STUDIES ON THE BIOSYNTHESIS, DEGRADATION AND SYNTHESIS OF OLIVACINE-ELLIPTICINE TYPE INDOLE ALKALOIDS

BY

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June, 1975

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ABSTRACT

Part I of this thesis describes the isolation of representatives of a class of indole alkaloids, lacking the 3-β-ethylamino side chain, from two plant sources *Aspidosperma australe*, and *Aspidosperma vargasii*. A preliminary investigation of the biosynthesis of several of these compounds was conducted in *Aspidosperma vargasii*. From crude extracts of *Aspidosperma australe* the pyridocarbazole alkaloids olivacine (16) and guatambuine (25) were isolated. From *Aspidosperma vargasii* uleine (18), apparicine (19), desmethyluleine (85) and the pyridocarbazoles 9-methoxyolivacine (82) and guatambuine (25) were isolated. Aromatic tritium labelled tryptophan (27) and stemmadenine (13) were shown to be incorporated into 9-methoxyolivacine (82) and tryptophan (27) was also incorporated into guatambuine (25) in *Aspidosperma vargasii*. Neither precursor was incorporated into uleine (18).

In part II a degradation scheme was developed for the isolation of the C-1 methyl, C-2 methyl(N-methyl) and C-3 methylene groups of the "D" ring of the olivacine (16) and ellipticine (17) systems. Both ellipticine (17) and olivacine (16) were converted to their N-methyl tetrahydro derivatives guatambuine (25) and N-methyltetrahydroellipticine (26) *via* formation of the methiodide salts of 16 and 17 followed by reduction with sodium borohydride. Compounds 25 and 26 were converted to their corresponding methiodides 86 and 95 and reacted under Hofmann reaction conditions. Olefins 88 and 97 were obtained from guatambuine methiodide (86) and olefin 102 was obtained from 95. Olefins 88 and 102 were reacted with ozone and the formaldehyde produced was isolated as the bisdimedone derivative.

The C-2 vinyl compound 97 was elaborated into the C-3 vinyl compound 112 by hydrogenation of 97 to 103, formation of the methiodide 111 and reaction of 111 with sodium hydride in dimethylformamide.

The methiodides 86 and 95 were also ring opened to 89 and 107 by reaction with lithium aluminum hydride. These compounds were in turn converted to their methiodides 90 and 108 and reacted with potassium t-butoxide in t-butanol. The trimethylamine produced during the reactions was isolated as the tetramethylammonium iodide salt. The efficiency of the N-methyl group isolation was determined by degrading (N-^{14}C methyl)-guatambuine methiodide (86) and N-methyltetrahydroellipticine methiodide (95) via the lithium aluminum hydride ring-opening sequence.

Guatambuine (25) was also ring-opened to a C-3 vinyl derivative 125 by reaction with acetic anhydride and sodium acetate.

Part III was concerned with the synthesis of olivacine (16). Two approaches were developed; in sequence A the reaction of tryptophyl bromide (207) with methylacetoacetate (205) gave 3-carbomethoxy-5-(3-indolyl)-2- pentanone (204). Cyclization of 204 led to an equal mixture of 1-methyl-2-carbomethoxycarbazole (134) and 1-methyl-2-carbomethoxy-1,2,3,4-tetrahydrocarbazole (209) formed by disproportionation of the initially formed 208. Dehydrogenation of the mixture of 134 and 209 over Pd/C gave 134. The carbazole ester 134 was also obtained directly from 204 by cyclization in the presence of chloranil as the hydrogen acceptor. Compound 134 was reduced to the alcohol 157 with lithium aluminum hydride and the alcohol 157 was oxidized to the aldehyde 152 with Jones reagent. The aldehyde 152 was converted to olivacine (16) and guatambuine

(25) by a known procedure.

In sequence B., when 9-benzyltetrahydrocarbazole (217) was reacted under Vilsmeier-Haack conditions 1-methyl-3-formyl-9-benzylcarbazole (219) was formed. Compound 219 was elaborated to the aminoacetal 224 by two routes; condensation with aminoacetaldehyde diethylacetal (171) led to the imine acetal 221 which was alkylated with methylmagnesium chloride to give 224. Alternatively 219 was alkylated to give the α-hydroxyethyl carbazole 222 which was converted to its corresponding acetate 223. The acetate group was displaced by aminoacetaldehyde diethylacetal (171) to give 224. The cyclization of 224 to 6-benzo-olivacine (225) followed by debenzylation to olivacine (16) was not attempted, however the conditions necessary for the cyclization have been worked out for the synthesis of the closely related molecule, ellipticine (17).

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INTRODUCTION PART 1

The plant kingdom plays a paramount role in both the existence and maintenance of all animal life on this planet. The existence of the present life-sustaining oxygen-rich atmosphere is considered in large part to be a consequence of the photosynthetic production of oxygen by primitive plant forms during the early stages of the development of the earth.[1,2,3] This same process, harnessing the power of the sun has sustained life by enabling plants to produce consumable energy-containing compounds vital to the function of the animal organism.

A considerable number of these consumable plants have been found over the ages to be beneficial to man in a medicinal or maintenance manner, aiding in his fight against the diseases which threaten the longevity of his life. The use of such herbal remedies to cure ailments is generally considered to precede even the origins of agriculture. Accounts of their reported use are found in the inscriptions and writings of the ancient Egyptian, Babylonian and Chinese civilizations. Rivalled perhaps only by the Ayurvedic medicine in India, Chinese herbal medicine is the oldest continuous surviving tradition in their culture. The SHANG-HAN LUN ("Treatise on Fevers") written by Chang Chung-Ching (ca A.D. 195) is a classic of Chinese clinical medicine right down to the present day.[4]

In modern times the scientific world has come to view folklore and tribal medicines with considerable interest. Ignoring the shrouds of mystic and

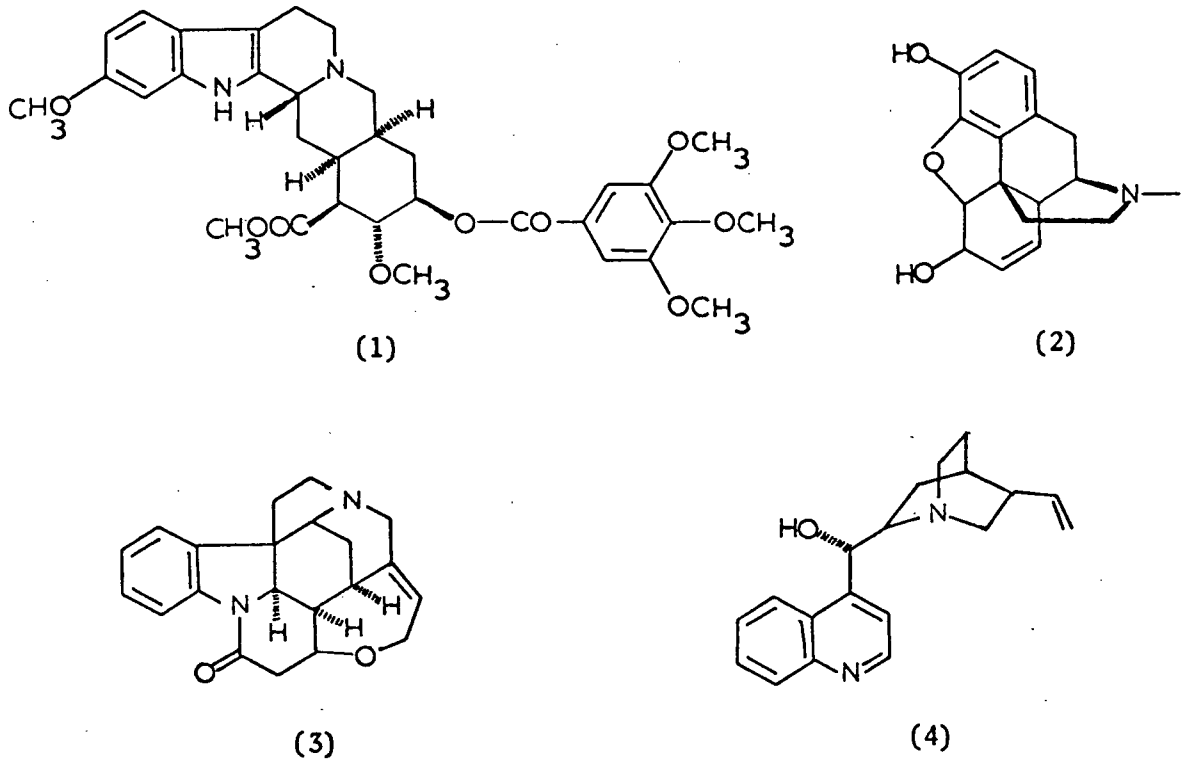


Figure 1. Compounds of Major Pharmacological Interest.

