# STUDIES RELATED TO THE BIOSYNTHESIS OF INDOLE ALKALOIDS

BY

RATTAN SAGAR SOOD

B.Sc. Honours, Panjab University, India, 1964
M.Sc., The University of British Columbia, 1968

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ABSTRACT

The Strychnos skeleton (e.g. preakuammicine, 56) has been postulated in the literature to rearrange to Aspidosperma (e.g. vindoline, 5) and Iboga (e.g. catharanthine, 6) bases via the intervention of $\Delta^{4,21}$-dehydrosecodine (76). Part A of the thesis describes the syntheses and biosynthetic evaluation of two close relatives, 16,17-dihydrosecodin-17-ol (90) and secodine (107), of the fugitive acrylic ester (76).

In the synthetic sequence, condensation of 3-ethylpyridine with 2-carboethoxy-3-(β-chloroethyl)-indole (80) followed by the reduction of the resulting pyridinium chloride (82) gave N[β-{3-(2-hydroxymethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (84). The benzoate ester (85) of alcohol (84) was treated with potassium cyanide to afford N-[β-{3-(2-cyanomethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (86). This latter compound upon treatment with methanol and hydrogen chloride gas gave N-[β-{3-(2-carbomethoxymethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (88). Formylation of the ester (88) with methyl formate followed by reduction of the resulting enol (89) gave 16,17-dihydrosecodin -17-ol (90). Feeding of [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90) into Vinca minor L. revealed no significant activity into the isolated alkaloids. The substance in fact appeared to be a toxic component with marked deterioration of the plant occurring within 24 hours.

In another investigation, synthetic 16,17-dihydrosecodin-17-ol (90) was dehydrated to secodine (107). Feeding of [ar-$^{3}H$]-secondine (107) into Vinca minor L. showed low but positive incorporation into vincamine

(72) and minovine (73). "Blank" experiments revealed that after the maximum period required for the plant to absorb a solution of the labelled compound, 61% remained as monomer (107) while 32% had been converted to the dimers (presecamine and secamine).

In conclusion this study while providing some preliminary information on the later stages of indole alkaloid biosynthesis has also created an entry into more sophisticated biosynthetic experiments. This situation will hopefully lead to a better understanding of the manner in which this large family of natural products is synthesized in the living plant.

In Part B of the thesis some preliminary studies leading to the biosynthesis of vincamine (eburnamine family) are described. The intermediacy of a tetracyclic pyruvic ester (12) was invoked by Wenkert several years ago to rationalise the rearrangement of Aspidosperma skeleton to vincamine (2). To confirm this speculation a short synthesis of a close relative (i.e., 24) of the postulated precursor was contemplated.

In the synthetic sequence reaction of tryptophyl bromide (18), produced by the action of phosphorus tribromide on tryptophol (17), with 3-acetylpyridine ethylene ketal (16) gave N-[β-(3-indolyl)-ethyl]-3'-acetylpyridinium ethylene ketal bromide (19). The pyridinium bromide (19) on catalytic reduction and acid hydrolysis furnished N-[β-(3-indolyl)-ethyl]-3'-acetylpiperidine (21). Alkylation of the ketone (21) using trityl sodium and allyl bromide gave N-[β-(3-indolyl)-ethyl]-3'-allyl-3'-acetylpiperidine (22). Osmylation of the allylic double bond in (22) did not give the desired diol (23). Tentative assignment is given in structure (34) to the polar compound obtained in this manner.

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## ACKNOWLEDGEMENTS

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# PART A

# STUDIES RELATED TO THE BIOSYNTHESIS OF INDOLE ALKALOIDS

# INTRODUCTION

During the past fifty or sixty years, tremendous advances have been made in the laboratory preparation of complex naturally occurring organic substances. As early as the first part of this present century pioneering work was being carried out in various laboratories. Among the early successes in this vastly challenging field may be cited the syntheses of numerous terpenes, alkaloids including nicotine, dihydroquinine and the simple opium bases; porphyrins including the blood pigments, the common hexoses, as well as many amino acids and peptides.

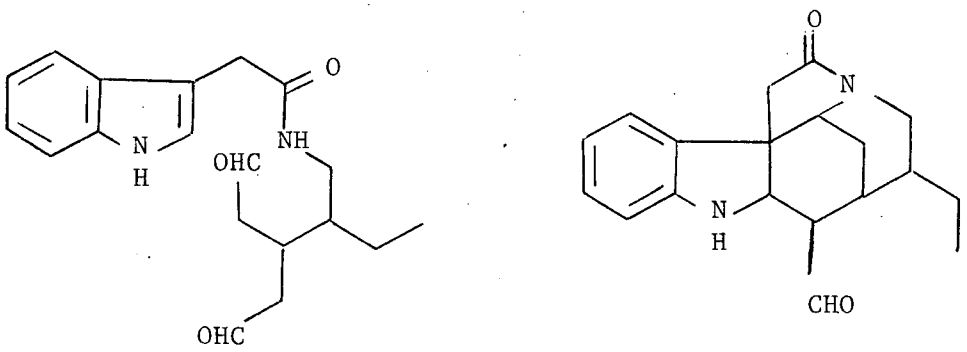
Yet, while the dramatic announcements of multistage syntheses appeared successively during the last several decades, a fainter appeal could be heard emanating from a smaller group of individuals interested in knowing how these complex natural products are formed in nature. It seemed that with the structure of so many natural products having been established, it should be possible to perceive some relations between them. Such considerations brought numerous suggestions about the possible biosynthetic pathways which may be involved.

The first forays into the biogenetic speculations began with the recognition of common structure features among the compounds produced by closely related natural organisms. This recognition led to infer-

ences of a relatively simple common origin for these compounds. Fortunately, it appears that this fantastic array of naturally occurring compounds are indeed built up from a relatively small number of fundamental templates. For example, in the field of alkaloids the common building blocks are acetic acid, ornithine and lysine for the reduced systems and tyrosine, phenyl alanine, 3,4-dihydroxyphenylpyruvic acid, and tryptophan for the many bases containing aromatic nuclei. Once the fundamental building block for a certain group of compounds is established this allows one to speculate on the sequence of transformations regarded as feasible in the living cell. Most fruitful among the efforts to verify these speculations have been the feeding experiments with radioactive precursors. This is followed by isolation of radioactive natural compound and chemical degradation to isolate particular atoms and examine their radioactivity. It is a tribute to the authors of these biogenetic schemes that they have been so often proved correct.

When we examine how the study of biogenesis of natural products has helped in our understanding, the following two gratifying features always come to one's mind: a) first of all, with the emergence of pathways of biogenesis, it became possible to organise and divide the natural products into families according to their biogenetic groups. For example, steroids and terpenes, which at one time looked a bewildering array of compounds, now allow convenient correlation through their isoprenoid derivation. Although the present division of natural products into biogenetic groups is sometimes rough and arbitrary and often speculative, it provides a convenient organization

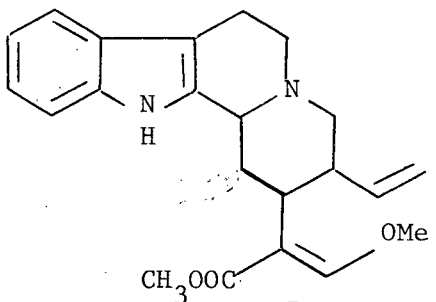
for learning and orientation which is better than has been possible before; b) the biogenetic speculations led to the successful construction of some remarkably simple laboratory syntheses of complex natural compounds. These syntheses were modeled on biogenetic lines and the synthetic schemes are to many chemists, more esthetically pleasing and satisfying. More important however, the biogenetic syntheses are often neater, shorter and more efficient than normal routes in which no attention is paid to the natural processess. Indeed it is sometimes found that the most satisfactory route to a particular natural product is the biogenetic type. The Robinson tropinone synthesis is an early but still an excellent example of this approach. A direct method of constructing the complex strychnine skeleton,[2] summarized in the following scheme, is another illustration.




It must be emphasized here that our real biosynthetic evidence from tracer and enzyme study is yet in its infancy and some of the biogenetic schemes could be discredited or seriously altered. It is undeniable that with the study of biogenesis, the science of natural products has raised to a new level.

In the annals of biogenetic theory perhaps no single class of natural products has enjoyed more ingenious speculations from the organic chemists than the family of indole alkaloids, which are formally derived from tryptamine and a "$C_9$-$C_{10}$" unit (for reviews see references 3-5). Not only the biochemical origin of the latter species but its appearance in the well known Corynanthe-Strychnos pattern (10, Figure 1) has provoked stimulating comments ever since Barger[6] drew attention to a possible biogenesis of yohimbine in 1934.

Recent structural studies have increased the number of these alkaloids to more than 800.[7] A tryptamine residue (2) appears almost invariably and in the few cases examined by the tracer method,[8,9] this residue has been found to be derived in the expected way from tryptophan (1). Tryptamine[10-12] (2) has recently been shown to be specifically incorporated into several alkaloids of Vinca rosea with considerable variation in efficiency, suggesting that decarboxylation may be delayed in some cases.

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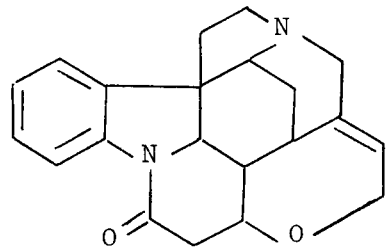


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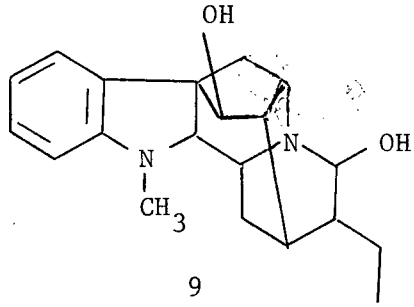


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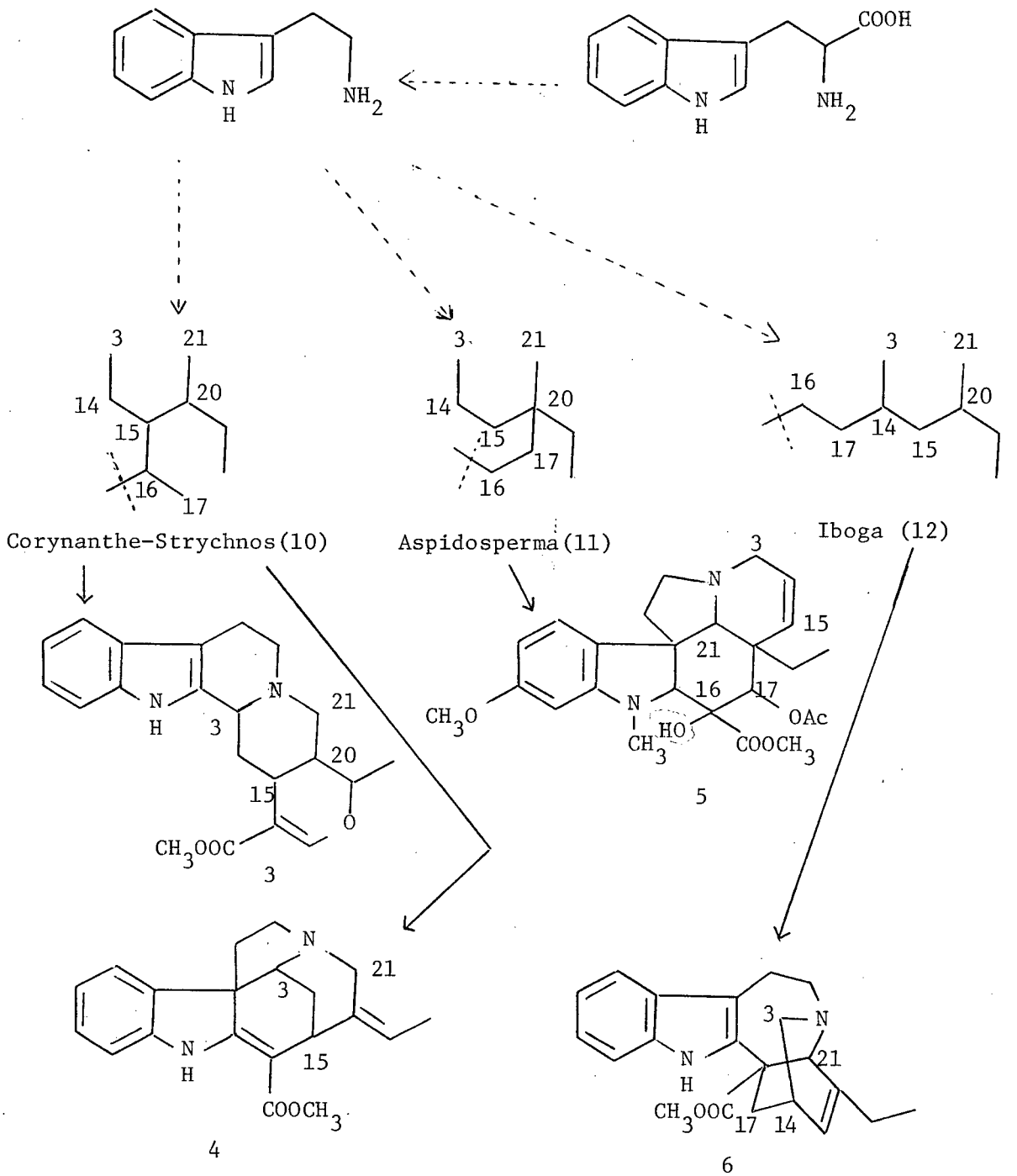



Figure 1. Scheme portraying the proposed relationship between three main classes of indole alkaloids.

The remaining nine or ten carbon atoms ($C_9$-$C_{10}$ unit) appear in what at first sight seems a bewildering variety of different arrangements but closer inspection allows three main groups to be discerned.[13] Together these three main groups account for the vast majority of indole alkaloids. We can conveniently refer to them as (a) Corynanthe-Strychnos type which possess the $C_9$-$C_{10}$ unit as (10) e.g. corynantheine (7), ajmalicine (3); (b) the Aspidosperma type having the $C_9$-$C_{10}$ unit as (11) e.g. vindoline (5) and (c) the Iboga series where the $C_9$-$C_{10}$ unit appears as (12) e.g. catharanthine (6). In those alkaloids where only nine carbon atoms are present in addition to the tryptamine residue, it is invariably the carbon atom indicated by the dotted line that has been lost (Figure 1).

## Origin of $C_9$-$C_{10}$ Unit

In contrast to the general agreement by different workers with regards to the "tryptophan" portion of the indole alkaloids, the biogenetic origin of the "non-tryptophan" or $C_9$-$C_{10}$ unit, has been the subject of much controversy. A number of theories dealing with this aspect have been proposed over the years.

It was suggested many years ago by Barger[6] and Hahn[14] that the indole alkaloids such as yohimbine (13) are formed by a Mannich reaction between tryptamine and 3,4-dihydroxyphenylacetylaldehyde. The initial product of this reaction (14) then undergoes a second Mannich reaction with formaldehyde yielding the pentacyclic system (15). It was then suggested[1] that the extra carbon atom, which is often at $C_{16}$ in ring E (Figure 2), is also derived from formaldehyde. A contribution

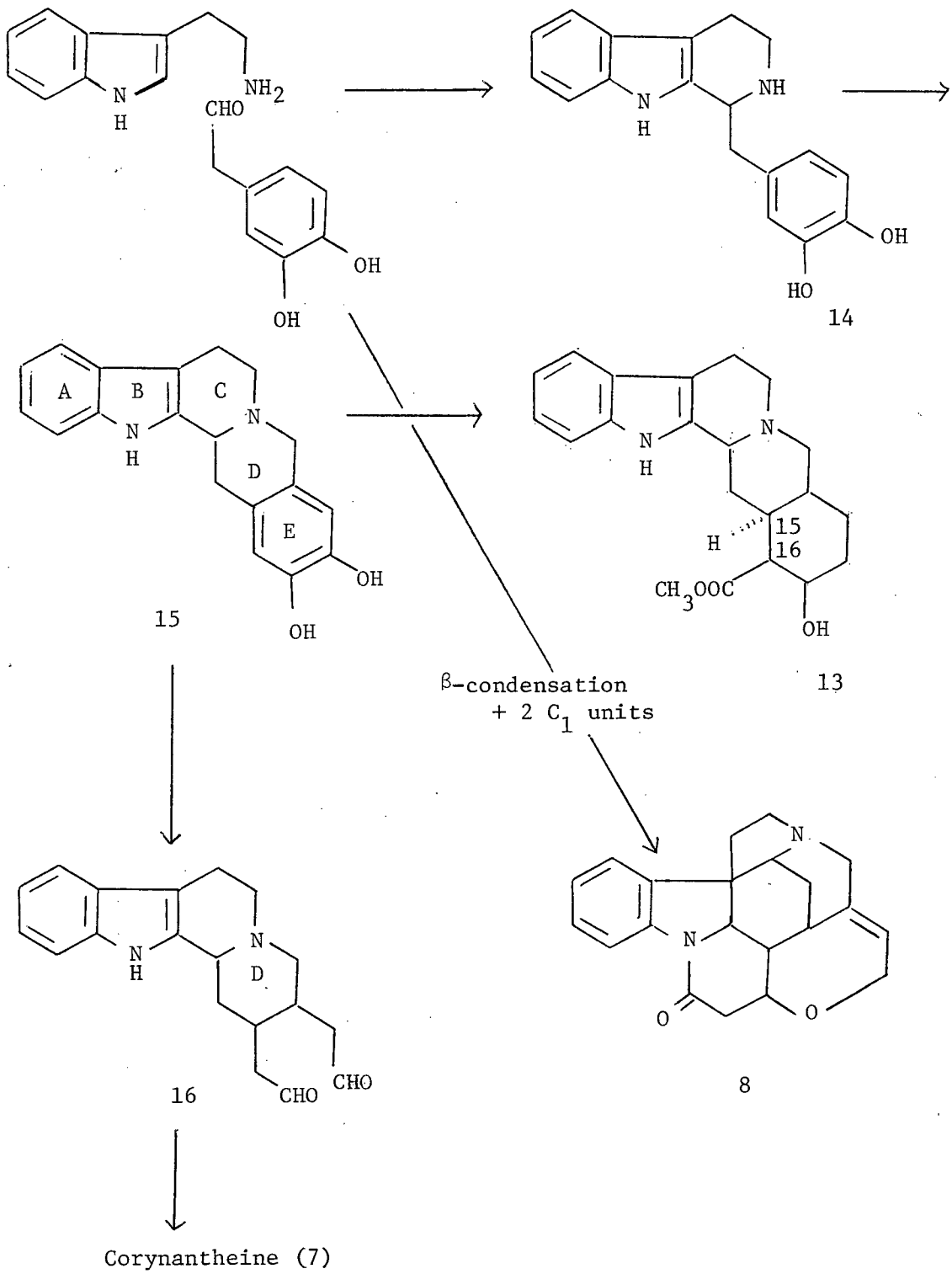



Figure 2. Barger-Hahn-Robinson-Woodward hypothesis.

of Woodward[15,16] was the suggestion that the catechol type ring E could undergo fission to yield an intermediate such as (16). The two side chains attached to ring D can then undergo plausible condensations with each other (e.g. ajamlicine, 3; corynantheine, 7) or with other parts of the molecule (e.g. ajamline 7) to give rise to various structural types. With this scheme Woodward was also able to rationalise the biogenesis of strychnine (8, Figure 2).

However, a number of deficiencies arose with the above theory and in 1959 Wenkert[17-19] proposed an elegant alternative. He suggested that prephenic acid (16) acts as a progenitor of the indole alkaloids. The latter rearranges according to the scheme shown in Figure 3 to afford a crucial intermediate, the seco-prephenate-formaldehyde (SPF) unit (20), which can be incorporated into yohimbine (13) and corynantheine

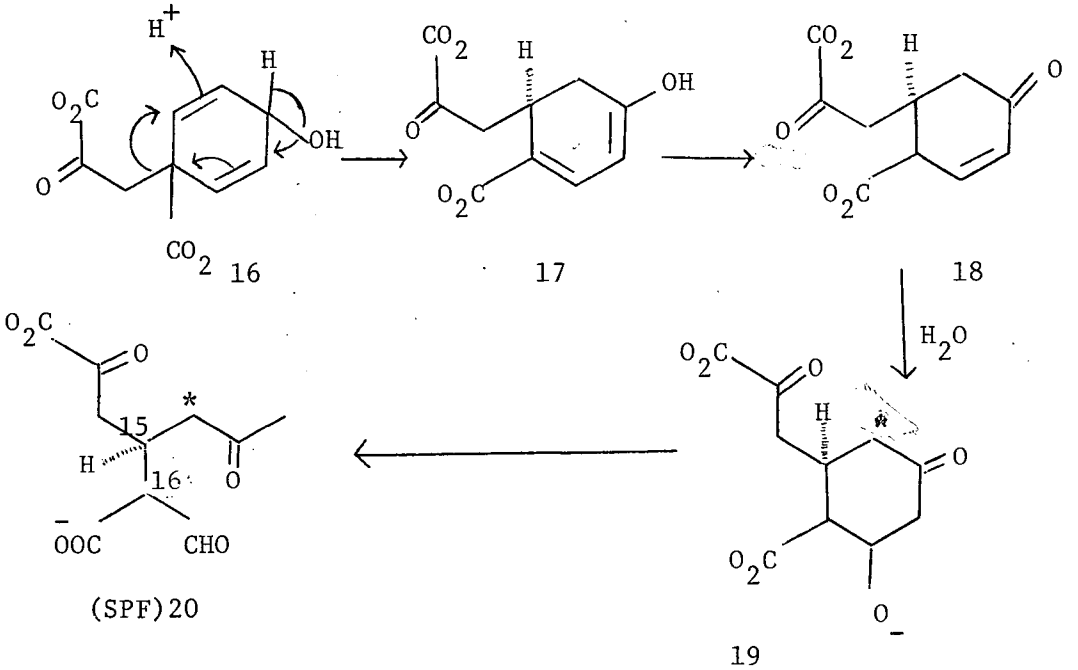



Figure 3. Wenkert's prephenic acid hypothesis.

(7). The most attractive features of this hypothesis are that it accounted for the presence of carboxyl group at $C_{16}$ and also rationalises the fact that the hydrogen attached at $C_{15}$ in yohimbine (13) and related alkaloids almost always has an α-configuration. Wenkert suggested that the α-configuration of the hydrogen atom at $C_{15}$ in the intermediate (17) is the result of stereospecific migration of the pyruvate side chain in compound (16). Furthermore, there is little chance for randomization at $C_{15}$ in the subsequent modifications of (17) to yield the various indole alkaloids.

Schlitter and Taylor[13] in 1960 and Leete[20-22] in 1961 postulated that the "non-tryptophan" portion of the indole alkaloids was derived via the acetate pathway. The suggestion was that a six carbon chain derived from three acetate units, condenses with malonic acid and a one carbon unit (biologically equivalent to formaldehyde) yielding the desired $C_{10}$ unit (Figure 4).

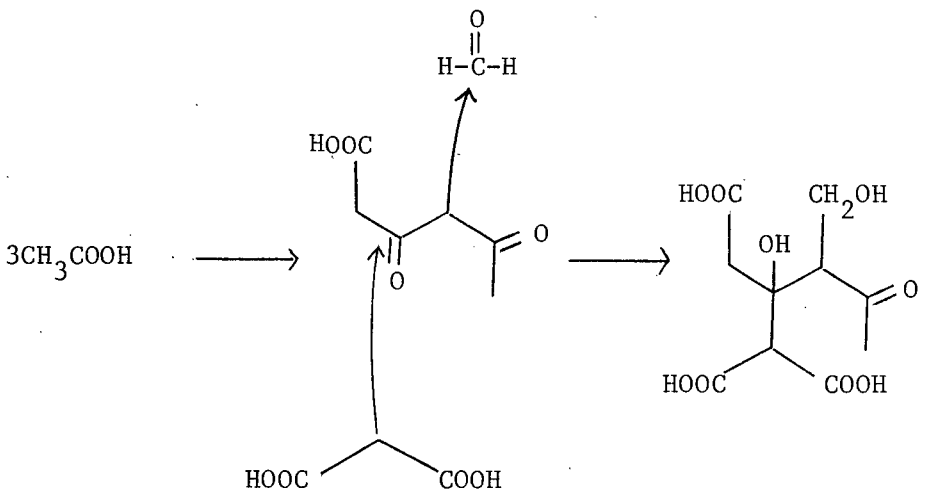
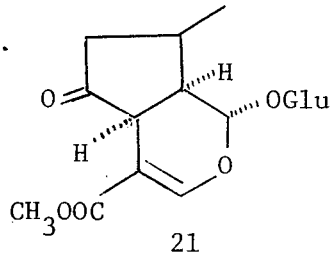



Figure 4. The acetate hypothesis.

Another hypothesis based on structural relationships was suggested independently by Wenkert[17-19] and Thomas.[23] The striking similarities between the skeletal features of various monoterpenes, verbenalin (21), gentiopicrin (22), bakankasin (23), swertiamarin (24), genipin (25), aucubin (26) andthe seco-prephenate-formaldehyde unit (20, the "$C_9$-$C_{10}$" unit) led these authors to suggest that the non-tryptophan portion of the indole alkaloids is "monoterpenoid" in origin.

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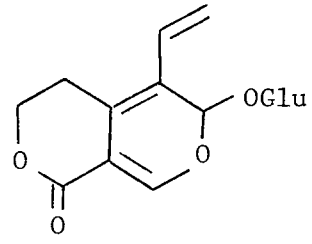


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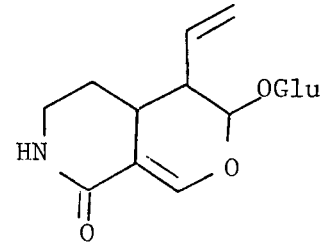


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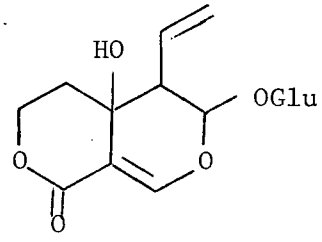


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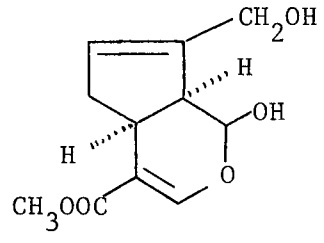


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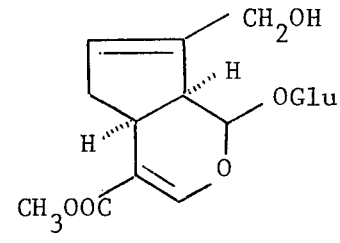


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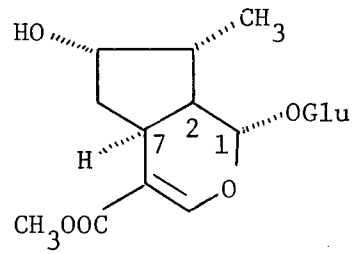
The initial biosynthetic experiments using radioactive precursors disproved all the hypotheses concerning the genesis of "$C_9$-$C_{10}$" portion of the indole alkaloids. It was not until 1965 that Scott[24] and coworkers were the first to report a successful incorporation of mevalonate (27) into vindoline (5). Subsequent publications by several groups of workers[25-28] established that specifically labelled mevalonic acid was incorporated into the indole alkaloids in a manner consistent with the monoterpene hypothesis. The next logical precursor

geraniol (28) was found to be incorporated[29-32] as an intact unit into vindoline (5), catharanthine (6) and ajmalicine (3) in Vinca rosea L. shoots. Each of these alkaloids is representative of one of the three types of alkaloid families found in this and other plants.

These findings allowed Battersby[4] to rationalise the three categories of indole alkaloids as shown in Figure 5. The Corynanthe skeleton (10) is first derived formally by cleavage of the generalized iridoid pattern (29). Loss of one carbon atom (indicated by broken line in 10) rationalises the Strychnos "$C_9$" unit (30), as found in akuammicine (4). The Corynanthe skeleton is schematically related to the Aspidosperma (11) and the Iboga series (12) by cleavage of the $C_{15}$-$C_{16}$ bond in (10) and formation of the $C_{17}$-$C_{20}$ (path A) or $C_{17}$-$C_{14}$ (path B) bonds. The formation of the yohimbine class (31) is also reached from 10, this time by ring closure via $C_{17}$-$C_{18}$ bond formation.

The first evidence for the cyclopentane intermediate in the pathway was obtained by Battersby and coworkers[33] who showed that loganin (32) was incorporated into ajmalicine (3), vindoline (5) and catharanthine (6) in Vinca rosea L. plants. These findings were later confirmed by other workers in Vinca rosea[34-36] and Rauwolfia serpentina

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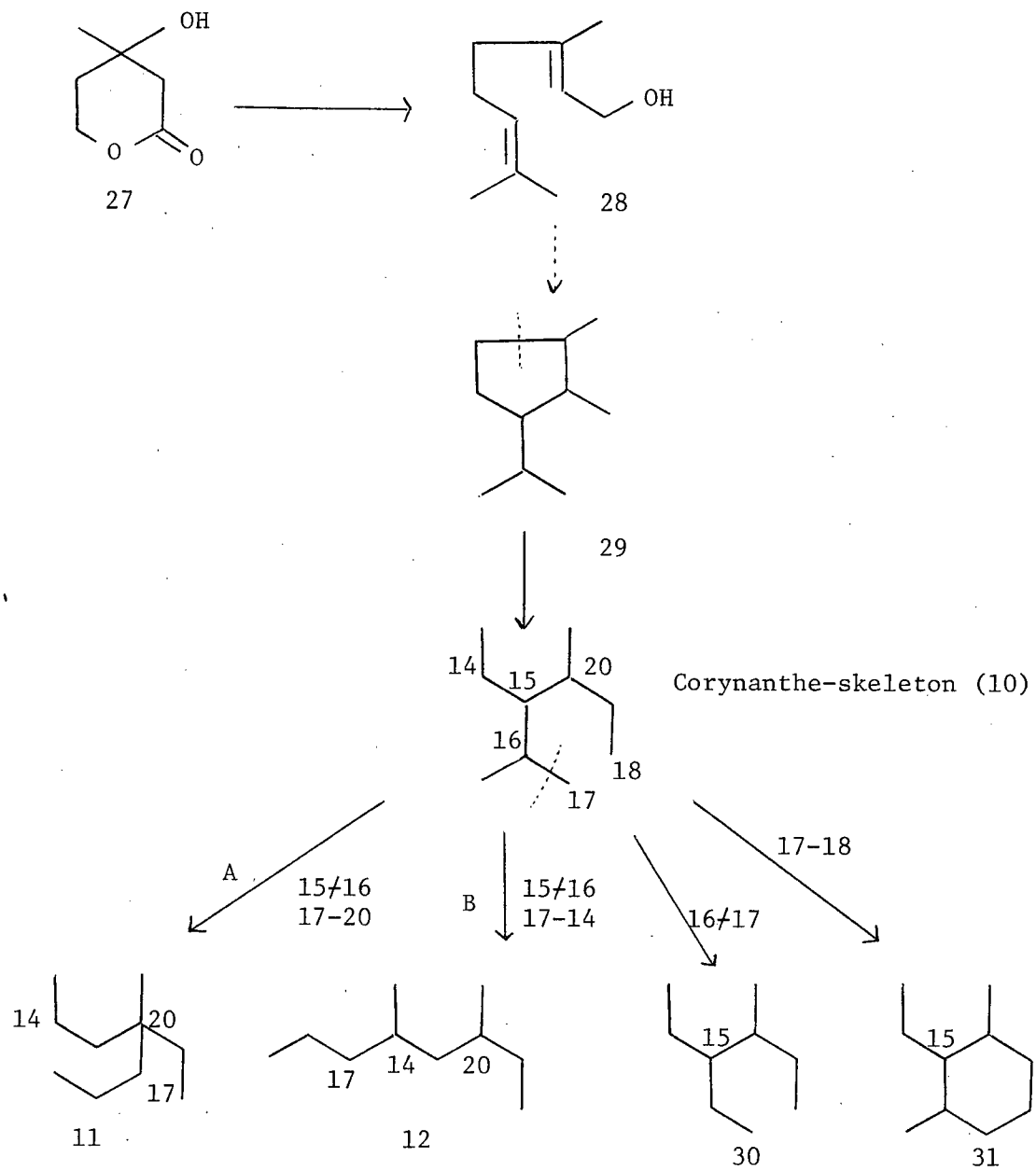
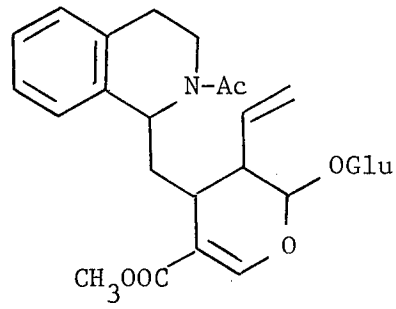



Figure 5. The Thomas-Wenkert hypothesis.

plants.[34] The next step forward in the continuing search to unfold the biosynthetic pathway of indole alkaloids, had to wait until the structure of another alkaloids, ipecoside (33) was unraveled.[37] The non-nitrogenous portion of ipecoside was also found to be derived from

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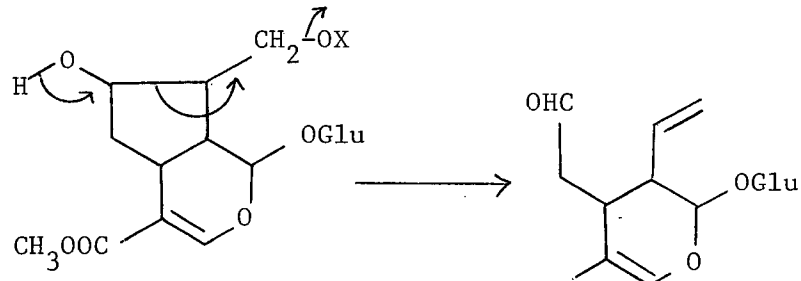
loganin (32).[4] This led Battersby to suspect that loganin is cleaved to yield secologanin (36) and the illustrated process by way of hydroxy loganin (34), perhaps as its phosphate ester (35), is a plausible one.[4,38]




34, X = H

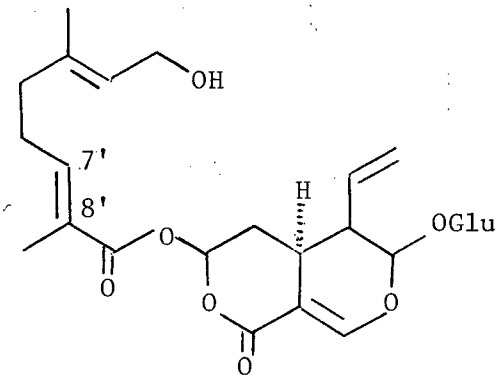
35, X = phosphate

36

Confirmatory evidence for the existence of the hypothetical intermediate (36) came with the isolation[39,40] of three new glucosides, foliamenthin (37), dihydrofoliamenthin (38), and menthiafolin (39). Not only are these glucosides of great biosynthetic interest in their own right, but they also contain secologanin (36) in a masked lactol form. It is interesting to point out that secologanin is a biointermediate corresponding to (10) in the Thomas-Wenkert hypothesis (Figure 5).

The synthesis of radioactive (doubly-labelled) secologanin and its

feeding to Vinca rosea resulted in the positive incorporation without scrambling[38,41] of label. A most gratifying outcome of all these elegant labelling experiments was the finding that the stereochemical

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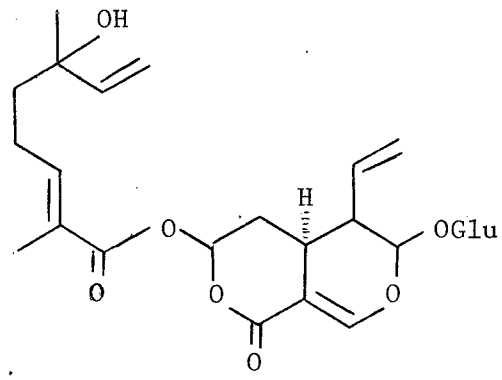



38, 7',8'-reduced

39

integrities of $C_7$ in the loganin (32) and its secoderivative (36) are maintained at $C_{15}$ in the Corynanthe alkaloids. Furthermore $C_1$ of the loganin (32) is carried through to the alkaloids at the aldehyde level, i.e., the proton marked with an asterisk in geraniol (28) (see Figure 6) survives all subsequent rearrangements. The latter requirement assumes prime importance in formulating and testing the mechanisms developed below. The summary of the geraniol → seco-loganin pathway[38] is shown in Figure 6 and it includes the recently described iridoid (40)[42] as well as the "secolactone" sweroside (41)[43] which has been biologically converted into the three main classes of indole alkaloids.

It has been further argued some years ago[4] that if secologanin (36) condenses with tryptamine, a β-carboline (e.g. 42) would be formed

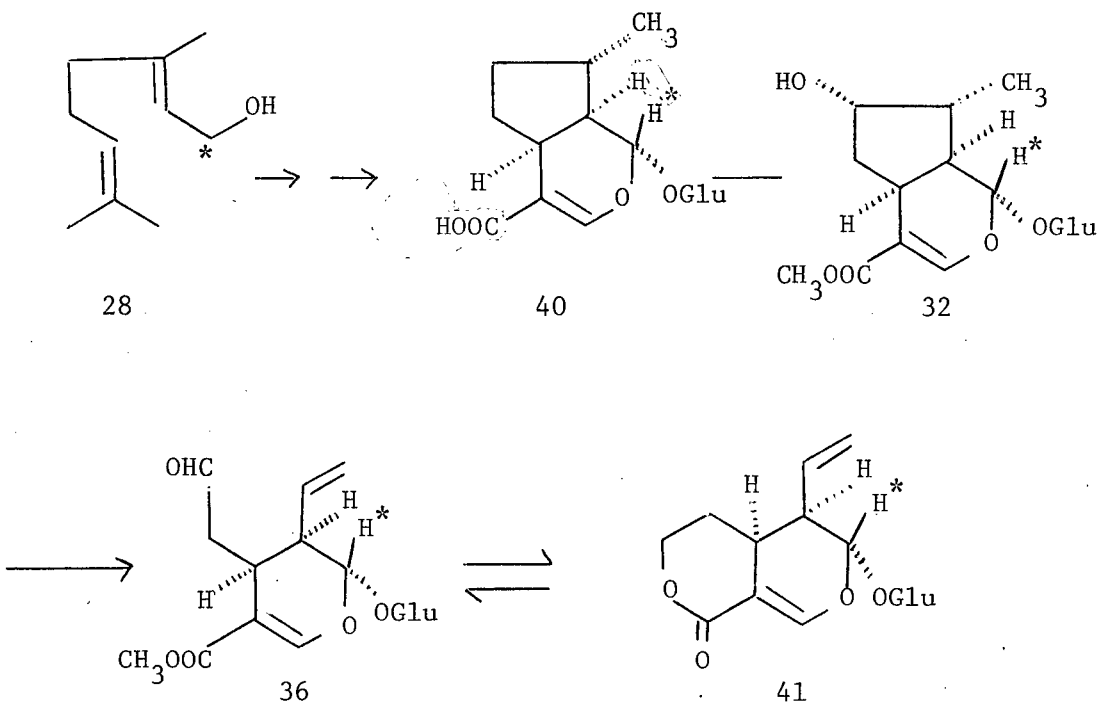
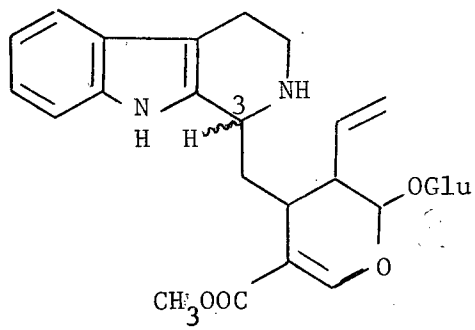



Figure 6. A summary of geraniol → secologanin pathway.

and this latter substance was suggested as the first nitrogenous intermediate in the biosynthetic sequence leading to indole alkaloids. Evidence for this kind of intermediate was obtained with the discovery of strictosidine (42) (stereochemistry not established) in Rhazya species[44] and its presence was subsequently demonstrated in Vinca rosea by dilution with radioactive tryptophan and loganin.[45]

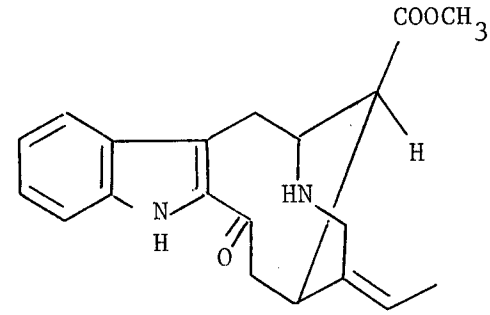
A mixture of the radioactive isomers, vincoside (43) and isovincoside (44) (enantiomeric at $C_3$) prepared from secologanin of known stereochemistry and tryptamine, when fed to Vinca rosea resulted in the isolation of radioactive ajmalicine (3), vindoline (5), catharanthine (6) and perivine (45).[46,47] Dilution analysis in Vinca rosea




42

43, $\alpha$-$C_3H$

44, $\beta$-$C_3H$

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plants which had previously taken up [5-$^3$H]-loganin, confirmed that secologanin (36), vincoside (43) and isovincoside (44) are natural products of the plant. Subsequently N-acetylvincoside was isolated from the glycoside fraction of Vinca rosea[48] in good yield (19 mg/ 1.5 kg) and vincoside (43) was shown to be converted to the three types of indole alkaloids. Isovincoside (44) was not an effective precursor.[47]

With this knowledge in hand that indole alkaloids are in fact elaborated monoterpenoids, the next problem was to find out how β-carboline (42) is converted into various indole alkaloids. A major problem inherent in these unknown steps concerns the timing and mechanisms of transformations whereby the β-carboline system is sequentially transformed not only to form Corynanthe and Strychnos alkaloids but how it rearranges to the Aspidosperma and Iboga bases. In this regard it is relevant to mention that Scott's study of the sequential appearance of various alkaloids in short term (1-300 hours) germinating Vinca rosea has helped considerably in suggesting the dynamics of the biosynthetic mechanisms. An interesting account of this work has been

reviewed very recently by Scott[5] and a summary of these results is shown in Table I.

