# STUDIES RELATED TO SYNTHESIS AND BIOSYNTHESIS OF INDOLE ALKALOIDS

by

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B.Sc. Honours, University of British Columbia, 1965

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

in the
Department of Chemistry

We accept this thesis as conforming to
the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1969

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## ABSTRACT

In Part I of this thesis, an investigation of the reaction of the alkaloid, catharanthine (12), with zinc in glacial acetic acid is presented. Four isomeric carbomethoxydihydrocleavamines (60-63) have been isolated and fully characterized. It is also shown that heating catharanthine in a mixture of acetic acid and sodium borohydride provides a very convenient method for the preparation of the previously unknown 18β-carbomethoxy-cleavamine (64). A similar investigation is presented for some related chemistry of the Aspidosperma series. Minovine (72), when heated in a mixture of acetic acid and sodium borohydride, is readily converted to vincaminoreine (71) and vincaminorine. The reversibility of the latter reaction is demonstrated by the transannular cyclization of vincaminoreine to afford minovine.

In Part II, Section A, of this thesis, the synthesis of possible precursors of the Aspidosperma, Vinca, and Iboga alkaloids are described. The synthesis of the methyl cyanoacetate adduct (52) and dimethyl malonic ester adduct (53) was accomplished without difficulty. The other synthetic precursor, 96, was prepared by treating the chloroindolenine, 94, of the tetracyclic indole, 93, with methyl cyanoacetate and triethylamine.

In Part II, Section B, of this thesis are also described the biosynthetic studies in Vinca rosea L. and Vinca minor L. plants. The biogenetic importance of the transannular cyclization reaction is described by evaluating appropriate nine-membered ring alkaloids as possible precursors of the Aspidosperma, Vinca, and Iboga alkaloids. To confirm that the transannular cyclization reaction is not important in the plant, a sequential incorporation of DL-tryptophan-3-$^{14}$C into Vinca minor L. plants is presented. The synthetic precursors, 52, 53 and 96, were also evaluated for their biogenetic importance.

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# ACKNOWLEDGEMENTS

I wish to express my thanks to Professor James P. Kutney for his continual encouragement and excellent guidance throughout the course of my research.

I am also grateful to the British Columbia Sugar Refining Company Limited and the National Research Council of Canada for scholarships which I received during my study.

# PART I

# THE PARTIAL SYNTHESIS OF SOME NINE-MEMBERED RING INDOLE ALKALOIDS AND RELATED DERIVATIVES

## Introduction

Nature has provided a vast array of organic compounds of varying degrees of complexity and interest. A large group of these compounds, known as alkaloids, have been of considerable interest, even since before the onset of science in the modern sense of the word. Alkaloids are nitrogenous bases that usually occur in plants, and are localized in the bark, roots, seeds or leaves. The taxonomic distribution of alkaloids cannot be fixed with any certainty as the chemistry of the flora of only a few regions of the world have been intensively studied and the greater majority of plants still remain to be examined. It has been estimated that 10-20% of all plants contain alkaloids [1].

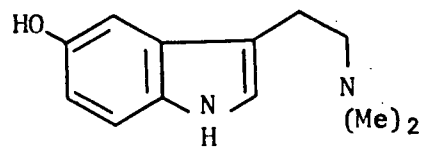
Certain alkaloids have been long known to have characteristic and profound effects on the biological systems, and this observation undoubtedly has provided the stimulus to their examination in ever increasing detail. These compounds tend to upset the balance of endogenous amines associated with the chemistry of the central nervous system [2]. The range of effects produced by alkaloids is detailed in pharmacological collections [3] and many have been medically useful.

A number of alkaloids have been isolated from animals such as the toad poison, bufotenine (1). Samandarine [4] (2) occurs with several related alkaloids as the skin poison of two salamanders (S. maculosa Laurenti and S. atra Laurenti) of European habitat, whereas batrachotoxinin A [5] (3) is isolated from the skin of the Columbian arrow poison

frog (Phyllobates aurotaenia).

The indole alkaloids constitute a large class of compounds ranging in complexity from simple derivatives such as psilocybine (4), the hallucinogenic principle of Mexican mushrooms, to intricate ring systems such as those found in strychnine (5), the well-known poison. Strychnine has also been used as a stimulant for the heart. Over forty alkaloids which bear striking resemblances to strychnine have been isolated in recent years from the particularly potent Calabasch curare. Yohimbine (6) was employed as an aphrodisiac in veterinary medicine. Reserpine (7), isolated from the Indian snakeroot, Rauwolfia serpentina, was widely used in native medicine, usually as a sedative. It is useful in treatment of hypertension and of various mental disorders. The alkaloids of Ergot, which is a fungus parasitic on cereal grasses, especially rye, have an oxytocic effect useful in childbirth. Of the Ergot alkaloids, the synthetic derivative of lysergic acid (8), in the form of the diethylamide, produces symptons like those of schizophrenia when administered in extremely small doses [6]. Vinblastine [7] (9), a dimeric alkaloid produced by Vinca rosea Linn, is used clinically as a potent anti-leukemic agent.

During investigations by the Lilly group [8] on the dimeric Vinca alkaloids, vinblastine or vincaleukoblastine (VLB), leurosine, and leurocristine, they found that each was cleaved by concentrated hydrochloric acid to an indole compound and a vindoline derivative. In the instances of VLB and leurosine, the latter was desacetylvindoline (10), whereas leurocristine gave des-N-methyl-desacetylvindoline (11). Both VLB and leurocristine afforded the same indole derivative, velbanamine, but the corresponding compound with leurosine was cleavamine. Velbanamine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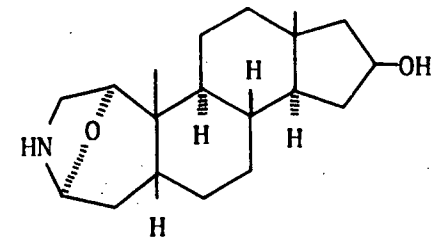
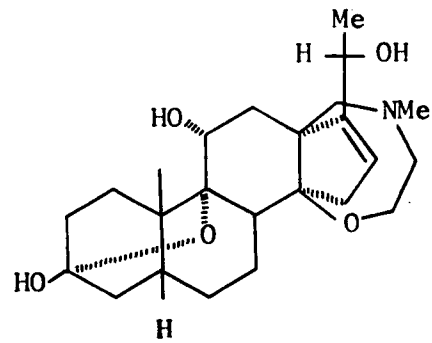
H
N
(Me)2

1


OH
H
HN
O
H
H
H

2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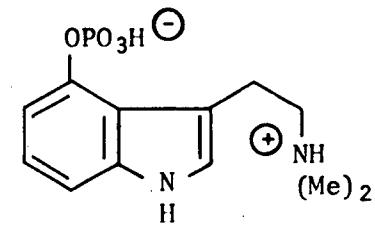
OH
HO
NMe
O
O
HO
H

3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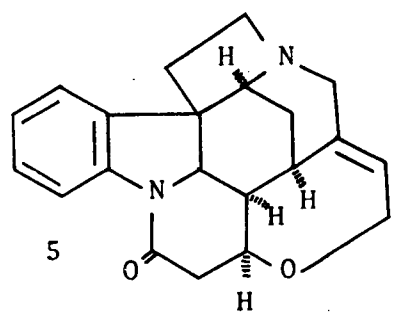
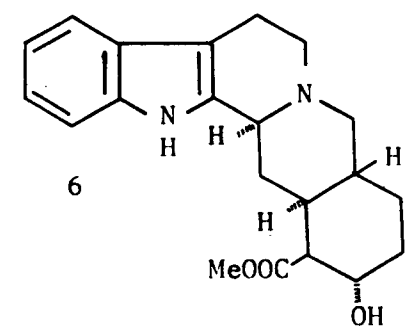
(Me)2
N
H

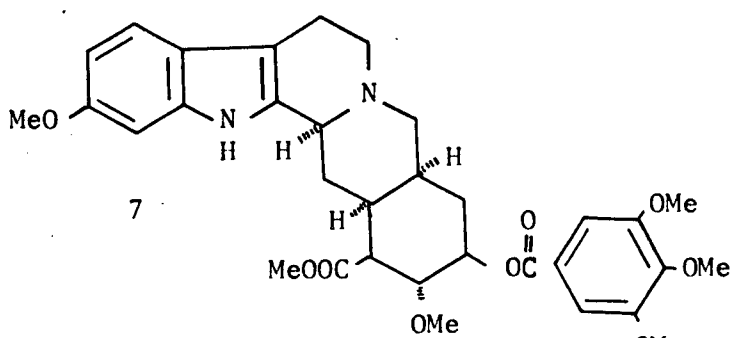
4


H
N
N
H
O
O
H

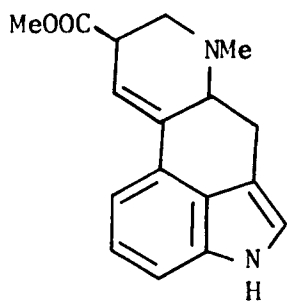
5


N
H
N
H
H
H
MeOOC
OH

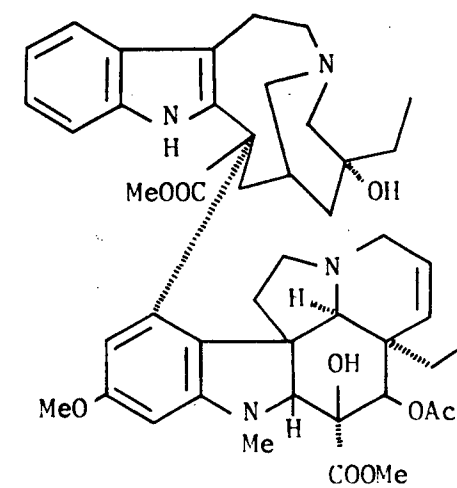
6


MeO
N
H
N
H
H
H
MeOOC
OMe
O
OC
OMe
OMe
OMe

7

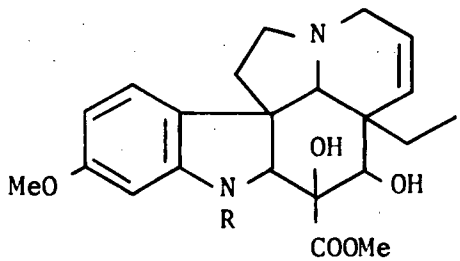

MeOOC
NMe
N
H

8


N
N
H
MeOOC
OH
N
H
OH
MeO
N
H
OAc
Me
COOMe

9

was considered to be a hydroxy-dihydrocleavamine, since it yielded some




10, R = Me

11, R = H

cleavamine on prolonged heating with acid [9]. When catharanthine was subjected to the same acid treatment, one of the products was found to be cleavamine, which suggested that the dimeric alkaloids were constituted of vindoline and catharanthine-like moieties. Moreover, the infrared spectrum of VLB could be approximated by an equimolar mixture of vindoline and catharanthine [10]. Therefore, the Lilly research group postulated a partial structure for VLB which consisted of vindoline attached to a hydroxycatharanthine. However, it was soon realized that VLB must contain a carbomethoxycleavamine rather than a catharanthine unit as the indole portion of the molecule. On the basis of this and other evidence, a revised structure (9) was proposed for VLB [9].

From the structure determination of VLB, considerable knowledge of the chemistry of catharanthine and cleavamine was accumulated. When catharanthine (12) was treated with concentrated hydrochloric acid in the presence of tin and stannous chloride, two of the products obtained were descarbomethoxycatharanthine (16) and cleavamine (18) in addition to two dihydrocleavamine derivatives [11,12]. The structure (18) of cleavamine had been suggested mainly on the basis of a comparison of the mass spectral cracking patterns of cleavamine and dihydrocleavamine with that of quebrachamine [13] (30), and was finally established by an x-ray analysis of cleavamine methiodide [14,15].

The formation of all the products can be rationalized on the basis of

the mechanism shown in Figure 1. The lone pair of electrons on $N_{(b)}$ in 12 can participate in a rearrangement to form an iminium ion, with concurrent ring cleavage and protonation at the β-position of the indole. The resulting ring-opened intermediate, 13, is stabilized by two factors: (a) the conjugated nature of the iminium ion, and (b) the conjugation of the newly generated double bond between C-17 and C-18 with both the ester and anilino functions. After acid hydrolysis of the ester, decarboxylation may occur via 14 in a manner analogous to the mechanism proposed for the Iboga alkaloids [16,17]. This ring-opened intermediate renders the molecule more flexible and the decarboxylated intermediate, 15, is obtained. The intermediate, 15, may follow either of two reaction paths. If the original electron flow is reversed, then the C-5/C-18 bond is regenerated and the product is descarbomethoxycatharanthine (16). But if the α-methylene indoline system in 15 merely rearranges to the indole, the intermediate, 17, from which the ring-opened tetracyclic compounds must ultimately be formed, is obtained. If one assumes that 17 is the actual intermediate, 1,2-reduction of the iminium ion will give cleavamine directly. On the other hand, 1,4-reduction can also occur to afford an enamine, 19, which rearranges in the well-known manner to the iminium compound, 20, with subsequent reduction to provide the two isomeric dihydrocleavamines (21). A mixture of 4α- and 4β-dihydrocleavamines was obtained, because the approach of the proton to C-4 in 19 can occur from above or below the plane of the ring with equal facility.

The last steps of the above mechanism explain the formation of cleavamine and its dihydro derivatives when catharanthine is reacted with acid in the presence of a reducing agent (stannous chloride, mossy tin). Cleavamine is also formed (although in lower yield) by the action

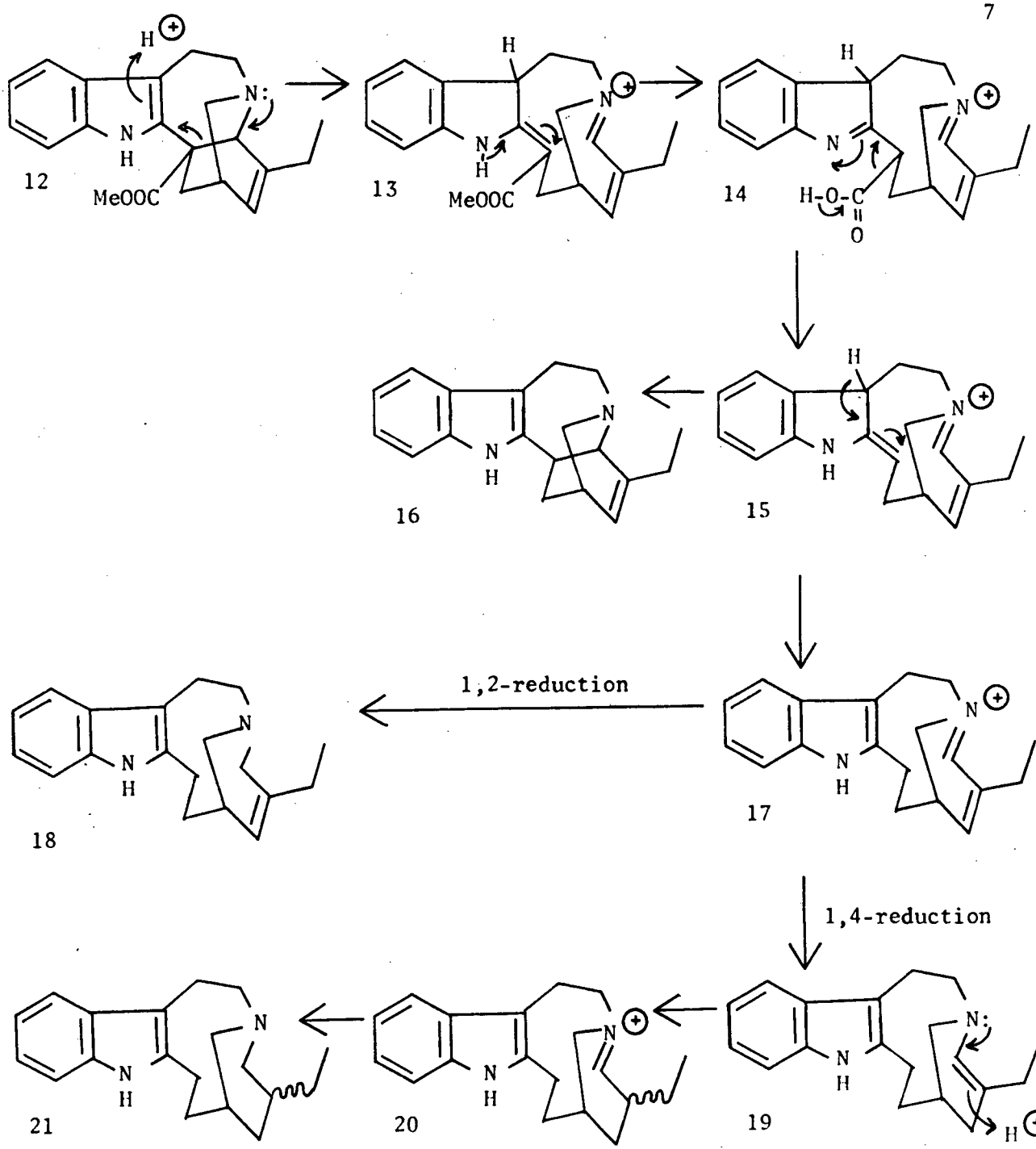
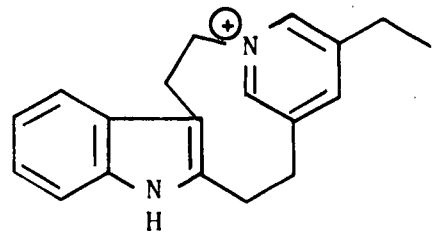



Figure 1. Reaction mechanism proposed for the formation of cleavamine, descarbomethoxycatharanthine and the epimeric dihydrocleavamines.

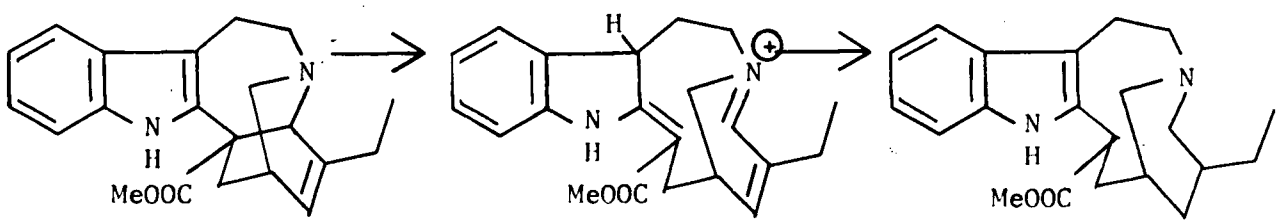
of concentrated hydrochloric acid alone [18]. In the absence of an inorganic reducing agent, the reduction step possibly takes place via an intramolecular redox reaction wherein two molecules of the intermediate, 17, react to yield one molecule of cleavamine and one of a pyridinium compound, 22. The increase in yield of cleavamine in a reducing medium is thus



22

explicable on the grounds that 17 is reduced directly to cleavamine and no pyridinium compound is formed.

Additional evidence for the formation of the tetracyclic intermediate, 13, was furnished from the reaction of pentacyclic alkaloids with glacial acetic acid in the presence of zinc dust. The isolation of a carbomethoxydihydrocleavamine (23) with zinc in acetic acid [9] provided evidence that the reduction of the iminium system can occur before the loss of the ester group.



12 13 23

One of the crucial steps in the mechanism of the acid-catalyzed rearrangement of catharanthine implies a transannular cyclization of intermediate 15 to provide a route to descarbomethoxycatharanthine. The

use of such transannular cyclizations involving iminium salt intermediates is particularly interesting, since Kutney and coworkers have demonstrated that such reactions are of synthetic importance and have led to syntheses of Iboga and Aspidosperma-type systems. They were the first to demonstrate the feasibility of such cyclizations under laboratory conditions. They converted dihydrocleavamine [8,11,12,19] (21) in two steps to 7-ethyl-5-desethylaspidospermidine [20] (24). Likewise, they [21] were able to convert carbomethoxydihydrocleavamine [9,12] (23) into 7-ethyl-5-desethylvincadiffomine (28) having the Aspidosperma skeleton and the Iboga alkaloids, coronaridine [22] (29), and its C-4 epimer, dihydrocatharanthine [23]. Oxidation of carbomethoxydihydrocleavamine in the $N_b$-C-19 direction led to the iminium ion, 26, which cyclized in the reaction media to provide 28. Alternately, oxidation of carbomethoxydihydrocleavamine in the $N_b$-C-5 direction led to an iminium ion, which, by virtue of equilibration with the enamine, 27, resulted in the mixture of C-4 epimeric iminium ions, 25. On this basis, one epimer cyclized to coronaridine, the other, to dihydrocatharanthine. Since new asymmetric centres are generated during the course of these transannular cyclizations, one might anticipate that several diastereoisomers would be formed. However, the reaction proceeded in a stereospecific manner. This result was not unexpected [20,24], since it was apparent (from examination of molecular models) that effectively only one conformation of each iminium ion would permit the reacting centres to be in close proximity and at the same time not be highly strained. Furthermore, the marked rigidity of the cyclic products limits rather severely the stereochemistry of the various asymmetric centres.

Kutney and coworkers [14,24] and, independently, Schmid and coworkers [25] successfully carried out a conversion in the natural series. They found

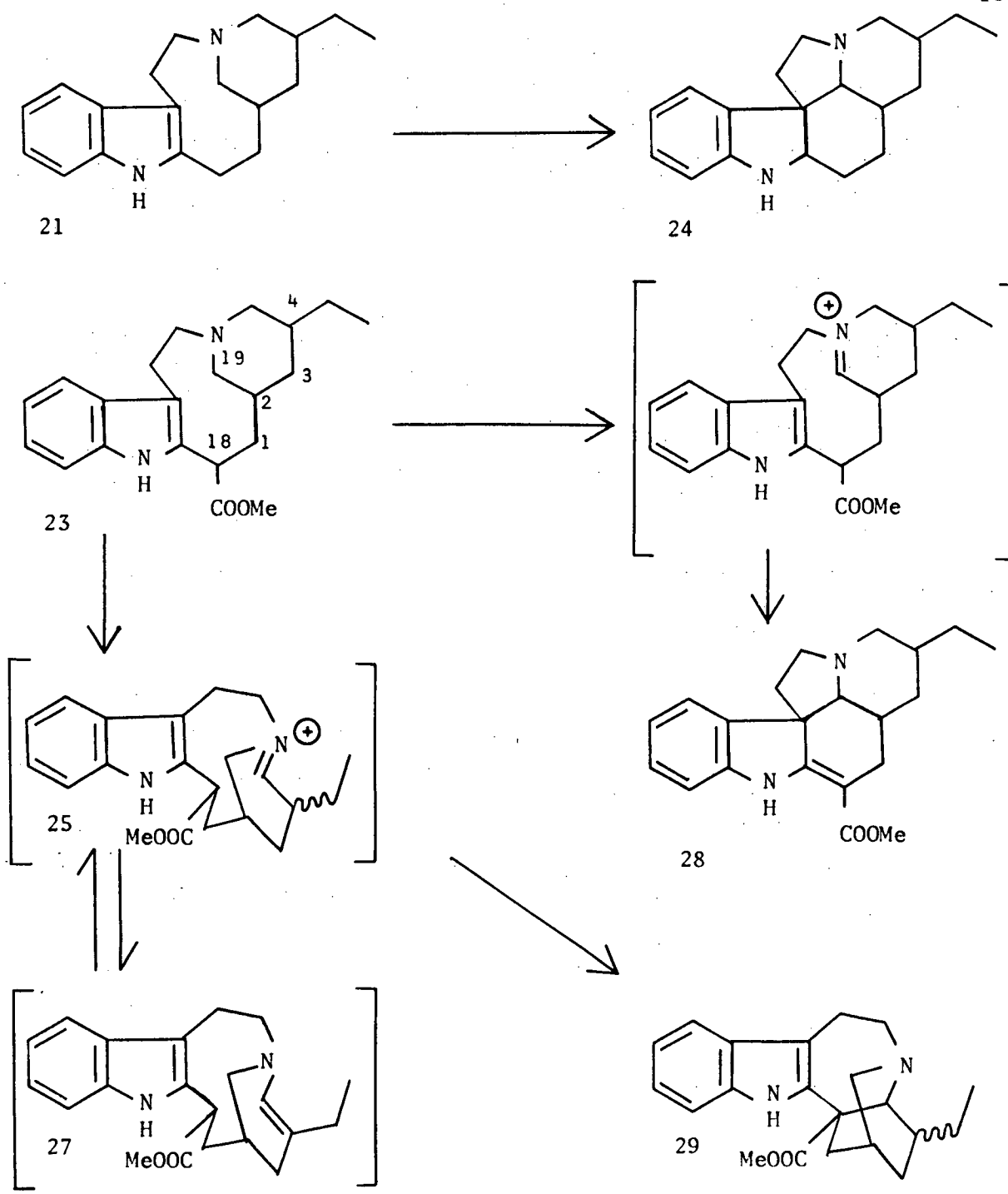
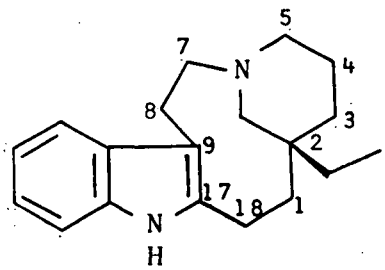
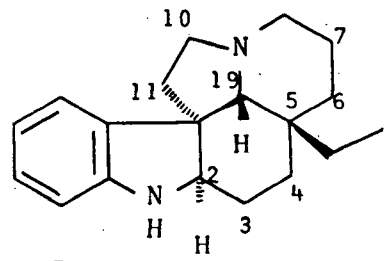



Figure 2. The transannular cyclization and its synthetic application in the Aspidosperma and Iboga series.

that (-)-quebrachamine (30) was transformed into (+)-aspidospermidine [26,27] (31) under suitable conditions. Thus, the cyclization led to the natural diasterioisomer. The situation with regard to the absolute configuration of the pseudo-Aspidosperma and Iboga compounds produced via the transannular cyclization was not unambiguous because the optical activity of the C-2 carbon in the dihydrocleavamine molecule would conceivably have been destroyed in the process. However, Kutney and coworkers showed that the asymmetry of the C-2 carbon atom was retained in the transannular cyclization reaction. 7-Ethyl-5-desethylaspidospermidine obtained from




30

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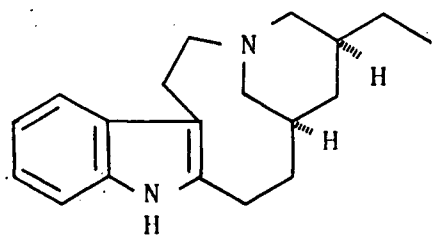
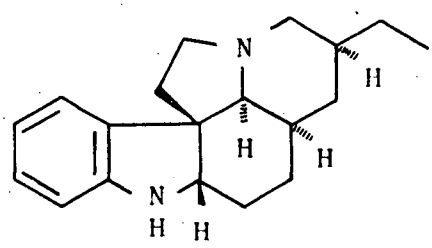



32

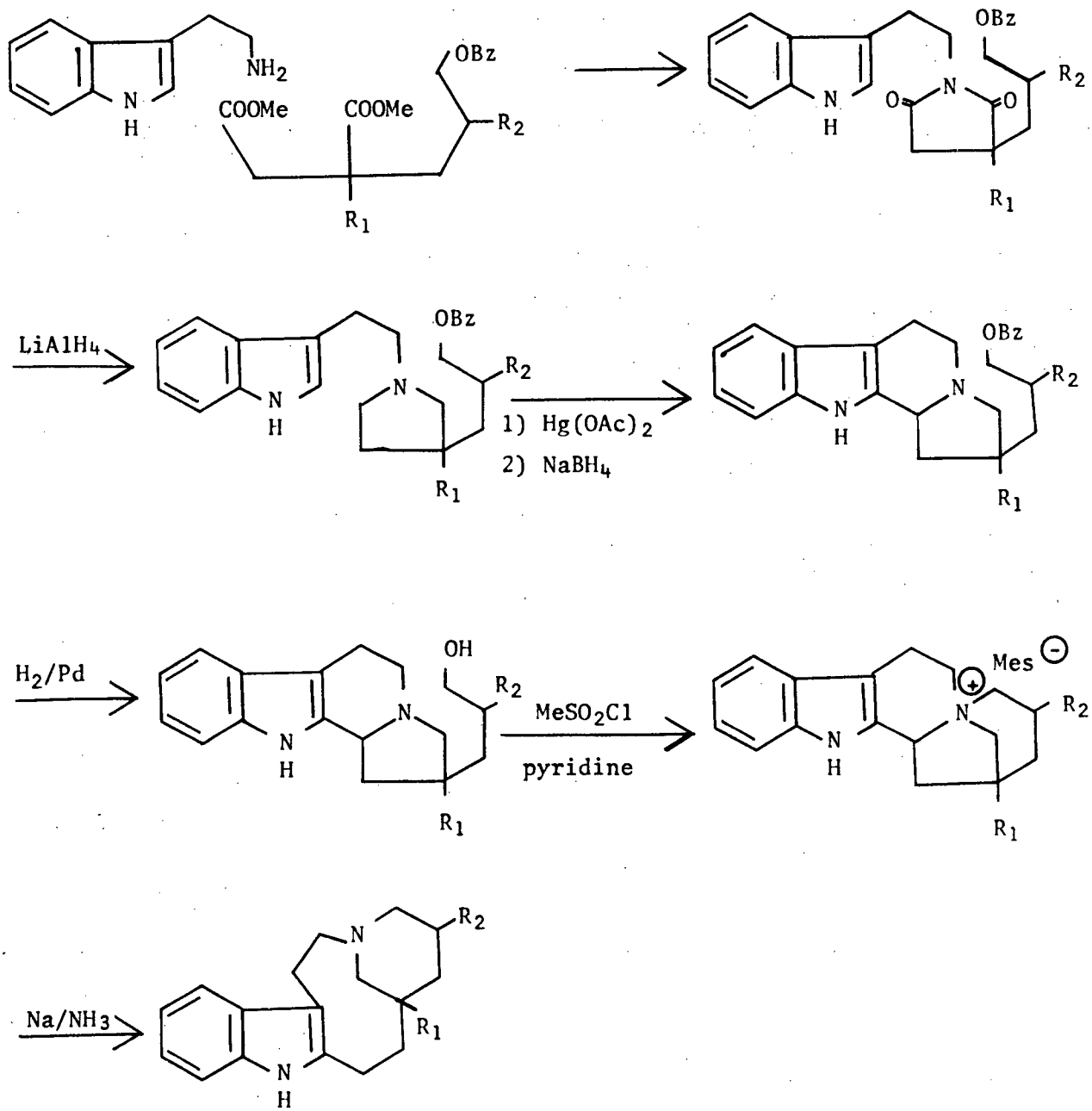



33

4β-dihydrocleavamine (32) was established to have the absolute configuration as shown in 33 by x-ray diffraction of its $N_a$-acetyl-$N_b$-methiodide derivative [15]. The configuration of the C-2 carbon atom [14,28] in 4β-dihydrocleavamine thus remained unaltered during the course of the conversion reaction. The configuration of the C-19, C-12 and C-2 carbon atoms relative to the configuration of the C-5 carbon atom of 7-ethyl-5-desethylaspidospermidine was that expected from conformational consideration.

In addition, the configuration of the C-4 carbon atom of 4β-dihydrocleavamine was unaltered during the course of the reaction. Since the transannular cyclization of 4β-dihydrocleavamine to 7β-ethyl-5-desethylaspidospermidine took place in a stereospecific manner, it followed that the transannular cyclization of carbomethoxydihydrocleavamine to provide 7-ethyl-5-desethylvincadifformine, dihydrocatharanthine and coronaridine was also a stereospecific process.

The transannular cyclization,in addition to its value in the biogenetic postulates of Wenkert, had an important application for synthesis of alkaloids. The transannular cyclization step was incorporated into a general total synthesis of a number of alkaloids bearing the Aspidosperma or Iboga skeletons. Since this step was shown to be completely stereospecific with the configuration of each new asymmetric centre being completely determined by the configuration at C-2 in the nine-membered ring intermediates, the total synthesis based on this step was not beset with stereochemical problems during the various stages of the synthetic sequence. Also, parallel total syntheses of the dihydrocleavamine and quebrachamine systems were feasible. Thus, the total synthesis of alkaloids bearing the Aspidosperma skeleton on the one hand and the Iboga skeleton on the other complemented each other with obvious advantages over synthetic approaches that required a completely different method for each skeletal type. The important features of the synthesis of dl-quebrachamine [29] (34) and dl-dihydrocleavamine [30] (35), as carried out by Kutney and coworkers, are shown in Figure 3. The extension [30] of the synthesis from dl-dihydrocleavamine (35) involved introduction of a carbomethoxy function at C-18 to provide a carbomethoxydihydrocleavamine (36) (Figure 4). This latter step also in turn completed the total




34, $R_1$ = Et; $R_2$ = H

35, $R_1$ = H; $R_2$ = Et

Figure 3. Kutney's synthesis of dl-quebrachamine, dl-4α-dihydrocleavamine, and dl-4β-dihydrocleavamine.

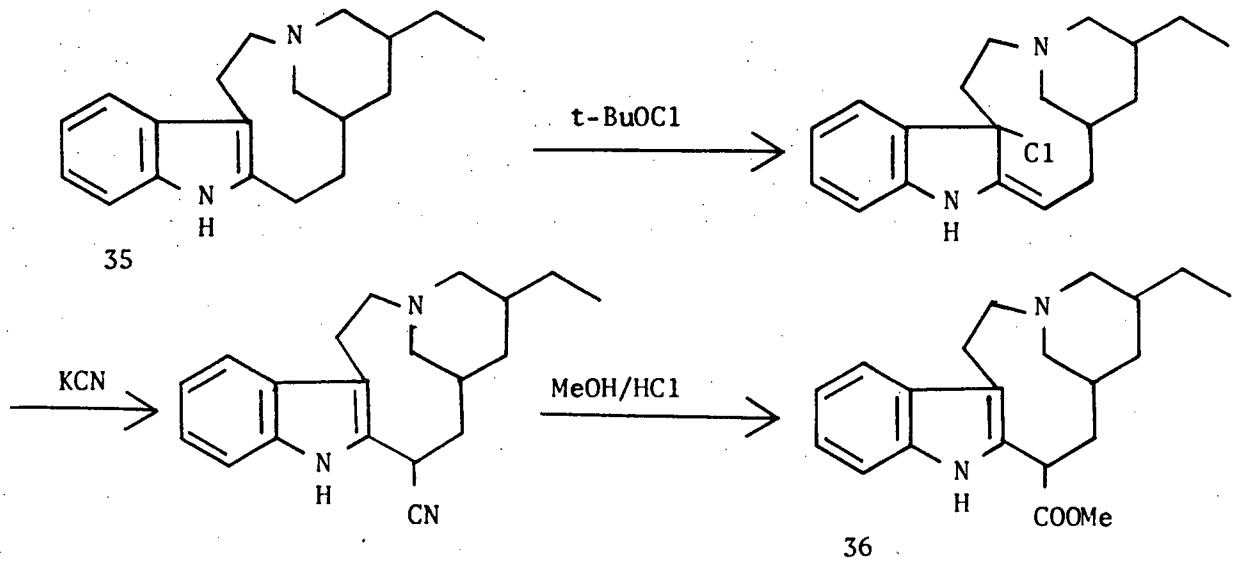
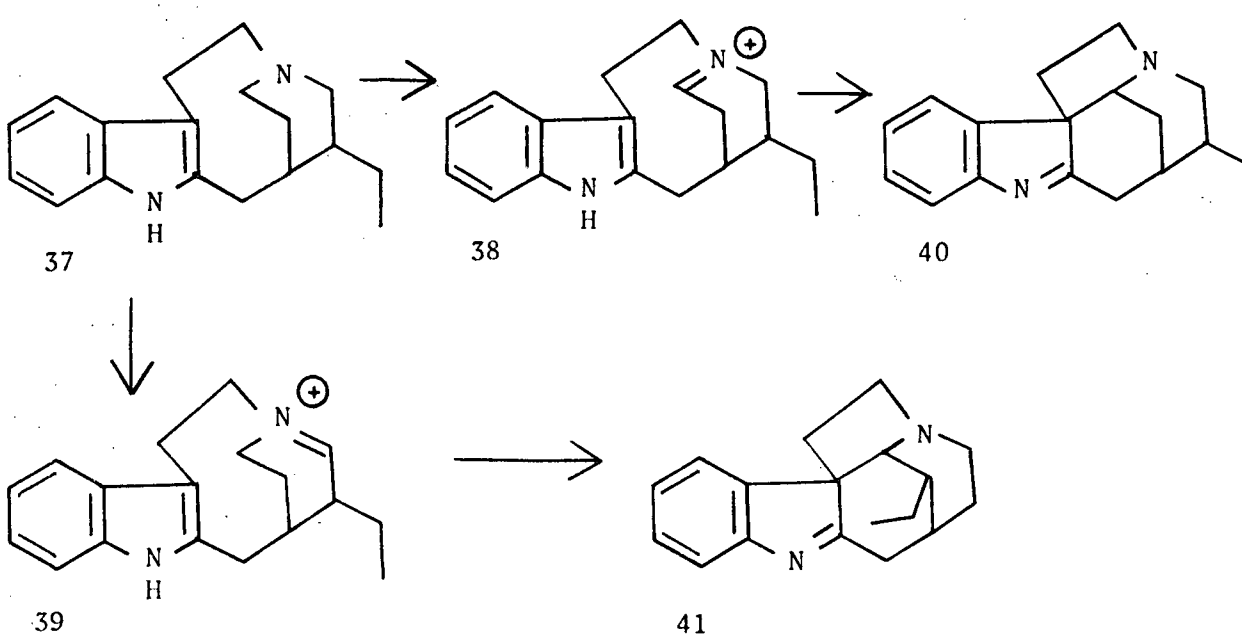



Figure 4. Kutney's synthesis of carbomethoxydihydrocleavamine.

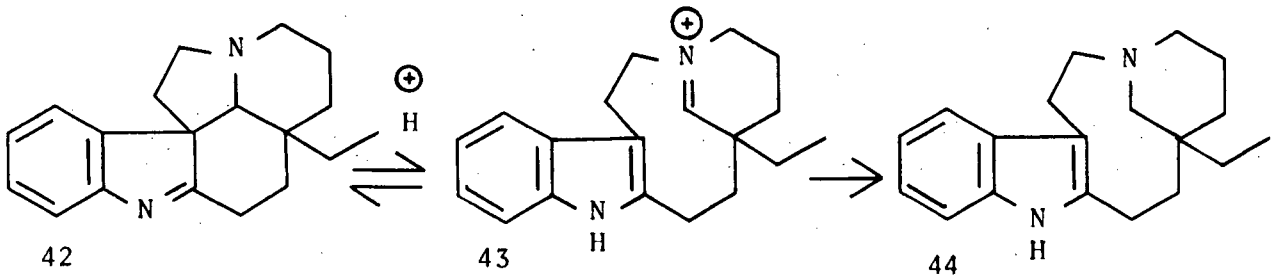


synthesis of dl-coronaridine and dl-dihydrocatharanthine in view of the previous transannular cyclization studies [21]. Introduction of a carbomethoxy function at C-3 in quebrachamine would provide a carbomethoxyquebrachamine or vincadine [31], which is a naturally occurring Vinca alkaloid. A successful transannular cyclization of a carbomethoxyquebrachamine in the manner described previously would provide vincadifformine, another Vinca alkaloid.

Similar transannular cyclizations have also been accomplished with the Akuamma class of alkaloids. Oxidation of the tetracyclic indole, 37, with either oxygen and platinum or potassium permanganate gives the two iminium intermediates, 38 and 39, which undergo transannular cyclization to afford tubifoline (40) and condifoline (41), respectively [32]. It was also found that condifoline was converted into tubifoline under a variety of conditions (e.g. neat at 117-120°, in tetralin at 160-180°, in triethylamine, in ammonia-isopropanol, or in potassium t-butoxide–t-butanol).