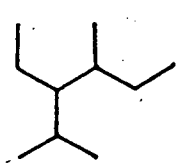
considering the possible curative effects of these herbal extracts, it has been realized that many possess pharmacological activity. Such investigations have often led to the isolation of the biologically active component. This work has been spurred on by such medically important discoveries as: Reserpine (1) for the treatment of mental disorders, isolated from the Indian snake root plant Rauwolfia serpentina; morphine (2) an analgesic from the opium poppy Papaver somniferum; strychnine (3) the cardiac principle and stimulant from Strychnos nux vomica; and quinine (4) an antimalarial from Cinchona bark (Figure 1).

These land mark discoveries have provided the incentive for the presently active and intense worldwide investigation of the plant kingdom for compounds of possible medicinal value. The subsequent efforts towards the synthesis of these

molecules has been the foundation of the modern drug industry. Although a tremendous number of synthetically prepared drugs are in use today, it is estimated that approximately 50% of the medicines prescribed are still derived from natural plant sources.[5,6]

It was recognized early in the investigations that some indole alkaloids possess beneficial medicinal properties. They have been found to occur widely among flowering plants particularly of the Apocynaceae, Loganiaceae, and Rubiaceae plant families.[7] These three families stand close together in the phylogenetic charts of the taxonomists.[8] The family Apocynaceae is particularly rich in alkaloids, especially the genera Rauwolfia, Vinca (Catharanthus), Alstonia and Aspidosperma.

The great majority of complex indole alkaloids can be visualized to consist of a tryptamine unit and a C_9-C_{10} unit which is monoterpene derived. Approximately 1000 indole alkaloids are known to date.[9] These compounds in general offering minor variations in functionality, oxidation state and stereochemistry lend themselves to a formal division into four main groups according to their skeletal features. These four groups, the corynanthe (5), strychnos (5), aspidosperma (6) and iboga (7) are represented by three distinct skeletal arrangements of the carbon atoms in the C_9-C_{10} unit as illustrated below.



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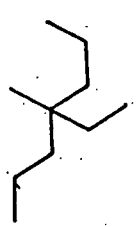

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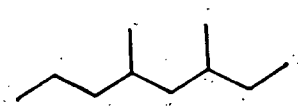


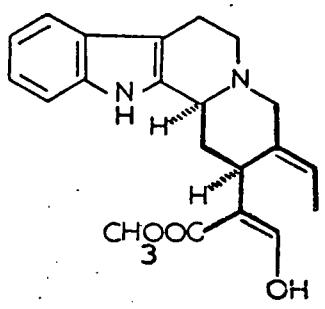
(7)

Examples of these groups are the ring closed forms: geissoschizine (8), preakuammicine (9), vindoline (10) and catharanthine (11) and the corresponding ring opened forms: picraphylline (12), stemmadenine (13), vincadine (14) and 18-carbomethoxycleavamine (15) (Figure 2).

As a result of an intensive search of the Apocynaceae family for both biological and taxonomical purposes, it has been found that many plants of the genera Aspidosperma and Ochrosia contain complex alkaloids possessing novel structures.[7,9,10] These compounds are exemplified by the structures of olivacine (16), ellipticine (17), uleine (18), apparicine (19) and vallesamine (20). They are novel in that they do not possess the 3-β-ethylamino side chain (tryptamine bridge) indigenous to the majority of naturally occurring indole alkaloids. It can be seen that uleine (18), apparicine (19), and vallesamine (20), have only a single carbon atom separating the 3-position of the indole nucleus from the basic nitrogen (Nb), whereas, olivacine (16), and ellipticine (17), being substituted 6H-(4,3-b)-pyrido carbazoles[11*] exhibit a three carbon bridge (Figure 3).

In terms of formality, these compounds can be considered to possess the corynanthe-strychnos skeleton 5, however by virtue of their almost exclusive presence in Aspidosperma plants, they are considered to be aspidosperma alkaloids. They are generally found to co-occur in many of these plant systems and are not, therefore, simply isolated examples of biological peculiarities. They are also found to co-occur with a small number of normal tryptamine bridged alkaloidal systems such as N-acetylaspidospermidine (21), aspidocarpine (22), N-acetyl-11-hydroxyaspidospermatidine (23), and 11-methoxy-4,19-dihydrocondylocarpine (24)[10] (Figure 3).

* Two numbering systems have been used to number the pyridocarbazole skeleton. The system used to label this system and the Uleine (18) and apparicine (19) skeletons in this thesis was that accepted by Chem. Abstracts (Ref. 11).



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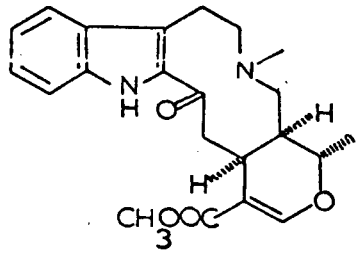

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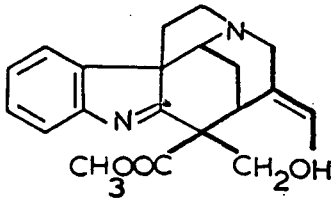

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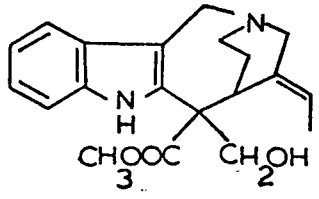

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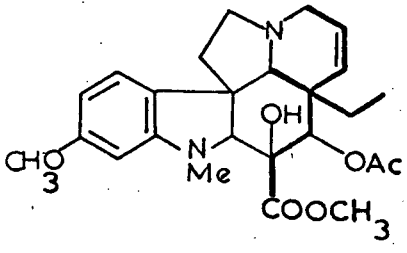

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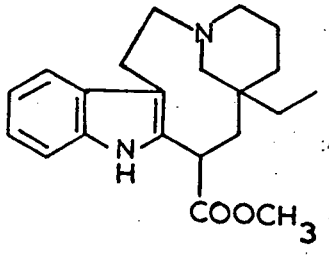

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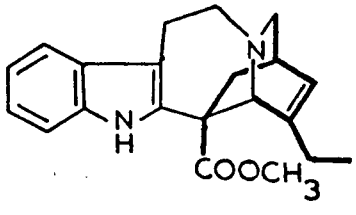

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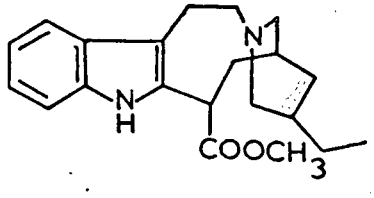

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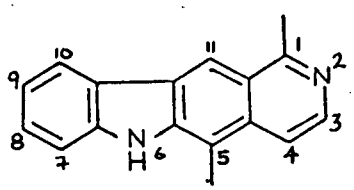
Figure 2. Some Representative Indole Alkaloids.



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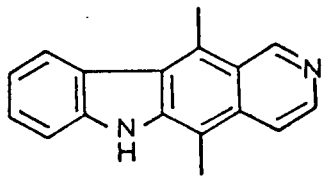

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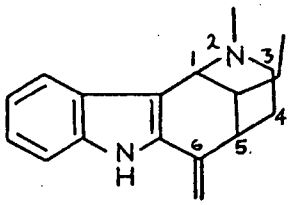

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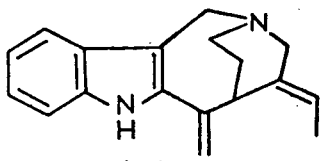

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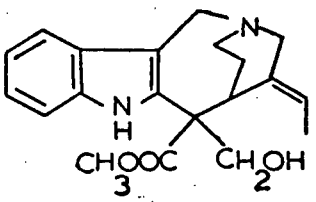

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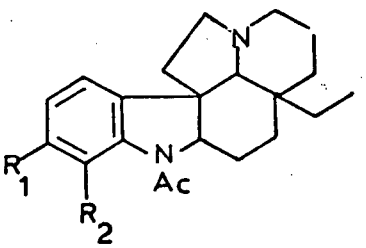
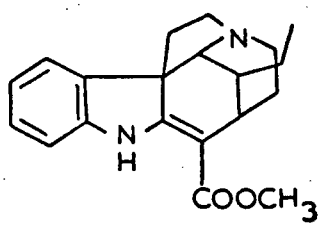


(21) $R_1=R_2=H$

(22) $R_1=OCH_3$, $R_2=OH$



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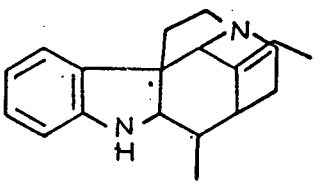


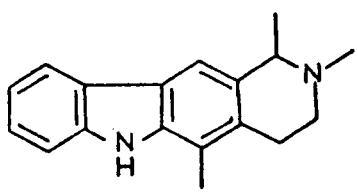
(23)

Figure 3. Indole Alkaloids Occurring in Genus Aspidosperma.