Table I. Isolation of Alkaloids from Vinca rosea Seedlings

| Germination time, hr | Alkaloids isolated | Type |
|---|---|---|
| 0 | None | |
| 26 | Vincoside (43)<br>Ajmalicine (3)<br>Corynantheine (7) | "Corynanthe" |
| 28-40 | Corynantheine aldehyde (50)<br>Geissoschizine (51)<br>β-Hydroxyindolenine (57)<br>"Diol" (58) | Corynanthe |
| | Geissoschizine Oxindole (59) | Corynanthe |
| 40-50 | Preakuammicine (56)<br>Akuammicine (4)<br>Stemmadenine (55)<br>Tabersonine (71) | "Corynanthe-Strychnos"<br>Strychnos<br>"Corynanthe-Strychnos" |
| 72 | 11-Methoxytabersonine (77) | Aspidosperma |
| 100-160 | Catharanthine (6)<br>Coronaridine (78) | Iboga |
| 200 | Vindoline (5) | Aspidosperma |

The biological conversion of vincoside (43) into various indole alkaloids could reasonably involve cleavage of the glucose unit to form vincoside aglucone (46) which would be in equilibrium with, or convertible into aldehyde (47,48). Ring closure to N(b) (see 49, Figure 7) and reduction could then lead to corynantheine aldehyde (50) and/or geissoschizine (51). Ajmalicine (3), an abundant alkaloid of Vinca rosea

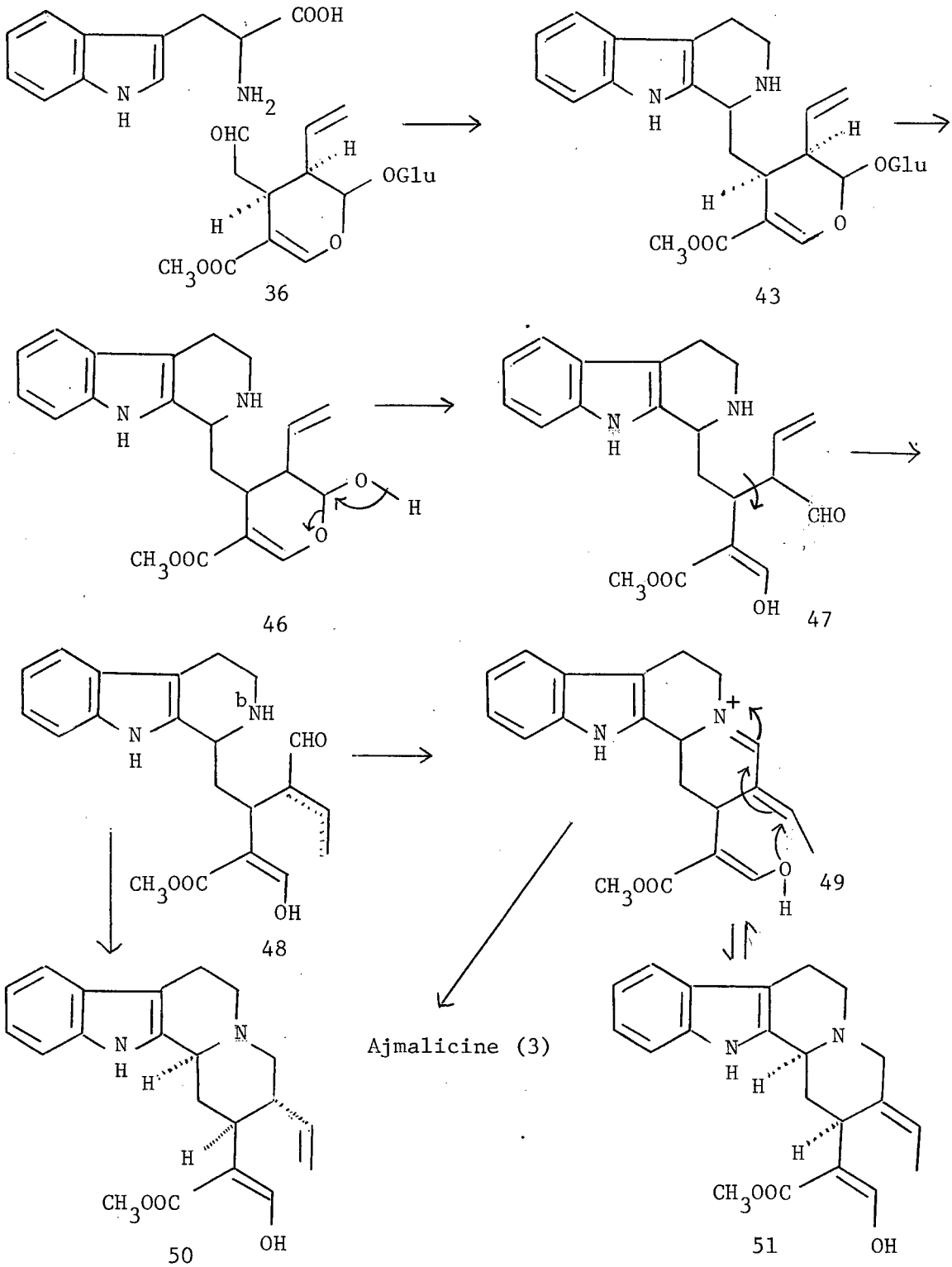



Figure 7. A summary of the secologanin → geissoschizine pathway.

could be reached by cyclization of the species 51 or 49 since the $C_{20}$ proton of the ajmalicine (3) is not labelled by loganin-2-$^{3}H$.[49]

Earlier feeding experiments in Vinca rosea[50] established the intact incorporation of geissoschizine (51) into ajmalicine (3), akuammicine (4), vindoline (5), and catharanthine (6). The incorporation of corynantheine aldehyde (50) into vindoline (5) and catharanthine (6) has been reported in Vinca rosea seeds.[51] This finding is in sharp contrast to the insignificant incorporation of this substance in mature plants.[50,51] However Battersby,[50] in trying to reconcile these observations, has suggested that it could be possible that seedings are able to convert (50) to (51). Recently geissoschizine (51) has been shown to be a component present both in Vinca rosea plants[50] and Vinca rosea seeds (28-40 hours fraction).[52] This study therefore suggests that geissoschizine (51) stands as a key Corynanthe alkaloid beyond vincoside (43) on the biosynthetic pathway.

A most gratifying outcome of these elegant labelling studies was the rearrangement ($\alpha \rightarrow \beta$) of geissoschizine (51) to form the Strychnos skeleton of akuammicine (4).[50] This observation was particularly important because this rearrangement generates the bond between $C_2$ and $C_{16}$ (see 51 and 56 in Figure 8). Since it is necessary to oxidize the Corynanthe series in order to reach the Strychnos level, it was suggested[19,53] that this process when applied to geissoschizine (51) involves one electron oxidative coupling to give strictamine (52, R = CHO). Precedent for the rearrangement of compounds such as (53) to the Strychnos representative, akuammicine (4) is available,[54] so that the indolenine (54) or its reduced form (56) (Figure 8) could be

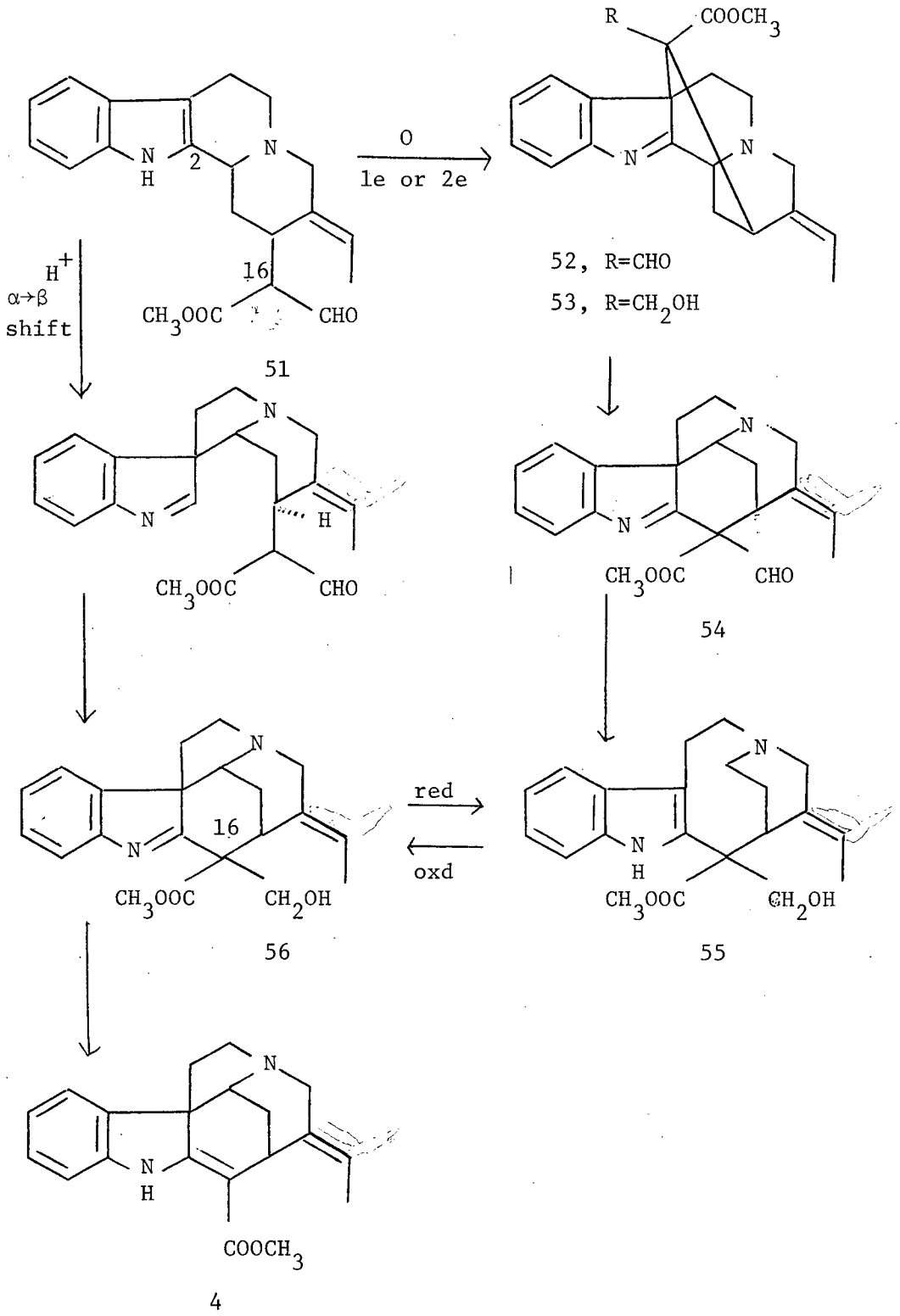



Figure 8. The rearrangement of Corynanthe → Strychnos skeleton.

reached by such a mechanism. An alternative to this mechanism, also an *in vitro* analogy,[55] is α-protonation of the indole nucleus followed by the α → β rearrangement summarized in Figure 8. Consistent with this study was the isolation of "$C_{10}$" and "$C_9$" Strychnos alkaloids preakuammicine (56) and akuammicine (4) in the 45-50 hours fraction of *Vinca rosea* seeds.[56]

More recently Scott[56] has suggested a third mechanism (Figure 9) which imputes an intermediary role to an unknown alkaloid geissoschizine oxindole (59). The formation of such an oxindole from geissoschizine (51) has ample *in vitro* precedence and might take place *in vivo* by the steps indicated in Figure 9, where the β-hydroxy indolenine (57) is rearranged directly or via the dihydroxyindoline ("diol" 58) to (59). Conversion of 59 to the imino ether (60, R = alkyl or enzyme bound functionality) would endow 60 with reactivity required[57] to form preakuammicine (56), as shown. In support of this mechanism Scott[56] was very gratified to find geissoschizine oxindole (59) (identical with the synthetic material) in the 45-hour fraction of *Vinca rosea* seeds.

To summarise all the work up to here, it is safe to say that all the steps involved in the biochemical conversion of secologanin to Corynanthe alkaloids have become clear. With the study of sequential isolation of various biointermediates, the steps involved in the rearrangement of Corynanthe skeleton to Strychnos skeleton have just begun to unfold themselves.

## The Biogenesis of Aspidosperma and Iboga Alkaloids

A most ingenious idea was adduced by Wenkert[19,58] to rationalise the transformation of the Corynanthe skeleton to the Aspidosperma and

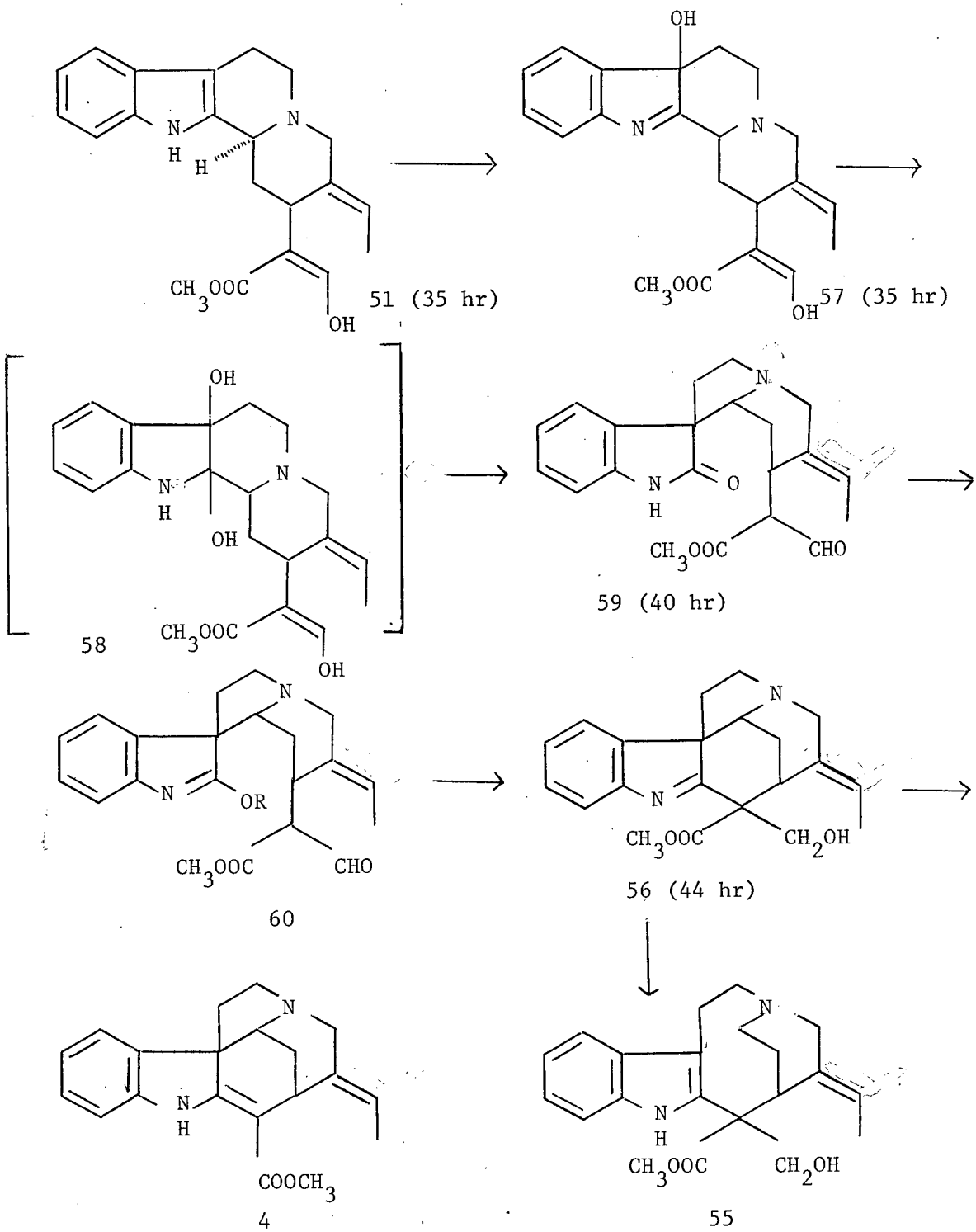
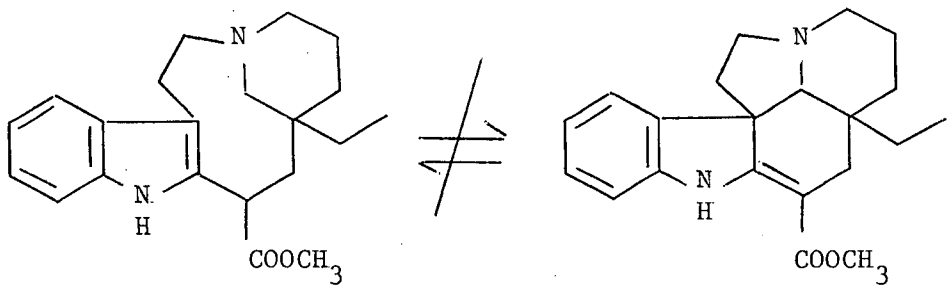



Figure 9. Scott's scheme for the rearrangement of Corynanthe → Strychnos skeleton.

Iboga type (Figure 10) (type A and B transformations at the alkaloid level in Figure 5). The rearrangements suggested for paths A and B required the presence of the 1,5-dicarbonyl function in order to operate the reverse Michael reaction implicit in the cleavage of $C_{15}$-$C_{16}$. Thus the cleavage product (63) undergoes ordinary oxidation-reduction changes and in this manner piperidines of various oxidation states are formed. Intramolecular Michael and Mannich reactions of the latter lead to Aspidosperma (65) and Iboga like (68) skeletons. However, feeding experiments with radioactive precursors raised some serious doubts regarding some of the steps depicted in Figure 10. For example transannular cyclization utilized by Wenkert to provide an entry into Aspidosperma (64 → 65) and Iboga (67 → 68) skeletons, inspite of having excellent analogies <u>in vitro</u>[59-62] was shown by Kutney[63,64] to be an insignificant biochemical reaction. This latter study suggested that the genesis of pentacyclic alkaloids (Aspidosperma type) e.g. vincadifformine (70) is completely independent of the nine-membered alkaloids e.g. vincadine (69).

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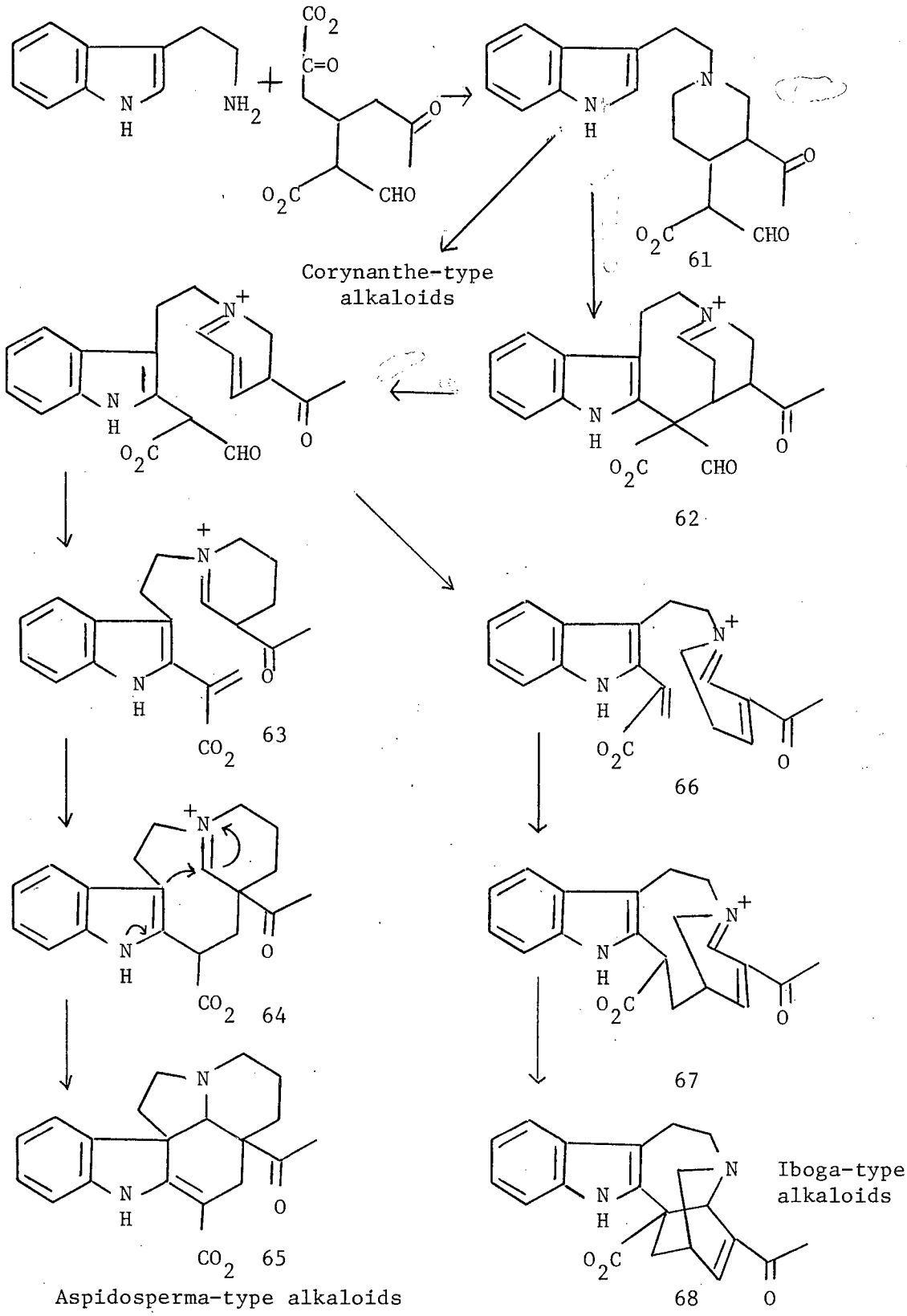
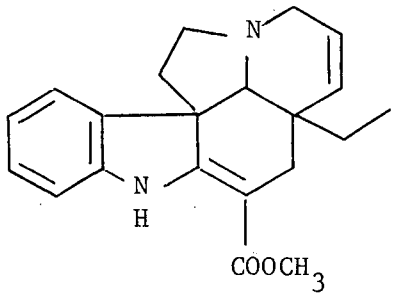



Figure 10. Wenkert's proposal for the biosynthesis of Aspidosperma and Iboga alkaloids.

Returning to the chronological isolation of various alkaloids from Vinca rosea seeds (Table I) Scott[51] found another alkaloid stemmadenine (55) in the 50-hour experiment. It is very interesting to point out here that the possible intermediacy of units similar in structure to stemmadenine e.g. 62 was invoked in the sequence between the Corynantheinoid and Aspidosperma bases by Wenkert[19,58] (Figure 10) many years ago. When the germination was allowed to proceed further (72 hours), it led to the isolation of an Aspidosperma alkaloid, tabersonine (71). Catharanthine (6), the principal Iboga alkaloid of Vinca, although isomeric with tabersonine, does not appear to be formed until the germination has proceeded for 100 hours. This was a vital piece of evidence in suggesting a rough sequence of alkaloid formation in nature i.e. stemmadenine (55) → tabersonine (71) → catharanthine (6). The biochemical conversion of tabersonine (71) to vindoline (5) and most interestingly to the Iboga alkaloid catharanthine (6) has been demonstrated in our laboratories[64] and independently by Scott[51] in Vinca rosea seeds. These latter results suggest a possible relationship between the Aspidosperma and Iboga alkaloids. Similarly belief in stemmadenine (55) as a true biointermediate was further strengthened by its incorporation into tabersonine (71) and catharanthine (6) in Vinca rosea seeds[51] and into vincamine (72) and minovine (73) in Vinca minor plants in our laboratories.[65] Scott and Qureshi[66] reported the rearrangement of tabersonine (71) to (±)-catharanthine (6) and (±)-pseudocatharanthine (74) in refluxing acetic acid. Stemmadenine (55) under similar conditions (refluxing acetic acid)rearranged to (±)-tabersonine (71), (±)-catharanthine (6),



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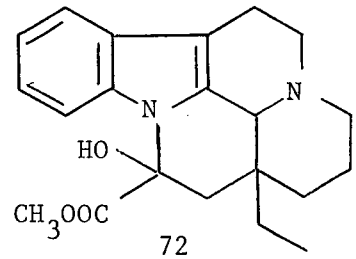

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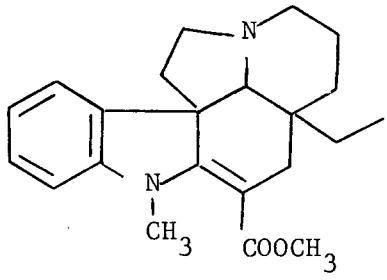

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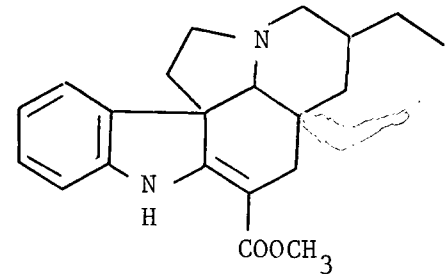


74

and (±)-pseudocatharanthine (74). These results were portrayed as a laboratory simulation of the biochemical results described above. However, lately Smith[67] inspite of many repeated attempts failed to duplicate Scott's in vitro results.

A most attractive mechanism linking stemmadenine (55), tabersonine (71), and catharanthine (6) was advanced by Kutney.[64] This involves the achiral intermediate 76a≡76b, which can, in principle be generated by migration of the double bond in stemmadenine (55) to (75), followed by the illustrated fragmentation (Figure 11). A similar postulate for the formation of the acrylic ester (76) has been independently advanced by Scott[66]. In order to explain the observed

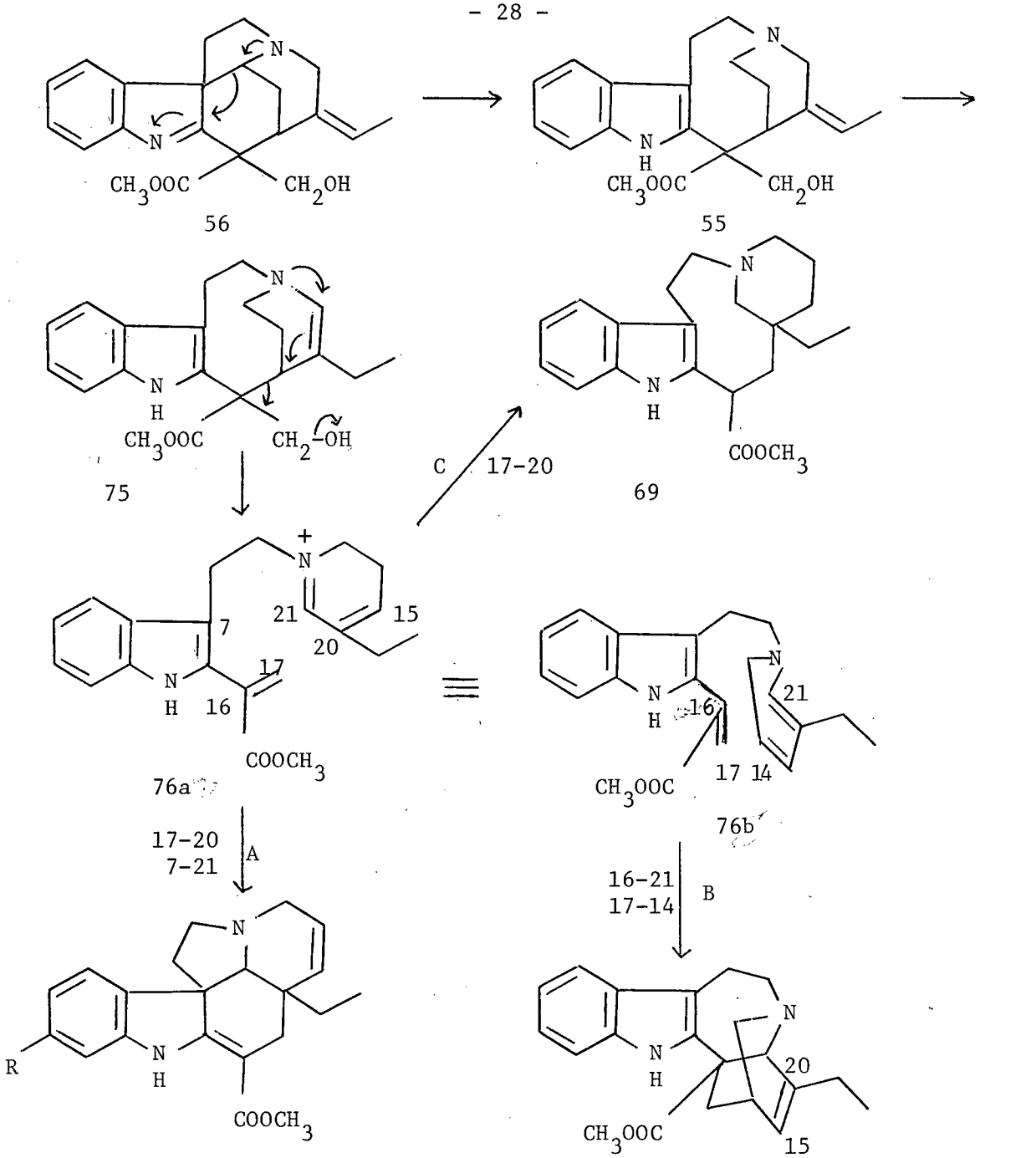
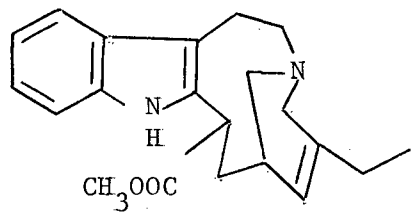



Figure 11. Some later stages of indole alkaloid biosynthesis.

sequence, it is suggested that the enzymatic folding of 76 in mode A would give tabersonine (71), and later, at an other enzymatic site, cyclization in mode B forms catharanthine (6). Yet a third possibility C explains the genesis of the vincadine (69) series. The relative insignificance of the transannular cyclization[63,64] now suggest that the process 7 → 21 occurs prior to or simulataneously with 17 → 20 in the elaboration of the putative intermediate (76) to vincadifformine (70). In a similar fashion the conversion of the unit (76) to the alkaloid catharanthine (6) is unlikely to proceed initially via the process 17 → 14, since this would lead to a carbomethoxycleavamine system (79). Previous results[63] in our laboratory have suggested that

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carbomethoxycleavamine (79) is not a progenitor of this Iboga alkaloid (6). This theory therefore places stemmadenine (55) in a key position between the Strychnos and other families not only in Vinca rosea but predictably in all species, and furthermore rationalizes the formation of racemic Aspidosperma alkaloids such as (±)-vincadifformine (70) mediated by the achiral ester (76). The absolute minimum of functionality has been used for all of these postulated interconversions, and

it is generally believed that the proposed biogenesis is common to all species. Thus it so turned out that the galaxy of complex, oxygenated, fragmentated, and rearranged structures which constitutes the complex series of indole alkaloids in fact stem from these few fundamental alkaloids. It must be emphasized here that although all the evidence indicated to develop the above theory represent an important modification of Wenkert's original theory particularly with regards to sequence, oxidation level and mechanisms, these results do not detract from the essential correctness of his views on the interrelationship of the main classes of indole alkaloids.

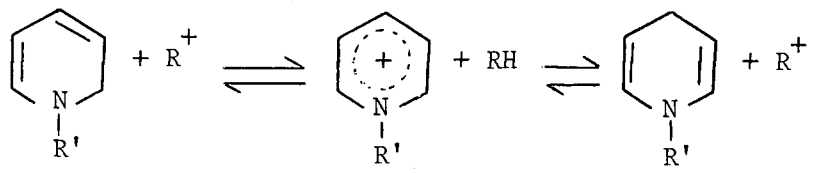
In summary, all the available results suggest very emphatically that the acylic ester (76) would fulfil a pivotal role in the genesis of various families of indole alkaloids. Laboratory analogies for almost all of the suggested processes are now available. With the establishment of the Corynanthe-Strychnos-Aspidosperma-Iboga relationship (based on sequential isolation and feeding experiments) the various further subclasses should fall into place. In 1967 Battersby[4] made the statement "The problem is at a most fascinating stage where the researcher can see that the precise detail of the pathways to the indole alkaloids cannot now escape him". The rapidly evolving scene as summarized above provides ample proof in support of this view.

## DISCUSSION

With the knowledge that indole alkaloids are in fact elaborated monoterpenoids, we were intrigued by the second major problem posed by the structures before us; how are the rearrangement of Corynanthe to Aspidosperma and Iboga skeletons carried out in nature (summarized in Figure 11), and where would we begin to test the virtual myraid of possible substrates designed to undergo the A and B transformations? It was fully revealed in the introduction that Wenkert's[53] speculations on the mechanisms of rearrangements involve the acrylic acid (66) and its dihydro-derivative (63) as intermediates, whilst Kutney's[64] and Scott's[66] results led them to propose a different mechanism making use of acrylic ester (76) and the corresponding enamine. The intermediacy of acrylic ester (76) was again invoked by Scott[66] to explain the *in vitro* transformation of tabersonine (71) to (±)-catharanthine (6) and (±)-pseudocatharanthine (74); and of stemmadenine (55) to (±)-tabersonine (71), (±)-catharanthine (6) and (±)-pseudocatharanthine (74) (as already mentioned earlier, these later transformations have now been questioned[67]). In summation, all these results provided a strong suggestion that the acrylic ester (76) may be a true biointermediate. We decided that in spite of many problems associated with the synthesis and feeding of this putative intermediate (76), the knowledge gained by its evaluation as a biogenetic intermediate would be of greatest value in suggesting the dynamics of the biosynthesis of Aspidosperma and

Iboga alkaloids.

To examine the biogenetic role of any intermediate, the first step involves the synthesis of the postulated precursor. This is then followed by feeding the active precursor into the appropriate plant system and isolation of the alkaloids after a certain period of time to examine the amount of radioactivity in them. It was clear to us at the outset of our synthetic work that (76) because of the presence of a dihydropyridine segment in it would be a very unstable compound. There was considerable precedent available in the literature which could lend support to our initial doubts. For example, it is well known that dihydropyridines readily oxidize to the corresponding pyridines. This process occurs so rapidly that even contact with atmospheric oxygen is sufficient to bring about the transformation. This property therefore makes the characterization and study of the properties of the dihydropyridines rather difficult.[68] Another reaction characteristic of dihydropyridines is their rapid isomerization. Although complete experimental details are not known, any reagent that can assist in the removal of a proton, hydrogen atom, or hydride ion may cause isomerization.[68] The following scheme illustrates the isomerization of 1,2- and 1,4-dihydropyridines through a pyridinium ion.



Investigations directed towards elucidating the structures and mode of biological action of coenzymes NAD and NADP have frequently engendered studies on the chemistry of dihydropyridines. On several occasions, the latter have been found to dimerize.[69]

With all this knowledge in hand, it became obligatory to make some model compound as our synthetic target. We understood that this model compound while sufficiently stable in its own right (to allow characterization) should be capable of transformation in vivo to the putative intermediate (76) via biologically feasible reactions. One such compound which met all these prerequisites was 16,17-dihydrosecodin-17-ol (90, see Figure 12) (the name for this compound was suggested by Battersby,[85] numbered according to biogenetic principles[70]). This compound upon dehydration ($COOCH_3$-C(H)-C(OH) $\longrightarrow$ $COOCH_3$-C=$CH_2$) and oxidation in the piperidine ring (H-C-N- $\longrightarrow$ -C=N$^+$-) could generate the desired intermediate. Since both these reactions are biogenetically feasible, we made the alcohol (90) as our initial synthetic target.

For the sake of convenience and ease of presentation, the discussion has been divided into two parts. The first part describes the synthesis of 16,17-dihydrosecodin-17-ol (90) as well as syntheses of [ar-$^3$H]-16,17-dihydrosecodin-17-ol (90) and [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90). Finally feeding of the $^{14}$C-precursor into Vinca minor L. is presented. The second part describes the syntheses of secodine (107, the name for this compound was suggested by Smith[96]), [ar-$^3$H]-secodine (107) and feeding of the active precursor into Vinca minor L.

PART I

The desired alcohol (90) was amenable to synthesis by the route shown in Figure 12. The choice of this route was dictated by the fact that it was possible to synthesize 2-carboethoxy-3-(β-chloroethyl)-indole (80) in the laboratory in reasonable quantities. This material intrinsically incorporated all the structural requirements needed to start our sequence. For example the presence of the α-carboethoxy group acted as a handle in allowing us to expand the side chain at the α-position of the indole in (80) to the desired functionality. On the other hand presence of chlorine in the 3-(β-chloroethyl) side chain allowed us to attach the appropriated substituted pyridine to the indole nucleus.

The synthesis of the desired chloroindole (80) was devised a few years ago in our laboratories in connection with some other work on the total synthesis of indole alkaloids[71] and the sequence is fully revealed in Figure 13. Diethyl-γ-chloropropylmalonate[72] (91) was prepared in 70% yield by treating the monosodium salt of the diethyl-malonate with 1,3-bromochloropropane. The chloromalonate derivative (91) was converted into the corresponding arylhydrazone (93) through the agency of a Japp-Klingemann reaction.[73,74] This procedure involved the slow addition of anhydrous benzenediazonium chloride[75] to the anion of (91) in ethanol at -5°. The reaction mixture was allowed to stand overnight inthe refrigerator and the crude reaction product was subjected to a Fischer indole synthesis[76] using sulfuric acid as catalyst.