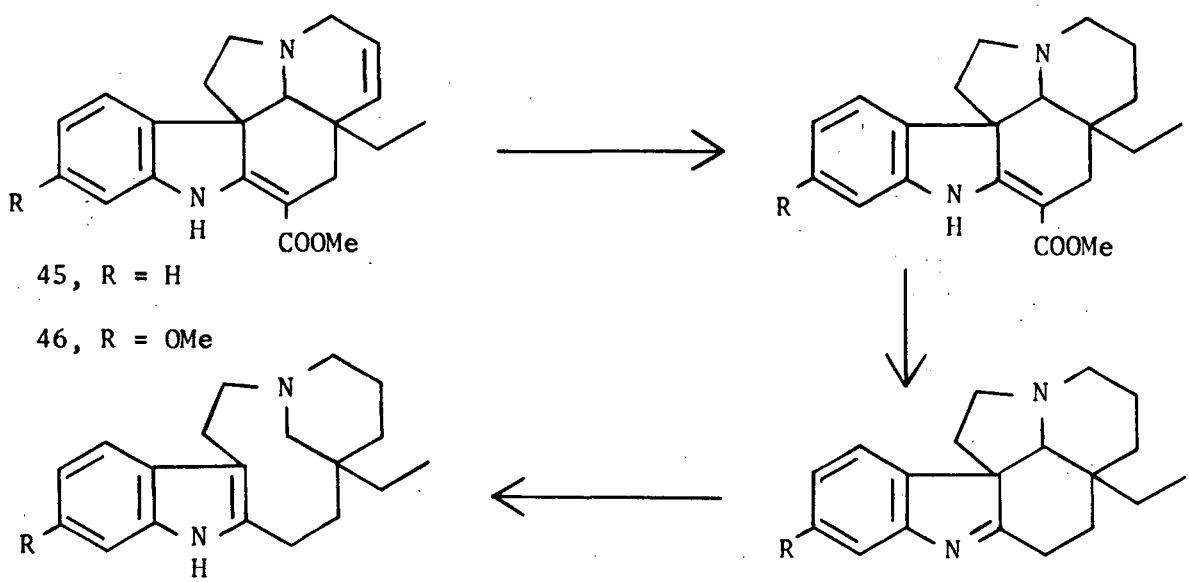
Even though the transannular cyclization is an important step in the synthesis of alkaloids, the reverse of this process has also provided some interesting chemistry in addition to the aspects already discussed with respect to the ring-opening reactions in the catharanthine system. Stork and coworkers [33] used such an opening reaction when they converted dl-1,2-dehydroaspidospermidine (42) to dl-quebrachamine (44) by bringing about the equilibrium, 42 $\rightleftharpoons$ 43, in methanol and selectively reducing the very reactive iminium function in the tetracyclic cation with potassium borohydride.

An analogous opening of the pentacyclic system was an important step in the structure determination of the naturally occurring alkaloids, (-)-tabersonine [34] (45) and 16-methoxy-(-)-tabersonine [35] (46). Figure 5



42 43 44

outlines the reaction sequence for 16-methoxy-(-)-tabersonine. Hydrogenation followed by decarboxylation afforded 16-methoxy-1,2-dehydro-(-)-aspidospermidine (47) which, on treatment with sodium borohydride in 10% ethanolic sodium hydroxide, afforded the tetracyclic 16-methoxy-(+)-quebrachamine (48).



45, R = H

46, R = OMe

48, R = OMe

47, R = OMe

Figure 5. Structure determination of 16-methoxy-(-)-tabersonine.

The reverse of the transannular cyclization is also postulated to occur when akuammicine is heated with methanol. Akuammicine (49) is remarkable in the ease with which it breaks up into aromatic optically

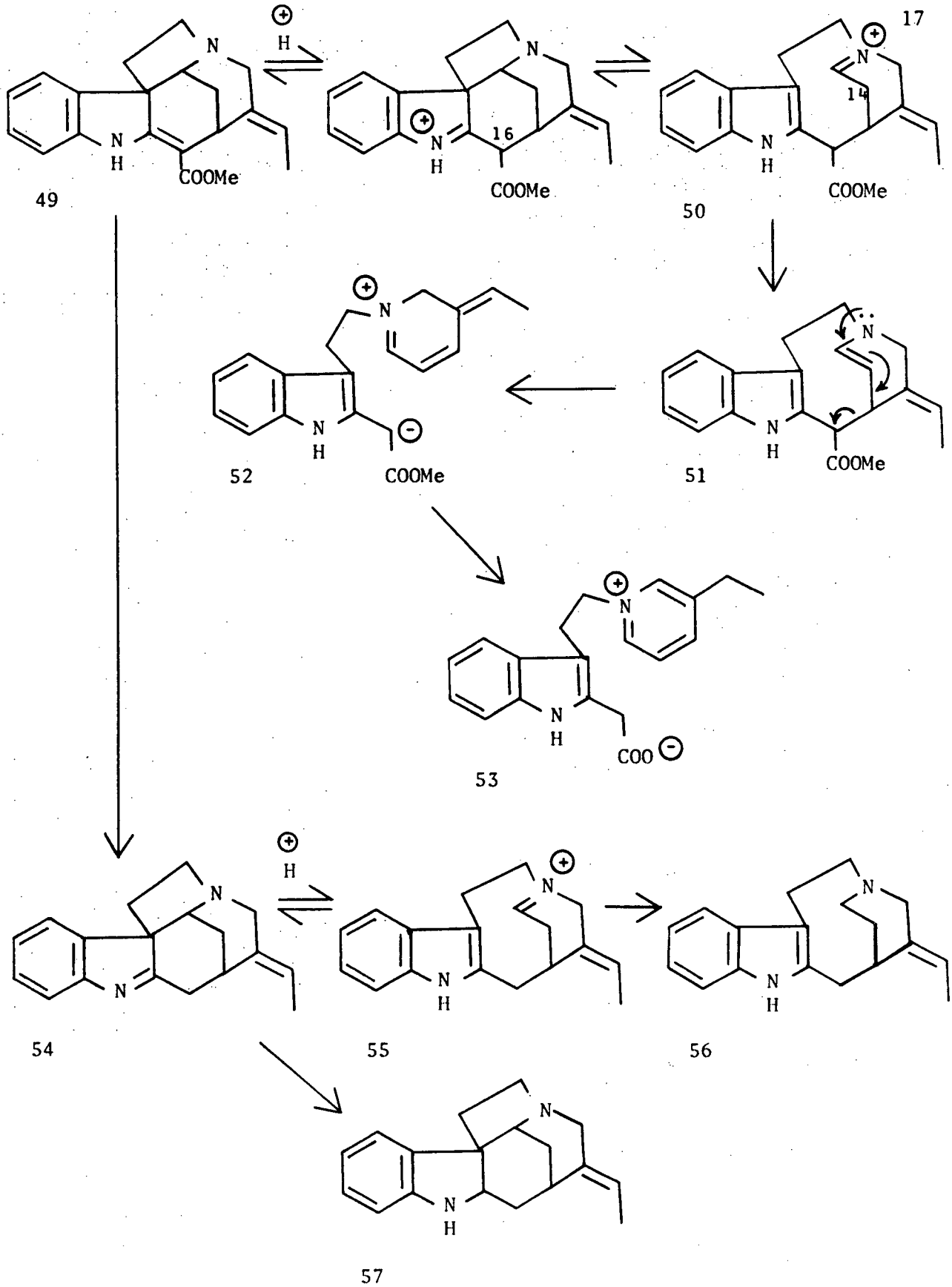



Figure 6. Some properties of akuammicine.

inactive components (Figure 6). Upon heating in a sealed tube in methanol at 80°, the "betaine" (53) is formed. The formation of 53 from akuammicine is explained by the interconversion of a C-16-protonated species to 50, followed by proton removal at C-14 to give an intermediate enamine (51) that can cleave by an essentially irreversible retro-Michael reaction to the ester (52). Subsequent aromatization of the latter leads to 53. This interpretation of the decomposition of akuammicine was consistent with the observations that 2,16-dihydro-akuammicine and akuammicine methiodide do not break down under comparable conditions; the former is simply epimerized at C-16, and the latter remains unaffected [36].

Upon acid hydrolysis, akuammicine, like a β-ketoester, suffered decarboxylation. The resulting indolenine (54) exists in equilibrium with the indole iminium salt (55). In the absence of a proton donor, the indolenine form is favoured---e.g., reduction with lithium aluminum hydride in ether gave the indoline (57), whereas, in methanol (proton donor) reduction with potassium borohydride, it afforded the indole (56) [37].

The transannular cyclization has also been postulated as an important step for the biosynthesis of indole-type alkaloids. In order to gain a better knowledge of this enamine-imine chemistry of indoles, and hopefully to provide intermediates suitable for biosynthesis in this area, the reaction mediated by acid with various indole alkaloids must be examined in greater detail. The purpose of the first section of this thesis is to discuss a detailed investigation of the acid-catalyzed reactions in the catharanthine system, as well as some related chemistry of the Aspido-sperma series.

## Discussion

Cleavamine (18) was initially obtained by the Lilly group in their investigations on Vinca alkaloids [8,12], and its derivation from the alkaloid, catharanthine, provided an example of a novel acid-catalyzed rearrangement which had little or no precedent in indole alkaloid chemistry. A further study by Kutney and coworkers on the reaction of this alkaloid with hydrochloric acid in the presence of stannous chloride allowed the isolation of two dihydrocleavamine derivatives in addition to cleavamine [11]. None of the products possessing the cleavamine skeleton still retained the ester function and were, therefore, of little interest to our immediate requirement.

The reaction of catharanthine with zinc in acetic acid, on the other hand, was shown to yield a carbomethoxydihydrocleavamine [9,12], and it became of immediate importance to our investigations. It was necessary for us to study this reaction in more detail in the hope that other carbomethoxydihydrocleavamine or carbomethoxycleavamine derivatives could be isolated.

In our hands, catharanthine, on reaction with zinc dust in refluxing glacial acetic acid, provided a complex mixture from which four compounds (representing approximately 40% of the crude reaction mixture) could be isolated and characterized. The major compound (isomer C[a]), mp 172°,

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a For the sake of clarity, the compounds are designated A, B, C, D in order of increasing polarity on silica gel chromatoplates.

obtained by column chromatography on alumina, followed by crystallization from methanol, was identical with the previously reported carbomethoxydihydrocleavamine [9,12]. The other three compounds were obtained pure after preparative thin-layer chromatography (tlc) on silica gel.

Isomer A, mp 144-147°, appeared to be another carbomethoxydihydrocleavamine derivative when high resolution mass spectrometry established the molecular formula, $C_{21}H_{28}N_2O_2$. The molecular ion (m/e 340) was accompanied by fragments which were immediately reminiscent of the quebrachamine and cleavamine fragmentation process [12] (Figure 7). Thus, the fragment at m/e 215 may arise from cleavage at "a" and "b" as shown in Figure 7, while loss of the ester group (d) from the latter would generate the species at m/e 156. The accompanying piperidine fragment arising from this cleavage would be seen at m/e 124. Alternate fission at "b", "c" and "d" would give rise to the fragments at m/e 144, 143 and 138. Conclusive evidence for the structure of this compound was obtained when the known carbomethoxydihydrocleavamine (isomer C), on treatment with boron trifluoride, was converted to isomer A. This experiment clearly established that these compounds were merely isomeric at C-18. A discussion on the stereochemistry is deferred to a later section.

Isomer B, mp 146-149°, was the next compound obtained in the tlc purification. High resolution mass spectrometry again established that this substance was also a carbomethoxydihydrocleavamine derivative, and analysis of the fragmentation pattern confirmed this assignment. Hydrolysis and decarboxylation of B (dilute hydrochloric acid) provided 4β-dihydrocleavamine.

With regard to the latter experiment, reaction of isomer C with dilute acid gave rise to a dihydrocleavamine identical to that previously

obtained by the Lilly workers [12], but not identical to 4β-dihydrocleavamine as mentioned above.

Isomer D, mp 226-229°, was the fourth compound isolated from the zinc-acetic acid reaction. Evidence for a carbomethoxydihydrocleavamine formulation was obtained as above. Reaction of D with boron trifluoride provided B, and consequently, the relationship between these isomers was apparent.

The above results established the gross structures of the four compounds obtained, but clearly, insufficient evidence has been presented to differentiate between them. The data which allows complete structural and stereochemical assignments to isomers A, B, C and D will now be discussed.

X-ray evidence on cleavamine methiodide [14,28] established the absolute configuration at C-2 in this molecule. On this basis, the stereochemistry at C-2 in the dihydrocleavamine, obtained by catalytic reduction of cleavamine [12], is also defined. Furthermore, as shown by the x-ray method [15], the cyclization product derived from this compound is 7β-ethyl-5-desethylasidospermidine (Aspidosperma numbering). It is, therefore, established that this dihydrocleavamine isomer can now be termed as 4β-dihydrocleavamine (58)[b,c]. An extension of this argument allows the assignment, 4α-dihydrocleavamine (59), to the isomer obtained by removal of the ester function in isomer C. In consideration of the experiments mentioned earlier, it is clear that A and C now belong to the 4α-dihydrocleavamine series and differ only in stereochemistry at C-18. Similarly, isomers B and D are in the β series and merely differ at C-18.

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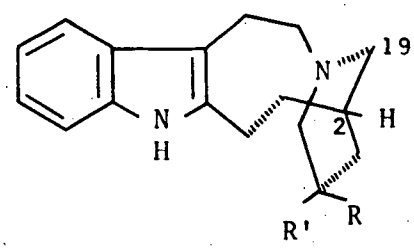
b It must be noted that C-7 in the conventional Aspidosperma numbering system is C-4 in the Iboga system.

c For the sake of convenience, the "β" orientation is designated to the C-4 ethyl group which is trans to the hydrogen atom at C-2 as indicated in the structures 58 and 59.

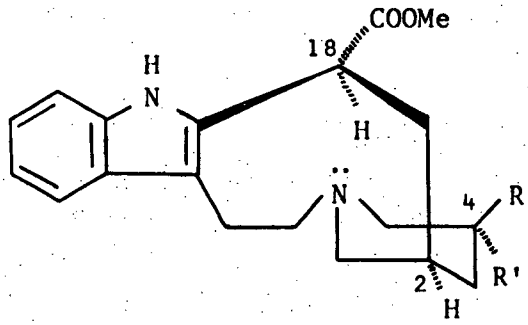



58, R' = Et; R = H

59, R' = H; R = Et

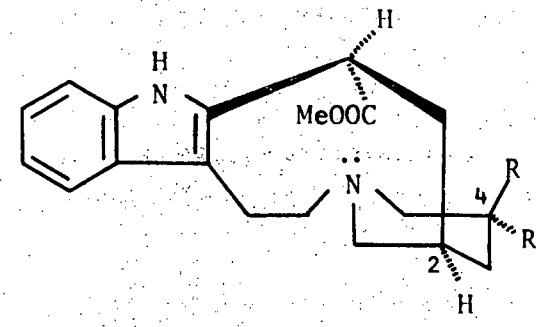
The remaining question of stereochemistry at C-18 in the two series was settled by nmr spectroscopy. Figures 8, 9, 10, and 11 illustrate the nmr spectra for isomers A-D, respectively. Isomers A and B possess multiplets in the region τ 4.5-5.0 for C-18-H, whereas the corresponding proton absorbs at higher field in compounds C and D (τ 6.0-6.2). This rather dramatic difference in the resonance frequency is readily explicable in terms of the appropriate conformational structures which are possible in these two series (60-63). In 60 and 61, the proton at C-18 is in close proximity to the basic nitrogen atom of the piperidine moiety, and it would be expected to absorb at a lower frequency. Such a situation does not prevail in 62 and 63, and a more normal resonance frequency for C-18-H would be anticipated. On this basis, and in conjunction with the previous arguments presented above, isomer A is now completely defined as 18β-carbomethoxy-4α-dihydrocleavamine (60), while B is the 18β isomer in the 4β series (61). Isomer C, the major component obtained previously in another laboratory [9], is the 18α-carbomethoxy compound in the 4α series (62), while D can now be assigned structure 63.

It is appropriate to mention here that similar nmr arguments have been employed by Mokry and Kompis in establishing the stereochemistry of the structurally related Vinca alkaloids, vincaminoreine (71) and the epimeric vincaminorine [38].




60, R = H; R' = Et

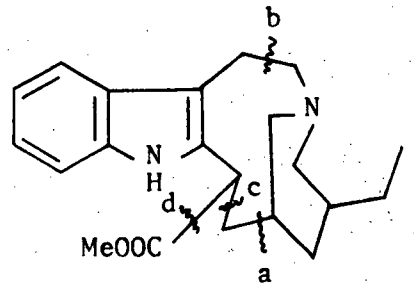
61, R = Et; R' = H




62, R = H; R' = Et

63, R = Et; R' = H

Our further interest in finding a route to the unknown carbomethoxy-cleavamine (64) series led to a consideration of the hydride reduction of the intermediates derived from the acid-catalyzed ring opening of catharanthine. It had been previously postulated [11,12] that dihydropyridine salts were formed in the initial stages of this reaction, whereas a pyridinium derivative may well explain the formation of cleavamine or its dihydro analogues, particularly in the absence of any reducing agent. Indeed, either one of these intermediates would be expected to reduce with hydride reagents. When catharanthine hydrochloride dissolved in hot glacial acetic acid was treated with sodium borohydride, a surprisingly good yield (63%) of a crystalline compound, mp 121-123°, was obtained. Elemental analysis of this compound was in accord with the molecular formula, $C_{21}H_{26}N_2O_2$, but in particular, the nmr spectrum (Figure 12) was immediately indicative of a cleavamine system. A multiplet centered at $\tau$ 4.76, along with a clear triplet at $\tau$ 8.96, the latter occurring at lower field than in the dihydro-cleavamine derivatives, provided strong evidence for the double bond at the 3,4 position. The C-18 proton resonating at $\tau$ 4.89 indicated that the ester group in this molecule was in the β-orientation in accord with the



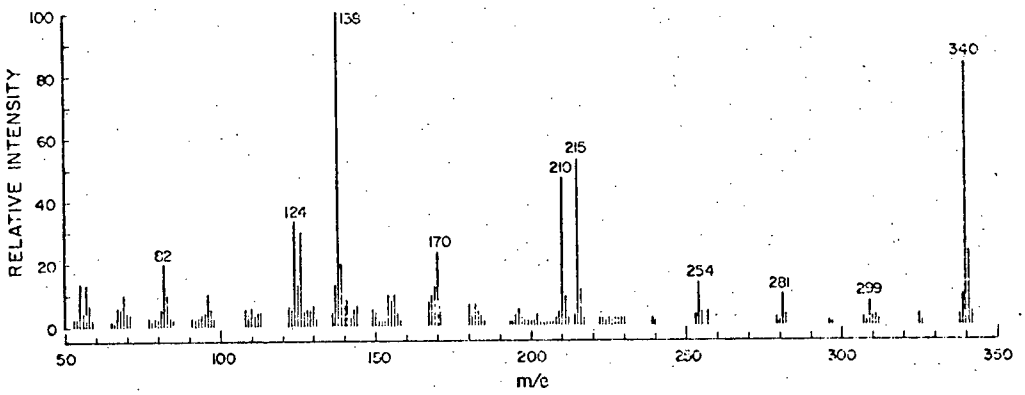




Figure 7. Mass spectrum of carbomethoxydihydrocleavamine (isomer A) (60).

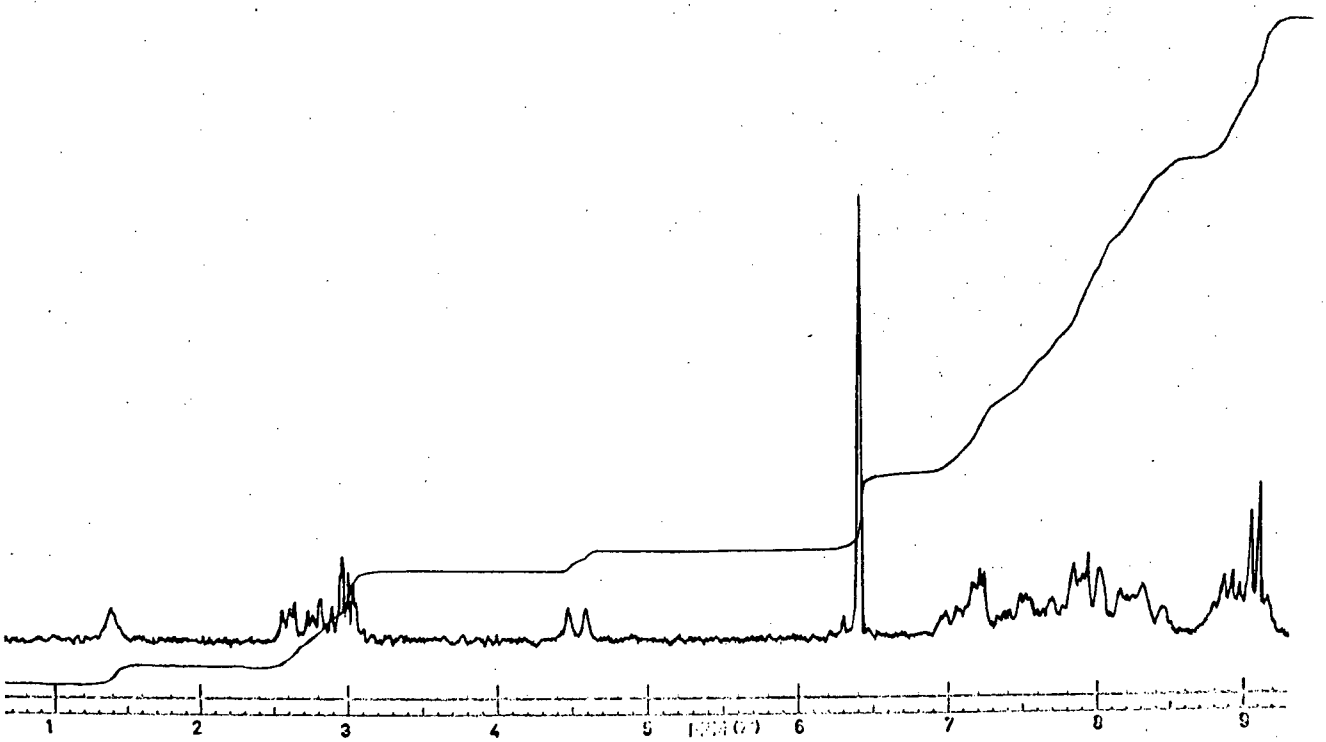



Figure 8. Nmr spectrum of carbomethoxydihydrocleavamine (isomer A) (60).

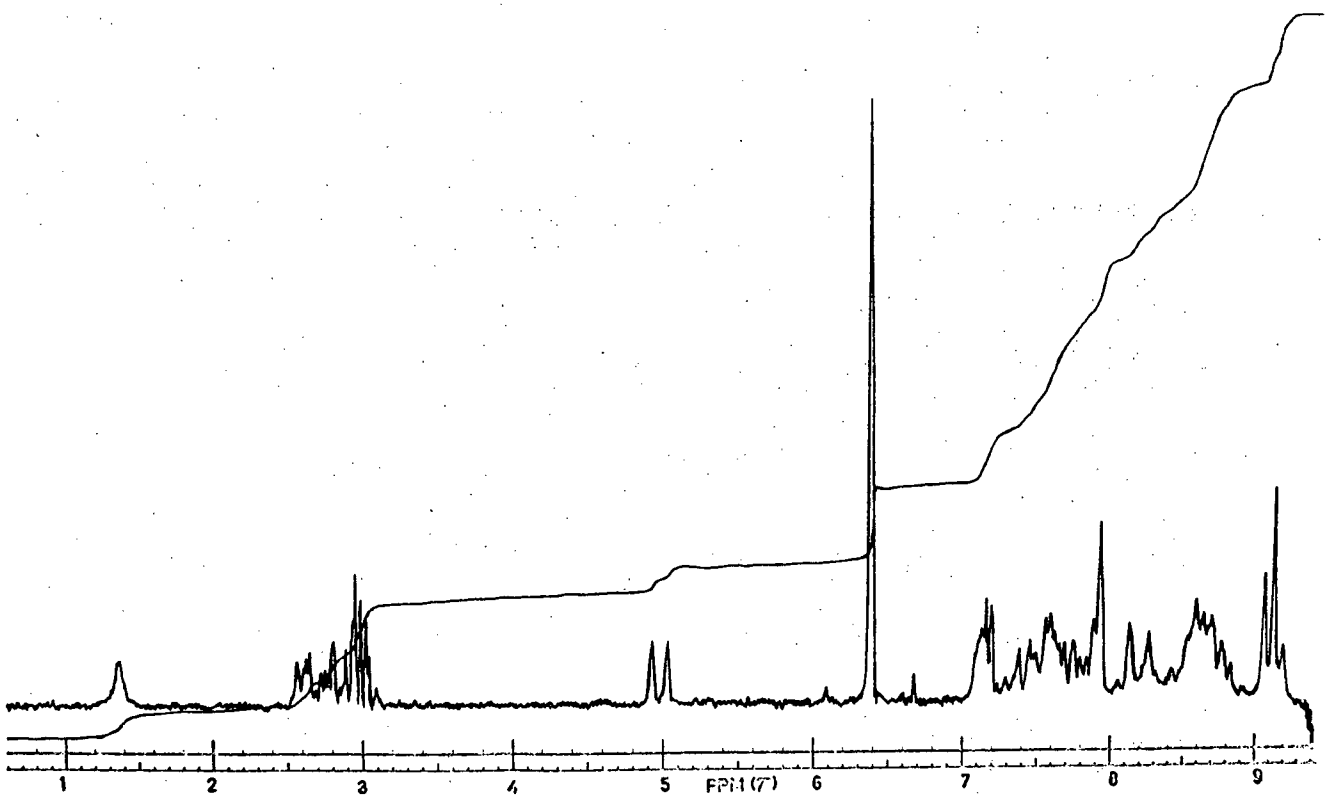



Figure 9. Nmr spectrum of carbomethoxydihydrocleavamine (isomer B) (61).

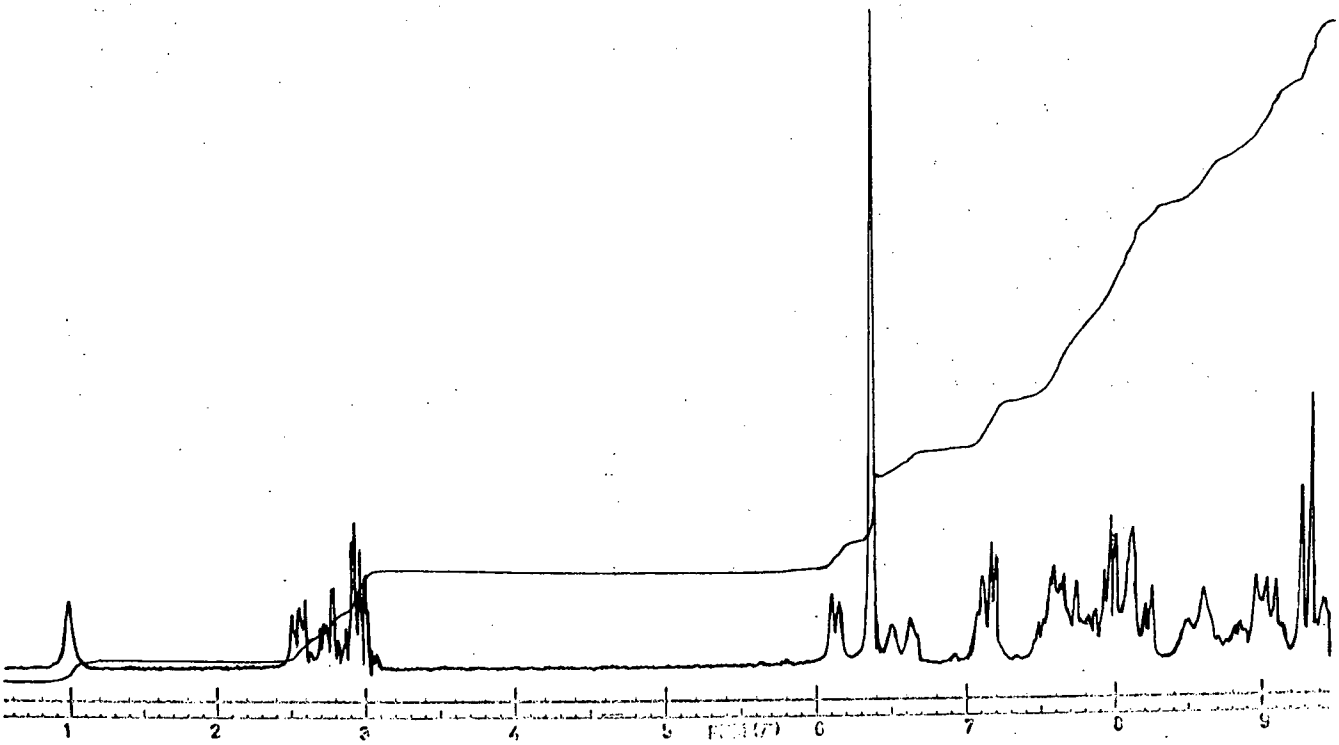



Figure 10. Nmr spectrum of carbomethoxydihydrocleavamine (isomer C) (62).

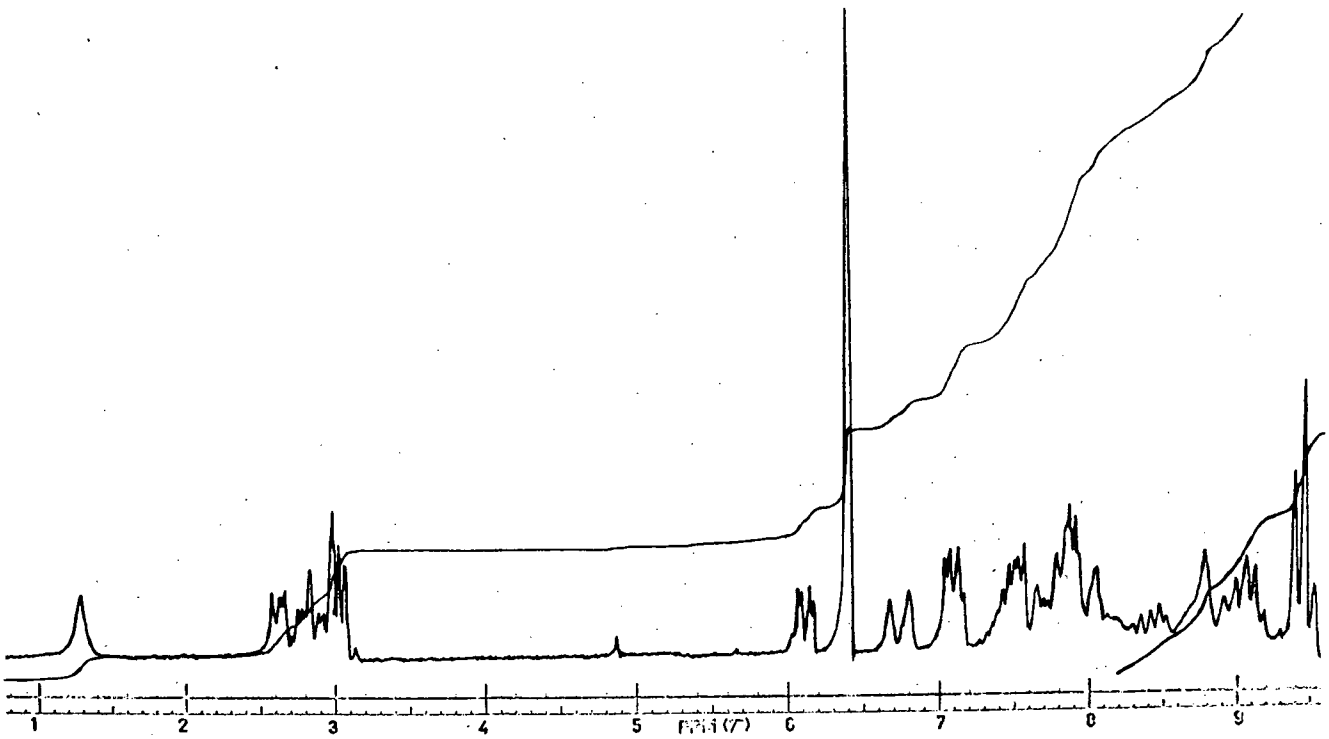


Figure 11. Nmr spectrum of carbomethoxydihydrocleavamine (isomer D) (63).

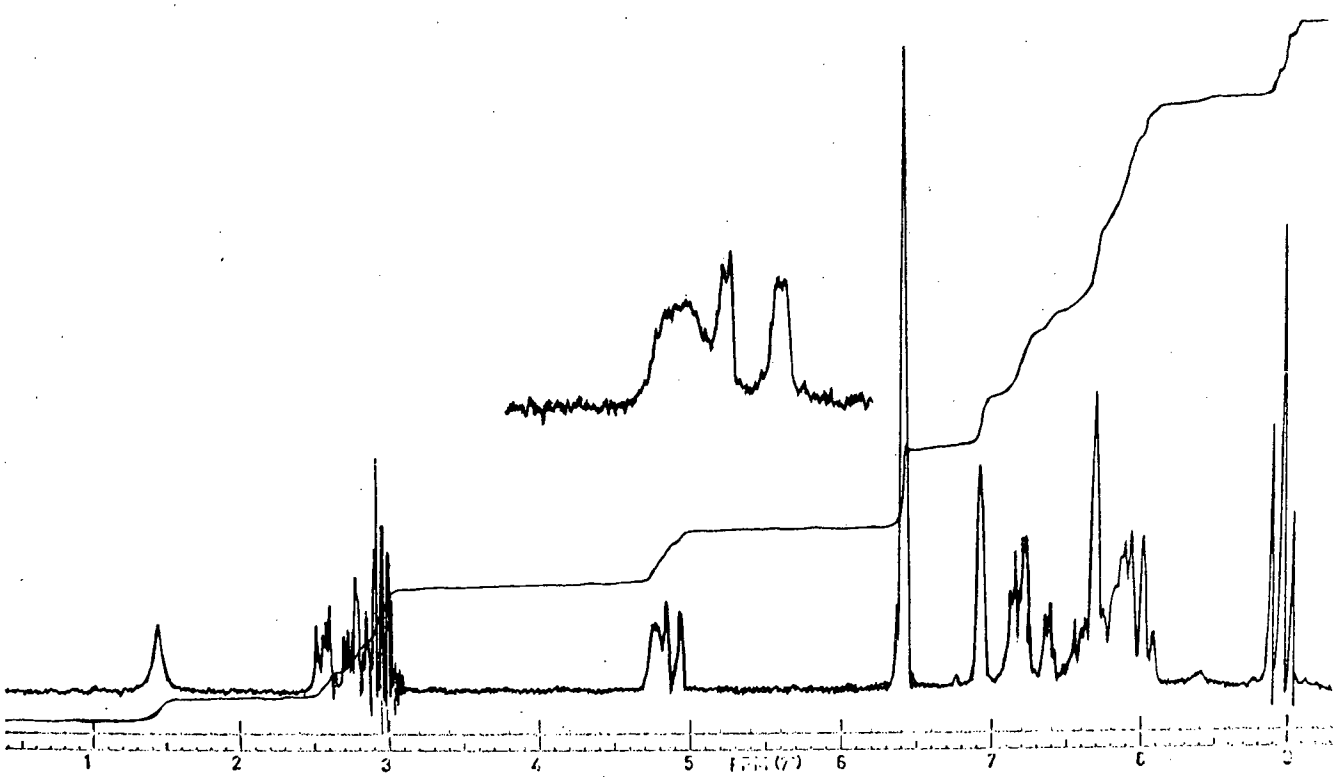


Figure 12. Nmr spectrum of carbomethoxycleavamine (64).

previous assignment indicated above. Finally, acid hydrolysis and decarboxylation of this crystalline compound yielded cleavamine. The structure of this product was,thus, established to be 18β-carbomethoxycleavamine (64) [39].

The formation of the four carbomethoxydihydrocleavamines and carbomethoxycleavamine which have been isolated from the reaction of catharanthine with glacial acetic acid and a reducing agent can be rationalized on the basis of the mechanism shown in Figure 13. The lone pair of electrons on $N_b$ in catharanthine can participate in a rearrangement to form an iminium ion, with concurrent ring cleavage and protonation at the β-position of the indole. The resulting ring-opened intermediate 65 is stabilized by two factors: (a) the conjugated nature of the iminium ion, and (b) the conjugation of the newly generated double bond between C-17 and C-18 with both the ester and the anilino functions. The crucial intermediate, 65, may follow either of two reaction paths. If the original electron flow is reversed, then the C-5/C-18 bond is regenerated and catharanthine is regenerated. But if the α-methylene indoline system in 65 merely rearranges to the indole, the intermediate, 66, from which the ring-opened tetracyclic compounds must ultimately be formed is obtained. If one assumes that 66 is the actual intermediate, 1,2-reduction of the iminium ion occurs readily in the presence of sodium borohydride and carbomethoxycleavamine is obtained directly. On the other hand, 1,4-reduction can also occur to afford an enamine, 67, which rearranges in the well-known manner to the iminium compound, 68, with subsequent reduction to provide the four isomeric carbomethoxydihydrocleavamines (60-63).

The cleavamine-type nine-membered ring compounds could now be evaluated in plants as possible precursors in the biosynthesis of Iboga alkaloids.

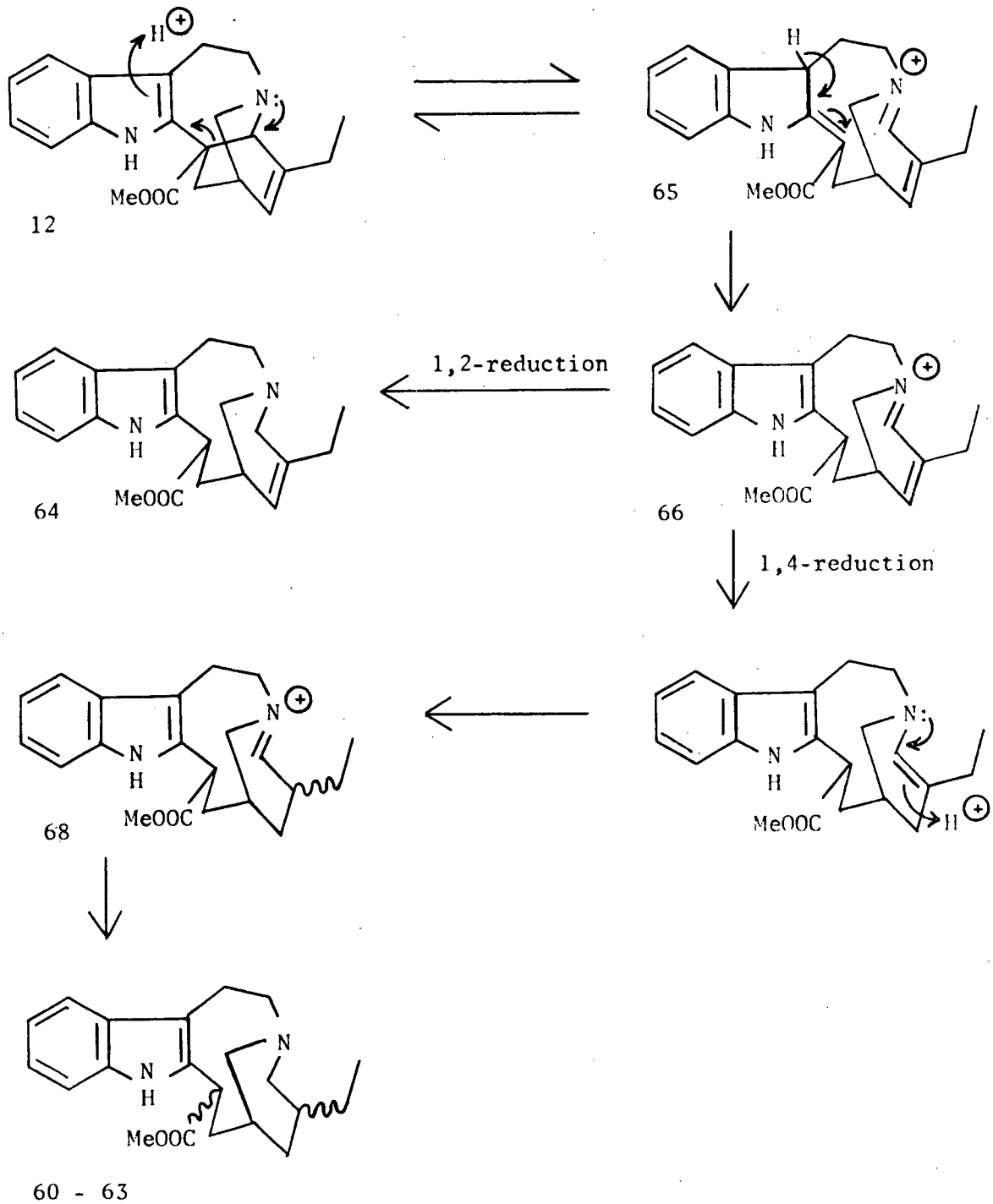
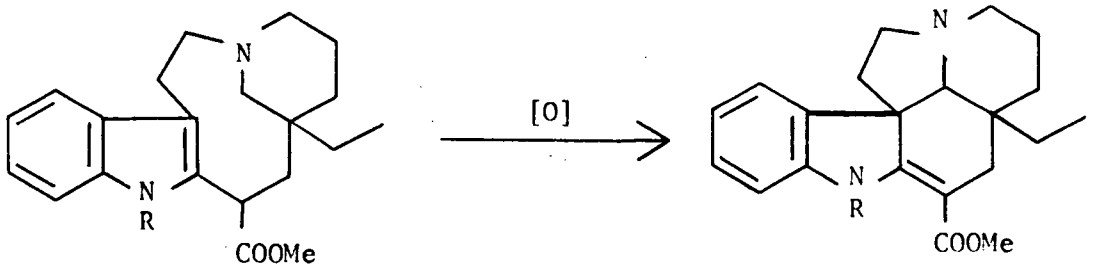



Figure 13. Formation of carbomethoxydihydrocleavamines and carbomethoxycleavamine.

Chemically, in the laboratory, these nine-membered ring compounds are readily converted to the Iboga-type systems. Carbomethoxydihydrocleavamine (isomer C) has been shown to be converted [21] into coronaridine and its C-4 epimer, dihydrocatharanthine (Figure 2). Also, recently, carbomethoxy-cleavamine has been converted [40] to catharanthine using mercuric acetate in acetic acid.