In the latter two cases it is tempting to envisage the build-up of the uleine skeleton by extension of the two carbon tryptamine bridge.[114]

Both olivacine (16) and ellipticine (17) have received considerable interest in recent years as a result of their antitumor activity, however our interest in these compounds relates to their biosynthetic build-up in the plant system. At the initiation of the present work, the occurrence of these compounds posed the important questions: What is the mechanistic pathway of their in vivo synthesis and is there some connection between their occurrence and that of the normal tryptamine bridged alkaloids found in the plant. In other words, are these alkaloids closely related in terms of the presently accepted indole alkaloid biosynthetic scheme? The answers to these questions, it was hoped, would be found through a study of the biosyntheses of these compounds in Aspidosperma plants.

A considerable number of reviews have been published concerning different aspects of indole alkaloid biosynthesis. A detailed discussion of the present state of the biosynthesis and occurrence of this class of compounds is contained in the recent reviews by Schmid[12] and Cordell[13]. As a consequence, therefore, only a brief overview of the biosynthesis of indole alkaloids will be discussed in this presentation with emphasis on the present knowledge concerning the biosynthesis of olivacine (16), ellipticine (17), uleine (18), apparicine (19), and the N-methyltetrahydro derivatives of the pyrido carbazoles, guatambuine (21) and N-methyltetrahydroellipticine (22).



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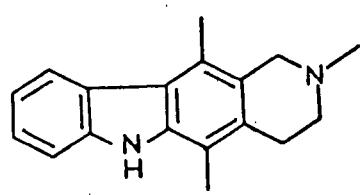

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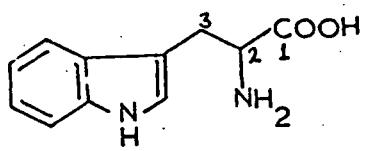
There is general agreement that the tryptamine segment of the indole alkaloids is derived from tryptophan (27), in accord with the postulate of Pictet[14] in 1906. With the development of tracer techniques in the last decade, this hypothesis has received ample justification.[15-18] Labelled tryptophan (27) has been found to be successfully incorporated into a large variety of indole alkaloids. Tryptamine, (28) however, the other logical



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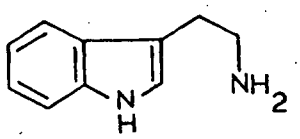


(28)

precursor has been incorporated with mixed success.[18-20] The exact nature of this result is difficult to interpret, it could suggest that the decarboxylation is delayed in some cases, or that tryptamine (28) is not transported to the site of biosynthesis as is the amino acid.

The biogenetic origin of the remaining C_9-C_{10} unit, in contrast to the source of the β-aminoethylindole moeity, was the subject of much controversy. The earliest speculations as to the origin of this non-tryptophan unit were based upon the structural similarities of indole alkaloids.[20]

Prior to the first tracer experiments concerned with the origin of this unit, there were three main postulates in existence. The original Barger-Hahn[21-2] postulate had been elaborated by Robinson[23] and Woodward[24-6] to the point where it could accommodate the formation of alkaloids of the strychnos and corynanthe, as well as the original yohimbe, alkaloid skeletons as shown in figure 4. The combined hypothesis involved the condensation of 3,4-dihydroxyphenylacetaldehyde (29) derived from tyrosine with tryptamine (28) either at the 3-position of the indole system to yield strychnine (3), or at the 2-position

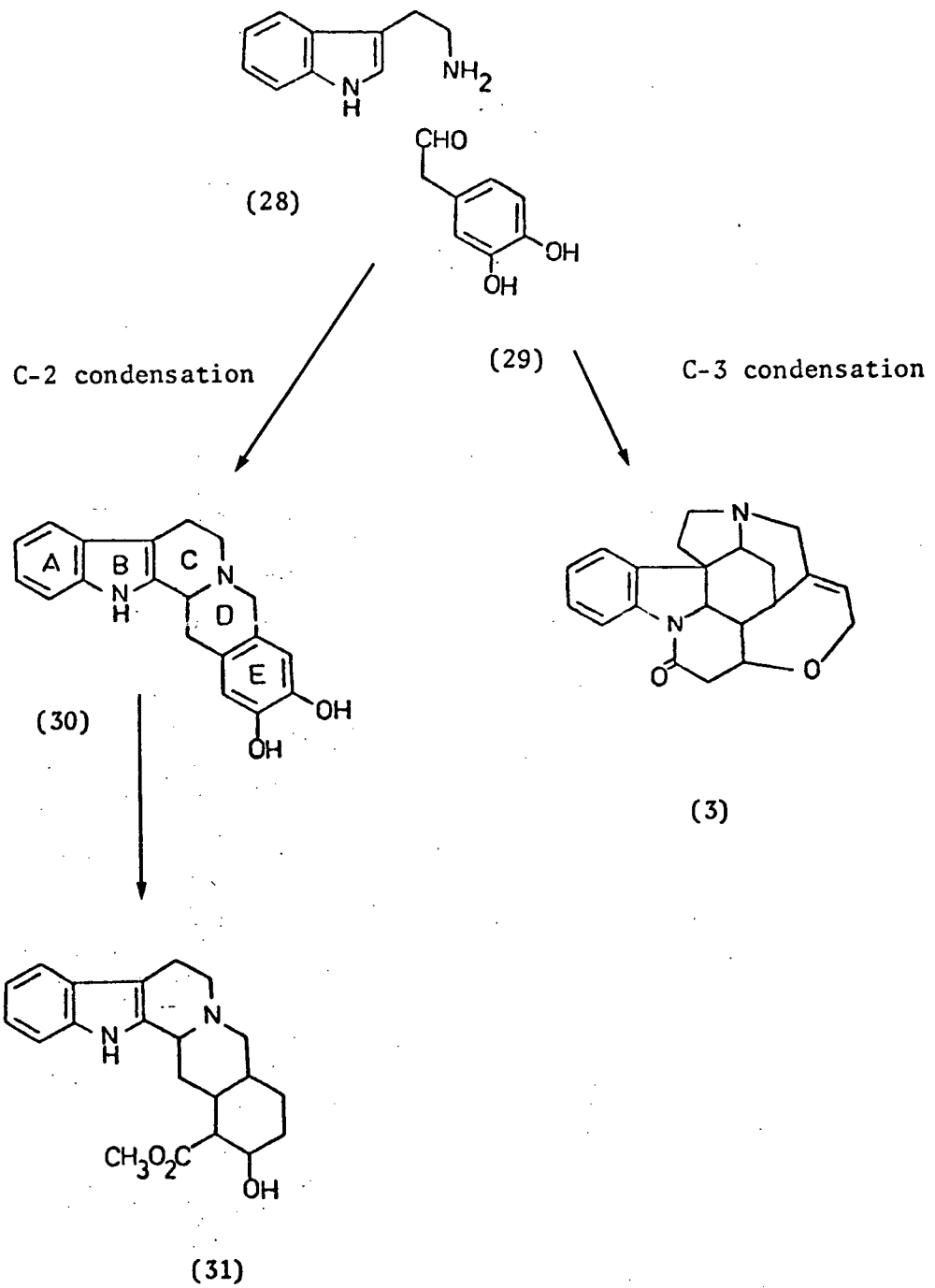


Figure 4. The Barger - Hahn - Robinson - Woodward Hypothesis.

