the numbering system used by Bernauer et. al.

Since, compounds possessing the basic Strychnos skeleton were readily available from literature preparations we considered the possibility of synthesizing preakuammicine and stemmadenine derivatives from one of these structures. The advantages in using a curan derivative (21) as starting material were three-fold. Firstly, the stereochemistry at positions C-3, C-7 and C-15 of the piperidine ring was known and fixed. Secondly, known chemistry in this area could possibly be used to convert the ring-closed Strychnos skeleton to the ring-opened stemmadenine system. Thirdly, the availability of various derivatives of the curan type provided an opportunity to introduce a radio-label in various positions in the molecule near the end of the synthetic sequence.

The first requirement of a scheme to convert the Strychnos system to the desired ten-membered ring system of the stemmadenine series was to introduce stereoselectively an oxygenated carbon functionality at C-16. As mentioned earlier, the stereochemistry at C-16 in preakuammicine (8) and stemmadenine (4) was unknown. It would, therefore, be advantageous to synthesize both epimers to (a) ascertain the natural configuration, and (b) administer both epimers in plant feeding experiments to evaluate the biosynthetic importance of the stereochemistry at C-16. It was felt that the stereochemistry during introduction of the C unit at C-16 could be controlled and predicted because the stereochemistry of the piperidine part of the curan system (21b) created considerable steric crowding over the β-face the molecule. An examination of molecular models left no doubt that an incoming electrophile or nucleophile at C-16 must react from the α-face.
Therefore, by selecting the appropriate oxidation level of the C\textsubscript{1}-unit, conversion to both C-16 epimers of the preakuammicine (8) and stemmadenine (4) systems would be possible.

The curan derivative that became available to our laboratories was 18-hydroxy-28,16a-cur-19-ene-17-ol, also known as caracurine VII and Wieland-Gumlich aldehyde (22).\textsuperscript{20} This compound, which was of pivotal importance in the chemistry of the curan alkaloids had also been synthesized from strychnine (29) thus linking these two large families of alkaloids via established chemical routes. This compound (22), hereafter referred to as Wieland-Gumlich aldehyde (WGA) in the interest of simplicity, was made readily available from the degradation of strychnine (29), by a modification of the original degradative work\textsuperscript{21,22}, developed in the laboratories of Schmid and Karrer and published in 1969.\textsuperscript{23} Due to the availability of strychnine (29), and hence WGA (22) the latter
compound was an attractive intermediate for the synthesis of preakuammicine (8), stemmadenine (4) and their derivatives.

In one area of these investigations in our laboratories, (Figure 4) Fuller was successful in achieving the synthesis of 16-epi-stemmadenine (28) from the intermediate (23), the latter being available from WGA (22) by Smith's procedure. The crucial step in this sequence was the conversion (25) to (26) in which the C-1-unit was introduced at position C-16. Although the mechanism of this reaction is unknown, it apparently involves anion formation at C-16, therefore it should be possible, in a similar manner, to introduce a C-1-unit of higher oxidation level to achieve the other epimer. Such a study will form part of the following discussion.

The degradation scheme that was used to convert strychnine (29) to WGA (22) is shown in Figure 5. Strychnine (29) was treated with excess sodium ethoxide and isoamyl nitrite to give iso-nitrosostrychnine (30) which was isolated as its hydrochloride salt. A Beckman rearrangement of this product using thionyl chloride followed by hydrolysis yielded 1-cyanoformyl-Wieland-Gumlich aldehyde (32) as the hydrochloride salt. Treatment of an aqueous solution of (31) with steam permitted hydrolysis
Figure 4. Synthesis of 16-epi-stemmadenine (28).
Figure 5. The degradation of strychnine (29) to Wieland-Gumlich aldehyde (22)
of the cyanoformyl group to give WGA (22) in an overall yield of 35-40%. This material had physical and spectroscopic properties identical with those of an authentic sample of WGA (22).

The next step in the synthesis of the desired curan system was the removal of the C-18 oxygen functionality in structure (22). Two methods for this objective were already available in the literature. The first method involved the sodium borohydride reduction of the hemiacetal (22) to Wieland-Gumlich diol (33). The latter was then converted to the corresponding allylic bromide (34) using 3% saturated solution of hydrogen bromide in glacial acetic acid. Zinc dust treatment of the latter in acetic acid gave the acetate (35). Basic hydrolysis of this material resulted in the alcohol derivative, 2β,16α-cur-19-ene-17-ol (36). The identity of this product was confirmed by comparison of its spectroscopic data with that which was published. Of particular note was the UV spectrum \( \lambda_{\text{max}}^{243,297} \) showing the presence of an indoline chromophore and the mass spectrum which confirmed the molecular weight as being 296 mass units.

This technique for deoxygenating C-18 was considered as a means of introducing a radio-label into C-18 of structure (36) and hence into the ethyldiene side-chain of preakuammicine (8) and stemmadenine (4) if these compounds could be synthesized from this starting material. By dissolving the anhydrous allylic bromide (34) in tritiated acetic acid followed by treatment with zinc dust in an inert atmosphere, the introduction of tritium at C-18 could be accomplished.
A second method\textsuperscript{26} for deoxygenation of C-18 involved catalytic hydrogenolysis of Wieland-Gumlich diol (33). The latter was dissolved in aqueous acidic medium and treated with palladium-on-charcoal catalyst under an atmosphere of hydrogen gas. In this way selective reduction at C-18 afforded the alcohol (36) in high yield and purity.

![Chemical structures](image)

Figure 6. Two methods for deoxygenation of C-18 of Wieland-Gumlich aldehyde (22).
A third method which achieved this deoxygenation was discovered during the current investigation in a related study which will be described shortly. WGA (22) was dissolved in liquid ammonia, employing 1,2-dimethoxyethane and anhydrous t-butanol as co-solvents, at -78°. Excess lithium metal was added. A blue colour was soon achieved indicating an excess of dissolved metal. After maintaining this blue colour for 15-30 minutes (as required from thin-layer chromatographic monitoring) the reaction was quenched with water. Subsequent work-up and chromatography yielded the alcohol (36) in good yield (75-82%). This method is more convenient and provides a better overall yield than the two previously described procedures. A possible mechanism by which this hydrogenolysis occurred is shown in Figure 7. Probably the basic media promotes ring-opening of the hemiacetal to give structure (22a)

![Figure 7](image)

Figure 7. A possible mechanism for the two-step reduction of Wieland-Gumlich aldehyde (22) to 26,16α-cur-19-ene-17-ol (36).
which then accepts two electrons followed by protonation to give the alcohol (36).

To further explore the utility of this reduction, Wieland-Gumlich diol (33) was subjected to the same experimental conditions as just described. It was found that this material could also be efficiently hydrogenolyzed to give the alcohol (36).

The reaction just described was discovered during the course of an investigation directed at developing a method for an efficient conversion of WGA (22) to 18-deoxy Wieland-Gumlich aldehyde (37). The latter transformation was desirable in view of the anticipated difficulties associated with the oxidation of the C-17 hydroxyl in the curenol (36) to the aldehyde (37). Fritz, Besh and Wieland\textsuperscript{27} first reported that the Woodward modification of the Oppenauer oxidation of (36), using potassium \(t\)-butoxide and benzophenone, yielded only the unsaturated aldehyde, nor-fluorocurarine (39). Their rational for this unusual conversion was that the saturated aldehyde (37) underwent facile air oxidation to give the...
unsaturated system. A later study by the same group\textsuperscript{26} showed that this conversion could be achieved using lithium \textit{t}-butoxide in nitrobenzene solvent but no increases in yield of either (37) or (39) was reported. In 1964, Boekelheide et. al.\textsuperscript{28} reported a 40% yield of nor-fluorocurarine (39) from Oppenauer oxidation of (36) using potassium \textit{t}-butoxide with 5 moles of benzophenone as the hydride acceptor. Only small quantities of the saturated aldehyde (37) were obtained. They proposed that $\alpha,\beta$-unsaturation occurred by a second hydride transfer to the oxidizing agent, rather than by air oxidation, to give the indolenine intermediate (38), which tautomerized to nor-fluorocurarine (39).

Figure 8. The oxidation of alcohol (36) to nor-fluorocurarine (39).
Our first attempts to obtain quantities of the saturated aldehyde (37) involved varying the Boekelheide conditions for the Oppenauer oxidation of alcohol (36). When the reaction was performed at room temperature rather than refluxing benzene the conversion required 4 hours to complete rather 0.5 hours. Also, decreasing the quantity of oxidizing agent from 5 moles to 2 moles prevented the reaction from going to completion and starting material was recovered. In these studies, the progress of the oxidation was monitored by thin-layer chromatography (tlc) and by working up aliquots of the reaction mixture at regular time intervals. The identification of nor-fluoro-curarine (39) was made on the basis of its ultraviolet (uv) spectrum which showed a characteristic strong absorption at 363 nm and its infrared (ir) spectrum which exhibited a carbonyl absorption at 1640 cm\(^{-1}\). Of significance in the proton magnetic resonance (pmr) spectrum of (39) was a singlet absorption at \(\tau1.0\) which was attributed to the aldehydic proton of the unsaturated carbonyl group.

Other means of oxidizing alcohol (36) to the saturated aldehyde (37) were studied. The Ratcliffe modification\(^{30}\) of the Collin's oxidation was unsuccessful. Six moles of the pyridine-chromate complex were stirred with alcohol (36) for 1 hour, 6 hours and 24 hours at room temperature while a second similar study was made at 30-33°. At no time could the desired reaction product be detected by tlc. In all cases only small amounts of the starting material could be isolated from the reaction mixture. The latter was worked up using a variety of conditions in an attempt to recover organic material. Besides using aqueous sodium
bicarbonate solution, the reaction mixture was washed with IN sodium hydroxide solution in one attempt and IN hydrochloric acid solution followed by neutralization in another attempt. The variations in work-up procedure did not yield better recovery of organic material. A possible explanation for these results is that a chromium VI ion could coordinate with the indoline nitrogen atom and the hydroxyl group in compound (36) to form a very stable complex (40), which would not be isolated under the conditions used. Related to this problem

is the observation by Smith\textsuperscript{25} that the similar structure N-acetyl-Wieland-Gumlich aldehyde (41) could not be oxidized with chromic acid at 100°.

A third study involved attempts to oxidize alcohol (36) using anhydrous dimethylsulphoxide as a reagent. When dicyclohexylcarbodimide\textsuperscript{30} or sulphur trioxide-pyridine\textsuperscript{31} were used as dehydrating agents no reaction products could be detected and a nearly quantitative yield of starting material was recovered. However, when phosphorus pentoxide\textsuperscript{32} was used a very complex product mixture was obtained. Examination of this mixture by tlc and pmr revealed that none of the desired aldehyde (37) was present.
At a later time, a fourth study of the oxidation of alcohol (36) was initiated and resulted in the achievement of good yields of both the saturated aldehyde (37) and the unsaturated nor-fluorocurarine (39). It was found that when alcohol (36), in a benzene solution with 2 moles of benzophenone at 65°, was treated with excess potassium hydride the aldehyde (37) could be obtained in 70-78% yield. This material was identical with that previously obtained in small amounts from the present study of the Oppenauer oxidation and a similar study in our laboratories. Of particular importance in the characterization of this product was its indoline UV spectrum ($\lambda_{max}$ 242, 298), a carbonyl absorption at 1715 cm$^{-1}$ in its IR spectrum and an aldehydic proton absorbing as a singlet at $\tau$0.23 in its PMR spectrum.

When alcohol (36) was first treated with potassium hydride in a benzene solution at 65°-70° and then treated with 4 moles of nitrobenzene over a 15 minute time period a good yield of nor-fluorocurarine (39) was obtained. Only trace quantities of the saturated aldehyde (37) could be detected by TLC.

Thus, for the first time both aldehydes (37) and (39) could be obtained in good yields by reliable procedures. Good yields could only be obtained if precautions to exclude moisture were exercised. In both procedures it was found useful to distil about 10% of the benzene from the reaction mixture, before potassium hydride or nitrobenzene were added, to ensure that any traces of moisture were removed.

In order to obtain anion formation at position C-16 of 18-deoxy-Wieland-Gumlich-aldehyde (37) it would be necessary to protect the indoline nitrogen atom since the indoline NH is also weakly acidic.
Two routes were developed to obtain N-functionalized derivatives of aldehyde (37).

In the first route, the curenol (36) was treated with acetic-formic anhydride at 0° for 30 minutes. After removal of the reagent in vacuo and purification by chromatography a high yield of the N-formyl-0-formyl derivative (42) was obtained. The uv spectrum ($\lambda_{max}$ 247, 288, 289) of this product indicated that an N-acylated indoline chromophore was present. In the ir spectrum a carbonyl absorption at 1660 cm$^{-1}$ indicating an amide group and a second carbonyl absorption at 1720 cm$^{-1}$ which was attributed to an O-formyl group provided further supporting evidence. The pmr spectrum of this product showed the presence of two aldehydic protons. A singlet absorption of 1.51 was assigned to the proton of an N-formyl group and a singlet absorption at 1.99 was attributed to the proton of an O-formyl group.

The product (42) was refluxed in methanol for 30 minutes to yield a new compound. This product had a uv spectrum similar to the starting material (42) but exhibited only one carbonyl absorption (1660 cm$^{-1}$) in the ir spectrum which corresponded to the presence of an amide group. The latter was confirmed by a singlet absorption at 1.24 in the pmr spectrum. A molecular ion which appeared at m/e 324 in the mass spectrum of this product allowed the assignment of structure (43).