In order to continue the study of the transannular cyclization reaction, the study of natural nine-membered ring compounds such as quebrachamine (30) was undertaken. Note, the distinction between the quebrachamine and the cleavamine systems is that quebrachamine has the piperidine ethyl group attached at position 2, whereas cleavamine has the ethyl group attached to the 4 position of the alkaloid (see quebrachamine (30)).

Quebrachamine itself is readily converted into 1,2-dehydroaspidosper-midine having the Aspidosperma skeleton using either mercuric acetate or oxygen in the presence of a catalyst [24,25]. Recently, vincadine (69) was also converted [41] to the Aspidosperma alkaloid, vincadifformine (70), using oxygen in the presence of a catalyst. To complete this series and to confirm that the transannular cyclization is a truly general reaction of nine-membered ring indole alkaloids, a N-methyl derivative must be converted to the corresponding Aspidosperma alkaloid. For this purpose, the N-methyl vincadine derivative or vincaminoreine (71) was chosen. A mixture of vincaminoreine and 5% platinum on powdered charcoal in ethanol was shaken under an atmosphere of oxygen. After one hour, the mixture was filtered and the filtrate concentrated to dryness. The crude product was purified on a preparative tlc plate using alumina as the adsorbent to afford a pure sample of minovine (72) [41]. This substance was identical in every respect (infrared, tlc) with an authentic sample of minovine [42,43].




69, R = H

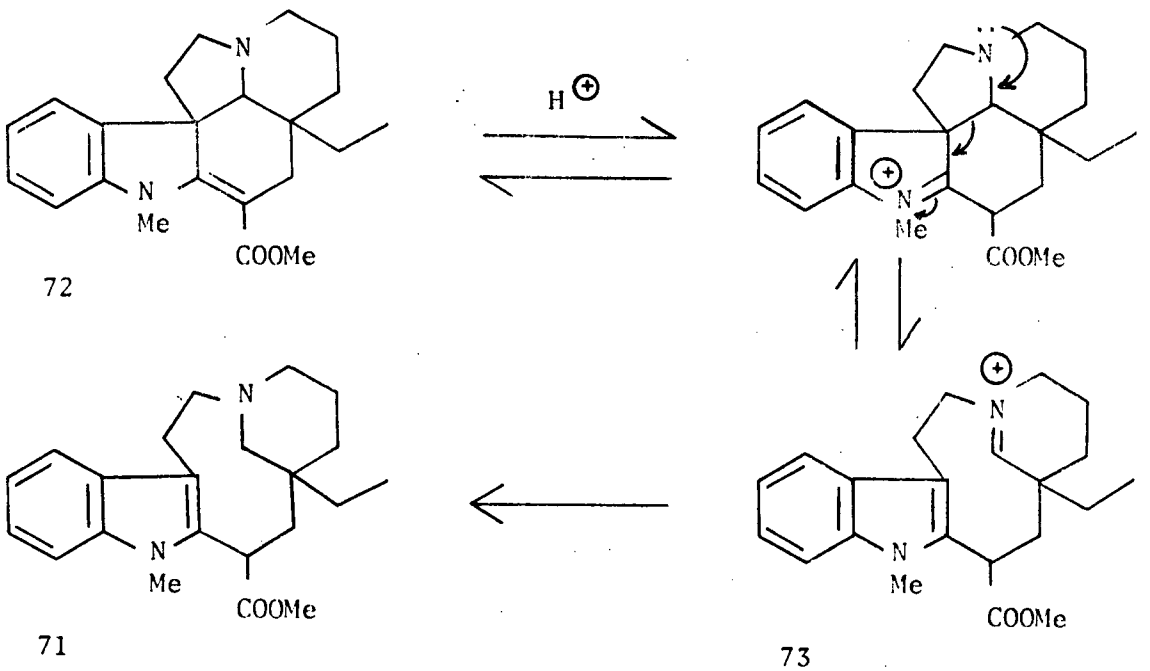
71, R = Me

70, R = H

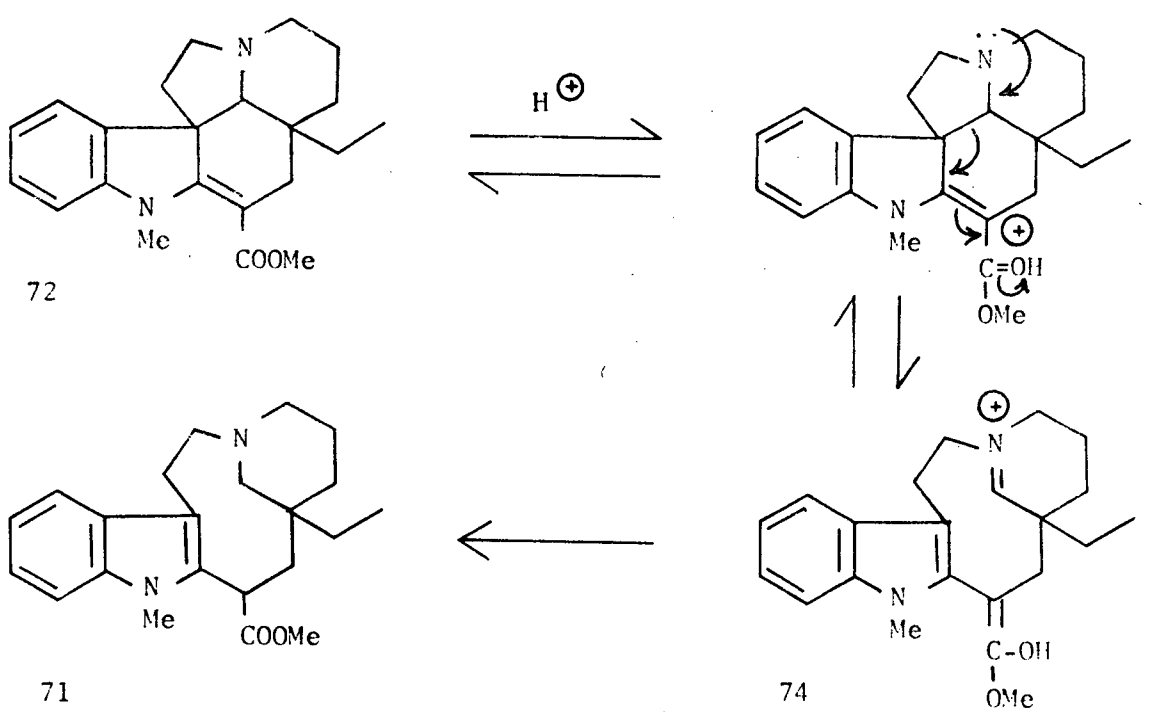
72, R = Me

The reverse of the transannular cyclization reaction, which led to minovine (72) and vincadifformine (70), was the next consideration. In effect, the reverse of this cyclization has been already discussed in the Iboga series, since the conversion of catharanthine to the various cleavamine derivatives invokes a similar process. With regard to the Aspidosperma alkaloids, some precedent in the literature was also available. Stork and coworkers [33] were able to convert dl-1,2-dehydroaspidospermidine to dl-quebrachamine by creating the equilibrium 42 $\rightleftharpoons$ 43 and selectively reducing the very reative iminium function in the tetracyclic cation with potassium borohydride.

It was felt that if the equilibrium 72 $\rightleftharpoons$ 73 could be brought about, then the reactive iminium function in 73 could be readily reduced with hydride reagent. The expected product, vincaminoreine (71), would be stable under these conditions and, thereby, the equilibrium would shift in the desired direction. An alternative pathway which does not invoke the above equilibrium is equally plausible. If protonation of the ester occurs, the equilibrium 72 $\rightleftharpoons$ 74 will be brought about and vincaminoreine (71) would be formed. When minovine (72) dissolved in hot glacial acetic acid was treated with an excess of sodium borohydride, two compounds exhibiting



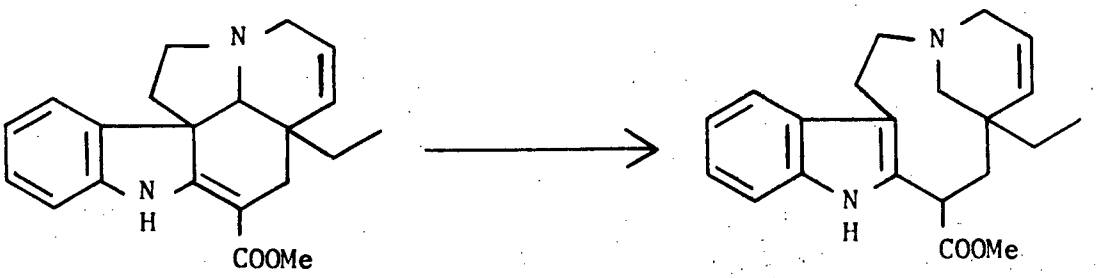

72 71 73




72 71 74

typical indole ultraviolet spectra were isolated. Purification on preparative silica gel tlc plates allowed the isolation of two compounds. The major component (60% yield), mp 138-139°, possessed the formula, $C_{22}H_{28}N_2O_2$, as determined by high resolution mass spectrometry. This compound was found to be identical with authentic vincaminoreine [38,44] (71) by appropriate comparison (tlc, melting point, nmr). The other component (10% yield), mp 129-131°, had also the molecular formula, $C_{22}H_{28}N_2O_2$. Thus, this compound is epimeric at the carbomethoxy-bearing carbon atom and must be vincaminorine [38,45]. The spectral data and melting point of this compound corresponded identically with that in the literature. Unfortunately, an authentic sample could not be obtained for purposes of comparison.

Thus, these investigations established the chemical inter-relationships between the nine-membered ring systems of the quebrachamine and cleavamine families with their cyclic Aspidosperma and Iboga relatives. The usefulness of these results in subsequent biosynthetic studies will be discussed later. For example, in connection with a biosynthetic study in Vinca rosea Linn plants, a comparison of a nine-membered ring compound with the corresponding cyclic system was necessary. In order to trace the biogenetic pathway in these plants, the Aspidosperma alkaloid, tabersonine (45), had to be compared with the unknown nine-membered ring compound, 6,7-dehydrovincadine (75). A small amount of tabersonine hydrochloride, when dissolved in hot glacial acetic acid and treated with sodium borohydride, yielded a pure compound after purification on thin-layer chromatoplates. No attempt was made to obtain this compound in crystalline form because of the small amounts involved. The high resolution mass spectrum was in accord with the molecular formula, $C_{21}H_{26}N_2O_2$. The ultraviolet spectrum was that of a typical




45 75

indole ($\lambda_{max}$ 228, 286, and 293 mμ), while the infrared exhibited a normal ester absorption at 1715 $cm^{-1}$. The nmr spectrum, although weak, was significant in that a one-proton signal was observed at τ 4.11 and a two-proton signal at τ 4.72. Thus, the two olefinic protons and the proton adjacent to the carbomethoxy function are accounted for, although the position of each proton could not be assigned with certainty. Since the C-18 proton resonates in the region τ 4.11-4.72, the ester group in this molecule must be in the β-orientation in accord with the previous assignments described earlier. On this basis, the reaction product is indeed the desired 6,7-dehydrovincadine (75).

The above investigations provide ample evidence for the versatility of enamine-imine intermediates in the area of indole alkaloids. Whether such intermediates are involved in alkaloid biosynthesis is clearly an interesting question. Indeed, the transannular cyclization reaction has been postulated as being an important step in the biosynthesis of several families of indole alkaloids. However, no experiments had been done to confirm this postulate. Part II of this thesis deals with some experiments designed to evaluate this reaction in terms of its significance in the plant.

## Experimental

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet (uv) spectra were recorded in methanol on a Cary 11 recording spectrometer, and the infrared (ir) spectra were taken on Perkin-Elmer Model 21 and Model 137 spectrometers. Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform at 100 megacycles per second (unless otherwise indicated) on a Varian HA-100 instrument and the chemical shifts are given in the Tiers $\tau$ scale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution molecular weight determinations were determined on an AE-MS-9 mass spectrometer. Analyses were carried out by Mr. P. Borda of the microanalytical laboratory, the University of British Columbia. Woelm neutral alumina and Silica Gel G (acc. to Stahl) containing 2% by weight of General Electric Retma p-1, Type 188-2-7 electronic phosphor were used for analytical and preparative thin-layer chromatography (tlc). Chromatoplates were developed using the spray reagents, carbon tetrachloride-antimony pentachloride 2:1 or 35% sulfuric acid saturated with ceric sulfate. Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise indicated).

### Cleavamine (18)

A mixture of catharanthine hydrochloride (12, 10 g), stannous chloride (11 g), and mossy tin (1 g) in concentrated hydrochloric acid (130 ml) was heated under reflux in a nitrogen atmosphere for 75 min. By the end of this time, an orange-red gum had formed in the reaction mixture. The

acidic solution was decanted from the gum and washed with methylene chloride (3 x 25 ml). The washings were combined with the gum, and then methanol (15 ml) and methylene chloride (25 ml) were added so that a clear solution was obtained. This solution was shaken with 1N aqueous sodium hydroxide (150 ml), separated, and washed with water (50 ml). The sodium hydroxide solution was washed with ether (2 x 25 ml) and the ethereal extract added to the methylene chloride solution. After drying over sodium sulfate, the organic solution was evaporated to leave a reddish oil (7 g), which was taken up in benzene and chromatographed on alumina (300 g). Cleavamine was eluted in the initial benzene-petroleum ether 30/60 1:1 fractions and recrystallized from methanol to give needles (1.7 g), mp 117-119° (Lit [11,12] mp 117-119°).

4β-Dihydrocleavamine (58)

A solution of cleavamine (18, 0.75 g) in ethyl acetate (10 ml) was hydrogenated over Adam's catalyst (0.007 g). Uptake of hydrogen ceased after 68 min, at which time one mole of hydrogen had been absorbed. Filtration and evaporation of the filtrate gave 4β-dihydrocleavamine (58), which was recrystallized from methanol as prisms (0.59 g); mp 136-138° (Lit [11] mp 136-138°).

Zinc-Acetic Acid Reduction of Catharanthine (12)

A mixture of catharanthine (4.9 g) and zinc dust (34 g) in glacial acetic acid (125 ml) was heated under reflux, with vigorous stirring, in a nitrogen atmosphere for 4 hr. The hot mixture was filtered and the filtrate was evaporated under reduced pressure until most of the acetic acid was removed. The residue was made basic by addition of dilute aqueous ammonia, and the resulting mixture was extracted thoroughly with ether. The combined extracts were washed with saturated brine and dried over

anhydrous sodium sulfate. Removal of the ether afforded a gummy residue which was subjected to chromatography on alumina (150 g). Elution with petroleum ether (bp 30-60°) (1200 ml) afforded 1.13 g of crystalline 18α-carbomethoxy-4α-dihydrocleavamine (62) (isomer C). Recrystallization from methanol gave colorless blocks: mp 169-171°; $[\alpha]_D^{23}$ +100° ($CHCl_3$); $\lambda_{max}$ (log ε): 227 (4.50), 286 (3.89), 293 (3.86) mμ; $\nu_{max}^{KBr}$: 3375 (-N-H), 2755 (Bohlmann band), 1709 ($COOCH_3$) $cm^{-1}$; nmr: τ 1.00 (singlet, 1H, N-H), 2.76 (diffuse, 4H, aromatic), 6.13 (doublet, 1H, C-18 proton), 6.37 (singlet, 3H, $COOCH_3$), 9.33 (triplet, 3H, $-CH_2\underline{CH_3}$). This compound was found to be identical (mp and mixture mp, infrared, nmr and tlc $R_f$ value) with the carbomethoxydihydrocleavamine reported previously [9,12,46].

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: M.W. 340.215. Found: 340.214 (mass spectrometry).

Further elution in the above chromatography with petroleum ether (bp 30-60°) provided a mixture (1.14 g) of the three remaining isomeric carbomethoxydihydrocleavamines which were separated by preparative tlc. Silica gel plates (20 x 60 cm, 0.5 mm thickness) were used, with 150 mg of the mixture being applied to each plate. After development with 3:1 chloroform-ethyl acetate, each desired band was scraped off the plate and eluted with warm methanol. Evaporation of the eluants gave the desired crystalline carbomethoxydihydrocleavamines.

18β- Carbomethoxy-4α-dihydrocleavamine (60, 0.28 g) (isomer A) was recrystallized from methanol, affording prisms: mp 144-147°; $[\alpha]_D^{23}$ +18° ($CHCl_3$); $\lambda_{max}$ (log ε): 227 (4.54), 286 (4.02), 294 (3.97) mμ; $\nu_{max}^{KBr}$: 3340 (-N-H), 2760 (Bohlmann band), 1707 ($COOCH_3$) $cm^{-1}$; nmr: τ 1.40 (singlet, 1H, N-H), 2.81 (diffuse, 4H, aromatic), 4.53 (doublet, 1H, C-18 proton), 6.40 (singlet, 3H, $COOCH_3$), 9.09 (triplet, 3H, $-CH_2\underline{CH_3}$).

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: M.W. 340.215. Found: 340.215 (mass spectrometry).

18β-Carbomethoxy-4β-dihydrocleavamine (61, 0.25 g) (isomer B) was obtained as prisms from methanol: mp 146-149°; $[\alpha]_D^{23}$ -66° ($CHCl_3$); $\lambda_{max}$ (log ε): 227 (4.54), 286 (4.02), 294 (3.98) mμ; $\nu_{max}^{KBr}$ 3300 (-N-H), 2755 (Bohlmann band), 1695 ($-COOCH_3$) $cm^{-1}$; nmr: τ 1.37 (singlet, 1H, N-H), 2.83 (diffuse, 4H, aromatic), 4.98 (doublet, 1H, C-18 proton), 6.39 (singlet, 3H, $-COOCH_3$), 9.12 (triplet, 3H, $-CH_2\underline{CH_3}$).

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: M.W. 340.215. Found: 340.215 (mass spectrometry).

18α-Carbomethoxy-4β-dihydrocleavamine (63, 0.22 g) (isomer D) was recrystallized from acetone, giving small blocks: mp 226-229°; $\lambda_{max}$ (log ε): 226 (4.50), 286 (3.92, 294 (3.90) mμ; $\nu_{max}^{KBr}$: 3335 (-N-H), 2760 (Bohlmann band), 1720 ($COOCH_3$) $cm^{-1}$; nmr: τ 1.30 (singlet, 1H, N-H), 2.84 (diffuse, 4H, aromatic), 6.12 (pair of doublets, 1H, C-18 proton), 6.40 (singlet, 3H, $-COOCH_3$), 9.45 (triplet, 3H, $-CH_2\underline{CH_3}$).

Anal. Calcd. for $C_{21}H_{28}O_2N_2$: M.W. 340.215. Found: 340.215 (mass spectrometry).

Epimerization of 18α-Carbomethoxy-4α-dihydrocleavamine (62) (Isomer C)

To a solution of compound 62 (500 mg) in dry benzene (10 ml) was added boron trifluoride etherate (1 ml) and the resulting solution was refluxed under an atmosphere of nitrogen for 6 hr. After cooling, the solution was poured into saturated aqueous sodium bicarbonate and the resulting mixture was extracted thoroughly with dichloromethane. The combined extracts were dried (anhydrous sodium sulfate) and evaporated under reduced pressure. The residual material was purified by preparative tlc (silica gel, chloroform), affording 200 mg of starting material (62), as shown by mp, mixture

mp, infrared and tlc, and 175 mg of 18β-carbomethoxy-4α-dihydrocleavamine (60) (isomer A). The latter was identical (mp and mixture mp, infrared, tlc) with compound 60 prepared previously (see above).

Epimerization of 18α-Carbomethoxy-4β-dihydrocleavamine (63) (Isomer D)

Compound 63 (22 mg) was treated with boron trifluoride etherate in benzene solution under conditions identical with those described above for compound 62. Purification of the crude product by preparative tlc (silica gel, 3:1 chloroform-ethyl acetate) afforded 7 mg of starting material (63), as shown by mp, infrared and tlc, and 8 mg of 18β-carbomethoxy-4β-dihydrocleavamine (61) (isomer B). The latter was identical (mp and mixture mp, infrared, tlc) with an authentic sample obtained previously (see above).

Decarbomethoxylation of 18β-Carbomethoxy-4α-dihydrocleavamine (60) (Isomer A)

A solution of compound 60 (33 mg) in 5N hydrochloric acid (1 ml) was heated, under an atmosphere of nitrogen, at 95° for 8 hr. The solution was cooled in ice and made basic by addition of dilute aqueous ammonia. The resulting mixture was extracted with dichloromethane and the combined extracts were dried over anhydrous sodium sulfate. Removal of solvent afforded amorphous material which was identical, as shown by infrared and tlc (silica gel, ethyl acetate), with an authentic sample of 4α-dihydrocleavamine (59) [11].

Decarbomethoxylation of 18β-Carbomethoxy-4β-dihydrocleavamine (61) (Isomer B)

Compound 61 (30 mg) was decarbomethoxylated under conditions identical with those described above. The product, which was crystallized from methanol, gave mp 136-138°, and was found to be identical, as shown by mp and mixture mp, infrared, and tlc (silica gel, 1:1 chloroform-ethyl acetate), with 4β-dihydrocleavamine (58) [11].

Decarbomethoxylation of 18α-Carbomethoxy-4α-dihydrocleavamine (62) (Isomer C)

Decarbomethoxylation of compound 62 (500 mg) under conditions identical with those described above, gave 4α-dihydrocleavamine (59), identical (infrared and tlc) with an authentic sample.

18β-Carbomethoxycleavamine (64)

A solution of catharanthine hydrochloride (5.5 g) in glacial acetic acid (250 ml) was heated to 90° in a 1 litre 3-necked flask equipped with a mechanical stirrer and a reflux condenser. Sodium borohydride was added at intervals to keep the solution gently refluxing. After 1 hr, the reaction mixture was cooled to 10°, poured into aqueous ammonia, and the resulting mixture was extracted thoroughly with dichloromethane. The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residual light brown oil was dissolved in hot methanol (7 ml) and the product was allowed to crystallize, giving 1.9 g of pure 18β-carbomethoxycleavamine (64), mp 121-123°. The mother liquor was evaporated and the residual oil was subjected to column chromatography on Woelm silica gel, activity I, (75 g). Elution with chloroform produced a further 1 g of crystalline 18β-carbomethoxycleavamine (64): mp 121-123°; yield = 2.9 g (63%); $\lambda_{max}$ (log ε): 225 (4.55), 277(sh) (3.89), 287 (3.95), 294 (3.92) mμ; $\nu_{max}^{CHCl_3}$: 3415 (N-H), 1708 ($COOCH_3$) $cm^{-1}$; nmr: τ 1.45 (singlet, 1H, N-H), 2.47-3.10 (diffuse, 4H, aromatic), 4.76 (multiplet, 1H, olefinic H), 4.89 (doublet, 1H, C-18 proton), 6.42 (singlet, 3H, $-COOCH_3$), 7.97 (quartet, 2H, $-C\underline{H}_2CH_3$), 8.96 (triplet, 3H, $-CH_2C\underline{H}_3$).

Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.35; H, 7.80; N, 8.50.

Decarbomethoxylation of 18β-Carbomethoxycleavamine (64)

A solution of compound 64 (50 mg) in 5N hydrochloric acid (2 ml) was heated at 90° for 3 hr. The solution was cooled, poured into aqueous ammonia and the resulting alkaline mixture was extracted thoroughly with dichloromethane. The combined extracts were washed with water, dried (anhydrous sodium sulfate), and evaporated under reduced pressure, producing 30 mg of a crystalline material. The latter was shown by mp, mixture mp, and infrared spectra to be identical with an authentic sample of cleavamine (18) [11].

Hydrogenation of 18β-Carbomethoxycleavamine (64)

A small sample of compound 64 was hydrogenated (room temperature and atmospheric pressure) in ethyl acetate over Adam's catalyst. Filtration, followed by evaporation of the filtrate under reduced pressure, afforded 18β-carbomethoxy-4β-dihydrocleavamine (61), identified by comparison (mp and mixture mp, infrared) with an authentic sample (see above).

Transannular Cyclization of Vincaminoreine (71)

A mixture of vincaminoreine (71, 0.024 g) and 5% platinum on charcoal (0.050 g) in ethanol (10 ml), under an atmosphere of oxygen, was shaken vigorously on a wrist-action shaker. After 1 hr, the mixture was filtered and the filtrate concentrated to dryness under reduced pressure. The residue was purified by preparative tlc (alumina, chloroform-benzene 1:3) to yield vincaminoreine (71, 0.003 g) and minovine (72, 0.0018 g): $\lambda_{max}$: 308(sh), 337 mμ; $\lambda_{min}$: 267 mμ; $\nu_{max}$ ($CHCl_3$): 1660 $cm^{-1}$. This minovine was found to be identical (ir, uv and various tlc systems) with an authentic sample [42,43].

Sodium Borohydride Reduction of Minovine (72)

A solution of minovine (72, 0.11 g) in glacial acetic acid (12 ml), under an atmosphere of nitrogen, was heated at 90° with an excess of

sodium borohydride for 30 min. The hot thick solution was poured into ice-cold aqueous ammonia and the resulting mixture was extracted thoroughly with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residual oil (0.119 g) was purified by preparative tlc (silica gel, chloroform) to afford two pure compounds. The less polar compound was vincaminoreine (71, 0.063 g): mp 138-139° (Lit [42,44] mp 138-139°); $\lambda_{max}$ (log ε): 229 (4.54), 288 (3.87), 295 (3.86) mμ; $\nu_{max}^{KBr}$: 1722 $cm^{-1}$ ($COOCH_3$); nmr: τ 2.50-3.08 (diffuse, 4H, aromatic), 6.17 (pair of doublets, 1H, C-18 proton), 6.36 (singlet, 3H, $-CH_3$), 6.53 (singlet, 3H, $-CH_3$).

Anal. Calcd. for $C_{22}H_{30}N_2O_2$: M.W. 354.231. Found: 354.231 (mass spectrometry).

The more polar compound was vincaminorine (0.010 g): mp 129-131° (Lit [45] mp 130-131°); $\lambda_{max}$ (log ε): 230 (4.57), 288 (3.89), 295 (3.88)mμ; $\nu_{max}^{KBr}$: 1715 $cm^{-1}$ ($COOCH_3$); nmr: τ 2.46-3.03 (diffuse, 4H, aromatic), 3.81 (pair of doublets, 1H, C-18 proton), 6.38 (singlet, 3H, $-CH_3$), 6.41 (singlet, 3H, $-CH_3$).

Anal. Calcd. for $C_{22}H_{30}N_2O_2$: M.W. 354.231. Found: 354.230 (mass spectrometry).

Sodium Borohydride Reduction of Tabersonine (45)

A solution of tabersonine hydrochloride (0.008 g) in glacial acetic acid (10 ml), under an atmosphere of nitrogen, was heated at 90° with an excess of sodium borohydride for 30 min. The hot thick solution was poured into ice-cold aqueous ammonia and the resulting mixture was extracted thoroughly with dichloromethane. The combined extracts were dried (sodium sulfate) and evaporated under reduced pressure. The residual oil (0.008 g) was purified by preparative tlc (silica gel, chloroform) to

afford the ring-opened product (75, 0.005 g) as an oil: $\lambda_{max}$ 228, 286, 293 mμ; $\nu_{max}$ ($CHCl_3$) 3440 (-NH), 1715 ($COOCH_3$) $cm^{-1}$; nmr: τ 1.44 (singlet, 1H, -NH), 2.47-2.98 (diffuse, 4H, aromatic), 4.11 (diffuse, 1H), 4.72 (diffuse, 2H), 6.39 (singlet, 3H, $COOCH_3$).

Anal. Calcd. for $C_{21}H_{26}N_2O_2$: M.W. 338.199. Found: 338.199 (mass spectrometry).

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42. We are very grateful to Dr. I. Kompis, Institute of Chemistry, Bratislava, Czechoslovakia, for providing us with samples of vincadine, vincaminoreine, vincadifformine and minovine.

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46. We are very grateful to Dr. M. Gorman, Lilly Research Laboratories, for an authentic sample of this compound.

# PART II

# STUDIES RELATED TO SYNTHESIS AND BIOSYNTHESIS OF INDOLE ALKALOIDS

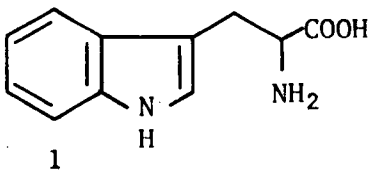
## Introduction

The study of the biosynthesis of the indole alkaloids has intrigued and interested workers in the field for many years. The early studies involving biosynthesis were based on natural compounds whose structures resembled proposed intermediates and the many chemical reactions which were believed to be of biogenetic significance. It was not until radioactive labelled precursors became readily available that the biogenetic hypothesis could be tested.

The amino acid, tryptophan (1), has a structural similarity to the indole alkaloids, and has long been felt to be the precursor to the indole portion of the molecule. Radioactive labelled tryptophan has been shown by a variety of workers to be incorporated into serotonin (2), ajmaline (3), serpentine (4), reserpine [1] (5) and gramine (6), as well as vindoline[2,3] (7), ibogaine [4] (8), catharanthine [2] (9) and ajmalicine (10).

In contrast to the general agreement by different workers with regard to the "tryptophan" portion of the indole alkaloids, the biogenetic origin of the "non-tryptophan", or $C_{9-10}$ unit, has been the subject of considerable controversy. With regard to the latter, a number of theories have been proposed over the years.

The earliest theory was proposed by several workers and was based upon structural similarities of indole alkaloids. This Barger [5]-Hahn [6,7]-Robinson [8]-Woodward [9,10] hypothesis involved dihydroxyphenylacetaldehyde (11) or an equivalent compound and two $C_1$ units in the non-tryptamine

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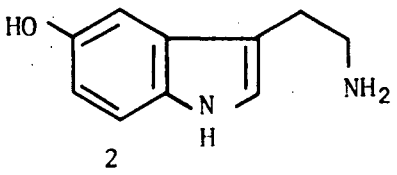


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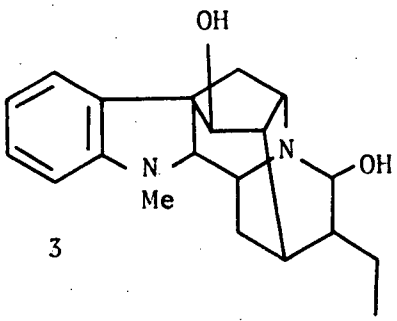


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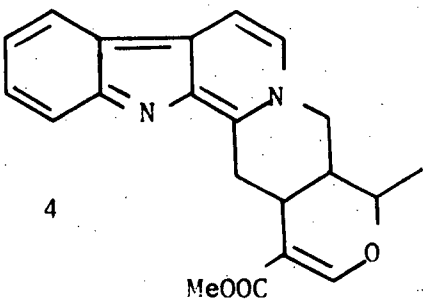


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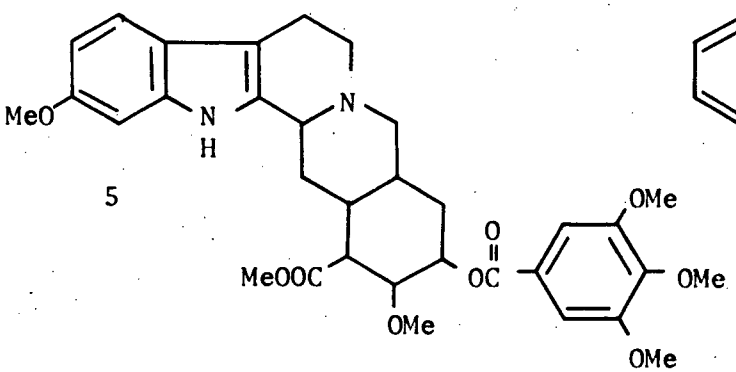


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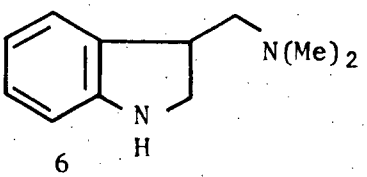


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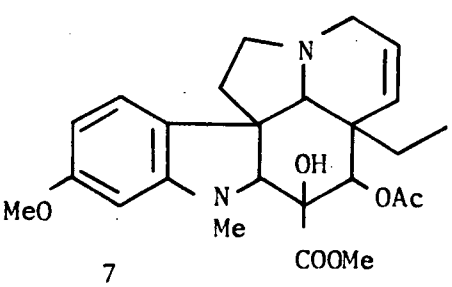


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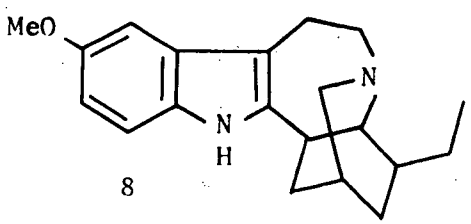


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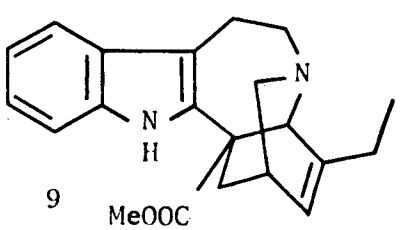


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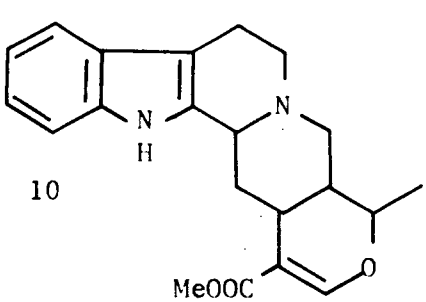


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portion of the alkaloids. The formation of yohimbine (12) and strychnine (13) is illustrated in Figure 1.

A number of deficiencies arose with the theory, and in 1959, Wenkert and Bringi [11,12] proposed an elegant alternative. They initially proposed that a hydrated prephenic acid (14) was the crucial intermediate, but later Wenkert [13] modified his hypothesis so that prephenic acid itself was the direct progenitor of these alkaloids. Various rearrangements and condensations afford a crucial intermediate, the seco-prephenate-formaldehyde (SPF) unit (15), which can be incorporated into yohimbine (12) and corynantheine (16) (Figure 2).

Schlittler and Taylor in 1960 [14] and Leete in 1961 [15-17] postulated that the non-tryptophan portion of the indole alkaloids was derived via the acetate pathway. The suggestion was that the relevant precursor might be formed by condensation of an open chain six carbon acetate unit, a one carbon unit and a three carbon unit. However, this hypothesis was soon withdrawn.

Another hypothesis based on structural relationships was proposed by Wenkert [11-13] and Thomas [18]. They suggested that the non-tryptophan moiety of the indole alkaloids was monoterpenoid in origin. Wenkert noted the structural identity of the carbon skeleton of the monoterpenes, verbenalin (17), gentiopicrin (18), bakankasin (19), swertiamarin (20), genipin (21) and aucubin (22), with the seco-prephenate-formaldehyde unit (15).

When Wenkert [13] proposed his monoterpenoid origin of the non-tryptamine portion of the complex indole alkaloids, he also postulated a biosynthetic scheme for alkaloids of the Aspidosperma and Iboga families (Figure 3). Condensation of the SPF unit (15) with tryptamine was envisaged as being followed by a retro-Michael reaction providing a cleavage product. If

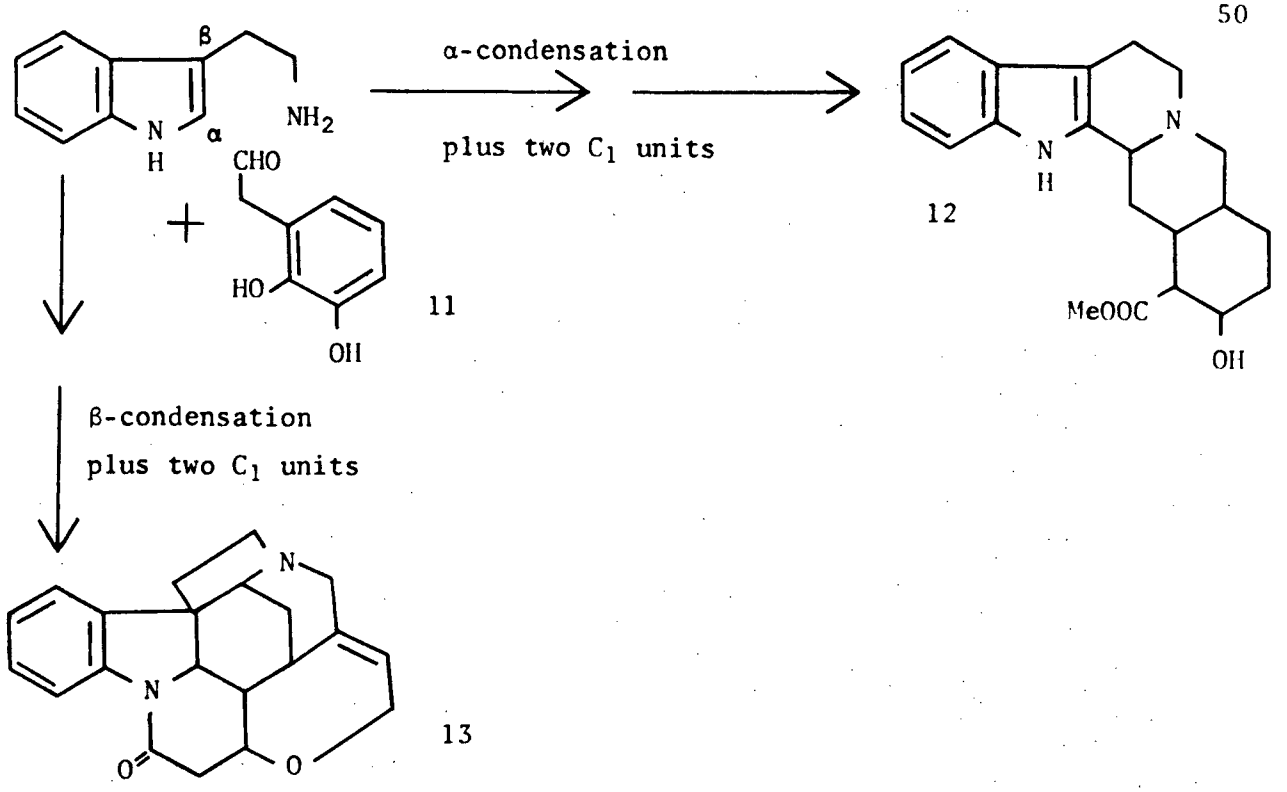



Figure 1. Barger-Hahn-Robinson-Woodward hypothesis.

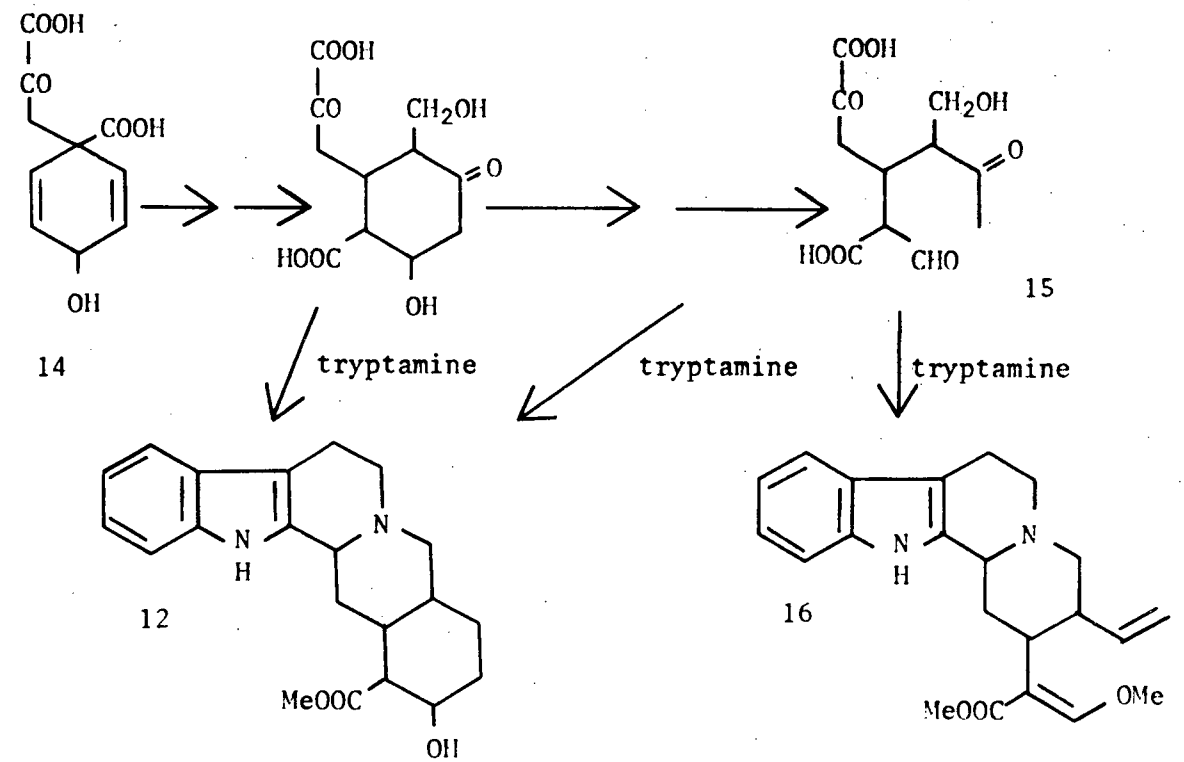
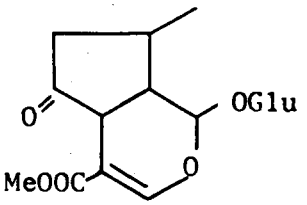



Figure 2. Prephenic acid hypothesis for the biosynthesis of Corynantheine alkaloids.