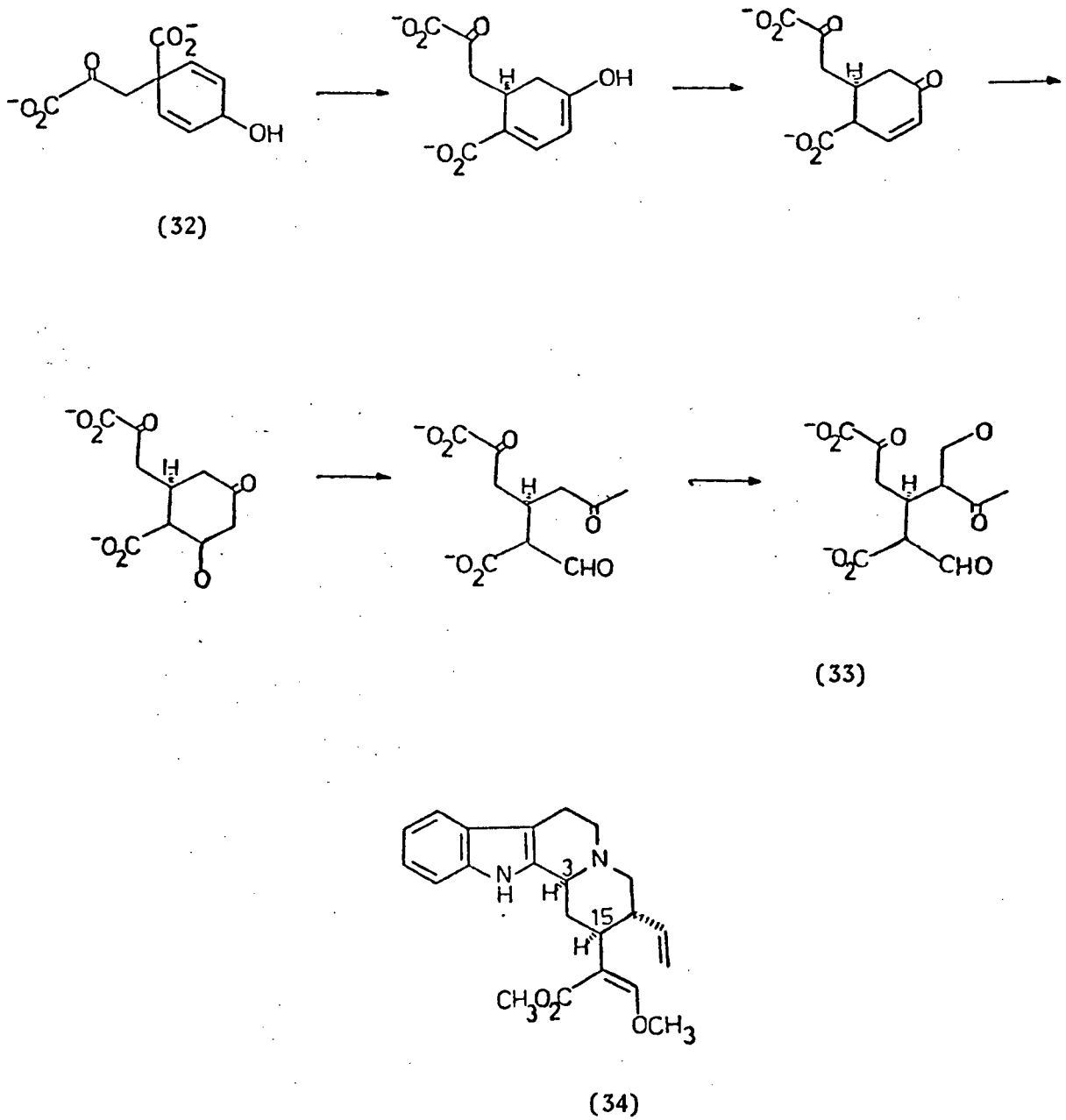


Figure 5. The Wenkert Prephenate Postulate.

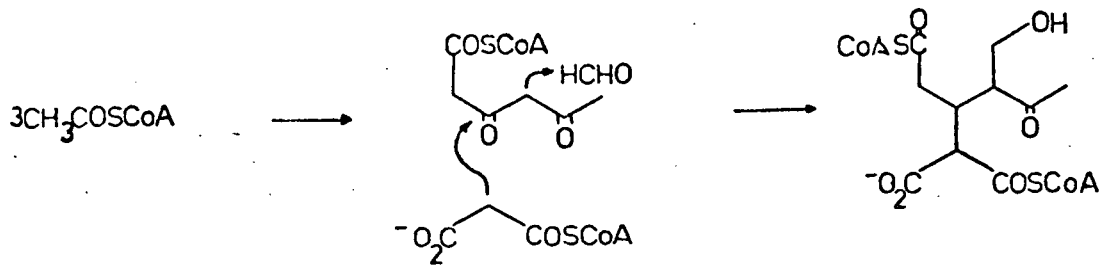


Figure 6. The Acetate Postulate.

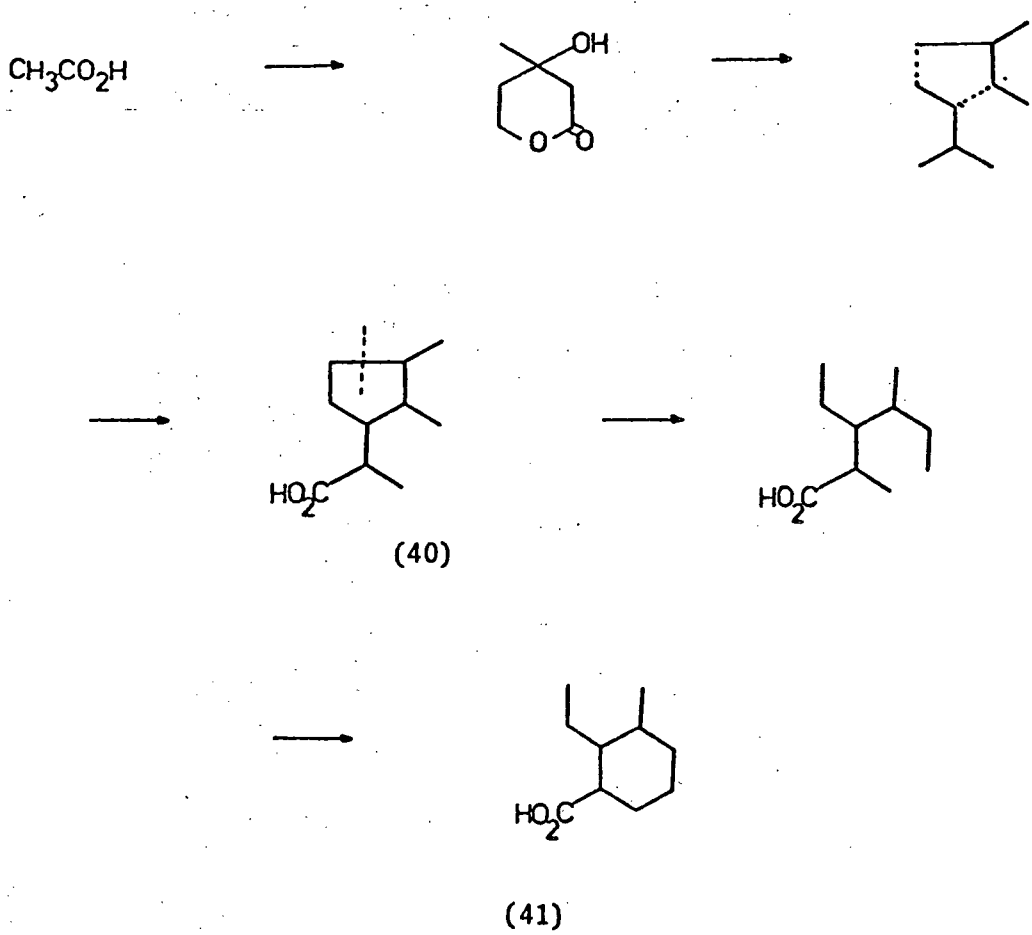


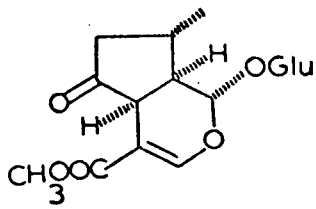
Figure 7. The Thomas-Wenkert Monoterpene Postulate.

to yield 30 which contains an aromatic ring E. Fission of the aromatic ring E between the two hydroxyl groups and subsequent combination with appropriate C_1 units leads to yohimbine (31).

A number of deficiencies were immediately evident in these speculations and in 1959 Wenkert and Bringi[27] proposed an elegant alternative. They initially proposed that a hydrated prephenic acid was the intermediate, but later Wenkert[28] modified this hypothesis so that prephenic acid (32) itself was the direct progenitor of the indole alkaloids. The latter rearranges according to the scheme shown in figure 5 to afford a crucial intermediate, the seco-prephenate-formaldehyde (SPF) unit (33) which can be elaborated into yohimbine (27) and corynantheine (34). This ingenious alternative scheme uses the alicyclic precursors of phenylalanine directly to account for the oxidation state of the E ring. A key step in this hypothesis, the 1,2-migration of the pyruvate side chain of prephenic acid with retention of configuration, explains the absolute configuration at C-15 in corynanthe-strychnos alkaloids.[29] The carbomethoxy group is an integral part of the hydroaromatic progenitor instead of being attached whenever necessary.