The synthesis of N-formyl-18-deoxy-Wieland-Gumlich aldehyde (44) was achieved by Collins oxidation of N-formyl alcohol (43). The treatment of the latter with 6 moles of chromic anhydride-pyridine complex for 45 minutes followed by a work-up with saturated sodium bicarbonate solution
afforded the desired aldehyde in 40% yield. The assignment of structure (44) was made on the basis of the characteristic spectral data. The ir spectrum showed in addition to the N-formyl carbonyl absorption at 1660 cm$^{-1}$, a second carbonyl absorption at 1720 cm$^{-1}$ while close examination of the

Figure 9. The synthesis of N-formyl-18-deoxy-Wieland-Gumlich aldehyde (44).
pmr spectrum of this product revealed a doublet absorption at \( \tau 0.24 \) and a singlet absorption at \( \tau 0.38 \) with a total integral for one aldehyde proton. Similarly, singlet absorption at \( \tau 1.14 \) and \( \tau 1.38 \) provided evidence for a proton of an N-formyl group. This multiplicity of signals in the low field region of the spectrum indicated the presence of both C-16 epimers of the N-formyl aldehyde (44). There appeared, however, to be only one product by tlc. No attempts were made to separate these isomers since the stereochemistry at C-16 would not alter the following reaction sequence.

Further evidence for structure (44) was obtained from its mass spectrum which exhibited a characteristic fragmentation pattern (Figure 10). The loss of the aldehyde group from the molecular ion (m/e 322) would yield ion (i) at m/e 293. A complex fragmentation of the molecular ion or ion (i) resulted in a significant ion(ii) which appeared at m/e 279. This fragmentation is very characteristic in the Strychnos series\(^{34,35}\) and is of particular diagnostic value for N-\( \alpha \)-functionalized derivatives. A second important fragmentation in this series is the retro-Diels-Alder-type fragmentation which yields an important ion(iii) corresponding to the piperidine portion of the molecule. In this example, this ion (iii) appeared at m/e 164 indicating the presence of the aldehyde group in the piperidine portion of the structure.

Other means of oxidizing the N-formyl alcohol (43) to the desired aldehyde were explored in order to increase the yield. In one study, the alcohol was treated with freshly prepared silver carbonate on celite in
Figure 10. A rationale for the fragmentation of aldehyde (44) in the mass spectrometer.

Although significant quantities of the desired product were obtained, the reaction always failed to go to completion and the desired product was difficult to isolate from minor components.
In another study, the alcohol (43) was treated with 2 moles of dimethylpyrazole-chromate complex in dichloromethane according to a procedure by Corey. This oxidation method was successful in that good conversion to the aldehyde (44) was apparent. However, pure quantities of the aldehyde could only be obtained by repeated chromatographic separations to in order to remove the dimethylpyrazole. This tedious procedure made it desirable to investigate another oxidation reaction.

The N-formyl alcohol (43) was treated with another reagent developed by Corey, pyridinium chlorochromate. This approach achieved the desired conversion in 78% yield.

A second synthesis of the N-formyl aldehyde (44) was later accomplished. After the conversion of alcohol (36) to aldehyde (37) was perfected as previously described, the latter was acylated with acetic-formic anhydride to give the N-formyl-18-deoxy-Wieland-Gumlich aldehyde (44) in a comparable overall yield. This synthesis served to further confirm the structure (44).

With the desired functionalized curan system in hand, a study was initiated to introduce a carbomethoxy group into the C-16 position. The plan, illustrated in Figure 11, was to synthesize the aldehydo-ester (45) having the stereochemistry shown for reasons previously discussed. Removal of the protecting group and subsequent oxidation of the indoline nitrogen atom would lead to the preakuammicine series. The latter could then be ring-opened to stemmadeninine (4) by a known reaction which will be discussed in detail later.

In the first attempt to introduce the desired ester functionality, compound (44) was treated with sodium hydride in dimethoxyethane and
Figure 11. Attempted functionalization of aldehyde (44) and the proposed plan for its conversion to preakuammicine and stemmadenine derivatives.
methylchloroformate at 80°. A tlc examination of the reaction mixture after 1 hour revealed almost complete conversion to a less polar material. This product had a uv spectrum similar to starting material, but the ir spectrum showed additional absorption in the carbonyl region near 1680 cm\(^{-1}\). A three-proton singlet absorption which appeared in its pmr spectrum at \(\tau 6.24\) showed the presence of a carbomethoxy group. In the mass spectrum of this product there appeared two molecular ion peaks at m/e 446 and 448 in a pattern which suggested the presence of an atom of chlorine. It therefore appeared that under these reaction conditions the methylchloroformate reacted with the more basic nitrogen (N\(^{+}\)) to give structure (47). This type of reaction has previously been observed in strychnine chemistry. A similar reagent, cyanogen bromide, was found to cause fission of the five-membered ring of strychnine to give structure (48).

Clearly, elevated temperatures could not be used to achieve conversion to structure (45), therefore a selection of other more powerful bases were used at lower temperatures. Table 1 summarizes a study in which reaction of the N-formyl aldehyde (44) was attempted using excess sodium hydride to form the anion followed by quenching which methylchloroformate. In each experiment the progress of the reaction was followed by tlc. When dimethylformamide or dimethylsulphoxide were used, small aliquots of the reaction were worked up and examined to determine the progress of the reaction. In all cases the reactions were worked up and the product mixture was separated and all components were examined spectroscopically.

Starting material (44) was recovered in every experiment shown except Experiment 3 in which complete decomposition had occurred.
In Experiment 7 in which the effective base is the dimethyl anion, some deformylation of the starting material had occurred and small amounts of aldehyde (37) were isolated. This observation was not unexpected since N-formyl groups have been deformylated under similar conditions.

Further attempts to functionalize the C-16 position of aldehyde (44) with methylchloroformate are summarized in Table 2. In Experiments 1-3 potassium hydride dispersed in oil was used and starting material plus the deformylated aldehyde (37) was recovered. When a 2 molar excess of potassium t-butoxide (Exp.4-6) or the "harpoon base" lithium diisopropyl amine (LDA) were used to generate the anion of aldehyde (44) only starting material was recovered in addition to tarry decomposition products.

There are two steps involved in the normal functionalization of a carbonyl group. The first is anion formation at the α-carbon and the second is the reaction of the anion with the electrophilic reagent. Therefore, when such a reaction fails the first question which has to be asked is whether or not anion formation has occurred during the reaction conditions used. In order to try to obtain some information about this, a parallel study was made in which deuterium oxide was used to quench the reaction instead of methylchloroformate. The expected products from such a reaction would have a deuterium atom in the position in which the anion was formed. The position of and the extent of deuteration could then be ascertained by examination of the mass spectrum of the product.

In the present study, the reaction conditions for Experiments 2, 4, 5 and 7 in Table 1 were duplicated quenched with D₂O and the recovered starting
Table 1. Attempted C-16 functionalization of aldehyde (44) using an excess of sodium hydride and quenching with methylchloroformate

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Reaction Time (hrs)</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{a}</td>
<td>6</td>
<td>THF</td>
<td>rt\textsuperscript{e}</td>
<td>(44) plus decomposition</td>
</tr>
<tr>
<td>2\textsuperscript{a}</td>
<td>2</td>
<td>-&quot;-&quot;</td>
<td>50\textdegree</td>
<td>-&quot;-&quot; -&quot;-&quot; -&quot;-&quot;</td>
</tr>
<tr>
<td>3\textsuperscript{a}</td>
<td>2</td>
<td>DMF</td>
<td>rt</td>
<td>Complete decomposition</td>
</tr>
<tr>
<td>4\textsuperscript{a,b}</td>
<td>1</td>
<td>-&quot;-&quot;</td>
<td>50\textdegree</td>
<td>(44) plus decomposition</td>
</tr>
<tr>
<td>5\textsuperscript{c}</td>
<td>4</td>
<td>-&quot;-&quot;</td>
<td>rt</td>
<td>-&quot;-&quot; -&quot;-&quot; -&quot;-&quot;</td>
</tr>
<tr>
<td>6\textsuperscript{c}</td>
<td>0.5</td>
<td>-&quot;-&quot;</td>
<td>55\textdegree</td>
<td>-&quot;-&quot; -&quot;-&quot; -&quot;-&quot;</td>
</tr>
<tr>
<td>7\textsuperscript{d}</td>
<td>1</td>
<td>DMSO</td>
<td>50\textdegree</td>
<td>(44) plus aldehyde(37)</td>
</tr>
<tr>
<td>8\textsuperscript{d}</td>
<td>3</td>
<td>-&quot;-&quot;</td>
<td>rt</td>
<td>(44)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 10-Fold excess of sodium hydride.
\textsuperscript{b} Granular rather than oil-despersed sodium hydride was used.
\textsuperscript{c} 1.5 Moles of sodium hydride.
\textsuperscript{d} 2 Moles of sodium hydride.
\textsuperscript{e} Room temperature.
Table 2. Further studies on the functionalization of aldehyde (44)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Reaction Time (hrs)</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>5 moles KH</td>
<td>THF</td>
<td>rt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(44) plus aldehyde(37)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-&quot;-&quot;</td>
<td>-&quot;-&quot;</td>
<td>60&lt;sup&gt;°&lt;/sup&gt;</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>-&quot;-&quot;</td>
<td>DMSO</td>
<td>35&lt;sup&gt;°&lt;/sup&gt;</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2 moles t-BuOK</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(44)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>-&quot;-&quot;</td>
<td>DMSO</td>
<td>rt</td>
<td>(44) plus decomposition</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-&quot;-&quot;</td>
<td>-&quot;-&quot;</td>
<td>50&lt;sup&gt;°&lt;/sup&gt;</td>
<td>(44)</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>2 moles LDA</td>
<td>THF</td>
<td>-78&lt;sup&gt;°&lt;/sup&gt;</td>
<td>(44)</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>-&quot;-&quot;</td>
<td>-&quot;-&quot;</td>
<td>0&lt;sup&gt;°&lt;/sup&gt;</td>
<td>(44) plus decomposition</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>-&quot;-&quot;</td>
<td>-&quot;-&quot;</td>
<td>rt</td>
<td>&quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

a. Room temperature

b. Refluxed for 30 minutes before cooling to 55<sup>°</sup> for addition of methylchloroformate
material (44) was examined by mass spectrometry. In each case, no significant incorporation of deuterium was detected. Similarly, the reaction conditions in Experiments 2, 5, 7, 8 and 9 in Table 2 were used in a deuteration study. Again, no incorporation of deuterium was detected. A similar study was also done by Lewis in our laboratories and similar results were obtained.

Due to the importance of this problem, it was decided that a similar study should be made on an aldehyde possessing another protecting group on the indoline nitrogen atom as the N-formyl group was complicating the product mixture in some experiments. It was found that when the alcohol (36) in Figure 12 was dissolved in tetrahydrofuran and treated with an excess of methylchloroformate a rapid reaction occurred. A tlc examination of the product mixture revealed the presence of two products. The ir spectrum of the mixture showed a broad carbonyl absorption (1730–1660 cm\(^{-1}\)). After this total product was stirred at room temperature for 30 minutes in a 2% methanolic potassium hydroxide solution only the more polar product could be detected. This material was identical to the single product which was obtained when the indoline alcohol (36) was dissolved in methanol and treated with methylchloroformate at room temperature for 15 minutes. The structure of this product (49) was assigned on the following data. Its uv spectrum (\(\lambda_{\text{max}}\) 241, 278, 287) showed the presence of an N-acylated indoline chromophore similar to that which was previously
Figure 12. The synthesis of N-carbomethoxy-18-deoxy-Wieland-Gumlich aldehyde (50) obtained for the N-formyl alcohol (42). In the ir spectrum, the N-H absorption at 3575 cm$^{-1}$ was absent but a strong carbonyl absorption at 1685 cm$^{-1}$ was found. The pmr spectrum of this material exhibited a three-proton singlet at $\tau 6.20$ which was assigned to the N-carbomethoxy
group. Also in this spectrum, the position of a one-proton multiplet at \( \tau 2.52 \), which was attributed to the aromatic proton at C-12, is indicative of an N-functionalized indoline system as it has shifted from \( \tau 2.8 \) in the corresponding N-H derivative. Further confirmation of this structure (49) was obtained from its mass spectrum which exhibited a molecular ion at m/e 354 as well as prominent fragments at m/e 309 (ion iv) and m/e 166 (ion v). The latter two ions represent a fragmentation pattern of these alkaloids, which was discussed earlier.

![Compound (49)](image)

Compound (49) was oxidized in good yield to the aldehyde (50) by pyridinium chlorochromate in a manner similar to that discussed in the N-formyl series. This product (50) exhibited a uv similar to that of the starting material. Its ir spectrum showed additional carbonyl absorption at 1720 cm\(^{-1}\). The pmr spectrum indicated the presence of an aldehydic proton appearing as a doublet absorption at \( \tau 0.40 \) (J=7Hz). The presence of the aldehyde group was also evident by the significant loss of 29 mass units from the molecular ion at m/e 352 in its mass spectrum.
Further confirmation of structure (50) was later obtained when quantities of aldehyde (37) became available. The latter compound was dissolved in tetrahydrofuran and treated with methylchloroformate to yield a single product. This product had physical and spectroscopic properties identical with those of N-carbomethoxy-18-deoxy-Wieland-Gumlich aldehyde (50).

Attempts to introduce the carbomethoxy group at C-16 of aldehyde (50) were carried out in a manner similar to that in the study discussed for the N-formyl series and are summarized in Table 3. In each experiment anion formation was attempted followed by quenching with methylchloroformate. In this series, no new products were detected by tlc examination of the reaction mixture or by spectroscopic examination of the worked-up reaction product. Starting material (50) was isolated along with various quantities of tarry decomposition products.