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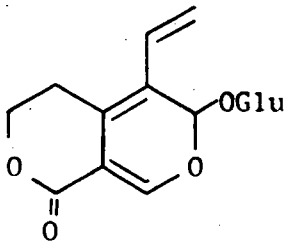


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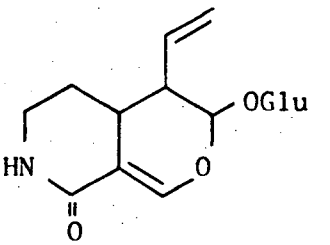


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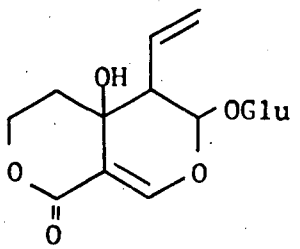


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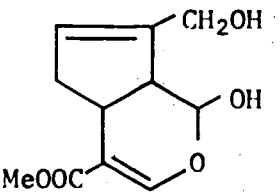


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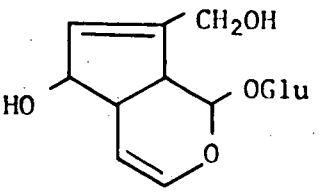


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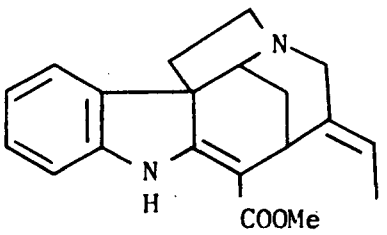
this intermediate undergoes ordinary oxidation-reduction changes, piperidines of various states of oxidation are formed whose intramolecular Michael and Mannich reactions lead to the Aspidosperma and Iboga-like skeletons, respectively. Wenkert [19] has also used iminium salt intermediates in his proposed biosynthetic pathway to the Akuamma (e.g., akuammicine (27)), Pleicocarpamine (e.g., pleiocarpamine (28)), and Hunteria (e.g., vincamine (29)) -like bases.

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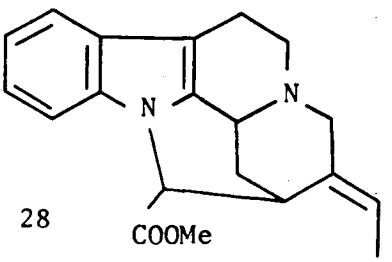


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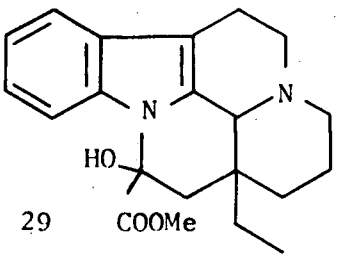


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The initial biosynthetic experiments, using radioactive precursors,

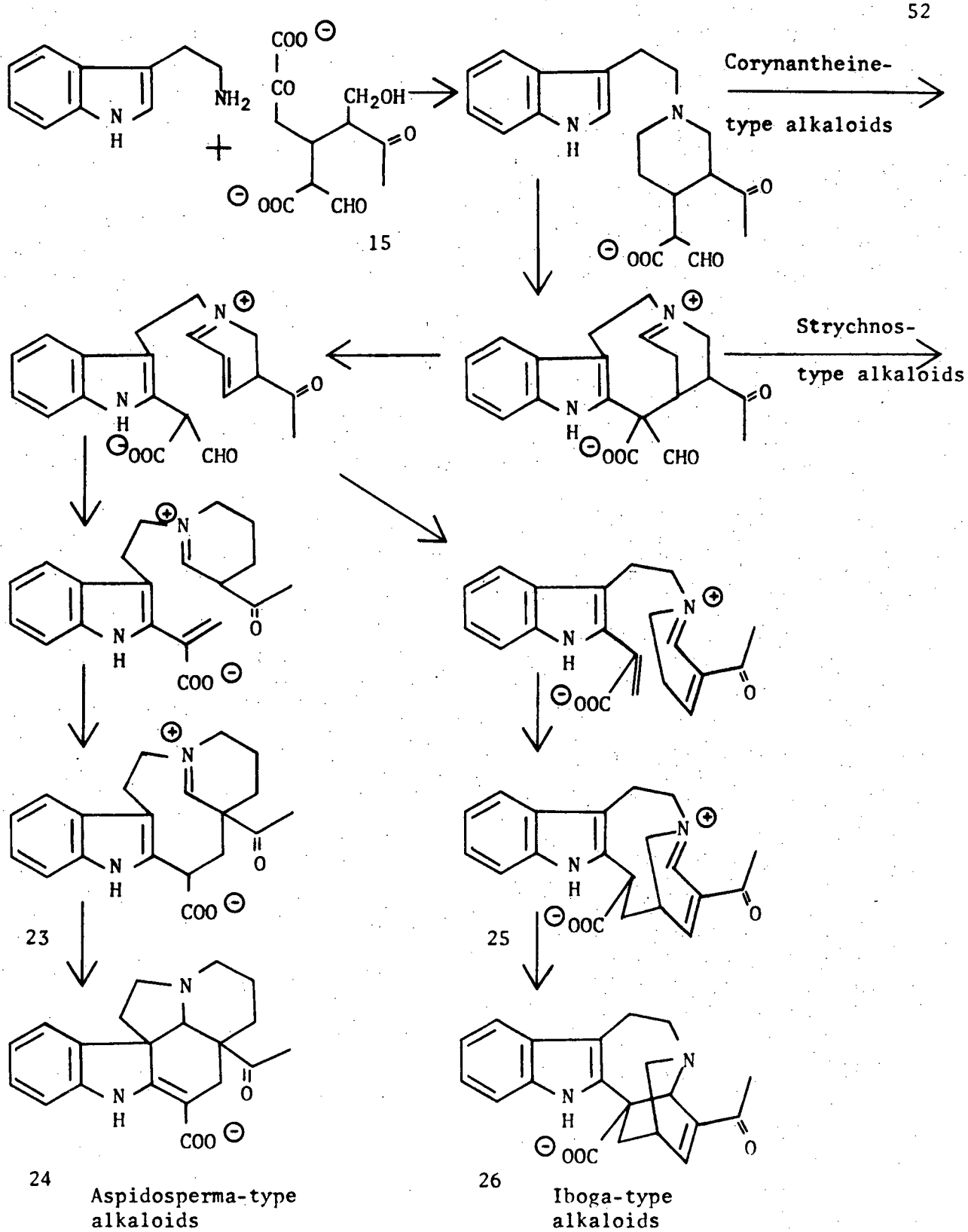



Figure 3. Wenkert's proposal for the biosynthesis of Aspidosperma and Iboga alkaloids.

disproved rather than proved all the hypotheses concerning the biosynthesis of the $C_{9-10}$ portion of the indole alkaloids. It was not until 1965 that Scott and coworkers [20] were the first to report a successful incorporation of mevalonate into an alkaloid of the tryptamine + $C_{9-10}$ type. Subsequent publication by several groups of workers [21-24] established that specifically labelled mevalonic acid was incorporated into indole alkaloids in a manner consistent with the monoterpenoid hypothesis. Not long after, the monoterpenoid, geraniol (30),was found to be incorporated [25-28] as an intact unit into vindoline (7), catharanthine (9), and ajmalicine (10) in Vinca rosea L. plants (Figure 4). Each of these alkaloids are representative of one of the three types of tryptamine + $C_{9-10}$ alkaloids.

The first evidence for cyclopentane intermediates in the pathway was obtained by Battersby and coworkers [29] who showed that loganin (31) was incorporated into ajmalicine, vindoline and catharanthine by Vinca rosea L. plants (Figure 4). Recently, loganin was again successfully incorporated into the indole alkaloids of Vinca rosea L. [30-33] plants and of Rauwolfia serpentina [34] plants. Degradation of the labelled alkaloids has led to the scheme shown in Figure 5. The structures represent the $C_{10}$ units of the Corynanthe, Iboga, and Aspidosperma groups of alkaloids which together account for the majority of the indole alkaloids. As an extension of his loganin work, Battersby [35] reported evidence from experiments in vivo which impose strict requirements on the mechanism of the formation of loganin and its conversion into the three classes of indole alkaloids. The results were obtained from the incorporation of various doubly labelled specimens of geraniol and of other substrates into young plants of V. rosea L. He concluded that: (a) the stereospecificity established for the formation of the two geraniol double bonds in other biological systems [36] also

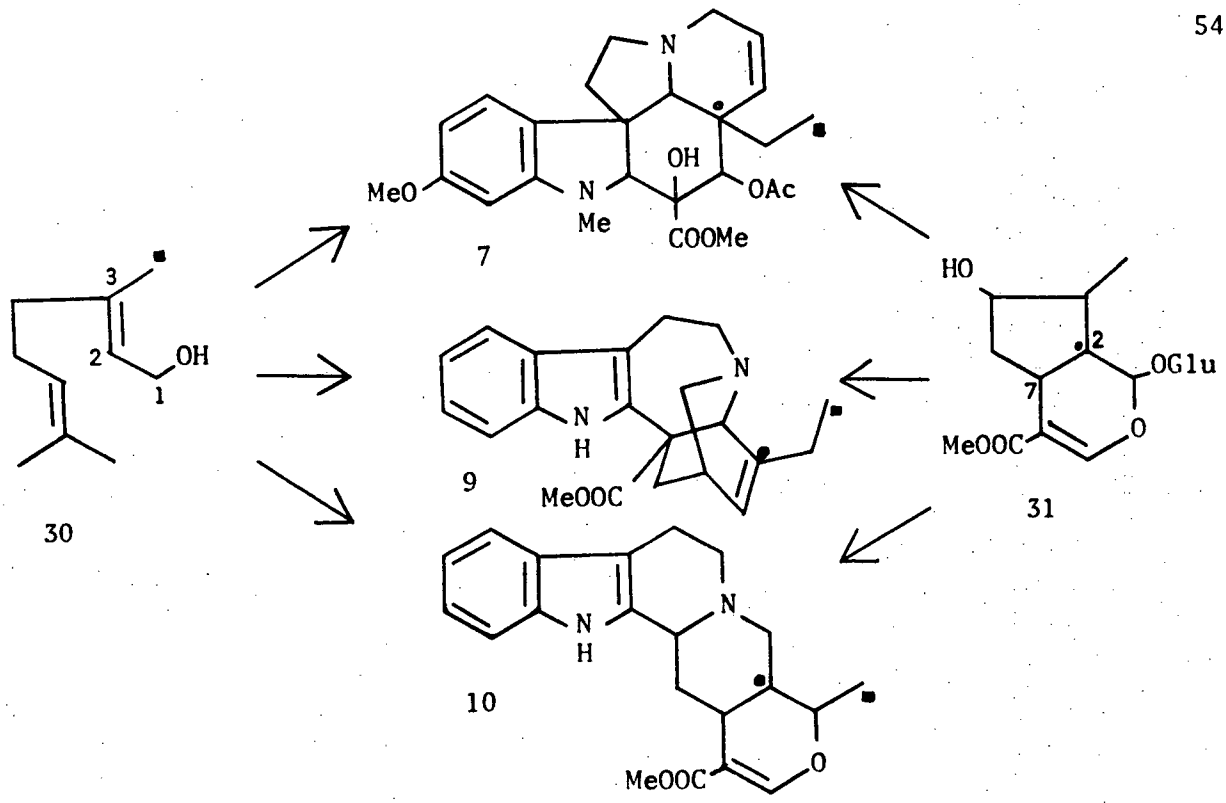



Figure 4. Incorporation of geraniol (30) and loganin (31) into Indole alkaloids.

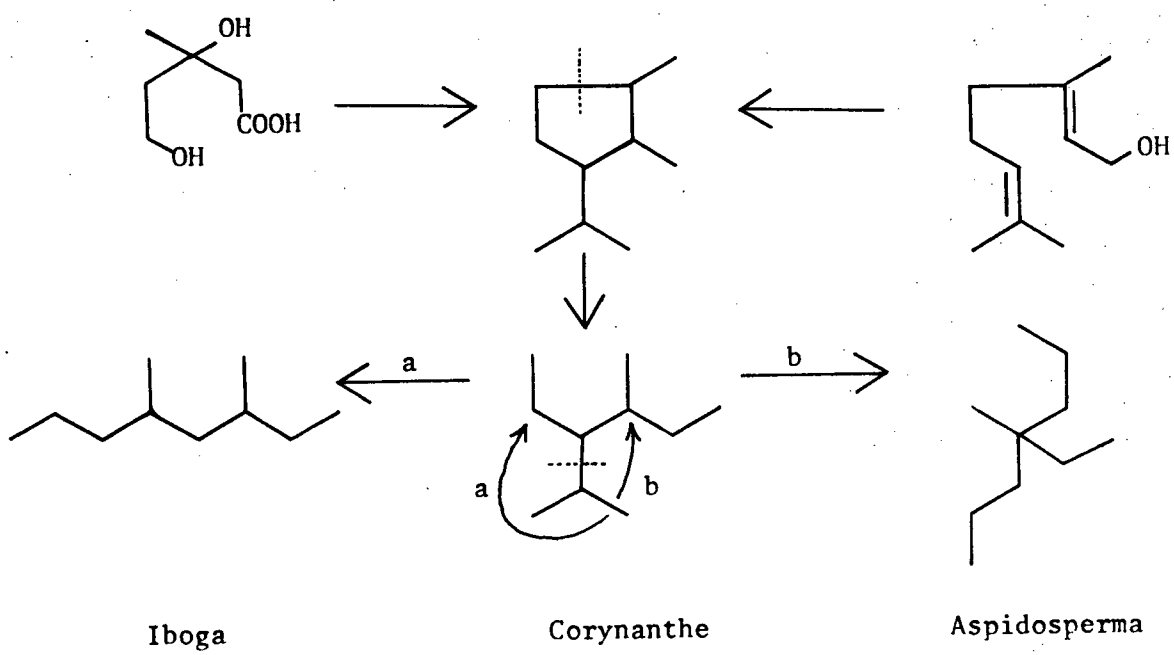
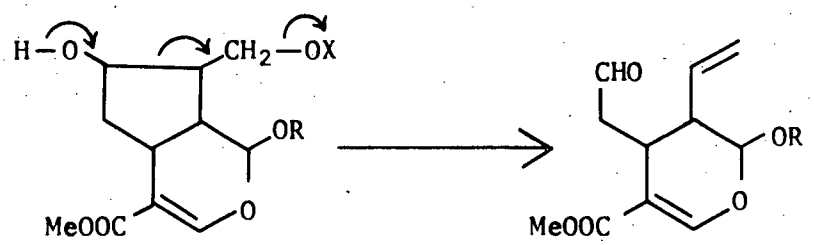



Figure 5. Scheme for the rearrangement of the monoterpene unit.

holds good in V. rosea L.; (b) if saturation of the 2,3-double bond of geraniol is a prerequisite for the formation of loganin, then both the reduction process and the subsequent removal of the proton from C-2 must occur in a stereospecific fashion; (c) in accord with the previous suggestion [13], the configuration of loganin at C-7 is determining for the stereochemistry of the corresponding centre in ajmalicine (10), and by extension for all other Corynanthe and Strychnos compounds; and (d) the stereochemical correlation of C-2 of loganin with the corresponding centre of ajmalicine is fortuitous; the observed loss of a proton from this position supports the idea of an enamine intermediate.

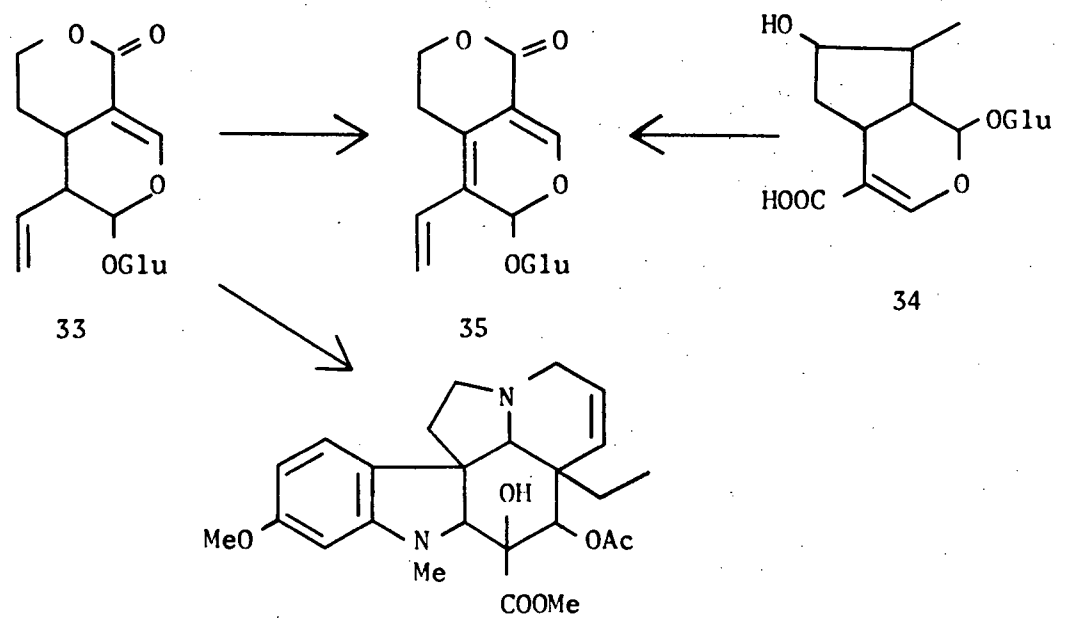
Battersby [37] had proposed a reasonable pathway from mevalonate to the indole alkaloids of the tryptamine + $C_{9-10}$ type. He was careful to emphasize "that several similar schemes could be written in which the sequence of operations is altered. For example, though at present it is attractive to consider cleavage of the cyclopentane ring before the nitrogenous portion of the molecule is introduced, the evidence is indirect. Plausible schemes reversing the order can be written." He also stated that "the conversion of the corynantheine-strychnine $C_{9-10}$ unit occurs after introduction of the nitrogen." Battersby [37] in the same paper describes an attractive mechanism for the fragmentation of the cyclopentane ring of loganin. A hydroxyloganin with a phosphate residue as a good leaving group could generate the desired aldehyde (32).

Recently, the monoerpenic glucoside, sweroside (33) was incorporated [38] into vindoline (7) in V. rosea L. plants. Sweroside bears a striking resemblance to the hypothetical condensing unit (32), having the same stereochemistry at C-2 and C-7. The incorporation of sweroside into vindoline was more efficient than the incorporation of loganin into



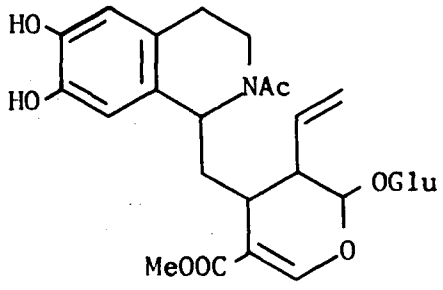

32, R = Glu

vindoline and this would tend to suggest that sweroside is further along the biosynthetic pathway than loganin. In this regard, there was good evidence that loganic acid (34) is a precursor of gentiopicroside (35) in Swertia carboliniensis plants [39] and sweroside is readily incorporated into gentiopicroside in Gentiana scabra plants [38]. A few alkaloids have been




isolated which have the hypothetical condensing unit (32) occurring as an intact or nearly intact unit. One of these, ipecoside (36), has the monoterpenic portion derived [40] from loganin. Another alkaloid having a

similar monoterpene unit is the indole alkaloid, cordifoline [41] (37). Recently, the tetrahydro-β-carboline monoterpenoid glycoside, strictosidine (38) was isolated [42] from Rhazya supp., and was also shown to be

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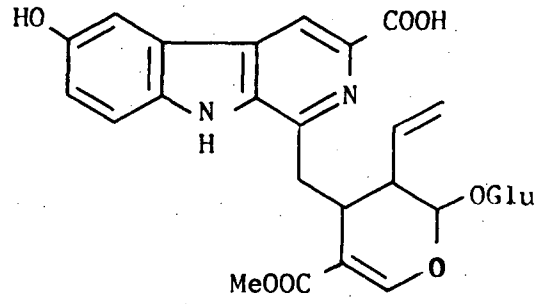


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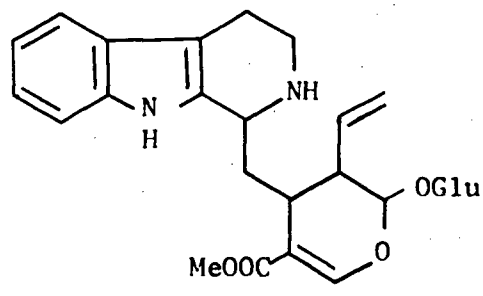
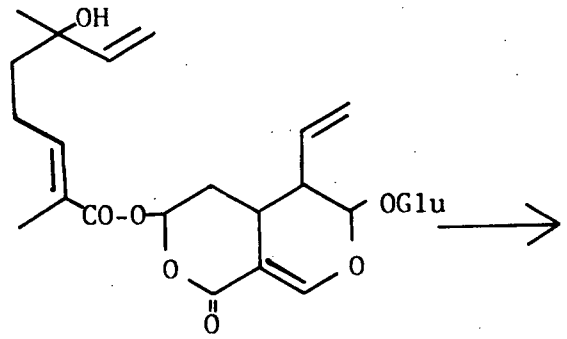



38

present [43] in V. rosea L. plants. The monoterpenoid portion of strictosidine has been shown [43] to be derived from loganin.

Using the reasoning that sweroside (33) and some other monoterpenoid derivatives are masked lactol forms of the desired aldehyde (32), such an aldehyde, secocyclopentanoid monoterpene or secologanin (32), has been synthesized [44]. Menthiafolin (39) was converted in good yield to secologanin (32). Indeed, when secologanin (32) was condensed with 3,4-dihydroxyphenethylamine (40), the major product formed was identical with the natural product, ipecoside (36). The radioactive [O-methyl-$^{3}$H]-secologanin was subsequently taken up by Vinca rosea L. shoots to yield

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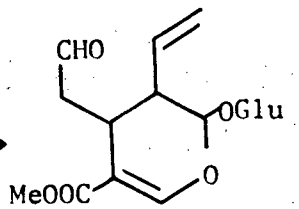


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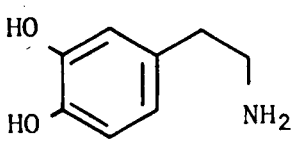


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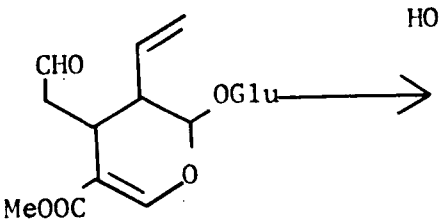


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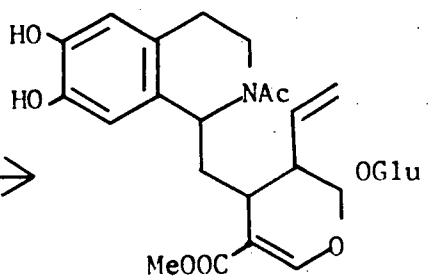


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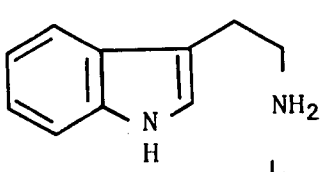


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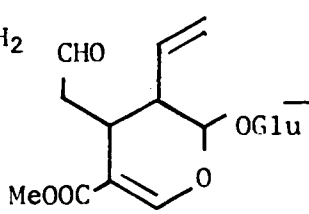


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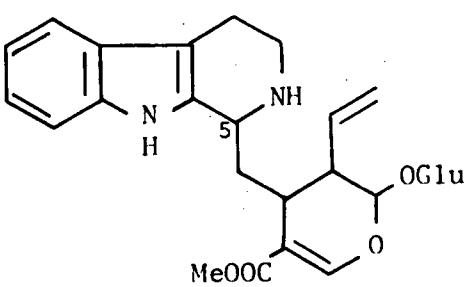
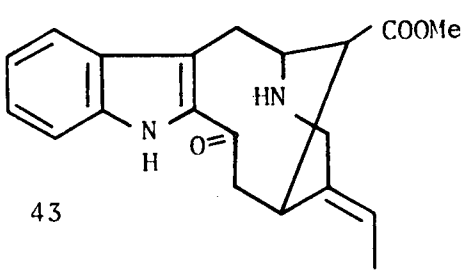



38, $C_5$-H(α)

42, $C_5$-H(β)




43

the following radioactive alkaloids: ajmalicine (10), vindoline (7), catharanthine (9), perivine (43) and serpentine (4).

The main features are now known of the pathway from mevalonate through geraniol and loganin to secologanin which then serves as precursor of the non-tryptamine units present in the three large classes of indole alkaloids [37]. Attention was then focused on the process whereby the tryptamine unit is introduced for assembly of these families by reactions taking place with or without rearrangement. Secologanin (32) was reacted with tryptamine to generate the β-carbolines, vincoside (strictosidine (38)) and isovincoside (42) [45,46]. When vincoside (strictosidine) was fed to V. rosea L. shoots, a good incorporation into alkaloids representing all three classes of indole alkaloids was obtained. Also, in connection with this work, dilution analysis in V. rosea L. plants which had previously taken up [5-$^3$H]loganin confirmed that secologanin (32), vincoside (strictosidine (38)) and isovincoside (42) are natural products of this plant. A summary of the pathway from acetate to indole alkaloids of tryptamine + $C_{9-10}$ type is shown in Figure 6.

The relatively high incorporation of a β-carboline system into unrearranged [ajmalicine (10)] and rearranged [vindoline (7) and catharanthine (9)] is of considerable interest. Conversion of vincoside (38) into the Corynanthe group [e.g., ajmalicine (10)] requires straightforward steps. However, the rearrangement processes leading to the Aspidosperma [e.g., vindoline (7)] and Iboga [e.g., catharanthine (9)] systems must still be elucidated.

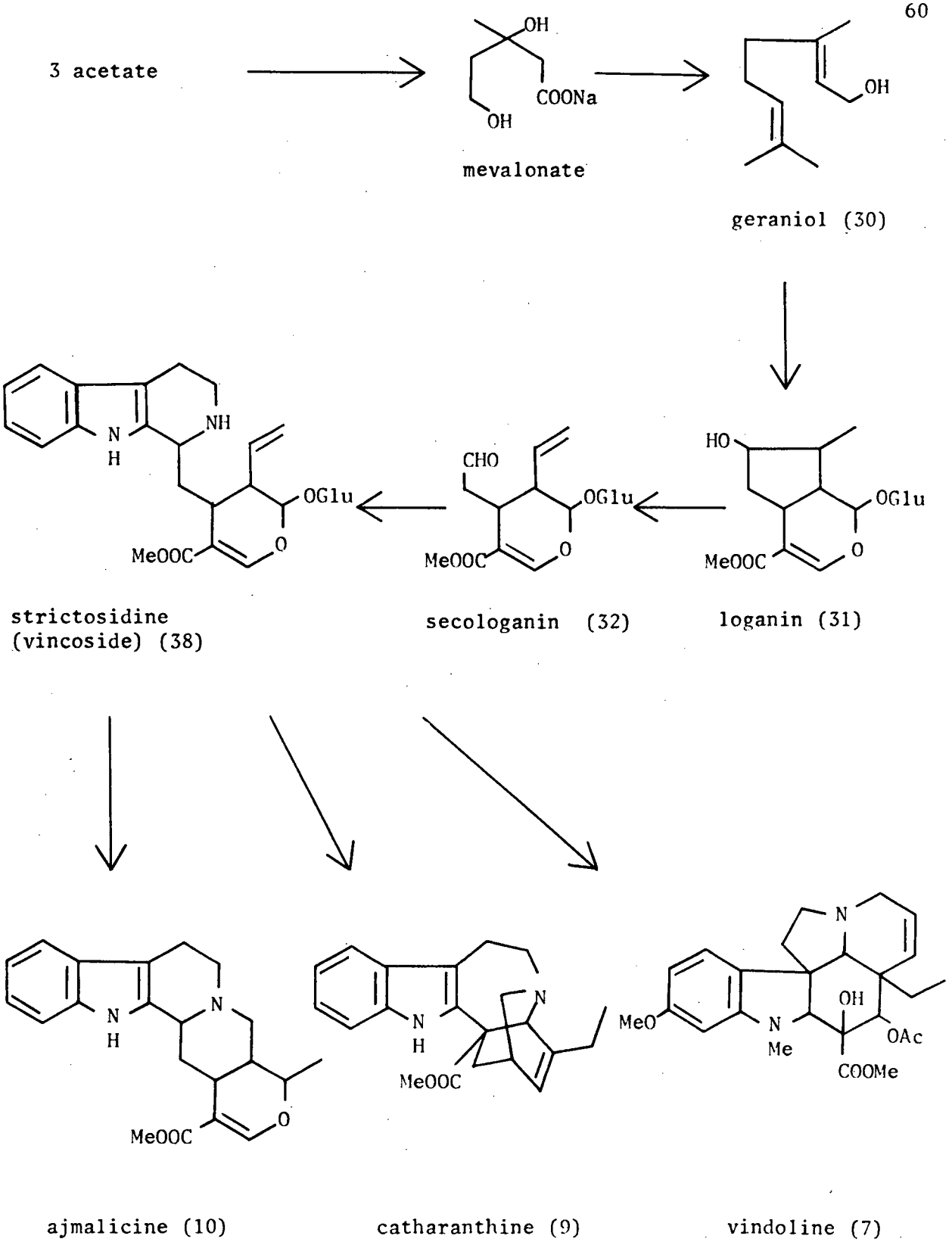



Figure 6. Summary of the pathway from acetate to Indole alkaloids of tryptamine + $C_{9-10}$ type.

## Discussion

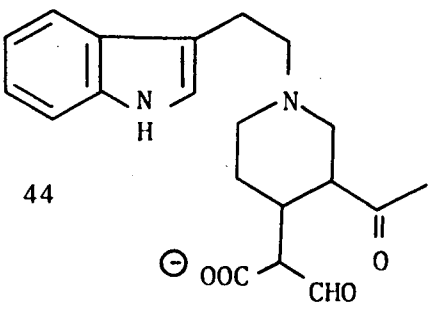
The Introduction has revealed that in recent years the biosynthesis of indole alkaloids has stimulated considerable interest in various laboratories. Almost without exception these investigations have concentrated on the nature of the "non-tryptophan" unit necessary in the biosynthesis, and numerous elegant experiments are now in hand which establish the monoterpene, loganin, as playing an important role in this regard. Our own interests in this area have been concerned with the later stages of the biosynthetic pathway, i.e., the steps involved after the tryptophan-$C_{10}$ "complex" has been formed. Such questions as (a) the structure of this "complex(es)" and (b) the pathways which it follows to elaborate the various families in the indole and dihydroindole series were of prime consideration.

In Section A of this discussion, the syntheses of possible precursors of the Aspidosperma and Vinca alkaloids are described, and in Section B, the biosynthetic studies are presented.

### Section A

Of the various postulates which were available, the one proposed by Wenkert [13] and outlined in Figure 3 of the Introduction was of particular interest in our initial considerations. We have already mentioned in Part I of this thesis that the transannular cyclizations, 23 → 24 and 25 → 26, as shown in Figure 3 of Part II, have found some parallel in our own chemical studies. The possible significance of these cyclizations in alkaloid biosynthesis will be discussed in later sections of this thesis.

At this time, I would like to present some studies on the synthesis of possible precursors prior to the formation of the nine-membered ring intermediates, 23 and 25. Figure 3 illustrates the possible importance of intermediates such as 44 and 45 in this connection, and we were, therefore, prompted to consider the laboratory synthesis of these types of systems.

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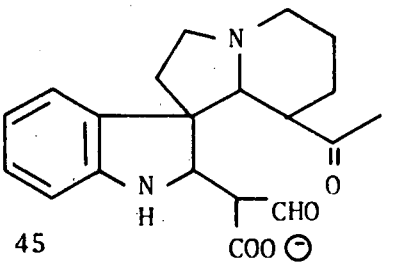


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The synthesis of the carbon skeleton present in 44 was facilitated by the reported synthesis of the α,β-unsaturated ketone (51) [47]. Although this synthesis was reported, the experimental procedures of some of the steps were not completely clear and first attempts to repeat the synthesis met with certain minor difficulties. However, these problems were rapidly eliminated and the experimental procedures followed in the present work are in accord with the sequence as shown in Figure 7. The ketalization of 3-acetylpyridine (48) proceeded to give a good yield (78%) of the ketal (49). Tryptophyl bromide (47) was obtained from tryptophol (46) in 80% yield and was used immediately (owing to instability of 47) for reaction with 49 to give the salt, 50, in 86% yield. Sodium borohydride reduction of 50, followed by acid hydrolysis, gave the α,β-unsaturated ketone, 51, in 50% yield after crystallization from methanol. All the data obtained for this substance was in agreement with the published information [47].

A Michael reaction of this α,β-unsaturated ketone with some suitable

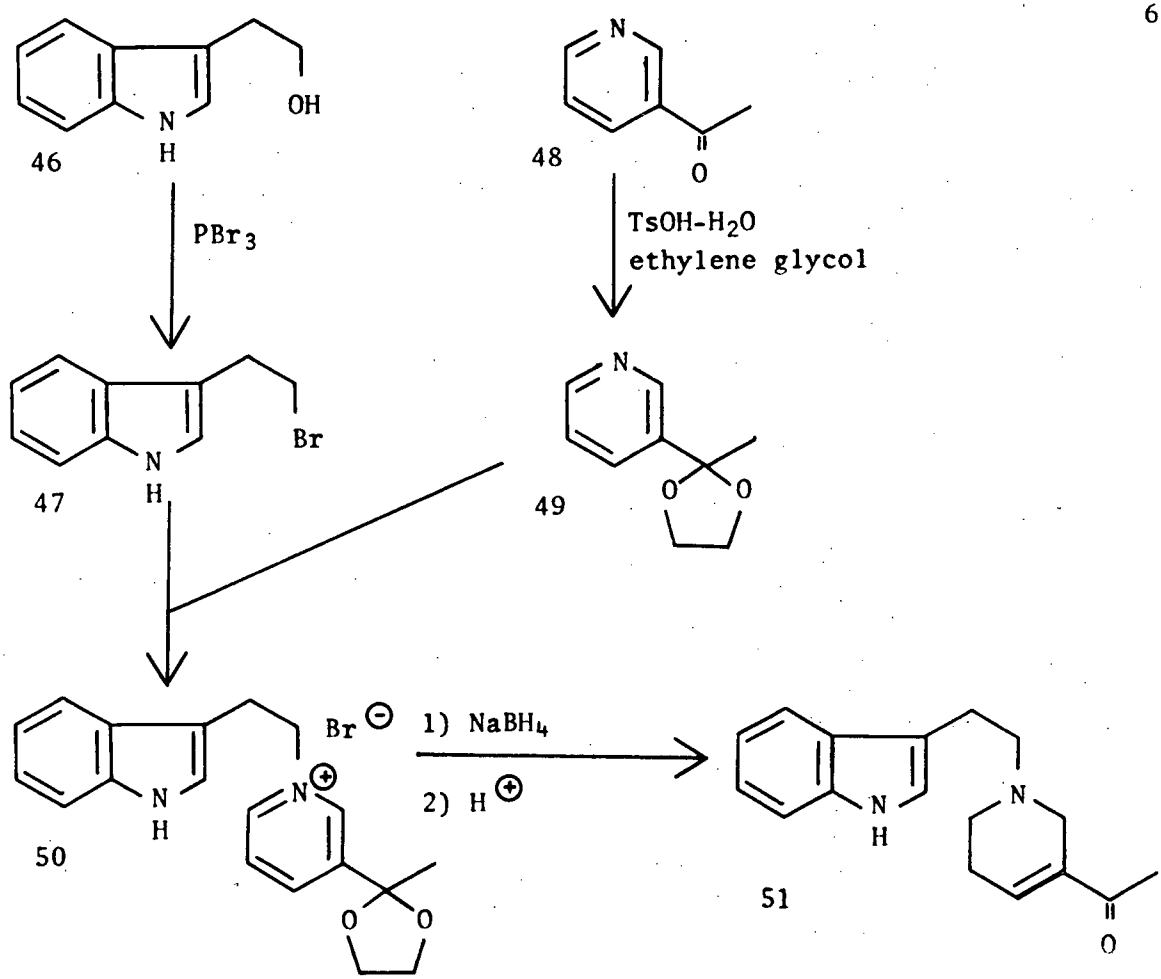
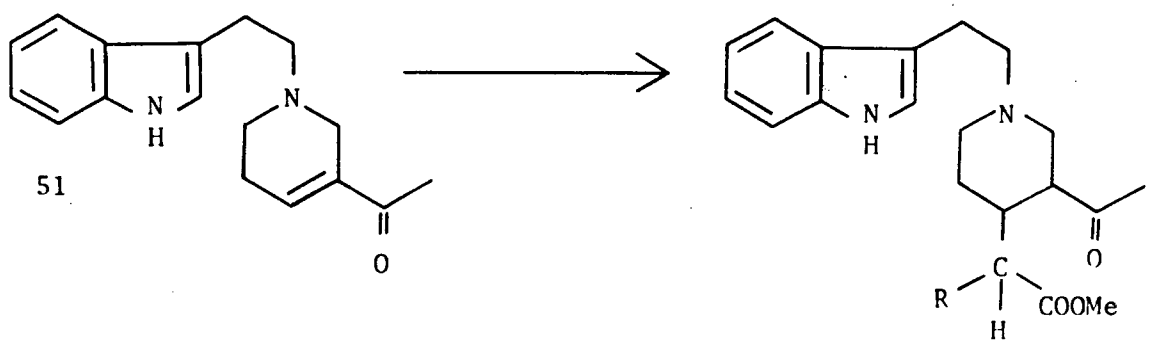



Figure 7. Preparation of α,β-unsaturated ketone 51.

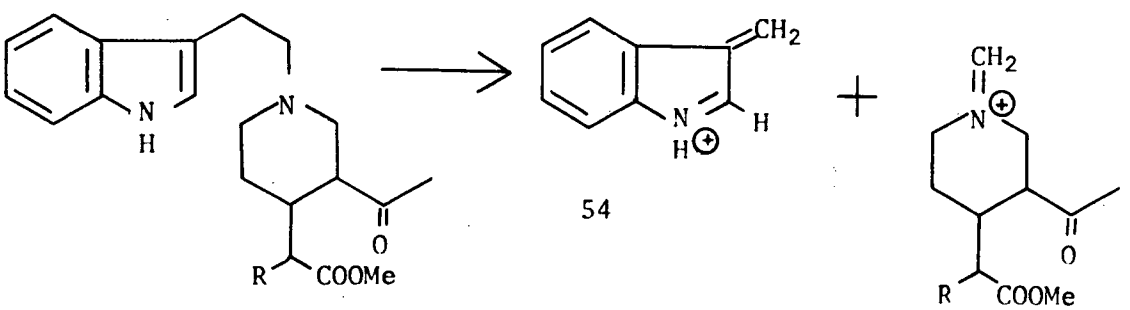



52, R = CN

53, R = COOMe

three carbon unit would then afford the desired carbon skeleton, 44. Since the exact oxidation state of the biological intermediate was not known, an attempt was made to synthesize various compounds with this carbon skeleton but having different oxidation states.

An obvious choice for this purpose was methyl cyanoacetate, and therefore, a mixture of this reagent, the ketone, 51, and triethylamine was allowed to stir at room temperature for 5 days. After purification, a mixture of epimers possessing the functionality shown in 52 was isolated in good yield. No attempt was made to separate these epimers at this time because this was not necessary for the biosynthetic studies. The infrared spectrum exhibited carbonyl absorption at 1700 $cm^{-1}$ for the methyl ketone, 1737 $cm^{-1}$ for the methyl ester and weak nitrile absorption at 2235 and 2195 $cm^{-1}$. The molecular formula was established as $C_{21}H_{25}N_3O_3$ by high resolution mass spectrometry (Found: 367.190; Calcd.: 367.190). The mass spectrum (Figure 8) showed a molecular ion peak at m/e 367, as well as two significant peaks at m/e 130 and 237 which are attributed to the simple fragmentation of the parent molecule to the ions, 54 and 55, respectively. The nmr spectrum established that two epimeric compounds had formed in about equal yield. The




52, R = CN
53, R = COOMe

55, R = CN
56, R = COOMe

two strong signals at τ 7.92 and 7.97 arise from the protons of the methyl ketone, whereas the other strong signals at τ 6.24 and 6.28 are from the

protons of the methyl ester. The remainder of the nmr spectrum corresponded to the desired structure. A more detailed presentation of the appropriate signals is given in the experimental section.