Schlittler and Taylor[30] in 1960 and Leete[31-33] in 1961 postulated that the non-tryptophan portion of the indole alkaloids was derived via the acetate pathway. The suggestion utilized a six carbon chain derived from three acetate units which condensed with malonic acid and a one carbon unit (biologically equivalent to formaldehyde) yielding the desired C_{10} unit (Figure 6).

At this time the structure elucidation of a number of cyclopentane monoterpenic glucosides (iridoids) had been achieved,[34-37] exemplified by verbenalin (35), genipin (36), aucubin (37), and asperuloside (38).



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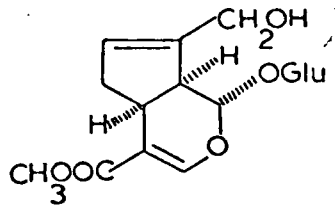

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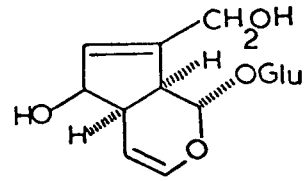

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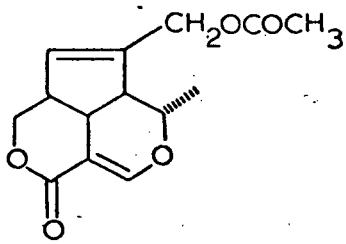

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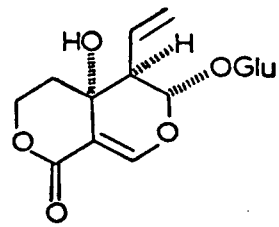


(39)

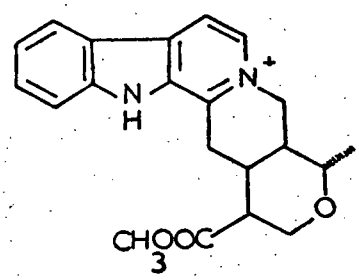
Realizing that these glucosides, and in particular the seco-cyclopentane unit of swertiamarin (39) had the corynanthe-strychnoslike skeleton 5 as well as the same stereochemistry in the appropriate position, as C-15 in the alkaloids[29], and that the carbomethoxy function (or derivative thereof) also appeared at the corresponding position, led Wenkert[28] and simultaneously and independently Thomas[38] to propose a monoterpenoid based hypothesis for the origin of the C_{9-10} unit (Figure 7).

The key intermediates in their pathway are the cyclopentanoid unit 40 having the corynanthe skeleton 5 and its cyclic form 41 analgous to swertiamarin (39). The unit 41 is derived intact without the need for an additional carbon from formaldehyde or glycine as required by the earlier hypotheses.

The cyclopentanoid monoterpenes genipin (36) and aucubin (37) have a related biosynthetic pathway and have been shown to co-occur with the alkaloids in the genus Strychnos[38].

The initial biosynthetic experiments using radioactive precursors disproved all the hypotheses concerning the genesis of the C_9-C_{10} unit.[39,40] However, further experiments by Battersby and co-workers with (2-^{14}C)-mevalonate afforded low incorporations of activity into the ubiquitous non-tryptophan unit.[40,43] These results were rapidly confirmed by two other research groups working with the plant species *Vinca rosea* and *Vinca major*.[41,42] The Thomas-Wenkert monoterpene postulate consequently became widely accepted.

More experiments by these same workers utilizing specifically labelled mevalonates coupled with degradative data demonstrated the intact incorporation of this unit into the representative alkaloids catharanthine (11), vindoline (10), ajmalicine (44), serpentine (42) and perivine (43).[43-6] Subsequently it was shown that geraniol pyrophosphate (45)[44,46-52] and nerol[44,50] (46) could also



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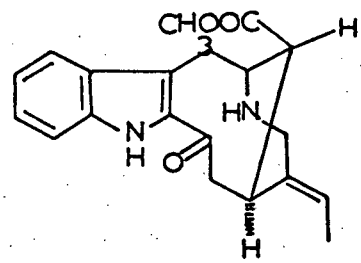


(43)

serve as precursors of these alkaloids (Figure 9). In addition, deuterium labelled mevalonolactone fed to *V. rosea* produced alkaloids whose mass spectral fragmentation patterns substantiated the radioactive label findings.[47-8]

From these experiments a pattern emerged for the rearrangement of the monoterpene unit into the three skeletal units 5, 6, and 7 for each of the alkaloidal

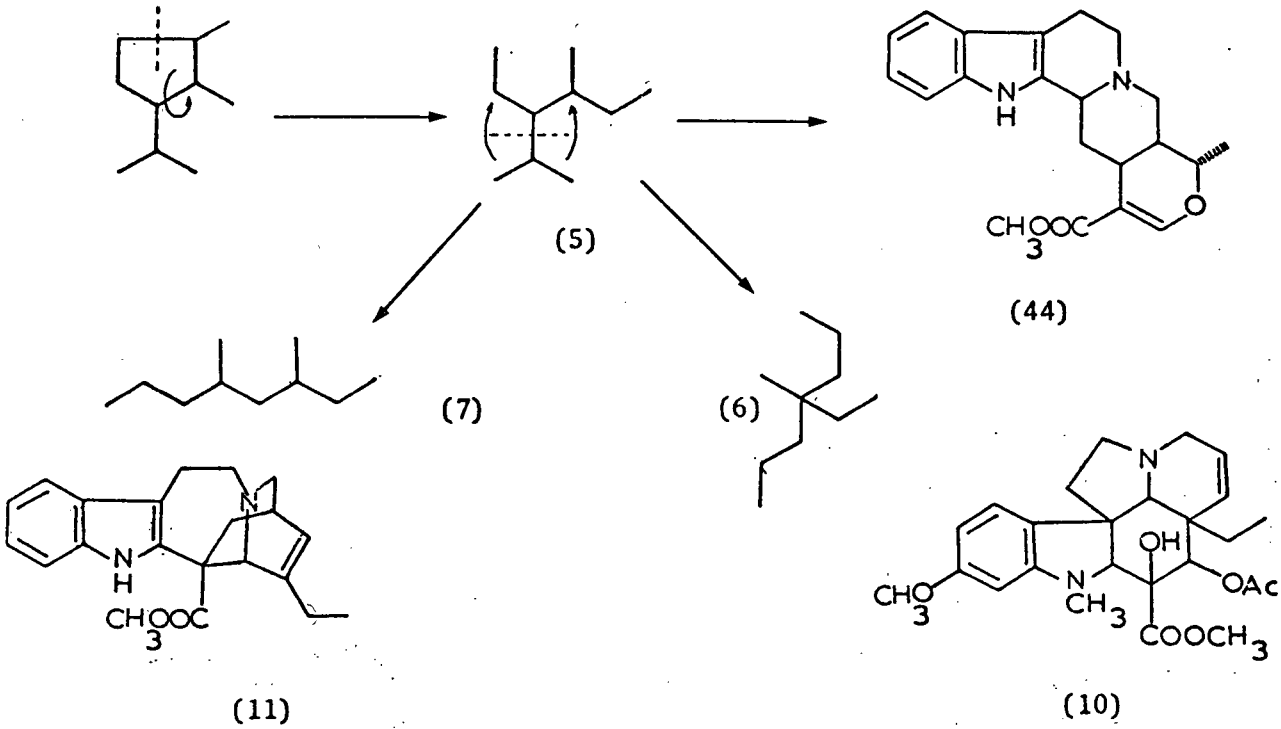


Figure 8. Rearrangement of Monoterpene Unit into the Three Alkaloid Skeletons

families, which was consistent with the position of the radiolabel in the various isolated alkaloids, (Figure 8). It appeared as though the corynanthe skeleton was formed first during the biosynthesis and it subsequently rearranged by the manner shown to the aspidosperma (6) and iboga (7) skeletons. It was not known however, how this process occurred in the plant or whether there were terpenoid glycosides, as yet not isolated, having skeleta of types 6 and 7 which independently reacted with tryptamine (28).