Table 3. Attempted C-16 functionalization of N-carbomethoxy aldehyde (50)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Reaction Time(hr)</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>10 moles NaH</td>
<td>THF</td>
<td>40°</td>
<td>(50) plus decomposition</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td><em>&quot;-</em>&quot;</td>
<td>DMSO</td>
<td>53°</td>
<td><em>&quot;-</em>&quot;   <em>&quot;-</em>&quot;   <em>&quot;-</em>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2 moles KH</td>
<td>C6H6</td>
<td>80°</td>
<td><em>&quot;-</em>&quot;   <em>&quot;-</em>&quot;   <em>&quot;-</em>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1 mole KH</td>
<td>DMF</td>
<td>50°</td>
<td>(50) plus complex mixture</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>2 moles LDA</td>
<td>THF</td>
<td>0°</td>
<td>(50)</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td><em>&quot;-</em>&quot;</td>
<td><em>&quot;-</em>&quot;</td>
<td>20°</td>
<td><em>&quot;-</em>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td><em>&quot;-</em>&quot;</td>
<td>THF</td>
<td>35°</td>
<td><em>&quot;-</em>&quot;</td>
</tr>
</tbody>
</table>
In this study also, a parallel deuteration study was made. The experimental conditions of Experiments 1, 2, 6 and 7 were duplicated and each reaction was quenched with deuterium oxide. Examination of the mass spectra of isolated starting material (50) revealed that no significant incorporation of deuterium had occurred.

The failure to detect anion formation or obtain functionalization at C-16 by treating the two curan derivatives (44) and (50) with a base and methylchloroformate is not fully understood. Three possibilities may be considered. Firstly, anion formation may not be occurring at all under the conditions employed due to the very hindered environment of position C-16. Secondly, anion formation may be occurring, but methylchloroformate may be too bulky to react in such a sterically unfavourable environment. And thirdly, given this second possibility, reaction may be occurring on the oxygen atom via the resonance shown. If that was the case, the product would not be expected to be detected or isolated under the conditions employed as it would rapidly hydrolyse to starting material.

A different approach to the functionalization of position C-16 was initiated. It was discovered in our laboratories that when the
unsaturated aldehyde, nor-fluorocurarine (39) was treated with sodium borohydride in methanol at room temperature the ring-opened indole alcohol (52) could be obtained in 40% yield. This reductive ring-opening reaction which was first developed by Smith and Wrobel in this series of compounds probably proceeds via the seco-immonium derivative (51). In the present study, it was found that an increased yield of 60-65% could be obtained if this ring-opening reaction was done in 2% methanolic potassium hydroxide solution at 40°. The plan to convert product (52) to stemmadenine is shown in Figure 13.

A well known reaction of indole alkaloids is chloroindolenine formation. This reaction is accomplished by treating the indole derivative with a source of positive chlorine ions such as sodium hypochlorite, t-butyl hypochlorite or N-chlorobenztriazole. The chloroindolenines are normally unstable molecules and only a few have ever been isolated. Therefore, these derivatives are usually made in situ and treated with the appropriate reagent. The common reaction for which chloroindolenines have been used is the introduction of a nucleophile α to the indole (in this case position C-16) via the tautomeric form (represented by structure (54)). This type of reaction has been widely used in the Iboga alkaloids to introduce various nucleophiles such as cyanide ion and vindoline. However, this reaction appeared not to have been used in the Strychnos series. It was felt that the stereochemistry of the C₁ introduction at C-16 could still be controlled in the ring-opened series since examination of molecular models revealed that again a great deal of steric crowding on the β-face was present in these derivatives.
The indole alcohol (52) was treated with 1.1 equivalents of t-butylhypochlorite at 0° for 15 minutes. A tlc examination of the reaction mixture revealed the presence of very polar material and absence of starting material. The uv spectrum (λ<sub>max</sub> 310) indicated that the indole chromophore had been converted to a chloroindolenine chromophore. Attempts to isolate this material by preparative layer chromatography (plc), not unexpectedly, failed to give pure material. For the remainder of the study, the chloroindolenine (53) was formed in situ and treated with cyanide ion directly. A representative summary of results from this study is shown in Table 4. In each experiment, chloroindolenine formation was confirmed by uv examination before addition of the cyanide reagent. The reaction was then monitored by tlc and uv spectroscopy. At the end of the reaction time, the product mixture was isolated and separated by plc. In every experiment, the main component of the mixture was a tarry decomposition product remaining on the base-line of the chromatoplate. A large number of products in very small amounts was also apparent. However, a uv spectroscopic study of these complex mixtures revealed that no indole chromophores were present. A prohibitively large scaled-up reaction would have been required to obtain sufficient quantities of any one of these components for characterization. Since no indolic material was found this series of experiments was abandoned.
Figure 13. The plan to synthesize stemmadenine (4) via the chloroindolenine of des-carbomethoxy stemmadenine (52).
Table 4. Attempted C-16 introduction of cyanide via the chloroindolenine of indole (52)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Nucleophile</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time(hrs)</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KCN</td>
<td>CH₂Cl₂</td>
<td>rt¹</td>
<td>24</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2</td>
<td>&quot;-&quot;</td>
<td>DMF</td>
<td>rt</td>
<td>6</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>80°</td>
<td>0.5</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>4</td>
<td>ZnCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>24</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>5</td>
<td>&quot;-&quot;</td>
<td>DMF</td>
<td>50°</td>
<td>1.5</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>6</td>
<td>AgCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>48</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>7</td>
<td>&quot;-&quot;</td>
<td>DMF</td>
<td>55°</td>
<td>1.5</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>8</td>
<td>Bu₄NCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>48</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>9</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>35°</td>
<td>12</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>10</td>
<td>&quot;-&quot;</td>
<td>DMF</td>
<td>35°</td>
<td>5</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>11</td>
<td>Et₂AlCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>5</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>12</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>36°</td>
<td>12</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>13</td>
<td>&quot;-&quot;</td>
<td>&quot;-&quot;</td>
<td>5°</td>
<td>5 days</td>
<td>&quot;-&quot;</td>
</tr>
</tbody>
</table>

a. Room temperature
Perhaps one reason for the failure to introduce cyanide into position C-16 was due to interference of the hydroxyl group in compound (52). It was, therefore, decided to protect the hydroxyl as its acetate and study the functionalization of the chloroindolenine of this derivative.

The treatment of indole alcohol (52) with acetic anhydride and pyridine (10%) at 5° overnight yielded a product mixture of two components which could be separated by plc. Surprisingly, the major product exhibited a uv spectrum ($\lambda_{\text{max}}$ 248, 282, 290) corresponding to an N-acyl indole (56). This structural assignment was supported by its ir spectrum which showed an acetate carbonyl absorption at 1725 cm$^{-1}$ and an amide carbonyl absorption near 1640 cm$^{-1}$. Two three-proton singlet absorptions at $\tau$ 7.79 and 7.88 indicated the presence of both an N-acetate and an O-acetate group, respectively. Further confirmation of the structural assignment of this product (56) was evident in its mass spectrum which showed a molecular ion at m/e 380 with a prominent loss of 59 mass units which corresponded to the loss of an acetate fragment. Preliminary data on the minor component from this reaction suggested that it was the desired mono-acetate (57).

Since it is generally more difficult to acylate an indole nitrogen atom in comparison with an hydroxyl group, and not known to occur under standard acetylating conditions, it was felt that this reaction could be controlled to give the mono-acetate (57) as the major product. Treatment of the alcohol (52) with acetic anhydride and 1 drop of pyridine at -10° yielded the desired material as the major product. Good yields were obtained when this reaction was monitored by tlc. The product (57)
exhibited the normal indole chromophore in the uv spectrum ($\lambda_{\text{max}}$ 224, 283, 290) and a carbonyl absorption in the ir spectrum (1725 cm$^{-1}$) which corresponds to the presence of an O-acetate group. A three-proton singlet absorption at $\tau$7.89 as well as an N-H absorption at $\tau$1.69 in the pmr spectrum, and a molecular ion at m/e 338 in the mass spectrum provided clear evidence for the assignment of structure (57).

Figure 14. The plan to synthesize stemmadenine (4) via the chloroindolenine acetate (58).
With the indole acetate (57) in hand, a study designed to introduce cyanide into the C-16 position was initiated in a manner similar to that made in the free hydroxyl series previously discussed. As before chloroindolenine formation was clearly achieved when compound (57) was treated with a slight excess of t-butyl hypochlorite in dichloromethane or dimethylformamide. Presented in Table 5 are the results found which represent the study of the introduction of cyanide into position C-16 in the chloroindolenine acetate (58). As in the previous study, the progress of the reaction was monitored by tlc and uv spectroscopy, and the isolated product mixtures were examined for the presence of indolic material. In each experiment, a great deal of decomposition was obtained along with a small amount of a complex mixture of products. At no time could any indolic material be detected. Nor could any

Table 5. Attempted C-16 introduction of cyanide via the chloroindolenine of indole acetate (57).

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Nucleophile</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time(hrs)</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>20</td>
<td>Decomposition</td>
</tr>
<tr>
<td>2</td>
<td>-&quot;-</td>
<td>DMF</td>
<td>rt</td>
<td>8</td>
<td>-&quot;-</td>
</tr>
<tr>
<td>3</td>
<td>-&quot;-</td>
<td>&quot;-&quot;</td>
<td>68°</td>
<td>1</td>
<td>-&quot;-</td>
</tr>
<tr>
<td>4</td>
<td>Bu₄NCN</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>24</td>
<td>-&quot;-</td>
</tr>
<tr>
<td>5</td>
<td>-&quot;-</td>
<td>DMF</td>
<td>rt</td>
<td>8</td>
<td>-&quot;-</td>
</tr>
<tr>
<td>6</td>
<td>-&quot;-</td>
<td>DMF</td>
<td>75°</td>
<td>1</td>
<td>-&quot;-</td>
</tr>
</tbody>
</table>
pure material be obtained in quantities sufficient for characterization.

With the failure to introduce cyanide into either compound (52) or compound (57) this approach was abandoned. Similar results were obtained in our laboratories by Lewis in which cyanide introduction via the chloroindolenine of the indole ester (59) was studied.

Clearly a new approach to the functionalization of the C-16 position of the curan skeleton (21) was required. Another curan derivative which had become available and which was potentially useful for elaboration to the preakuammicine derivatives was nor-fluorocurarine (39). This structure may be regarded as having a rigid cis-enaminocarbonyl system. The nucleophilic reactions of such conjugated systems may be classified into four types (Figure 15). Reaction types I, II and III are well known in such systems and are applicable in the current study,
but type VI reactions \(^{42}\) in which the \(\delta\)-carbon is nucleophilic is not relevant here because in the curan derivative this position is fully substituted. The factors which govern reaction at 0, C and N in such systems are not well understood and have been a subject of recent interest. The products that would be expected from these three reaction types if nor-fluorocurarine (39) was reacted with the electrophile, methylchloroformate, are shown in Figure 16. The plan was to enhance reaction conditions to favour type II reaction. The C-functionalized product would be of the desired preakuamicine series (60). It was felt that the type I N-substituted product (61) could easily be distinguished from the latter by its uv spectrum which was expected to be very different. The O-functionalized product (62) would be expected to hydrolyse back to starting material.

\[\text{Figure 15. The nucleophilic reactions of enaminocarbonyl systems.}\]
An examination of the literature revealed that two factors favoured C-functionalization of enaminocarbonyl systems. Firstly,
Meyers et al. reported that in the cis series (see partial structure 63) aprotic solvents favoured C-alkylation of a variety of cyclic enamino-ketones which were treated with methyl iodide, while the trans series (partial structure 64) O-alkylation predominated and was not influenced by choice of solvent. Secondly, a study by Kozerski which involved the acylation of acyclic enamino-ketones having an N-H bond and using acetyl chloride revealed that C-acylation was favoured in the presence of a base such as pyridine or triethylamine. This study also showed that C-acylation was favoured in the cis series.

When the lithium salt of nor-fluorocurarine (39) was quenched with methylchloroformate at 0° a single product was obtained. The uv spectrum (λmax 233, 268, 305 ) of this product indicated the presence of an indolenine chromophore (Figure 17). The wavelength of the electron-transfer band at 268 nanometers (nm) compares favourably with that reported for similar structures such as preakuammicine (8)(λmax 263)², precondylocarpine (65)(λmax 280)⁴⁵ and rhazinaline (66)(λmax 266).⁴⁶ Such a uv spectrum would not be expected for an N-functionalized derivative (61). Although
Figure 17. The uv spectrum of the product from the reaction of norfluorocurarine (39) with methylchloroformate.

No literature example of the chromophore in structure (61) could be found, in all examples in which there is a double bond α to the indoline nucleus the principal band in the uv spectrum appears at wavelengths greater than 300 nm. This band in the uv spectrum of the starting material norfluorocurarine (39) appeared at 363 nm.
The carbonyl region in the ir spectrum of this product showed three absorption bands: 1720, 1670 and 1640 cm\(^{-1}\). The band at 1720 cm\(^{-1}\) was assigned to a C-carbomethoxy group. The bands at 1670 and 1640 cm\(^{-1}\) could be a doublet absorption of an aldehyde group geminal to a carbomethoxy group. Due to the steric and electronic environment near the C-16 position such an aldehyde group may have sufficiently restricted rotation so that two rotomers would each contribute a band in the ir spectrum. This rationale is illustrated by the partial structures (67) and (68). Carbonyl absorptions of different wavelengths may be expected.

Figure 18. The carbonyl region of the infrared spectrum of product (60)
for the alignment (67) and non-alignment (68) of the \( \pi \)-orbitals of
the two systems.