The preparation of the malonic ester adduct (53), was first attempted using the same procedure as for 52 but only starting material, 51, was recovered. A solution of the anion of dimethyl malonate was then prepared in dry tetrahydrofuran and the α,β-unsaturated ketone (51) was added to it. After purification, a mixture of epimers of 53 was obtained in good yield. The infrared spectrum exhibited broad carbonyl absorption at 1745-1695 $cm^{-1}$ attributed to the methyl ketone and the two ester groups. The molecular formula was established as $C_{22}H_{28}O_5N_2$ by high resolution mass spectrometry (Found: 400.198; Calcd.: 400.200). The mass spectrum (Figure 9) was dominated by intense peaks at m/e 130 and 270 which arise from the simple fragmentation of the parent molecule to the ions, 54 and 56, respectively. This fragmentation mode is analogous to that observed for the coupling product, 52, mentioned earlier. Again, the nmr spectrum (Figure 10a) established that two epimeric compounds had formed in about equal yield. The methyl ester protons appeared as a series of singlets in the region τ 6.34, corresponding to six protons, and the methyl ketone protons appeared at τ 7.84 and 7.96. This epimeric mixture of malonic ester adducts (53) was similar in many respects to the mixture of cyanoacetate adducts (52). Thus, both series of compounds were unstable because of the ease of the retro-Michael reaction with the subsequent regeneration of the α,β-unsaturated ketone (51) and dimethyl malonate or methyl cyanoacetate. This feature along with the fact that these compounds possessed similar $R_f$ values on various tlc systems did not allow complete separation of the pure components without extensive loss of material. However, after careful

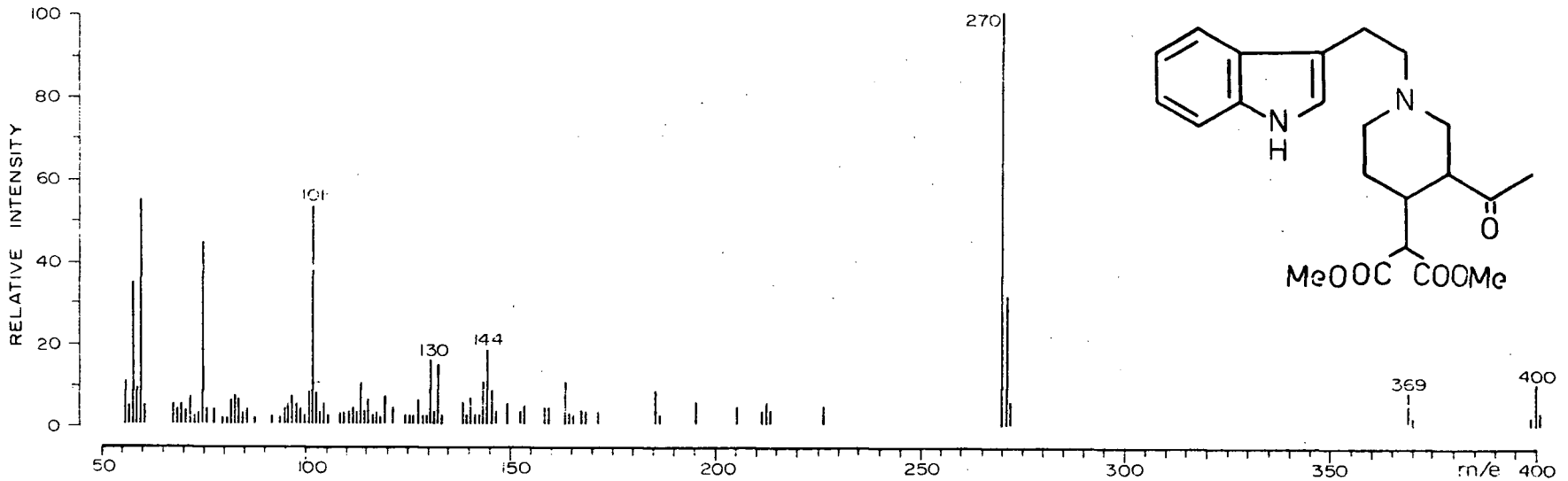



Figure 9

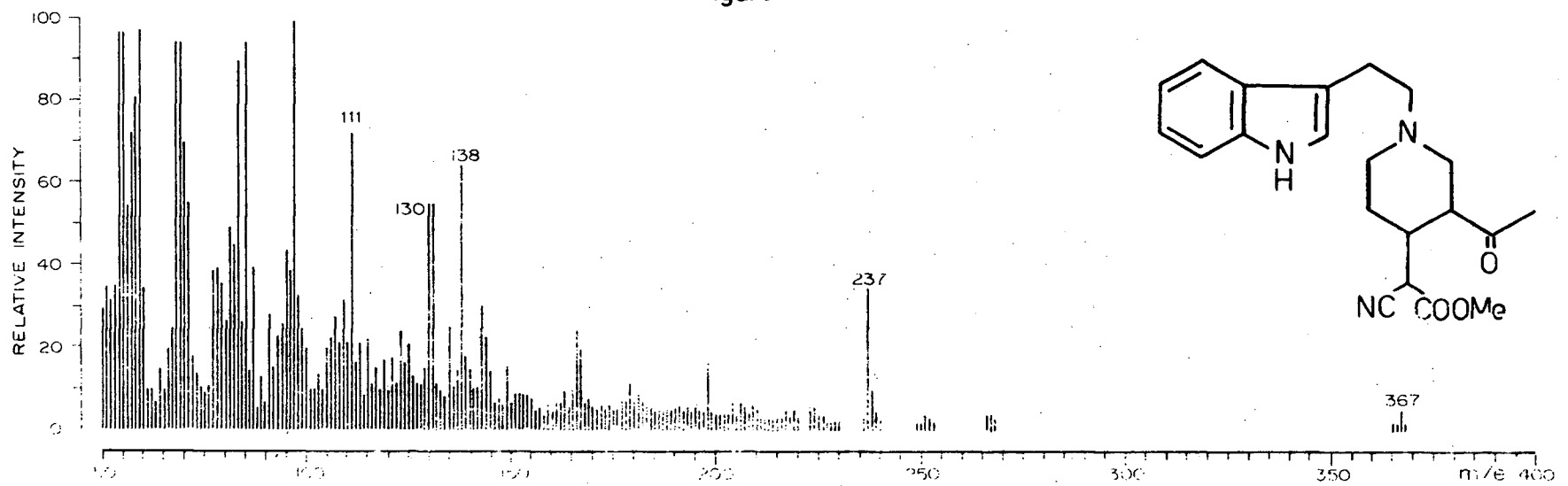



Figure 3

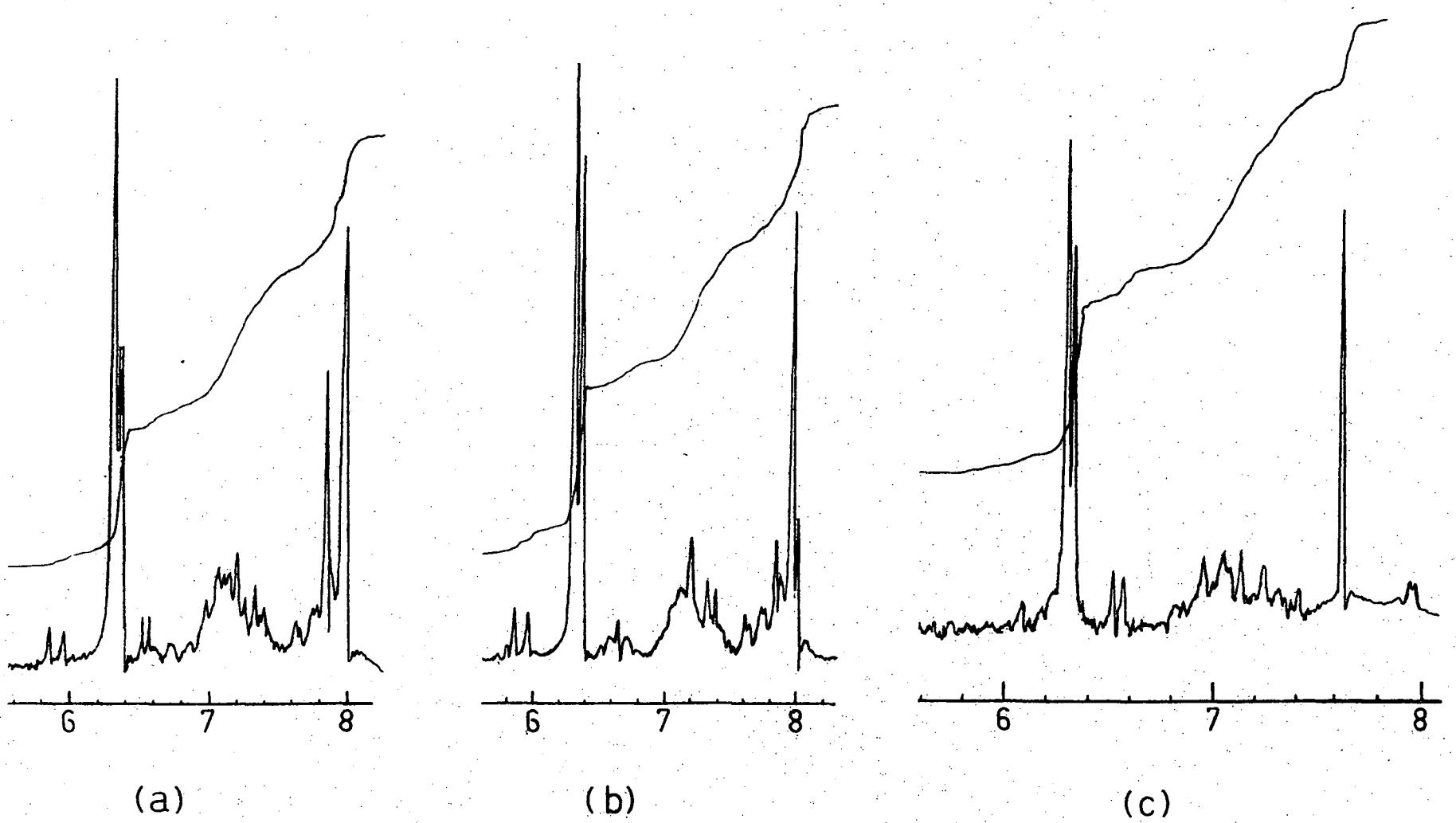
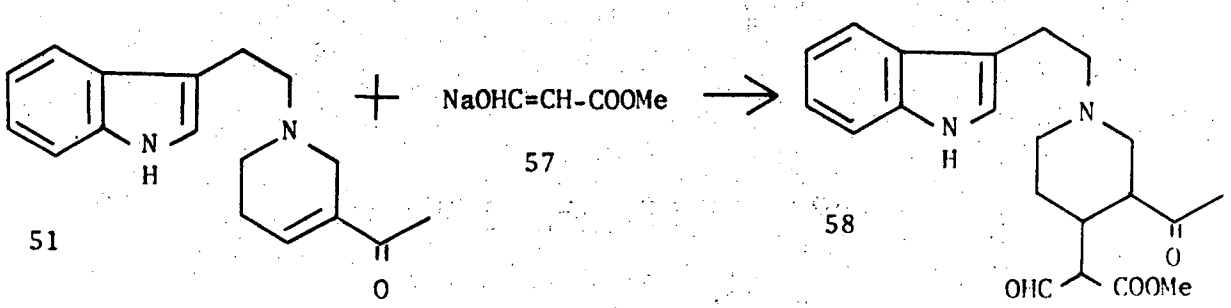



Figure 10. (a) Partial nmr spectrum of a mixture of malonic ester adducts 53.
(b) Partial nmr spectrum of the less polar malonic ester adduct 53.
(c) Partial nmr spectrum of the more polar malonic ester adduct 53.

preparative tlc separation using silica gel, a partial separation of the epimers of the malonic ester adducts, 53, was obtained. Now, a series of nmr signals could be assigned to each epimer. The methyl ester protons of the less polar epimer appeared as three proton singlets at τ 6.31 and 6.36 while the methyl ketone protons absorbed at τ 7.96 (Figure 10b). The one proton doublet, J = 10 cps, at τ 5.91 can be assigned to the proton on the carbon adjacent to the ester groups. The nmr spectrum (Figure 10c) of the more polar epimer possessed a similar series of signals with the methyl ester protons absorbing at τ 6.31 and 6.34 and the methyl ketone protons at τ 7.84. The proton on the carbon adjacent to the ester groups appeared as a doublet, J = 4 cps, centered at τ 6.54.

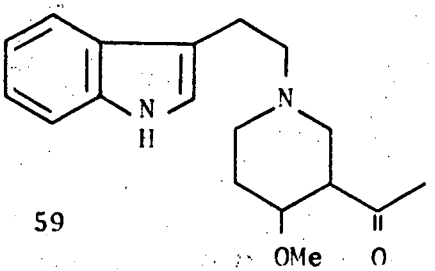
Having prepared the two derivatives, 52 and 53, attention was directed towards the synthesis of yet another member in this series. The aldehydo-ester adduct, 58, would have the functionality most closely related to the intermediates in Wenkert's postulate. This compound could be formed from the condensation of the α,β-unsaturated ketone (51) with the anion derived from α-formyl methyl acetate (57).




The preparation of this anion (57) has been reported as the product of the Claisen condensation between methyl formate and methyl acetate [48]. It is acknowledged that sodium formate and the sodium salt of acetoacetic

methyl ester are by-products of this reaction [49]. However, the nmr spectrum (in $D_2O$) of the product obtained when the two esters were added to an ethereal suspension of sodium methoxide showed three singlets at $\tau$ 1.49, 6.65 and 8.07. This was not consistent with the desired product and was explicable in terms of a mixture of sodium formate ($\tau$ 1.49) and the salt of methyl acetoacetate ($\tau$ 6.65 and 8.07), assuming exchange of the methylene protons of the latter compound.

In view of the possibility that the above mixture may contain a small proportion of 57, numerous attempts were made to add it to the $\alpha,\beta$-unsaturated ketone, 51, using a large excess of the mixture of sodium salts. The use of tetrahydrofuran, triethylamine, or dimethylsulfoxide as solvents gave rise to no reaction and 51 could be re-isolated in quantitative yield from all reactions. In methanol, however, it at first appeared that an adduct had been formed. This tentative conclusion was based on the infrared spectrum, which showed several carbonyl absorptions at 1697 and 1665 $cm^{-1}$. The properties of this reaction mixture, however, were later explicable in terms of a mixture of the starting material, 51 ($\nu_{max}$ 1665 $cm^{-1}$) and the product, 59 ($\nu_{max}$ 1697 $cm^{-1}$). The latter substance is merely the result of the addition of methoxide ion to the starting ketone. The nmr spectrum was also consistent with this observation in that signals at $\tau$ 7.40 ($\underset{\underset{O}{\|}}{C}$-$CH_3$) and 6.31

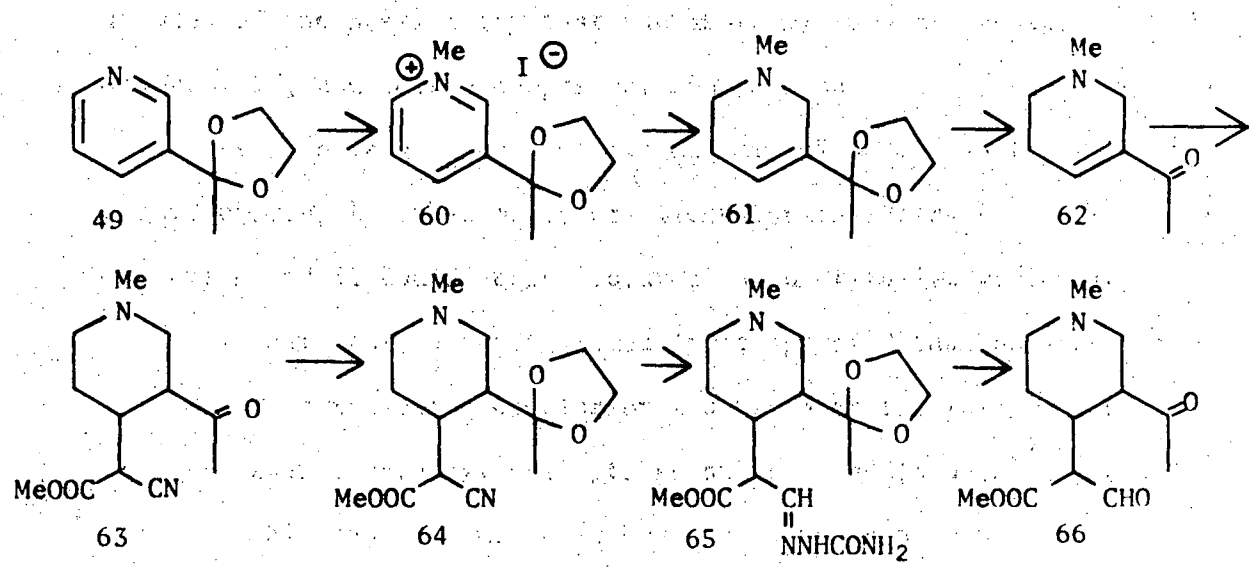



59

($OCH_3$) were evident. The mass spectrum with a peak at m/e 300 supported the formation of 59. These conclusions were confirmed in a reaction of

sodium methoxide with 51, under which conditions the same mixture was obtained.

In view of the lack of success in the synthesis of 58 by direct Michael addition of formyl acetic ester to the α,β-unsaturated ketone, 51, the less direct route of addition of cyanoacetate followed by reduction of the nitrile group was considered. The following pilot route on model piperidine compounds was investigated.

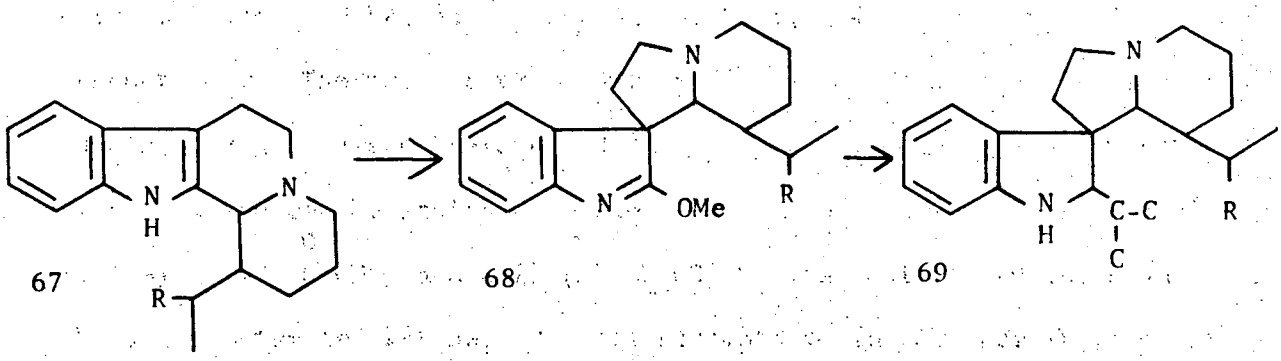



Accordingly, 3-acetylpyridine (48) was converted to the known ketal, 49, and this compound was treated with methyl iodide in ether to give the crystalline salt, 60, in 95% yield. Sodium borohydride reduction of this salt yielded the liquid ketal, 61, which on hydrolysis provided N-methyl-3-acetyl-$\Delta^3$-piperidine (62). The formation of the α,β-unsaturated ketone in the latter was confirmed by the strong carbonyl absorption at 1670 $cm^{-1}$ in the infrared. The vinyl proton in the nmr spectrum of this compound occurred as a one proton triplet at unusually low field (τ 3.10), whereas the three proton singlets for the N-methyl and methyl ketone occurred in the expected region (τ 7.63 and 7.73). Conversion of 62 to the methyl

cyanoacetate adduct, 63, was successful, but separation from methyl cyanoacetate proved difficult. The separation was finally accomplished by conversion to the picrate salt followed by regeneration of the base by filtration through an Amberlite IRA-400 ($HCO^-$) column [50] to afford a mixture of the starting material, 62, and the cyanoacetate adduct, 63. The presence of the adduct, 63, was indicated in the infrared spectrum with characteristic nitrile, ester and ketone absorptions at 2221, 1747, and 1709 $cm^{-1}$, respectively. The nmr spectrum confirmed that the methyl cyanoacetate adduct and the α,β-unsaturated ketone, 62, were present in about equal amounts. The signals which can be assigned to the adduct were: τ 6.17 ($COOCH_3$), 7.60 ($N\text{-}CH_3$), 7.78 ($C\text{-}CH_3$) and 6.21 ($NC\text{-}\underline{CH}\text{-}COOCH_3$) in addition to the signals of the α,β-unsaturated ketone, 62. All attempts to further purify this mixture by column chromatography and preparative tlc always resulted in the formation of additional amounts of the α,β-unsaturated ketone, 62, with the corresponding loss of the methyl cyanoacetate adduct. This latter situation again reveals the facile <u>retro</u>-Michael reaction to which reference was already made in the compounds, 52 and 53. Attempted sodium borohydride reduction again resulted in the formation of the α,β-unsaturated ketone, 62. Finally, attempts to form the ketal, 64, met with failure and further investigations of this approach were, therefore, abandoned.

Since several compounds bearing the skeleton as shown in 44 were now available, we turned our attention towards the synthesis of carbon skeleton 45 having an appropriate three carbon side chain attached to the indole system. It will be noted that this system would represent a different rearrangement of $C_{10}$ "non-tryptophan" unit and would be of interest to the biosynthetic postulated as outlined in Figure 3. The crucial step in our synthetic considerations for this series focus on the oxidative rearrangement

of α,β-disubstituted indole derivatives to their β,β-substituted spiro relatives. The particular reaction which was initially considered involved the rearrangement, 67 → 68. The resulting imino ether (68) would be expected to combine with nucleophiles, and thereby, provide an entry into the desired system. Thus, reaction with a suitable three carbon unit would generate the desired carbon skeleton as represented by 69.




The oxidative rearrangement of indoles to spiro compounds is a well-known reaction and has found use in the alkaloid field. A number of examples of its application to alkaloids of particular interest to this study have appeared in the literature and deserve comment. The rearrangement reaction is normally preceded by the oxidative step using t-butyl hypochlorite. For example, the treatment of the chloro derivative, 71, arising from t-butyl hypochlorite oxidation of the indole system (Figure 11) with aqueous methanol at pH 6 resulted in rearrangement to give the spiro oxindole analogue, 73 [51]. However, treatment of the chloro derivative, 71, with methanolic alkali gave the corresponding spiro imino ethers, 72, which, when subsequently refluxed with dilute acid gave the oxindole analogue, 73 [52]. The imino ethers are known to react with several nucleophiles. However, their reactivity is still considerably less than that of the imino chlorides and only strong nucleophiles are expected to undergo smooth reactions with

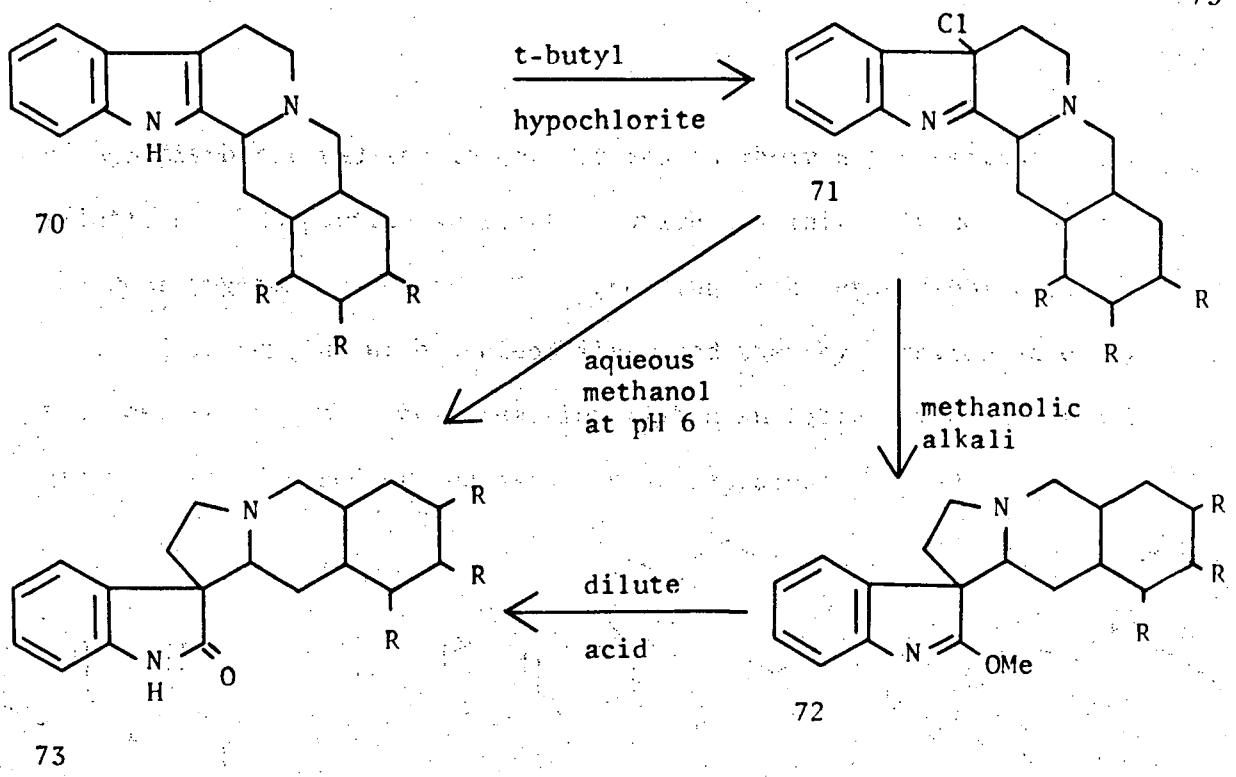
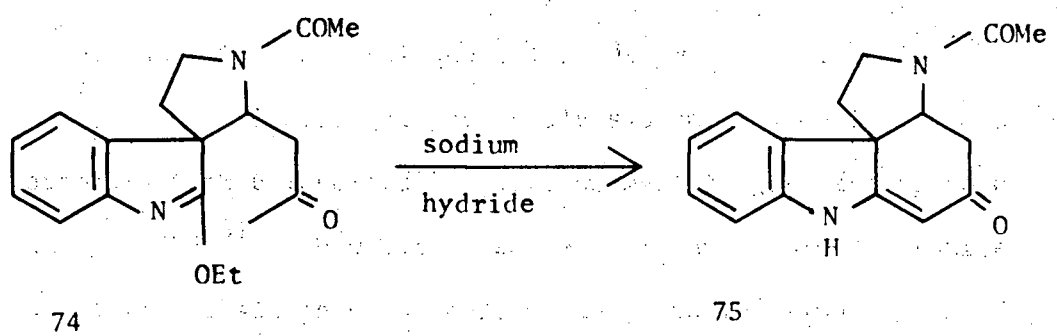



Figure 11. Oxidative rearrangement of Indole alkaloids.

them [53]. Ban [54] has recently reported such a study, in which he was able to convert the imino ether, 74, to the vinylogous amide, 75, using sodium hydride in dimethylsulfoxide.




The starting material for the synthetic sequence directed towards the carbon skeleton, 69, was the known tetracyclic ketone, 78. The synthesis of this tetracyclic ketone [19] presented no problems and is outlined in

Figure 12. Tryptophyl bromide (47) was obtained from tryptophol (46) and used immediately (owing to instability of 47) for reaction with 3-acetylpyridine (48) to give the salt, 76, in 94% yield. Catalytic hydrogenation of 76 using palladium on charcoal in ethanol gave the $\Delta^2$-piperidine, 77.

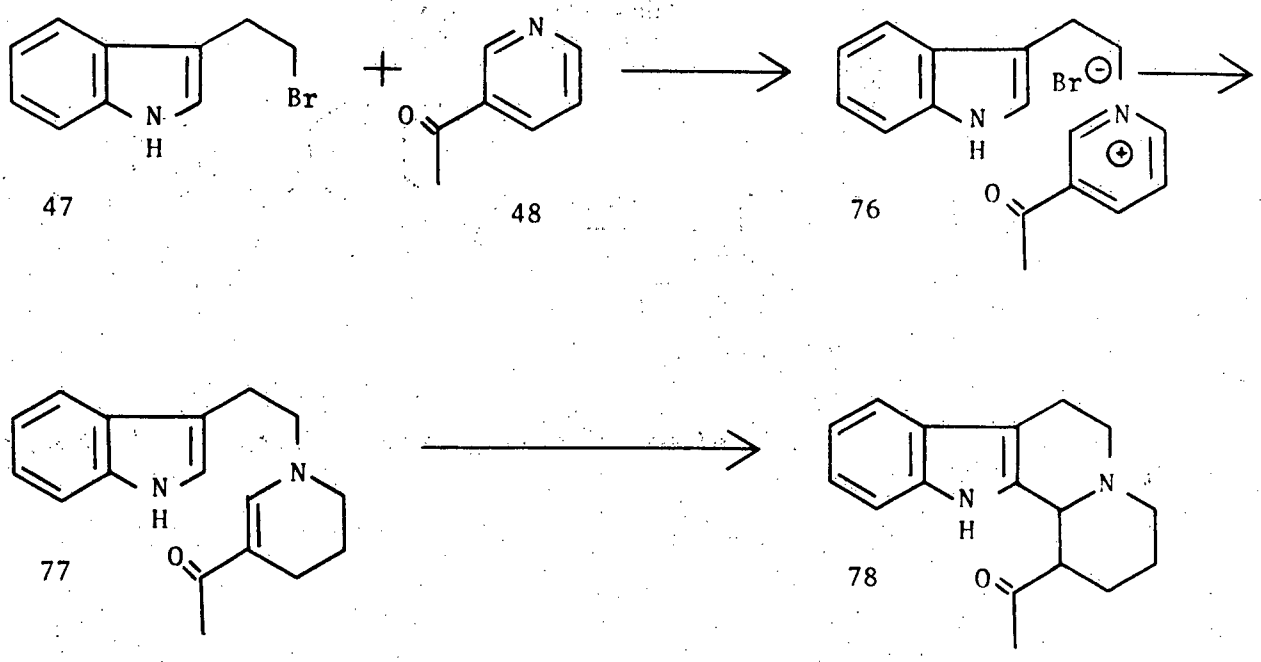
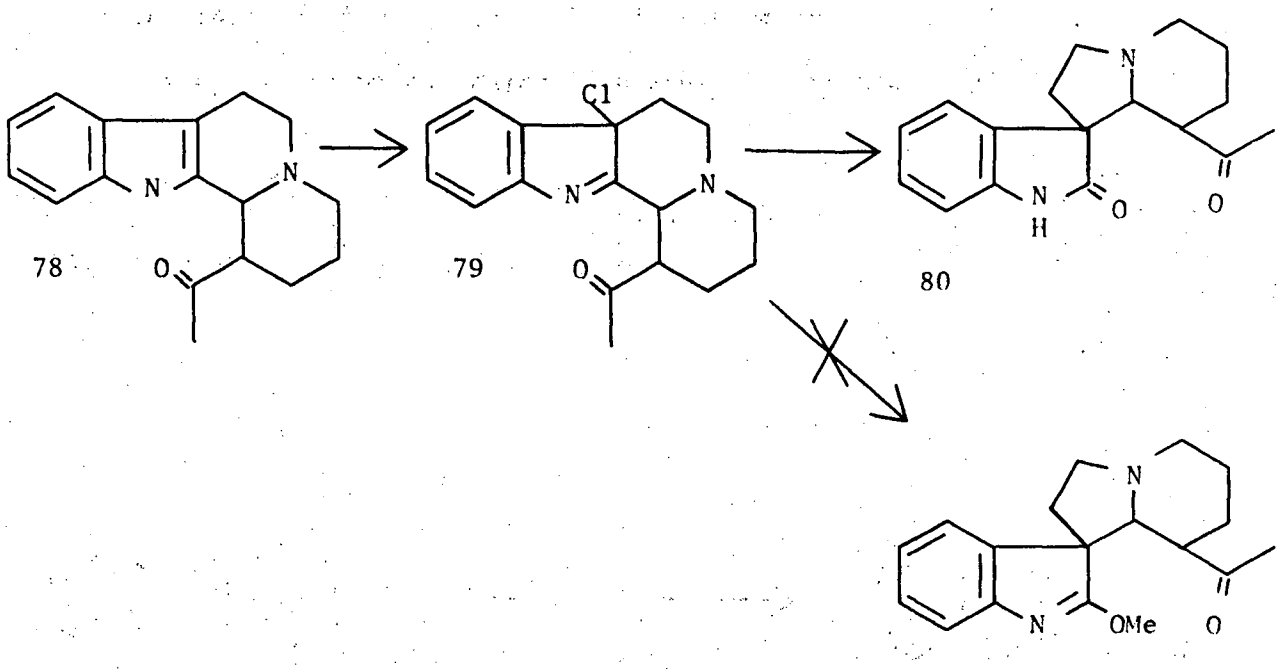



Figure 12. Preparation of tetracyclic ketone 78.

Without purification, the latter was exposed to Pictet-Spengler cyclization. Treatment of 77 with acid yielded two epimeric tetracyclic ketones, 78. Equilibration using sodium methoxide in methanol afforded the more stable tetracyclic ketone in 75% yield from the salt, 76.

Formation of the chloroindolenine (79) was accomplished using molar quantity of t-butyl hypochlorite in carbon tetrachloride at -12°. Attempted purification of this chloro compound always resulted in decomposition. However, the infrared spectrum possessed indolenine absorption at 1585 $cm^{-1}$ and a normal ketone absorption at 1700 $cm^{-1}$.

Treatment of the chloroindolenine (79) with methanolic alkali afforded a complex mixture of compounds. The major compound was obtained in 40% yield



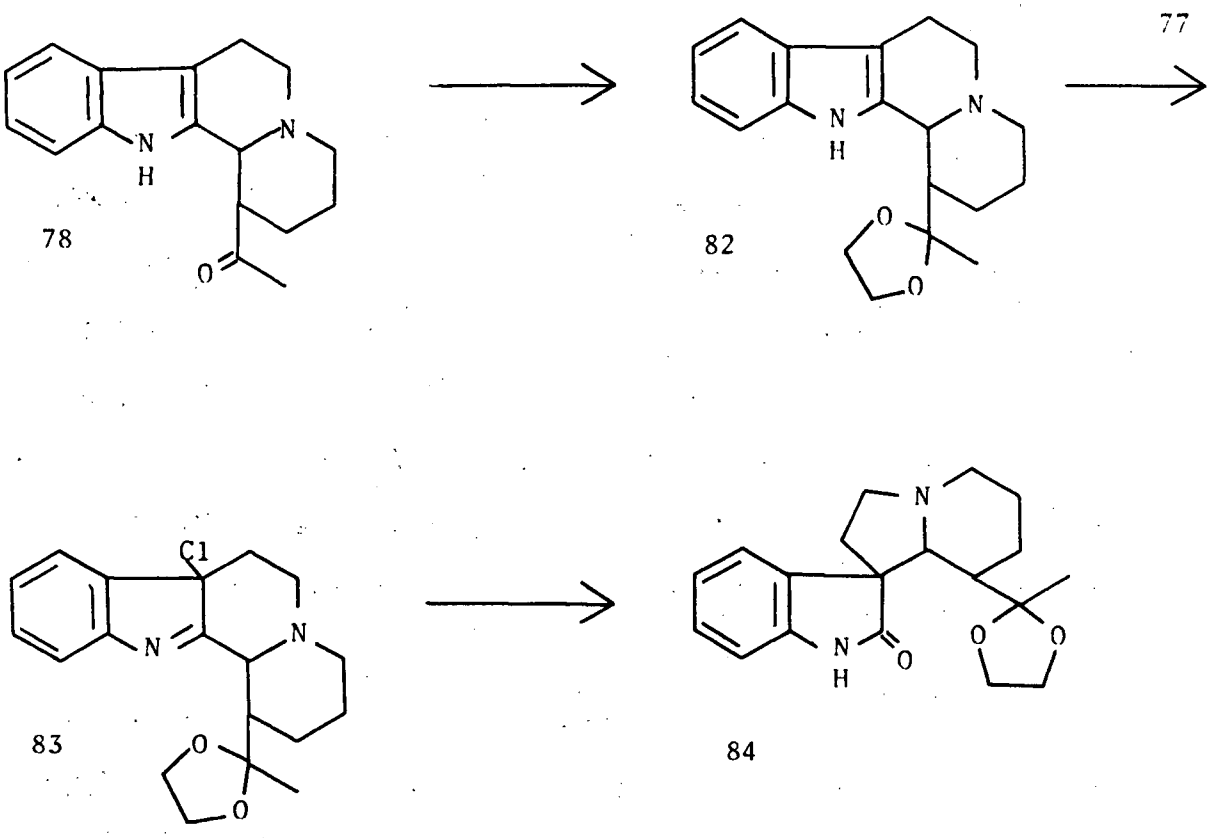

after a combination of column chromatography and preparative tlc purifications. Crystallization of this product from methanol afforded colorless prisms, mp 205-208°, which analyzed for $C_{17}H_{20}N_2O_2$. The compound possessed a typical indolenine absorption in the ultraviolet region at 210, 253 and 280 mμ. In addition to the absorption band at 3122 $cm^{-1}$ for a proton on the indolic nitrogen in the infrared spectrum,there appeared sharp bands at 1721 $cm^{-1}$ for the methyl ketone and another carbonyl absorption at 1695 $cm^{-1}$. The significant features of the nmr spectrum were a one proton singlet at τ 1.00, a four proton multiplet centred at τ 2.94 due to the aromatic protons of the benzene ring and a three proton signlet at τ 7.30 for the protons of the methyl ketone. Clearly, from the spectral data and analysis, this compound was not the desired imino ether (81). The data suggested that the

oxindole (80) had formed. The infrared spectrum showed the strong carbonyl absorption at 1695 $cm^{-1}$, characteristic of oxindole, whereas this spectrum did not have the strong absorbance in the region of 1575 $cm^{-1}$ which characterizes the imino ether system. The nmr evidence confirmed that an oxindole had formed. The one proton signal at $\tau$ 1.00 could be assigned to the proton of the indolic nitrogen and the absence of a strong three proton signal in the region $\tau$ 6.0-6.5 confirmed that the methyl imino ether had not formed. An attempt was made to convert the oxindole, 80, to an imino ether using Meerwein's reagent, triethyloxonium tetrafluoroborate. However, examination of the crude reaction product by tlc (silica gel) showed that a complex mixture of compounds had formed and as a result purification was not attempted on this mixture.

It was now obvious that the tetracyclic ketone was capable of providing various side products, some of which could be the result of the reaction with the carbonyl group itself. Thus, the formation of a derivative of the ketone was considered. Conversion of the ketone to the ethylene ketal, 82, was accomplished without difficulty and the spectral data confirmed its formation. The ethylene ketal protons produced a four proton signal in the nmr spectrum at $\tau$ 6.07 and no carbonyl absorption was present in the infrared.

The chloroindolenine, 83, was prepared and immediately reacted with potassium hydroxide in methanol. However, the major compound formed (45% yield) was not the desired imino ether but the oxindole, 84. The infrared spectrum was characteristic of an oxindole system with the NH absorption at 3100 $cm^{-1}$ and ketone absorption at 1700 $cm^{-1}$. Again, the nmr did not possess a sharp three proton signal in the region $\tau$ 6.0-6.5 for the methyl protons of a methyl imino ether system.

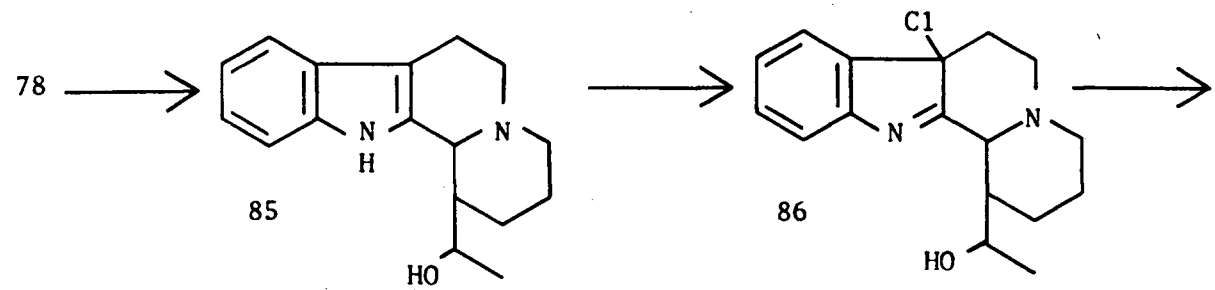
In the hope that yet another derivative of the ketone group would

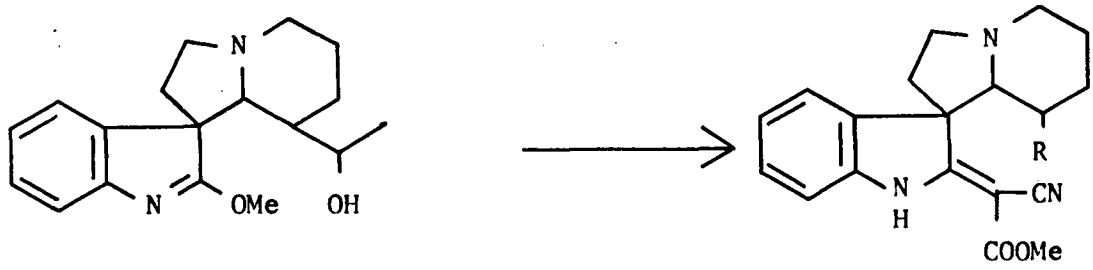



generate the imino ether system, the conversion to an alcohol was considered. Reduction of the ketone to the alcohol, 85, using sodium borohydride was accomplished without difficulty [19]. In addition to the elemental analysis, the molecular formula was established by high resolution mass spectrometry as $C_{17}H_{22}N_2O$ (Found: 270.172; Calcd.: 270.173). The significant feature of the nmr spectrum was a three proton doublet (J = 6 cps) at $\tau$ 8.74 due to the methyl protons on the carbon adjacent to the carbon bearing the alcohol group. In addition to the lack of any carbonyl absorption in the infrared spectrum, there appeared broad bands at 3130 and 3030 $cm^{-1}$ for NH and OH functions.