With the monoterpene hypothesis clearly substantiated, an elegant series of experiments by Battersby and co-workers determined the exact nature of the sought after cyclopentanoid intermediate. The iridoid compounds genipin (36) and verbenalin (35) and others proposed by Wenkert[28] were found not to be incorporated into any of the alkaloids studied.[50,53] However a very similar monoterpene loganin (49) exhibiting essentially the correct oxidation level and stereo-

chemistry anticipated for the non-tryptophan precursor was found to be specifically incorporated into the alkaloids catharanthine (11), vindoline (10), ajmalicine (44), serpentine (42) and perivine (43) in V. rosea[53-5], as well as into the alkaloids in Rauwolfia serpentina[55], and Cephaelis ipecacuanha.[64] Furthermore, through isotopic dilution studies it was shown that loganin (49) co-occurred with the indole alkaloids in V.rosea[53-4,57] and Strychnos nux vomica.[57] It has since been shown that loganin (49) occurs in many alkaloid containing plants.[13]

The isoprenoid origin of loganin (49) was subsequently demonstrated by feeding specifically labelled forms of mevalonate, geraniol, and nerol to V.rosea[53-4,56-8], M. trifoliata[54-5,59-61], S. nux vomica[57-8,62], and S. carliensis.[62-3]

Battersby demonstrated also the incorporation in V. rosea of 10-hydroxygeranial and 10-hydroxynerol (47) into both loganin (49) and the aforementioned alkaloids[65-6] (Figure 9). Randomization of the label at the positions marked 2,6 indicated that oxidation at both of these carbons is a necessary part of the sequence to deoxyloganin (48).

Deoxyloganin (48) was found to be a constituent of V. rosea and S. nux vomica and was shown to be a precursor of loganin (49) and the indole constituents of the former plant system.[67-8]

The cleaved monoterpene derivative of loganin (49), secologanin (50) was isolated from V. rosea and found once more to be specifically incorporated into the alkaloids in the plant. Loganin (49) was shown also, to be a precursor of this compound.[69]

An interesting observation is that the central intermediate of Wenkert's prephenic acid hypothesis,[28] the SPF unit (33) is almost identical in structure, stereochemistry and oxidation level to the critical monoterpene intermediate

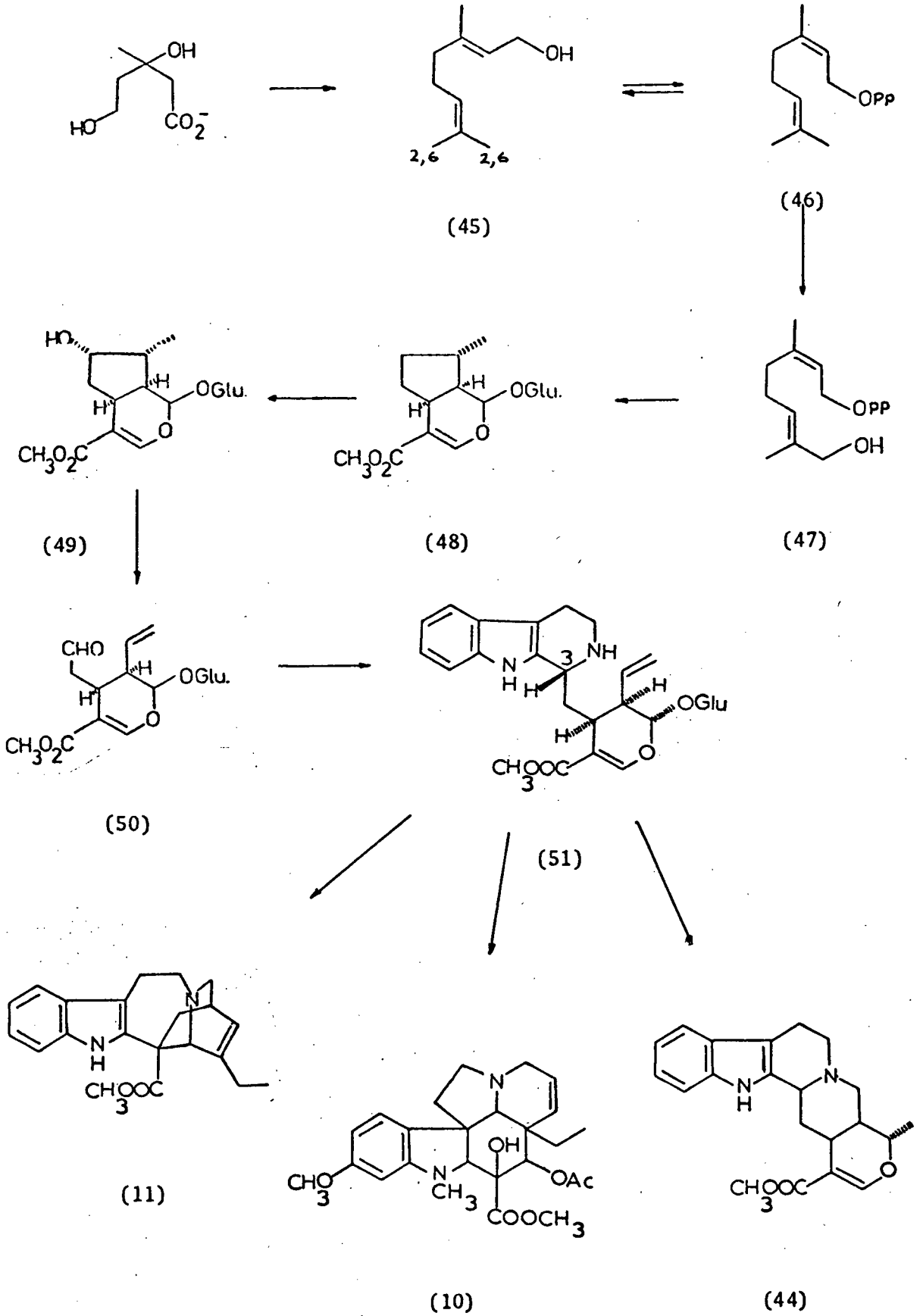
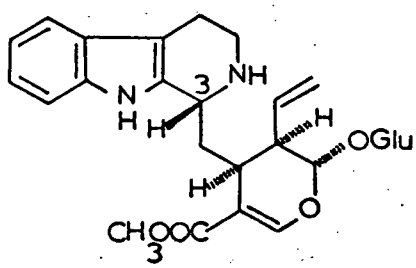


Figure 9. The Early Stages of Indole Alkaloid Biosynthesis.

secologanin (50), although it was unsupported by experimental evidence.

With the origin of the C_9-C_{10} unit firmly supported by experiment, efforts were directed towards determining how secologanin (50) was utilized by the plant system in the biosynthesis of indole alkaloids.

Simultaneously and independently, vincoside (51) and isovincoside (52) were isolated from V. rosea[70], and strictosidine (52) from Rhazya stricta[71]. X-ray analysis[72-3] has proven the relative configurations about C-3 to be as shown. In addition it has been shown that vincoside (51) is specifically incorporated by V. rosea plants into all three types of indole alkaloids,[70,74-5] and that it is itself derived from tryptophan (27) and loganin (49)[70] (Figure 9). Isovincoside (52) was not incorporated into any of the alkaloids studied[74]



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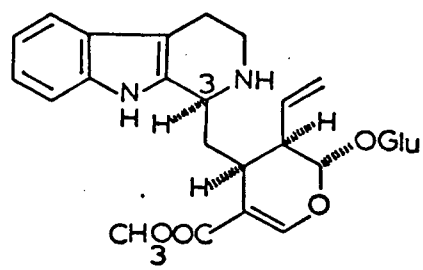


(52)

which was surprising since it rather than vincoside (51) exhibits the configuration at C-3 found in the corynanthe alkaloids.

To date it has been demonstrated that the hydrogen at C-3 is epimerized on incorporation into the alkaloids and that through incorporation of (5-^{3}H) loganin (corresponding to C-^{3}H) into the alkaloids that retention of tritium occurs in the requisite epimerization[76]. Much remains to be done however to

determine the fate of the vincoside molecules during biosynthesis.

These results in V. rosea at least strongly suggest that a crucial intermediate, vincoside (51), is formed by convergent pathways involving separate biosystheses of tryptophan (27) and secologanin (50), and that this intermediate then undergoes the appropriate rearrangements to the various families of complex indole alkaloids. Vincoside (51), then, serves as a convenient dividing line, its biosynthesis may be thought of as the early stages of biosynthesis, while the subsequent rearrangements make up the latter stages of the biosynthesis.

The bioconversion of vincoside (51) into the corynanthe alkaloids entails an unexceptional enzymic hydrolysis of the glucosidic residue followed by reductive condensation of the nascent aldehyde (53) as shown in figure 10. Corynantheine aldehyde (54), corynantheine (34) and geissochizine (8) are the immediate products of the cyclization while ajmalicine (44), an abundant corynanthe compound in V. rosea is subsequently reached by cyclization of 8. The former three compounds have been detected to be actively involved in the biosynthesis through sequential feeding and deuterium labelling experiments in V. rosea[77-79], thus lending support to the proposed transformations.