In the pmr spectrum (Figure 19) of this product an aldehydic
proton absorption appeared as a singlet at \( \tau 0.02 \) which represents a
paramagnetic shift of 0.6 ppm from the corresponding absorption in the
spectrum of the starting material (39). A three-proton singlet
absorption at \( \tau 6.08 \) confirmed the presence of a carbomethoxy group.
The latter chemical shift compares favourably with the corresponding
chemical shift of the carbomethoxy groups in preakuammicine (8) and
precondylocarpine (65) which were \( \tau 6.14 \) and 6.15, respectively.

Of particular interest was the remarkable resemblance between
the mass spectrum of this product and that published for precondylo-
carpine (65). Both compounds fragment to lose the carbomethoxy group
from the molecular ion to give a peak at \( M^+ - 58 \) rather than the more usual
\( M^+ - 59 \). Due to the lack of information on the complex fragmentations
which indolenines appear to undergo in the mass spectrometer, little
more structural assignment could be made at this time based on the mass
spectrum of this product.
Figure 19. The pmr spectrum of compound (60).
Based on the above described data we assigned the structure (60) to this product. Studies made on model systems suggested that nor-fluorocurarine (39) should react with methylchloroformate according to pathway II (Figure 17) under the conditions used. It was later found that this same reaction could be achieved in comparable yield by heating nor-fluorocurarine (39) with excess methylchloroformate to 65°, either with tetrahydrofuran as a co-solvent, or just using the reagent itself as the solvent. The latter became the usual method for the preparation of the aldehydo-ester (60). The stereochemistry at C-16 was assigned from steric considerations. Due to the steric crowding over the 3-face of the molecule (39)(discussed earlier) an incoming electrophile should be α-oriented. It was the latter argument upon which the stereochemistry of 16-epi-stemmadenine (28) and hence stemmadenine (4) was assigned.²⁴

Further evidence for the assignment of structure (60) to the above product was obtained from its stability in basic media. This material (60) was found to be stable in 5% methanolic potassium hydroxide solution for more than 6 hours at room temperature. This observation tends to eliminate the carbamate (61) from consideration, as it is well known that compounds of this type easily decarboxylate under milder conditions. When product (60) was heated to 65° in 5% methanolic potassium hydroxide solution, complete hydrolysis to the starting material, nor-fluorocurarine (39), occurred in 2 hours.

With the aldehydo-ester (60) in hand, a study for the conversion of this material to the preakuammicine (8) and stemmadenine (4) series
was begun. Two approaches were initially considered. Firstly, it would be desirable to simply reduce the aldehyde (60) to the alcohol (69) and compare the latter with a sample of natural preakuammicine (8).

Figure 20. Some expected reactions of preakuammicine derivatives.
Secondly, the synthesis of stemmadenine series may be achieved by either direct reductive ring-opening of preakuammicine aldehyde series (60) or by ring-opening of the corresponding alcohol (69). The latter conversion was claimed to have been done by Scott and Quereshi but few experimental details were given. These workers also claim to have converted preakuammicine (8) to akuammicine (5) in basic media.

Compound (60) was treated with recrystallized sodium borohydride in dry methanol at room temperature for 30 minutes. An examination of this reaction by tlc revealed the presence of one major product. This product was isolated by plc and found to have the following spectral data. The uv spectrum ($\lambda_{\text{max}}$ 228, 292) indicated the possible presence of an indolenine chromophore conjugated to a carbon-carbon double bond. The ir spectrum showed that no carbonyl groups were present in the molecule. This observation was confirmed by the pmr spectrum. Both the aldehyde proton absorption and the methyl absorption of the carboxymethoxy group of the starting material (60) were absent from the pmr spectrum of the product. However, two one-proton singlet absorptions at $\tau$3.89 and 4.54 suggested the presence of an exocyclic methylene group. The mass spectrum revealed a molecular ion at m/e 276 which was also the base peak. A high resolution mass measurement on this peak confirmed the molecular formula of this molecule to be $C_{19}H_{20}N_2$. The structure which best fits this data is that of 1,2-dehydro-16,17-dehydro-2β-cur-19-en (72).

A possible mechanism by which compound (72) could arise from treatment of the aldehydo-ester (60) with sodium borohydride is shown
in Figure 21. The reduction of a carbomethoxy group geminal to an aldehyde group under similar conditions is well known (for examples see the synthesis of secodinol described in Part I and the reduction of rhazinaline (66) in ref. 46).

Figure 21. A possible mechanism for the formation of compound (72).
The problem now became one of either reducing the aldehyde group in compound (60) to achieve the preakuammicine series or to achieve the reductive ring-opening of (60) to give the stemmadenine series, while preventing decarboxylation. In order to evaluate these two possibilities compound (60) was treated with sodium borohydride under various conditions of pH and temperature. The representative results from this study are summarized in Table 6. In Experiments 1-7 the main product of each reaction was the deoxygenated indolenine (72). A study of the minor components of the product mixtures by uv and mass spectroscopy failed to show the presence of preakuammicine or stemmadenine derivatives. In Experiments 8-10, very complex product mixtures were obtained and no identifiable components could be isolated.

The results from a study of the reduction of compound (60) with other metal hydrides are shown in Table 7. In each experiment, a complex mixture of products was obtained with the major product being the indolenine (72). At no time could indolic material be detected.

Another approach to the reduction of product (60) was considered. The aldehydo-ester was dissolved in ammonia and dimethoxyethane at -78° and treated briefly with lithium metal. The possible products that may be expected under these conditions would be the alcohol (69), its 1,2-dihydro-derivative or akuammicine (5). However, only very complex product mixtures could be obtained. Careful examination of these materials by uv and mass spectroscopy failed to demonstrate the presence of any of the desired compounds.
Table 6. Reactions of Compound (60) with NaBH₄

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Time(hr)</th>
<th>Temp</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>0.5</td>
<td>rt</td>
<td>(72)</td>
</tr>
<tr>
<td>MeOH</td>
<td>2</td>
<td>-30°</td>
<td>(72)</td>
</tr>
<tr>
<td>MeOH + 10% KOH</td>
<td>1</td>
<td>rt</td>
<td>(72) plus minor products</td>
</tr>
<tr>
<td>MeOH + 10% KOH</td>
<td>0.5</td>
<td>65°</td>
<td>&quot;</td>
</tr>
<tr>
<td>MeOH + 1% HoAc</td>
<td>1</td>
<td>rt</td>
<td>&quot;</td>
</tr>
<tr>
<td>MeOH + 10% HoAc</td>
<td>1</td>
<td>rt</td>
<td>&quot;</td>
</tr>
<tr>
<td>MeOH + 50% HoAc</td>
<td>1</td>
<td>rt</td>
<td>&quot;</td>
</tr>
<tr>
<td>HoAc</td>
<td>0.5</td>
<td>rt</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>HoAc</td>
<td>0.3</td>
<td>90°</td>
<td>&quot;</td>
</tr>
<tr>
<td>THF</td>
<td>.5</td>
<td>rt</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Table 7. Further studies on the metal hydride reduction of Compound (60)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBH₄</td>
<td>MeOH</td>
<td>rt</td>
<td>1</td>
<td>(72)</td>
</tr>
<tr>
<td>&quot;</td>
<td>MeOH+10%KOH</td>
<td>35°</td>
<td>0.5</td>
<td>(72) plus complex mixture</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>MeOH+10%HoAc</td>
<td>rt</td>
<td>1</td>
<td>(72) &quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>HoAc</td>
<td>rt</td>
<td>1</td>
<td>Complex mixture</td>
</tr>
<tr>
<td>Red-Al</td>
<td>THF</td>
<td>rt</td>
<td>0.2</td>
<td>(72) plus complex mixture</td>
</tr>
</tbody>
</table>
The failure to obtain from product (60) the alcohol (69) of the preakuammicine series or a ring-opened stemmadenine derivative by metal hydride reducing agents was attributed to a facile reduction-elimination process shown in Figure 21. In order to gain further insight into the chemistry of this structure it was decided to protect the aldehyde group in order to eliminate its participation in further attempts to ring-open the molecule.

When the aldehydo-ester (60) was treated with ethane dithiol and a catalytic amount of boron trifluoride etherate in dichloromethane at 5°, a good yield of the thioacetal derivative was obtained. This material was assigned structure (73) on the basis of the following spectral data. The uv spectrum ($\lambda_{max}$ 238, 275, 283 sh) was very similar to that of the starting material. In the ir spectrum, there appeared one carbonyl absorption bond near 1710 cm$^{-1}$ and an indolenine absorption at 1560 cm$^{-1}$. The pmr spectrum of this material, exhibited a 3-proton singlet absorption at $\tau$ 6.09 indicating the presence of the carbomethoxyl functional group.
group. The thioacetal group was evident by the appearance of a one-proton singlet absorption at \( \tau 4.19 \) which was attributed to the C-17 proton, and a four-proton multiplet signal near \( \tau 6.68 \) was assigned to the methylene protons of the thioacetal group. Further confirmation of this structure was given by its mass spectrum which showed a molecular ion at m/e 426. A high resolution mass measurement on the latter confirmed the molecular formula \( \text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_2\text{S}_2 \).

A study designed to achieve ring-opening of the thioacetal derivative (73) was initiated. When this material was treated with sodium borohydride in refluxing methanol for 48 hours a small quantity of indolic \( (\lambda_{\text{max}} 228,284,291) \) material was obtained. The ir spectrum indicated the absence of carbonyl absorptions. This observation was supported by the absence in the pmr spectrum of the carbomethoxyl methyl absorption. However, present in this spectrum was a one-proton doublet absorption at \( \tau 4.82 \) (\( J=8 \text{Hz} \)) which was attributed to the C-17 proton, and a multiplet absorption near \( \tau 6.7 \) confirmed the presence of the thioacetal methylene protons. The mass spectrum of this material revealed a molecular ion at m/e 370. Based on these data, this reduction product was assigned structure (75) or (76). The stereochemistry at C-16 could at this point not be assigned with certainty.

Two pathways must be considered for the course of this reaction and the failure to detect the stemmadenine thioacetal derivative (74). The carbomethoxy thioacetal (73) could undergo decarboxylation to give the \( \alpha \)-vinyl indoline (77) which would then undergo ring-opening to product. Or, the ring-opening reaction may have proceeded first to give the desired material (74) which could also decarboxylate under these reaction conditions.
However, at no time during the course of this reaction could any intermediate products be detected by tlc monitoring. It was also found that this same conversion could be achieved in 2 hours in refluxing 5% methanolic potassium hydroxide and sodium borohydride.

In connection with another approach to this problem some quantities of the α-vinyl thioacetal (77) were required and its synthesis from the carbomethoxy thioacetal (73) was achieved by treatment of the latter with hot 10% methanolic potassium hydroxide. The subsequent ring-opening of this material to the indole thioacetal (75) or (76) was done in refluxing methanol with sodium borohydride. Therefore, since this pathway could be achieved, the question as to the course of the reductive ring-opening of compound (73) remains unanswered.

The above results may indicate that the ring-opening of the indolenine (73) is base-catalysed but the presence of hydroxyl ions may have caused decarboxylation of compound (73) or intermediate (74). Therefore, it was decided to attempt this conversion under anhydrous conditions in the presence of sodium methoxide. The carbomethoxy thioacetal (73) was dissolved in a 0.2 molar solution of sodium methoxide in methanol and treated with anhydrous sodium borohydride. After 2 hours two major products were detected by tlc. Subsequently, these materials were isolated by plc and the following data was obtained. The less polar material exhibited an indolic uv spectrum ($\lambda_{max} 228, 284, 291$) and no carbonyl absorption in the ir spectrum. Except for the position of one absorption the pmr spectrum of this material was very similar to the spectrum of product (75) or (76) previously obtained. A one
proton doublet appeared at \( \tau 5.32 \) which was assigned to the C-17 proton of the other C-16 epimer of the ring-opened thioacetal (75) or (76). The unambiguous assignment of the stereochemistry at C-16 is at this point tentative. An examination of molecular models revealed the possibility that when the thioacetal group is \( \beta \)-oriented (structure 75) the C-17 proton is in a less shielding environment than if the thioacetal group was \( \alpha \)-oriented (structure 76).

These results and subsequent work on this approach to achieve the synthesis of stemmadenine derivatives made it clear that decarboxylation could not be prevented under basic conditions. The ring-opening of
thioacetal (73) was then attempted under various conditions in acetic acid solvent. The thioacetal (73) was treated with sodium borohydride in glacial acetic acid at room temperature for one hour in one study and at 90° for 15 minutes in a second study. In both studies a complex mixture of products was obtained. Only small amounts of starting material could be identified. A uv spectroscopic study of the other components from these reactions failed to show the presence of indolic material. A third study in which the thioacetal (73) was dissolved in methanol with 1%, 10% and 50% acetic acid and treated with sodium borohydride also failed to give indolic material. At this point this approach was abandoned.

The results described in this Part indicate that the synthesis of preakuammicine (8) and stemmadenine (4) derivatives will require a great deal of further investigation. We believe, however, that the synthesis of preakuammicine aldehyde (7) has been achieved and that the latter is identical with product (60). Preakuammicine aldehyde (7) is at this point a hypothetical intermediate in the proposed biogenetic conversion of the Corynanthe alkaloids to the Strychnos family of alkaloids, but may in the future be isolated as a natural product.