The chloroindolenine (86) of the tetracyclic alcohol, 85, was formed as before using t-butyl hypochlorite. The ultraviolet spectrum of the crude product exhibited the expected absorption ($\lambda_{max}$ 253 and 290 (sh) mμ), while no indole absorption could be seen. Without purification, this crude

product was immediately reacted with a methanolic alkali solution at reflux temperature for two hours. The crude product was purified by column chromatography on alumina and final purification was accomplished by preparative tlc on silica gel to afford the crystalline imino ether (87), mp 140-143°, in 33% overall yield from the tetracyclic alcohol. In addition to high resolution mass spectrometry, the elemental analysis established the molecular formula, $C_{21}H_{28}N_2O_2$.



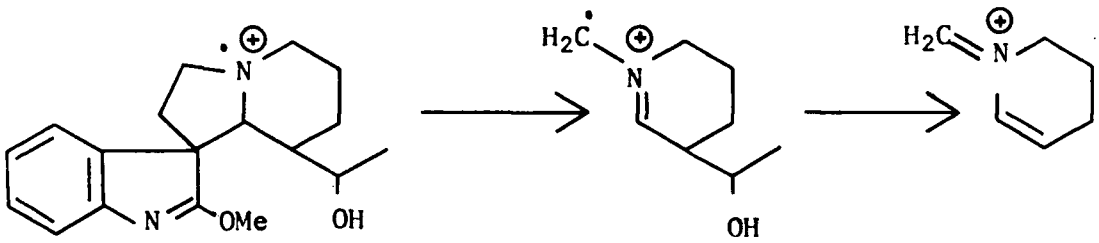

88, R = $CHOHCH_3$

89, R = H

The imino ether lent itself to a straightforward structural analysis due to the presence of certain very characteristic spectroscopic features. The compound possessed indolenine absorption in the ultraviolet region ($\lambda_{max}$ 213 and 253-258 mμ). In addition to the broad absorption band at 3300 $cm^{-1}$ (OH) in the infrared spectrum, there appeared a strong band at 1575 $cm^{-1}$ characteristic of the imino ether function. The nmr spectrum

(Figure 13) exhibited a four proton multiplet centred at τ 2.82 for the aromatic protons of the benzene ring and the protons of the methyl imino ether appeared as a three proton singlet at τ 5.94. A three proton doublet at τ 9.29 (J = 6 cps) was assigned to the methyl protons on the carbon adjacent to the alcohol group.

The mass spectrum of the imino ether (Figure 15) provided further structural evidence. The molecular ion peak was the base peak at the desired value of m/e 300 and the spectrum was dominated by peaks at m/e 141

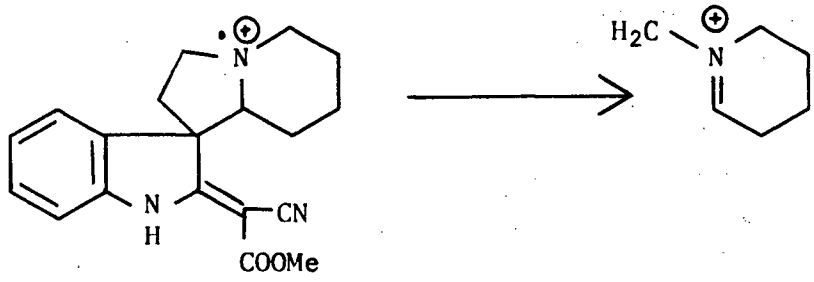



m/e 300 m/e 141 m/e 96

and 96. The fragment at m/e 141 arises from cleavage of the indole system from the piperidine system, while loss of the side chain from the latter would generate the species at m/e 96. Two other significant peaks in the mass spectrum at m/e 282 and 255 are the loss of water and side chain, respectively, from the parent molecule.

Now that the synthesis of the imino ether, 87, was successful, we considered its reaction with appropriate nucleophiles. Thus, 87 was treated with methyl cyanoacetate and triethylamine at 60-65° in a sealed tube for 100 hr. After removing the excess methyl cyanoacetate by distillation at reduced pressure, examination of the crude product showed that it was a complex mixture of compounds. The major compound was isolated with the aid of column chromatography on alumina and crystallization from hexane afforded

crystals, mp 185-188°. The initial spectroscopic evidence, ultraviolet and infrared, suggested that the desired adduct, 88, had formed. The ultraviolet spectrum was that of a typical α-methylene indolenine with maxima at 234 and 334 mμ and the minimum at 258 mμ. In addition to the absorption band at 3190 $cm^{-1}$ (NH) in the infrared spectrum, there appeared two strong absorptions at 2190 and 1680 $cm^{-1}$ for the α,β-unsaturated nitrile and ester groups, respectively. However, the nmr spectrum (Figure 14) indicated this was not the desired adduct, 88, but appeared to be a substance which lacked the side chain on the piperidine ring (e.g., 89). Most importantly, there were no signals which could be attributed to the protons of the side chain. However, the remainder of the spectrum confirmed that the three carbon adduct resulting from condensation with methyl cyanoacetate had formed. Thus, the indolic nitrogen proton absorbed at τ -1.03 and the methyl ester protons were observed as a three proton singlet at τ 6.19. The loss of the side chain was indeed confirmed by the mass spectrum (Figure 16); the molecular ion exhibited a significant peak at




m/e 323 m/e 97

m/e 323, whereas the base peak was at m/e 97 for the piperidine fragment. This fragmentation pattern arose in a manner analogous to that already described for the imino ether, 87, the latter having the corresponding fragments at m/e 141 and 96. The elemental analysis along with a high

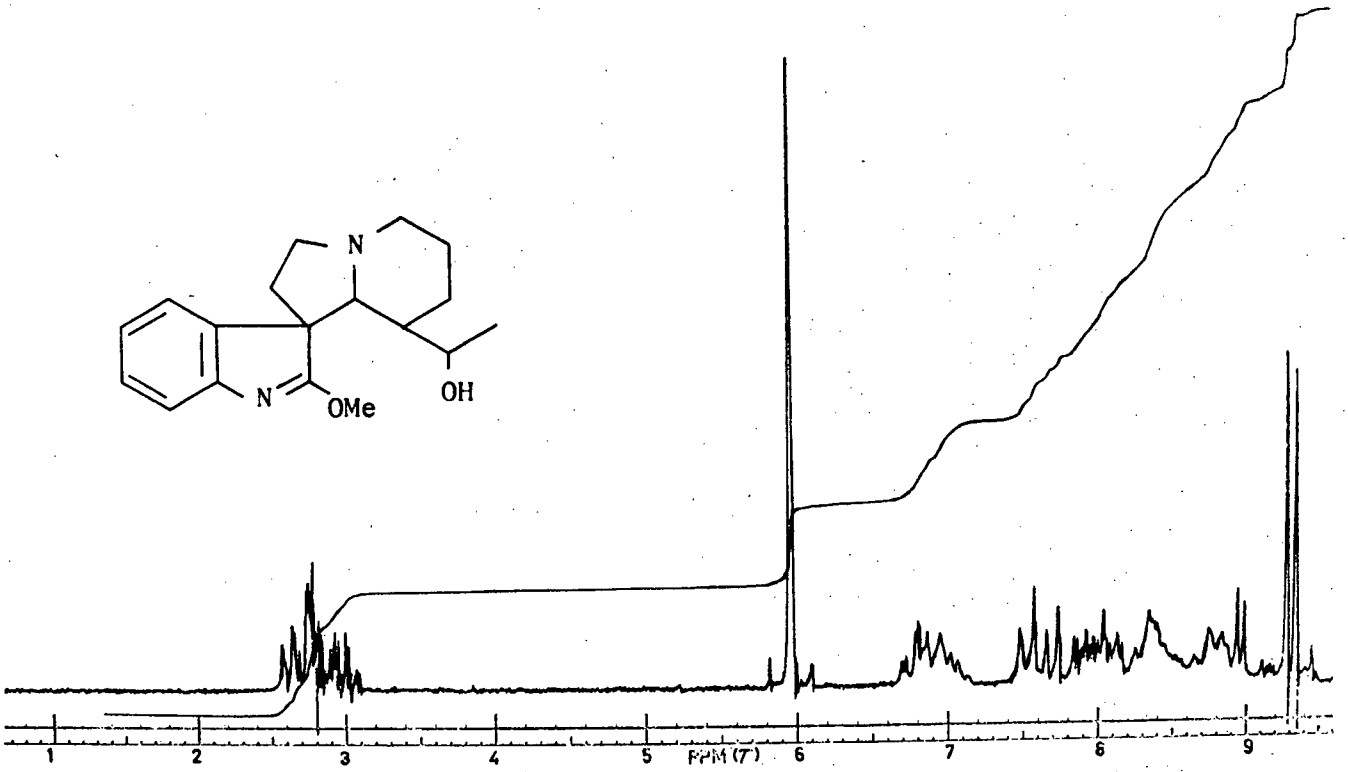



Figure 13. Nmr spectrum of imino ether 87.

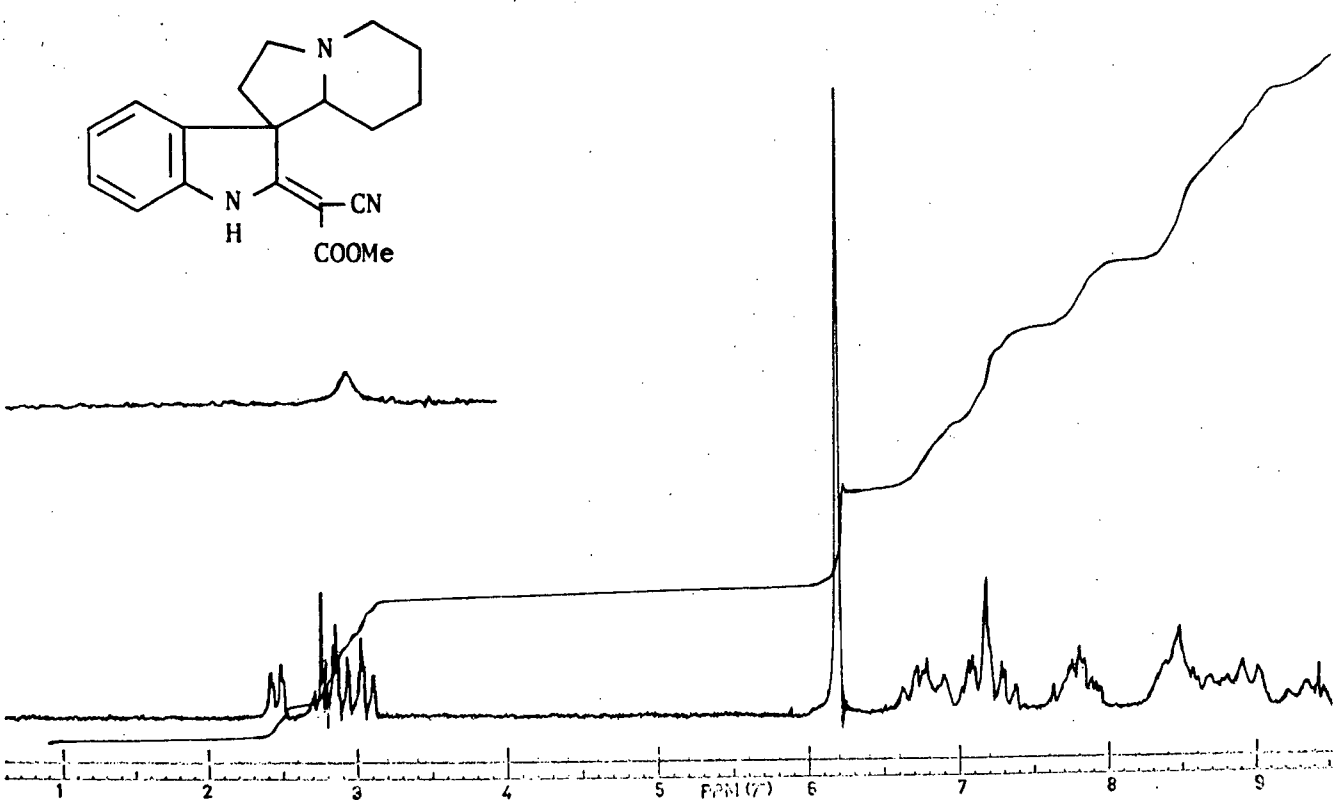



Figure 14. Nmr spectrum of 89.

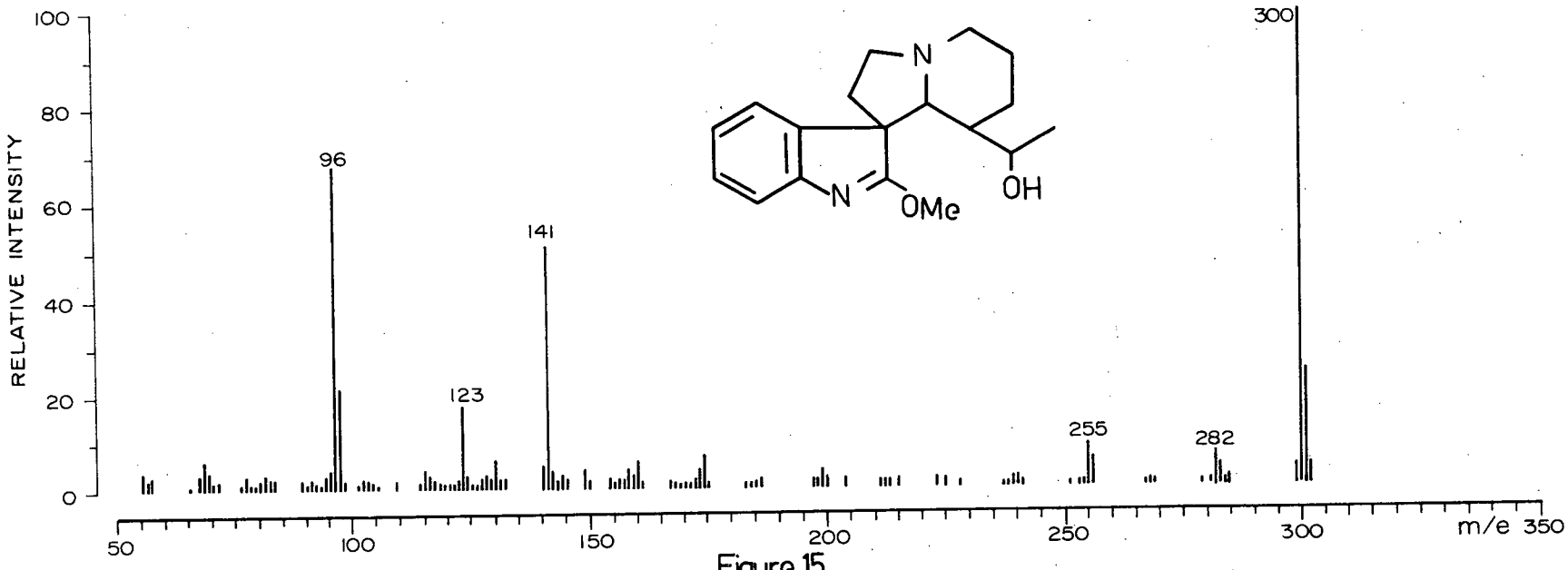



Figure 15

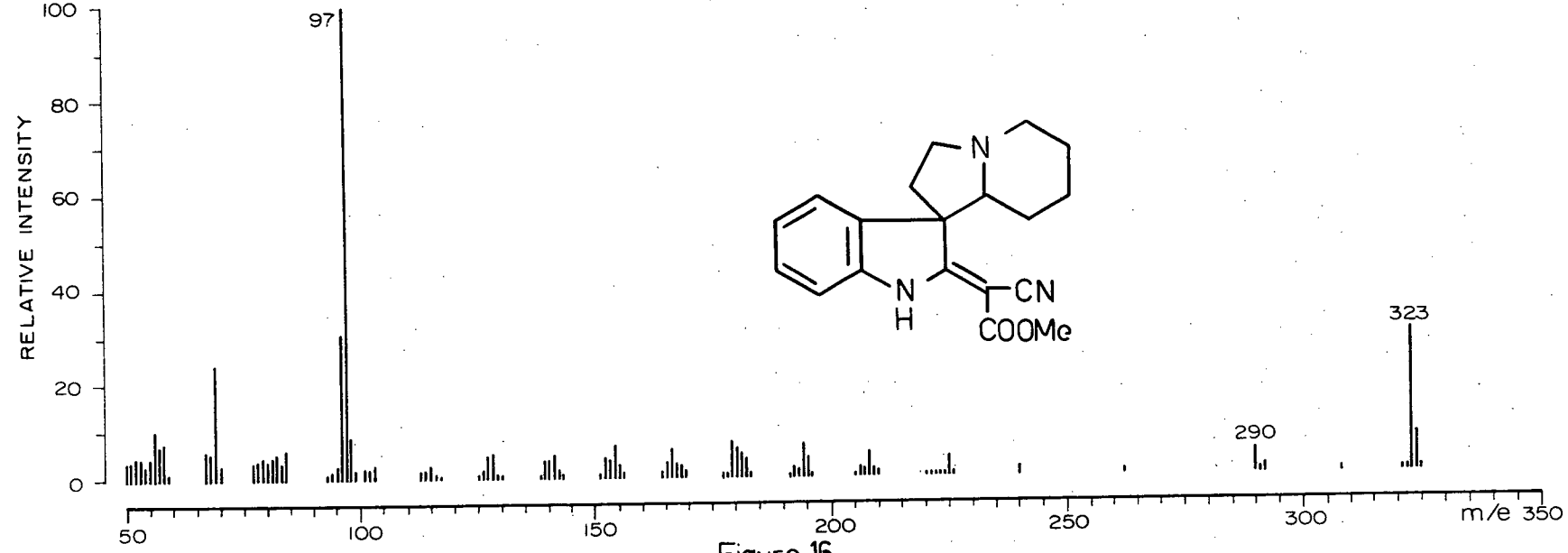



Figure 16

resolution mass spectrum established the molecular formula, $C_{19}H_{21}N_3O_2$, for the adduct, 89.

Two plausible mechanisms can account for the loss of the side chain on the piperidine ring from the desired adduct, 88 (Figure 17). If the loss of this substituent occurs with concurrent opening of the cyclopentane ring, then the indole-enamine intermediate, 90, is generated. This same intermediate, 90, can also arise if the ring opening of the cyclic system involves the nitrogen atom as shown in 88 → 91. The subsequent loss of the side chain is explicable in terms of a retro-Aldol, i.e., 91 → 90. The enamine, 90, could readily rearrange to afford the iminium ion, 92, and the latter undergoes cyclization to provide the final product (89). The cyclization step, 92 → 89 has precedent from our own work, since a similar mechanism is involved in the transannular cyclization of nine-membered ring alkaloids to the corresponding Aspidosperma system, i.e., conversion of vincaminoreine to minovine (Part I of this thesis).

In the hope of isolating the desired adduct, 88, before any loss of the side chain could occur, the sealed tube reaction was conducted in the same manner as before except the time of reaction was reduced. Unfortunately, this investigation was complicated by the fact that the reaction between methyl cyanoacetate and triethylamine afforded a thick oily mixture of compounds whose spectral characteristics were similar to those of the desired adduct. For example, when methyl cyanoacetate and triethylamine were allowed to react in a sealed tube at 60° for 50 hr, the infrared spectrum exhibited absorption bands corresponding to unsaturated nitrile and ester functions, while the ultraviolet had absorption maxima at 265 and 335 mμ. The desired adduct also absorbs at 335 mμ in the ultraviolet region. In addition, this reaction of imino ether, methyl cyanoacetate and triethylamine was extremely difficult to purify as the last traces of methyl

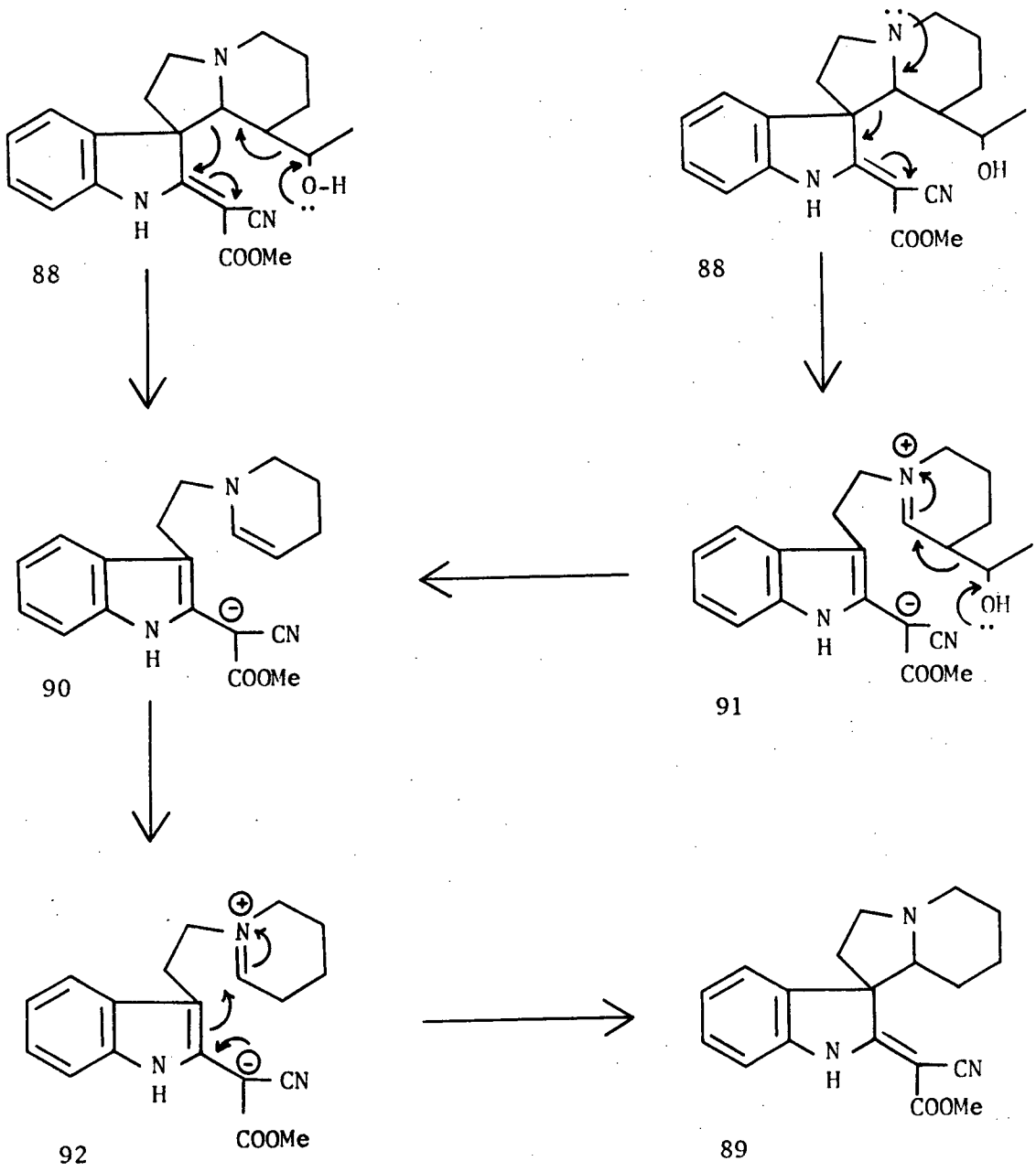
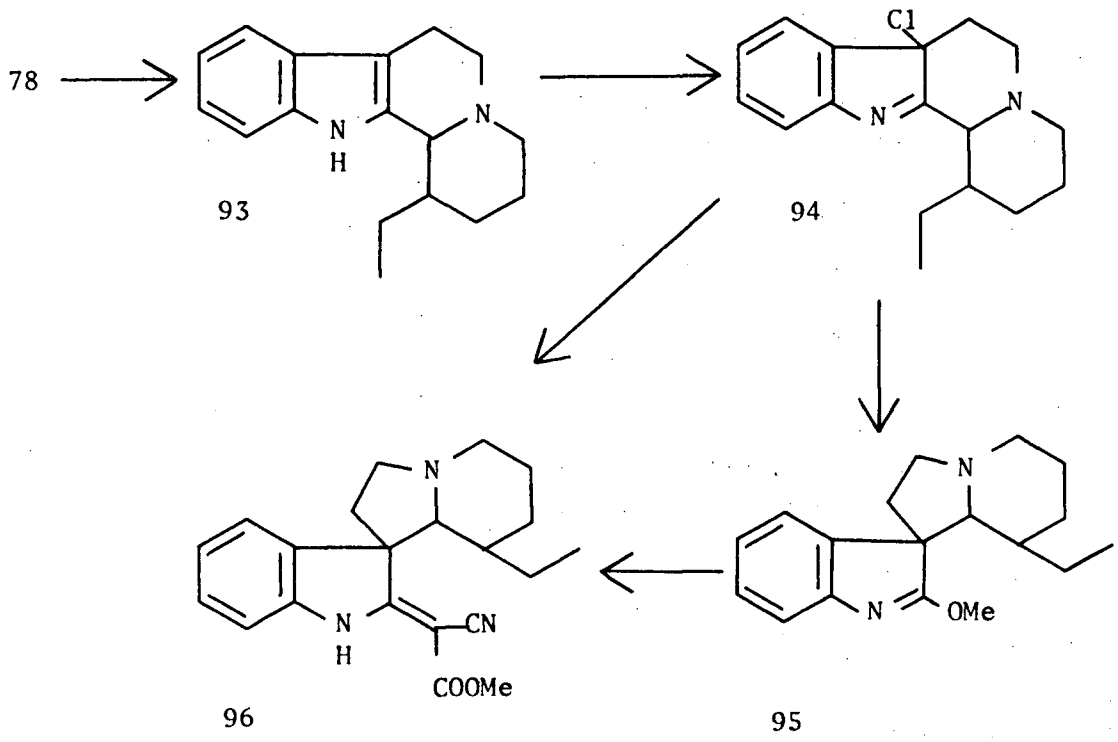



Figure 17. Mechanisms to account for the loss of the side chain of the piperidine substituent.

cyanoacetate were difficult to remove. A number of reactions of different time duration were carried out using sufficient amounts of the imino ether, 87, so that the reaction products could be examined by nmr spectroscopy. However, after purification of the various reaction products, no trace of the desired adduct, 88, could be isolated. The only noticeable feature was the consistently lower yields of the adduct, 89, as the reaction time was reduced.

Since the presence of the oxygen function on the piperidine system was causing numerous problems with the synthetic sequence, its removal became necessary. A number of different methods were studied in order to obtain the most efficient conversion. In one approach, the tetracyclic alcohol, 85, was converted to its tosylate and the latter was subsequently reduced with lithium aluminum hydride. The resulting product from this sequence was the tetracyclic component bearing the ethyl side chain [19]. Another method used was to convert the tetracyclic alcohol to its bromide using 48% aqueous hydrogen bromide and subsequent hydrogenation of the bromide [19]. Finally, a modified Wolff-Kishner reduction of the tetracyclic ketone, 78, was accomplished. This latter procedure was by far the best reaction affording the tetracyclic compound, 93, in 70% yield. This substance corresponded in all respects to that recorded in the literature [19].

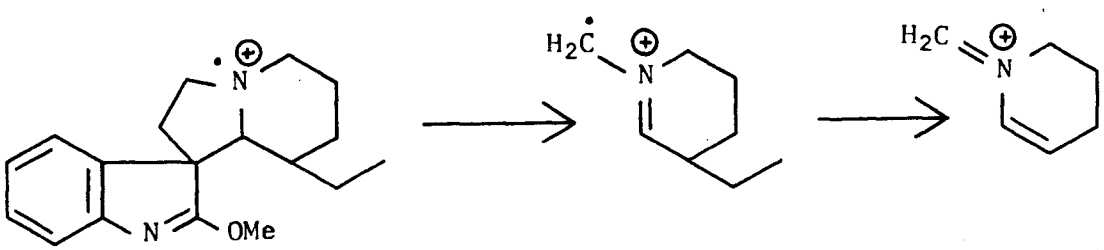
The tetracyclic compound, 93, was treated with a molar amount of t-butyl hypochlorite to give the chloroindolenine, 94. The chloroindolenine was exceptionally stable and could be purified on preparative tlc plate (silica gel) to afford a pure sample in 75% yield. When a methylene chloride solution of this chloro derivative was concentrated to dryness, a yellow amorphous solid was obtained, mp 95-100°, whose infrared spectrum exhibited the expected imine absorption at 1588 $cm^{-1}$ and the ultraviolet spectrum was




characteristic of an indolenine ($\lambda_{max}$ 225, 266 and 293 mμ). The nmr spectrum was significant in that the indolic nitrogen proton was absent while the remainder of the spectrum corresponded to the assigned structure. The mass spectrum of the chloroindolenine had small molecular ion peaks at m/e 288 and 290 in the approximate ratio 3:1 which is in accord with the relative abundance of chlorine. The base peak at m/e 253 corresponded to the fragment remaining after the loss of chlorine. As final evidence that the desired chloroindolenine, 94, had formed without any skeletal rearrangements, this compound was treated with lithium aluminum hydride in ether and the tetracyclic indole, 93, was obtained in quantitative yield.

The chloroindolenine, 94, was treated, as in previous instances, with potassium hydroxide in methanol; however, no imino ether, 95, could be isolated even when the reaction times were extended. The only products obtained were unreacted 94 and the tetracyclic indole, 93. In order to

obtain a more drastic medium, a methanolic solution of sodium methoxide was added to a solution of the chloro derivative in methanol. A number of runs were conducted in order to obtain the optimum reflux time for the formation of the imino ether, 95. After 11 hr at reflux temperature, a complex mixture of compounds was obtained from which the desired imino ether, 95, was isolated as a mixture of isomers in 13% yield. These two isomers behaved identically on all chromatographic systems and the actual presence of isomers could only be determined by nmr spectroscopy. The characterization of the imino ether, 95, was carried out on this mixture of isomers. The infrared spectrum contained no NH absorption while the imino ether system showed strong absorption at 1568 $cm^{-1}$. The ultraviolet spectrum was that of an indolenine system with the maxima at 215 and 254-258 mμ. The significant feature of the nmr spectrum was the presence of a strong three proton signal at τ 5.97 with a much smaller signal at τ 5.92, both attributable to the methyl protons of the methyl imino ether function. As in the case of the other imino ethers prepared,a similar fragmentation pattern in the mass

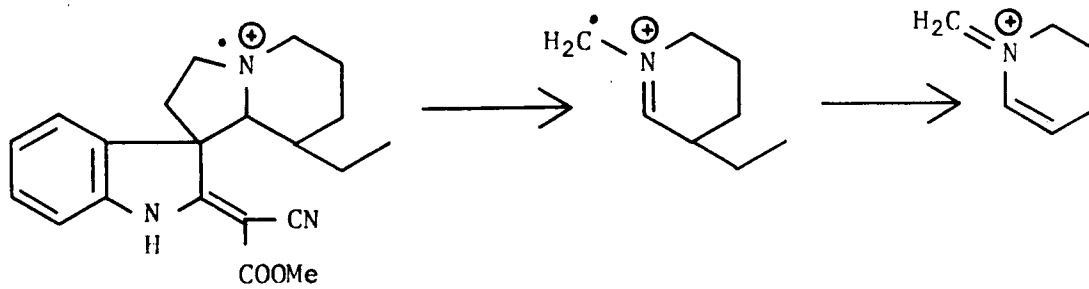



m/e 284 m/e 125 m/e 96

spectrum was evident here: a strong molecular ion at m/e 284 along with the corresponding piperidine fragments at m/e 125 and 96.

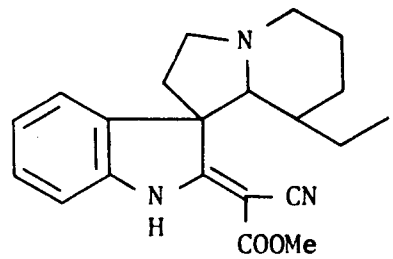
The conversion of the imino ether, 95, using methyl cyanoacetate and triethylamine in a sealed tube was accomplished as before. After purification

by column chromatography followed by preparative tlc on silica gel, the desired adduct, 96, was crystallized from methanol to afford colorless blocks, mp 165-168°. This adduct lent itself to a straightforward spectral analysis. Thus, high resolution mass spectrometry established the molecular formula, $C_{21}H_{25}N_3O_2$ (Found: 351.195; Calcd.: 351.195). The infrared spectrum possessed the NH absorption at 3300 $cm^{-1}$ along with unsaturated nitrile and ester bands at 2210 and 1666 $cm^{-1}$, respectively. The ultraviolet spectrum was that of a normal α-methylene indolenine with maxima at 236, 295 and 335 mμ. The nmr spectrum (Figure 18) showed a broad one proton singlet at τ -1.08, the normal four proton multiplet centred at τ 2.72 for the aromatic protons, a three proton singlet at τ 6.18 for the methyl ester protons and a three proton triplet at τ 9.53 for the methyl protons of the ethyl group. The mass spectrum (Figure 19) was in accord with the structure of the desired adduct having a molecular ion at m/e 351 and the piperidine fragments at m/e 125 and 96.




m/e 351 m/e 125 m/e 96

Now that the desired adduct, 96, was available, attention was directed towards improving the yield of the last steps of the sequence. It was felt that a one-step conversion of the chloroindolenine to the adduct, 96, might be feasible. Thus, reaction of a mixture of the chloroindolenine, methyl cyanoacetate and triethylamine under sealed tube conditions was attempted and encouraging results were noted. A number of other conditions were employed in order to further investigate this reaction. In the hope that a



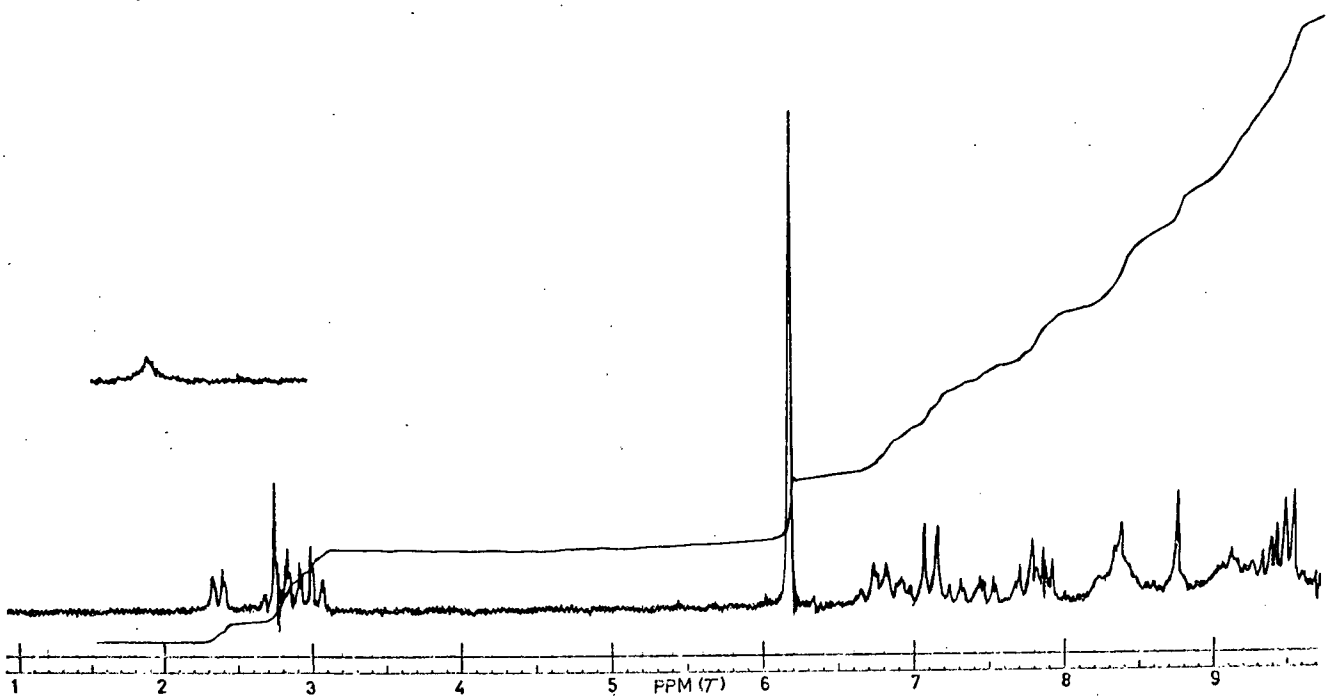




Figure 18. Nmr spectrum of 96.

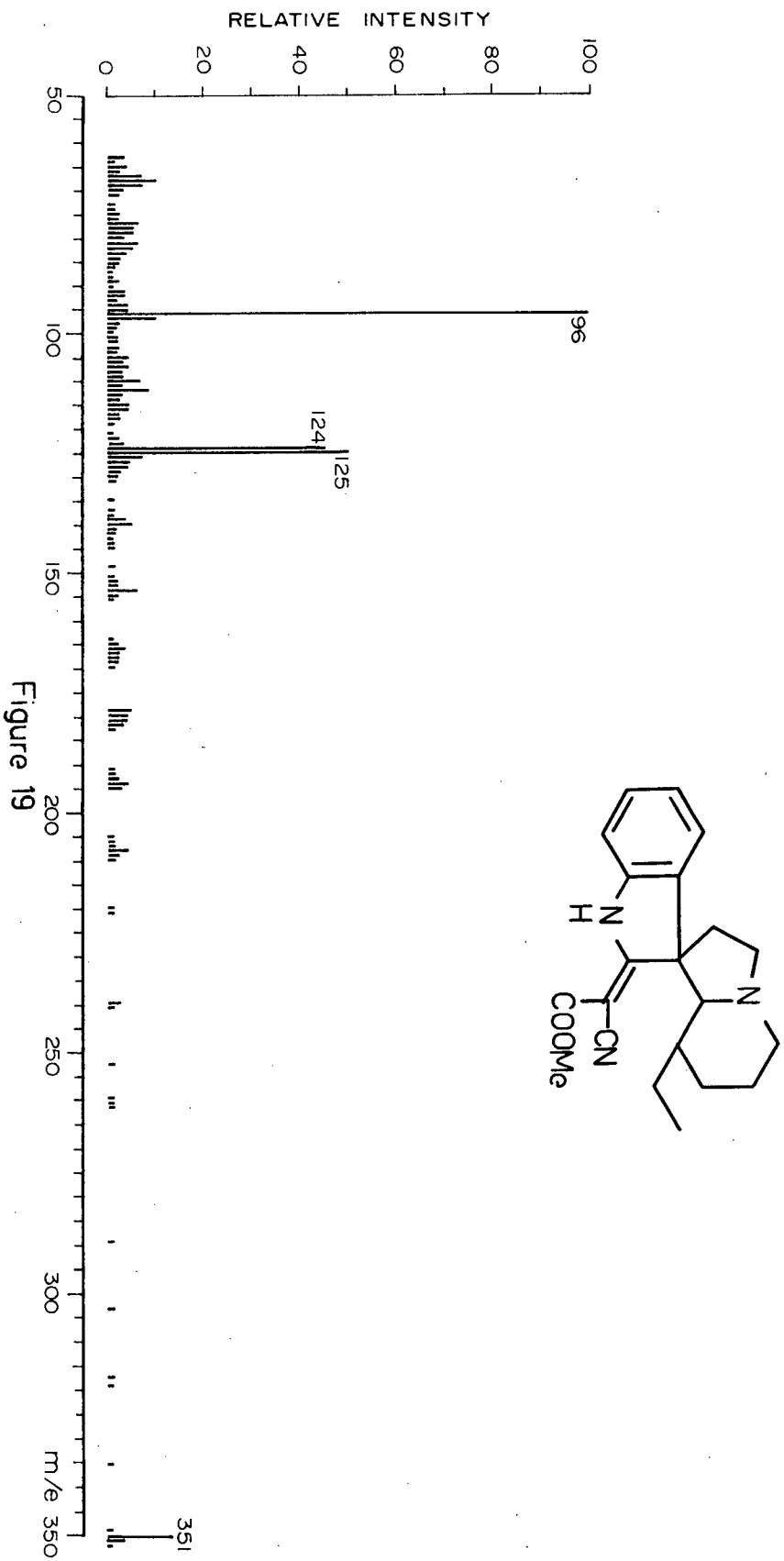

RELATIVE INTENSITY
0
20
40
60
80
100
50
100
150
200
250
300
m/e 350
96
124
125
351
H
N
CN
COOMe
N

Figure 19

polar solvent would assist the condensation, dimethylsulfoxide was used with sodium hydride as the base to form the anion of the methyl cyanoacetate. However, no desired material could be detected. Another series of reactions involved the formation of the sodium salt of methyl cyanoacetate in methyl cyanoacetate and reacting this with the chloroindolenine. These reactions provided only small amounts of the adduct. From all of these experiments, the tetracyclic indole, 93, was the major product (yield 50-80%) with varying small amounts of the chloroindolenine. The maximum yield (8%) of the desired adduct was obtained by heating at 76° a solution of the chloroindolenine in methyl cyanoacetate and triethylamine in a sealed tube for 144 hr. Heating for a shorter period resulted in lower yield, whereas heating for longer periods (454 hr) did not increase the yield. Although this last step of the synthetic sequence proceeded in low yield, sufficient quantities of the adduct, 96, were prepared so that biosynthetic studies could be performed with it. Some of the latter experiments are discussed in the next section of this thesis.