The strychnos alkaloids differ from the corynanthe formally only in the position of attachment of the same C_{10} unit 5 to tryptamine. Two pathways have been proposed by Wenkert[80] and Scott[81] respectively depicting the rearrangements involved in transforming the corynanthe into the strychnos alkaloids as illustrated in figure 11 for the major strychnos compounds akuammicine (59) and stemmadenine (13). geissochizine (8) containing a reactive β-aldehydo ester

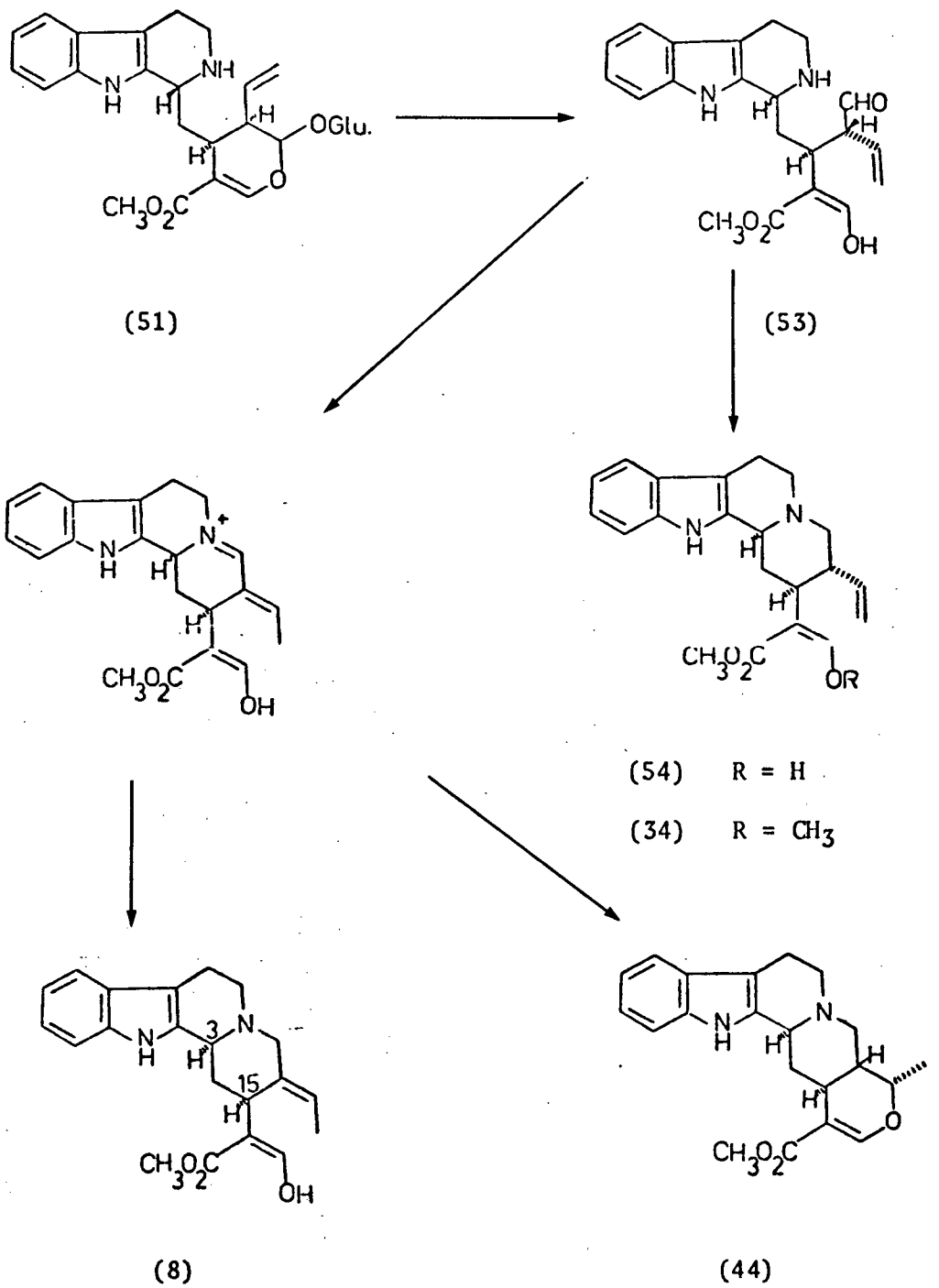
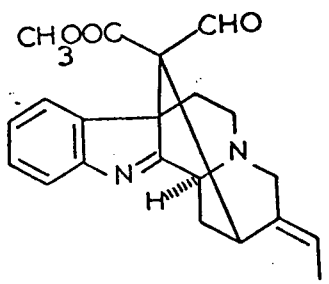


Figure 10. The Biogenesis of the Corynanthe Family.

function is the central intermediate in both pathways.

Credence but not precedence is given to the Wenkert scheme (A) by the presence of the formylstrictamine 55 along with pleiocarpamine (56) in Rhazya stricta.[82,13]



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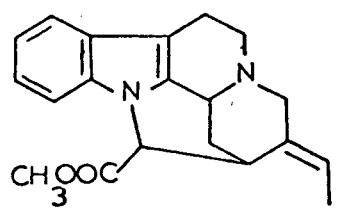


(56)

By the same token Scott's postulate (B) is supported by the isolation of the β-hydroxyindolenine 57, geissochizine oxindole (58) and preakuammicine (9) from sequential feedings in V. rosea[79] and the recently reported formation of oxindole alkaloids in Mitragyna parvifolia.[83-4] Also, the incorporation of geissochizine oxindole (58) into akuammicine (59)[79] lends credence to the postulate.

Much remains to be done however, to determine whether one or both pathways are important in the plant, and whether or not the pathway taken depends upon the individual plant.

Throughout the investigations of indole alkaloid biosynthesis a major concern has been to determine the manner in which the aspidosperma and iboga families are derived through rearrangement of the monoterpene portion of the corynanthe skeleton 5 as depicted in figure 8.