Future work in this area should be directed toward three objectives. One objective is the reduction of the aldehyde group in product (60) to achieve the preakuammicine derivative (69) and compare the latter with naturally occurring preakuammicine (8). Another objective should be the conversion of product (60) to stemmadenine (4) directly or via alcohol (69). The third objective that should be included in this investigation
is the conversion of stemmadenine (4) to iso-stemmadenine (10).
Migration of the double bond from position C-19,20 to position C-20,21 has already been achieved in the Strychnos area using Raney nickel. The evaluation of the biosynthesis and the biomimetic chemistry of iso-stemmadenine (10) would be of great importance to a more complete understanding of this fascinating area of natural products.
EXPERIMENTAL

Thin layer chromatographic (tlc) studies were carried out using EM Reagents GF254 Silica gel or Woelm neutral alumina. The chromatoplates used for analysis were 0.3 mm in thickness and those used for preparative separations were 1.0 mm thick. In all cases, electronic phosphor (about 2% by weight) was added to the absorbent to aid visualization. The chromatoplates were developed in one of the following solvent systems: eluent A consisted of chloroform plus 1% methanol, eluent B consisted of chloroform plus 2% methanol, and eluent C consisted of dichloromethane plus 10% methanol and 0.1% triethylamine. The chromatoplates were examined under a long and short wavelength ultraviolet scanning lamp and visualized further with a ceric sulphate spray or iodine vapors.

Column chromatography was performed using Shawinigan neutral alumina in large scale preparations and Woelm neutral alumina when more exact separations were required. The dimensions of the columns were generally maintained at the accepted optimum ratio of diameter to height as 1:10. Throughout this work, all solvents were distilled before use.

Ultraviolet spectra were recorded in methanol on a Cary 15 spectrophotometer. Absorption values ($\lambda_{\text{max}}$) are given in nanometers (nm). Infrared spectra were measured in chloroform on a Perkin-Elmer model 137 double beam instrument, and absorption bands ($\nu_{\text{max}}$) are quoted in wavenumbers (cm$^{-1}$).
Proton magnetic resonance (pmr) spectra were recorded in deuterochloroform at room temperature at 100 MHz on a Varian HA-100 or a Varian XL-100 spectrometer. The latter was also used to record spectra in the Fourier transform mode. Chemical shifts are given in the Tier's $\tau$ scale using tetramethylsilane as the internal standard at $\tau$10.00. Coupling constants (J-values) are given in hertz (Hz). $\tau$-values given to multiplet signals refer to the center of the signal.

Mass spectra were recorded on an AEI-MS-902 or an Atlas CH-43 mass spectrometer. Fragmentation data is given in mass to charge ratios (m/e) followed by percent relative abundance. High resolution mass measurements were determined on the AEI-MS-902 or an AEI-MS-50 using suitable standards.

Melting points were determined on a Kofler block and are uncorrected. Elemental analyses were performed by Mr. P. Borda of the Microanalytical Laboratory, University of British Columbia, Vancouver.

The Conversion of Strychnine (29) to Wieland-Gumlich Aldehyde (32)

The conversion of strychnine (29) to Wieland-Gumlich aldehyde has been described in detail$^{21-23}$ and since an authentic sample$^{20}$ of the latter was available no data need be given here for these intermediates. The procedures used are appropriately referenced and differ little from those published.

23-Isonitrosostrychnine Hydrochloride (30)$^{23}$

A three-necked round bottom flask equipped with a thermometer
and a dry ice-acetone condenser was charged with strychnine (29) (50g, dried over P₂O₅) in absolute ethanol (800 ml). Iso-pentynitrite (98g, freshly distilled under reduced pressure) was added. While being stirred mechanically the temperature of the reaction mixture was raised to, and maintained at, 70° during the addition of sodium metal (16g) dissolved in ethanol (500 ml). Stirring at 70° was continued for 4 hours. The resulting dark brown solution was allowed to cool and stirring was continued overnight.

The solution was evaporated in vacuo treated with cold water (100 ml) and evaporated again to remove unreacted iso-pentynitrite. The reduced bulk was dissolved in water (500), glacial acetic acid (100) and conc. hydrochloric acid (40). To this solution was added Norit (3g) and the mixture was brought to a boil and filter. After standing at 5° overnight a crystalline material was obtained by vacuum filtration (43.5g). A small sample was recrystallized from methanol, m.p. 218° (decomp) (lit. m.p. 220°, decomp).²³

N(a)-Cyanoformyl Wieland-Cumlich Aldehyde Hydrochloride (32)

After drying thoroughly in vacuo the above salt (40g) was slowly added to thionyl chloride (120 ml) previously cooled in an ice-water bath. This solution was stirred at room temperature. After 4 hours, the solution was cautiously poured onto ice, with vigorous stirring, to yield a gray precipitate. Crystallization of this material occurred after 30 minutes and it was then filtered, washed with cold water and dried in vacuo to give a hydrochloride salt (31g). Recrystallization from methanol yielded colourless material, m.p. 220° (decomp)
The crude salt (29g) from the above reaction was added to a two-necked flask charged with distilled water (400 ml). This mixture was heated in an oil bath (120°). After boiling began, steam was rapidly injected into the boiling mixture for 1-2 hours. The progress of the reaction was monitored by working up small aliquots which were examined by tlc on alumina (eluent B).

When the hydrolysis appeared to be complete, the mixture was cooled and made alkaline with cold ammonium hydroxide. Repeated extraction with chloroform and evaporation in vacuo yielded a brown residue (19g). This material was chromatographed on alumina (500g, act.III) using benzene-ethylacetate gradient. Pooling of the appropriate fractions as determined by tlc yielded a colourless crystalline material (17g) which had physical and spectroscopic properties identical with an authentic sample of Wieland-Gumlich aldehyde. Recrystallization from methanol-acetone yielded colourless needles, m.p. 212-215° (lit. m.p. 212.5-215°).

Wieland-Gumlich Aldehyde (32)

Wieland-Gumlich aldehyde (5g) was dissolved in methanol (50 ml) and sodium borohydride (0.5g) was added over 30 minutes at room temperature. The solution was stirred for a further 30 minutes after which time water (10 ml) was added and stirring continued for a further 15 minutes. This
solution was then evaporated in vacuo and the residue was taken up in chloroform containing 10% methanol and chromatographed on alumina (100g, act. III). Elution with ethylacetate yielded a material (4.1g) which was recrystallized from methanol-water to give colourless crystals. After drying thoroughly in vacuo, this compound had m.p. 250-254° (lit. m.p. 251-253)\(^{22}\); \(\lambda_{\text{max}}\) 243, 297; \(\nu_{\text{max}}\) 3600 (–OH), 3300 (ONH), 1600 (indoline); pmr signals: 2.9-3.4 (multiplets, 4H, aromatic C-H), 4.27 (triplet, J=7, 1H, C-19H), 5.88 (multiplet, J=2 and J=7; 2H, C-18H\(_2\)); mass spectrum: \(M^+\) 312 (82), 182 (43), 144 (100); high resolution mass measurement: Calc. for \(\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_2\): 312.1837. Found: 312.1841.

28-16α-17-acetoxy-cur-19-en (35)\(^{22}\)

Wieland-Gumlich glycol (33, 3.6g) was added to a solution of hydrogen bromide in glacial acetic acid (250 ml, 3% saturated). The flask was sealed and let stand at room temperature for 2 days.

Most of the solvent was then removed in vacuo and more glacial acetic acid (400 ml) was added. Freshly activated zinc powder (50g)* was added and the mixture was shaken vigorously for 5 hours. The zinc was removed by filtration and washed with acetic acid and then ethanol. These washings were combined with the filtrate and reduced in volume in vacuo. The residue was dissolved in dilute ammonium hydroxide and extracted with chloroform, dried over sodium sulphate and evaporated to

* Powdered zinc was activated by washing twice with 5% aqueous hydrochloric acid, followed by successive washings with methanol and ether, and then dried in vacuo.
give a light brown gum (1.2g). Examination of this material by tlc on alumina (eluent B) revealed the presence of one major component, \( \nu_{\text{max}} \) 1720 (acetate). This material was used as such in the next reaction.

\[\text{28,16\textalpha-Cur-19-en-17-ol (36)}\]

The above acetate (1g) was refluxed in methanolic potassium hydroxide (10%) for 1 hour. With cooling, this reaction mixture was neutralized with dilute hydrochloric acid, reduced in volume and extracted with chloroform. The combined organic washings were dried with anhydrous sodium sulphate and evaporated in vacuo to give a brown amorphous material (910 mg).

This material was chromatographed on alumina (act.IV). Elution with a benzene-ethylacetate gradient yielded a pure compound (850 mg) which could be crystallized from benzene, m.p. 172-175\(^\circ\) (lit. m.p. 177-178\(^\circ\))\(^{26}\); \( \lambda_{\text{max}} \) 243, 297, \( \nu_{\text{max}} \) 3575(N-H), 3350(O-H), 1600(indoline); pmr signals: 2.8-3.5 (multiplets, 4H, aromatic-H), 4.58 (quartet, J=7, 1H, C-19H), 8.50 (multiplet, J=7 and J=2, C-18CH\(_3\)\(_3\)); mass spectrum: \( M^+ \) 296(89), 281(17), 279(15), 265(12), 166(100), 144(43), 130(31); high resolution mass measurement; Calc. for C\(_{19}\)H\(_{24}\)N\(_2\)O: 296.1887, Found: 296.1888.

Catalytic Hydrogenolysis of Wieland-Gumlich Glycol (33)\(^{26}\)

The glycol (300 mg) was dissolved in a solution of water (20 ml), acetic acid (12 ml) and hydrochloric acid (0.2 ml). After addition of
palladium-charcoal catalyst (10%, 75 mg) the reaction mixture was flushed with hydrogen and let stir in a slightly positive pressure hydrogen atmosphere. The progress of the reaction was monitored by volume of gas up-take.

The reaction mixture was then filtered, cooled in an ice-water bath and made alkaline with aqueous potassium hydroxide resulting in white precipitate. The total mixture was extracted repeatedly with chloroform (500 ml). The organic washings were combined, dried with anhydrous sodium sulphate and evaporated \textit{in vacuo} to give a light yellow foam (285 mg). Chromatography of this product on alumina gave a compound (270 mg) which had physical and spectroscopic properties identical with 2β,16α-cur-19-en-17-ol (36) which had previously been obtained.

\textbf{Birch Reduction of Wieland-Gumlich Aldehyde (32)}

The aldehyde (1g) was suspended in dry dimethoxyethane (75 ml) and dry \textit{t}-butanol (0.5 ml) in a round bottom flask equipped with a dry ice-acetone condenser, a source of ammonia gas and a magnetic stirring bar. This mixture was cooled in a dry ice-acetone bath (−78°) and then filled with ammonia (100 ml). Freshly cut chips of lithium metal were added until a dark blue colour could be maintained for 30 minutes. Ammonium chloride was then added to destroy the excess lithium (i.e. blue colour was removed). The reaction mixture was then allowed to warm as the ammonia evaporated.

The residue was evaporated further \textit{in vacuo} before partitioning between water and chloroform. The combined chloroform washings were
dried with anhydrous sodium sulphate and evaporated in vacuo to give a light yellow foam (915 mg). This material was passed through a column of alumina (20g, act.III) with a benzene-ethylacetate solvent gradient to yield a compound (890 mg) which had physical and spectroscopic properties identical with 2β,16α-cur-19-en-17-ol (36) which had been previously obtained.

Birch Reduction of Wieland-Gumlich Glycol (33)

The glycol (150 mg) was hydrogenolysed under the same conditions described above for Wieland-Gumlich aldehyde (32). After chromatography, a compound (121 mg) was obtained which had physical and spectroscopic properties identical with 2β,16α-cur-19-en-17-ol (36) which had been previously obtained.
Oppenauer Oxidation of 2β,16α-cur-19-en-17-ol (36) by Boekelheide's Procedure

The alcohol (36) (300 mg) was dissolved in refluxing benzene (100 ml, freshly distilled from lithium aluminum hydride) with benzophenone (910 mg, sublimed). Potassium t-butoxide (560 mg) was added and the reaction was stirred for 2 hours under an inert atmosphere. After cooling in an ice-bath, water (100 ml) was added and the mixture was partitioned. The aqueous layer was extracted three times with dichloromethane (250 ml) and the combined organic extracts were washed with water before drying over anhydrous sodium sulphate. After removal of the solvent, the residue was chromatographed on alumina (30 g, act. III) using a petroleum ether-benzene solvent gradient. Pooling of the appropriate fractions yielded the major product (118 mg) as a yellow foam. This material was identified as nor-fluorocurarine (39) based on the following data: m.p. 182-185°C (lit. m.p. 185-186°C)26; \( \lambda_{\max} \) 244 290 sh, 298, 363; \( \nu_{\max} \) 3300 (N-H), 1640 (CHO), 1605 (indoline); pmr: -0.2 (broadsinglet, 1H, N-H), 0.70 (singlet, 1H, CHO), 2.7 - 3.2 (multiplets, 4H, aromatic-H), 4.68 (quartet, J=7, 1H, =CH-CH\(_3\)), 8.48 (doublet of doublets, J=7 and 2, 3H, =CH-CH\(_3\)); mass spectrum: M\(^+\) 292 (63), 263 (15), 249 (21), 243 (26), 222 (17), 208 (15), 194 (18), 167 (22), 121 (100); high resolution mass measurement: Calc. for C\(_{19}\)H\(_{20}\)N\(_2\): 292.1575. Found: 292.1567.