We would like here to make certain remarks regarding the preparation

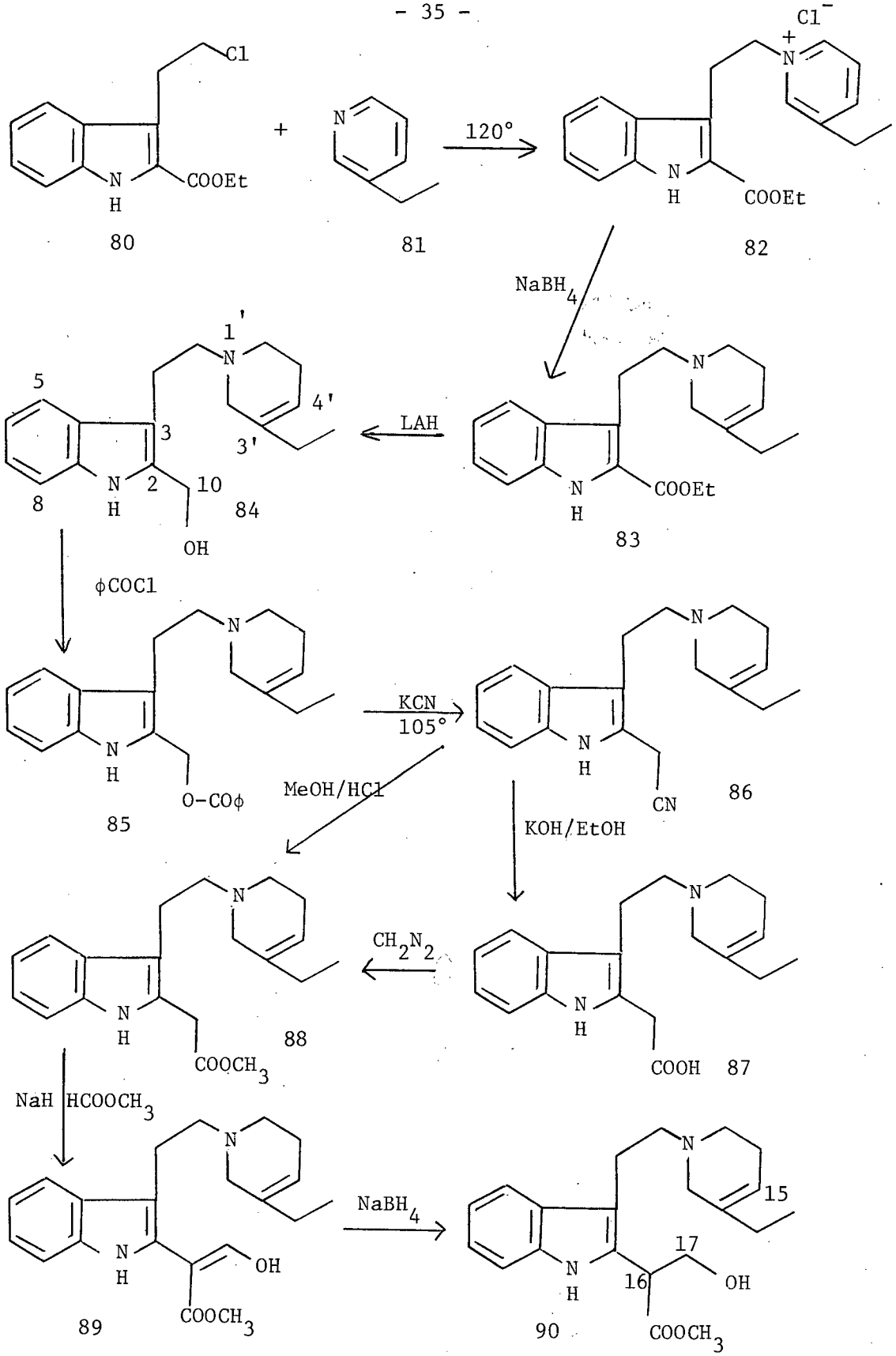



Figure 12. Synthesis of 16,17-dihydrosecodin-17-ol (90).

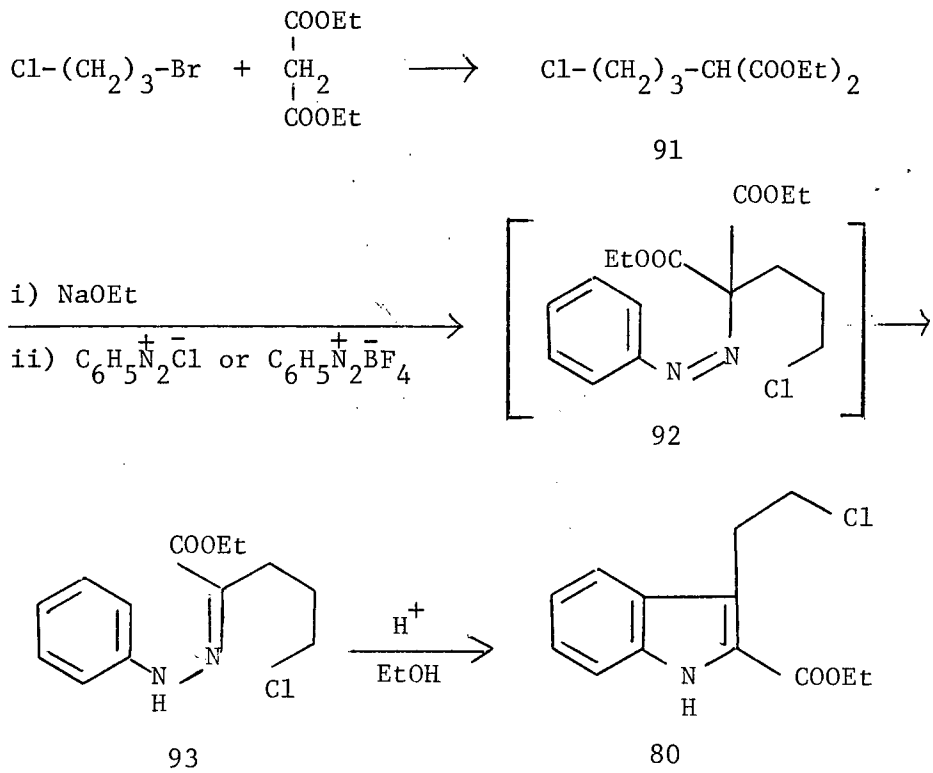



Figure 13. Synthesis of 2-carboethoxy-3-(β-chloroethyl)-indole (80).

of anhydrous benzenediazonium chloride. The usual method for making this diazo salt is given by Smith and Waring.[75] These authors report that their method gives the salt in crystalline form. However our experience with this procedure is in sharp contrast to this claim. In spite of having followed the reported procedure very carefully, we continually ended up with lumps of the solid diazonium chloride. Since the reaction conditions demanded that we add the diazonium salt in small portions, the lumps had to be broken into small pieces (under nitrogen). This did not pose much of a problem in small scale reactions, but it started raising its ugly head when the reaction was

scaled up to obtain larger quantities of the chloroindole (80). In one of the instances the crushing of the lumps into small pieces resulted in a very violent explosion. All of these unfortunate incidents kept on thwarting our progress for a long time as the supply of chloroindole (80) remained very limited. In the meantime a new synthesis of 6,7-diazasteroid (95, see Figure 14) appeared in the literature[77] and the use of m-methoxybenzenediazonium fluoroborate was reported. This led us to wonder if we could also use the more stable[78] benzenediazonium fluoborate in our sequence. Indeed coupling of the anion of (91) with benzenediazonium fluoborate[78,79] gave a deep red oil. This product was

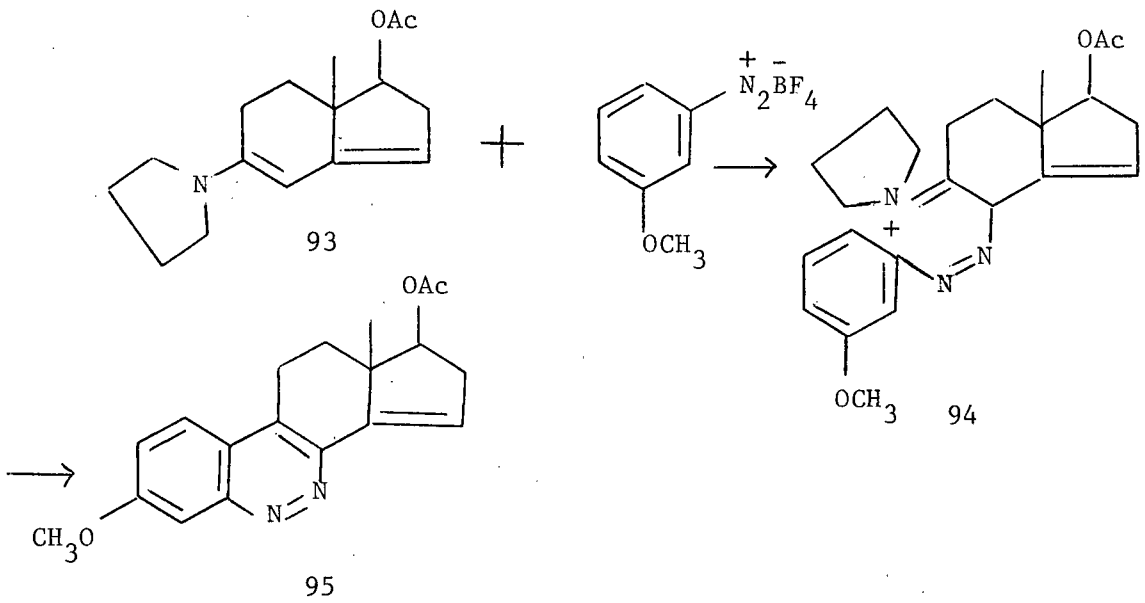



Figure 14. Synthesis of 6,7-diazasteroid (95).

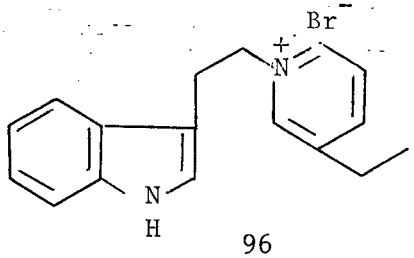
subjected to a Fischer indole synthesis. Purification of the crude product by chromatography on alumina gave a crystalline compound which showed the same $R_f$ value on thin layer chromatography (t.l.c.) and

spectral properties as the chloroindole (80) obtained when benzenediazonium chloride was used. The most gratifying outcomes of this undertaking were that: (a) benzenediazonium fluoborate, when dry was always a very fine powder (like talcum face powder), a situation which remarkably simplified our technical problem in the Japp-Klingemann reaction namely the slow addition of diazonium salt to the anion of (91); (b) the fact that the fluoborate salt was fairly stable and easy to handle, allowed us to scale up the preparation of this salt to 120 gm/batch, thereby allowing large scale preparation of the chloroindole (80). With all these problems unraveled, we were able to start our synthetic sequence with confidence.

For various reasons we thought the best reaction to start initially was the coupling of 3-ethylpyridine to the chloroindole (80). For this purpose 3-ethylpyridine (81, bp 162-163°) was readily obtained from commercially available 3-acetylpyridine by means of Wolff-Kishner reduction.[80] Condensation of chloroindole (80) with 3-ethylpyridine gave a white amorphous solid (82) in 91% yield. In general this salt was used directly for the succeeding step. However a small amount of material was crystallized for analytical purposes, mp 87-89°. The spectral data compared favourably with the assigned structure (82). The infrared spectrum indicated a strong ester absorption at 1701 $cm^{-1}$. In the nmr spectrum the resonances of the two ethyl groups ($-\underline{CH_2}-CH_3$, $-COO\underline{C_2H_5}$) were clearly separated; two triplets at $\tau$ 8.98 (3H, $-CH_2-\underline{CH_3}$) and 8.63 (3H, $-COOCH_2-\underline{CH_3}$); two quartets at $\tau$ 7.40 (2H, $-\underline{CH_2}CH_3$) and 5.74 (2H, $-COO\underline{CH_2}CH_3$). In the spectrum the resonances corresponding to the aromatic protons of the pyridinium nucleus (4H, $\tau$ 1.4-2.34) and of

the indole nucleus (4H, $\tau$ 2.56-3.18) were also clearly discerned. Because of the overlapping absorptions of the pyridinium and indole nuclei, the ultraviolet spectrum was not too informative. Finally the molecular formula, $C_{20}H_{23}N_2O_2Cl$, was supported by mass spectrometry ($M^+$ 358).

With the pyridinium salt (82) in hand, reduction to the tetrahydropyridine (83) was then considered. At this stage it was thought that if we could use lithium aluminum hydride (LAH) for this purpose, reduction of the pyridinium segment in salt (82) to the tetrahydropyridine stage would be accompanied by conversion of the ester function to the desired primary alcohol (84). However a survey of the literature revealed that while there is a general agreement between various workers that sodium borohydride always reduces N-alkylpyridium salts to their corresponding tetrahydropyridines,[81-83] the results from the LAH reductions are at variance. For example, it was found by Panouse[81] that N-alkylpyridinium salts are reduced by LAH to 1-alkyl-1,2-dihydropyridines. Reduction of N-[β-(3-indolyl)-ethyl]-pyridinium bromide (96) by means of sodium borohydride or LAH has been reported[82] to lead exclusively to the tetrahydropyridine (97). Later Wenkert[83] reported the formation of three compounds (97-99) upon reduction of

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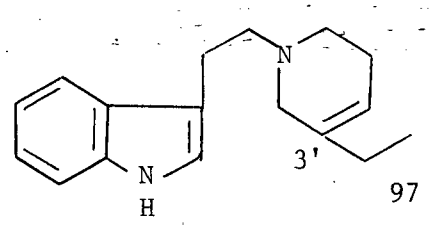


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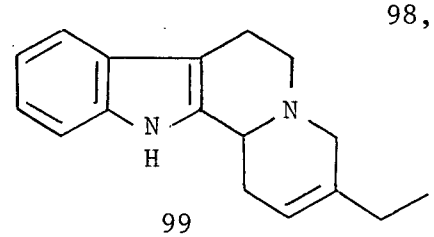
98, $\Delta^{3'}$-reduced




99

(96) with LAH. All these contradictory findings diminished our enthusiasm for LAH reduction. Indeed when a small amount of pyridinium chloride (82) was exposed to LAH, we were not surprised to find that the crude product was a complicated mixture of several components. A more successful alternative involving two reductive steps provided the desirable results. Thus sodium borohydride reduction of the salt (82) to the tetrahydropyridine (83) followed by LAH reduction of the carbo-ethoxy group in the latter yielded alcohol (84).

The nmr spectrum of the crude sodium borohydride product was very informative. The resonance corresponding to the four protons of the pyridinium nucleus in (82) ($\tau$ 1.2-2.34) had completely disappeared and instead a broad one proton singlet at $\tau$ 4.5 was reconcilable with the presence of an olefinic proton at $C_{4'}$. In this tetrahydropyridine (83), the isolated double bond has been tentatively placed in the $\Delta^{3',4'}$. Its position would be made unambiguous in the next step.

The crude tetrahydropyridine ester (83) obtained above was subjected directly to the lithium aluminum hydride reduction. The resultant light yellow gum which was purified by chromatography on alumina provided a crystalline compound (84), mp 108-110°, in an overall yield of 70% from the chloroindole (80). The structure (84) was assigned on the basis of the following spectra data. In the infrared, there was no absorption in the ester region and instead a broad band at 3340 $cm^{-1}$ ($-CH_2\underline{O}H$) was now evident. The nmr spectrum (Figure 15) showed the newly formed hydroxymethylene group as a sharp singlet at $\tau$ 5.17. The olefinic proton ($C_{4'}$-H) was located at $\tau$ 4.45 in good agreement with the assignment reported by Wenkert.[83] The mass spectrum

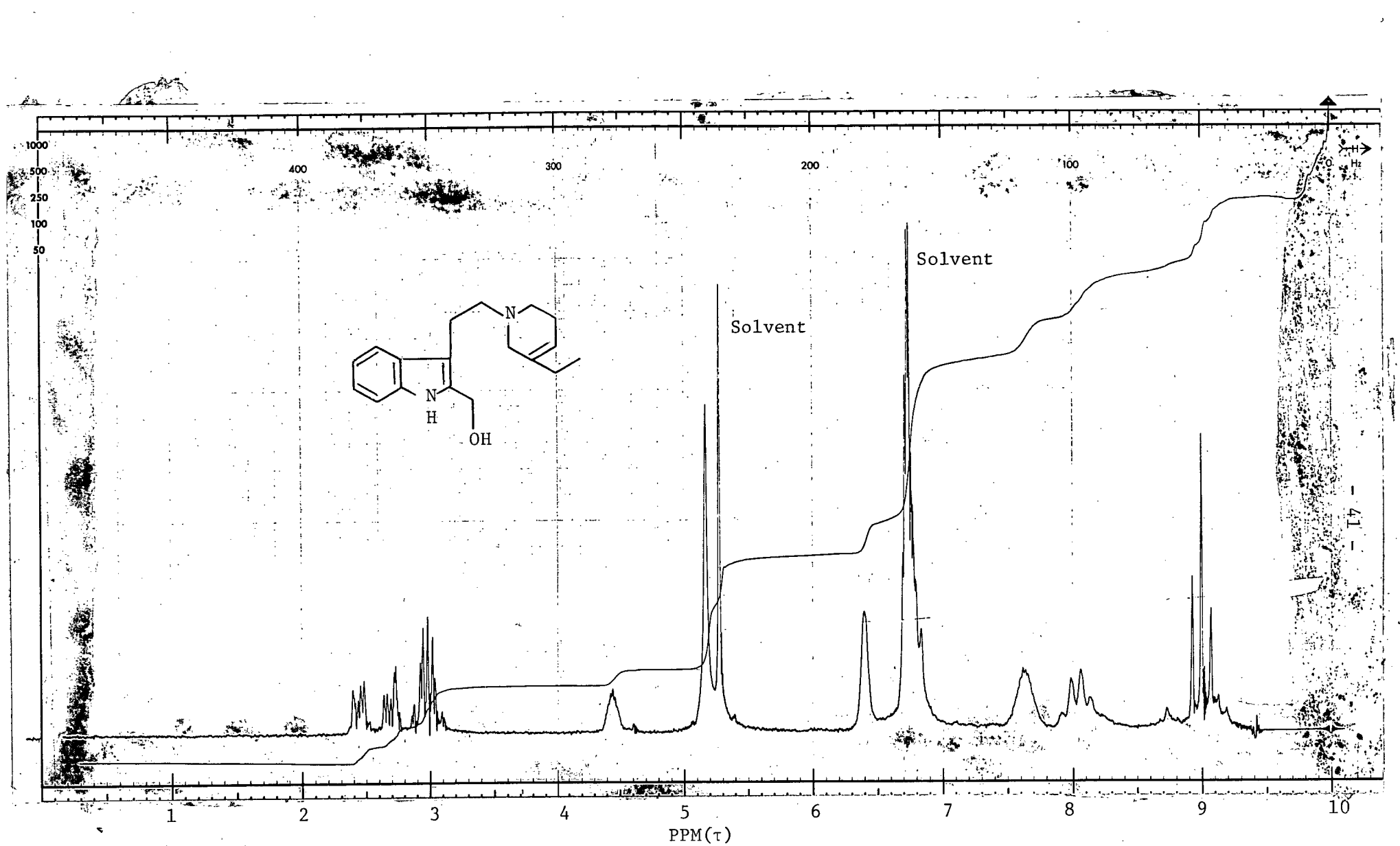
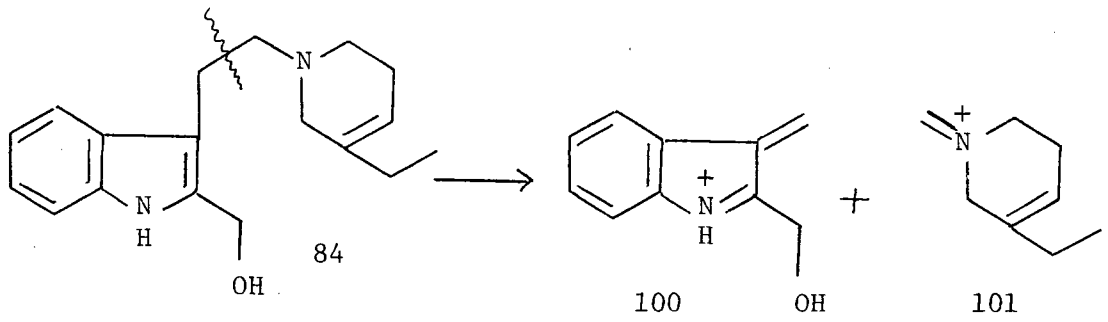



Figure 15. Nmr spectrum of alcohol 84.

(Figure 16) showed a molecular ion peak at m/e 284 and was dominated by two significant peaks at m/e 160 and m/e 124. These peaks were attributed to the simple fragmentation of the parent molecule to the ions (100) and (101) respectively. Finally the molecular formula,




$C_{18}H_{24}ON_2$, was confirmed by high resolution mass spectrometry (Found: 284.184; Calc.: 284.188) and elemental analysis.

Now to elaborate the side chain at the α-position of the indole in alcohol (84) to the desired functionality, it was necessary at this stage to incorporate an extra carbon atom. The sequence, 84 → 86, proved most desirable for this purpose. The conversion of the alcohol (84) to benzoate (85) was accomplished by dissolving the alcohol in dry pyridine and treating it with benzoyl chloride. The whole reaction was over within three hours and the crude product obtained was homogeneous on tlc. This allowed its utilization in the succeeding step without any purification. However for analytical purposes a small amount of material was further purified by chromatography on alumina and recrystallization from methylene chloride and petroleum ether, mp 110.5-112.5°. The spectral data of the benzoate (85) was in complete accord with the formulation. The infrared showed the carbonyl of the benzoate ester at 1715 $cm^{-1}$ while in the nmr spectrum, the aromatic

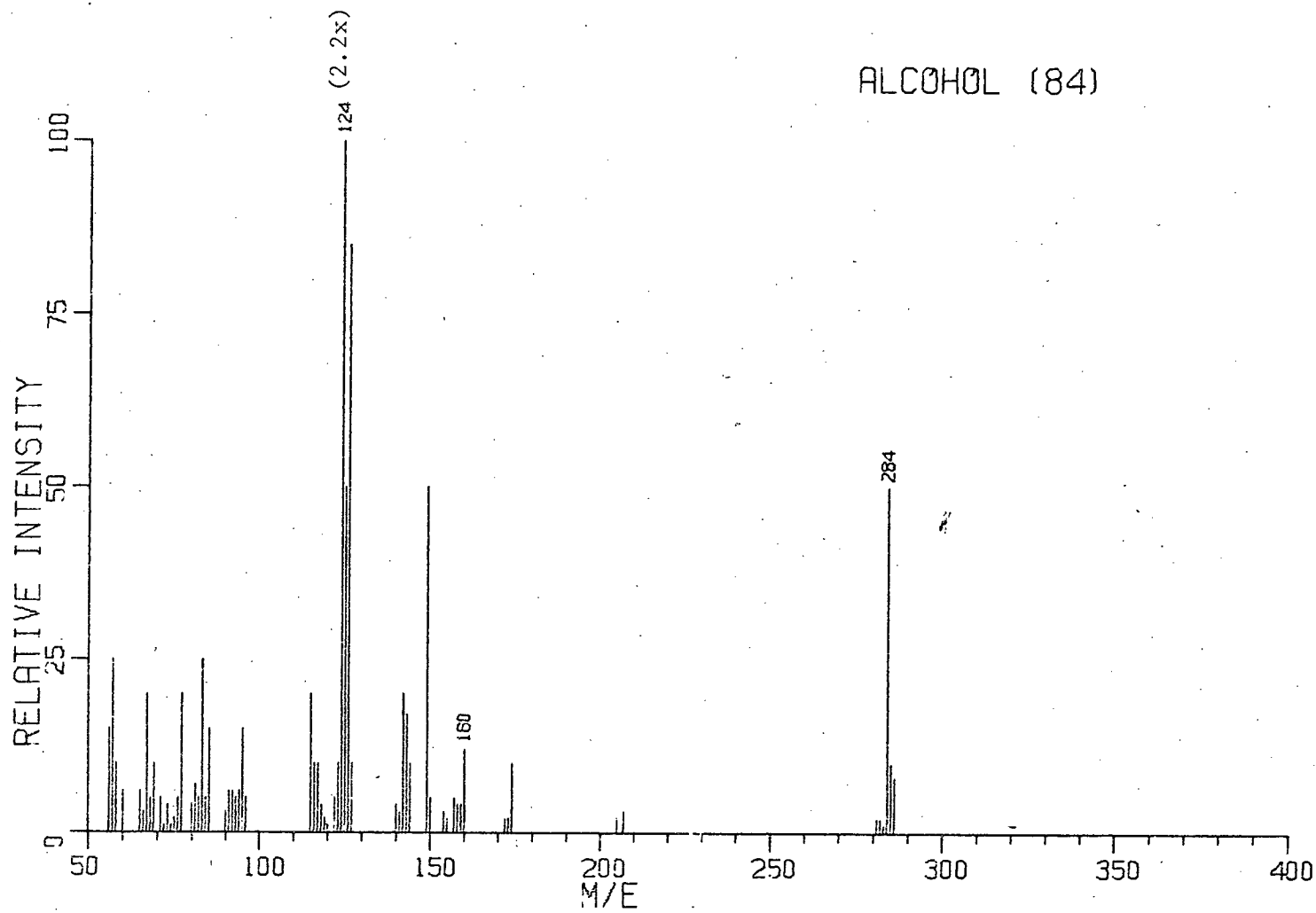



Figure 16. Mass spectrum of alcohol 84.

protons of the benzoyl group although overlapping with the protons of the indole nucleus, appeared between τ 2-3 (9H). The mass spectrum indicated a molecular ion peak at m/e 388. Finally the molecular formula, $C_{28}H_{25}O_2N_2$, was confirmed by high resolution mass spectrometry (Found: 388.215; Calc.: 388.216) and elemental analysis.

Although the above procedure for making the benzoate derivative (85) was quite satisfactory, it was observed that if all the pyridine (used as solvent) was not carefully removed from the crude product, the yield in the succeeding reaction was somewhat lower. In view of the known susceptibility of benzoates to heat, the pyridine had to be removed at room temperature in vacuo. This process was tedious and generally required leaving the compound under vacuo for considerable period of time. In order to obviate this difficulty several alternative procedures were studied. The optimum conditions involved dissolving the alcohol (84) in tetrahydrofuran and treating the mixture with benzoyl chloride in the presence of solid potassium carbonate. The crude product thus obtained was chromatographed on alumina. Elution with chloroform gave a crystalline compound which had the same $R_f$ and spectral properties as the benzoate obtained above. The overall conversion of alcohol (84) to benzoate (85) by this second procedure was essentially quantitative and the quality of the product obtained was much superior.

With benzoate (85) in hand, the next step forward demanded the nucleophilic displacement of the benzoate group with cyanide anion. This reaction contrary to our expectation, turned out to be a very temperamental process. This conversion posed many problems and some

of them are delineated below: (a) the reaction proved extremely sensitive to temperature. After running a few small-scale trial reactions it was clear that the temperature had to be raised very slowly from room temperature to 105° otherwise considerable tarring of the reaction mixture occurred; (b) we found that the desired compound (86) was also very sensitive to temperature. This implied that when the temperature of the reaction mixture reached 105°, it had to be maintained at this elevated temperature for a minimum amount of time. If the reaction mixture was left longer than required at high temperature (105°), an overall yield of 20% as compared to 65% was obtained. Furthermore the cyano compound (86) obtained was also contaminated with many spurious side products. At this stage it is not possible to define the various side reactions that occurred at this high temperature. For the purpose of control in this reaction thin layer chromatography played an important role. Fortunately it so happened that the benzoate (85) and the cyano compound (86) showed very distinct colors when the tlc plate was sprayed with antimony pentachloride. Benzoate (85) appeared as a dark blue spot while the cyano compound (86) appeared as light green. Upon disappearance of the benzoate in the mixture (as monitored by tlc) the reaction was immediately terminated; (c) finally we experienced some trouble in the purification of the cyano compound (86) by chromatography. It was found that when a large amount of alumina was used (ratio of compound to alumina 1:100) in anticipation of achieving better separation, it led to considerable polymerization of the desired compound. This fact was even more pronounced when the columns employed were slow running or in other words when the cyano compound (86) was left on the

column for a longer period of time. In all these cases only very dark polar gums were obtained from the columns. However when the columns employed were very short and fast running, the cyano compound (86) was eluted in crystalline form and in a highly satisfactory yield. In summation, it could be said that this displacement reaction was very critical (in terms of temperature, time, and purification by chromatography) but the whole process turned out to be fairly efficient when properly executed.

The optimum conditions for the above reaction required dissolving the benzoate (85) in dimethylformamide and adding solid potassium cyanide ( 10 fold excess) to it. This heterogeneous mixture was stirred at room temperature for one hour and then the temperature of the oil bath was gradually raised to 105° over a period of 45 minutes. The reaction mixture was maintained at this elevated temperature for about one hour. At this time tlc indicated no more starting material. The reaction was immediately stopped and the pure cyano compound (86) was isolated by chromatography on alumina. The initial elutions with benzene-petroleum ether (1:1) provided a crystalline compound (55%) while the latter fractions (benzene elution) were gummy (10%). Tlc examination of this gum indicated that this portion of the cyano compound contained a very minute impurity but the compound was of reasonable quality to be utilized in the next reaction. For analytical purposes a small amount of the material was recrystallized from dichloromethane-petroleum ether, mp 135-137°, and later sublimed at 100°/0.01 mm. The spectral data compared favourably with the assigned structure (86). The infrared was diagnostic for the presence of a nitrile group (2256 $cm^{-1}$)

while the strong ester peak present in the benzoate (85) at 1715 $cm^{-1}$ had completely disappeared. In the nmr spectrum (Figure 17) the methylene group adjacent to the nitrile ($-CH_2-CN$) appeared as a sharp singlet at $\tau$ 6.2. The mass spectrum indicated a molecular ion peak at m/e 293 and was dominated by two peaks at m/e 124 and m/e 169. Finally the molecular formula, $C_{19}H_{23}N_3$, was confirmed by high resolution mass spectrometry (Found: 293.186; Calc.: 293.189) and elemental analysis.

Now that all the problems associated in obtaining the cyano compound (86) had been unraveled, we considered its conversion to the carbomethoxy ester (88). Two reactions utilised most widely for this purpose are: (a) hydrolysis of nitriles to corresponding carboxylic acids and subsequent esterification of the latter to esters; (b) treating the nitriles with methanol and hydrochloric acid. Both of these procedures were tried for our own purpose.

Treatment of the nitrile (86) with alcoholic potassium hydroxide gave the corresponding carboxylic acid (87). We initially had some fears that the carboxylic acid (87), because of its amphoteric character might pose some problem during its workup. But contrary to our expectation the reaction products could be extracted quantitatively from the aqueous layer once the pH of the reaction medium was carefully brought to 7. The impure acid (87) obtained in this way was esterified with diazomethane. Unfortunately tlc of the crude product showed it to be a mixture of many components. However, the desired compound was obtained pure by very careful chromatography on alumina (yield 25-30%). When the carboxylic acid (87) was esterified with methanol and sulfuric acid, again a yield of 25% was obtained.

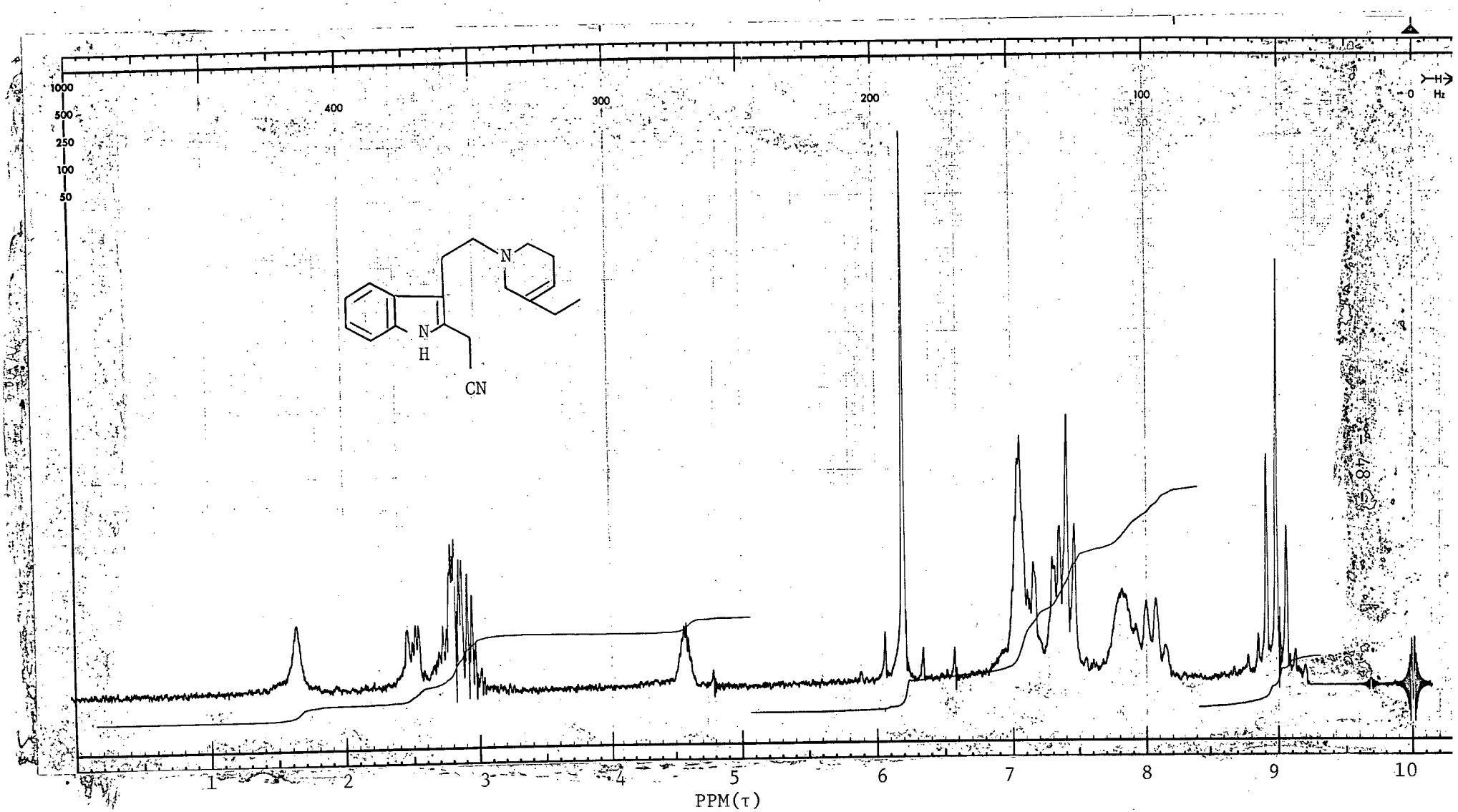
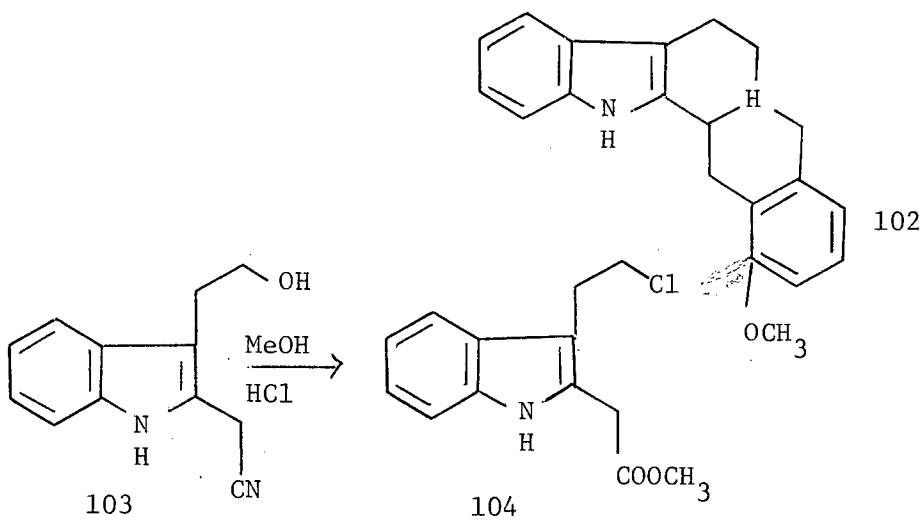



Figure 17. Nmr spectrum of nitrile 86.

The poor yield obtained above necessitated an investigation of the alternative procedure mentioned earlier, namely the methanolysis of nitriles. For this purpose the cyano compound (86) was dissolved in a mixture of methanol and 12 N HCl (1:1) and the contents were stirred at room temperature for three days. We were very happy when the crude product indicated essentially one spot on tlc. But when this product was flushed through a small alumina column to get rid of what was apparently a minor polar baseline contaminant, there was a considerable loss of material. The pure compound obtained represented only a 30% yield in the conversion, 86 → 88. This implied that the baseline material was either the bulk in the crude product or else decomposition of ester was occurring on the column.

All our initial attempts to improve the yield of the conversion (86 → 88) failed completely. A considerable amount of time was spent with no apparent success. Fortunately during this time Wenkert[84] published a synthesis of dl-dihydrogambirtannine (102). The most interesting part of the synthesis, which was pertinent to our own work, was the conversion of nitrile (103) to chloroester (104) by treatment



with methanolic hydrochloric acid. The high yield of chloroester (104) obtained intrigued us to utilize the same reaction condition as indicated by Wenkert. So the cyano compound (86) was dissolved in methanol containing 1% water. This solution was saturated with HCl gas and the resulting mixture was stirred at room temperature for 60 hours. The crude product indicated essentially one spot on tlc (alumina, chloroform/ethyl acetate, 1:1). When this product was flushed through an alumina column, we were very surprised as well as gratified to find the carbomethoxy ester (88) obtained as white crystalline needles instead of the dark brown gum obtained earlier. It is important to emphasize the fact that in the above reaction, the amount of water present in the methanol was very critical in terms of yield. It just happened that 1% water gave the optimum yield. If more water was present in the reaction mixture the yield was considerably lowered. We found the best way to obtain reproducible results was to dilute absolute methanol with 1% water. For analytical purposes a small amount of material was recrystallized from dichloromethane and petroleum ether, mp 85-87.5°. The spectral data compared favourably with the assigned structure (88). The presence of an ester group was indicated by the infrared (1728 $cm^{-1}$) and a sharp singlet in the nmr (Figure 18) at $\tau$ 6.34. The methylene group adjacent to the ester carbonyl ($-\underline{CH}_2-COOCH_3$) appeared as a sharp singlet at $\tau$ 6.27. The mass spectrum (Figure 19) indicated the molecular ion at m/e 326 and was dominated by two significant peaks at m/e 124 and m/e 202. Finally the molecular formula, $C_{20}H_{26}N_2O_2$, was confirmed by high resolution mass spectrometry (Found: 326.202; Calc.: 326.199) and elemental analysis.