Section B

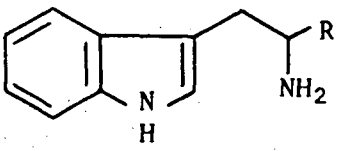
As already mentioned, there are various postulates involving the biogenesis of Indole alkaloids, but the one proposed by Wenkert [13] was of particular interest in our initial considerations, since it relates directly to previous synthetic work in this area. The transannular cyclization reaction developed by Kutney and coworkers, and mentioned in Part I of this thesis, provides a general entry into Iboga, Aspidosperma and Vinca alkaloids[55-58]. The fundamental similarity of this latter process to the later steps in Wenkert's postulates (Figure 3, Part II of this thesis) provided the stimulus for its evaluation as a biosynthetically significant reaction.

For this purpose, the appropriate nine-membered ring intermediates represented by quebrachamine (97) and vincaminoreine (99) were evaluated as

possible precursors of the Aspidosperma and Vinca alkaloids, while the corresponding carbomethoxydihydrocleavamine (105) and carbomethoxycleavamine (106) derivatives were studied for their possible role in the biosynthesis of the Iboga family. Numerous experiments were conducted in Vinca rosea Linn and Vinca minor Linn plants, and a brief resume of the results is presented in Tables I and II.

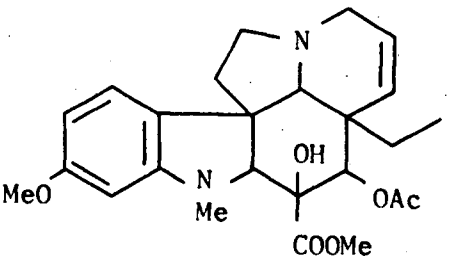
The preparation of the tritium labelled radioactive precursor from the inactive alkaloid was accomplished by exchanging the aromatic protons of the indole system with radioactive tritium atoms. The method developed in these laboratories proved to be very successful for this purpose. Tritium labelled trifluoroacetic acid was used for this acid catalyzed exchange of the aromatic protons and was prepared by reacting molar quantities of trifluoroacetic anhydride and tritium labelled water. A simple vacuum transfer system was used to bring the tritium labelled trifluoroacetic acid into contact with the alkaloid and it was subsequently removed after reaction was complete. It was soon realized that this method for the formation of radioactive alkaloids possessed some significant features: (a) the alkaloids were recovered virtually unchanged from the acidic medium, (b) the method appears general to essentially all indole alkaloids, (c) since a large excess of acid was used, the dilution of the radioactivity in the reaction was very small and the recovered tritium labelled trifluoroacetic acid was suitable for reuse, and (d) the experimental procedure was very simple in its operation.

The experimental method associated with the incorporation of large molecular weight compounds in terms of permeability, etc., was appreciated, and the initial experiments dealt with an evaluation of various techniques for the incorporation of such compounds. Table I illustrates the results from the various methods of feeding. It soon became apparent that no



1, R = COOH

41, R = H



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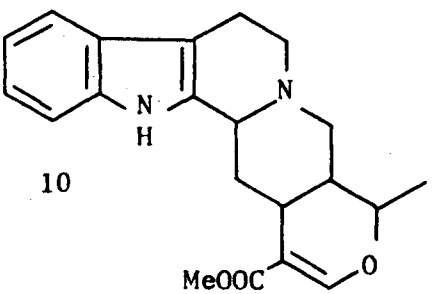

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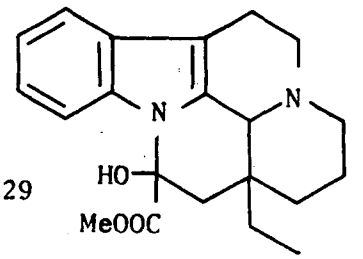

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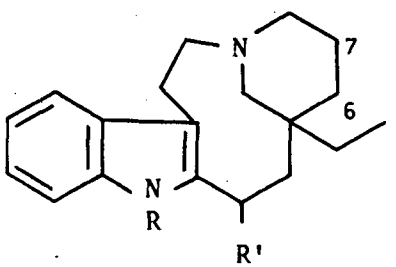
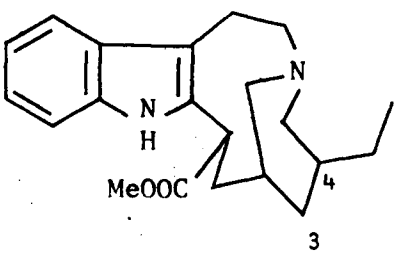


97, R = R' = H

98, R = H; R' = COOMe

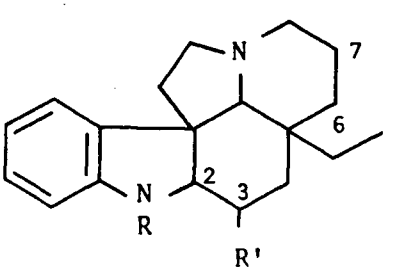
99, R = Me; R' = COOMe

100, R = H; R' = COOMe;
6,7-double bond



105

106, 3,4-double bond

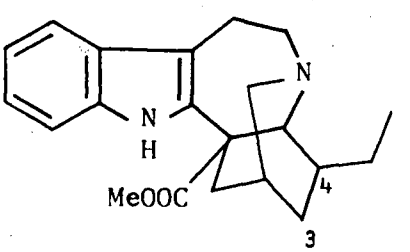


101, R = R' = H

102, R = H; R' = COOMe;
2,3-double bond

103, R = Me; R' = COOMe;
2,3-double bond

104, R = H; R' = COOMe;
2,3- and 6,7-double bonds



107

9, 3,4-double bond

Table I. Results of incorporation of nine-membered ring intermediates into V. rosea L. and V. minor L. by various techniques.

| Exp. No. | Compound Fed | Plant | Feeding Method | Alkaloid Isolated | % Incorporation |
|---|---|---|---|---|---|
| 1 | [ar-$^{3}$H]-carbomethoxycleavamine (106, HCl salt) | V. rosea L. | Cotton wick into stems, 8 days | Catharanthine (9) | <0.015 (0.04)[a] |
| 2 | [ar-$^{3}$H]-carbomethoxycleavamine (106, HCl salt) | V. rosea L. | Vacuum infiltration of leaf discs, 42 hours | Catharanthine | <0.008 |
| 3 | [ar-$^{3}$H]-carbomethoxycleavamine (106, acetate salt) | V. rosea L. | Leaf vein injection, 6 days | Catharanthine | <0.05 |
| 4 | [ar-$^{3}$H]-carbomethoxycleavamine (106, HCl salt) | V. rosea L. | Hydroponic, cut stems, 46 hours | Catharanthine | <0.011 |
| 5 | 18β-carbomethoxy($^{14}$C)-4α-dihydrocleavamine (105, HCl salt) | V. rosea L. | Hydroponic, cut stems, 7 days | Coronaridine (107) | <0.015 |
| 6 | 18α-carbomethoxy($^{14}$C)-4α-dihydrocleavamine (105, HCl salt) | V. rosea L. | Hydroponic, cut stems, 5 days | Coronaridine | inactive |
| 7 | [ar-$^{3}$H]-quebrachamine (97) [59] | V. minor L. | Hydroponic, cut stems, Tween 20 emulsion | Aspidospermidine (101) | 0.48 (3.4)[a] |
| 8 | [ar-$^{3}$H]-quebrachamine (97, HCl salt) [59] | V. minor L. | Hydroponic, cut stems, 20 days | Aspidospermidine | 0.08 (0.03)[a] |
| 9 | [ar-$^{3}$H]-vincaminoreine (99, acetate salt) [59] | V. minor L. | Absorption through leaf sections, 4 days | Vincamine (29)<br>Aspidospermidine<br>Minovine (103) | <0.001<br><0.008<br>0.7 (0.3)[a] |

Table I. Results of incorporation of nine-membered ring intermediates into V. rosea L. and V. minor L. by various techniques (continued).

| Exp. No. | Compound Fed | Plant | Feeding Method | Alkaloid Isolated | % Incorporation |
|---|---|---|---|---|---|
| 10 | [ar-$^{3}$H]-vincadine (98) [59] | V. minor L. | Absorption through leaf sections, 4 days | Vincamine<br>Aspidospermidine<br>Minovine | 0.08 (0.06)[a]<br><0.003<br>inactive |

[a] Values in parentheses refer to blank experiments which were conducted under similar conditions to those involved in the plant feedings.

particular technique showed any obvious advantage over the others. In experiments 1-4, carbomethoxycleavamine (106) was administered to V. rosea L. plants by a number of different methods and one of the major alkaloids, catharanthine (9), was isolated. Experiments 5 and 6 were conducted in order to compare the incorporations of the isomeric carbomethoxydihydrocleavamines into coronaridine (107) by V. rosea plants. Again the incorporations were not significant. The most frustrating aspect of these results was the inability to delineate what might be construed as a "positive" demonstration of the transannular cyclization process from the rather trivial oxygen-catalyzed conversion of the intermediates to the alkaloids during the period of incorporation. The conversion of these compounds to the appropriate alkaloids by oxygen in the presence of a metal catalyst has already been discussed in Part I of this thesis. It was hoped that a much higher level of incorporation in the plants relative to the blank experiment could be obtained.

In an attempt to obtain internally consistent data which may shed more light on the cyclization reaction, our attention was turned to a series of experiments in which identical conditions were maintained throughout the entire series. For this purpose, 6-month old V. rosea L. plants were selected and the incorporation of the appropriate precursor was administered by the cotton wick technique into the stems of the plant. In each instance, the number of plants fed was sufficient to provide the direct isolation of the alkaloids which, without any further dilution with "cold" material could be crystallized to constant activity. Conversion of each of these into the corresponding hydrochloride salts confirmed the level of radioactivity. By this sequence, even very low levels of incorporation could be easily detected. The pertinent results for catharanthine (9), vindoline (7), and ajmalicine (10) are summarized in Table II, and a brief analysis of these

is appropriate.

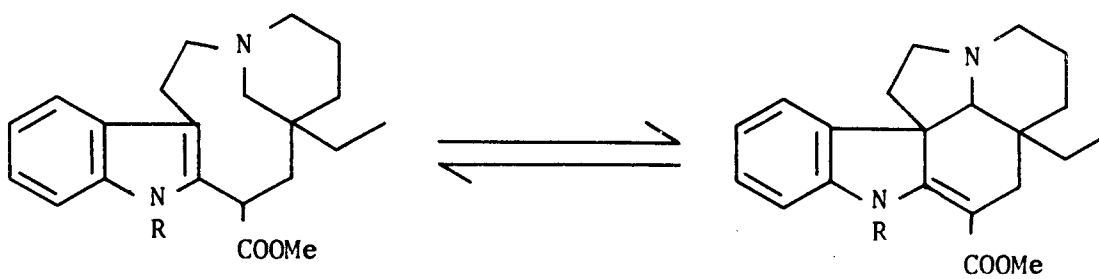
Table II. Results of incorporation of various intermediates into V. rosea L. under identical conditions.

| Expt. No. | Compound Fed | % Incorporation | | |
|---|---|---|---|---|
| | | Catharanthine | Vindoline | Ajmalicine |
| 11 | DL-tryptophan-[3-$^{14}$C] (1) | 0.05 | 0.15 | 0.8 |
| 12 | [ar-$^{3}$H]-tryptamine (41) | 0.01 | 0.003 | 0.4 |
| 13 | [ar-$^{3}$H]-100 | <0.001 | <0.001 | <0.001 |
| 14 | [ar-$^{3}$H]-tabersonine (104) | 0.05 | 0.03 | <0.001 |
| 15 | [ar-$^{3}$H]-carbomethoxy-cleavamine (106) | 0.03 | <0.001 | inactive |
| 16 | [ar-$^{3}$H]-carbomethoxy-cleavamine (106)--- blank experiment | 0.04 | | |

Experiments 11 and 12 illustrate that (a) the age of the plants selected for this study was suitable for biosynthesis, and (b) the experimental method chosen at least provides positive incorporation of established precursors. Experiments 13 and 14 provide an important comparison between two closely related compounds in their role as potential precursors in the biosynthetic pathway. While the alkaloid, tabersonine (104), is converted into catharanthine and vindoline, the 6,7-dehydrovincadine derivative (100) is not incorporated. The latter compound is the immediate precursor of this alkaloid (104) in the laboratory conversion which utilizes the trans-annular cyclization reaction. The incorporation of tabersonine into these alkaloids furthermore establishes that the experimental method employed allows the incorporation of higher molecular weight "precursors" into the

plant system. The level of incorporation of the cleavamine derivative, 106, into the Iboga system is also negligible, as shown in experiments 15 and 16. All of these experiments [60] strongly suggest that the transannular cyclization reaction is probably not significant in either Aspidosperma or Iboga biosynthesis, although it is clear that negative results must be interpreted with caution.

In the hope of obtaining more distinctly positive results, a completely different approach to this problem was studied. It is clear that the transannular cyclization process as illustrated in the conversion of the alkaloid, vincadine (98), to vincadifformine (102), a reaction easily accomplished in the laboratory [61], is only one of a number of alternative pathways in the plant elaboration of Aspidosperma alkaloids. An equally attractive and plausible scheme could invoke the reverse process, namely the ring opening of the pentacyclic system to yield the nine-membered ring alkaloids (i.e., 102 → 98). Indeed, such a ring-opening process readily occurs in the




98, R = H
99, R = Me

102, R = H
103, R = Me

laboratory as discussed in Part I of this thesis. This latter process would imply that Aspidosperma alkaloids of type 102 are biosynthetic precursors of type 98. In order to obtain information on the relationship, if any, between these alternatives, a study was initiated in Vinca minor L., a plant which possesses a wonderful array of Aspidosperma alkaloids [62].

Table III. Results of incorporation of DL-tryptophan-3-$^{14}$C into Vinca minor L. at various time intervals.

| Time | Total % Incorporation: Vincadine (98) + vincaminoreine (99) (A) | Vincadifformine (102) + minovine (103) (B) | B/A |
|---|---|---|---|
| 4 hr | 0.003 | 0.057 | 19 |
| 1 day | 0.015 | 0.24 | 16 |
| 2 days | 0.010 | 0.21 | 21 |
| 4 days | 0.010 | 0.22 | 22 |
| 7 days | 0.009 | 0.13 | 14 |
| 14 days | 0.003 | 0.06 | 20 |

A detailed investigation involving the incorporation of DL-tryptophan-3-$^{14}$C into V. minor L. over different time intervals was undertaken, and some of the results are summarized in Table III. The method involved incorporation of a solution of the amino acid in 0.1 N acetic acid containing a few drops of methanol,and after the appropriate time, the isolation of the alkaloids was carried out by chromatographic techniques. In each time interval reported, the experiment was repeated at least twice. There was surprisingly good agreement between the results obtained in the individual experiments, and Table III gives the average values obtained in these studies. For the purposes of this discussion, the total percent incorporation into the nine-membered ring alkaloids, vincadine (98) and vincaminoreine (99), and their respective cyclic relatives, vincadifformine (102) and minovine (103), is presented. The fourth column in Table III shows the relative ratio of activities between these two groups.

A critical analysis of these results reveals several interesting

features. These are (a) activity in the alkaloids is noted even after a short exposure of 4 hr, (b) the activity in the pentacyclic alkaloids (102 and 103) is consistently higher than in the nine-membered ring system, and (c) the relative ratio of activities (B/A) is remarkably similar over the time interval, 4 hr - 2 weeks. This latter finding is certainly the most important in terms of providing information about the later stages of Aspidosperma alkaloid biosynthesis. The lack of any tendency for the ratio B/A to progressively increase or decrease with time speaks strongly against any biosynthetic relationship between the two groups of alkaloids. In other words, the previous suggestion that the transannular cyclization is not biosynthetically important is now strongly supported by the above results. Furthermore, the ring-opening process (102 → 98) as mentioned above is similarly unimportant in providing a pathway to the nine-membered ring alkaloids found in V. minor L. It is clear that such an interpretation of the results is valid only if an equilibrium in the plant between the two alkaloid groups can be excluded. The rather constant B/A could be obtained if an equilibrium mixture were enriched with respect to the pentacyclic alkaloids, vincadifformine (102) and minovine (103), to the extent of approximately 20:1. An evaluation of this process was, thereby, in order. The incorporation of various nine-membered ring alkaloids into V. minor L. was studied, and some experiments in this direction have been already discussed. An approach from the opposite direction was also studied when the radioactive alkaloid, minovine (103), was incorporated into this plant (Feeding Experiment No. 17). In a typical experiment, during which radioactive 103 was administered over a one-week period, the subsequent investigation of the isolated plant alkaloids showed essentially no activity in the nine-membered ring compounds (B/A >2500). On this basis, the above equilibration process is not significant in V. minor L. In turn, the

conclusion must be made that the genesis of the quebrachamine-vincadine family is independent of the pathway leading to the rigid pentacyclic Aspidosperma series (102, 103, etc.) [63].

The question as to how the present results fit into the biosynthetic pattern which is rapidly evolving from the combined data of other investigations is worthy of comment. Wenkert [19] suggested that "all indole alkaloids of the tryptamine + $C_{10}$ structure type may be derived from corynantheinoid or closely related progenitors". Based on elegant experiments in his and other laboratories, Battersby [37] was able to postulate the possible role of structures such as 38 in the biosynthesis of corynantheinoid as well as Aspidosperma and Iboga bases. From very recent reports this latter postulate has been supported by the isolation [42,43,45] of such a structure, strictosidine (vincoside) (38), and its incorporation into the Aspidosperma and Iboga bases [45,46]. The possible intermediacy of units similar in structure to the alkaloid, stemmadenine (109), was involved in the sequence between the corynantheinoid and Aspidosperma bases [13,19], and again recent work [64] on germinated Vinca rosea L. seeds supports its role in this regard. The conversion of the alkaloid, tabersonine (104), to vindoline and most interestingly to the Iboga alkaloid, catharanthine, has been demonstrated in V. rosea L. plants in our laboratory and also by Scott [64] in germinated seeds. This latter result suggests a possible relationship between the Aspidosperma and Iboga alkaloids. Scott in the same paper studied the technique of short-term germination of V. rosea L. seeds and found that the order of formation of identifiable alkaloids was Corynanthe, Aspidosperma and Iboga. In summation, all of these results strongly suggest, but do not prove, that the sequential formation of the indole alkaloids may follow the order: Corynanthe → Aspidosperma → Iboga.

The results of the present investigation relate to the possible structural units which bear on the Corynanthe → Aspidosperma pathway. An attractive sequence (Figure 20) which is in accord with present findings may be postulated (strictosidine (38) → 108 → 109 → 110 → vincadine (98), vincadifformine (102), catharanthine (9), etc.). The intermediates and mechanisms involved represent an important modification of Wenkert's original theory [13,19], particularly with regard to sequence, oxidation level and mechanism, without, however, detracting from the essential correctness of his views on the interrelationships of the main classes of indole alkaloids. The rearrangement of the double bond in stemmadenine (109) and subsequent bond fission will provide the intermediate, 110. A similar postulate for the formation of 110 has been advanced by Scott [64]. This latter intermediate may then elaborate to the Aspidosperma and Iboga bases by the formal bond formations as indicated. The relative order of the latter processes relates directly to the results presented in this thesis. From the tryptophan incorporations discussed above, it is attractive to postulate that the independent biosynthetic pathways which lead to the vincadine and vincadifformine groups may initiate from a common intermediate such as 110. The eventual elaboration of the alkaloid systems depends merely on the relative order in which the bonds are formed. Thus, the process a → b converts 110 to vincadine, etc. The relative insignificance of the transannular cyclization process now suggests that the process c → d occurs prior to or simultaneously with a → b in the elaboration of 110 to vincadifformine. In similar fashion, the conversion of unit 110 to the alkaloid, catharanthine, is unlikely to proceed initially via the process a → e, since this would lead to a carbomethoxycleavamine (106) system. Our results have shown that we were unable to demonstrate the conversion of the latter to this Iboga alkaloid.

Having investigated the transannular cyclization reaction with respect

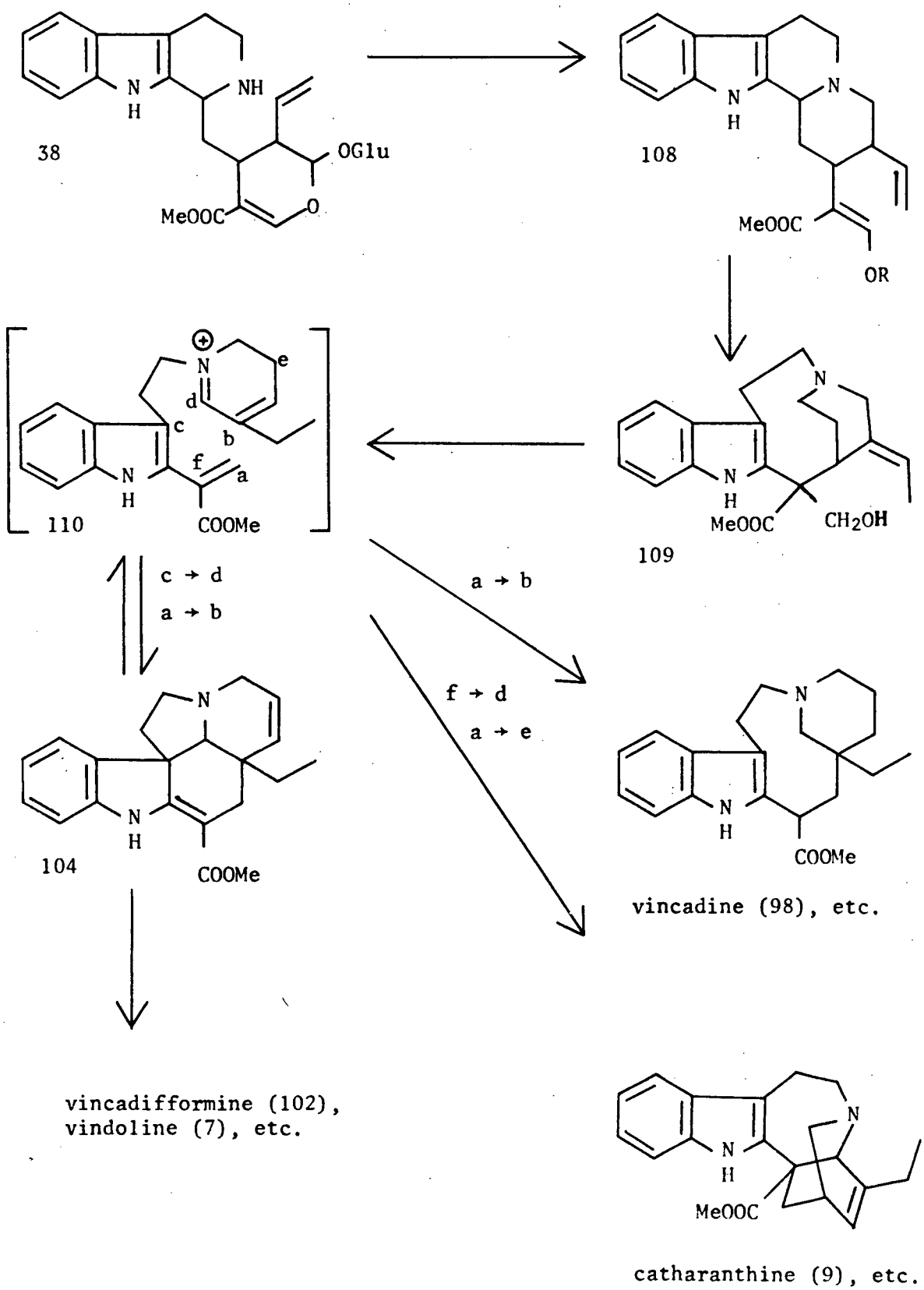
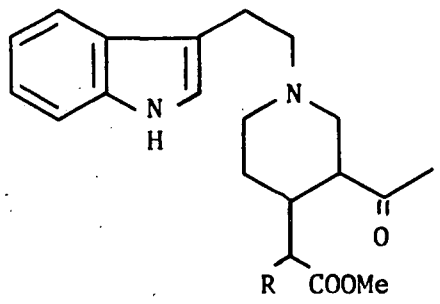
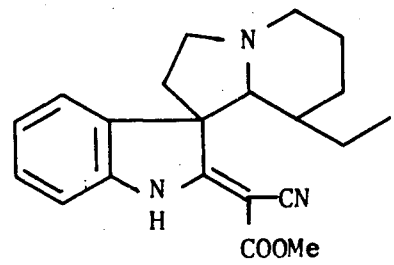



Figure 20. Some later stages of Indole alkaloid biosynthesis.

to its role in indole alkaloid biosynthesis, our attention was directed towards studying other aspects of the later stages of indole alkaloid biosynthesis using the totally synthetic substances discussed in Section A as potential precursors. The initial studies using the structural skeleton represented by the cyanoacetic ester analogue, 52, and malonic ester intermediate, 53, were conducted so that another feature of the biogenetic postulates of Wenkert (Figure 3) could be tested. Wenkert had postulated that structurally related intermediates could be the precursors of all the indole alkaloids of the tryptamine + $C_{9-10}$ type. Experiments 18-24 (Table IV) reveal briefly the results of the investigations which utilize the synthetic substances, 52 and 53, as potential precursors. From experiments 23 and 24,




96

52, R = CN
53, R = COOMe

it can be noted that the cyanoacetic ester analogue, 52, was incorporated at a low level into ajmalicine, while the malonic ester intermediate, 53, was not utilized. Clearly, the former substance cannot be seriously considered as a precursor, although the nitrile function can probably be converted into an aldehyde or similar grouping in the plant.

The other synthetic substance used as a potential precursor is represented by the structure, 96. Its possible relevance stems from a consideration of the previous biosynthetic results already discussed. These results showed that the transannular cyclization process is not biosynthetically important

Table IV. Results of incorporation of synthetic intermediates into V. rosea L. and V. minor L. by various techniques.

| Exp. No. | Compound Fed | Plant | Feeding Method | Alkaloid Isolated | % Incorporation |
|---|---|---|---|---|---|
| 18 | [ar-$^{3}$H]-52 (HCl salt) | V. minor L. | Hydroponic, cut stems, 5 days | Vincamine (29) | inactive |
| | | | | Quebrachamine (97) | inactive |
| | | | | Vincadine (98) | inactive |
| | | | | Minovine (103) | inactive |
| 19 | [ar-$^{3}$H]-53 (HCl salt) | V. minor L. | Hydroponic, cut stems, 7 days | Vincamine | inactive |
| | | | | Quebrachamine | inactive |
| | | | | Vincadine | inactive |
| | | | | Minovine | inactive |
| 20 | [ar-$^{3}$H]-53 (HCl salt) | V. rosea L. | Dissolved in 10% DMSO in ethanol and painted on leaves, 5 days | Catharanthine (9) | inactive |
| 21 | [ar-$^{3}$H]-53 | V. rosea L. | Dissolved in 10% DMSO in ethanol and painted on leaves, 6 days | Catharanthine | inactive |
| | | | | Ajmalicine (10) | inactive |
| | | | | Vindoline (7) | inactive |
| 22 | DL-Tryptophan-[3-$^{14}$C] (1) | V. rosea L. | Dissolved in 10% DMSO in ethanol and painted on leaves, 9 days | Catharanthine | 0.009 |
| | | | | Ajmalicine | 0.003 |
| | | | | Vindoline | 0.005 |
| 23 | [ar-$^{3}$H]-52 (acetate salt) | V. rosea L. | Cotton wick into stems, 9 days | Vindoline | <0.001 |
| | | | | Catharanthine | <0.001 |
| | | | | Ajmalicine | 0.004 |
| 24 | [ar-$^{3}$H]-53 (acetate salt) | V. rosea L. | Cotton wick into stems, 9 days | Vindoline | <0.001 |
| | | | | Catharanthine | <0.001 |
| | | | | Ajmalicine | <0.001 |

Table IV. Results of incorporation of synthetic intermediates into V. rosea L. and V. minor L. by various techniques (continued).

| Exp. No. | Compound Fed | Plant | Feeding Method | Alkaloid Isolated | % Incorporation |
|---|---|---|---|---|---|
| 25 | [ar-$^{3}$H]-96 (acetate salt) | V. minor L. | Hydroponic, cut stems, 2 days | Vincamine<br>Minovine | inactive<br>inactive |
| 26 | [ar-$^{3}$H]-96 (acetate salt) | V. rosea L. | Cotton wick into stems, 9 days | Vindoline<br>Catharanthine<br>Ajmalicine | inactive<br>inactive<br>inactive |

in either Aspidosperma or Iboga biosynthesis. Furthermore, the ring-opening process is similarly unimportant in providing a pathway to the nine-membered ring alkaloids found in V. minor L. Thus, the conclusion must be made that the genesis of the quebrachamine-vincadine family is independent of the pathway leading to the rigid pentacyclic Aspidosperma and Iboga series. From this result, it can be postulated that these independent pathways may initiate from a common intermediate such as 110 (Figure 20). The eventual elaboration of the alkaloid systems depends merely on the relative order in which the bonds are formed. The relative insignificance of the transannular cyclization process suggests that the process c → d occurs prior to or simultaneously with a → b in the conversion of 110 to the Aspidosperma system. In order to test this theory, a study was initiated in which totally synthetic substances, representing an intermediate having the c → d bond formed in 110, could be evaluated as potential precursors. Experiments 25 and 26 (Table IV) using V. minor L. and V. rosea L. plants, respectively, reveal briefly the results of some of our initial investigations using the synthetic precursor, 96. This synthetic precursor has the c → d bond preformed in 110 and may be of interest as a precursor of the Aspidosperma family of alkaloids, even though its functionality is not completely appropriate. Although our initial results are negative with respect to incorporation of this structural system, the conclusion cannot be made that intermediates possessing the skeletal features inherent in 96 are not on the biosynthetic pathway. Clearly, the structure, 96, can be modified so that a more plausible precursor of the same carbon skeleton can be obtained. Such a modification would be to introduce a degree of unsaturation into the piperidine ring and conversion of the nitrile function into an aldehyde or an olefin. Some of these investigations are now underway in our laboratory.

## Experimental

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet (uv) spectra were recorded in methanol on a Cary 11 recording spectrometer, and the infrared (ir) spectra were taken on Perkin-Elmer Model 21 and Model 137 spectrometers. Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform at 100 megacycles per second (unless otherwise indicated) on a Varian HA-100 instrument and the chemical shifts are given in the Tiers $\tau$ scale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution molecular weight determinations were determined on an AE-MS-9 mass spectrometer. Analyses were carried out by Mr. P. Borda of the microanalytical laboratory, the University of British Columbia. Woelm neutral alumina and Silica Gel G (acc. to Stahl) containing 2% by weight of General Electric Retma p-1, Type 188-2-7 electronic phosphor were used for analytical and preparative thin-layer chromatography (tlc). Chromatoplates were developed using the spray reagents, carbon tetrachloride-antimony pentachloride 2:1 or 35% sulfuric acid saturated with ceric sulfate. Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise indicated).

Radioactivity was measured with a Nuclear-Chicago Mark 1 Model 6860 Liquid Scintiallation counter in counts per minute (cpm). The radioactivity of a sample in disintegrations per minute (dpm) was calculated using the counting efficiency which was determined for each sample by the external standard technique [65] utilizing the built-in barium-133 gamma source.

The radioactivity of the samples was determined using a scintillator solution made up of the following composition: toluene (1 litre), 2,5-diphenyloxazole (4 g) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (0.05 g). In practice, a sample of an alkaloid as the free base was dissolved in benzene (1 ml) in a counting-vial,or in the case of the salt of an alkaloid, the sample was dissolved in methanol (1 ml) in a counting-vial,and then in both cases, the volume was made up to 15 ml with the above scintillator solution. For each sample counted, the background was determined for the counting-vial to be used by filling the vial with one of the above scintillator solutions and counting (3 x 100 min) to determine the background cpm. The counting-vial was emptied, refilled with the sample to be counted and the scintillator solution and counted (3 x 200 min). The difference in cpm between the background count and the sample count was used for subsequent calculations.

Tryptophyl bromide (47)

A solution of phosphorus tribromide (0.56 ml) in ether (10 ml) was added (46, 2.8 g) dropwise to an ice-cold solution of tryptophol in ether (140 ml). After 15 hr, the supernatant was decanted, washed with sodium bicarbonate solution (2 x 100 ml), water (100 ml) and dried over sodium sulfate. Removal of the solvent yielded the product as white crystals (3.1 g), mp 101-102° (Lit [47] mp 90-95°).

3-Acetylpyridine ethylene ketal (49) [66]

A solution of 3-acetylpyridine (48, 60 g), ethylene glycol (40.5 g) and p-toluene sulfonic acid hydrate (105 g) in benzene (250 ml) was heated under reflux for 17 hr with a Dean-Stark apparatus to remove water. The mixture was poured into excess aqueous sodium bicarbonate solution, the layers separated and the aqueous phase extracted with benzene (3 x 150 ml).

The combined extracts were washed with sodium bicarbonate solution (2 x 150 ml), water (150 ml) dried (sodium sulfate) and evaporated. Distillation gave the product (63.3 g): bp 165°/88 mm; nmr: τ 2.5 (multiplet, 4H, aromatic), 6.1 (multiplet, 4H, ketal) and 8.35 (singlet, 3H, C-$CH_3$).

N-[β-(3-Indolyl)ethyl]-3-acetylpyridinium ethylene ketal bromide (50)

Tryptophyl bromide (47, 3.1 g) and 3-acetylpyridine ethylene ketal (49, 6.3 ml) were heated at 80° under nitrogen for 8 hr. Addition of ether (20 ml) to the cooled reaction mixture yielded a precipitate whose crystallization from methanol afforded pure 50 (3.9 g), mp 210-211° (Lit [47] mp 209-210°).

N-[β-(3-Indolyl)ethyl]-3-acetyl-$\Delta^3$-piperideine (51)

A suspension of sodium borohydride (10 g) in methanol (150 ml) was added to a solution of the salt (50, 3.3 g) in methanol (50 ml). After 2 hr at room temperature, the solution was evaporated almost to dryness and water was added (400 ml). The solution was acidified with hydrochloric acid to pH 2, and stirred at room temperature for 24 hr. The solution was neutralized with sodium bicarbonate and extracted with methylene chloride (3 x 100 ml). The combined extracts were washed with water (100 ml), dried over sodium sulfate, and evaporated to give an orange oil (2.3 g) which was crystallized from methanol to afford colorless crystals of 51 (1.1 g): mp 185-190°; $\nu_{max}$ ($CHCl_3$): 3465 (NH), 1662 (C=O) $cm^{-1}$; $\lambda_{max}$ (log ε): 222 (4.48), 275 (3.60), and 283 (3.63) mμ; nmr: τ 1.9 (singlet, 1H, NH), 2.8 (multiplet, 6H, aromatic and olefine) and 7.72 (singlet, 3H, CO-$CH_3$) (Lit [47] mp 185-190°).

N-[β-(3-Indolyl)ethyl]-3-acetyl-4-(2-methyl cyanoacetate)-piperidine (52)

α,β-Unsaturated ketone (51, 0.10 g) was added to a solution of methyl

cyanoacetate (0.185 g), methanol (0.03 ml) and triethylamine (0.01 ml), and the mixture was stirred at room temperature, under an atmosphere of nitrogen for 5 days. As much as possible of the solvent was removed under vacuum and the residue chromatographed on alumina (7 g). Elution with benzene afforded a mixture of two epimeric methyl cyanoacetate adducts and a small amount of the starting material. Final purification by preparative tlc (silica gel, ethyl acetate) afforded an inseparable mixture of two epimeric methyl cyanoacetate adducts, 52 (0.090 g): $\lambda_{max}$ 223, 283, 290 mμ; $\nu_{max}$ ($CHCl_3$): 3455 (N-H), 2235 and 2195 ($C\equiv N$), 1737 ($COOCH_3$), 1700 (C=O) $cm^{-1}$; nmr: τ 1.96 (singlet, 1H, N-H), 2.74 (multiplet, 5H, aromatic), 5.74 (multiplet, 1H, NC-CH-$COOCH_3$), 6.24 and 6.28 (singlets, 3H, $COOCH_3$), 7.92 and 7.97 (singlets, 3H, CO-$CH_3$); mass spectrum (intensity): m/e 367 (5), 237 (35), 130 (55).

Anal. Calcd. for $C_{21}H_{25}N_3O_3$: M.W. 367.190. Found: 367.190 (mass spectrometry).

N-[β-(3-Indolyl)ethyl]-3-acetyl-4-(2-dimethyl malonate)-piperidine (53)

Sodium was cut into fine pieces (0.035 g) and suspended in dry tetrahydrofuran (10 ml, freshly distilled from sodium). Dimethyl malonate (0.23 g) was added and the mixture heated at reflux for 2 hr,when all the sodium had dissolved giving a cloudy solution. α,β-Unsaturated ketone (51, 0.055 g) was added with stirring under dry nitrogen. After stirring for 3 hr at reflux, the solution was cooled and poured into dilute ammonium hydroxide solution. The aqueous solution was extracted with methylene chloride (3 x 25 ml); the combined organic extracts were dried (sodium sulfate) and evaporated to yield an oil (0.056 g). Purification by preparative tlc (silica gel, ethyl acetate) afforded a mixture of the two epimeric dimethyl malonate adducts, 53 (0.034 g): $\lambda_{max}$ 223, 283, 292 mμ; $\nu_{max}$ ($CHCl_3$): 3455 (N-H), 1745-1695 (broad, C=O and $COOCH_3$) $cm^{-1}$; mass

spectrum (intensity): m/e 400 (8), 270 (100), 130 (55).

Anal. Calcd. for $C_{22}H_{28}O_5N_2$: M.W. 400.200. Found: 400.198 (mass spectrometry).

Using the above tlc system, a partial separation of the two epimers was obtained. The nmr of the less polar adduct was: τ 1.90 (singlet, 1H, N-H), 2.70 (multiplet, 5H, aromatic), 5.91 (doublet, J = 10 cps, 1H, $CH_3OOC$-CH-$COOCH_3$), 6.31 and 6.36 (singlets, 6H, $COOCH_3$), 7.96 (CO-$CH_3$) and the nmr of the more polar adduct was: τ 2.03 (singlet, 1H, N-H), 2.70 (multiplet, 5H, aromatic), 6.31 and 6.34 (singlets, 6H, $COOCH_3$), 6.54 (doublet, J = 4 cps, 1H, $CH_3OOC$-CH-$COOCH_3$), 7.84 (singlet, 3H, CO-$CH_3$).

Reaction of α,β-unsaturated ketone (51) with sodium methoxide in methanol

A solution of the α,β-unsaturated ketone (51, 0.023 g) in methanol (5 ml) was added to a solution of sodium methoxide (0.400 g) in methanol (10 ml). After heating at reflux for 2 hr, the cooled solution was poured into saturated sodium bicarbonate solution (60 ml) and the basic components extracted with methylene chloride (3 x 10 ml). The combined extracts were washed with water (15 ml), dried over sodium sulfate and evaporated to give a white solid (0.024 g): mp 175-178°, $\nu_{max}$ $CHCl_3$): 1697 and 1665 $cm^{-1}$; $\lambda_{max}$: 222, 286, 291 mμ; nmr (DMSO-$d_6$): τ 6.31 ($OCH_3$) and τ 7.40 (CO-$CH_3$); mass spectrum: highest m/e was 300 and the remainder of the spectrum was consistent with a mixture of starting material, 51, and the methoxy adduct (59).

1-Methyl-3-acetylpyridinium iodide ethylene ketal (60)

A solution of methyl iodide (20 g) in ether (50 ml) was added to a stirred ice-cold solution of 3-acetylpyridine ethylene ketal (49, 20 g) in ether (50 ml). The reaction mixture was left at room temperature overnight and the precipitated salt, 60, was filtered (36.8 g) and purified by recrystallizations from methanol, mp 190°.

1-Methyl-3-acetyl-$\Delta^3$-piperideine ethylene ketal (61)

A solution of the salt (60, 1.0 g) in methanol (50 ml) was treated with

sodium borohydride (5 g) in methanol (75 ml). After 2 hr, the solution was evaporated to dryness and water was added (200 ml). The solution was neutralized with 2N hydrochloric acid and then basified with sodium carbonate prior to extraction with chloroform (4 x 50 ml). The combined extracts were washed with water (2 x 50 ml), dried (sodium sulfate) and evaporated to give the product, 61, as a pale yellow liquid (0.373 g); nmr: $\tau$ 4.18 (multiplet, 1H, vinyl), 6.18 (multiplet, 4H, ketal), 7.68 (singlet, 3H, N-$CH_3$), 8.60 (singlet, 3H, C-$CH_3$).