A minor product (14 mg) was later identified as 2β,16α-cur-19-en-17-ol (37).
Modified Oppenauer Oxidation of 2β,16α-cur-19-en-17-ol (36) to give 2β,16α-cur-19-en-17-al (37)

The alcohol (36)(145 mg) was dissolved with benzophenone (270 mg) in benzene (50 ml, freshly distilled from lithium aluminum hydride). Approximately 5 ml of benzene were then distilled from the reaction mixture before potassium hydride (200 mg of a 22% oil suspension) was added. This mixture was refluxed for 1 hour. After cooling in an ice-water bath, water (50 ml) was added and the mixture was partitioned. The aqueous layer was extracted with dichloromethane and the combined organic extracts were washed with water and dried over an hydrous sodium sulphate. After removal of most of the solvent in vacuo, the residue was chromatographed on alumina (8 g, act.III) using a petroleum ether-benzene solvent gradient. The major product (105 mg) obtained by pooling the appropriate fractions was identified as 2β,16α-cur-19-en-17-al (37) based on the following data, 

\[ \lambda_{\text{max}} 247, 297; \nu_{\text{max}} 3450 (\text{N-H}), 1720 (\text{CHO}), 1600 \text{(indoline)}; \text{pmr}: 0.24 (\text{singlet, 1H, CHO}), 2.8 - 3.5 (\text{multiplets, 4H, aromatic-H}), 4.60 (\text{quartet, J=7, 1H, =CH-CH}_3), 8.40 (\text{doublet of doublets, J=7 and 2, 3H, =CH-CH}_3); \text{mass spectrum: } M^+ 294 (42), 251 (16), 199 (15), 164 (97), 144 (100), \text{high resolution mass measurement: Calc. for } C_{19}H_{22}N_2O: 294.1731. \text{ Found: 294.1747.}

This material was compared spectroscopically with that prepared by others in our laboratory for another study. 

Modified Oppenauer Oxidation of 2β,16α-cur-19-en-17-ol (36) to give Nor-fluorocurarine (39)

The alcohol (36)(295 mg) was dissolved in benzene (70 ml, freshly
distilled from lithium aluminum hydride) and about 7 ml of benzene were distilled from the reaction mixture. After the temperature of the oil bath had been lowered to 60–65°, potassium hydride (350 mg of a 22% oil suspension) was added. Stirring in an inert atmosphere was continued for 10 minutes. Nitrobenzene (0.45 ml) was added dropwise over 15 minutes and stirring was continued for a further 15 minutes. The reaction vessel was then cooled in an ice-water bath and water (50 ml) was added. This mixture was partitioned and the aqueous layer was extracted repeatedly with dichloromethane (250 ml). The combined organic extracts were dried over anhydrous sodium sulphate and most of the solvent was removed in vacuo. The highly coloured residue was chromatographed on alumina (30 g, act.III) using a petroleum ether-benzene solvent gradient. The appropriate fractions were pooled to yield yellow foam (179 mg) which crystallized from benzene. This material had physical and spectroscopic properties identical with those of nor-fluorocurarine (39) previously synthesized.
l-Formyl-2β-16α-cur-19-en-17-0-formate (42)

The indoline alcohol (36)(900 mg) was dissolved in acetic-formic anhydride (10 ml) and stirred at room temperature for 15 minutes. The solvent was then removed in vacuo at ambient temperature. The residue was chromatographed on alumina (20 g, act.III) using benzene as the eluent to yield, after removal of solvent, a light yellow foam (940 mg), \( \lambda_{\text{max}} 247, 282, 287; \nu_{\text{max}} 1720 (\text{O-CHO}), 1660 (\text{N-CHO}), 1600 (\text{indoline}) \); pmr: 1.51 (singlet, 1H, N-CHO), 2.00 (singlet, 1H, O-CHO), 4.4 (quartet, J=7, 1H, =CH-CH\(_3\)), 8.40 (doublet of doublets, J=7 and 2, 3H, =CH-CH\(_3\)) \( \text{mass spectrum: } M^+ 352 (81), 337(37) 324 (49), 279 (100), 144 (81) \).

l-Formyl-2β-16α-cur-19-en-17-ol (43)

The diformate (42)(940 mg) was refluxed in methanol (15 ml) for 30 minutes. After the removal of the solvent in vacuo, a tlc examination (alumina, eluent A) revealed the presence of a single component. This material was chromatographed on alumina (20 g, act.III) to yield a light yellow foam (780 mg), \( \lambda_{\text{max}} 247, 282, 287, \nu_{\text{max}} 1660 (\text{N-CHO}), 1600 (\text{indoline}) \); pmr: 1.24 (singlet, 1H, N-CHO), 4.48 (quartet, J=7, 1H, =CH-CH\(_3\)), 8.34 (doublet of doublets, J=7 and 2, 3H, =CH-CH\(_3\)) \( \text{mass spectrum: } M^+ 324 (77), 309 (49), 293 (31), 279 (100); \text{high resolution mass measurement: } \text{Calc. for } C_{20}H_{24}N_{2}O_{2} 324.1837. \text{ Found: } 324.1826. \)

Oxidation of N-formyl Alcohol (43) with Collin's Reagent

Collin's reagent was prepared in situ in dichloromethane (30 ml, freshly distilled from P\(_2\)O\(_5\)) using chromium trioxide (200 mg,
dried overnight in high vacuum at 100°) and pyridine (0.4 ml, distilled from KOH) at 0°. To this solution was added the alcohol (43)(100 mg). After stirring for 45 minutes, saturated aqueous sodium bicarbonate solution (30 ml) was added and the resulting mixture was stirred for a further 30 minutes before partitioning. After repeated extraction of the aqueous layer with dichloromethane, the organic extract was washed with water and dried over anhydrous sodium sulphate. The dark coloured residue obtained after removal of the solvent \textit{in vacuo} was chromatographed on alumina (5 g, act.III). The appropriate benzene fractions were combined to yield a light yellow amorphous material (39 mg), which was assigned structure (44) based on the following data:

- $\lambda_{\text{max}}$ 242, 282, 288;
- $\nu_{\text{max}}$ 1720 (C-CHO), 1665 (N-CHO), 1590 (indoline);
- pmr: 0.22 (doublet, $J=4$, 0.5H, C-CHO), 0.37 (singlet, 0.5H, C-CHO), 1.14 (singlet, 0.5H, N-CHO) 1.40 (singlet, 0.5H, N-CHO), 2.0 (multiplet, 1H, C-12N), 4.46 (multiplet, 1H, =CH-CH$_3$), 5.18 (multiplet, 1H, C-16H), 8.45 (multiplet, 3H, =CH-CH$_3$); mass spectrum: $M^+$ 322 (41), 293 (50), 279 (74), 180 (44), 164 (100) high resolution mass measurement: Calc. for C$_{20}$H$_{22}$N$_2$O$_2$ 322.1680. Found: 322.1678.

**Oxidation of N-formyl Alcohol (43) with Silver Carbonate**

Silver carbonate on celite was prepared according to Fetizone's procedure. 36 This reagent (0.4 mmoles) was refluxed with the N-formyl alcohol (43) (96 mg, 0.3 mmoles) in benzene overnight. After filtration and removal of the solvent the residue was examined by tlc (alumina, eluent A) and found to contain a mixture of components. This mixture (95 mg) was chromatographed on alumina (6.5 g, act.III) using a benzene-
ethyl acetate solvent gradient. Pooling of the appropriate chromatographic fractions yielded a light yellow amorphous material (24 mg), which had physical and spectroscopic properties identical with those of the N-formyl aldehyde (44) which had previously been characterized. A second component (10 mg) was identified as starting material (43).

**Oxidation of N\textsubscript{a}-formyl Alcohol (43) with DMSO-SO\textsubscript{3}**

The alcohol (43)(100 mg) was dissolved in dry dimethyl sulphoxide (1 ml, freshly distilled from 4A molecular sieves) and treated with pyridine-sulphur trioxide complex (300 mg) and triethylamine (0.1 ml). After stirring at room temperature for 1 hour, 20 volumes of water were added to the reaction mixture before it was extracted with benzene. The organic washings were dried over anhydrous sodium sulphate and evaporated to yield a colourless amorphous material. Chromatography of this material yielded an amorphous compound (18 mg) which had physical and spectroscopic properties identical with the N\textsubscript{a}-formyl aldehyde (44) previously characterised. A second component (62 mg) from this product mixture had physical and spectroscopic properties identical with starting material (43).

**Oxidation of N\textsubscript{a}-formyl Alcohol (43) with 3,5-Dimethylpyrazole-chromium Trioxide Complex**

The alcohol (43)(90 mg, 0.3 mmoles) was added to a stirred solution
of 3,5-dimethylpyrazole (60 mg, 0.6 mmole) and carefully dried chromium trioxide (65 mg) in dichloromethane (25 ml, freshly distilled from P₂O₅). The resulting brown suspension was stirred at room temperature for 1 hour. Saturated aqueous sodium bicarbonate was added to the mixture and the latter was extracted with dichloromethane (100 ml). The organic extract was washed with water and dried over anhydrous sodium sulphate and evaporated in vacuo. The resulting residue was examined by tlc (alumina, eluent A) and found to contain three major components which appeared to be dimethylpyrazole, starting material and the desired aldehyde. Subsequent chromatography on alumina and silica gel yielded only samples of pure materials which had spectroscopic properties identical with the Nₐ-formyl aldehyde (44) and the starting material (43).

Oxidation of Nₐ-formyl Alcohol (43) with Pyridinium Chlorochromate

The Nₐ-formyl alcohol (43) (305 mg) was dissolved in dichloromethane (60 ml, freshly distilled from P₂O₅) and treated with pyridinium chlorochromate (480 mg) which had been prepared according to Corey's procedure. This coloured suspension was stirred at room temperature for 2 hours, after which time, an equal volume of saturated aqueous sodium bicarbonate solution was added and the mixture was stirred for a further 15 minutes.

This mixture was then extracted repeatedly with dichloromethane (250 ml). The organic extract was washed with aqueous sodium bicarbonate solution and once with water before drying over anhydrous sodium sulphate.
After removal of the solvent, the dark coloured residue was chromatographed on alumina (10 g, act.III). Fractions eluted with benzene yielded the desired aldehyde (218 mg) as a light yellow foam. The physical and spectroscopic properties of this material was identical to the N-formyl aldehyde (44) which had been previously characterized.

1-Formyl-2β,16α-cur-19-en-17-al (44) From Aldehyde (37)

18-Deoxy-Wieland-Gumlich aldehyde (37) (47 mg) was dissolved in tetrahydrofuran (12 ml) and treated with acetic-formic anhydride (0.5 ml) at room temperature for 1 hour. Saturated aqueous sodium bicarbonate was then added and resulting mixture was partitioned. After washing the aqueous layer with dichloromethane, the combined organic extracts were washed with water and dried over anhydrous sodium sulphate. The residue, obtained after removal of the solvent, was purified by plc on silica gel (eluent C) to yield an amorphous material (37 mg) whose physical and spectroscopic properties were identical with those of the aldehyde (44) which had been previously characterized, and later synthesized by others in our laboratories by a similar route.
Attempted C-16 Functionalization of 1-Formyl-2β,16α-cur-19-en-17αl (50)

The data relevant to the study of the functionalization of the C-16 position of the N-formyl aldehyde (44) using sodium hydride and methyl chloroformate is summarized in Table 8, and data relevant to a similar study using other bases is summarized in Table 9.

In all experiments in Table 8 and 9 the following general procedures were used. All glassware was flame-dried and all reactions were conducted in an atmosphere of dry nitrogen gas. In Experiments 1-8 of Table 8 and 1-3 of Table 9, the aldehyde (44) was dissolved in the appropriate solvent at the required temperature. The base was then added and the mixture was stirred for the required time period, after which excess methyl chloroformate (0.2-0.3 ml, distilled from 4Å molecular sieves) was added. Stirring was continued for 10-20 minutes before ice-water was added. The reaction mixture was then partitioned between water and dichloromethane. The organic extracts were washed with water and dried over anhydrous sodium sulphate before the solvent was removed in vacuo to give the reaction product. When dimethyl sulphoxide (DMSO) and dimethyl formamide (DMF) were used as solvents the work-up was simpler if, at the end of the reaction time, only a few drops of water were added and most of the solvent was removed in high vacuum before partitioning between water and dichloromethane.

In Experiments 4-6 of Table 9, a variation in the above described procedure was employed. Potassium t-butoxide was first transferred to the reaction vessel from a previously prepared stock solution in benzene. The benzene was then removed in high vacuum and the weight of the reagent
was determined. The appropriate solvent was then added, followed by the addition of the aldehyde (44).

In Experiments 7-9 of Table 9, the base lithium diisopropyl amine (LDA) was first prepared at 0° using equal molar quantities of diisopropyl amine (distilled from potassium hydroxide), n-butyl lithium and hexamethyolphosphoramide (HMPA). After 15 minutes, the aldehyde (44) was added and the above described procedure was employed.