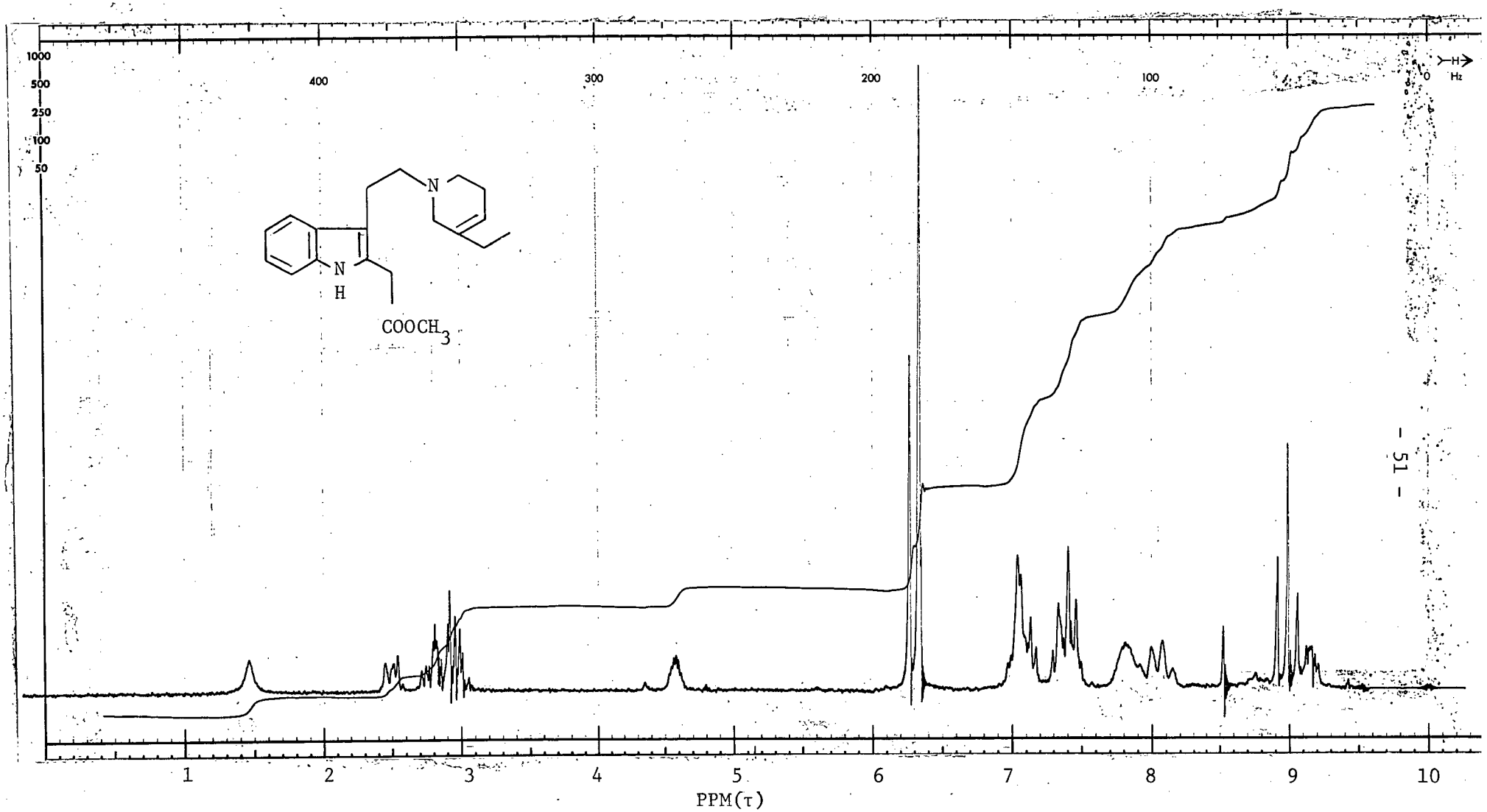



Figure 18. Nmr spectrum of carbomethoxy ester 88.

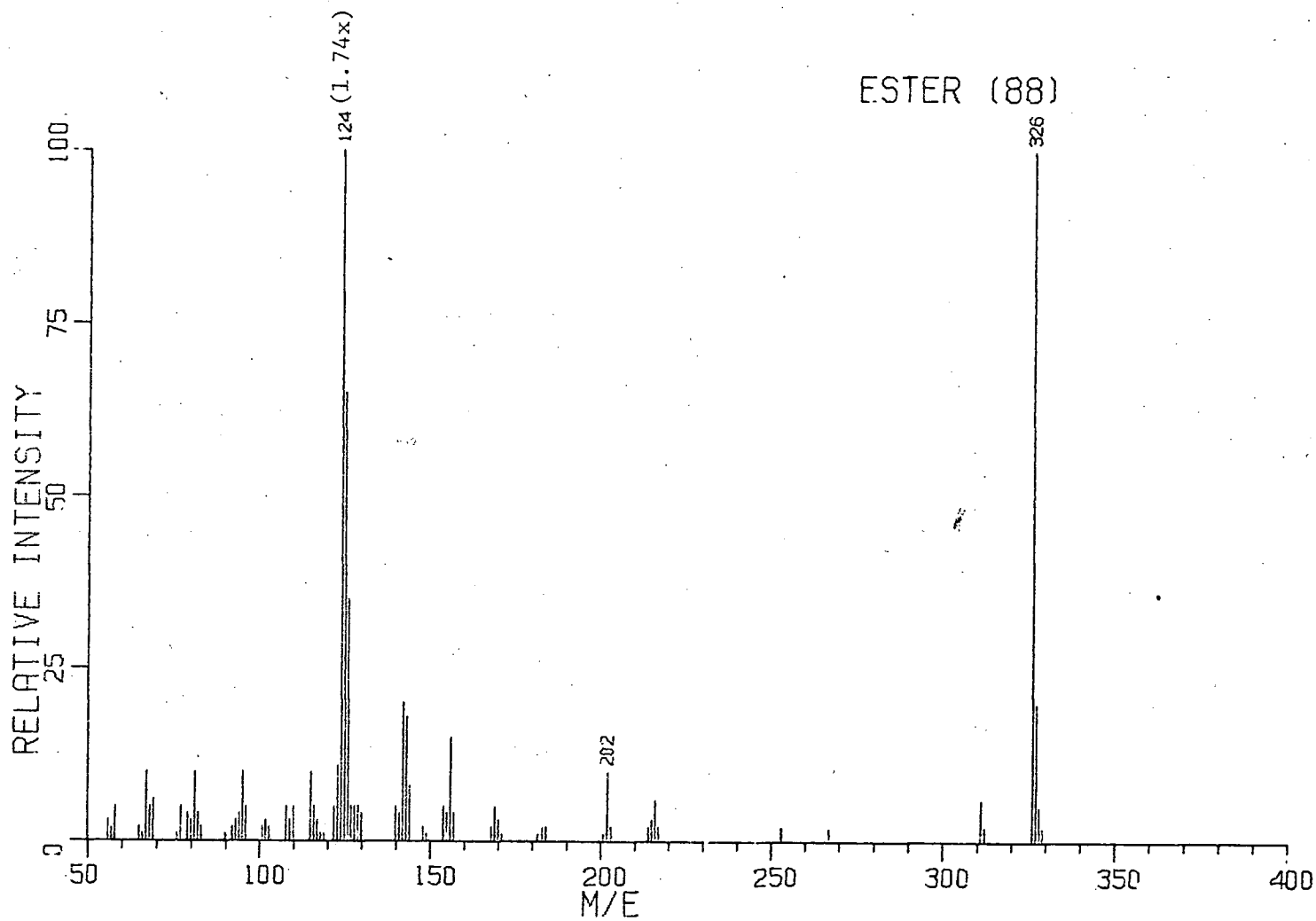
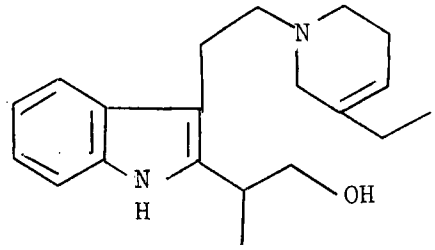



Figure 19. Mass spectrum of carbomethoxy ester 88.

With the synthesis of the basic ring skeleton accomplished, it remained to complete the synthesis of the desired 16,17-dihydrosecodin-17-ol (90) by incorporating the hydroxymethylene ($-CH_2OH$) side chain at $C_{10}$ in the ester (88). It was envisaged to put the said reaction into practice by formylating the carbomethoxy ester (88) to enol (89) and then reducing the latter with sodium borohydride. Formylation of the ester (88) was done using sodium hydride and methyl formate. Tlc examination of the crude product indicated extremely little starting material (< 5%) and one huge streak rising from just above the baseline. This heavy spot we believed was the desired enol (89). This crude product could be separated inefficiently (poor separation and considerable loss of material on column) into its components (88 and 89) by chromatography on silica gel. The purified enol (89) indicated a parent molecular ion peak at m/e 354 which was in agreement with the molecular formula, $C_{21}H_{26}N_2O_3$.

The difficulties mentioned above prompted us to utilize the crude enol in the next reaction, consequently the product obtained above was dissolved in methanol and the solution was exposed to sodium borohydride at 0°. We were surprised when the tlc examination of the crude product indicated the presence of one very polar compound along with the major component. It was found that the amount of this polar material eventually decreased to a minimum as the temperature of the reaction mixture was lowered to -30°. In view of the peripheral nature of this "polar component", we made no attempt to characterize it. However, Battersby[85] while working independently on the synthesis of 16,17-dihydrosecodin-17-ol (90) observed the same result (a complete discussion of

Battersby's work is deferred until a later portion of this thesis). According to him this polar compound is the "diol" (105). Recently[86] a



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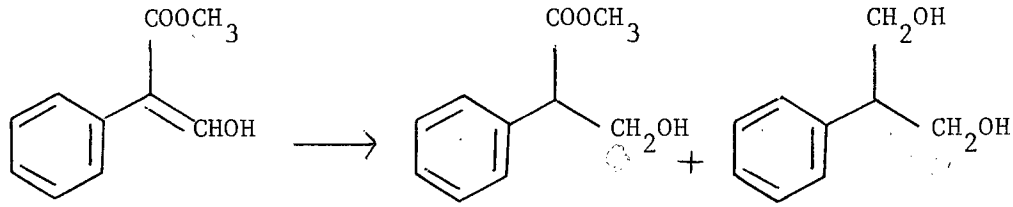
similar observation has been made during the reduction of enol (106). Thus the possible reduction of both the ester and enol functions in (89) cautioned us to exercise some care. Monitoring of the reaction by



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tlc was carefully conducted and the first appearance of "diol" (105) was a signal to immediately terminate the reduction by quenching the excess of borohydride with a few drops of 2 N HCl. The other precaution to be observed in this reaction was to always keep the temperature low (around -30°). Once the precautions indicated were observed, the whole process could be performed very efficiently. The crude alcohol obtained was purified by chromatography on alumina and after crystallization provided an analytical sample, mp 131.5-132°. The overall yield of the reaction i.e. 88 → 90 was 40%.

There are number of instances in the literature[87-89] where the formylation of activated methylene groups adjacent to ester carbonyl functions have been done using triphenylmethyl sodium (trityl sodium) as a base. This tempted us to substitute trityl sodium for sodium hydride in our sequence. It must be emphasized here that this investigation was undertaken to improve the yield of alcohol (90). However, it was found that formylation of ester (88) using triphenylmethyl sodium and methyl formate followed by reduction of the resulting enol (89) gave the same yield of alcohol (90) as obtained earlier. The complication of separating triphenylmethane from the reaction product etc. forced us to discontinue the use of this base.

The purified alcohol indicated spectral data which was in complete accord with the assigned structure (90). In the infrared a broad peak at 3050 $cm^{-1}$ implied the presence of a hydroxyl group. A sharp peak at 3400 $cm^{-1}$ was attributed to indolic-N<u>H</u> while the ester group appeared at 1718 $cm^{-1}$. The nmr spectrum (Figure 20) exibited the following resonances. The methyl group of the ester appeared as a sharp singlet at $\tau$ 6.37. Prominant features of this spectrum in comparison to the nmr spectrum of the ester (88) (Figure 18) was the appearance of a braod multiplet centered at $\tau$ 6.0. This multiplet integrated for four protons (-C<u>H</u>$_2$-O<u>H</u> + -C(<u>H</u>)-CH$_2$OH). In the mass spectrum (Figure 21) the alcohol (90) indicated a molecular ion at m/e 356. It readily lost a molecule of water to give the radical ion m/e 338, which corresponded to the molecular ion of secodine (107). As to be expected ion (107) fragmented to the ions (108, m/e 214) and to (101, m/e 124). Furthermore an ion m/e 326 (109) corresponding to loss of $CH_2O$ strongly

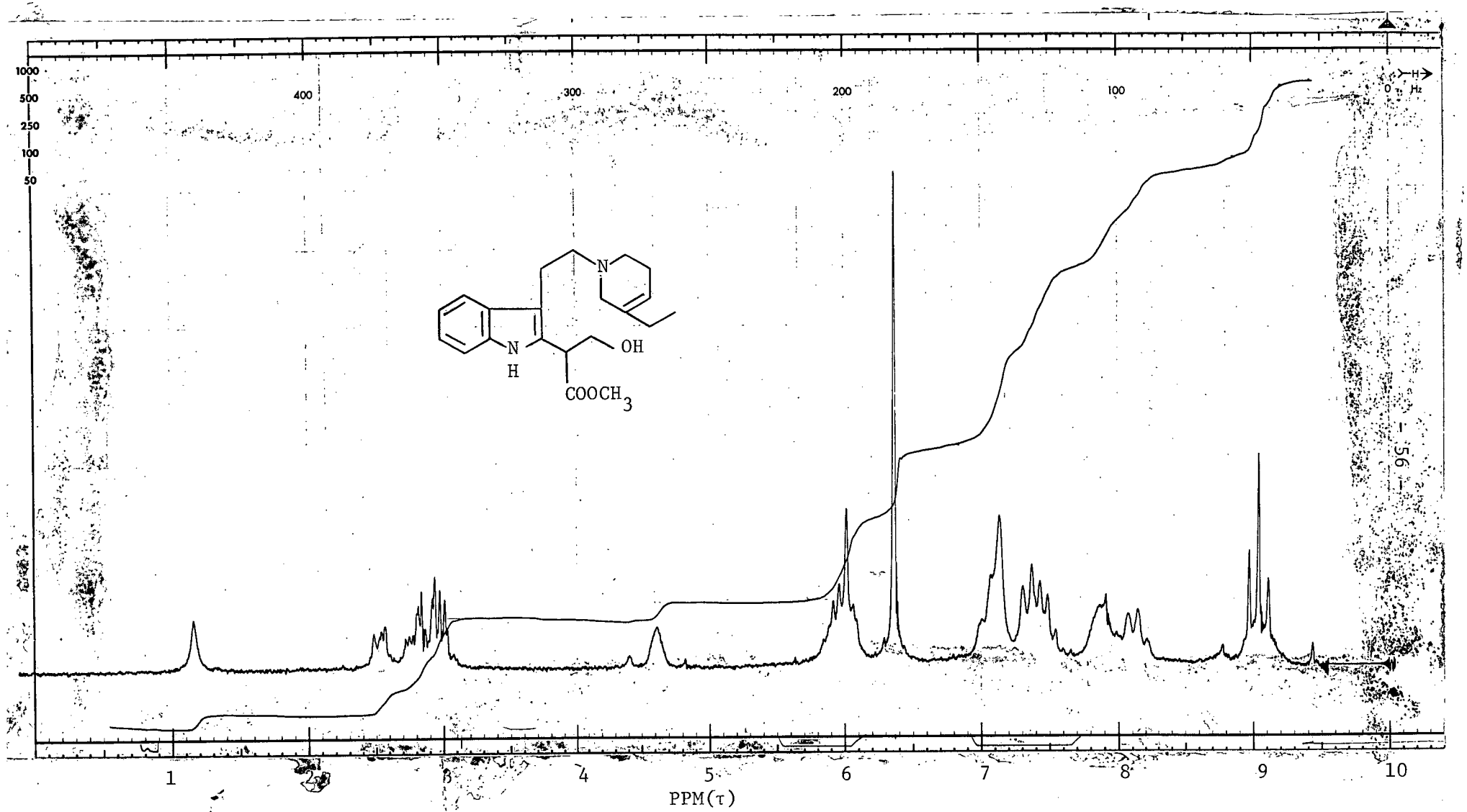



Figure 20. Nmr spectrum of 16,17-dihydrosecodin-17-ol (90).

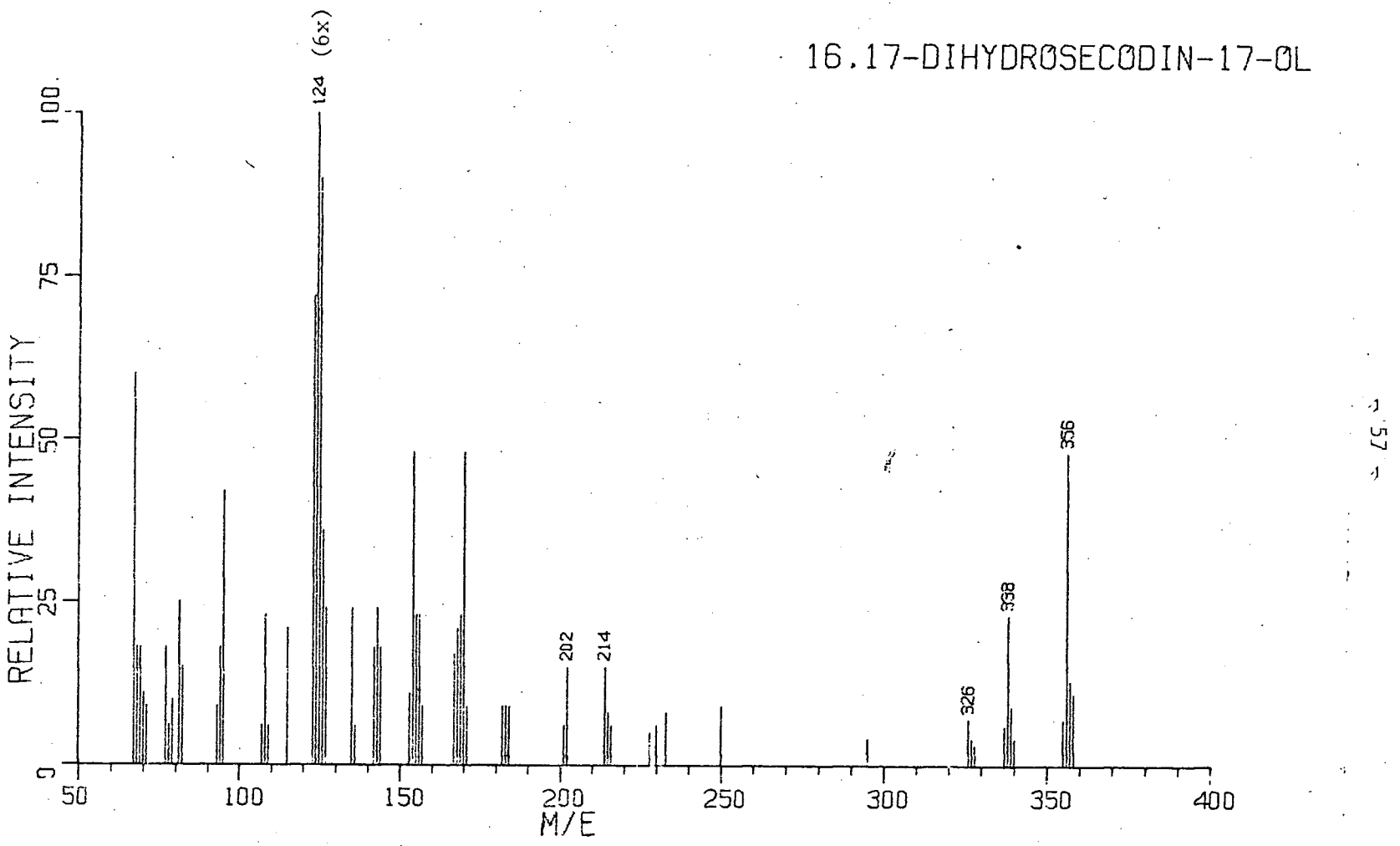



Figure 21. Mass spectrum of 16,17-dihydrosecodin-17-ol (90).

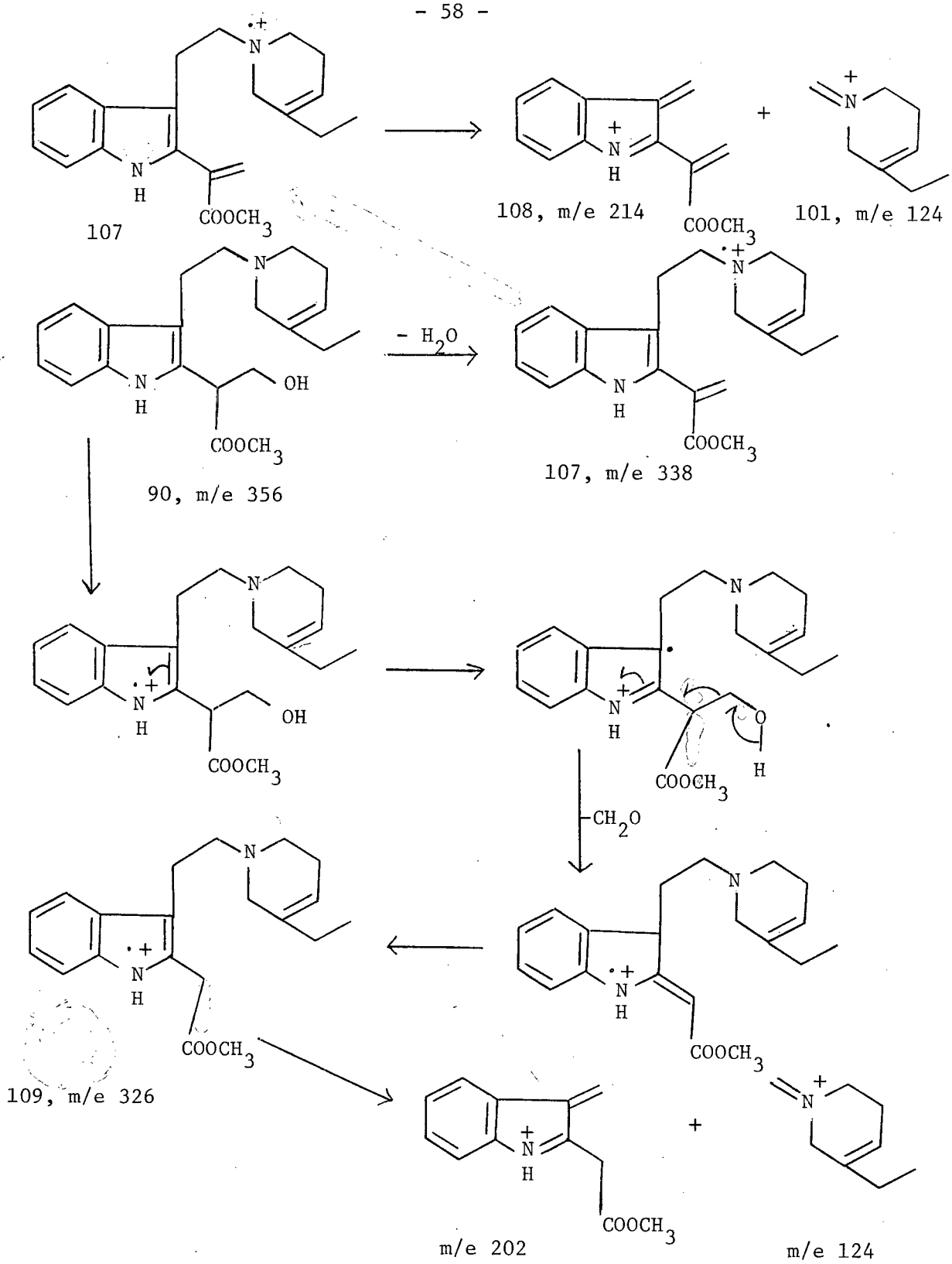



Figure 22. Postulated fragmentation of 16,17-dihydrosecodin-17-ol (90) in the mass spectrometer.

suggested position 17 for the hydroxyl group. A scheme portraying the mass spectrometric fragmentations has been summarized in Figure 22. Finally the molecular formula, $C_{21}H_{28}N_2O_3$, was confirmed by high resolution mass spectrometry (Found: 356.207; Calc.: 356.209) and elemental analysis.

This marked the end of our initial synthetic endeavor. We now were in a position to investigate whether (90) played a role in the biosynthesis of the various families of indole alkaloids mentioned previously.

Evaluation of 16,17-Dihydrosecodin-17-ol (90) as Bio-intermediate

Two radioisotopes most widely utilised in biosynthetic studies for making radioactive "precursors" from inactive alkaloids are tritium ($^3H$) and $^{14}C$. It is well known that tritium labelling although relatively less expensive can sometimes give erroneous results due to exchange between the protons and the tritium atoms *in vivo*. On the other hand $^{14}C$ labelling is very reliable in the sense that in general no exchange of the label can occur. However, the much higher costs often associated with the synthesis of $^{14}C$-labelled materials sometimes require at least initial reliance on tritium as the tracer. In our instance it was considered advantageous first to make the tritium labelled synthetic alcohol (90) since we believed that this could fulfil our immediate needs. If we were fortunate in obtaining positive incorporation with [ar-$^3H$]-alcohol (90), it was envisaged to check the extent of incorporation with [$^{14}COOCH_3$]-alcohol (90). For this purpose preparation of [ar-$^3H$]-16,17-dihydro-

secodin-17-ol (90) and [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90) were considered and the syntheses of both of these active compounds are described below.

The method utilised for making tritium labelled indole precursors was developed in our laboratories a few years ago.[90] It involves acid catalysed exchange of aromatic protons of the indole nucleus with tritium labelled trifluoroacetic acid. The latter reagent is prepared by reacting equimolar quantities of trifluoroacetic anhydride and tritium labelled water. A simple vacuum transfer system is used to bring the tritium labelled trifluoroacetic acid into contact with the alkaloid. The acid is subsequently removed after the reaction is complete. It was soon realised 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 appeared general to essentially all indole alkaloids, (c) since a large excess of acid was used, the dilution of radioactivity in the reaction was very small and the recovered trifluoroacetic acid was suitable for reuse, and (d) the experimental procedure was very simple in its operation.

With all this knowledge in hand, we exposed the synthetic 16,17-dihydrosecodin-17-ol (90) to tritium labelled trifluoroacetic acid. Unfortunately when the reaction mixture was worked up, we were very surprised to find the crude product as a complicated mixture of several components. It must be emphasised here that even before the above reaction was performed, we were a little skeptical that some of the alcohol (90) might dehydrate to the corresponding acrylic ester (107). But this latter reaction and then subsequent transformation of the

resulting acrylic ester (107) to other spurious products were not considered to be predominating under the mild conditions employed. In view of the small amount of alcohol (90) at hand during the course of the active synthesis, it was not possible to define the various products formed in the above reaction. However this problem was quickly unraveled. It was mentioned on page 50 that when a methanol solution of nitrile (86) is saturated with HCl gas, the former is transformed into the carbomethoxy ester (88) (Figure 12). This result suggested to us that the ester (88) was a relatively stable compound in acidic medium. We planned to capitalise on this observation by exchanging the aromatic protons of ester (88) with tritium. In order to secure the desired radioactive alcohol (90), it was then necessary to formylate the "hot" ester (88) and reduce the resulting enol. The postulated scheme (Figure 23) when put into practice proved highly satisfactory.

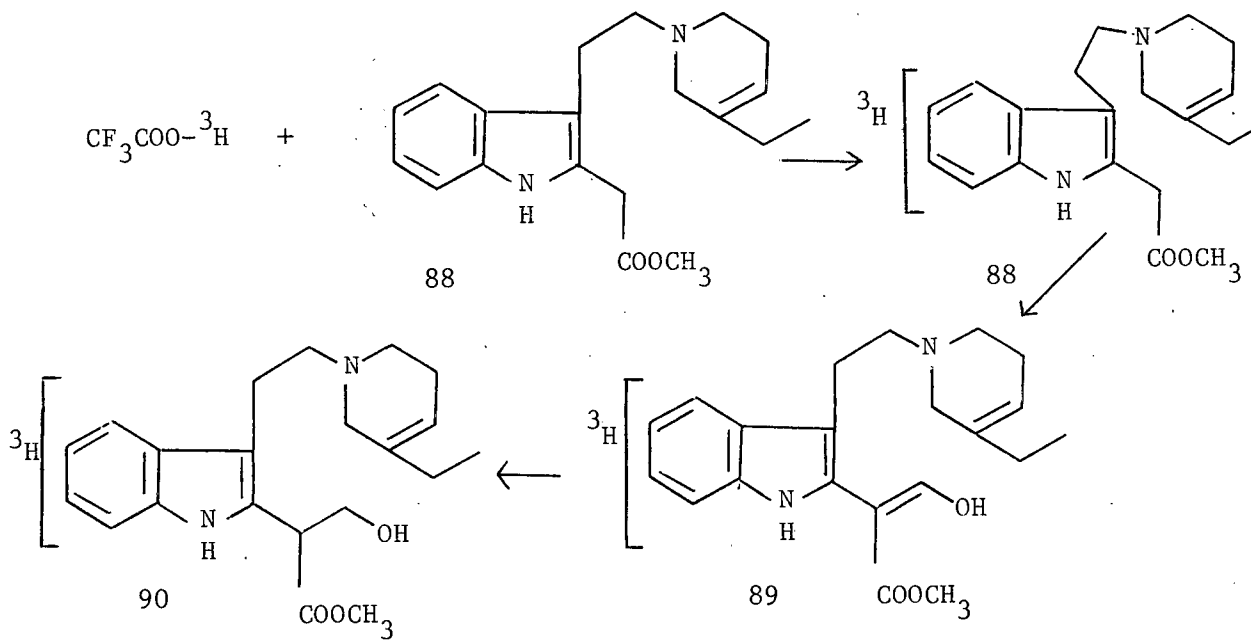



Figure 23. Synthesis of [ar-$^3$H]-16,17-dihydrosecodin-17-ol.

Before describing the active syntheses it should be mentioned here that while working with the radioactive compounds as presented in Figures 23 and 24, thin layer chromatography proved extremely helpful. Fortunately all the compounds starting from benzoate (85) to alcohol (90) showed very characteristic colors when tlc plates were sprayed with antimony pentachloride. Therefore while pursuing the active syntheses (Figures 23 and 24) it was not considered imperative to obtain any formal spectral data since it was sufficient to compare the $R_f$ values and colors of the radioactive compounds with their cold counterparts already available and completely characterized (Figure 12).

To start the sequence outlined in Figure 23, the carbomethoxy ester (88) was treated with $^{3}H$-trifluoroacetic acid. The crude product although homogeneous on tlc, showed some baseline material. The mixture was flushed through a small alumina column to afford the pure radioactive ester (88). This active ester was formylated using sodium hydride and methylformate and the resulting enol was reduced with sodium borohydride. Chromatography of the crude product on alumina afforded the desired [ar-$^{3}H$]-16,17-dihydrosecodin-17-ol (90). The most gratifying outcome of this venture was the fact that the obtained active alcohol (90) had a very high specific activity (dpm/mg). This result allowed us to conduct numerous experiments both in Vinca rosea and Vinca minor plants.

While contemplating the synthesis of [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90) our attention was obviously drawn to the nucleophilic displacement reaction where the benzoate group was displaced by cyanide

anion (85 → 86, Figure 12). For a while our preconceived goal looked very easy with the thought that by substituting radioactive potassium cyanide ($K^{14}CN$) for potassium cyanide in the above reaction, we could obtain the active-nitrile (86). In order to obtain the desired [$^{14}COOCH_3$]-alcohol (90), it would be merely necessary to carry the active nitrile (86) through a similar sequence of reactions as done previously on its cold counterpart in Figure 12. However, it was soon realised that in the conversion, 85 → 86, we were using approximately a ten-fold excess of potassium cyanide. In view of the high cost of radioactive potassium cyanide, it became imperative to reinvestigate this reaction. This investigation was directed at finding out the minimum amount of potassium cyanide required in the displacement of the benzoate while still maintaining a reasonable conversion to the nitrile (86). For this purpose a series of reactions were run with decreasing amounts of potassium cyanide. It immediately became apparent that the displacement reaction required a minimum of 5 fold excess of potassium cyanide. Under these conditions a 40% yield of nitrile (86) was obtained as compared to 65% when a 10 fold excess was employed. If the amount of potassium cyanide was reduced any further the yield of nitrile was cut down very drastically. For example a two fold excess of potassium cyanide gave less than 20% of nitrile.

In the displacement reaction (85 → 86, Figure 24) the benzoate (85) was dissolved in dimethylformamide and the solution was exposed to radioactive potassium cyanide ($K^{14}CN$). The pure active nitrile (86, specific activity $4.32 \times 10^6$ dpm/mg or $1.27 \times 10^9$ dpm/mmole) obtained by chromatography on alumina was dissolved in methanol

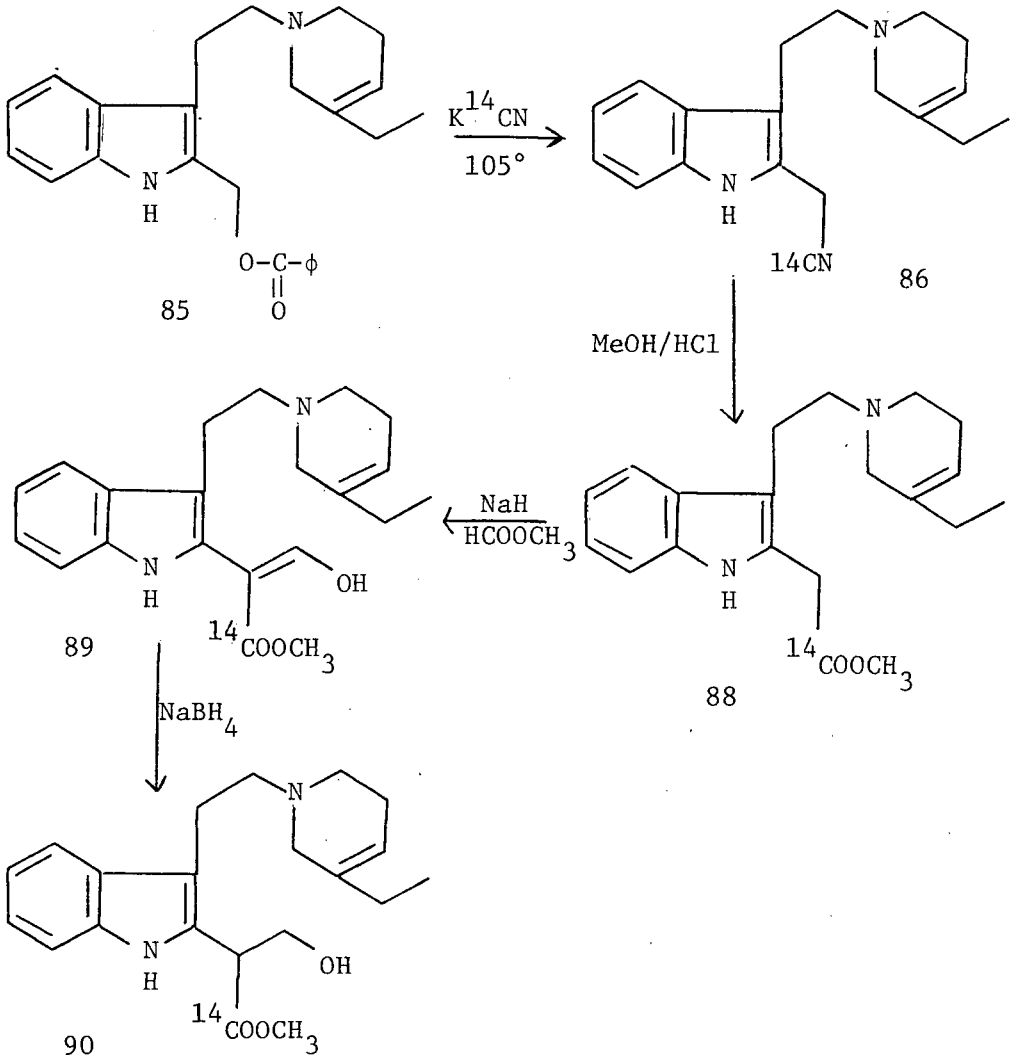



Figure 24. Synthesis of [$^{14}$COOMe]-16,17-dihydrosecodin-17-ol.

containing 1% water. The solution was then saturated with hydrogen chloride gas and the resultant crude product upon chromatography furnished the desired active carbomethoxy ester (88). The latter substance was formylated as before to yield the crude enol (89) which without further purification was reduced with sodium borohydride at ≒30°. Chromatography of the crude product on alumina furnished the desired [$^{14}$COOCH$_3$]-16,17-dihydrosecodin-17-ol (90).

With the completion of the synthesis of both tritium and $^{14}C$-alcohol (90), it became necessary to investigate the incorporation if any, of this substance into the appropriate plant systems.

Interest in Vinca rosea has been considerable since the discovery in it of antileukemic alkaloids. As a result of this finding an extensive investigation of its alkaloidal constituents has been conducted in various laboratories.[91-94] The structures of more than sixty alkaloids are known and these represent many structural types. Vindoline (5), catharanthine (6) and ajmalicine (3) are three of the major alkaloids present and possess the Aspidosperma (11), Iboga (12) and Corynanthe (10) systems respectively.

On the other hand the tiny green plant Vinca minor[95] possesses a wonderful array of Aspidosperma alkaloids. Of the more than twenty alkaloids isolated, structures of about twenty are known. Minovine (73, Aspidosperma type) and vincamine (72, eburnamine family) are two major alkaloids which could be isolated, purified and recrystallised with great ease. In addition Vinca minor grows in abundance around our campus. All these factors made this plant an excellent choice for our biosynthetic studies.

Before describing the feeding results it would be relevant to mention that at this stage our whole research project became very diversified. We realised that now the problem would involve feeding the tritium and $^{14}C$-labelled alcohols (90) to both Vinca rosea and Vinca minor for various intervals of time. Regardless whether the active substances showed positive or negative incorporation, these results would require repetition to confirm the initial findings. In order to

carry out these requirements with optimum accuracy and efficiency, the various incorporation experiments were performed simultaneously by three of us, John Beck, Neil Westcott and myself. In this thesis the result of only those experiments which were performed by me are described. Whenever relevant or necessary in the later discussion, the results of the other workers will be mentioned.

For the purpose of the biosynthetic study, [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90, total activity 9.89 x $10^6$ dpm) made soluble with 0.1 N acetic acid and a few drops of ethanol was incorporated via the hydroponic technique, to Vinca minor shoots. After four days, the plants were killed and the isolated alkaloidal material was shown to contain 31% of the total activity fed. Vincamine (72) and minovine (73) were isolated by a chromatographic separation developed earlier in these laboratories.[90] Vincamine showed one spot on tlc and in most of the various experiments conducted the isolated amount was sufficient to allow several crystallizations without the addition of cold material. Minovine (73) however required further purification by preparative layer chromatography and then further dilution with the cold alkaloid to allow crystallization to constant activity. Several crystallizations revealed that vincamine (72) possessed an activity of 102 dpm (total) corresponding to a specific incorporation of < 0.001%. Unfortunately this amount of radioactivity was so small that it was difficult to ascertain the significance if any of this result. A minute trace of a radioactive impurity present in the alkaloid could be responsible. On the other hand virtually no activity could be detected in the purified minovine (73).

In a parallel series of experiments, [ar-$^{3}$H]-16,17-dihydrosecodin-17-ol (90) was fed to Vinca minor by my colleague, John Beck. It is sufficient here to state that he also could not detect any significant activity in the two alkaloids, vincamine (72) and minovine (73).

In another concurrent investigation, [ar-$^{3}$H]-16,17-dihydrosecodin-17-ol (90) was fed to Vinca rosea by another colleague Neil Westcott, no significant activity could be detected in vindoline(5) and catharanthine (6).