1-Methyl-3-acetyl-$\Delta^3$-piperideine (62)

The ketal (61, 0.370 g) was dissolved in 2N hydrochloric acid (10 ml) and stirred at room temperature under nitrogen for 18 hr. The reaction mixture was basified with sodium bicarbonate and extracted with chloroform (4 x 10 ml). The combined extracts were washed with water (2 x 10 ml), dried (sodium sulfate) and evaporated to give 62 as a pale yellow liquid (0.230 g): $\nu_{max}$ (film): 1670 (C=O) $cm^{-1}$; nmr: $\tau$ 3.10 (triplet, 1H, C-4-H), 7.63 (singlet, 3H, N-$CH_3$) and 7.73 (singlet, 3H, C-$CH_3$).

N-Methyl-3-acetyl-4-(2-methyl cyanoacetate)-piperidine (63)

A solution of 1-methyl-3-acetyl-$\Delta^3$-piperideine (62, 0.20 g), methyl cyanoacetate (0.4 ml), triethylamine (3.0 ml) in methanol (10 ml) was heated under reflux for 5 hr. The reaction mixture was cooled and evaporated. Saturated sodium bicarbonate solution (10 ml) was added and extracted with chloroform (4 x 10 ml). The combined extracts were washed with water (15 ml), dried over sodium sulfate, and evaporated to give an orange liquid. This was dissolved in methanol (1 ml) and a saturated solution of picric acid in methanol (2.5 ml) was added. After an orange oil separated out, the supernatant liquid was decanted off. The oil was washed with an additional volume of methanol (0.5 ml), and dissolved in acetone-water

9:1 (30 ml). An Amberlite IRA-400 $HCO_3^-$ column (1 x 15 cm) was prepared by washing through with acetone-water 9:1 (30 ml) and the above solution was then filtered through the column, which was subsequently washed with a further 30 ml portion of acetone-water 9:1. The combined eluate and washings were evaporated, saturated sodium bicarbonate solution (10 ml) was added and the mixture extracted with chloroform (3 x 10 ml). The combined extracts were washed with water (10 ml), dried over sodium sulfate and evaporated to give a mixture of starting material (62) and the desired methyl cyanoacetate adduct (63) as a yellow oil (0.090 g): $\nu_{max}$ ($CHCl_3$): 2221 (CN), 1747 ($COOCH_3$), 1709 (saturated ketone), 1664 (unsaturated ketone) $cm^{-1}$; nmr: (methyl cyanoacetate adduct 63) $\tau$ 6.17 ($COOCH_3$), 6.21 (CH-CN), 7.60 ($N-CH_3$), 7.78 ($C-CH_3$) and (starting material 62) $\tau$ 3.10 (olefine proton), 7.60 ($N-CH_3$), 7.73 ($C-CH_3$).

N-[β-(3-Indolyl)ethyl]-3-acetylpyridinium bromide (76)

Tryptophyl bromide (47, 3.98 g) and 3-acetylpyridine (48, 6.5 ml) was heated at 70° for 12 hr and then at 100° for 4 hr under a nitrogen atmosphere. The crystalline mass was triturated with an ethanol-ether 2:1 solution (10 ml) and the crude product recrystallized from 60% aqueous methanol, giving N-[β-3-indolyl)ethyl]-3-acetylpyridinium bromide (76, 5.8 g), mp 214-217° dec (Lit [19] mp 214-216° dec).

N-[β-(3-Indolyl)ethyl]-$\Delta^2$-3-acetylpiperideine (77) [19]

A suspension of pyridinium salt (76, 5.8 g) and 10% palladium-charcoal (1.2 g) in ethanol (200 ml) and triethylamine (12.9 ml) was hydrogenated at room temperature and atmospheric pressure; the uptake ceased after 5 hr (hydrogen uptake 950 ml). Filtration and concentration of the filtrate under reduced pressure afforded a syrup that was dissolved in chloroform (50 ml). After washing with water (2 x 50 ml), the chloroform was dried

over potassium carbonate and concentrated under reduced pressure to give the impure tetrahydro ketone as a syrup: $\nu_{max}$ ($CHCl_3$): 3320 (NH), 1620 and 1561 (vinylogous amide) $cm^{-1}$. The crude tetrahydro ketone, 77, was immediately used for the next reaction.

Tetracyclic ketone (78)

Impure tetrahydro ketone (77) was treated with 1N hydrochloric acid (350 ml) under an atmosphere of nitrogen on a steam bath for 2 hr. After cooling to 0°, the aqueous phase was basified with potassium carbonate and extracted with chloroform (4 x 50 ml). The combined chloroform extracts were dried over potassium carbonate and concentrated to yield a syrup which was chromatographed on alumina (150 g). The product was eluted with benzene and subsequently crystallized from methanol to afford meedles of the tetracyclic ketone, 78 (2.24 g), mp 165-170°. The mother liquors were subjected to isomerizing conditions (2 hr reflux in 25 ml of 0.07 M sodium methoxide in methanol). The product was crystallized from methanol to give needles (1.51 g), mp 163-170°. The combined material was recrystallized from methanol to give pure tetracyclic ketone, 78: mp 174-177°; $\lambda_{max}$ (log ε): 222 (4.54), 286 (3.89), 291 (3.78) mμ; $\nu_{max}^{KBr}$: 3320 (NH), 1683 (C=O) $cm^{-1}$; nmr: τ 2.21 (singlet, 1H, NH), 2.78 (multiplet, 4H, aromatic), 7.77 (singlet, 3H, C-$CH_3$) (Lit [19] mp 171-173°).

Chloroindolenine (79) of tetracyclic ketone

t-Butyl hypochlorite (0.0845 g, 0.79 mmole) in carbon tetrachloride (15.7 ml) was added dropwise over 30 min to a stirred solution of 78 (0.20 g, 0.79 mmole) in anhydrous methylene chloride (10 ml) containing one drop triethylamine, cooled in an ice-salt mixture. After the addition was completed, stirring was continued for 30 min. The reaction mixture was washed with water (15 ml), dried (sodium sulfate) and evaporated to give the crude chloro derivative, 79, as a yellow viscous oil (0.254 g):

$\lambda_{max}$: 234, 297 mμ; $\nu_{max}$ ($CHCl_3$): 1700, 1585 $cm^{-1}$. The crude product was used directly for the next reaction.

Oxindole (80)

A solution of the above impure chloroindolenine (79) and potassium hydroxide (0.06 g) in methanol (12.5 ml) was refluxed for 2 hr under an atmosphere of nitrogen. A half of the solvent was evaporated off and the mixture was distributed between water and methylene chloride. The organic layer was separated and dried over sodium sulfate. The oil (0.222 g) obtained after the solvent was removed was chromatographed on alumina (20 g). Examination of the fractions showed that the reaction product was a complex mixture. The major component, eluted with benzene-chloroform 1:1, was further purified using preparative tlc (silica gel, ethyl acetate-ethanol 2:1) to afford the pure compound, 80 (0.045 g). Crystallization from methanol gave colorless prisms: mp 205-208°; $\lambda_{max}$ (log ε): 210 (4.44), 253 (3.85), 280 (3.21); $\nu_{max}$ (nujol): 3122 (NH), 1721 (C=O), 1695 (N-C=O) $cm^{-1}$; nmr (60 Mc/s): τ 1.00 (singlet, 1H, NH), 2.94 (multiplet, 4H, aromatic), 7.30 (singlet, 3H, $C-CH_3$).

Anal. Calcd. for $C_{17}H_{20}N_2O_2$: C, 71.80; H, 7.09. Found: C, 71.88; H, 7.51.

Formation of ethylene ketal (82)

A solution of ketone (78, 1.5 g) ethylene glycol (6 ml), p-toluene-sulfonic acid (1.2 g) and benzene (40 ml) was refluxed for 4 hr under an atmosphere of nitrogen. The resulting orange solution was cooled to 0° and slowly poured into 5% aqueous sodium hydroxide (50 ml) still maintaining a solution temperature of 0°. The benzene layer was separated and the aqueous phase extracted with additional amounts of benzene (3 x 15 ml). The combined benzene extract was dried over sodium sulfate and evaporated to yield an orange residue (1.88 g) which was chromatographed on alumina

(100 g). Elution of the column with benzene afforded the compound, 82 (1.272 g) which could not be induced to crystallize: $\lambda_{max}$: 222, 279, 290 mμ; $\nu_{max}$ ($CHCl_3$): 3330 $cm^{-1}$ (N-H); nmr: τ 0.28 (singlet, 1H, NH), 2.77 (multiplet, 4H, aromatic), 6.07 (multiplet, 4H, ethylene ketal), 8.62 (singlet, 3H, C-$CH_3$); mass spectrum (intensity): m/e 312 (100), 311 (100).

Further elution of the column with chloroform-benzene 1:4 afforded another epimer of 82 (0.256 g) which was crystallized from methanol: mp 187-188°; $\lambda_{max}$ (log ε): 222 (4.57), 281 (3.91), 290 (3.82) mμ; $\nu_{max}$ ($CHCl_3$): 3350 $cm^{-1}$ (NH); nmr: τ -0.03 (singlet, 1H, NH), 2.75 (multiplet, 4H, aromatic), 5.96 (multiplet, 4H, ethylene ketal), 8.52 (singlet, 3H, C-$CH_3$).

Anal. Calcd. for $C_{19}H_{24}N_2O_2$: C, 73.04; H, 7.74; N, 8.97. Found: C, 73.17; H, 7.83; N, 8.78.

Chloroindolenine (83)

The chloroindolenine (93) of the tetracyclic ethylene ketal (82, 0.09 g) was formed in the manner previously described to afford a yellow oil: $\lambda_{max}$: 253, 292 (sh) mμ. The crude product was used directly for the next reaction.

Oxindole (84)

A solution of the crude chloroindolenine (83) and potassium hydroxide (0.08 g) in methanol (4 ml) was refluxed for 2 hr under an atmosphere of nitrogen. A half of the solvent was evaporated off and the mixture was distributed between water and methylene chloride. The organic layer was separated and dried over sodium sulfate. After removal of the solvent, the oil was purified using preparative tlc (ethyl acetate-ethanol 2:1) to afford the oxindole, 84, as an oil (0.042 g): $\nu_{max}$ ($CHCl_3$): 3100 (NH),

1700 (C=O) $cm^{-1}$; nmr (60 Mc/s): $\tau$ 2.72 (multiplet, 4H, aromatic), 6.10 (multiplet, 4H, ethylene ketal), 8.64 (singlet, 3H, C-$CH_3$).

Tetracyclic alcohol (85) [19]

A solution of sodium borohydride (0.050 g, 1.3 mmole) in absolute methanol (10 ml) was added slowly to a stirred solution of 78 (0.20 g, 0.7 mmole) in absolute methanol (10 ml). After stirring for 2 hr at room temperature under an atmosphere of nitrogen, the mixture was diluted with water (5 ml) and the methanol removed under reduced pressure. The remaining aqueous solution was acidified with 2N hydrochloric acid until acid to litmus and then made basic with potassium carbonate solution. The aqueous solution was extracted with methylene chloride (3 x 10 ml). The combined extracts were dried over sodium sulfate and evaporated under reduced pressure to afford the alcohol, 85, as a white solid (0.204 g). This solid was crystallized from methanol: mp 228-230°; $\lambda_{max}$ (log $\varepsilon$): 224 (4.53), 282 (3.87), 290 (3.80) m$\mu$; $\nu_{max}^{KBr}$: 3130, 3030 $cm^{-1}$; nmr: $\tau$ 0.20 (singlet, 1H, NH), 2.78 (multiplet, 4H, aromatic), 8.74 (doublet, J = 6 cps, 3H, -CHOH$\underline{CH_3}$); high resolution mass spectrum: Calcd. for $C_{17}H_{22}N_2O$: M.W. 270.173. Found: 270.172.

Chloroindolenine (86) of tetracyclic alcohol (85)

t-Butyl hypochlorite (0.124 g, 1.1 mmole) in carbon tetrachloride (23 ml) was added dropwise over 30 min to a stirred solution of 85 (0.28 g, 1.04 mmole) in anhydrous methylene chloride (80 ml) containing one drop triethylamine, cooled in an ice-salt mixture. After the addition was completed, stirring was continued for 30 min. The reaction mixture was washed with water (20 ml), dried over sodium sulfate and evaporated to give the crude chloro derivative, 86, as a yellow viscous oil: $\lambda_{max}$: 253, 290 (sh) m$\mu$. The crude product was used directly for the next reaction because initial attempts at purification resulted in decomposition.

Imino ether (87)

A solution of the above crude chloroindolenine, 86, and potassium hydroxide (0.078 g) in methanol (19 ml) was refluxed for 2 hr under an atmosphere of nitrogen. A half of the solvent was evaporated off and the mixture distributed between water and methylene chloride. The organic layer was separated and dried over sodium sulfate. The oil (0.321 g) obtained after removal of the solvent was chromatographed on alumina (30 g). The major component was eluted with benzene-chloroform 4:1 and further purified using preparative tlc (silica gel, ethyl acetate-ethanol 2:1) to afford the pure imino ether, 87 (0.099 g). Crystallization from ether gave fine needles: mp 140-143°; $\lambda_{max}$ (log ε): 213 (4.38), 253-258 (3.72) mμ; $\nu_{max}^{KBr}$ 3300 (OH), 1575 (N=C-O) $cm^{-1}$; nmr: τ 2.82 (multiplet, 4H, aromatic), 5.94 (singlet, 3H, $OCH_3$), 9.29 (doublet, J = 6 cps, 3H, -CHOHCH$_3$); mass spectrum (intensity): m/e 300 (100), 282 (7), 255 (9), 141 (41), 96 (68); high resolution mass spectrum: Calcd. for $C_{18}H_{24}N_2O_2$: M.W. 300.184. Found: 300.186.

Anal. Calcd. for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05. Found: C, 71.97; H, 8.05.

Adduct (89)

A mixture of 87 (0.30 g), methyl cyanoacetate (2 ml) and triethylamine (0.1 ml), in a sealed tube was heated at 65° for 100 hr. The excess methyl cyanoacetate was distilled off using a bath temperature of 60° and a pressure of 0.01 mm. The red residue was chromatographed on alumina (25 g) and the major component (0.172 g) was eluted with petroleum ether-benzene 3:1. Crystallization from hexane gave the adduct, 89, as flakes: mp 185-188°; $\lambda_{max}$ (log ε): 234 (4.12), 334 (4.32);and $\lambda_{min}$ (log ε): 258 (2.97);$\nu_{max}^{KBr}$: 3190 (NH), 2190 (CN), 1680 ($COOCH_3$); nmr: τ -1.03 (singlet,

1H, NH), 2.78 (multiplet, 4H, aromatic), 6.19 (singlet, 3H, $COOCH_3$); mass spectrum (intensity): m/e 323 (30), 97 (100); high resolution mass spectrum: Calcd. for $C_{19}H_{21}N_3O_2$: M.W. 323.163. Found: 323.163.

Anal. Calcd. for $C_{19}H_{21}N_3O_2$: C, 70.56; H, 6.55; O, 9.90. Found: C, 70.85; H, 6.81; O, 9.78.

Wolff-Kishner reduction of tetracyclic ketone (78)

A solution of ketone (78, 1.0 g), 85% hydrazine (20 ml), and potassium hydroxide (3.0 g) in diethylene glycol (65 ml) was refluxed for 1.5 hr (bath temperature 165°) under nitrogen. The condenser was removed and the bath temperature was raised to 215° while water and excess hydrazine were removed by passing nitrogen through the reaction solution. Heating at 215° was continued for 5 hr. After cooling, the reaction product was diluted with water (10 ml) and extracted with methylene chloride (3 x 25 ml). The combined organic extracts were dried over sodium sulfate and evaporated to afford a yellow gum (1.5 g) which was chromatographed on alumina (110 g). Elution of the column with benzene afforded the pure tetracyclic indole, 93 (0.73 g) which was crystallized from methanol: mp 65-95° dec; $\lambda_{max}$ (log $\varepsilon$): 221 (4.54), 273 (3.87), 2.78 (3.86), 289 (3.76) mμ; $\nu_{max}$ ($CHCl_3$): 3450 $cm^{-1}$ (NH); nmr: $\tau$ 1.99 (singlet, 1H, NH), 2.75 (multiplet, 4H, aromatic), 9.02 (triplet, J = 7 cps, 3H, $CH_2$-$CH_3$) (Lit [67] mp 60-100°).

Anal. Calcd. for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72. Found: C, 79.90; H, 9.00.

Chloroindolenine (94)

t-Butyl hypochlorite (0.05 M in carbon tetrachloride, 10 ml) was added dropwise over 30 min to a stirred solution of 93 (0.114 g) in anhydrous methylene chloride (10 ml) containing one drop of triethylamine cooled in an ice-salt mixture. After the addition was completed, stirring was

continued for 30 min. The reaction mixture was washed with water (10 ml), dried over sodium sulfate and evaporated to give the crude chloro derivative as a yellow viscous oil (0.140 g). Purification by preparative tlc (silica gel, ethyl acetate-ethanol 2:1) afforded a pure sample (0.097 g) of the chloro derivative, 94. Concentration of a methylene chloride solution of the chloro derivative afforded a yellow amorphous powder: mp 95-100°; $\nu_{max}$ ($CHCl_3$): 1588 $cm^{-1}$ (-N=C-); $\lambda_{max}$ (log ε): 225 (4.30), 266 (3.29), 293 (3.21) mμ; nmr: τ 2.62 (multiplet, 4H, aromatic), 9.06 (triplet, 3H, $CH_2\underline{CH_3}$); mass spectrum (intensity): m/e 290 (2), 288 (5), 253 (100).

Lithium aluminum hydride reduction of chloroindolenine (94)

A solution of chloro derivative (94, 0.005 g) in ether (1 ml) was treated with excess lithium aluminum hydride. After 10 min at room temperature, water was added and the mixture extracted with ether (3 x 1 ml). The ether extracts were combined, dried over sodium sulfate and concentrated to dryness. Examination by tlc, ultraviolet and infrared spectrum of the product showed that it was identical to the tetracyclic indole (93).

Imino ether (95)

A small chip of sodium was dissolved in methanol (10 ml) and the solution added to a solution of the chloroindolenine (94, 0.31 g) in methanol (10 ml). After refluxing for 11 hr under an atmosphere of nitrogen two-thirds of the solvent was evaporated off and the mixture distributed between water and methylene chloride. The organic layer was separated and dried over sodium sulfate. The oil obtained after evaporation of the solvent was chromatographed on alumina (15 g). The desired imino ether was eluted with petroleum ether-benzene 2:1 and was subjected to further purification using preparative tlc on silica gel (ethyl acetate-ethanol 2:1) to afford a mixture of two imino ethers, 95 (0.032 g), in a ratio of 4:1 as determined by nmr. This mixture was inseparable using a variety

of tlc adsorbents and solvents and as a result the spectral characteristics were determined on the mixture: $\nu_{max}$ ($CHCl_3$): 1568 $cm^{-1}$ (-N=C-O); $\lambda_{max}$: 215, 254-258, 285 (sh), 292 (sh) mμ; nmr: τ 2.80 (multiplet, 4H, aromatic), 5.92 and 5.97 (singlets, 3H, $-OCH_3$), 9.50 (triplet, 3H, $-CH_2\underline{CH_3}$); mass spectrum (intensity): m/e 284 (21), 125 (59), 96 (100).

Anal. Calcd. for $C_{18}H_{24}N_2O$: M.W. 284.189. Found: 284.184 (mass spectrometry).

Adduct (96)

A solution of imino ether (95, 0.050 g), methyl cyanoacetate (0.5 ml) and triethylamine (0.1 ml) was heated at 76° for 120 hr in a sealed tube. The excess methyl cyanoacetate was distilled off using a bath temperature of 80° and a pressure of 0.7 mm. The red residue was chromatographed on alumina (7 g) and the desired adduct was eluted with petroleum ether-benzene 1:1 and further purification on preparative tlc (silica gel, ethyl acetate-ethanol 2:1) afforded the pure adduct, 96 (0.009 g). Crystallization from methanol afforded colorless blocks: mp 165-168°; $\nu_{max}$ ($CHCl_3$): 3300 (NH), 2210 (CN), 1666 ($COOCH_3$) $cm^{-1}$; $\lambda_{max}$ (log ε): 236 (4.08), 295 (3.79), 335 (4.30); $\lambda_{min}$ (log ε): 258 (2.91); nmr: τ -1.08 (singlet, 1H, NH), 2.72 (multiplet, 4H, aromatic), 6.18 (singlet, 3H, $COOCH_3$), 9.53 (triplet, 3H, $-CH_2\underline{CH_3}$); mass spectrum (intensity): m/e 351 (13), 125 (50), 124 (45), 96 (100).

Anal. Calcd. for $C_{21}H_{25}N_3O_2$: M.W. 351.195. Found: 351.195 (mass spectrometry).

Adduct 96 was also prepared by the following procedure.

A mixture of chloroindolenine (94, 0.110 g) methyl cyanoacetate (1 ml) and triethylamine (0.1 ml) in a sealed tube was heated at 76° for 144 hr. The excess methyl cyanoacetate was distilled off using a bath temperature of 65° and a pressure of 0.1 mm. The red residue was chromatographed on

alumina (5 g) and the desired adduct was eluted with petroleum ether-benzene 1:1 followed by further purification on preparative tlc (silica gel, ethanol-ethyl acetate 1:2) afforded the pure adduct, 96 (0.008 g). Crystallization from methanol afforded colorless blocks, mp 165-168°. This adduct was identical in all respects (mixed mp, tlc, ir, uv, nmr) to that obtained from the reaction of the imino ether, 95, with methyl cyanoacetate.

Extraction of alkaloids from Vinca minor Linn

The following procedure was developed in order to extract and purify the alkaloids of Vinca minor Linn plants. This procedure was used for all extractions of V. minor L. plants and was scaled according to the wet weight of plants used.

Vinca minor Linn plants (9 kg, wet weight) obtained from the gardens of the University of British Columbia were mascerated with methanol in a Waring Blender, filtered and re-mascerated until the filtrate was colorless. This green filtrate (8,000 ml) was concentrated to dryness under reduced pressure and the residue dissolved in 2N hydrochloric acid (4,500 ml). The acid layer was extracted with benzene (2 x 2,000 ml) and the benzene extracts were back extracted with 2N hydrochloric acid (2 x 500 ml). The combined aqueous phases were made basic with 15N ammonium hydroxide,taking care that the temperature of the solution did not rise above 25°,and extracted with chloroform (3 x 2,400 ml). The combined chloroform extracts were washed with water (2,000 ml), dried over sodium sulfate, and concentrated under reduced pressure. The resulting alkaloid residue (13.6 g) was dissolved in benzene-methylene chloride 1:1 (100 ml) and chromatographed on alumina (700 g). The column was eluted successively with petroleum ether 30/60°, benzene, chloroform, and methanol; fractions of 700 ml were taken. The fractions eluted with petroleum ether-benzene 2:1 were combined and subjected

to additional column chromatography on alumina and final purification using preparative tlc on silica gel (ethyl acetate-chloroform 3:7) to afford quebrachamine (97, 0.002 g), vincadine (98, 0.005 g) and vincaminoreine (99, 0.035 g). Continued elution of the column with petroleum ether-benzene 1:1 afforded vincadifformine (102, 0.010 g) and further elution with petroleum ether-benzene 1:4 afforded minovine (103, 0.41 g). The initial fractions eluted with benzene contained 1,2-dehydroaspidospermidine (101; 1,2-double bond, approx. 0.002 g), but no attempt was made to finally purify this compound. The later fractions eluted with benzene were combined and crystallized from methanol affording vincamine (29, 0.65 g). All the above alkaloids were compared on tlc with authentic samples [62] and in addition spectral comparison was made with literature values [68].

Extraction of alkaloids from Vinca rosea Linn (Catharanthus roseus G. Don)

The following procedure was developed in order to extract and purify the alkaloids of Vinca rosea Linn plants. This procedure was used for all extractions of V. rosea L. plants and was scaled according to the wet weight of plants used.

Vinca rosea Linn plants were obtained from the greenhouse at the University of British Columbia [69]. The plants (300 g, wet weight) were mascerated with methanol in a Waring Blender, filtered and re-mascerated until the filtrate was colorless. This green filtrate (combined volume was 3,000 ml) was evaporated to dryness, the residue taken up in 2N hydrochloric acid (2,000 ml) and washed with benzene (3 x 1,000 ml). The combined benzene extracts were back extracted with 2N hydrochloric acid (2 x 500 ml). The combined aqueous phases were made basic with 15N ammonium hydroxide and extracted with chloroform (3 x 1,000 ml). The combined chloroform extracts were washed with water (1,000 ml), dried over sodium sulfate and evaporated to give a

brown oil (1.003 g).

The oil was dissolved in benzene-methylene chloride 1:1 (3 ml) and chromatographed on alumina (100 g). The column was eluted successively with petroleum ether 30/60°, benzene, chloroform and methanol; fractions of 100 ml were taken. The later benzene-petroleum ether 1:1 fractions were combined and crystallized from methanol affording catharanthine (9, 0.044 g), the benzene fractions were combined and crystallized from methanol affording ajmalicine (10, 0.025 g), and the initial benzene-chloroform 4:1 fractions were combined and crystallized from ether giving vindoline (7, 0.029 g). When required, the hydrochloride salt of catharanthine and vindoline was formed by blowing hydrogen chloride gas on the surface of an ethereal solution of the alkaloid; and catharanthine hydrochloride was crystallized from methanol, whereas vindoline hydrochloride was crystallized from acetone. The hydrochloride salt of ajmalicine was prepared by adding concentrated hydrochloric acid (10 drops) to a concentrated methanolic solution of the alkaloid and crystallization of ajmalicine hydrochloride from methanol.

18α-Carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine (105) and 18β-carbomethoxy-($O^{14}CH_3$)-4α-dihydrocleavamine (105)

A solution of 18α-carbomethoxy-4α-dihydrocleavamine (105, 0.008054 g), benzene (1 ml), boron trifluoride etherate (0.2 ml) and methanol-$^{14}C$ (0.2 ml, $3.875 \times 10^7$ dpm) was refluxed for 12 hr and then stirred at room temperature for a further 12 hr. After pouring into saturated aqueous sodium bicarbonate, the aqueous solution was extracted with methylene chloride (3 x 10 ml). After drying over sodium sulfate, the organic extract was concentrated to dryness under reduced pressure to afford an oil (0.0091 g). This oil was purified using preparative tlc (silica gel, chloroform) to afford 18β-carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine (0.005 g, $3.55 \times 10^6$ dpm) and 18α-carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine (0.002 g, $1.24 \times 10^6$ dpm)

as oils. Inactive 18β-carbomethoxy-4α-dihydrocleavamine (0.0206 g) was added to the 18β-carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine and the hydrochloride salt was formed by blowing gaseous hydrogen chloride upon an ethereal solution and crystals (0.009 g, $4.63 \times 10^7$ dpm/mmole) were obtained from acetone. Inactive 18α-carbomethoxy-4α-dihydrocleavamine (0.0090 g) was added to the 18α-carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine and the hydrochloride salt was formed as above and crystals (0.0052 g, $4.62 \times 10^7$ dpm/mmole) were obtained from acetone.

Trifluroacetic acid-$^3H$

Trifluroacetic anhydride (1.17 g, 5.55 mmole) was added to water-$^3H$ (0.10 g, 5.55 mmole, 100 mcurie/g) using a vacuum transfer system. The resulting trifluroacetic acid-$^3H$ (1.27 g, 0.9 mc/mmole) was stored under an atmosphere of nitrogen at -10° until required.

Tritium labelled radioactive alkaloids for biosynthesis studies

The following procedure is typical for the formation of all the radioactive precursors utilizing tritium in the aromatic portion of the alkaloid molecule.

Trifluroacetic acid-$^3H$ (0.5 g, 0.9mc/mmole) was added to tryptamine (41) hydrochloride (0.048 g) using a vacuum transfer system. The solution was allowed to stand under an atmosphere of nitrogen at room temperature for 24 hr. The trifluroacetic acid-$^3H$ was removed using a vacuum transfer system. Concentrated ammonium hydroxide solution (10 ml) was carefully added to the residue and the organic components extracted with dichloromethane (10 x 15 ml). The organic extract was washed with water (10 ml), dried over sodium sulfate, and concentrated to dryness under reduced pressure to afford an oil (0.034 g). This oil was subjected to column chromatography on alumina (1.0 g) and [ar-$^3H$]-tryptamine (0.010 g, $6.16 \times 10^7$ dpm) was eluted with chloroform. Various tlc systems were used

in order to confirm that a pure material was recovered.

Feeding experiment no. 1

[ar-$^{3}$H]-Carbomethoxycleavamine (106) hydrochloride (0.009078 g, 2.28 x $10^{8}$ dpm/mmole) was dissolved in distilled water and the solution administered to V. rosea L. plants (wet weight 35 g) by the cotton wick method. After 8 days under intermittent fluorescent lamp illumination, the plants were extracted to afford the alkaloids as a brown gum (0.084 g). The alkaloid extract was purified to afford catharanthine (9) as an oil. Inactive catharanthine (0.050 g) was added and the catharanthine was crystallized to constant activity. The total activity in the catharanthine was 800 dpm representing a maximum incorporation of 0.015%.

Another portion of the above [ar-$^{3}$H]carbomethoxycleavamine hydrochloride was dissolved in water and allowed to remain in the open test-tube for 8 days. After made basic, the aqueous solution was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were dried (sodium sulfate), evaporated and was purified as above to afford radioactive catharanthine (9). The conversion of carbomethoxycleavamine to catharanthine in the test-tube was 0.04%.

Feeding experiment no. 2

[ar-$^{3}$H]-Carbomethoxycleavamine (106) hydrochloride (0.00652 g, 2.31 x $10^{8}$ dpm/mmole) was dissolved in distilled water (10 ml) and the solution administered to V. rosea L. leaf discs (15 g) by vacuum infiltration. After 42 hr under fluorescent lamp illumination, the leaf discs were extracted and the catharanthine (9) crystallized to constant activity as in feeding experiment no. 1. The total activity in the catharanthine was 350 dpm representing a maximum incorporation of 0.008%.

Feeding experiment no. 3

[ar-$^{3}$H]-Carbomethoxycleavamine (106) hydrochloride (0.00207 g,

2.31 x $10^8$ dpm/mmole) was dissolved in 0.1N acetic acid (0.4 ml) and the solution adminstered to growing V. rosea L. plants (wet weight 139 g) by placing fine glass capillaries containing the solution into the leaf veins of the plants. The solution was absorbed into the leaf veins by this method. After 6 days under intermittent fluorescent lamp illumination, the plants were extracted and the catharanthine (9) crystallized to constant activity as in feeding experiment no. 1. The total activity in the catharanthine was 630 dpm or a maximum incorporation of 0.05%.

Feeding experiment no. 5

18β-Carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine (105) hydrochloride (0.00278 g, 4.63 x $10^7$ dpm/mmole) was dissolved in distilled water (10 ml) and distributed equally among 10 test-tubes. V. rosea L. cuttings (91 g) were inserted into these test-tubes and placed under intermittent fluorescent lamp illumination with the aqueous level in the test-tubes maintained with distilled water. After 7 days, the cuttings were extracted to afford the alkaloids as a brown gum (0.187 g, 1.37 x $10^5$ dpm). The alkaloid extract was purified by preparative tlc (alumina, benzene-chloroform 3:1, and then silica gel, ethyl acetate-ethanol 2:1) to afford coronaridine (107) as an oil (0.001 g, 50 dpm) representing a maximum incorporation of 0.015%.

Feeding experiment no. 6

18α-Carbomethoxy($O^{14}CH_3$)-4α-dihydrocleavamine (105) hydrochloride (0.00356 g, 4.62 x $10^7$ dpm/mmole) was administered to V. rosea L. cuttings as in feeding experiment no. 5, except the time for incorporation was 5 days. After extraction and purification, the coronaridine (107) did not contain any radioactivity.

Feeding experiment no. 12

[ar-$^3$H]-Tryptamine (41, 0.010 g, 6.16 x $10^7$ dpm) was dissolved in 0.1N acetic acid (15 ml) and administered to growing 4-6 month old

V. rosea L. plants (wet weight 300 g). The administration was by the cotton wick method, where a wick of cotton string was passed through the plant stem about 1 inch above the ground and the ends of the string placed in a small test-tube near the plant. The test-tubes were filled with the solution of [ar-$^3$H]-tryptamine and after this solution was absorbed into the plant, further volumes of 0.1N acetic acid were added to the test-tubes in order to wash all the [ar-$^3$H]-tryptamine into the plant. The plants were placed under intermittent fluorescent lamp illumination and watered as required. After 9 days, the alkaloids (1.003 g, 1.2 x $10^7$ dpm) were isolated and purified by chromatography to afford the pure alkaloids: catharanthine (9, 0.044 g), ajmalicine (10, 0.025 g), and vindoline (7, 0.029 g). After crystallization of the free base and then its hydrochloride salt to constant activity, the following incorporations were obtained: catharanthine (0.01%), ajmalicine (0.4%), and vindoline (0.003%).

Feeding experiments no. 11-16, 23, 24, and 26

These feeding experiments were conducted in a manner similar to feeding experiment no. 12. The incorporations into catharanthine (9), ajmalicine (10), and vindoline (7) were obtained by crystallization of the free base and then its hydrochloride salt to constant radioactivity and these results are summarized in Tables II and IV.

Feeding experiment no. 17

[ar-$^3$H]-Minovine (103, 0.0142 g, 6.25 x $10^6$ dpm) was dissolved in 0.1N acetic acid (10 ml) and methanol (0.5 ml) and distributed equally among 10 test-tubes. V. minor L. cuttings (45 g) were inserted into these test-tubes and placed under intermittent fluroescent lamp illumination with the aqueous level in the test-tubes maintained with distilled water. After 7 days, the cuttings were extracted to afford the alkaloids as a brown gum (0.094 g, 7.7 x $10^5$ dpm). After purification, the vincaminoreine

(99) was isolated as an oil (0.009 g, 21.8 dpm) which represented a maximum incorporation of 0.0003%.

Feeding experiment no. 18

The epimeric mixture of [ar-$^{3}$H]-52 hydrochloride (0.0025 g, 1.35 x $10^{6}$ dpm) was dissolved in distilled water (10 ml) and distributed equally among 10 test-tubes. V. minor L. cuttings (46 g) were inserted into these test-tubes and placed under intermittent fluorescent lamp illumination with the aqueous level in the test-tubes maintained with distilled water. After 5 days, the cuttings were extracted to afford the alkaloids as a brown gum (0.155 g, 8.7 x $10^{4}$ dpm). After purification, the vincamine (29, 0.05 g) was obtained crystalline and did not contain any radioactivity. The fractions containing quebrachamine (97), vincadine (95), vincaminoreine (99) and minovine (103) also showed a lack of any radioactivity.

Feeding experiment no. 19

The epimeric mixture of [ar-$^{3}$H]-53 hydrochloride (0.00218 g, 2.4 x $10^{7}$ dpm) was dissolved in distilled water (10 ml) and distributed equally among 10 test-tubes. V. minor L. cuttings (41 g) were inserted into these test-tubes and placed under intermittent fluorescent lamp illumination with the aqueous level in the test-tubes maintained with distilled water. After 7 days, the cuttings were extracted and the alkaloids obtained as an oil (0.171 g, 5.6 x $10^{6}$ dpm). The alkaloids quebrachamine (97), vincadine (8), vincaminoreine (99), minovine (103) and vincamine (29) were purified and did not contain any radioactivity.

Feeding experiment no. 20

The epimeric mixture of [ar-$^{3}$H]-53 hydrochloride (0.00187 g, 2.06 x $10^{7}$ dpm) was dissolved in ethyl alcohol (3 ml) containing 10% dimethyl sulfoxide and painted on the leaves of growing V. rosea L.

plants (10.8 g). The plants were placed under intermittent fluorescent lamp illumination and the soil watered as necessary. After 5 days, the plants were extracted to afford the alkaloids as a brown gum (0.034 g, $5.8 \times 10^6$ dpm) from which catharanthine (9, 0.004 g) was isolated. Inactive catharanthine (0.04791 g) was added and after crystallization of the free base and then its hydrochloride salt, the catharanthine did not contain any radioactivity.

Feeding experiment no. 21

The epimeric mixture of [ar-$^3$H]-53 (0.009 g, $7.5 \times 10^7$ dpm) was dissolved in ethanol (5 ml) containing 10% dimethyl sulfoxide and painted on the leaves of growing V. rosea L. plants (385 g). The plants were placed under intermittent fluorescent lamp illumination and the soil watered as necessary. After 6 days, the plants were extracted to afford the alkaloids as a brown gum (1.223 g, $3.56 \times 10^7$ dpm). Purification provided the following alkaloids: catharanthine (9, 0.044 g), ajmalicine (10, 0.019 g) to which inactive ajmalicine (0.0498 g) was added, and vindoline (7, 0.022 g) to which inactive vindoline (0.0724 g) was added. After crystallization of the free base and then its hydrochloride salt, the above alkaloids were obtained without any radioactivity.

Feeding experiment no. 22

DL-Tryptophan-[3-$^{14}$C] (1, 0.0121 g, $5.38 \times 10^8$ dpm) was dissolved in ethanol (5 ml) containing 10% dimethyl sulfoxide and painted on the leaves of growing V. rosea L. plants (320 g). The plants were placed under intermittent fluorescent lamp illumination and the soil watered as necessary. After 9 days, the plants were extracted to afford the alkaloids which were subsequently purified. After crystallization of the free base and then its hydrochloride salt to constant activity, the following incorporations were obtained: catharanthine (9, 0.009%), ajmalicine (10, 0.003%) and

vindoline (7, 0.005%).

Feeding experiment no. 25

[ar-$^{3}$H]-Adduct (96, 0.014 g, 1.1 x $10^{6}$ dpm) was dissolved in 0.1N acetic acid (3 ml) and methanol (0.2 ml) and distributed equally among 9 test-tubes. V. minor L. cuttings (27.4 g) were inserted into these test-tubes and placed under intermittent fluorescent lamp illumination with the aqueous level in the test-tubes maintained with distilled water. After 2 days, the cuttings were extracted to afford the alkaloids as a brown gum (0.114 g, 6 x $10^{4}$ dpm). After chromatography, the isolated minovine (103, 0.003 g) was diluted with inactive minovine (0.040 g) and repeatedly recrystallized until the minovine did not contain any radioactivity. The isolated vincamine (29, 0.010 g) was repeatedly recrystallized until it did not contain any radioactivity.

Incorporation of DL-tryptophan-3-$^{14}$C into Vinca minor L. at various time intervals

The following procedure is typical of the manner in which the experiments were performed. Nine cuttings of Vinca minor Linn (wet weight 16.4 g) were taken from an outdoor garden and immediately placed in three separate test tubes (three cuttings per tube) containing tryptophan-3-$^{14}$C (0.00184 g, 6.37 x $10^{7}$ dpm) in 0.1N acetic acid (1.0 ml) with a few drops of methanol to cause solution.

The cuttings were allowed to take up the solution, the tubes being refilled as necessary, and after the solution had been taken nearly to dryness three times, the tube was filled with saline solution and left for a period of two days, being irradiated with fluorescent daylight-type lights for 36 out of 48 hr.

After 48 hr, the cuttings were extracted to afford an oil (0.0334 g,

2.52 x $10^6$ dpm) which was chromatographed on alumina (5.0 g) in the following manner.

| Fraction | Solvent | Volume (ml) | Weight of fraction (mg) |
|---|---|---|---|
| 1 | petroleum ether (40-60) | 30 | 1.20 |
| 2 | petroleum ether-benzene 18:7 | 25 | 0.65 |
| 3 | petroleum ether-benzene 18:7 | 50 | 0.35 |
| 4 | petroleum ether-benzene 1:1 | 50 | 1.65 |
| 5 | benzene | 50 | 5.40 |
| 6 | benzene | 50 | 1.90 |
| 7 | benzene-chloroform 4:1 | 50 | 4.30 |
| 8 | chloroform | 50 | 6.50 |
| 9 | methanol | 20 | 2.15 |

Vincaminoreine (99) and vincadine (98) were present in fraction 2, vincadifformine (102) in fraction 3, and minovine (103) in fraction 4. Appropriate aliquots of these fractions were run on a silica chromatogram sheet developed in chloroform-ethyl acetate 19:1. The sheet was then passed through a calibrated Nuclear-Chicago Actigraph II Model 1039 tlc counter connected to a recorder (Nuclear-Chicago Model 8416) and integrator (Nuclear-Chicago Model 8704). The incorporation of tryptophan into the various alkaloids was calculated and ratios determined. The experiment was performed in duplicate and the mean results tabulated in Table III of the discussion [70].

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68. We are grateful to Dr. I. Kompis, Institute of Chemistry, Bratislava, Czechoslovakia, for providing us with samples of vincadine, vincaminoreine, vincadifformine and minovine.

69. Appreciation is gratefully acknowledged to Mr. P. Salisbury for advice and for the cultivation of Vinca rosea L. plants.

70. Appreciation is gratefully acknowledged to Dr. C. Ehret and Dr. D.C. Wigfield for their assistance with this experiment.