In every experiment, the reaction product was separated by chromatography on alumina (act.III) and the components were examined spectroscopically. In every experiment, except number 3 in Table 8, unreacted starting material, aldehyde (44), was recovered. In Experiments 7 of Table 8 and 1-3 of Table 9, samples of 18-deoxy-Wieland-Gumlich aldehyde (37) were isolated and identified by spectroscopic comparison with material previously synthesized. In all experiments, quantities of very polar tar-like material was obtained.
Table 8. Attempted C-16 functionalization of aldehyde (44): additional data associated with Table 1.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Aldehyde (44) (mg, mmoles)</th>
<th>Sodium Hydride (mg, mmoles)</th>
<th>Solvent (ml)</th>
<th>Temperature</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48, 0.15</td>
<td>36, 1.5</td>
<td>THF(30)</td>
<td>rt&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>50, 0.16</td>
<td>39, 1.6</td>
<td>THF(25)</td>
<td>50°</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>82, 0.25</td>
<td>60, 2.5</td>
<td>DMF(20)</td>
<td>rt</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>50, 0.16</td>
<td>40°, 1.7</td>
<td>DMF(20)</td>
<td>50°</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>85, 0.26</td>
<td>10, 0.4</td>
<td>DMF(30)</td>
<td>rt</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>85, 0.26</td>
<td>10, 0.4</td>
<td>DMF(20)</td>
<td>55°</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>48, 0.15</td>
<td>7, 0.3</td>
<td>DMSO(20)</td>
<td>50°</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>50, 0.16</td>
<td>7, 0.3</td>
<td>DMSO(20)</td>
<td>rt</td>
<td>3</td>
</tr>
</tbody>
</table>

a. Calculated based on 55% oil suspension
b. Granular sodium hydride
c. Room temperature
Table 9. Attempted C-16 functionalization of aldehyde (44): additional data associated with Table 2.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Aldehyde(44) (mg, mmoles)</th>
<th>Base (mg, mmoles)</th>
<th>Solvent (ml)</th>
<th>Temperature</th>
<th>Reaction Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 , 0.15</td>
<td>KH(32,0.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>THF(30)</td>
<td>rt&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>45 , 0.14</td>
<td>KH(28,0.7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>THF(30)</td>
<td>60°</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>65 , 0.20</td>
<td>KH(40,1.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DMSO(20)</td>
<td>35°</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>38 , 0.12</td>
<td>_t-_BuOK(28,0.25)</td>
<td>C₆H₆(50)</td>
<td>55°&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>50 , 0.15</td>
<td>_t-_BuOK(34,0.30)</td>
<td>DMSO(25)</td>
<td>rt</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>85 , 0.26</td>
<td>_t-_BuOK(62,0.55)</td>
<td>DMSO(25)</td>
<td>50°</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>46 , 0.14</td>
<td>LDA(0.28)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>THF(20)</td>
<td>-78°</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>132, 0.41</td>
<td>LDA(0.80)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>THF(45)</td>
<td>0°</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>68 , 0.21</td>
<td>LDA(0.42)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>THF(20)</td>
<td>rt</td>
<td>3</td>
</tr>
</tbody>
</table>

a. Calculated based on 22% oil suspension
b. Plus 1 equivalent of HMPA
c. Room temperature
d. Refluxed for 30 minutes before cooling to 55° for the addition of methyl chloroformate.
l-Carbomethoxy-2β,16α-cur-19-en-17-ol (49)

The alcohol (36)(100 mg) was dissolved in methanol (10 ml) and treated with methylchloroformate (0.2 ml) and let stir at room temperature for 15 minutes. The solvent was then removed \textit{in vacuo} and the residue was partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The combined organic washings were dried and evaporated \textit{in vacuo} to give a light yellow amorphous material (110 mg), \( \lambda_{\max} \) 241, 278, 287; \( \nu_{\max} \) 3350 (OH), 1685 (N-CO\(_2\)CH\(_3\)), 1600 (indoline); pmr: 2.52 (multiplet, 1H, C-12H), 2.65-3.0 (multiplets, 3H, aromatic-H), 4.50 (multiplets J=7, 1H, =CH-CH\(_3\)), 5.68 (multiplet, 2H, -CH\(_2\)-OH) 6.20 (singlet, 3H, N-CO\(_2\)CH\(_3\)), 8.33 (doublet of doublets, J=7 and 2, =CH-CH\(_3\)); mass spectrum: \( M^+ \) 354(86), 323(21), 309(100), 257(21), 202(63), 166(85); high resolution mass measurement: Calc. for C\(_{21}\)H\(_{26}\)O\(_3\)N\(_2\): 354.1942 Found: 354.1916.

l-Carbomethoxy-2β,16α-cur-19-en-17-al (50)

The N-carbomethoxy alcohol (49)(450 mg) was dissolved in dichloromethane (100 ml, freshly distilled from P\(_2\)O\(_5\)) and treated with pyridinium chlorochromate (750 mg) which had been prepared according to Corey's\(^{38}\) procedure. The resulting coloured suspension was stirred at room temperature for 2.5 hours, after which time, an equal volume of saturated aqueous sodium bicarbonate solution was added and the mixture was stirred for a further 15 minutes.

This mixture was extracted repeatedly with dichloromethane (300 ml) and the combined organic extracts were washed with water.
before being dried over anhydrous sodium sulphate. After removal of the solvent, the residue was chromatographed on alumina (20 g, act III) using a benzene-ethyl acetate solvent gradient. The desired aldehyde was obtained as a light yellow foam (325 mg), λ<sub>max</sub> 242, 282, 288; ν<sub>max</sub> 1720(CHO), 1690(N-CO₂CH₃) 1600 (indoline); pmr: 0.40 (doublet, J=4, 1H, -CHO), 2.38 (multiplet, 1H, C-12H), 2.6-3.0 (multiplets, 3H, aromatic-H), 4.45 (quartet, J=7, 1H, =CH-CH₃), 6.23 (singlet, 3H, N-CO₂CH₃), 8.45 (doublet of doublets, J=7 and 2, 3H, =CH-CH₃); mass spectrum: M⁺ 352(58), 323(22), 309(78), 202(47), 164(100); high resolution mass measurement: Calc. for C₂₁H₂₄N₂O₂: 352.1742. Found: 352.1737.

1-Carbomethoxy-2β,16α-cur-19-en-17-al (50) From Aldehyde (37)

18-Deoxy-Wieland-Gumlich aldehyde (37)(55 mg) was dissolved in tetrahydrofuran (10 ml) and treated with excess methylchloroformate. After stirring at room temperature for 1.5 hours the solvent was removed in vacuo and the residue was partitioned between saturated aqueous sodium bicarbonate solution and dichloromethane. The organic extract was washed with water and dried over anhydrous sodium sulphate before being evaporated in vacuo. The amorphous product (41 mg) was chromatographed by plc (silica gel, eluent C) to yield a product (35 mg) which had physical and spectroscopic properties identical with previously synthesized N<sub>a</sub>-carbomethoxy-18-deoxy-Wieland-Gumlich aldehyde (50).
Attempted C-16 Carboxylation of 1-Carbomethoxy-28,16α-cur-19-en-17-al (50)

The data relevant to the study of the functionalization of the C-16 position of the N-carbomethoxy aldehyde (50) is summarized in Table 10. In this study, the same general procedures described above for the previous study of the N-formyl aldehyde (44) were employed for similar experiments.

At no time could any products other than the starting material (50) or very polar decomposition material be detected spectroscopically after chromatography on alumina (act.III).

Table 10. Attempted C-16 functionalization of aldehyde (50): additional data associated with Table 3.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Aldehyde (50) (mg, mmoles)</th>
<th>Base (mg, mmoles)</th>
<th>Solvent (ml)</th>
<th>Temperature (°C)</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68 , 0.19</td>
<td>NaH(50, 2.0)</td>
<td>THF(30)</td>
<td>40</td>
<td>6</td>
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<tr>
<td>2</td>
<td>75 , 0.21</td>
<td>NaH(50, 2.0)</td>
<td>DMSO(20)</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>45 , 0.13</td>
<td>KH(10, 0.25)</td>
<td>C₆H₆(60)</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>50 , 0.14</td>
<td>KH(6, 0.15)</td>
<td>DMF(20)</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>50 , 0.14</td>
<td>LDA(0.28)</td>
<td>THF(20)</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>46 , 0.13</td>
<td>LDA(0.25)</td>
<td>THF(25)</td>
<td>rt</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>35 , 0.10</td>
<td>LDA(0.20)</td>
<td>THF(15)</td>
<td>35</td>
<td>1</td>
</tr>
</tbody>
</table>

a. Calculated based on 55% oil suspension
b. Calculated based on 22% oil suspension
c. Plus 1 equivalent of HMPA.
Des-carbomethoxy-stemmadenine (52)

Nor-fluorocurarine (39)(660 mg) was dissolved in methanolic potassium hydroxide (35 ml, 2%) at 40° and treated with NaBH₄ (0.5 g) which was added over a 0.5 hour period. Stirring was continued for a further 1.5 hours before the mixture was reduced in volume in vacuo and partitioned between water and dichloromethane. The aqueous layer was repeatedly extracted with dichloromethane and the combined extracts were washed with water before drying over anhydrous sodium sulphate. After removal of the solvent, the residue (470 mg) was chromatographed on alumina (15 g, act.IV) using a benzene-ethyl acetate solvent gradient. After pooling of the appropriate fraction a light yellow amorphous material (395 mg) was obtained: \( \lambda_{\text{max}} 226, 282, 288; \; \nu_{\text{max}} 3350 \) (N-H), 3250 (O-H); pmr: 1.0 (broad singlet, 1H, N-H), 2.4-3.0 (multiplets, 4H, aromatic-H), 4.5 (quartet, J=7, 1H, =CH-CH₃), 5.72 (doublet, J=5, 2H, -CH₂-OH), 6.15 (multiplet, 1H, C-16H), 8.3 (multiplet, 3H, =CH-CH₃); mass spectrum: \( M^+ 296(57), 265(12), 251(15), 166(61), 144(86), 130(32), 123(100), 122(50); \) high resolution mass measurement: Calc. for C₁₉H₂₄N₂O: 296.1887. Found: 296.1887.

1,17-Diacetyl-des-carbomethoxy-stemmadenine (56)

Des-carbomethoxy-stemmadenine (53)(60 mg) was dissolved in acetic anhydride (5 ml) and pyridine (0.5 ml) and let stand at 5° overnight. The solvent was then removed in vacuo and the residue was partitioned between saturated aqueous sodium bicarbonate solution and dichloromethane. The aqueous layer was extracted further and the organic extracts were washed
with water before drying over anhydrous sodium sulphate. After removal of most of the solvent this material was separated by plc on silica gel (eluent C) to yield two amorphous materials. The major product (45 mg) was assigned structure (56) based on the following data:

$$
\lambda_{\text{max}} 248, 282, 290 ; \nu_{\text{max}} 1725 (\text{O-Ac}), 1640 (\text{N-Ac}), \text{pmr}: 2.4-2.9 \text{ (multiplets, 4H, aromatic-N)}, 4.47 \text{ (quartet, J=7, 1H, } =\text{CH-CH}_3), 5.3 \text{ (multiplet, 2H, } -\text{CH}_2\text{-OAc}), 7. \text{ (singlet, 3H, } N\text{-C}(0)\text{CH}_3), 7.89 \text{ (singlet, 3H, } -\text{O}_2\text{CCH}_3), 8.26 \text{ (multiplet, 3H, } =\text{CH-CH}_3); \text{mass spectrum: } M^+ 380(27), 321(21), 293(53), 208(16), 186(17), 144(100); \text{high resolution mass measurement: Calc. for } C_{23}H_{28}N_2O_3: 380.2100. \text{Found: 380.2108.}
$$

**17-Acetyl-des-carbomethoxy-stemmadenine (57)**

Des-carbomethoxy-stemmadenine (52) (190 mg) was dissolved in acetic anhydride (5 ml) with 1 drop of pyridine and let stand at -10° for 6 hours. The solvent was then removed in vacuo and the residue was partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The organic extract was washed with water and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was carefully chromatographed on alumina (10 g, act.III) using a benzene-ethyl acetate solvent gradient. The less polar fractions yielded an amorphous material (6 mg) which was identified as the diacetate (56). The major product was identified as 17-acetyl-des-carbomethoxy-stemmadenine (57) (176 mg) based the following data: $$\lambda_{\text{max}} 224, 282, 288 ; \nu_{\text{max}} 1730 (-\text{OAc}); \text{pmr: 1.69 (broad singlet, 1H, N-H), 2.4-2.9 (multiplets, 4H, aromatic-H), 4.49 (quartet, J=7, 1H, } =\text{CH-CH}_3), 5.3 \text{ (multiplet, 2H, } -\text{CH}_2\text{-OAc}), 7.89 \text{ (singlet, 3H, } -\text{O}_2\text{CCH}_3), 8.26 \text{ (multiplet, 3H, } =\text{CH-CH}_3);$$
mass spectrum: \( M^+ \) 338(9), 293(7), 279(14), 170(25), 144(39), 123(100);

high resolution mass measurement: Calc. for \( C_{21}H_{26}O_2N_2 \cdot 338 \cdot 1976 \); Found: 338.1972.

**Attempted C-16 Cyanide Introduction in Alcohol (52) via Chloroindolenine (53)**

The data relevant to the study of the introduction of a cyano group into the C-16 position of indole alcohol (52) *via* the chloroindolenine (53) is summarized in Table 11.

In all experiments in this study, the following general procedure was employed. The indole alcohol (52) was dissolved in dichloromethane (freshly distilled from \( P_2O_5 \) at 0°). \( \tau \)-Butyl hypochlorite (1.1 equivalents from a 0.05 M stock solution in \( CC_1_4 \)) was added and the reaction mixture was stirred for 15 minutes. The reaction was monitored by tlc on alumina (eluent B) and uv spectroscopy. When the uv chromophore of the starting material was absent and the characteristic indolenine chromophore (\( \lambda_{\text{max}} \) 260, 310) was present, the nucleophile was added and the mixture was stirred at the required temperature. However, when even dimethylformamide (DMF) was used as solvent the dichloromethane was removed in vacuo after chloroindolenine formation.

All experiments were monitored by tlc on alumina and by uv spectroscopy. At the end of the reaction time, the mixture was partitioned between dichloromethane and water. The organic extracts were dried over anhydrous sodium sulphate before the solvent was removed in vacuo. The residues were partitioned by plc on silica gel (eluent C).

In every experiment, a very complex mixture of products were obtained. The major products were dark coloured tar-like material which remained near
the base-line of the chromatoplate. At no time could new indolic
cmaterial be detected by uv or mass spectroscopy.