The most frustrating aspect of all these results was the inability to delineate what might be construed as a positive incorporation of alcohol (90) into any of those alkaloids isolated by us. We, of course, were fully aware of the fact that negative results in biosynthetic studies have to be interpreted with great care. It is well known that success in a biosynthetic experiment depends upon such factors as absorption and permeability in the plant as well as the ability of the plant to carry out the desired biosynthesis. Thus the age of the plant, length of incorporation, the method of feeding etc. become very critical factors. In this regard it is pertinent to mention that the earlier workers in our laboratories had established conditions during which large molecular weight substances were incorporated into the plant systems.[63,64] This situation therefore reinforced our prevailing impression that the apparently negative incorporations of alcohol (90) were not due to technical difficulties with the experimental method.

It was indicated in the early part of this discussion (page 33) that we prepared 16,17-dihydrosecodin-17-ol (90) as our synthetic target only with the hope that it would be transformed in vivo to the

putative intermediate (76) by appropriate dehydration and oxidation (in the piperidine ring). However negative incorporation of alcohol (90) suggest that the plant systems utilised may be incapable of carrying out either one or both of these reactions.

After we had completed the above results, Battersby[85] reported the presence of 16,17-dihydrosecodin-17-ol (90) in the plant Rhazya orientalis. In these experiments he fed to the shoots [O-methyl-$^{3}$H]-loganin and from the isolated active alcohol, 90, was able to show an incorporation of 0.013%. In the same manner, when the experiment was repeated with shoots of Vinca rosea, radioactive (90) was again isolated but of very low specific activity. To use it as a carrier Battersby[85] synthesised the alcohol (90) by the general route outlined in Figure 25. Although Battersby's route is quite different from ours in the earlier stages (see Figure 12), the later steps are essentially identical.

In summary of his independent study Battersby stated that "16,17-dihydrosecodin-17-ol (90) is a natural product present in Rhazya orientalis probably arising from a biosynthetic intermediate blocked by reduction (e.g. 113) or by hydration and reduction (e.g. 76)".

Recently Smith[96] reported the isolation of tetrahydrosecodine (110) and 16,17-dihydrosecodine (111) from Rhazya stricta and tetrahydrosecodin-17-ol (112) from Rhazya orientalis. These alkaloids were isolated in very small amounts and their structures were derived mainly from mass spectrometric measurements. The presence of tetrahydrosecodine (110) was again demonstrated in Rhazya orientalis by dilution studies when [2-$^{14}$C]-tryptophan was administered to Rhazya orientalis.[97] The

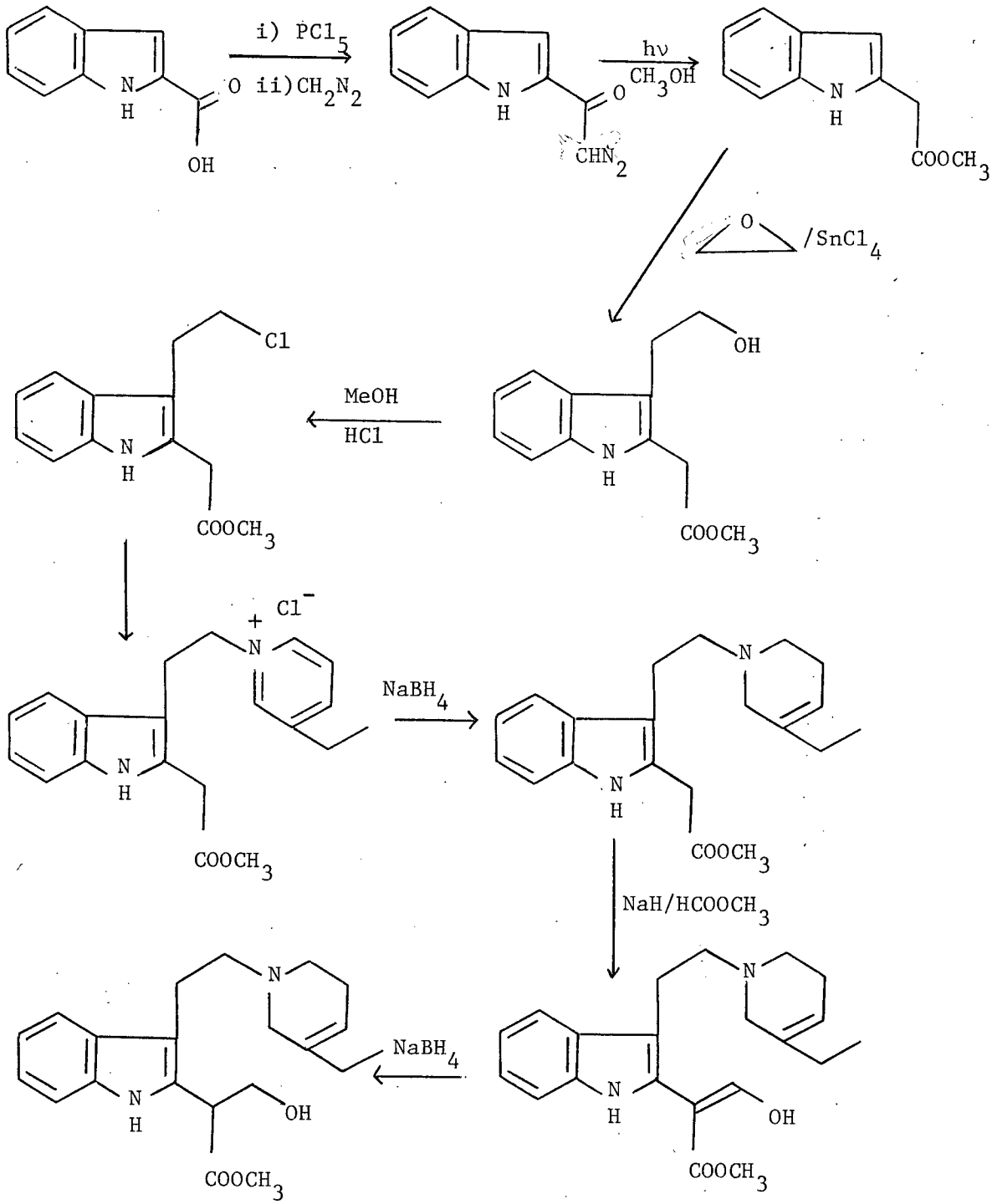



Figure 25. Battersby's synthesis of 16,17-dihydrosecodin-17-ol.

plants were worked up for the alkaloids with the addition of synthetic (110) as carrier (Figure 26). The constant activity found for the

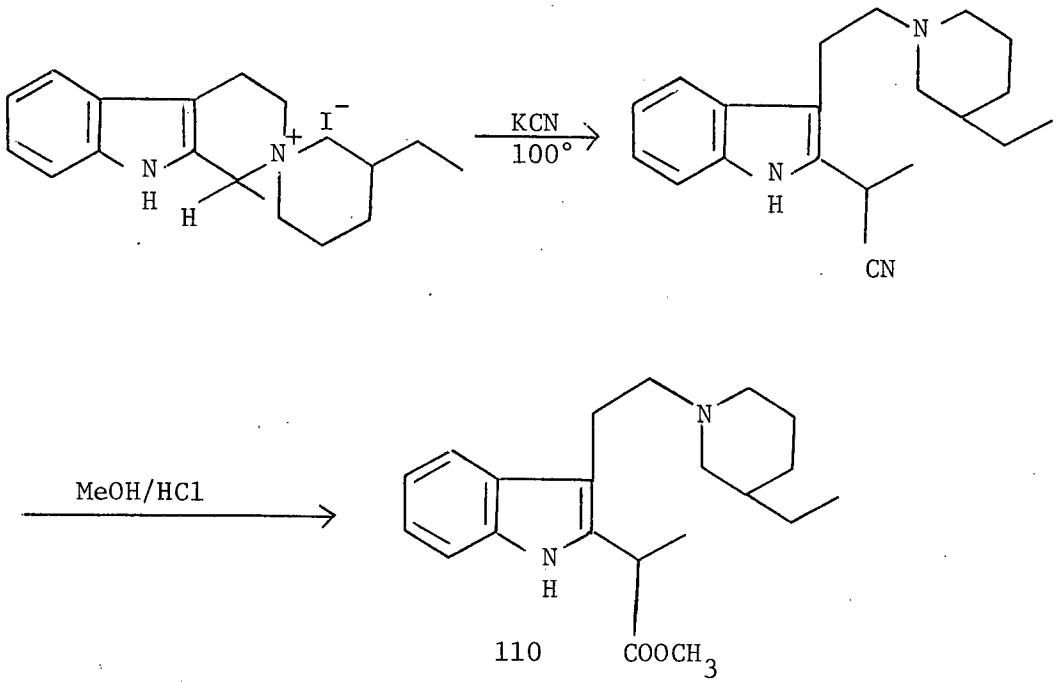
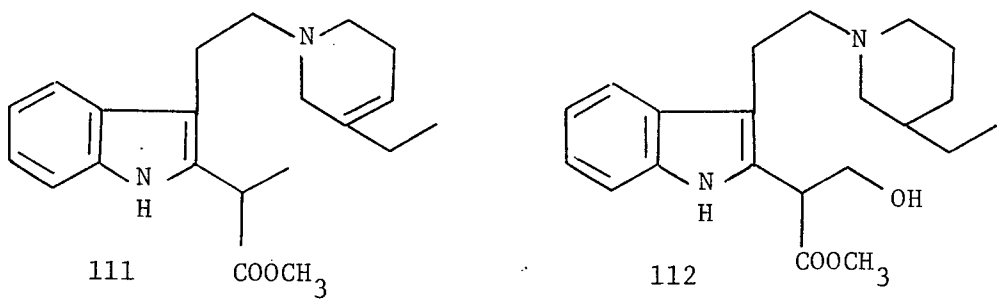



Figure 26. Smith's synthesis of tetrahydrosecodine (110).

rigorously purified tetrahydrosecodine (110) corresponded to 0.5% incorporation. This high incorporation was quite remarkable and this




led Smith to suggest that tetrahydrosecodine (110) is on a metabolic side track very close to the main alkaloid biosynthetic route. This fact fits well with the notion that simple reduction of the putative acrylic ester (76) takes tetrahydrosecodine (110) out of circulation. It is interesting to note that a similar type of explanation (i.e.

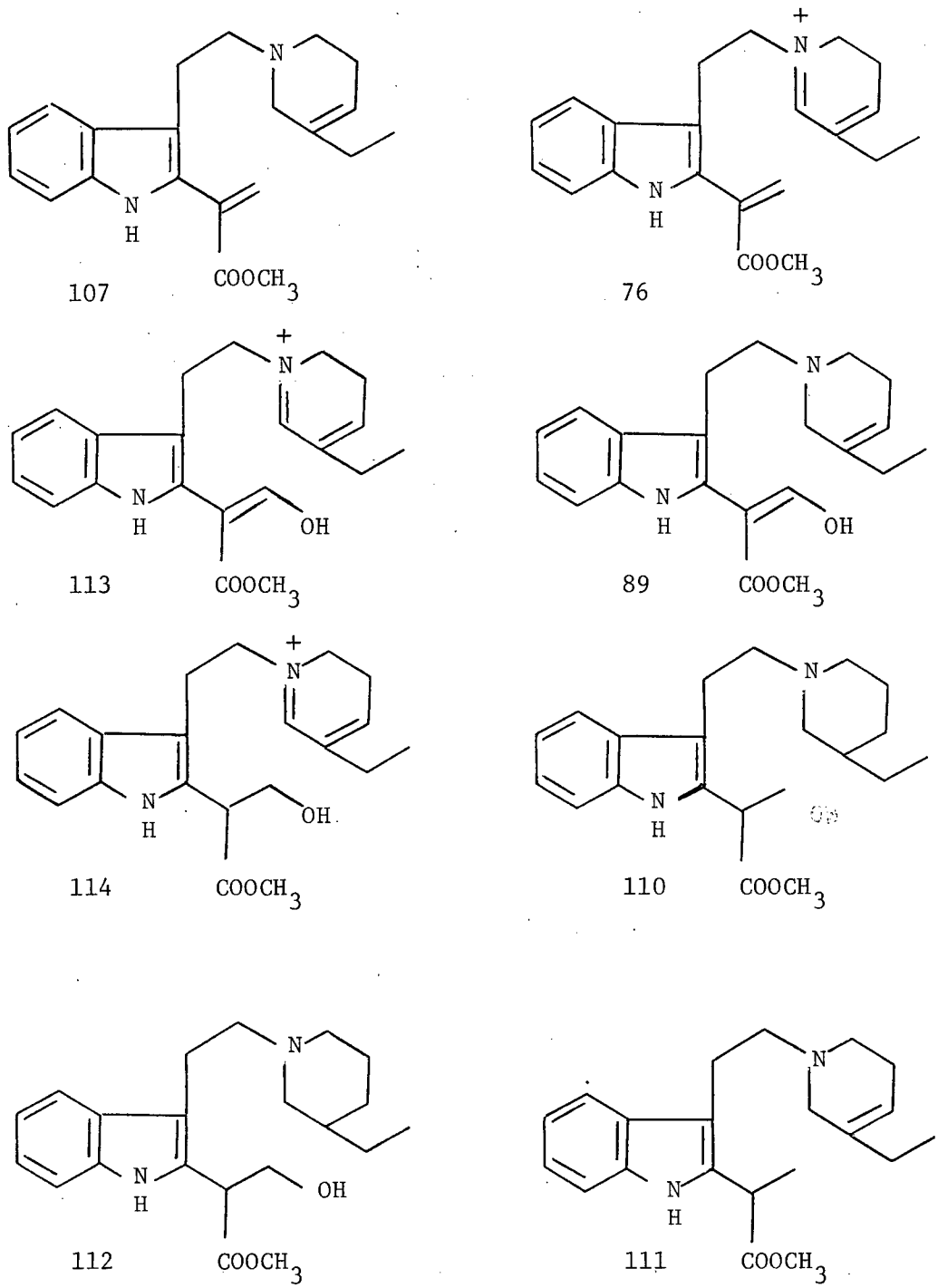



Figure 27. Some of the compounds derivable in vivo from 16,17-dihydro-secodin-17-ol (90).

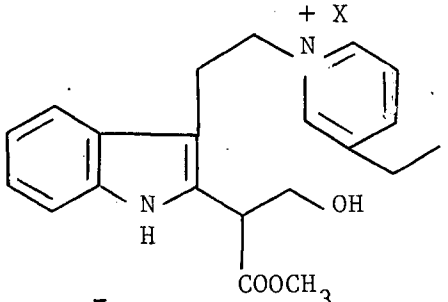
hydration and reduction of acrylic ester, 76) was used by Battersby[85] when 16,17-dihydrosecodin-17-ol (90) was isolated from the plants.

The question now is how the above results fit into the biosynthetic story which is rapidly evolving from the combined data of the various laboratories: First of all, these results give further support to the suggested cleavage process for the biosynthesis of indole alkaloids in the Aspidosperma and Iboga families as mentioned previously. It is further evident that compounds 107, 76, 89, 113, 114 (not yet isolated) 110, 111, 112 and the dimeric secamine (119) and presecamine (116),[98] all are derivable in principle from the alcohol (90) in vivo Whether any of these compounds (Figure 27) will turn out to be the correct biointermediate remains an open question. Some of our own experiments which could be readily extended into this area are discussed in the next section of this thesis.

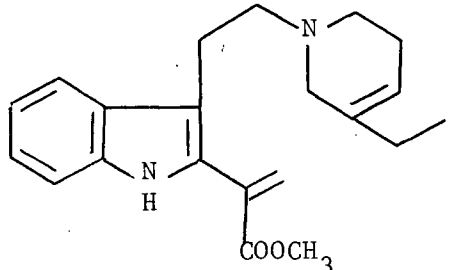
PART II

The negative incorporation obtained by feeding the synthetic 16,17-dihydrosecodin-17-ol (90) into Vinca minor and Vinca rosea plants suggested to us that the former may not be capable of acting as a progenitor of the acrylic ester (76) in the plant. It therefore became evident that to persue our preconceived goal the alcohol (90) required synthetic modification to some other model compound. At this stage our whole research project entered a very perplexing phase. Among the many avenues which were available, it was very difficult to decide unequivocally which route to explore first. In this part of the discussion we will portray some of our attempts to obtain some of the other close relatives of the fugitive acrylic ester (76).

The two compounds bearing structures 107 and 115 appeared to us to represent templates which could under reasonable biochemical modification convert to the acrylic ester (76) and thereby in turn to the Aspidosperma and Iboga bases. Our choice was obviously dictated by the fact that these compounds (107 and 115) were amenable to syntheses from




115 $X^-$ = Cl, OAc




107

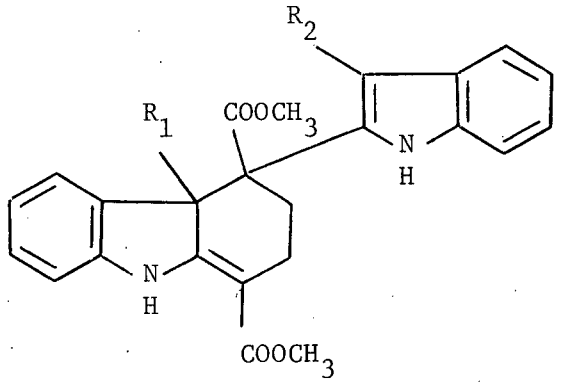
the available alcohol (90). It should be noted that in comparison to the alcohol (90), the pyridinium alcohol (115) which would be sufficiently stable for isolation represented a much higher level of oxidation in the piperidine ring. It seemed reasonable that some

reducing system in the plant like $NADPH^+$ would convert the pyridinium ring to the desired dihydropyridine system. On the other hand the tetrahydropyridine derivative, 107, named secodine by Smith[96] who has recently isolated the close relatives of this compound in his work on Rhazya species, was in a lower level of oxidation than the acrylic ester (76). Perhaps an oxidative process in the plant would lead in vivo to 76. The discussion which follows describes our attempts in the laboratory syntheses of these substances.

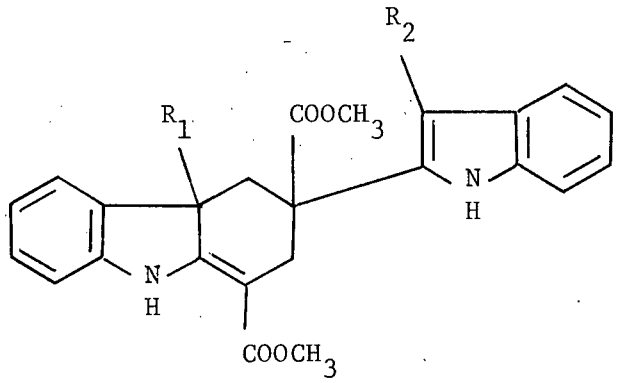
In connection with transannular cyclization work in our laboratories,[59-62] mercuric acetate was found to be an excellent reagent for oxidising the piperidine ring of several alkaloids to the corresponding tetrahydropyridines (i.e. $\text{C-N-} \rightarrow \text{C=}\overset{+}{\text{N}}\text{-}$). We also envisaged to utilise the same reaction for oxidising 16,17-dihydrosecodin-17-ol (90) to the pyridinium alcohol (115). This aspect of the problem was undertaken by two of my colleagues, Neil Westcott and John Beck. Although the details of all this work cannot be properly discussed here, suffice it to say that all our attempts with mercuric acetate oxidation reaction were very disappointing. In all instances poor yields and products of little utility were obtained. These results therefore left us little alternative except to concentrate our efforts in obtaining secodine (107) for biosynthetic evaluation.

To secure secodine (107) from alcohol (90) it was obligatory to dehydrate the latter substance. For several reasons to be presented later we preferred the base catalysed dehydration rather than the alternative of acid catalysis. For this purpose a small amount of alcohol (90) dissolved in benzene was exposed to sodium hydride as the base. After the reaction was over, the excess of the hydride was

destroyed by the addition of a few drops of 2 N hydrochloric acid. Tlc examination of the crude product indicated two spots with very similar $R_f$ values. The whole crude product was rapidly flushed through a small column of alumina and the mixture exposed to a spectroscopic examination. The nmr spectrum indicated two signals for NH ($\tau$ 0.63 and 1.23) and two ester ($COOCH_3$) peaks at $\tau$ 6.29 and 6.49 respectively. Similarly in the infrared two ester peaks were evident and these could be conveniently assigned to saturated (1730 $cm^{-1}$) and unsaturated (1680 $cm^{-1}$) ester groups. This result immediately suggested that we were dealing with a mixture which contained at least one dimeric compound. At a time when we were still entangled in this problem, Smith[96-98] published a series of papers which quickly unravelled our problems. He reported the isolation of three new dimeric alkaloids, presecamine (116a or 116b), dihydropresecamine (117a or 117b), tetrahydropresecamine (118a or 118b) from Rhazya stricta and tetrahydropresecamine from Rhazya orientalis. Smith[98] further observed that presecamine (116a or 116b) rearranges quantitatively at room temperature in 2 N hydrochloric acid to one of the secamines (119, Figure 28; 120 Figure 29). This important observation represented one of the main arguments for the structures suggested and would tend to favour structure type (a). The type (b) dimers was however not excluded on mechanistic grounds (Figure 29) since it could (although less plausibly) lead to the other possible secamine structure (120). It should be noted that for convenience in the subsequent discussion presecamine and secamine will be considered in terms of structures 116a and 119 respectively.



Type a

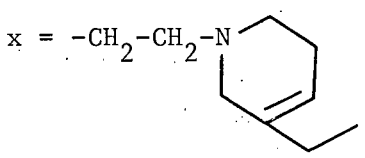


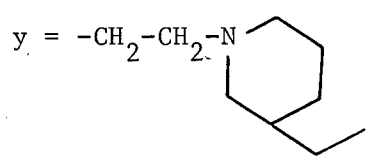
Type b

116 $R_1 = R_2 = x$

117 $R_1 = x$, $R_2 = y$ or $R_1 = y$, $R_2 = x$

118 $R_1 = R_2 = y$





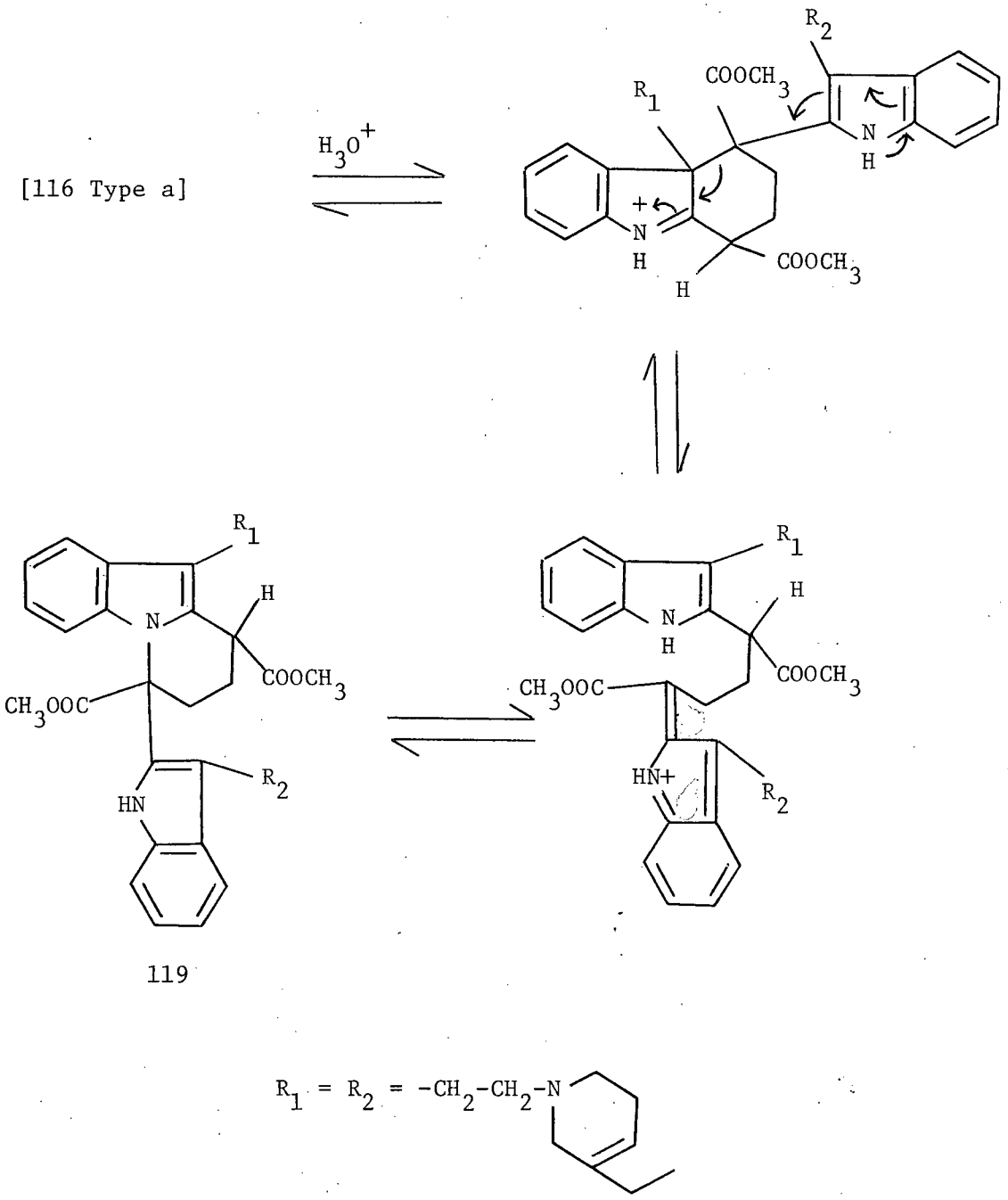



Figure 28. Rearrangement of presecamine (type a) to secamine (119).

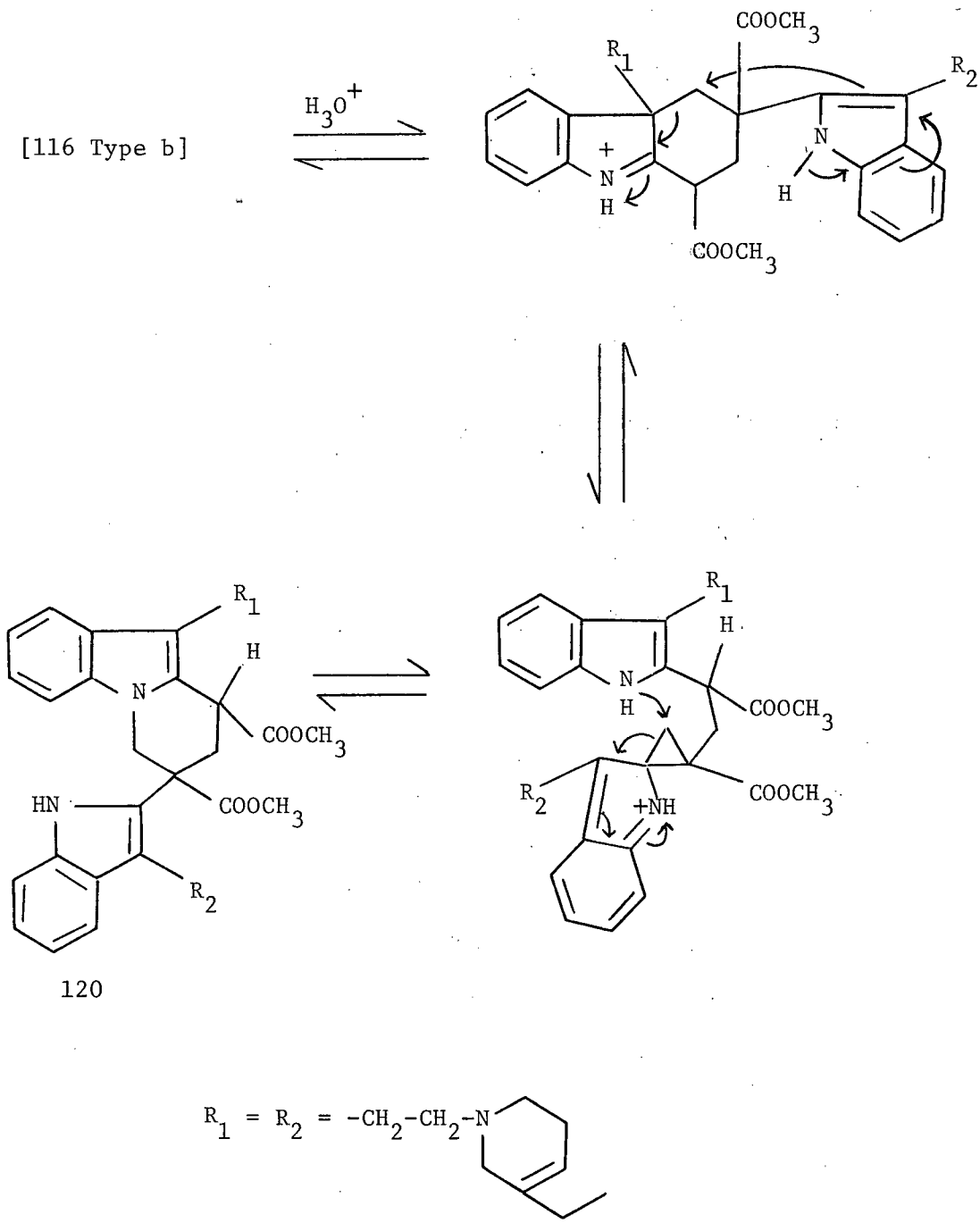



Figure 29. Rearrangement of presecamine (type b) to secamine (120).

It was further observed that on attempted sublimation presecamine (116) undergoes a facile retro-Diels-Alder reaction to yield secodine (107). A brief resume of the data obtained by Smith[98] in support of structure, 107, for secodine is fully revealed in Figure 30. Not only was this data of great importance in its own right but it was directly pertinent to our work as well.

We shall now try to portray how our work on dehydration of 16,17-dihydrosecodin-17-ol (90) converges with Smith's experiments in a most gratifying way. Our crude mixture from the sodium hydride reaction indicated ultraviolet absorption ($\lambda_{max}$ 224, 285 (inf), 292, 326 mμ) and nmr signals (two ester singlets at τ 6.29 and 6.49) which was reminiscent of the spectroscopic properties ($\lambda_{max}$ 227, 228 (inf), 295, 329 mμ and singlet at τ 6.23 and 6.42) reported for the dimeric presecamine (116). With this knowledge in hand it became possible to speculate on the nature of at least three compounds present in the crude reaction mixture. The presence of the desired secodine (107) was indicated by a very pronounced unsaturated methoxycarbonyl singlet at τ 6.29 in the nmr spectrum. The ratio between the integrations of the unsaturated and saturated methoxycarbonyl singlets was 3:1 (in pure presecamine this ratio should be 1:1). The other two compounds present in the crude mixture were obviously presecamine (116) and secamine (119). The latter substance would arise from the rearrangement of presecamine (116) when 2 N hydrochloric acid was used in the work up of the reaction.

Returning to Smith's study it was clear that the reaction mixture should not be exposed to hydrochloric acid to avoid the rearrangement of

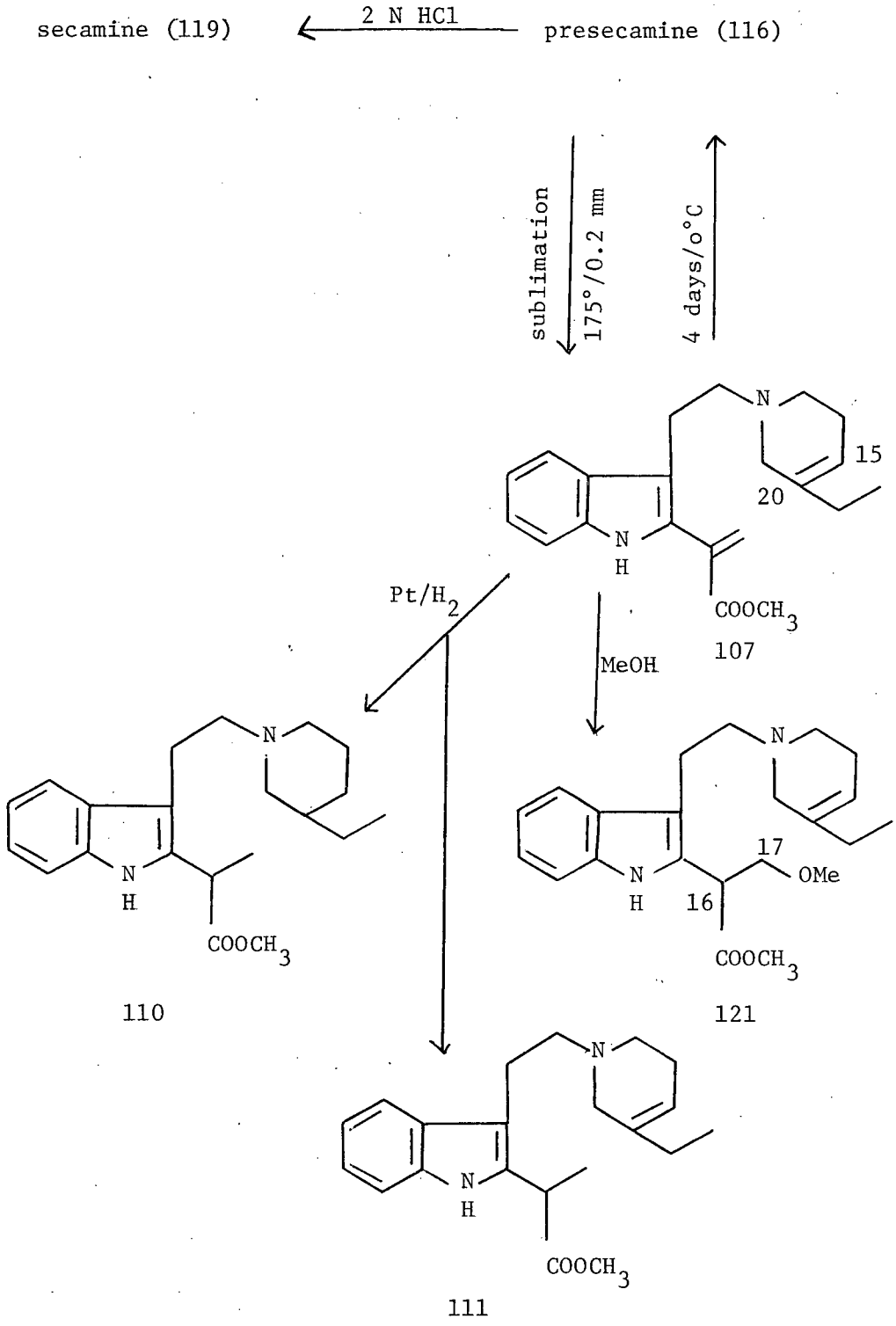
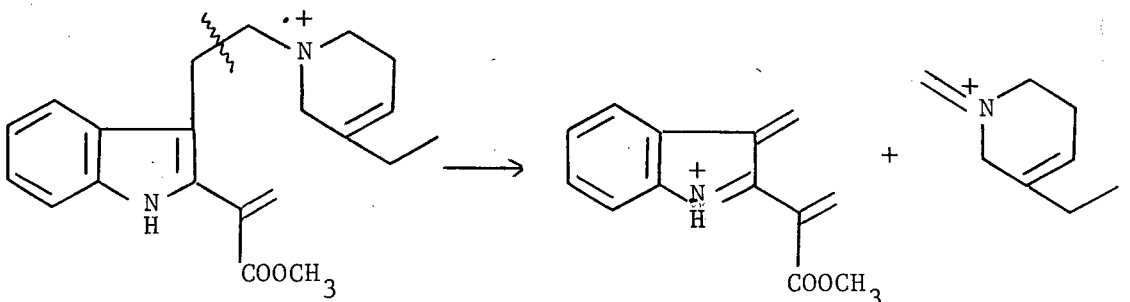



Figure 30. A summary of data supporting the structure of secodine (107).

presecamine (116) to secamine (119). Furthermore Smith[98] had found that secodine (107) reacted very slowly (over a period of 10 days) with methanol to give 17-methoxy-16,17-dihydrosecodine (121). Finally in the dimerization of secodine (107) to presecamine (116, Figure 30) it was specifically indicated that this reaction occurs at 0° in the absence of solvent. These observations clearly pointed to the fact that secodine (107) could perhaps be isolated free from dimer if the reaction mixture was kept cold and in solution. It was indeed found in our work that secodine (107) could be stored for several hours in dry benzene at 0° without any appreciable dimerization.

The optimum conditions for the dehydration of the alcohol (90) involved dissolving this substance in dry benzene and exposing it to sodium hydride. The reaction mixture was stirred under nitrogen at 40° for 15 minutes. At this time tlc examination of the mixture indicated three spots. The front running spot which seemed to represent the bulk of material, was due to secodine (107). The other two minor spots were due to the dimer presecamine (116) and the starting compound (90). The crude mixture was flushed through a small alumina column using benzene for the elution. The fraction collected was freeze-dried immediately under vacuum. We were very surprised as well as gratified to find that the gummy product obtained in this manner was homogeneous on tlc. Further elution of the column with chloroform afforded presecamine (116) and unreacted alcohol (90). In small scale reactions the yield in this dehydration procedure varied but the most favourable reaction provided 61% of secodine. It is now appropriate to discuss

some of the evidence in support of the structural assignment. In the nmr spectrum (Figure 31) the olefinic protons of the acrylic ester were represented as a pair of doublets at τ 3.55 (J = 1 Hz) and 3.91 (J = 1 Hz), respectively. The methoxycarbonyl was indicated by a sharp singlet at τ 6.20. These signals compared favourably with those reported for 15,20-dihydrosecodine (107, $\Delta^{15,20}$-reduced). In the latter the olefinic protons were indicated at τ 3.54 and 4.01; methoxycarbonyl at τ 6.18. The mass spectrum (Figure 32) indicated a molecular ion peak at m/e 338 in agreement with the molecular formula, $C_{21}H_{26}N_2O_2$. The spectrum was dominated by a significant peak at m/e 124(101) while a weak peak at m/e 214 showing a metastable peak at 135.5 was consistent with the fragment 108, both formed in the manner illustrated.