Table 11. Attempted C-16 introduction of cyanide: additional data
associated with Table 4.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Indole(52)(^a) (mg,mmoles)</th>
<th>Nucleophile (mg,mmoles)</th>
<th>Solvent (ml)</th>
<th>Temperature</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48, 0.16</td>
<td>KCN(52, 0.8)</td>
<td>CH(_2)Cl(_2)(15)</td>
<td>rt(^c)</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>35, 0.12</td>
<td>KCN(58, 0.9)</td>
<td>DMF(10)</td>
<td>rt</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>50, 0.17</td>
<td>KCN(98, 1.5)</td>
<td>DMF(15)</td>
<td>80(^\circ)</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>35, 0.12</td>
<td>Zn(CN)(_2)(140, 1.2)</td>
<td>CH(_2)Cl(_2)(20)</td>
<td>rt</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>36, 0.12</td>
<td>Zn(CN)(_2)(70, 0.6)</td>
<td>DMF(15)</td>
<td>50(^\circ)</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>50, 0.17</td>
<td>AgCN(228, 1.7)</td>
<td>CH(_2)Cl(_2)(30)</td>
<td>rt</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>40, 0.14</td>
<td>AgCN(200, 1.5)</td>
<td>DMF(10)</td>
<td>55(^\circ)</td>
<td>1.5</td>
</tr>
<tr>
<td>8</td>
<td>54, 0.18</td>
<td>Bu(_4)NCN(91, 0.9)</td>
<td>CH(_2)Cl(_2)(25)</td>
<td>rt</td>
<td>48</td>
</tr>
<tr>
<td>9</td>
<td>35, 0.12</td>
<td>Bu(_4)NCN(120,1.2)</td>
<td>CH(_2)Cl(_2)(25)</td>
<td>35(^\circ)</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>35, 0.12</td>
<td>Bu(_4)NCN(120,1.2)</td>
<td>DMF(10)</td>
<td>35(^\circ)</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>48, 0.16</td>
<td>Et(_2)AlCN(1.6)(^b)</td>
<td>CH(_2)Cl(_2)(25)</td>
<td>rt</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>37, 0.13</td>
<td>Et(_2)AlCN(1.3)</td>
<td>CH(_2)Cl(_2)(25)</td>
<td>36(^\circ)</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>62, 0.21</td>
<td>Et(_2)AlCN(2.1)</td>
<td>CH(_2)Cl(_2)(25)</td>
<td>5(^\circ)</td>
<td>5 days</td>
</tr>
</tbody>
</table>

\(^a\) Stirred with 1.1 equivalents of \(t\)-BuOCl at 0\(^\circ\) for 15 minutes before addition of nucleophile.

\(^b\) Available from Alfa Products.

\(^c\) Room temperature.
Attempted C-16 Cyanide Introduction in Acetate (57) via Chloroindolenine (58)

The data relevant to the study of the introduction of a cyano group into the C-16 position of the indole acetate via the chloroindolenine (58) is summarized in Table 12.

In all experiments, the same procedure as that described above for the study involving alcohol (52) was employed.

At no time in this study, could indolic material be detected by uv or mass spectroscopy.

Table 12. Attempted C-16 introduction of cyanide: additional data associated with Table 5.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Indole (57)</th>
<th>Nucleophile</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45, 0.13</td>
<td>KCN(85, 1.3)</td>
<td>CH$_2$Cl$_2$(30)</td>
<td>rt$^b$</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>35, 0.10</td>
<td>KCN(65, 1.0)</td>
<td>DMF(20)</td>
<td>rt</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>28, 0.08</td>
<td>KCN(52, 0.8)</td>
<td>DMF(15)</td>
<td>68°</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>34, 0.10</td>
<td>Bu$_4$NCN(50, 0.5)</td>
<td>CH$_2$Cl$_2$(30)</td>
<td>rt</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>26, 0.08</td>
<td>Bu$_4$NCN(49, 0.5)</td>
<td>DMF(20)</td>
<td>rt</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>30, 0.09</td>
<td>Bu$_4$NCN(91, 0.9)</td>
<td>DMF(20)</td>
<td>75°</td>
<td>1</td>
</tr>
</tbody>
</table>

a. Stirred with 1.1 equivalents of t-BuOCl at 0° for 15 minutes before addition of nucleophile

b. Room temperature
Reaction of Nor-fluorocurarine (39) with Methylchloroformate

Nor-fluorocurarine (39) (120 mg) was dissolved in tetrahydrofuran (2 ml, freshly distilled from lithium aluminum hydride). This solution was added to a previously prepared solution of lithium diisopropyl amine-hexamethylphosphoramide complex (1:1, 2 equivalents) in tetrahydrofuran (6 ml) at 0°. After stirring for 10 minutes, methylchloroformate (0.2 ml) was added and stirring was continued at 0° for 15 minutes. Cold water (10 ml) was added and the mixture was partitioned. The aqueous layer was extracted with dichloromethane and the combined organic extracts were washed three times with water before drying over anhydrous sodium sulphate. After removal of the solvent, the residue was chromatographed on alumina (8 g, act.III) using benzene. The appropriate fractions were combined to give a single product: \( \lambda_{\text{max}} \) 233, 268, 305 (sh); \( \upsilon_{\text{max}} \) 1720 (\(-\text{CO}_2\text{CH}_3\)), 1670 and 1640 (\(-\text{CHO}\)), 1600 (indolenine) pmr: 0.02 (singlet, 1H, \(-\text{CHO}\)), 2.3 (multiplet, 1H, C-12H), 2.6-3.0 (multiplets, 3H, aromatic-H), 4.56 (quartet, \( J=7 \), =CH-CH\(_3\)), 6.08 (singlet, 3H, -CO\(_2\)CH\(_3\)), 8.34 (multiplet, 3H, =CH-CH\(_3\)); mass spectrum: \( M^+ \) 350 (100), 321 (33), 307 (17), 292 (20), 121 (57); high resolution mass measurement: Calc. for C\(_{21}\)H\(_{22}\)O\(_3\)N\(_2\): 350.1629 Found: 350.1650.

Alternate Synthesis of Aldehydo-ester (60)

Nor-fluorocurarine (39) (180 mg) was suspended in methylchloroformate (5 ml, distilled from 4A molecular sieves) and stirred vigorously at 60-65° under a nitrogen gas atmosphere. After 20 minutes, the solvent was removed in vacuo and the residue was partitioned between saturated
aqueous sodium bicarbonate solution and dichloromethane. The organic extract was washed once with water and then dried over anhydrous sodium sulphate. After removal of the solvent, the residue was chromatographed on alumina (10 g, act.III) to give two compounds. The major compound (152 mg) had physical and spectroscopic properties identical with the aldehydo-ester (50) which had previously been characterized. The second component (12 mg) was identified as the starting material nor-fluorocurarine (39).

Alkaline Hydrolysis of Aldehydo-ester (60)

The aldehyde-ester (60)(35 mg) was dissolved in methanolic potassium hydroxide (5 ml, 5%) and heated to 65° for 2 hours. Cold water (20 ml) was added and the resulting mixture was extracted with dichloromethane. The organic extracts were combined, dried over anhydrous sodium sulphate and evaporated in vacuo to give an amorphous material (23 mg). This material was chromatographed on alumina (act.III) to give a light yellow foam which had physical and spectroscopic properties identical with those of nor-fluorocurarine (39) which had been previously synthesized.

1,2-Dehydro-16,17-dehydro-23-cur-19-en (72)

The aldehydo-ester (60)(50 mg) was dissolved in methanol (15 ml, distilled from magnesium) and treated with sodium borohydride (20 mg, recrystallized from iso-propyl amine). The reaction was stirred at room temperature for 30 minutes after which time most of the solvent was
removed *in vacuo*. The residue was dissolved in water and extracted repeatedly with dichloromethane (150 ml). The organic extract was washed once with water and dried over anhydrous sodium sulphate. The residue, after evaporation of the solvent, was chromatographed by plc on silica gel (elucent C) to yield a light yellow amorphous material (26 mg): $\lambda_{\text{max}}$ 228, 292; pmr: 2.28-2.8 (multiplets, 4H, aromatic-$H$), 3.89 (singlet, 1H, C-17$H$), 4.36 (quartet, $J=7$, 1H, =CH-CH$_3$), 4.54 (singlet, 1H, C-17$H$), 8.16 (doublet, 3H, =CH-CH$_3$); mass spectrum: $M^+$ 276 (100), 232 (39), 194 (45), 170 (32); high resolution mass measurement: Calc. for $C_{19}H_{20}N_2$: 276.1621. Found: 276.1618.

This indoline (72) was isolated in a variety of experiments in which conversion of product (60) to the prekaummicine - and stemmadenine-series was attempted. These results are summarized in Tables 6 and 7. In most experiments, only samples of (72) were isolated for identification since the product mixtures were very complex.

In all experiments in Tables 6 and 7, the aldehydo-ester (60)(40-110 mg) was dissolved in the solvent indicated at the required temperature and treated with excess reducing agent, with the exception of Exp. 9 in Table 6. In the latter, compound (60) was added to a solution of NaBH$_4$ in glacial acetic acid at 90°, followed by addition of more reagent. In all cases, the basic reactions were worked up as described above. Those performed under acidic conditions were first made basic with aqueous sodium bicarbonate solution.
Thioacetal Formation of Aldehyde-ester (60)

The aldehyde-ester (60) (200 mg) was dissolved in dichloromethane (15 ml), ethane dithiol (1 ml) and 2 drops of boron trifluoride etherate. This mixture was let stand at 5° overnight. The solvent was then removed in vacuo and the residue was partitioned between saturated aqueous sodium bicarbonate solution and chloroform. After several extractions with chloroform, the organic extracts were combined and dried over anhydrous sodium sulphate. The solvent was then removed and the residue was chromatographed on alumina (5 g, act.III) using a benzene-methylene chloride solvent gradient. Pooling of the appropriate fractions yielded a light yellow amorphous material (215 mg): \( \lambda_{\text{max}} \) 238, 275, 283 (sh); \( \nu_{\text{max}} \) 1710 (\(-\text{CO}_2\text{CH}_3\)); pmr: 2.26 (multiplet, 1H, C-12H) 2.65-2.8 (multiplets, 3H, aromatic-H), 4.19 (singlet, 1H, C-17H), 4.42 (quartet, J=7, 1H, =CH-CH\_3) 6.09 (singlet, 3H, \(-\text{CO}_2\text{CH}_3\)), 6.69 (multiplet, 4H, =S-\text{CH}_2\text{-CH}_2\text{-S-}), 8.23 (doublet, 3H, =CH-CH\_3); mass spectrum: \( M^+ \) 426 (41), 376 (11), 355 (14), 167 (100), 121 (58); high resolution mass measurement: Calc. for C\text{23}H\text{26}N\text{2}O\text{2}S\text{2}: 426.1436 Found: 426.1427.

Attempted Ring-Opening of Thioacetal (73)

The carbomethoxy thioacetal (73) (82 mg) was dissolved in a methanolic sodium methoxide solution (20, 0.2N) and refluxed under an inhert atmosphere for 2.5 hours. The reaction mixture was then cooled to 0° and ice-water was added. This mixture was extracted repeatedly with dichloromethane and the organic extract was washed with water before drying over anhydrous sodium sulphate. Evaporation of the solvent yielded a residue (76 mg).
Examination of this material by tlc on silica gel (eluent C) revealed the presence of two major products, which were subsequently isolated by plc. The less polar material (75) had the following spectral data:

$\lambda_{\text{max}}$ 228, 284, 291; $\nu_{\text{max}}$ 3300 (N-H); pmr: 2.37 (multiplet, 1H, C-12H), 2.65-2.8 (multiplets, 3H, aromatic-H), 4.14 (doublet, J=8, 1H, C-17H), 4.44 (quartet, J=7, 1H, =CH-CH$_3$), 6.71 (multiplet, 4H, -S-CH$_2$-CH$_2$-S-), 8.24 (doublet, 3H, =CH-CH$_3$); mass spectrum: M$^+$ 370 (30), 309 (16), 123 (100); high resolution mass measurement: Calc. for C$_{21}$H$_{26}$N$_2$S$_2$: 370.1537, Found: 370.1543.

The second component (76)(14 mg) had the following data: $\lambda_{\text{max}}$ 228, 284, 290; $\nu_{\text{max}}$ 3300 (N-H); pmr: 2.37 (multiplet, 1H, C-12H), 2.65-2.8 (multiplets, 3H, aromatic-H), 4.45 (quartet, J=7, 1H, =CH-CH$_3$), 5.32 (doublet, J=8, 1H, C-17H), 6.74 (multiplet, 4H, -S-CH$_2$-CH$_2$-S-), 8.25 (doublet, 3H, =CH-CH$_3$); mass spectrum: M$^+$ 370 (28), 309 (15), 123 (100); high resolution mass measurement: Calc. for C$_{21}$H$_{26}$N$_2$S$_2$: 370.1537, Found: 370.1511.

In a second experiment, the indolenine thioacetal (73)(47 mg) was refluxed in methanolic potassium hydroxide (10%, 10 ml) in the presence of sodium borohydride (20 mg) for 2 hours. Most of the solvent was removed in vacuo and the residue was diluted with water before it was extracted with dichloromethane. The organic extract was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo to give an amorphous material. This material was chromatographed on silica gel (eluent C) to yield a slightly yellow gum (18 mg) which had physical and spectroscopic properties identical with compound (76), previously isolated.
In a third study, the thioacetal (73)(38 mg) was dissolved in glacial acetic acid (5 ml) and treated with sodium borohydride (40 mg) at room temperature for 1 hour. The reaction mixture was then diluted with water, made basic with sodium bicarbonate and extracted with dichloromethane. The organic extract was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo to yield an amorphous material. This material was chromatographed by plc on silica gel (eluent C) to yield the starting material (73)(17 mg) and a tar-like material from the base-line of the chromatoplate.

The thioacetal (73)(45 mg) was dissolved glacial acetic acid (1 ml) and added to a solution of sodium borohydride in glacial acetic acid (15 ml) at 90°. Sodium borohydride was added frequently over 10 minutes and the reaction was allowed to stir for a further 5 minutes. The reaction was then worked up as described above. Examination of the product mixture by tlc revealed a complex mixture of components. Subsequent chromatography and a uv spectroscopic study showed that no indolic material was present.
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