107 108 101

It is pertinent to mention here that when sodium hydride in the dehydration reaction was substituted by trityl sodium (triphenylmethyl sodium), it led to products of little utility. TLC indicated that the crude product was a mixture of three components. Out of these, two compounds separated in pure form by chromatography on alumina indicated very strong end absorption in the uv spectrum. This was indicative of a second aromatic system. It was felt that these two compounds arise

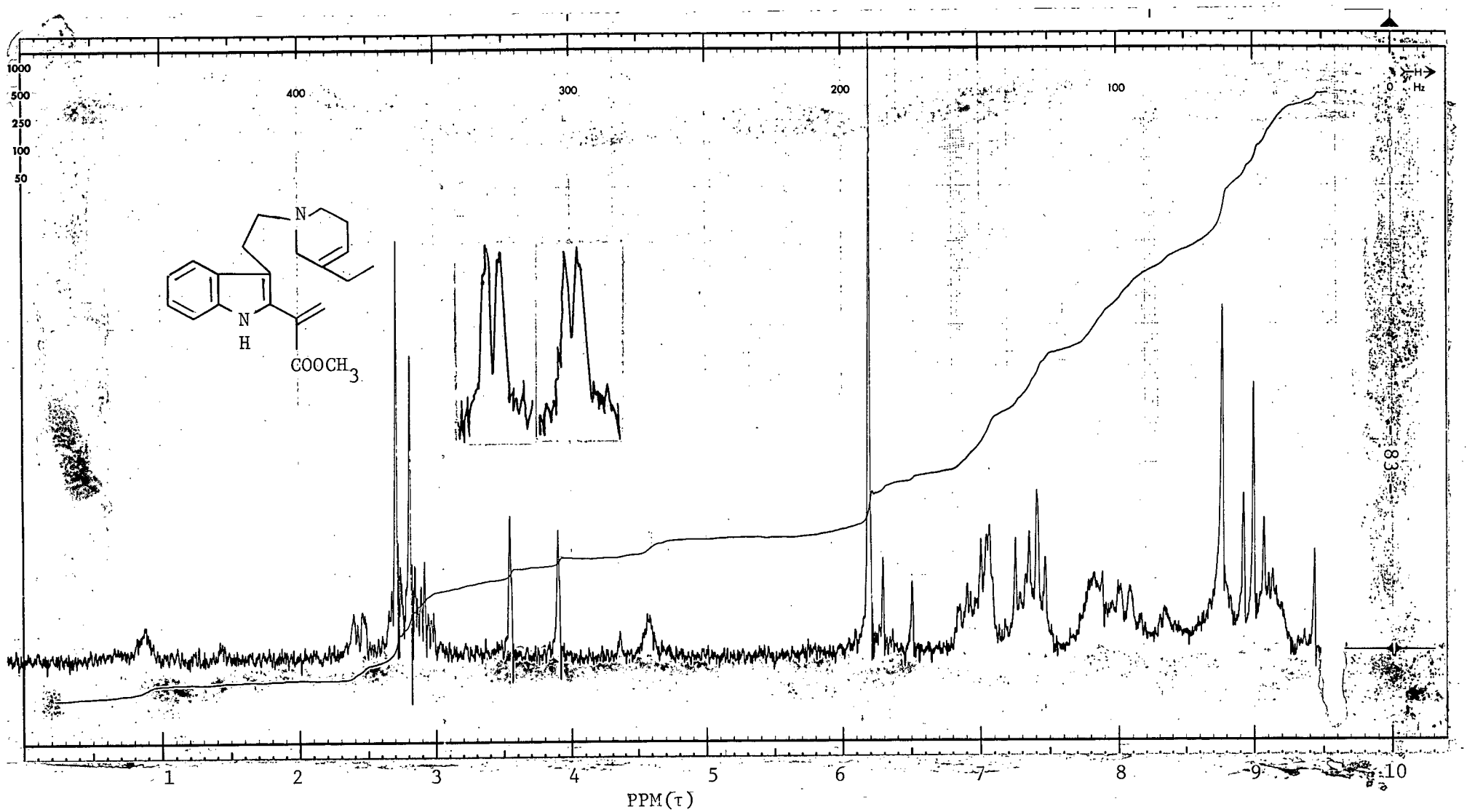



Figure 31. Nmr spectrum of secodine (107).

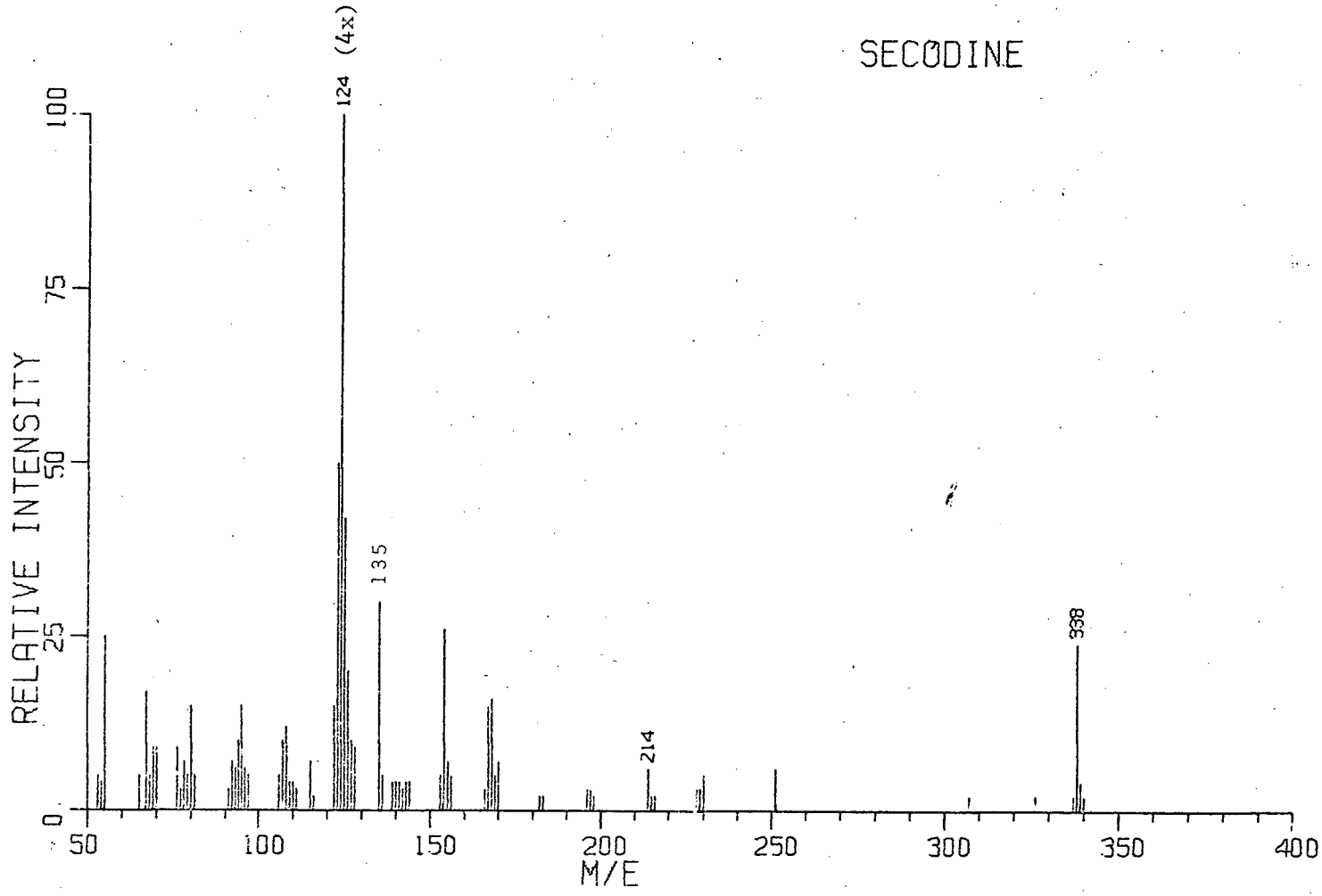
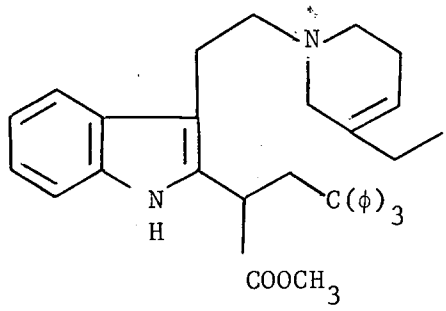



Figure 32. Mass spectrum of secodine (107).

from the condensation of the trityl anion with the acrylic ester functionality of secodine (107). Due to the very small amount of material at hand, no attempt was made to rigorously prove the structures of these compounds but tentative assignments are given in structures (122) and (123), respectively. Although this is not usual for triphenylmethyl sodium to react in this manner with an ester,[99] an example of

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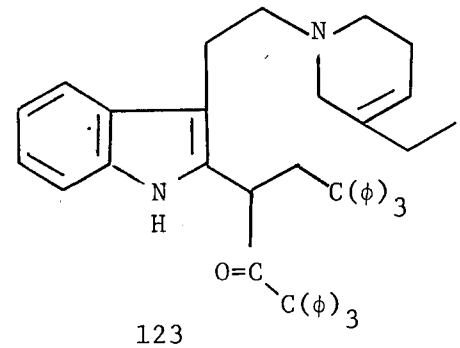


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the formation of a trityl ketone via a similar process has been observed.[100,101] In our case however, the molecule (107) because of the presence of the acrylic ester group was endowed with much greater reactivity to allow Micheal addition of the trityl anion.

With the completion of the long sought secodine (107) we now were in a position to investigate its possible role in the biosynthesis of the Aspidosperma and Iboga alkaloids.

Evaluation of secodine (107) as bio-intermediate

It should be mentioned here that some earlier workers in our laboratories have tentatively found that the best way to incorporate any given precursor into the plant system is to convert it into the acetate salt.[90] Normally the acetate salt is made by dissolving the compound

in 0.1 N acetic acid and a few drops of ethanol. So it was perfectly clear to us in the beginning that no matter how much care was taken in isolating pure secodine (107), the necessary conversion to the salt and eventual incorporation into the plant would allow some dimerization to presecamine (116). Under the influence of acid some of the presecamine (116) would then obviously rearrange to secamine (119). In summary we understood that we would be required under normal circumstances to incorporate a mixture of these three compounds. However it was envisaged that the presence of the dimeric compounds presumably would not jeopardise the feeding experiment provided the composition of the mixture could be determined at the time of feeding. For this purpose a "blank" experiment was conducted in such a manner that a clear distinction between the amount of secodine (107) and the dimeric compounds could be made. The details of this experiment are deferred until a later portion of the discussion. It is sufficient to emphasize presently that the procedure proved highly satisfactory.

For the biosynthetic investigation [ar-$^{3}$H]-16,17-dihydrosecodin-17-ol (90, specific activity 7.94 x $10^{7}$ dpm/mg) was dehydrated with sodium hydride in exactly the same manner as indicated previously. The pure [ar-$^{3}$H]-secodine (107, total activity 2.65 x $10^{8}$ dpm) was made soluble with 0.1 N acetic acid and a few drops of ethanol and the solution was administered to the shoots of Vinca minor L. The plants were allowed to grow for four days and then the alkaloids were isolated. The crude extract contained 22% of the total activity fed. Vincamine (72) and minovine (73) were isolated initially by chromatography and then further purified by preparative thin layer chromatography followed

by several crystallizations. Liquid scintillation counting revealed that the isolated alkaloids contained a very low level of radioactivity. Vincamine (72) showed an activity of 261 dpm/mg corresponding to a specific incorporation of 0.0013% while minovine (73) indicated an acitivity of 562 dpm/mg corresponding to a specific incorporation of < 0.001%. It is pertinent to call attention to the fact that in spite of the very low incorporation observed, the alkaloids were showing appreciable counts (cpm) above the normal background. In order to confirm the reliability of the above results another colleague of mine, John Beck, repeated this biosynthetic experiment employing a new series of plants. Fortunately his results for vincamine (72) and minovine (73) turned out to be in good agreement with the figures quoted above for these two alkaloids. In spite of the fact that the level of incorporation was extremely low, the most important observations to emerge from these experiments were that: (a) in comparison to our last biosynthetic experiment when 16,17-dihydrosecodin-17-ol (90) was fed, the Vinca minor shoots remained very healthy for the duration of the experiment (four days). In the former case the plants had started collapsing just after one day of feeding; (b) we were rather surprised when minovine (73) indicated almost twice as much radioactivity (dpm/mg) in comparison to vincamine (72). This factor was not self evident in considering the results of specific incorporation because minovine (73) is usually isolated in much smaller quantity than vincamine. This result could be due to the fact that for the secodine skeleton to incorporate into minovine (73), a few relatively straightforward ring closures are required (Figure 33) while for

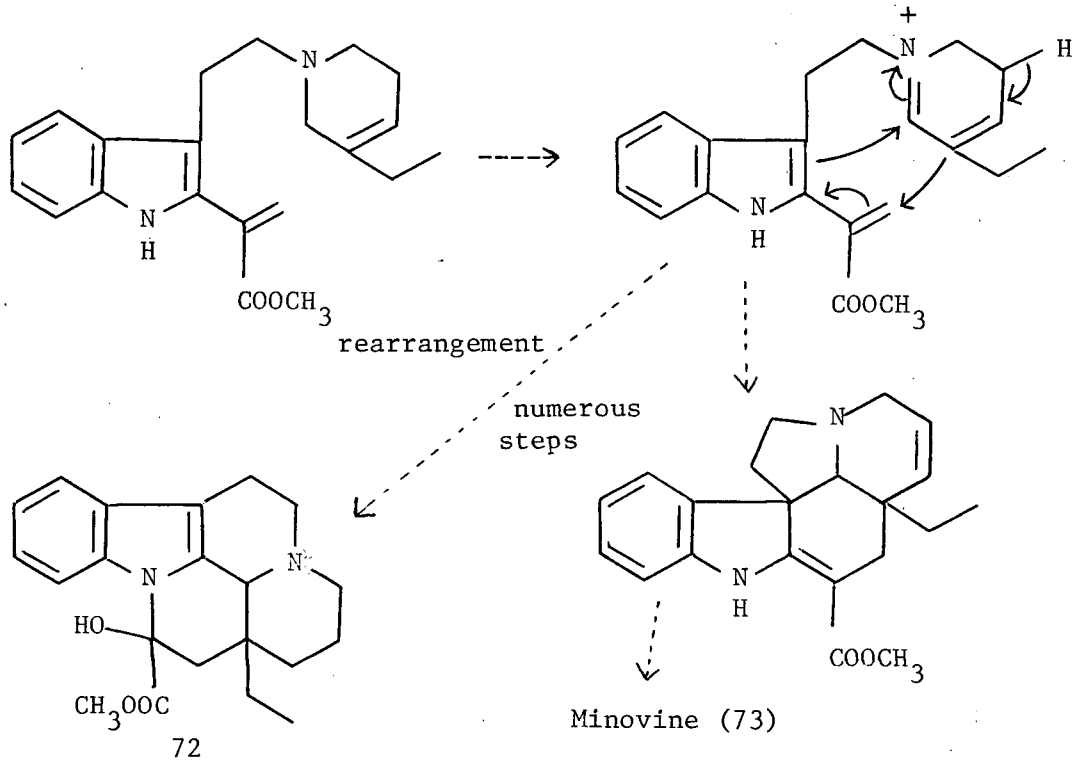
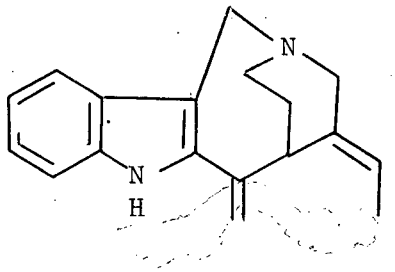



Figure 33. Proposed elaboration of secodine into vincamine and minovine.

vincamine (72) the secodine (107) molecule must undergo numerous rearrangements (Figure 33). A later discussion concerning studies on vincamine will present this aspect in more detail. We have no firm basis for this explanation and obviously additional experiments will be necessary before any more definite statement can be made.

In a complimentary series of experiments [ar-$^{3}$H]-secodine (107) was fed to Vinca rosea L. by John Beck in our laboratory. The radioactive vindoline (5) isolated (0.02% incorporation) was shown to be radiochemically pure by further conversion of this alkaloid into vindolinetriol having the same constant molar activity. Somewhat surprisingly Iboga alkaloid catharanthine (6) which co-occurs with vindoline (5) in

Vinca rosea indicated no activity. Similarly [ar-$^{3}$H]-secodine (107) was fed to Aspidosperma pyrricollum plants by Dr. Ken Stuart in our laboratory. Isolation of radioactive apparicine (124) indicated 0.01% incorporation.




124

All these incorporation results indicated above leave little doubt in our minds that secodine (107) or some closely related derivative which may be obtained by reaction of the enzyme systems on 107 may turn out to be a crucial bio-intermediate in indole alkaloid biosynthesis. It was now necessary to determine what percentage of the compound fed got into the plant in its monomeric state and what percentage of it was converted to the dimeric systems during the period of incorporation. A blank experiment was conducted in which [$^{14}COOCH_3$]-secodine (107) was converted into the acetate salt by dissolving it in 0.1 N acetic acid and a few drops of ethanol. The cloudy solution was left at room temperature for 2 hours (this is the maximum time the plants require to absorb the above solution). The contents were freeze-dried and a portion of the resulting gum was run on a Eastman Kodak neutral alumina strip plate employing a system which had been previously established for this purpose by means of the "cold" materials (for complete details see page 117 ). The activities in

the two spots corresponding to secondine (107) and the dimeric compounds (presecamine and secamine) were counted with a strip counter. It immediately became obvious that in the mixture the ratio between the secodine and the dimeric compounds was 61:32. Therefore the corrected specific incorporation into Vinca minor L. for vincamine (72) should be 0.002% and for minovine (73) < 0.0015%. This calculation assumes that the dimeric molecules do not convert back to the monomers in the plant.

All of the above results were very gratifying since they provided our first positive incorporation of a synthetic substance into the various plant species. The next important question as to secodine (107) is being incorporated as a intact unit will require the preparation of doubly labelled precursor i.e. [ar-$^{3}H$; $^{14}COOCH_3$]-secodine (107) or even better, secodine with one label in the indole unit and the other in the tetrahydropyridine portion. Such investigations are currently underway in our laboratories.

In conclusion it is clear that the above work has provided some preliminary information on the later stages of indole alkaloids biosynthesis. Most importantly it has created an entry into the more sophisticated experiments which will hopefully lead to a better understanding of the biosyntheses of this large family of natural products.

## 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 a Perkin Elmer Model 21, Model 137 and Model 457 spectrometers as KBr discs (unless otherwise stated). Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform (unless otherwise stated) at 100 megacycles per second (unless otherwise stated) on a Varian HA-100 instrument and the line positions or centre of multiplets are given in Tiers $\tau$ scale with reference to tetramethylsilane as the internal standard; multiplicity, integrated area and the type of protons are indicated in parentheses. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution molecular weight determinations were carried out 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 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 reagent carbon tetrachloride-antimony pentachloride (2:1).

Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise indicated).

Radioactivity was measured with a Nuclear Chicago Mark I Model 6860 Liquid Scintillation 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 utilising the built in barium-133 gamma source. The radioactivity of the sample was determined using a scintillation solution made up of the following composition: toluene (1 litre), 2,5-diphenyloxazole (4 gm) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (0.05 gm). In practice, a sample of an alkaloid as a free base was dissolved in benzene (1 ml) in a counting vial. In the case of the salt of an alkaloid, the sample was dissolved in methanol. Then in both cases, the volume was made up to 15 ml with the above scintillator solution. For each sample counted, the background (cpm) was determined for the counting vial to be used by filling the vial with the scintillator solution and counting (3 x 40 min). The counting-vial was emptied, refilled with the sample to be counted and the scintillator solution, and counted again (3 x 40 min). The difference in cpm between the background count and the sample count was used for the subsequent calculations.

For the sake of convenience and ease of presentation, the experimental has been divided into two portions. The first part describes the syntheses of 16,17-dihydrosecodin-17-ol (90) and secodine (107). The second portion describes the syntheses of radioactive precursors and their subsequent feeding into Vinca minor Linn.

PART I

Diethyl-γ-chloropropylmalonate (91)[72]

To a solution of sodium ethoxide prepared by dissolving sodium (23 gm, 1 mole) in ethanol (350 ml) was added in one portion a solution of diethyl malonate (160 gm, 1 mole) and 1,3-bromochloropropane (160 gm, 1 mole) in dry ether (200 ml). The reaction mixture was maintained at 35° for 4 hours and then allowed to stand at room temperature for 24 hours. Then the mixture was poured into water (700 ml) and extracted with ether. The extract was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting oil was distilled at reduced pressure to give the desired material (112 gm, 48%); bp 115°/0.5 mm, (lit. bp 142°/10 mm); $\nu_{max}$(film): 1730 ($-COOC_2H_5$) $cm^{-1}$; nmr (60 mc/s): τ 5.75 (quartet, 4H, 2 x $COO\underline{CH}_2CH_3$), 6.56 (triplet, 2H, $-CH_2-\underline{CH}_2-Cl$), 6.75 (triplet, 1H, $-CH_2-\underline{CH}-(COOEt)_2$), 8.10 (multiplet, 4H, $-\underline{CH}_2-\underline{CH}_2-CH_2-Cl$), 8.80 (triplet, 6H, 2x$-COOCH_2\underline{CH}_3$).

Benzenediazonium chloride[75]

Aniline hydrochloride (50 gm, 0.350 mole) was suspended in a mixture of glacial acetic acid (300 ml) and dry peroxide free dioxan (300 ml). The mixture was cooled in a ice-salt bath and isoamyl nitrile (50 gm, 0.420 mole) was added slowly, the temperature being held below 0°. After the addition was complete the mixture was stirred for 30 minutes during which the solid suspension dissolved. Dry dioxan (1500 ml) or dry ether (1500 ml) was added in one portion and the white precipitate of benzenediazonium chloride was collected, washed several times with fresh solvent and dried in a vacuum dessicator

(Yield 52 gm).

Synthesis of 2-carboethoxy-3-(β-chloroethyl)-indole (80) using benzenediazonium chloride

To a solution of sodium ethoxide, prepared by dissolving sodium (8.25 gm, 0.360 mole) in dry ethanol (1000 ml), was added diethyl-γ-chloropropymalonate (91, 85.0 gm, 0.360 mole) and the mixture was stirred under nitrogen for 30 minutes at room temperature. After cooling the reaction mixture in a ice-salt bath, the benzenediazonium chloride (52 gm, 0.370 mole) was added in small portions. During the addition the temperature of the reaction mixture was held below -2°. After the addition of the diazo salt was complete, the mixture was stirred for 30 minutes and then left in the refrigerator for 12 hours. The contents were poured into water (1000 ml) and the dark red oil thus separated was extracted into ether. The extract was washed with water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude reaction product (93, 106 gm) was immediately subjected to a Fisher indole synthesis described below.

The material obtained above was dissolved in dry ethanol (750 ml). To this concentrated sulfuric acid (100 ml) was added slowly and the mixture was refluxed for 12 hours. After cooling to room temperature, the volume of the reaction mixture was reduced to half under reduced pressure. The contents were poured onto ice and the resulting mixture was extracted with chloroform. The extract was washed several times with water, then with sodium carbonate solution, and again with

water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude dark semicrystalline material was purified by chromatography on alumina (Shawinigan, activity III, 3 kg). The desired material was eluted, first with benzene and later with benzene-chloroform (1:1), as a crystalline solid. Recrystallization from chloroform-petroleum ether gave a white crystalline solid (11.2 gm, 19%), mp 130-132°; $\nu_{max}^{nujol}$: 3250 (-NH), 1670 ($-COOC_2H_5$) $cm^{-1}$; $\lambda_{max}$ (log ε): 229 (4.40), 296 (4.27) mμ; nmr (60 mc/s): τ 0.75 (broad singlet, 1H, -NH), 2.60 (multiplet, 4H, aromatic), 5.54 (quartet, 2H, $-COOCH_2-CH_3$), 6.50 (multiplet, 4H, $-CH_2-CH_2-Cl$), 8.60 (triplet, 3H, $-COOCH_2-CH_3$).

Anal. Calc. for $C_{13}H_{14}O_2NCl$: C, 62.03; H, 5.57; N, 5.57; O. 12.75; Cl. 14.12. Found: C, 62.07; H, 5.53; N, 5.60; O. 12.64; Cl, 14.08.

Benzenediazonium fluoroborate[78,79]

Aniline hydrochloride (108 gm, 0.83 mole) was dissolved in water (275 ml) and conc. HCl (140 ml) in a three litre beaker. The solution was cooled to 0° and sodium nitrite (69 gm, 1 mole) in water (150 ml) was added dropwise, maintaining the temperature all the while below 5°. The addition of the sodium nitrite solution was stopped when a drop from the reaction mixture gave a blue coloration with starch iodide paper. A solution of 48% $HBF_4$ (183 ml) was cooled to 0° and added slowly to the diazonium salt solution. Precipitation was immediate but the stirring was continued for an additional 10 minutes. About half of this suspension was transferred to a sintered glass funnel and washed with ice cold water (50 ml), cold methanol (25 ml) and ether

(50 ml). The solid was sucked as dry as possible after each washing. The salt was transferred to a beaker and dried in a vaccum dessicator overnight. The other half was treated similarly. The total weight of solid material was (108 gm).

Synthesis of 2-carboethoxy-3-(β-chloroethyl)-indole (80) using benzenediazonium fluoroborate

To a solution of sodium ethoxide prepared by dissolving sodium (12 gm, 0.51 mole) in dry ethanol (1000 ml) was added diethyl-γ-chloropropylmalonate (91, 120 gm, 0.50 mole) and the mixture was stirred under nitrogen for 30 minutes at room temperature. After cooling the mixture in a ice-salt bath, the fluoroborate salt (105 gm, 0.55 mole) was added in small portions so that the temperature of the reaction mixture was always below -2°. After the addition of the diazo salt was complete, the mixture was stirred at 0° for 2 hours and then left in the cold room (-10°) for 12 hours. The contents were poured into water (1000 ml) and the dark red oil which separated out was extracted into ether. The extract was washed with water and with saturated brine solution, dried over anhydrous sodium sulfate and then concentrated under vacuum. The crude reaction product (93, 190 gm) was immediately subjected to a Fisher indole synthesis as described below.

The thick red oil was dissolved in dry ethanol (1000 ml). To this conc. sulfuric acid (200 ml) was added and the mixture was refluxed for 12 hours. After cooling to room temperature the reaction mixture was worked up in exactly the same manner as indicated on page 94 . The crude semicrystalline compound (140 gm) was purified by

chromatography on alumina (Shawinigan, activity III, 3 kg). Elution with benzene-chloroform (1:1) afforded the desired compound. This was recrystallised from chloroform-petroleum ether as white crystalline plates (14 gm, yield 13%), mp 131-132°. This material had the same $R_f$ value on tlc and spectral properties as the chloroindole (80) obtained earlier when diazonium chloride was used.

3-Ethylpyridine (81)[80]

A mixture of 3-acetylpyridine (60 gm), potassium hydroxide (50 gm), triethylene glycol (400 ml) and 85% hydrazine (90 ml) was heated for 1 hour at 110-125°. The reaction mixture was cooled and gradually reheated with a take-off condenser to a bath temperature of 180-190°. When the evolution of the nitrogen in the reaction mixture had ceased, the volume collected from the take-off condensor (about 200 ml) was extracted with ether. The extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting oil was distilled using an efficient fractionating column and 3-ethylpyridine was collected at 162-163° (lit. value 162-165°) (36 gm, yield 68%). $\nu_{max}^{film}$: no absorption in the carbonyl region.

N-[β-{3-(2-carboethoxy)-indolyl}-ethyl]-3'-ethyl-pyridinium chloride (82)

Chloroindole (80, 6.431 gm) was dissolved in 3-ethylpyridine (22 ml) and the mixture was heated in a sealed tube at 120° for 24 hours. After cooling to room temperature the sealed tube was opened and the contents were poured into anhydrous ether with stirring. The mixture was then left at room temperature for 3 hours. During this time all the occluded

3-ethylpyridine was extracted from the salt into the ether. The white amorphous solid was filtered under suction, washed several times with dry ether and finally dried in a vacuum dessicator (8.343 gm, yield 91%); mp 87-89° (recrystallised from methanol-ether); $\nu_{max}$: 3120-2880 (several bands, $\nu$C-H, aromatic), 1701 ($\nu$C=O) and 1250 ($\nu$C-O-C) $cm^{-1}$; $\lambda_{max}$(log $\varepsilon$): 296 (4.2), 276 (inf)(3.9), 226 (4.35) and 220 (4.3) m$\mu$; nmr ($CD_3OD$): $\tau$ 1.4-2.34 (multiplet, 4H, pyridinium protons), 2.56-3.18 (multiplet, 4H, indole protons), 5.74 (quartet, 2H, $-COO\underline{CH}_2-CH_3$), 7.40 (quartet, 2H, $-\underline{CH}_2-CH_3$), 8.63 (triplet, 3H, $-COOCH_2-\underline{CH}_3$), 8.98 (triplet, 3H, $-CH_2-\underline{CH}_3$); mass spectrum: $M^+$ 358; main peaks: m/e 215, 187, 169, 129.

N-[β-{3-(2-Carboethoxy)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (83)

To a solution of the pyridinium salt (82, 7.67 gm) in methanol (275 ml) and triethylamine (8 ml) was added slowly a solution of sodium borohydride (25 gm) in methanol (400 ml). The yellow color of the salt solution was discharged when the addition of the borohydride was complete. After stirring at room temperature for 2.5 hours, the reaction mixture was diluted with water (100 ml) and methanol was evaporated under reduced pressure. The remaining aqueous solution was acidified with 6 N HCl to pH 2, stirred at room temperature for 20 minutes and then made basic with 10% sodium carbonate solution. The basic solution was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated to give a thick gum (7.003 gm). This material was homogeneous on tlc and was used in the next reaction as such. Nmr (60 mc/s) of the crude

product: τ 4.5 (broad singlet, 1H, $C_3$,-H), 5.60 (quartet, ≃ 2H,-$COOCH_2$-$CH_3$).

N-[β-{3-(2-Hydroxymethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (84)

A solution of tetrahydropyridine (83, 7.003 gm) in dry THF (70 ml) was dropped slowly over a period of 40 minutes into a suspension of lithium aluminum hydride (9 gm) in THF (350 ml) under nitrogen. After the addition was complete, the reaction mixture was stirred at room temperature for 20 minutes and then refluxed for 2 hours. After cooling the mixture to ice temperature, the excess of the hydride was destroyed by careful addition of water (9 ml), 15% sodium hydroxide solution (9 ml) and water again (27 ml). The reaction mixture was filtered under suction and the precipitated hydroxides were washed several times with methylene chloride. The filtrate was evaporated under reduced pressure and the resulting gum was redissolved in methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to afford a light yellow gum (5.390 gm). This material was chromatographed on alumina (150 gm). Elution with benzene-chloroform (1:1) gave the desired alcohol (84, 4.40 gm). Yield of the reaction from chloroindole (80) was 70%. The alcohol was recrystallised from MeOH and later sublimed at 98°/0.01 mm, mp 108-110°; $\nu_{max}$: 3340 (νOH), 3180 (νNH) $cm^{-1}$; $\lambda_{max}$(log ε): 292 (3.73), 284 (3.81), 274 (shoulder)(3.76), and 223 (4.45) mμ; nmr ($CD_3OD$) (Figure 15): τ 2.40-3.09 (multiplet, 4H, indole protons), 4.45 (multiplet, 1H, $C_3$,-H), 5.17 (singlet, 2H, -$CH_2$-OH), 9.00 (triplet, 3H, -$CH_2$-$CH_3$); mass spectrum (Figure 16): $M^+$ 284; main peaks: m/e 174, 160, 142, 124; high resolution mass spectrometry: Calc. for $C_{18}H_{24}N_2O$: 284.188. Found: 284.184.

Anal. Calc. for $(C_{18}H_{24}N_2O)CH_3OH$: C, 72.09; H, 8.93; N, 8.85. Found: C, 72.05; H, 9.13; N, 8.22.

Benzoate ester of alcohol (84)

The alcohol (84, 1.50 gm, 5.4 mmole) was dissolved in dry pyridine (15 ml). The reaction mixture was cooled to 0° and benzoyl chloride (5.5 ml, 47 mmole) was added dropwise over a period of 10 minutes. The mixture was stirred at 0° for three hours, diluted with water (15 ml), made basic with 10% aqueous sodium carbonate solution and extracted with methylene chloride. The extract was washed several times with water, dried over anhydrous sodium sulfate and then evaporated very carefully (bath temperature not exceeding 40°) under reduced pressure to afford a thick gum (2.370 gm, contained traces of pyridine). This material was homogeneous on tlc and was used as such for the succeeding reaction. For analytical purposes, a small amount of benzoate (1 gm) was purified by chromatography on alumina (50 gm). Elution with benzene gave the desired material. This was recrystallised from methylene chloride-petroleum ether, mp 110.5-112.5°; $\nu_{max}$: 3000 (several bands, νC-H, aromatic), 1715 (νC=O of benzoyl group), 1455 (phenyl ring), 1260 (νC-O-C) $cm^{-1}$; $\lambda_{max}$(log ε): 293 (3.8), 284 (3.96), 274 (3.94), 2.24 (4.61) mμ; nmr: τ 1.36 (singlet, 1H, indole NH), 2-3 (multiplet, 9H, $C_6\underline{H}_5$-C=O + 4 indole protons), 4.58 (singlet, 3H, $C_3$,-H + $-\underline{CH}_2$-O-C-φ), 9.0 (triplet, 3H, $-CH_2-\underline{CH}_3$); mass spectrum: $M^+$ 388; main peaks: m/e 266, 170, 143, 124, 122; high resolution mass spectrometry: Calc. for $C_{25}H_{28}N_2O_2$: 388.216. Found: 388.215.

Anal. Calcd. for $C_{25}H_{28}N_2O_2$: C, 77.27; H, 7.28; N, 7.21. Found: C, 77.05; H, 7.29; N, 7.05.

Alternative synthesis of benzoate (85)

The alcohol (84, 2.82 gm, 0.01 mole) was dissolved in dry THF (50 ml) and the reaction mixture was cooled with ice. To this anhydrous potassium carbonate (5 gm) was added and the heterogeneous mixture was treated, dropwise, with benzoyl chloride (5 ml, 0.042 mole) under nitrogen. The mixture was stirred at 0° for 1 hour and then at room temperature for 3 hours. The reaction was worked up by adding water (50 ml) followed by mild warming of the mixture in warm water bath. A few minutes later saturated sodium carbonate solution (50 ml) was added and the mixture was extracted using benzene and methylene chloride. The organic phase was washed with water, dried over anhydrous sodium sulfate and evaporated. The resulting material was put on a column of alumina (100 gm). Elution with chloroform afforded the benzoate (85) as white crystalline solid (2.3 gm) as well as a yellowish foam (1.5 gm, one spot on tlc). The overall yield was 99%. This benzoate showed the same $R_f$ value and spectral properties as the benzoate obtained earlier.

N-[β-{3-(2-Cyanomethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperidenine (86)

The benzoate (85, 2 gm, .005 mole) was dissolved in dimethyl-formamide (60 ml). To this solid potassium cyanide (3.3 gm, 0.050 mole) was added and the heterogeneous mixture was stirred under nitrogen at room temperature for 1 hour. The temperature of the reaction mixture was now gradually raised to 105-110° over a period of 45 minutes. The reaction was monitored by tlc and after 1 hour at the elevated temperature, tlc indicated the completion of the reaction. The mixture was cooled down to room temperature, diluted with water (100 ml) and

extracted with methylene chloride. The extract was washed several times with water, dried over anhydrous sodium sulfate and evaporated to afford a dark thick oil. This oily material was left under vaccum until all the dimethylformamide was removed. The resultant dark crystalline compound (1.450 gm) was chromatographed on alumina. Elution with benzene-petroleum ether (1:1) and later with benzene furnished the pure nitrile (86) as a crystalline compound (0.825 gm, 55%). Later fractions of elution with benzene and benzene-chloroform (9:1) afforded a small amount of additional nitrile as gum (0.125 gm, 10%). This latter material was contaminated with a very minute red impurity but the compound was of reasonable quality to be utilised in the next reaction. The overall yield of the reaction was ≈65%. For analytical purposes, a small amount of nitrile (86) obtained was recrystallised from methylene chloride-petroleum ether and later sublimed at 100°/ .01 mm, mp 135-137°; $\nu_{max}$: 3160 (νN-H), ≈ 2900 (several bands, νC-H, aromatic), 2256 (νC≡N) $cm^{-1}$; $\lambda_{max}$(log ε): 291 (3.77), 281 (3.85), 274 (3.84), 221 (4.69) mμ; nmr (Figure 17): τ 1.63 (singlet, 1H, indole-N$\underline{H}$), 2.46-3.00 (multiplet, 4H, indole protons), 4.58 (multiplet, 1H, $C_3$-$\underline{H}$), 6.2 (singlet, 2H, -C$\underline{H}_2$-CN), 8.99 (triplet, 3H, -$CH_2$-C$\underline{H}_3$); mass spectrum: $M^+$ 293; main peaks: m/e 267, 169, 156, 124; high resolution mass spectrometry: Calc. for $C_{19}H_{23}N_3$: 293.189. Found: 293.186.

Anal. Calc. for $C_{19}H_{23}N_3$: C, 77.75; H, 7.92; N, 14.32. Found: C, 77.65; H, 7.86; N, 14.16.

N-[β-{3-(3-Carbomethoxymethylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (88)

Crystalline nitrile (86, 0.746 gm, 2.5 mmole) was dissolved in dry methanol (20 ml) and to this a small amount of water (0.2 ml or 1%) was added. The mixture was cooled in ice and saturated with HCl gas. After stirring at room temperature for 60 hours, the solution was taken to dryness under vacuum and the residue was treated with sodium bicarbonate solution. The basic solution was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The crude product so obtained was dissolved in a small amount of benzene and put on a column of alumina (40 gm). Elution with petroleum ether-benzene (2:8) and later with benzene furnished the pure carbomethoxy ester (88) as white crystalline compound. The compound was recrystallised from methylene chloride-petroleum ether (0.574 gm, 70%), mp 85-87.5°; $\nu_{max}$: 3000 (several bands, νC-H, aromatic), 1728 (νC=O of ester), 1460 (δC-H, $C\underline{H}_3$-CO-), 1245 (νC-O-C) $cm^{-1}$; $\lambda_{max}$(log ε): 292 (3.83), 283 (3.92), 274 (3.87), 223 (4.43) mμ; nmr (Figure 18): τ 1.46 (broad singlet, 1H, indole-N$\underline{H}$), 2.42-3.00 (multiplet, 4H, indole protons), 4.58 (multiplet, 1H, $C_3$,-$\underline{H}$), 6.27 (singlet, 2H, -$C\underline{H}_2$-$COOCH_3$), 6.34 (singlet, 3H, -$CH_2$-$COOC\underline{H}_3$), 9.00 (triplet, 3H, -$CH_2$-$C\underline{H}_3$); mass spectrum (Figure 19): $M^+$ 326; main peaks: m/e 267, 202, 156, 144, 124; high resolution mass spectrometry: Calc. for $C_{20}H_{26}N_2O_2$: 326.199. Found: 326.202.

Anal. Calc. for $C_{20}H_{26}N_2O_2$: C, 73.50; H, 8.04; N, 8.58. Found: C, 73.47; H, 8.05; N, 8.71.

Formylation of ester (88) using sodium hydride as base

A 25-ml three necked flask was equipped with a magnetic stirrer, a reflux condenser, a dropping funnel and a nitrogen inlet. All the glassware was flame dried then thoroughly flushed with dry nitrogen. To the reaction flask a 65% suspension of sodium hydride in paraffin oil (0.050 gm, 1.3 mmole) was added. This suspension was washed three times with 1-ml portions of dry benzene under nitrogen. The oil free sodium hydride was suspended in a fresh portion of dry benzene (2 ml) and to this freshly distilled methyl formate (dried first over calcium chloride and then over $P_2O_5$) (2 ml) was added. The carbomethoxy ester (88, 0.050 gm, 0.15 mmole) was dissolved in dry benzene (3 ml) and added dropwise to the above suspension. The reaction mixture was stirred at room temperature for 15 minutes and at 35° for 2 hours. At this time tlc indicated the completion of the reaction. The excess of hydride in the reaction mixture was destroyed by cooling the mixture to 0°, adding a few drops of methanol, followed by the addition of some crushed ice. The mixture was made acidic with 2 N HCl. The excess of the acid was neutralised with aqueous sodium bicarbonate solution and the heterogeneous mixture was extracted with methylene chloride. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated to afford the crude enol (89) as white foam (0.070 gm, contained some mineral oil). This material was used as such for the next reaction.

16,17-Dihydrosecodin-17-ol (90)

The crude enol (89) obtained above was dissolved in methanol (3 ml). The solution was cooled to -30° in a dry ice-acetone bath and sodium

borohydride (0.050 gm) was added to this in small portions. After stirring for 40 minutes at -30°, an additional amount of sodium borohydride (0.040 gm) was again added in small portions to the reaction mixture. Ten minutes later the mixture indicated no more unreacted enol on tlc and instead the polar "diol" (105) had just started appearing. The excess of borohydride in the cold reaction mixture was therefore immediately quenched by careful addition of 2-3 drops of 2 N HCl. The mixture was diluted with water (5 ml) and the methanol was evaporated under reduced pressure. The remaining mixture was acidified with 2 N HCl, made basic with sodium bicarbonate solution and extracted with chloroform. The organic phase after drying and evaporation left a white foam (68 mg). This material was dissolved in a small amount of benzene and put on a column of alumina (2.5 gm). Elution with benzene-chloroform in the order (9:1), (7:3), (1:1) and finally with chloroform afforded the pure alcohol (90, 22 mg). The yield of the reaction from the ester (88) was 40%. For analytical purposes the alcohol (90) was crystallised from dichloromethane, mp 131.5-132°; $\nu_{max}$: 3400 (sharp, νN-H), 3050 (broad, νO-H), ∿ 2900 (several bands, νC-H, aromatic), 1718 ( νC=O), 1465 (δC-H, $C\underline{H}_3CO$-), 1235 (νC-O-C) $cm^{-1}$; $\lambda_{max}$(log ε): 292 (3.86), 284 (3.93), 274 (shoulder)(3.87), 222 (4.49) mμ; nmr (Figure 20): 1.16 (singlet, 1H, indole N-$\underline{H}$), 2.48-3.00 (multiplet, 4H, indole protons), 4.61 (broad singlet, 1H, $C_{15}$-$\underline{H}$), 6.00 (multiplet, 4H, -$C\underline{H}_2$-OH + -$\underset{|}{C}$-$\underline{H}$), 6.37 (singlet, 3H, -COO$C\underline{H}_3$), 9.04 (triplet, 3H, -$CH_2$-$C\underline{H}_3$); mass spectrum (Figure 21): $M^+$ 356; main peaks: m/e 338, 326, 214, 202, 124; high resolution mass spectrometry: Calc. for $C_{21}H_{28}N_2O_3$: 356.209. Found: 356.207.

Anal. Calc. for $C_{21}H_{28}N_2O_3$: C, 70.24; H, 7.93; N, 7.86. Found: C, 70.20; H, 7.83; N, 7.35.

Formylation of carbomethoxy ester (88) using trityl sodium as base

A 25-ml three necked flask was equipped with a magnetic stirrer, a reflux condenser, a dropping funnel and a nitrogen inlet. All the glassware was flame dried and then thoroughly flushed with dry nitrogen. To a solution of the ester (88, 0.050 gm, 0.155 mmole) in dry tetrahydrofuran (3 ml) was added dropwise a solution of trityl sodium (2.2 ml, 0.18 N, 0.387 mmole) under nitrogen. The first half of the trityl sodium solution decolorized very rapidly as the proton from the indole nitrogen reacted. The other half of the solution decolorized very slowly until finally the last 1-2 drops were added, the red color of the base stayed in the reaction mixture. The solution was stirred at room temperature for about 2 minutes and then methyl formate (2 ml, dried first over calcium chloride and then freshly distilled over $P_2O_5$), was added dropwise. The red color of the base disappeared immediately and the resulting yellow solution was stirred at room temperature for 1.5 hours. The solution was evaporated to dryness under vacuum and the residue was acidified with 2 N HCl. The excess acid was neutralised with sodium bicarbonate solution and the mixture was extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude enol (89) was utilised as such for the next reaction.

Reduction of the crude enol (89) obtained by using trityl sodium as base

The crude product obtained above (containing crude enol and triphenylmethane) was dissolved in methanol. Some of the triphenylmethane did not dissolve in methanol and was left floating in the reaction medium. After cooling the heterogeneous mixture down to -30°, sodium borohydride (50 mg) was added in small portions over a period of 10 minutes. The mixture was stirred at -30° for 40 minutes. At this time an additional amount of sodium borohydride (40 mg) was again added to the reaction mixture. 10 minutes later tlc indicated the completion of the reaction. The excess of borohydride was immediately quenched by careful addition of 2-3 drops of 2 N HCl to the cold (-30°) reaction mixture. The mixture was worked up in exactly the same manner as indicated previously (page 105). The crude product was chromatographed on alumina (2.5 gm). Elution with benzene-petroleum ether (1:1) gave the triphenylmethane. The desired compound (90) was eluted with benzene-chloroform in the order (9:1), (7:3), (1:1) and finally with chloroform. The pure alcohol (90, 20 mg) obtained represented a yield of 37% from the ester (88).

Secodine (107)

A 10-ml flask was equipped with a magnetic stirrer, a reflux condenser, and a nitrogen inlet. All the glassware was first flame dried and then thoroughly flushed with dry nitrogen. To the reaction flask a 65% suspension of sodium hydride in mineral oil (25 mg, 0.65 mmole) was added. This suspension was washed three times with 0.5-ml portions of dry benzene and finally the oil free sodium hydride was

suspended in a fresh portion of dry benzene (0.5 ml). A solution of 16,17-dihydrosecodin-17-ol (90, 20 mg, 0.06 mmole) in dry benzene (2.5 ml) was dropped very rapidly into the above suspension under nitrogen. The reaction mixture was stirred at 40° for 15 minutes. In the meantime a column of alumina (2 gm, activity IV) was made in dry benzene. The crude reaction mixture was flushed through this column using benzene as eluent. This fraction (40 ml) which contained the desired material, was collected in a cold receiver. It was frozen with liquid nitrogen and freeze-dried under vacuum to afford secodine (107) as a light yellow gum (9.1 mg, 50%). Nmr (Figure 31): $\tau$ 0.89 (broad singlet, 1H, indole-N$\underline{H}$), 2.40-3.00 (multiplet 4H, indole protons), 3.55 (doublet, J 1 Hz, 1H, olefinic proton of the acrylic ester), 3.91 (doublet, J 1 Hz, 1H, olefinic proton of the acrylic ester), 4.58 (multiplet, 1H, $C_{15}$-$\underline{H}$), 6.20 (singlet, 3H, $CH_2$=$\underline{C}$-COO$\underline{CH_3}$), 9.00 (triplet, 3H, -$CH_2$-$\underline{CH_3}$); mass spectrum (Figure 32 ): $M^+$ 338; main peaks: m/e 307, 251, 214, 154, 124.

PART II

Trifluoroacetic acid-[$^3$H]

Trifluoroacetic anhydride (1.17 gm, 5.55 mmoles) was added to water-$^3$H (0.10 gm, 5.50 mmoles, 100 mcurie/gm) using a vacuum transfer system. The resulting trifluoroacetic acid-$^3$H (1.27 gm, 0.9 mcurie/mmole) was stored under an atmosphere of nitrogen at -10° until required.

[ar-$^3$H]-Carbomethoxy ester (88)

Trifluoroacetic acid-$^3$H (1.27 gm, 0.9 mcurie/mmole) was added to the crystalline ester (88, 0.1887 gm) using a vacuum transfer system. The solution was allowed to stand at room temperature for 48 hours under nitrogen atmosphere. After this time the trifuloroacetic acid-$^3$H was removed with a vacuum transfer system and concentrated ammonium hydroxide solution (10 ml) was added carefully to the above gummy residue. The mixture was extracted with dichloromethane. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The resulting gum was dissolved in methanol (10 ml) and then evaporated. This process was repeated four times to remove any labile tritium. The crude product was put on a column of alumina (15 gm) and the desired compound was eluted with benzene (0.152 gm, 80% yield, specific activity 1.10 x $10^8$ dpm/mg).

Formylation of [ar-$^3$H]-carbomethoxy ester (88)

The tritiated ester obtained above (0.152 gm) was formylated using sodium hydride (0.150 gm) and freshly distilled methyl formate (4 ml) (for complete details of the procedure see page 104). Temperature of the

reaction mixture was maintained at 35° and the reaction took 2 hours for completion. Evaporation of the solvent after work up gave the crude radioactive enol (89, 180.5 mg). This was used for the next reaction as such.

[ar-$^{3}$H]-16,17-Dihydrosecodin-17-ol (90)

The crude active enol (89, 180.5 mg) was dissolved in methanol (15 ml). After cooling the reaction mixture down to -30°, sodium borohydride (180 mg) was added in small portions over a period of 15 minutes. The mixture was stirred at -30° for 40 minutes. At this time an additional amount of sodium borohydride (100 mg) was again added in small portions. 15 Minutes later when the mixture indicated no more enol on tlc, the excess of borohydride was quenched with a few drops of 2 N HCl and the mixture was worked up in exactly the same manner as indicated on page 105. The crude product (152 mg) was chromatographed on alumina (8 gm). Elution with benzene-chloroform in the order (9:1), (7:3), (1:1) and finally with chloroform afforded the pure alcohol (90) which was crystallised from methylene chloride-petroleum ether (72 mg, 43% yield, specific activity 2.83 x $10^{10}$ dpm/mmole or 7.94 x $10^{7}$ dpm/mg).

N-[β-{3-(2-Cyano($^{14}$CN)methylene)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (86)

The benzoate (85, 1.09 gm, 0.25 mmole) was dissolved in dimethyl-formamide (25 ml). This was treated with a mixture of radioactive $^{14}$C-potassium cyanide (0.072 gm, total activity 8 mcurie) and potassium cyanide (0.753 gm, total potassium cyanide (0.825 gm) used in the reaction was 1.25 mmole or a five fold molar excess). The reaction was stirred at room temperature for 1 hour under nitrogen. The

temperature of the reaction mixture was now gradually raised to 105° over a period of 45 minutes. After 1 hour at this elevated temperature, tlc indicated the completion of the reaction. The mixture was cooled to room temperature and the crude readioactive nitrile (86) was isolated as a dark crystalline compound (for details of the work up see page 102). This crude material was chromatographed on alumina (30 gm). Elution with benzene and later with benzene-chloroform (9:1) afforded the pure active nitrile (86, 0.483 gm, specific activity $4.32 \times 10^6$ dpm/mg or $1.27 \times 10^9$ dpm/mmole). The yield of the reaction was 63%.

$[^{14}COOCH_3]$-Carbomethoxy ester (88)

Crystalline radioactive nitrile (86, 0.480 gm, 1.6 mmoles) was dissolved in dry methanol (10 ml). To this a small amount of water (0.1 ml or 1%) was added and the solution was saturated with HCl gas. After stirring at room temperature for 60 hours, the crude ester was isolated (for complete details of the work up see page 103). It was dissolved in a small amount of benzene and put on a column of alumina (25 gm). Elution with benzene-petroleum ether (2:8) and later with benzene furnished the pure radioactive ester (86, 0.302 gm). The yield of the reaction was 65%.

Formylation of $[^{14}COOCH_3]$-carbomethoxy ester (88)

Radioactive ester (88, 0.100 gm, 0.30 mmole) was formylated using sodium hydride (0.100 gm, 2.6 mmoles) and freshly distilled methyl formate (4 ml) (for complete details of the procedure see page 104).

The crude enol (89, 0.126 gm) isolated was used directly for the next reaction.

[$^{14}COOCH_3$]-16,17-Dihydrosecodin-17-ol (90)

The crude active enol (89, 0.126 gm) obtained above was dissolved in methanol. After cooling the solution down to -30°, sodium borohydride (100 mg) was added in small portions over a period of 15 minutes. The mixture was stirred at -30° for 40 minutes. At this time a small amount of sodium borohydride (0.040 gm) was again added slowly to the reaction mixture. Ten minutes later when tlc indicated the completion of the reaction, the crude alcohol was isolated (for details of the work up see page 105) and chromatographed on alumina (5 gm). Elution with benzene-chloroform in the order (9:1), (7:3), (1:1) and finally with chloroform afforded the pure alcohol (90, 0.044 mg, specific activity 3.56 x $10^6$ dpm/mg). The yield of the reaction from the ester (88) was 40%.

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 Vinca minor L. plants and was scaled according to the wet weight of the plants used.

Vinca minor Linn plants (9 kg, wet weight), obtained from the gardens of the University of British Columbia, were macerated with methanol in a Waring blender, filtered and re-macerated until the

filtrate was colorless. This green filtrate (8000 ml) was concentrated to dryness under reduced pressure and the residue was dissolved in 2 N HCl (4500 ml). The acid layer was extracted with benzene (2 x 2000 ml) and the benzene extracts were back extracted with 2 N HCl (2 x 500 ml). The combined aqueous phases were made basic with 15 N ammonium hydroxide, taking care that the temperature of the solution did not rise above 25°, and extracted with chloroform (3 x 2400 ml). The combined chloroform extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The resulting alkaloids residue (13.6 gm) was dissolved in benzene-methylene chloride (1:1) (100 ml) and chromatographed on alumina (700 gm). The column was eluted successively with petroleum ether, benzene, chloroform, and methanol; fractions of 700 ml were taken. The fractions eluted with petroleum ether and benzene were combined and subjected to an additional column chromatography on alumina (300 gm). Elution with petroleum ether-benzene (6:4) afforded minovine (73, 0.41 gm). Successive fractions, eluted with petroleum ether-benzene (2:8) and finally with benzene, were combined and crystallised from methanol affording vincamine (72, 0.65 gm). Both these alkaloids were compared on tlc with authentic samples.

Feeding of [$^{14}COOCH_3$]-16,17-dihydrosecodin-17-ol (90) to Vinca minor Linn. (feeding experiment no. I)

[$^{14}COOCH_3$]-16,17-Dihydrosecodin-17-ol (90, 0.0065 gm, 9.89 x $10^6$ dpm) was made soluble in 0.1 N acetic acid (1 ml) and ethanol (0.5 ml). The cloudy solution was diluted with water (5 ml). The resulting clear solution was distributed equally among ten test tubes and

three Vinca minor cuttings were placed into each of these test tubes (total weight of plants utilised was 43 gm). The plants were placed under fluorescent lamp illumination and the aqueous levels in the test tubes were maintained with distilled water. After four days the cuttings were extracted to afford the crude alkaloids as dark foam (0.092 gm, $3.06 \times 10^6$ dpm or 31% of the total activity fed). The crude extract was dissolved in a small amount of benzene-methylene chloride (≃ 2ml) and put on a column of alumina (15 gm). Elution with petroleum ether-benzene (1:1) and then further purification of the resulting gum by preparative thin layer chromatography (silica gel, ethyl acetate-methanol, 2:1) afforded pure minovine (73, 0.00545 gm). It was diluted further with an authentic sample of minovine (15.70 mg). Several crystallisations revealed that the alkaloid contained virtually no activity.

Further elution of the column with petroleum ether-benzene (4:6) afforded pure vincamine (72) as a crystalline compound (7.7 mg). After several crystallisations from methanol, vincamine indicated an activity of 102 dpm (total). This represented a maximum incorporation of < 0.001%.

Feeding of [ar-$^3$H]-secodine (107) to Vinca minor Linn. (feeding experiment no. 2)

In view of the rapid dimerization of secodine, the following procedure was developed and is typical of all the feeding experiments done whenever secodine was fed to the various plant species in our laboratories.

A 10-ml flask was equipped with a magnetic stirrer, a reflux condenser and a dry nitrogen inlet. The glassware was flame dried and then thoroughly flushed with nitrogen. A 65% suspension of sodium hydride in mineral oil (10 mg, 0.26 mmole) was added to the reaction flask. This suspension was washed three times with 0.5 ml-portions of dry benzene under nitrogen and the oil free sodium hydride was suspended in a fresh portion of dry benzene (0.5 ml). In a small dry test tube [ar-$^{3}$H]-16,17-dihydrosecodin-17-ol (90, 10 mg, 0.03 mmole, 79.4 x $10^{7}$ dpm) was dissolved in dry benzene (1.2 ml) by slightly warming the test tube in a hot water bath. This solution was dropped very rapidly (in about 35 seconds) into the suspension of sodium hydride. The mixture was stirred at 40° for 15 minutes and then quickly flushed through a small column of alumina (1.5 gm, activity IV) made up with benzene. The column was eluted with benzene and the eluted fraction was collected in a cold 25-ml volumetric flask. One ml of this solution was diluted to 100 ml with benzene and 1 ml of this latter solution (or 2.5 x $10^{-3}$ of the compound to be fed) was used for counting purposes. The remaining portion (24 ml) of the benzene fraction was transferred to a specially designed evaporator. The fraction was frozen with liquid nitrogen and freeze-dried under vacuum. This furnished secodine(107) as a light yellow gum (3.2 mg, 2.65 x $10^{8}$ dpm).

The gum obtained above was made soluble in 0.1 N acetic acid (1 ml) and ethanol (0.5 ml). The cloudy solution was diluted with water (5 ml) and the resulting clear solution was distributed equally among ten test tubes. Three Vinca minor cuttings were inserted into each of these test tubes (total weight of plants utilised was 40 gm) and the

plants were placed under fluorescent lamp illumination and the aqueous levels in the test tube were maintained with distilled water. After four days, the cuttings were extracted to afford the crude alkaloids as a dark foam (90.2 mg, $5.8 \times 10^7$ dpm representing a recovery of 22% of the total activity fed). The crude product was dissolved in a small amount of benzene-methylene chloride and chromatographed on alumina (20 gm). Elution with petroleum ether-benzene (7:3) and then subsequent purification of the resulting gum by preparative tlc (silica gel, ethyl acetate-methanol, 2:1) afforded pure minovine (73, 4.8 mg). This was diluted with cold minovine (10.4 mg) andthe total compound (15.2 mg) was crystallised to constant activity (562 dpm/mg). This represented a maximum incorporation of 0.001%. This incorporation was corrected for the amount of secodine(107) that dimerized at the time of feeding by running a "blank" experiment (for details see page 117). The corrected incorporation should be < 0.0015%.

Further elution of the column with petroleum ether-benzene (1:1) afforded crystalline vincamine (72, 12.7 mg). This was further purified by preparative tlc (alumina, ethylacetate-chloroform, 1:1). Pure vincamine (3.8 mg) obtained in this manner was diluted with cold vincamine (4.65 mg) andthe mixture was crystallised to constant activity (261 dpm/mg). This represented a maximum incorporation of 0.0013%. This extent of incorporation was again corrected for the amount of secodine(107) that dimerized at the time of feeding by correlation with the "blank" experiment (for details see page 117). The corrected incorporation should be 0.002%.

"Blank" for the feeding experiment no. 2

[$^{14}COOCH_3$]-16,17-Dihydrosecodin-17-ol (90, 10 mg, 20.7 x $10^6$ dpm) was dehydrated to [$^{14}COOCH_3$]-secodine (107, 3.4 mg, 7.03 x $10^6$ dpm) in exactly the same manner as indicated in the feeding experiment no. 2. The resulting gum was made soluble in 0.1 N acetic acid (3 ml) and ethanol (0.5 ml). The solution was left at room temperature for 2 hours (this is the maximum time the Vinca minor cuttings take to absorb the above volume of solution), frozen with liquid nitrogen, and finally freeze-dried under vacuum. The resulting solid was redissolved in methanol and a small portion of this solution was spotted horizontally on two Kodak neutral alumina chromatogram sheets. The sheets were developed in a mixture of benzene-chloroform (1:1) and passed through a calibrated Nuclear-Chicago Actigraph 11 Model 1039 tlc counter connected to a recorder (Nuclear-Chicago Model 8416) and integrator (Nuclear-Chicago Model 8704). The activities found in three spots corresponding to the baseline, dimeric compounds (presecamine and secamine) and secodine and their relative percentages in the initial mixture are summarized in Table 2.

Table 2. Results of the "blank" experiment

| Sheets | Secodine | | Dimeric Compounds | | Baseline Material | |
|---|---|---|---|---|---|---|
| | activity cpm | % | activity cpm | % | activity cpm | % |
| Sheet no. 1 | 21,693.94 | 61.22 | 11.313 | 32 | 2,394.46 | 6.78 |
| Sheet no. 2 | 32,650 | 61.52 | 16,871 | 32.03 | 3,422 | 6.45 |

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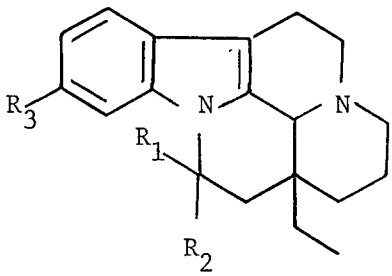
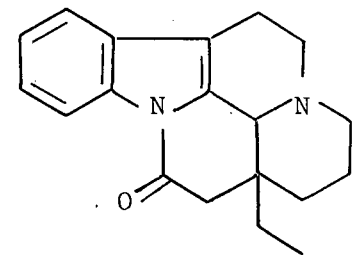
# PART B

# STUDIES RELATED TO THE BIOSYNTHESIS OF VINCAMINE

## INTRODUCTION

Some years ago during the course of an examination of the African apocynaceous plant Hunteria eburnea pichon[1] and the tiny evergreen plant Vinca minor L.[2], for substances of possible therapeutic value several new alkaloids were isolated. As the structure of these new



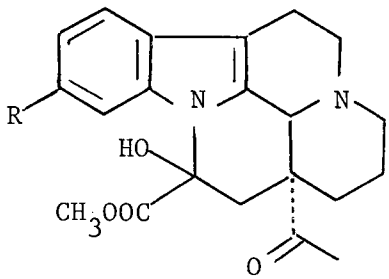

1 $R_3 = R_1 = H$, $R_2 = OH$ Eburnamine

2 Eburnamonine

3 $R_3 = H$, $R_1 = OH$, $R_2 = COOCH_3$ Vincamine

4 $R_3 = H$, $R_1 = COOCH_3$, $R_2 = OH$ Epivincamine

5 $R_3 = OCH_3$, $R_1 = OH$, $R_2 = COOCH_3$ Vincine




6 R = H Vincaminine

7 R = $OCH_3$ Vincinine

Figure 1. Various eburnamine-vincamine type alkaloids.

alkaloids were unraveled, it soon became evident that the unifying feature of all these compounds was the inheritance of a general pentacyclic structure (e.g. 1-7, see Figure 1). Since vincamine (3) and eburnamine (1) were the major alkaloids of the above mentioned plants, these pentacyclic alkaloids are now referred to as "eburnamine-vincamine" type alkaloids. A recent review of the chemistry of this family is now available.[3]

The striking similarity between the arrangement of the "non-tryptophan or $C_{10}$" portion of vincamine (3) and the Aspidosperma alkaloid (like vincadifformine, 8) (see Figure 2) led Wenkert[4] to

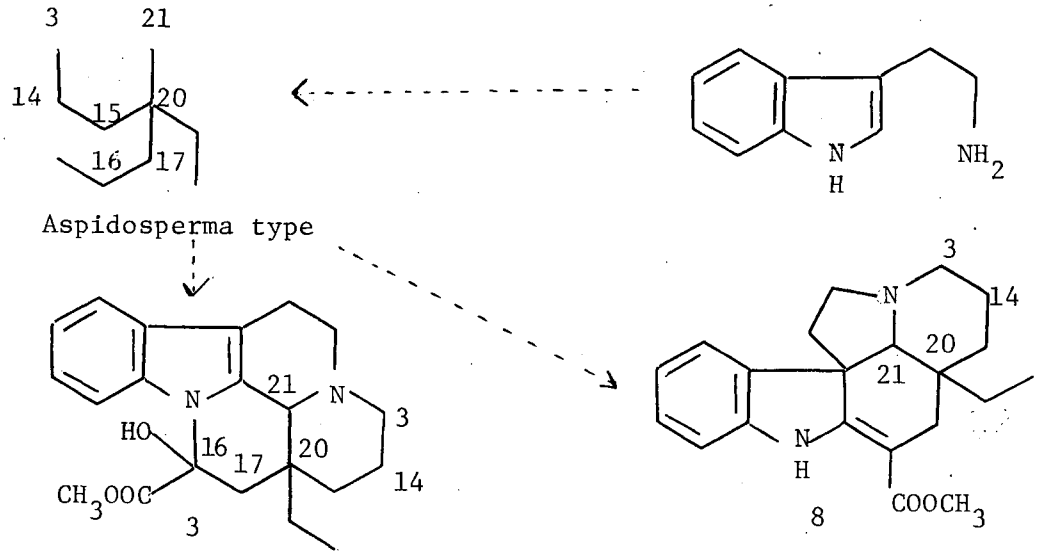



Figure 2. Scheme showing similar arrangement of non-tryptophan portion in vincamine and vincadifformine.

suggest that vincamine (3) is a rearranged Aspidosperma alkaloid. In attempting to bring this family into the main stream of the other groups (Corynanthe, Aspidosperma and Iboga) Wenkert advanced an

attractive mechanism which is shown in Figure 3. This rational would explain the biosynthesis of the entire family if it was assumed that

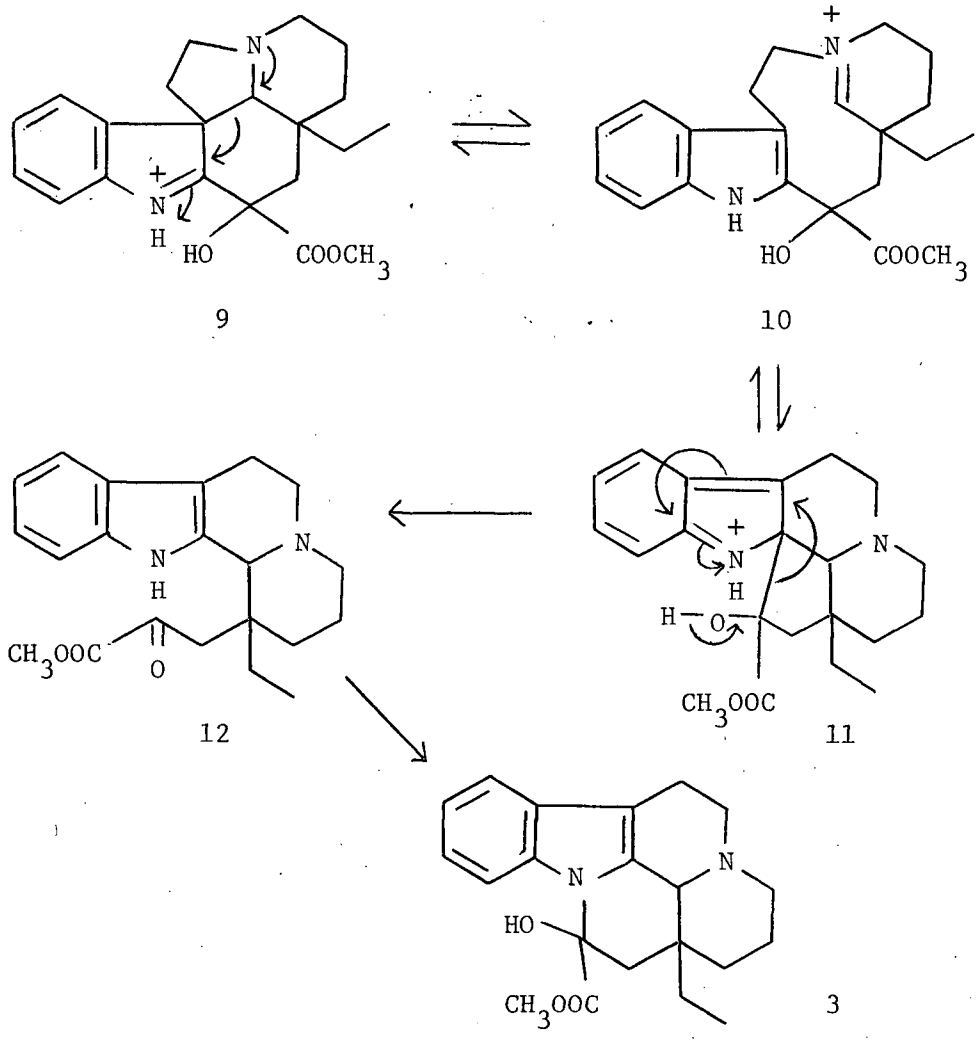



Figure 3. Wenkert's proposal for the rearrangement of Aspidosperma skeleton to vincamine.

vincamine (3) was the precursor of the alkaloids eburnamine (1), eburnamonine (2) etc. At a time when Wenkert put forward this proposal, the intermediate (12) had already been converted <u>in vitro</u> into vincamine (12-3, Figure 4)[5]. This latter conversion had another

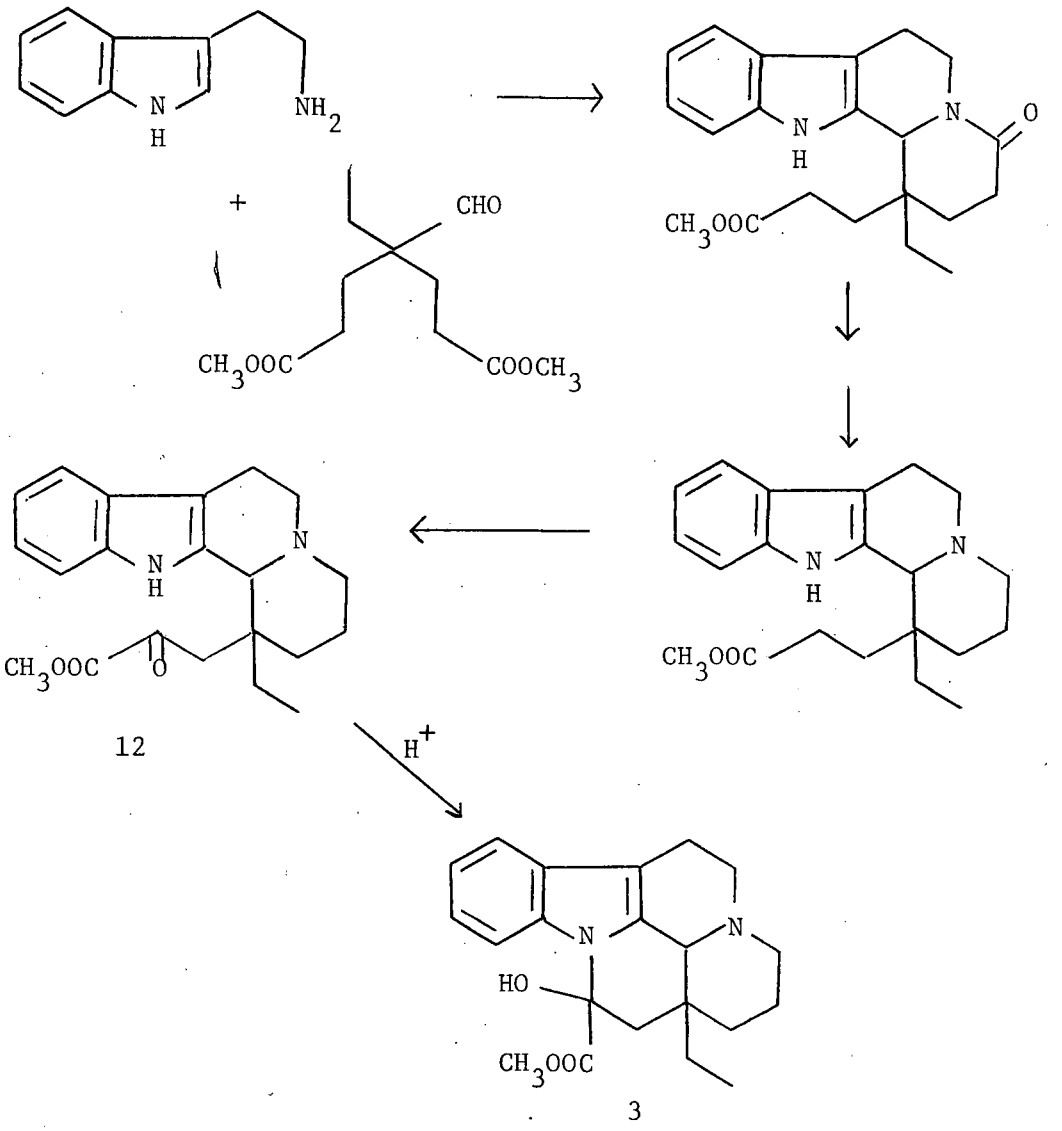
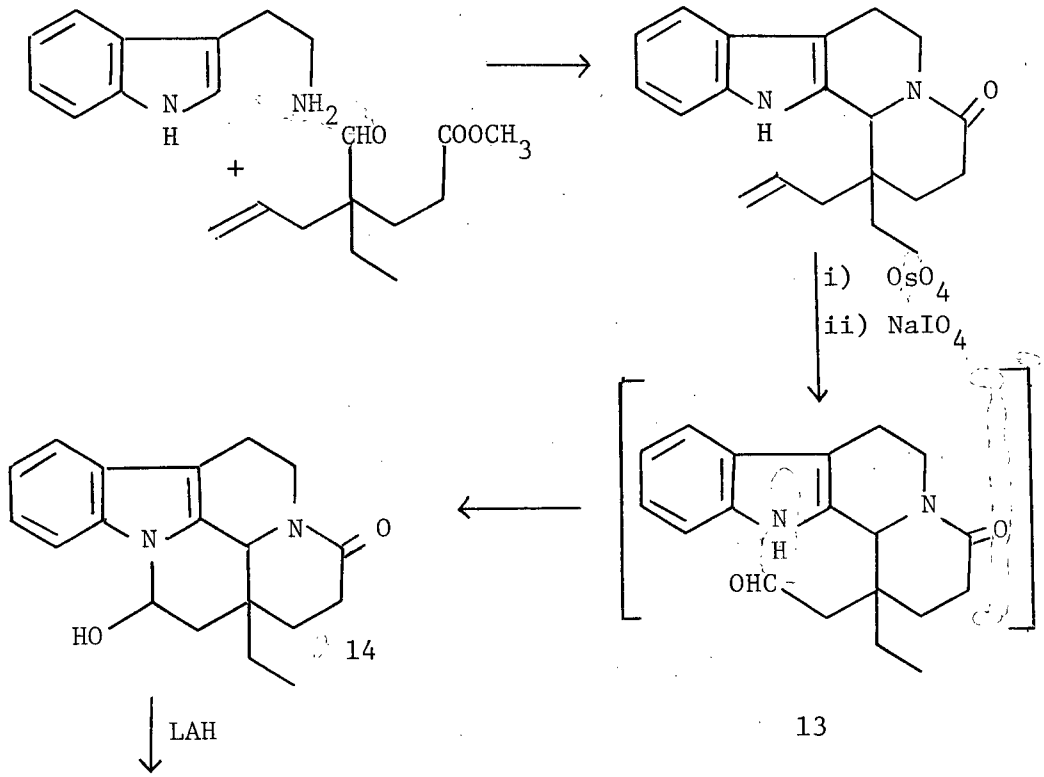



Figure 4. Synthesis of vincamine.

very close parallel in the synthesis of eburnamine (13-14, Figure 5)[6]. The laboratory syntheses however do not provide any real evidence for the biosyntheses of these alkaloids. It is the purpose of this portion of the thesis to present some preliminary studies which we hope will allow us to obtain some information in this direction.




13

14

dl-Eburnamine

Figure 5. Synthesis of eburnamine.

## DISCUSSION

In spite of the fact that the genesis of these pentacyclic alkaloids (Figure 1) was proposed several years ago, it is somewhat surprising that until now the biogenetic proposal has received no support from feeding experiments with radioactive precursors. Our previous experience with Vinca minor L. as already noted in the first section of this thesis stimulated us to initiate some biosynthetic studies in this direction.

Before describing the results of our experiments it is pertinent to call attention to the fact that it had already been established in our laboratory[7,8] that ring opening of the pentacyclic Aspidosperma type alkaloids to nine-membered intermediates (e.g. 9-10, Figure 3) and the transannular cyclization reaction (e.g. 10-11, Figure 3) are not significant biochemical reactions in Vinca rosea and Vinca minor plants. This study casts some doubt about some of the steps depicted in Wenkert's proposal. On the other hand there was no reason to doubt the possible validity of the other steps proposed.

While initiating the project we were faced with the problem as to which substrate we should select (Figure 3) for biosynthetic evaluation. It was indicated in the Introduction that (12), a bio-intermediate in Wenkert's proposal, has been converted into vincamine

(3) in vitro (Figure 4). We decided that the knowledge gained by evaluation of this type of transformation (12 → 3, Figure 4) in vivo while simulating the in vitro results would also be great value in suggesting the dynamics of the biosynthesis of vincamine (3). For this purpose a synthesis of a close relative (i.e. 24) of the proposed intermediate (12) was contemplated and the synthetic sequence is fully revealed in Figure 6. Our choice for making the compound (24) as our initial synthetic target was dictated by the fact that the proposed synthetic sequence was very short and it was easy to pursue the sequence from commercially available starting materials (3-acetylpyridine and tryptophol). It was assumed that the model compound (24) was capable of transformation in vivo to the putative intermediate (12) via biologically feasible reactions.

Before starting the synthetic sequence outlined in Figure 6, the following pilot route (Figure 7) on model piperidines was investigated. This work was undertaken to obtain optimum conditions for most of the reactions to be utilised later in the sequence in Figure 6. Accordingly 3-acetylpyridine (15) was converted into the known ketal (16),[10] and this compounds was treated with methyl iodide in ether to afford the crystalline salt (25) in 95% yield. Catalytic hydrogenation of the quaternary salt (25) yielded the piperidine ketal (26) which on acid hydrolysis furnished N-methyl-3-acetylpiperidine (27). The structure assigned was derived from the following spectral data. A sharp peak in the infrared (1710 $cm^{-1}$) and a three proton singlet in the nmr ($\tau$ 7.85) was reconcilable with the presence of a methyl ketone where as a three proton singlet for the N-methyl occurred in the expected

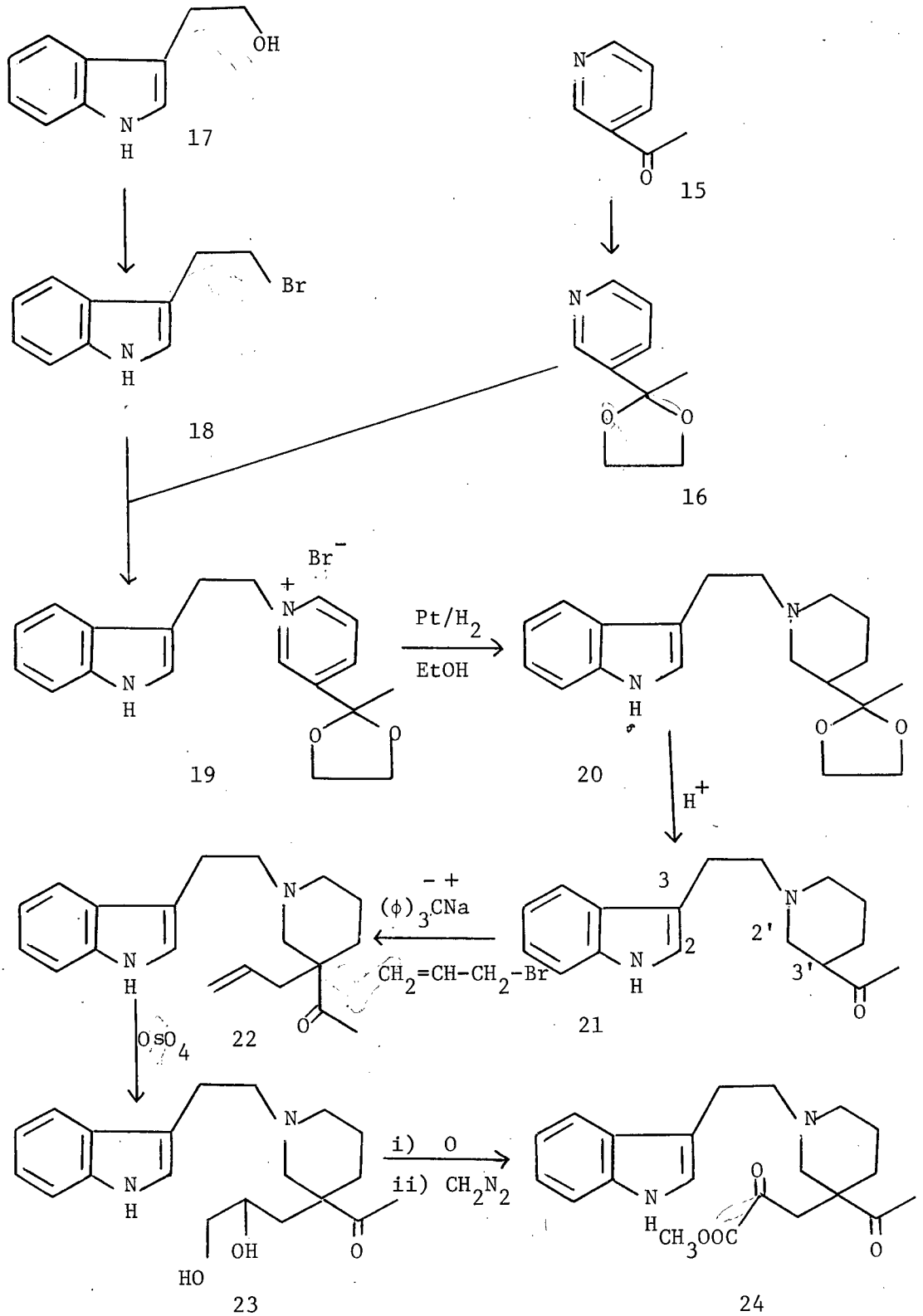



Figure 6. Proposed synthesis of intermediate 24.

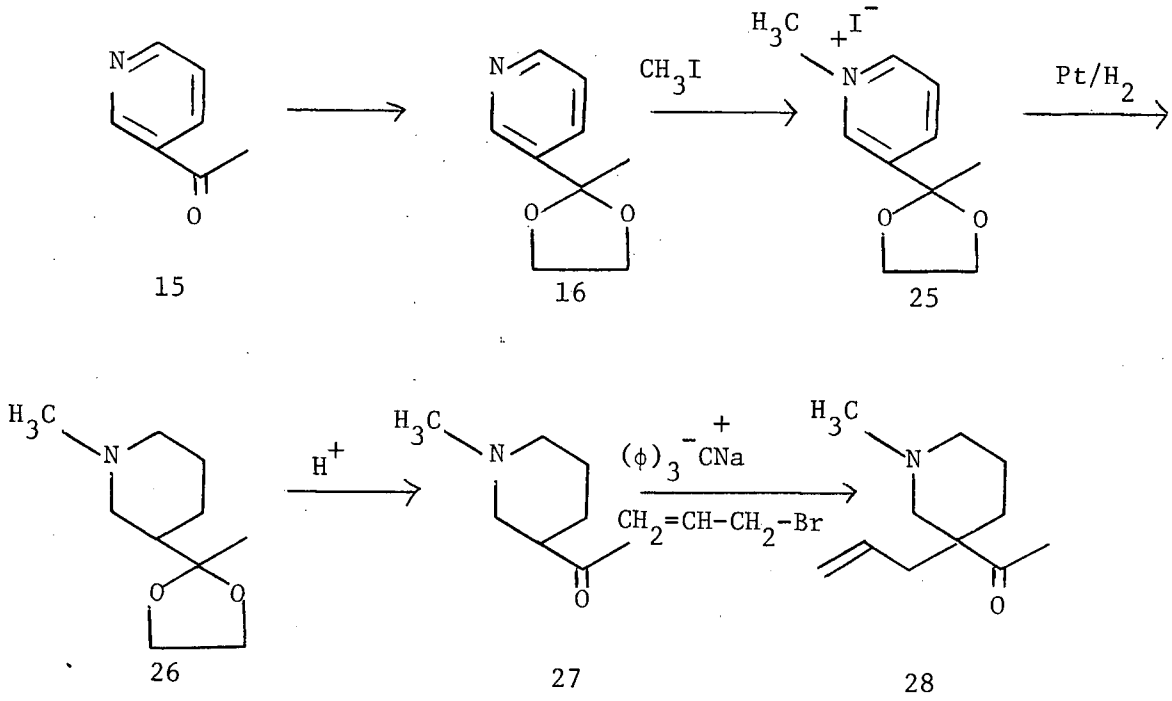



Figure 7. Synthesis of some model piperidine systems.

region (τ 7.72). Finally the molecular formula, $C_8H_{15}NO$, was confirmed by elemental analysis.

Attempts were now made to alkylate the ketone (27) using equivalent amounts of trityl sodium and allyl bromide. The chromatography of the crude product on alumina allowed the separation of two major components. The desired compound (28), eluted first from the column, indicated the following spectral data. In the nmr spectrum (Figure 8) the newly incorporated olefinic protons ($-CH_2-\underline{CH}=\underline{CH}_2$) were represented by a multiplet in the region τ 4.20-5.18 where as the three proton singlets for the methylketone and the N-methyl groups were still located in the expected region (τ 7.88 and 7.81). The molecular formula, $C_{11}H_{19}NO$, was confirmed by elemental analysis.

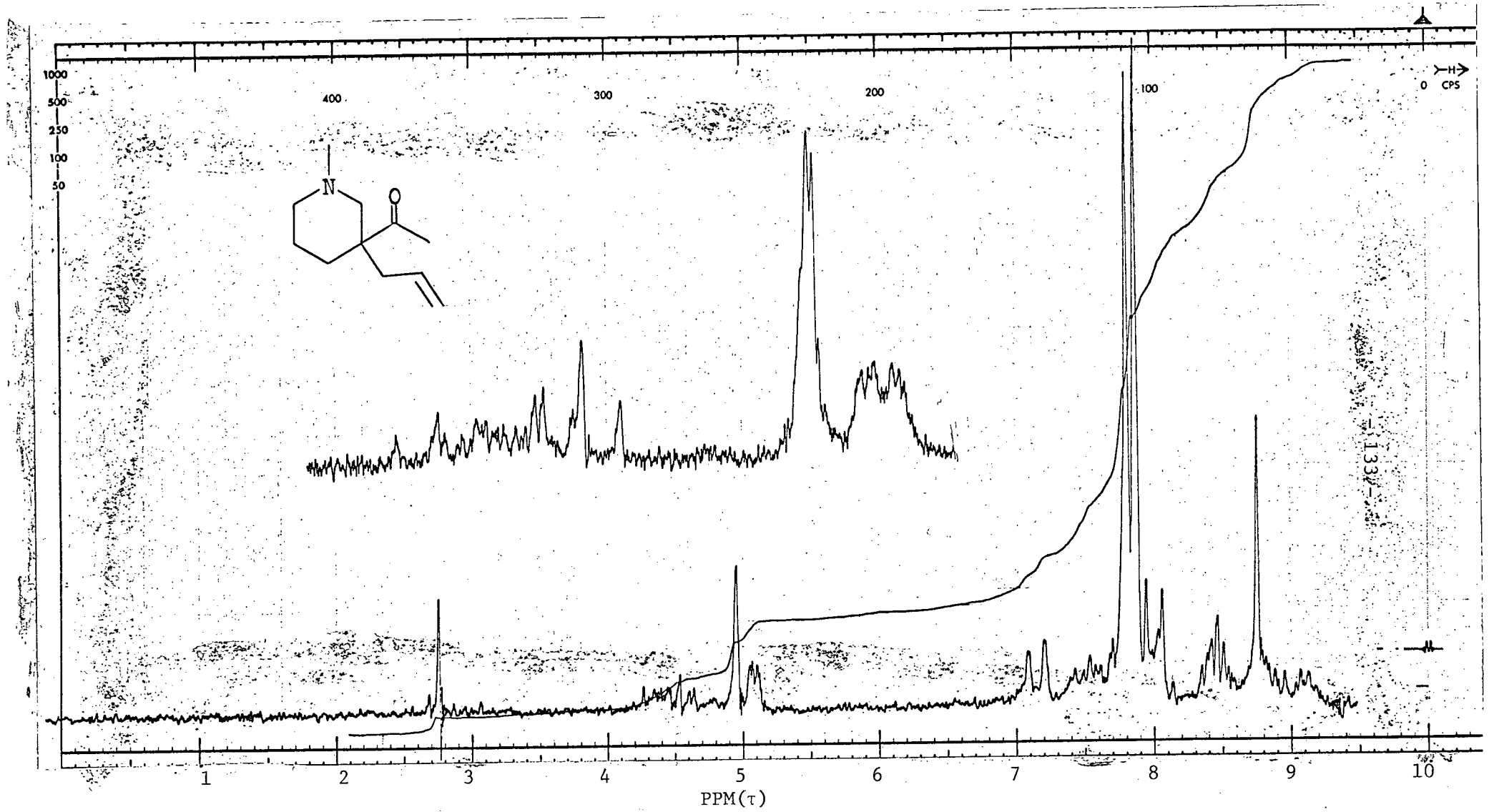
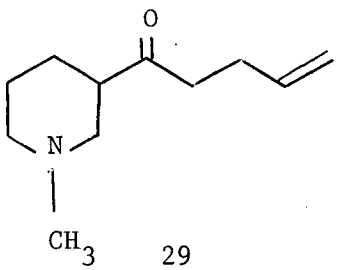



Figure 8. Nmr spectrum of 28.

The nmr spectrum (Figure 9) of the other compound isolated above showed the disappearance of the singlet corresponding to the methyl ketone group. With this result it immediately became obvious that in this compound the alkylation had occurred at the methyl group of the methyl ketone. Since the multiplet in the olefinic region integrated for three protons ($-CH_2-\underline{CH}=\underline{CH_2}$), the possibility that this was a dialkylated material was dismissed. Onthe basis of this limited information the compound has been tentatively assigned structure (29). Further work is necessary before a more definite structure could be put forth.




29

Encouraged by the success on the model compounds we started the sequence in Figure 6. The synthesis of the carbon skeleton present in (24) was facilitated by the reported synthesis of the ketone (21).[9] Although this synthesis was reported, the experimental procedure of some of the steps were not completely clear and the first attempts to repeat the synthesis met with certain minor difficulties. However these problems were quickly eliminated and the experimental procedures followed in the present work are in accord with the sequence indicated in Figure 6. The ketalization of 3-acetylpyridine (15) proceeded to give a good yield (78%) of the ketal (16).[10] Tryptophyl bromide (18)

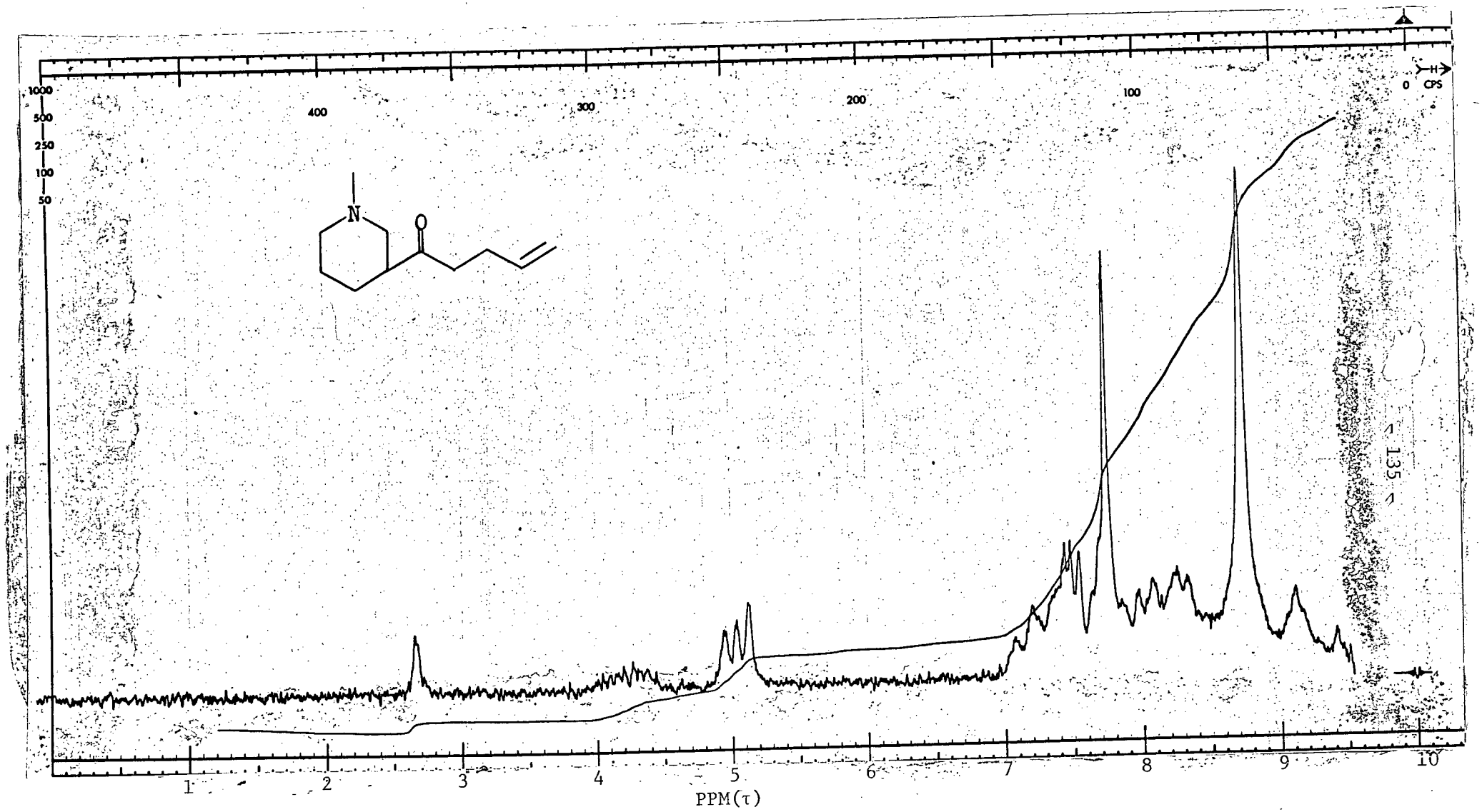



Figure 9. Nmr spectrum of 29.

was obtained from tryptophol (17) in 80% yield and was used immediately (owing to the instability of 18) for reaction with ketal (16) to give the salt (19) in quantitative yield. The salt was reduced catalytically over platinum oxide. The crude reduced product (20) on acid hydrolysis followed by chromatography on alumina and crystallization afforded the pure ketone (21), mp 132.5-134°. The spectral data of the ketone compared favourably with the assigned structure. A sharp peak in the carbonyl region of the infrared spectrum (1704 $cm^{-1}$) and a three proton singlet in the nmr ($\tau$ 7.91) were diagnostic for the presence of the methyl ketone in (21). The molecular formula, $C_{17}H_{22}N_2O$, was confirmed by mass spectrometry ($M^+$ 270).

In an attempt to alkylate the ketone (21), the anion of the ketone was made by means of trityl sodium and the anion so formed was immediately quenched with allyl bromide. The crude product on chromatography on alumina afforded the desired compound (22) in 30% yield. For analytical purposes a small amount of material was further purified by sublimation at 150°/0.05 mm. Supporting evidence for the assigned structure was derived from the following spectral data. The prominant feature of the nmr spectrum (Figure 10) in comparison to the nmr of the ketone (21) was the appearance of a multiplet in the olefinic region ($\tau$ 4.40-5.20, 3H, $-CH_2-\underline{CH}=\underline{CH}_2$) while the signals for the methyl ketone ($\tau$ 7.93) and the indolic-$\underline{NH}$ ($\tau$ 1.93) were still located at the same regions as they were in the ketone (21). This latter observation was a vital piece of evidence in indicating that the reaction has indeed taken the desired course. However final confirmation for the structure (22) came from mass spectrometry. In the mass spectrum (Figure 11) the

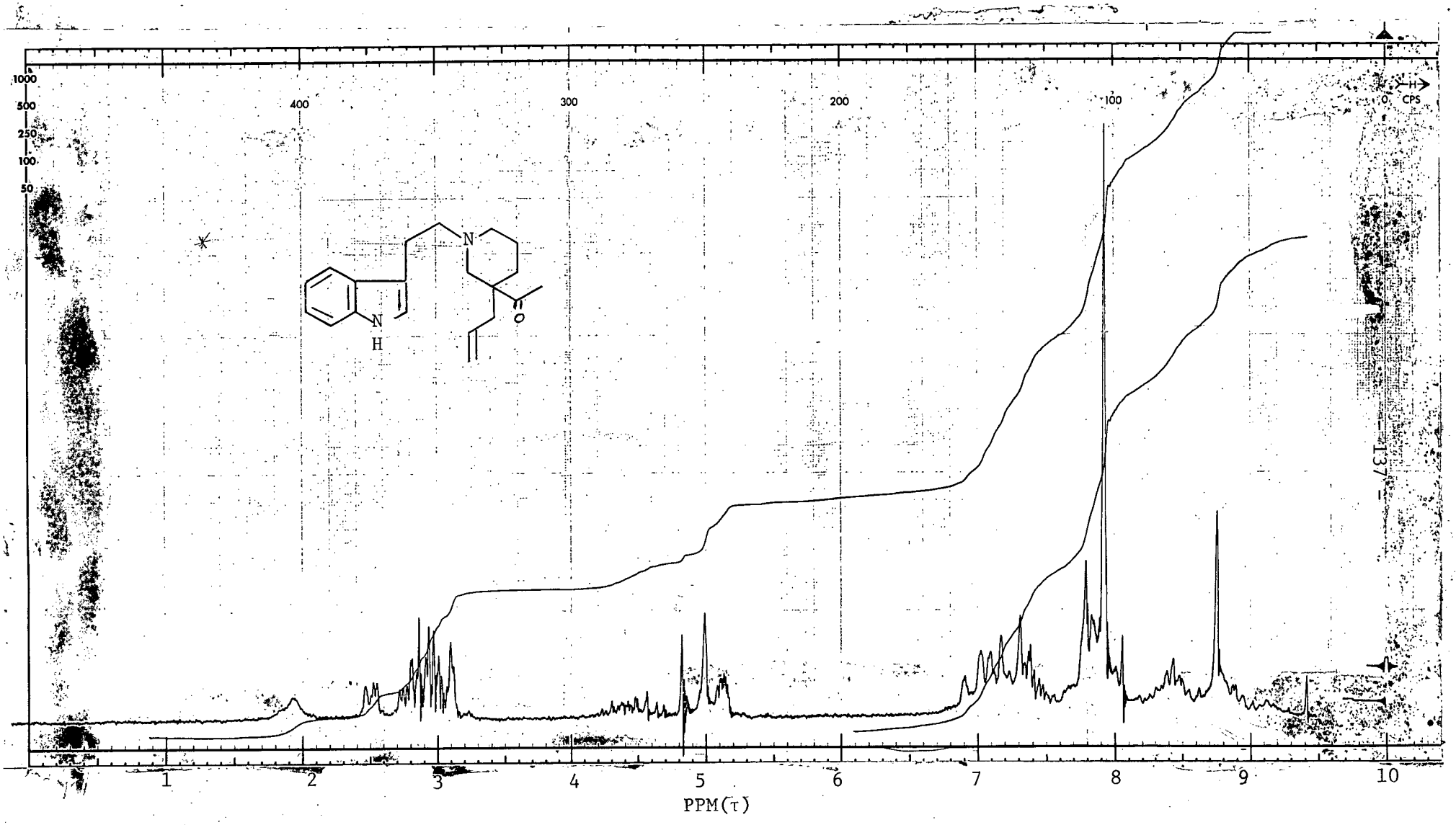



Figure 10. Nmr spectrum of olefin 22.

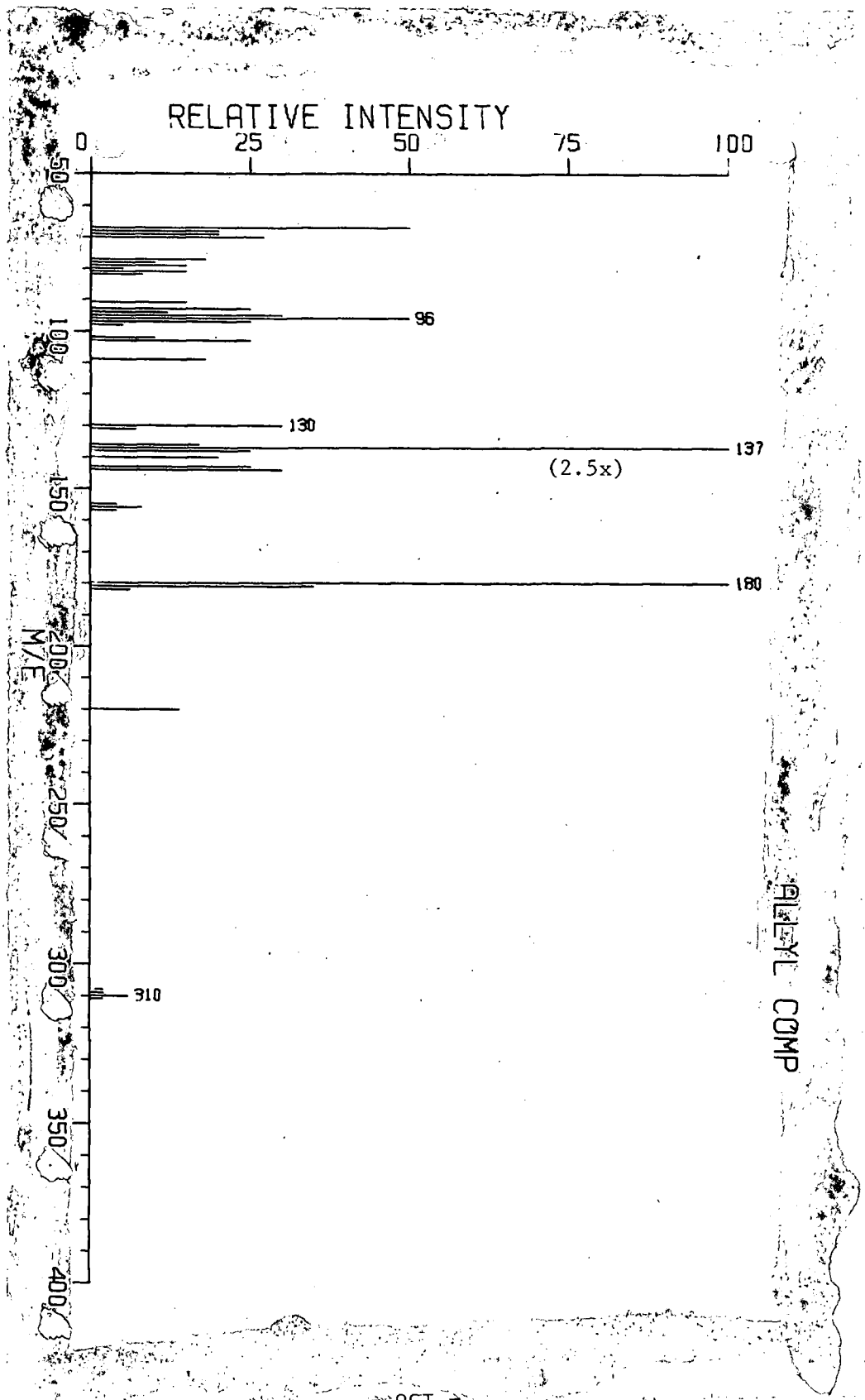



Figure 11. Mass spectrum of olefin 22.

olefin (22) indicated a molecular ion peak at m/e 310. This was in agreement with the molecular formula, $C_{20}H_{26}N_2O$. As to be expected the parent ion of (22) fragmented to the ions 30 (m/e 130) and 31 (m/e 180). Furthermore ion (31) readily lost the acetyl side chain to

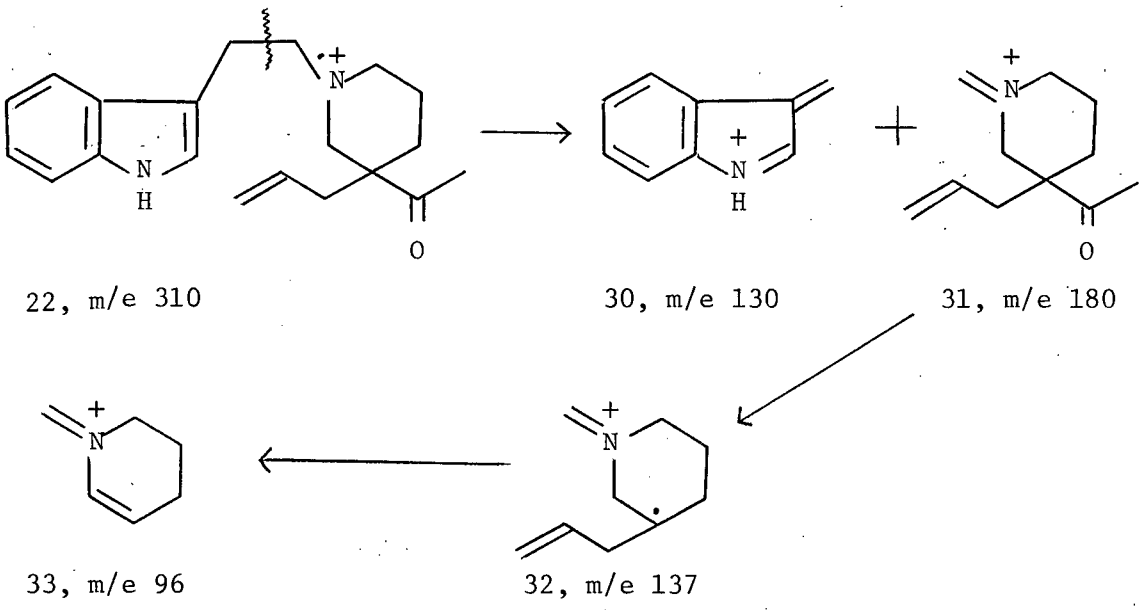
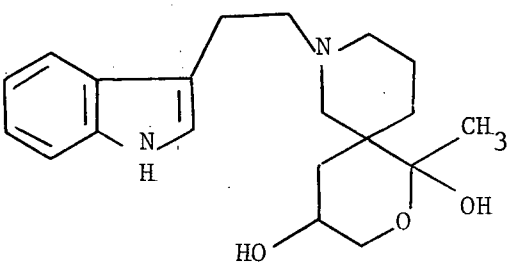



Figure 12. Postulated fragmentation of 22 in the mass spectrometer.

give the radical ion 32 (m/e 137). This latter ion further lost the allyl side chain to give the ion 33 (m/e 96, metastable peak at m/e 67). A scheme portraying the mass spectrometric fragmentations has been summarized in Figure 12. In spite of repeated attempts, the olefin (22) did not give the correct elemental analysis.

With the completion of the basic carbon skeleton, it was now considered appropriate to hydroxylate the allylic double bond in (22). Of the many reactions available for this purpose, osmylation enjoys a reputation for selectivity and was favoured a priori in the present

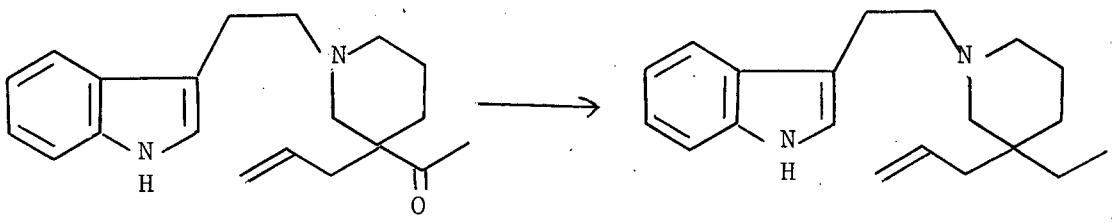
connection. Therefore the olefin (22) was dissolved in dry tetrahydrofuran and treated with osmium tetroxide.[11] The mixture was left in the dark at room temperature for 2 days. When the reaction mixture was worked up, unfortunately we observed a considerable loss of material. The crude product isolated represented a recovery of only 50% of the starting olefin (22). However the crude product was chromatographed on alumina. The small quantities of pure material so obtained in this particular investigation allowed only infrared and nmr spectral determinations. The spectral data immediately revealed that the above compound was not the desired diol (23). For example there was no evidence for the presence of a methyl ketone. The most interesting feature of the nmr spectrum was the finding there was no absorption in the olefinic region and instead a two proton multiplet and a three proton singlet were located at $\tau$ 6.5 and $\tau$ 8.64 respectively. These two signals being reconcilable with the presence of a hydroxymethylene group and a O-C-$\underline{CH}_3$ system suggested a tentative assignment of structure (34) to the above compound. However further work is necessary before a definite structure can be established.




34

This unexpected cyclization of the diol (23) to the hemiacetal (34) made it undesirable to continue the sequence as outlined in

Figure 6. It therefore became apparent that to obviate the above difficulty it was necessary to reduce the acetyl group in the olefin (22) to the corresponding ethyl group (i.e. 22-35) prior to osmylation of the olefinic linkage. In this manner the eventual completion of the



22 35

synthesis of intermediates bearing the desired skeleton as portrayed in 24 could be envisaged. Unfortunately time did not permit me to carry this work any further at this time but it will be continued by other workers 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 a Perkin Elmer Model 21 and Model 137 spectrometers. Nuclear magnetic resonance (nmr) spectra were recorded in deuteriochloroform at 100 megacycles per second (unless otherwise stated) on a Varian HA-100 instrument and the chemical shifts are given in Tiers $\tau$ scale with reference to tetramethylsilane as the internal standard; multiplicity, integrated area and type of protons are indicated in parentheses. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution molecular weight determinations were carried out 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 reagent carbon tetrachloride-antimony pentachloride (2:1) or iodine vapors. Woelm neutral alumina (activity III) was used for column chromatography (unless otherwise stated).

3-Acetylpyridine ethylene ketal (16)[10]

A solution of 3-acetylpyridine (15, 60 gm), ethylene glycol (40 gm) and p-toluenesulfonic acid hydrate (105 gm) 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 was extracted with benzene. The combined extracts were washed with sodium bicarbonate solution water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Distillation gave the product (63.3 gm); bp 165/88 mm; nmr: $\tau$ 2.5 (multiplet, 4H, aromatic), 6.1 (multiplet, 4H, ketal), 8.35 (singlet, 3H, $C-CH_3$).

N-Methyl-3-acetylpyridinium iodide ethylene ketal (25)

A solution of methyl iodide (20 gm) in dry ether (50 ml) was added to a stirred ice-cold solution of 3-acetylpyridine ethylene ketal (16, 20 gm) in ether (50 ml). The reaction mixture was stirred at room temperature overnight and the precipitated salt, 25, was filtered (34.5 gm, 95% yield) and purified by recrystallization from methanol, mp 190°.

Anal. Calc. for $C_{10}H_{14}NO_2I$: C, 39.11; H, 4.60; N, 4.60. Found: C, 39.12; H, 4.86; N, 4.55.

N-Methyl-3-acetylpiperidine ethylene ketal (26).

The salt (25, 10 gm, 32.6 mmoles) was dissolved in a mixture of water and ethanol (100 ml, 1:1). This pale yellow solution was added dropwise to a suspension of hydrogen-activated platinum oxide

(800 mg) in ethanol (200 ml) and the mixture was hydrogenated at atmospheric pressure. The absorption of hydrogen was complete (about 2400 ml, 66 mmole) after 8 hours. The catalyst was filtered off and the solvent removed in vacuo to afford a light yellow solid. This product was dissolved in 10% sodium carbonate solution and was extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to afford to pale yellow oil (5.8 gm); $\nu_{max}^{film}$: no absorption in the aromatic region; nmr (60 mc/s): $\tau$ 6.1 (singlet, 4H, ketal), 7.74 (singlet, 4H, N-C$\underline{H}_3$ + $\underline{H}$(?)), 8.75 (singlet, 3H, C-C$\underline{H}_3$).

N-Methyl-3-acetylpiperidine (27)

The crude ketal (26, 5.8 gm) was dissolved in 2 N hydrochloric acid (50 ml) and the mixture was stirred at room temperature overnight. The solution was made basic with 10% sodium carbonate solution and extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The resulting oil was distilled under reduced pressure, bp 116-117°/14 mm, to give a clear oil (4.48 gm). The yield of the reaction for reduction and removal of the ketal was 90%; $\nu_{max}^{film}$: 1710 ($\nu$C=O); nmr (60 mc/s): $\tau$ 7.72 (singlet, 3H, N-C$\underline{H}_3$), 7.85 (singlet, 3H, -COC$\underline{H}_3$).

Anal. Calc. for $C_8H_{15}NO$: C, 68.01; H, 10.63; N, 9.93; O. 11.34. Found: C, 67.80; H, 10.61; N, 10.08; O, 11.50.

N-Methyl-3-acetyl-3-allylpiperidine (28)

A 50-ml three necked flask was equipped with a magnetic stirrer, a reflux condensor, a dropping funnel and a nitrogen inlet. The glassware was flame dried and then thoroughly flushed with dry nitrogen. To a solution of triphenylmethyl sodium (23 ml, 0.17 N, 0.0038 mole) was added dropwise a solution of the ketone (27, 0.540 gm, 0.0038 mole) in anhydrous ether (2 ml). The formation of the carbanion was indicated by the instant disappearance of the red color of the base. The mixture was stirred at room temperature for 1 hour. Allyl bromide (0.34 ml, 0.0039 mole) was taken up in anhydrous ether and added dropwise to the above pale yellow solution over a period of 5 minutes. During the addition of allyl bromide, sodium bromide started separating out and the mixture became cloudy. The mixture was stirred for an additional 20 minutes. Water (10 ml) was added and the layers were separated. The aqueous layer was extracted with ether. The two ether layers were combined and evaporated in vacuo. The light yellow semi solid was taken up in benzene and treated with 10% aqueous acetic acid (20 ml). The two layers were separated, and the benzene layer was washed twice with water. The combined aqueous layers were combined, made basic with 10% aqueous sodium carbonate solution and extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The resultant light yellow oil (0.450 gm) was chromatographed on alumina (40 gm). Elution with benzene-petroleum ether (1:9) afforded the desired compound (28, 0.105 gm), bp 125°/6 mm; nmr (Figure 8): $\tau$ 4.20-5.18 (multiplet, 3H, $-C\underline{H}=C\underline{H}_2$), 7.81 (singlet, 3H, N-$C\underline{H}_3$), 7.88 (singlet, 3H, -$COC\underline{H}_3$).

Anal. Calc. for $C_{11}H_{19}NO$: C, 72.85; H, 10.48; N, 7.72. Found: C, 72.83; H, 10.72; N, 7.55.

Further elution of the column with petroleum ether-benzene (1:1) provided another compound, 3-(5'-keto-1'-pentenyl)-N-methylpiperidine (29, 60 mg). Nmr (Figure 9): τ 4.10-5.2 (multiplet, 3H, $-C\underline{H}=C\underline{H}_2$) and 7.78 (singlet, 3H, $N-C\underline{H}_3$). The loss of singlet corresponding to the methyl ketone was suggestive of alkylation at the methyl group, but the evidence at the time was insufficient to assign a definite structure to this compound.

Tryptophyl bromide (18)[9]

A solution of phosphorus tribromide (1 ml) in ether (20 ml) was added to an ice-cold solution of tryptophol (17, 4.8 gm) in ether (250 ml). After 15 hours, the supernatant was decanted, washed with sodium bicarbonate solution, water and dried with sodium sulfate. Removal of the solvent yielded the product as white crystals (4.5 gm, 80%), mp 95-100° (lit. mp 90-95°).

N-[β-(3-Indolyl)-ethyl]-3'-acetylpyridinium ethylene ketal bromide (19).[9]

Tryptophyl bromide (18, 4.5 gm) and 3-acetylpyridine ethylene ketal (16, 10 ml) were heated at 80° under nitrogen for 8 hours. Addition of ether (40 ml) to the cooled reaction mixture yielded a precipitate whose crystallization from methanol afforded pure salt (19, 7.2 gm), mp 208-211° (Lit. mp 209-210°).

N-[β-(3-Indolyl)-ethyl]-3'-acetylpiperidine ethylene ketal (20)

The pyridinium salt (19, 7.0 gm) was dissolved in ethanol (250 ml). This yellow solution was added dropwise to a suspension of hydrogen-activated platinum oxide (1 gm) in ethanol (100 ml) and the mixture was hydrogenated at atmospheric pressure. The uptake of hydrogen was complete after 10 hours. The catalyst was filtered off and the solvent removed *in vacuo* to afford a yellow gum. This was dissolved in 10% aqueous sodium carbonate solution and extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated to afford the piperidine ketal (20) as a light yellow gum.

N-[β-(3-Indolyl)-ethyl]-3'-acetylpiperidine (21)

The crude piperidine ethylene ketal (20, 7.5 gm) was dissolved in methanol (150 ml). The solution was acidified with 4 N HCl (100 ml) and the mixture was heated at 85° for 5 hours. After cooling to room temperature, methanol was evaporated from the reaction mixture. The resultant gum was made basic with sodium bicarbonate solution and extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated. The resulting gum (6.1 gm) was chromatographed on alumina (400 gm). Elution with benzene-petroleum ether (1:1) and benzene afforded the desired ketone (21). This was crystallised from benzene-petroleum ether (4.33 gm), mp 132.5-134°; $\nu_{max}$ ($CHCl_3$): 3400 (νN-H), 1704 (νC=O) $cm^{-1}$; nmr: τ 1.68 (singlet, 1H, N-H), 2.40-3.12 (multiplet, 5H, indole protons), 7.91 (singlet, 3H, $-COCH_3$); mass spectrum: $M^+$ 270; main peaks: m/e 140, 130, 103.

N-[β-(3-Indolyl)-ethyl)-3'-acetyl-3'-allylpiperidine (22)

A 500-ml three necked flask was equipped with a magnetic stirrer, a reflux condensor, a dropping funnel and an nitrogen inlet. All the glassware was flame dried and then thoroughly flushed with dry nitrogen. To a solution of the piperidine ketone (21, 4.00 gm, 0.014 mole) in dry tetrahydrofuran (150 ml) was added dropwise a solution of triphenylmethyl sodium until the red color of the base just stayed in the reaction mixture (150 ml, 0.2 N, 0.030 mole). The solution was stirred at room temperature for about 5 minutes and then allyl bromide (1.2 ml, 0.014 mole) in dry tetrahydrofuran (15 ml) was added to the above solution. The reaction mixture was stirred at room temperature for 2 hours and then evaporated to dryness. The residue was extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was dissolved in a small amount of benzene and put on a column of alumina (250 gm). Elution with benzene-petroleum ether (1:1) furnished the desired compound (22, 1.4 gm, yield 30%). For analytical purposes a small amount of allyl compound was sublimed at 140-150°/0.05 mm; $\nu_{max}(CHCl_3)$: 3367 (νN-H), 1701 (νC=O) $cm^{-1}$; nmr (Figure 10): τ 1.93 (broad singlet, 1H, indole-NH), 2.40-3.20 (multiplet, 5H, indole protons), 4.40-5.20 (multiplet, 3H, $-\underline{CH}=\underline{CH}_2$), 7.93 (singlet, 3H, $-COCH_3$); mass spectrum (Figure 11): $M^+$ 310; main peaks: m/e 180, 137, 130, 96, 67.

Osmylation of the olefin (22)

Osmium tetroxide (48 mg, 0.25 mmole) in dry purified dioxane (3 ml) was added to the olefin (22, 50 mg, 0.16 mmole) in the same solvent

(2 ml). The solution was left at room temperature for 48 hours and then saturated with hydrogen sulfide gas. The black precipitate was filtered off and the dioxan solution was evaporated to dryness under reduced pressure. The residue (13 mg) was dissolved in a small amount of benzene and put on a column of alumina (1 gm, activity IV). Elution with ether-methanol (95:5) afforded a very polar compound. Spectral data indicated that this was not the desired diol (23). $\nu_{max}$ ($CHCl_3$): no absorption in the carbonyl region; nmr: $\tau$ 1.8 (broad singlet, 1H, indole N-H), 2.40-3.20 (multiplet, 5H, indole protons), 6.50 (multiplet, 2H, $-CH_2O-$) and 8.64 (singlet, 3H $-O-C-CH_3$).

On the basis of this spectral data, the polar compound isolated above has been tentatively assigned structure 34. Further work is necessary before a definite structure can be established.

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