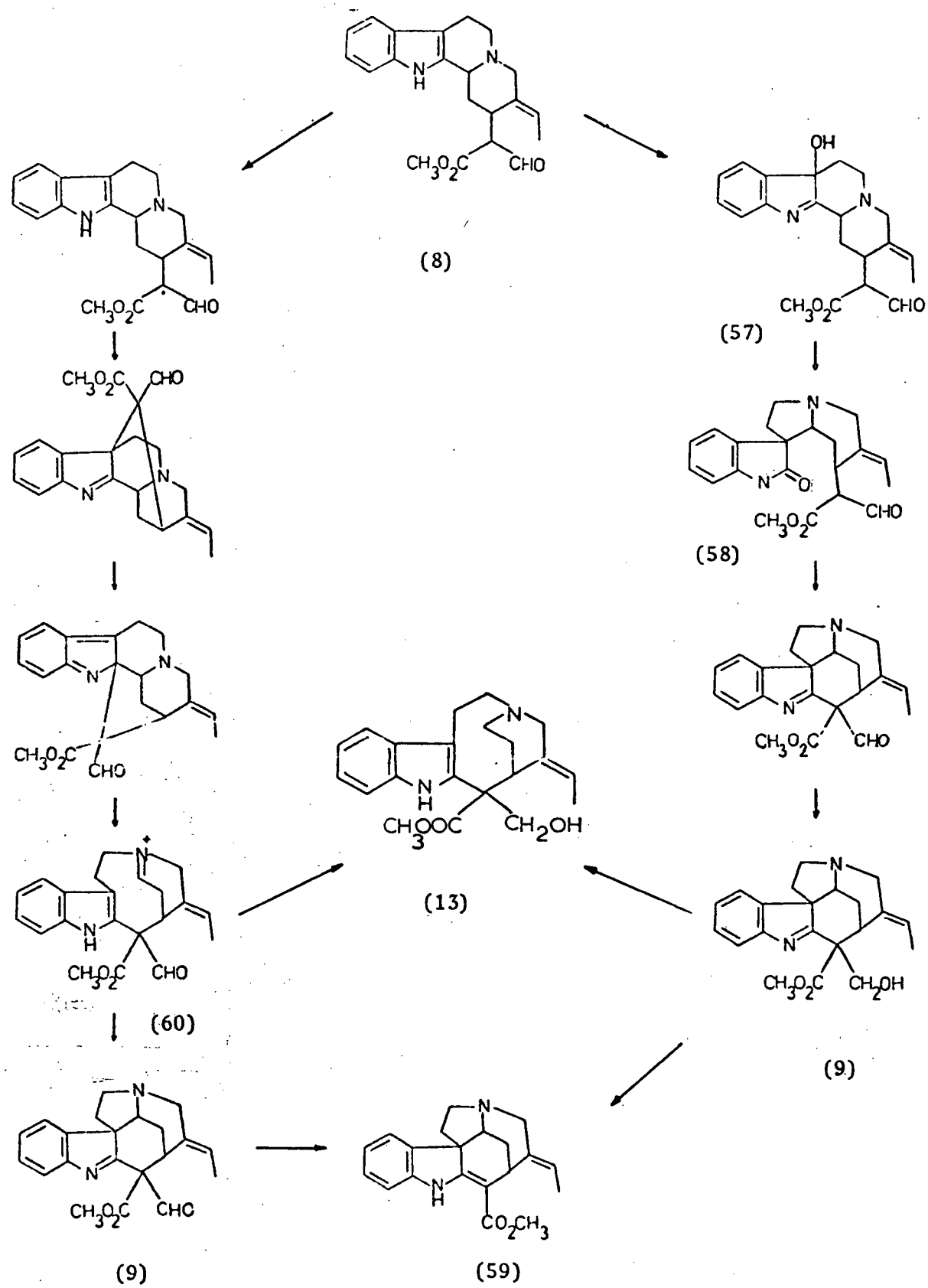


Figure 11. The Postulated Origins of the Strychnos Family.

Through the large body of incorporation experiments dealing with the early stages of biosynthesis, vincoside (51) has been shown to be a precursor of both the aspidosperma and iboga alkaloids. Subsequent incorporations of labelled forms of geissochizine (8)[85,79] corynantheine aldehyde[77-79] (54) and particularly stemmadenine[77] (13) into vindoline (11) and catharanthine (10) has demonstrated that the corynanthe-strychnos alkaloids are also precursors of the two families. Sequential feeding experiments by Scott[77,86] in V. rosea seedings further confirms that the strychnos alkaloids are the immediate precursors of the aspidosperma and iboga and that the postulated sequence: corynanthe → strychnos → aspidosperma → iboga is correct.

The isolation[87] and incorporation of stemmadenine (13) in V. rosea was of particular significance because it was recognized that it occurs furthest along the strychnos pathway towards the aspidosperma and iboga bases.

Wenkert[28] initially introduced the concept that a seco C_3-C_7 intermediate derived from the strychnos skeleton could act as a pivotal precursor to both the aspidosperma and iboga alkaloids. The Wenkert postulate portrayed in figure 12 suggests that via intramolecular Michael and Mannich condensations the seco C_3-C_7 piperidiene 62 obtained from a 1,5-dicarbonyl stemmadenine-like compound 61 (analogous to the iminium intermediate 60) is converted into the nine membered ring systems 63 and 64. Subsequent transannular cyclization of these components leads to the six membered ring bases 65 and 66 from which the aspidosperma and iboga alkaloids are derived.

A similar intermediate 67 was proposed by Levy[88,13] to account for the biosynthesis of the quebrachamine (68) as well as the aspidosperma and iboga bases.

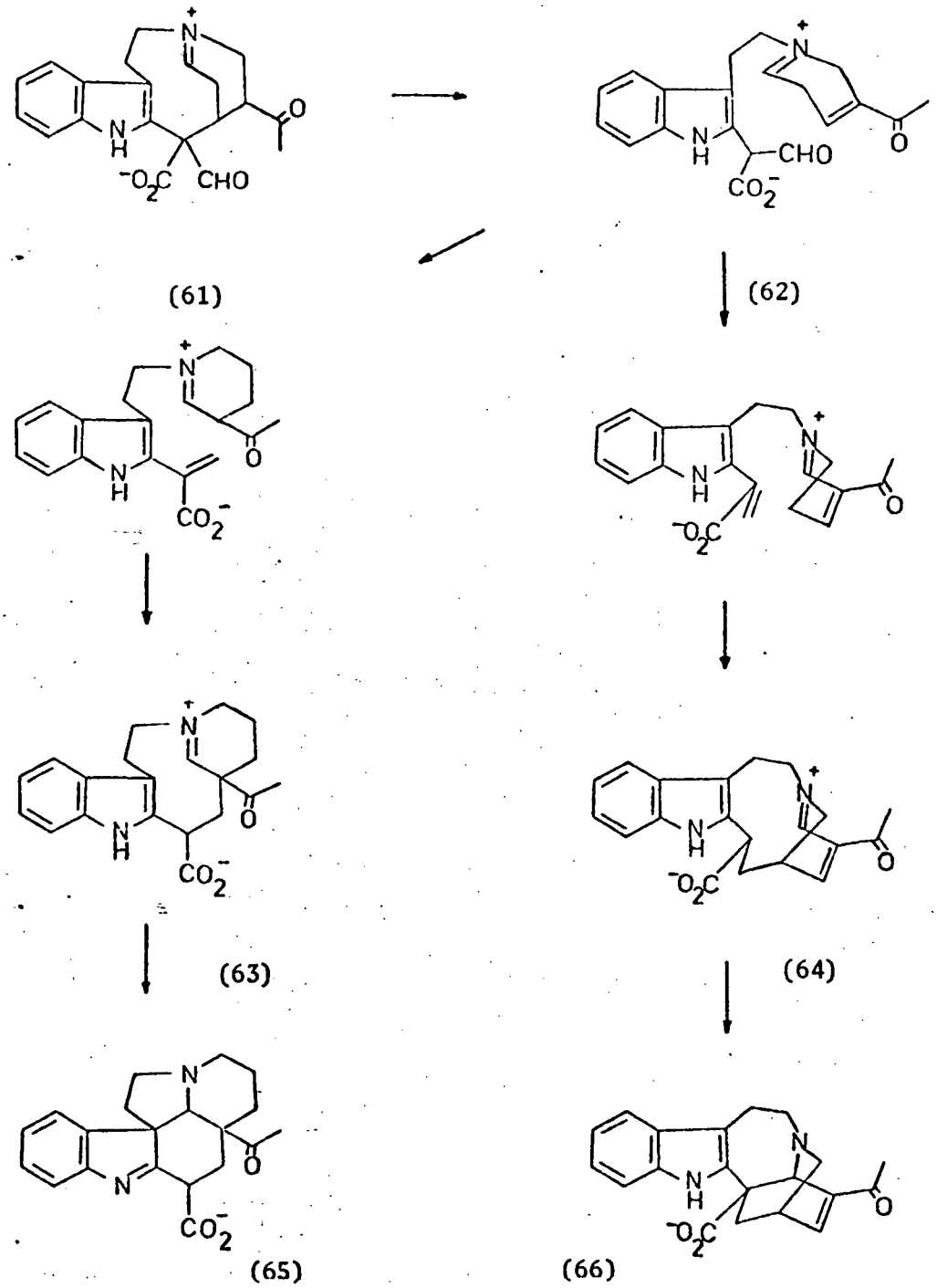
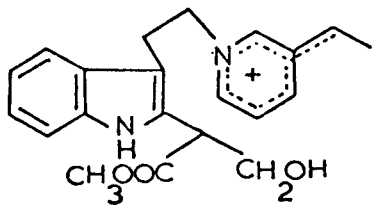
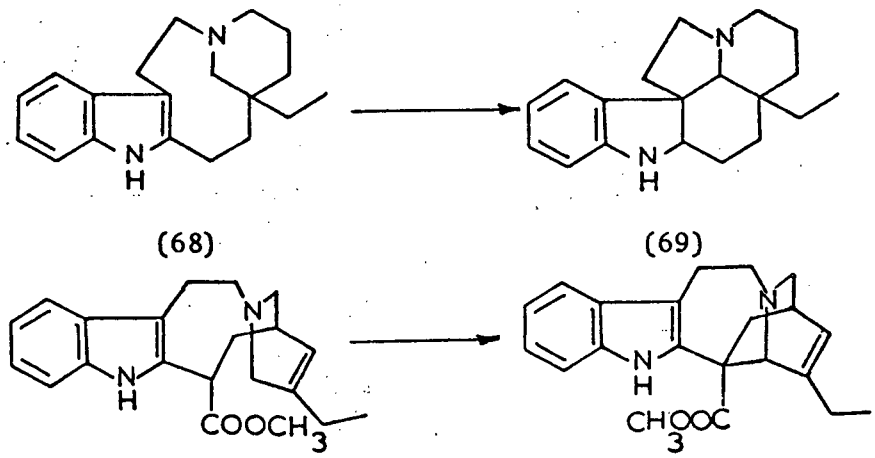


Figure 12. The Wenkert Postulate for the Biosynthesis of the Iboga and Aspidosperma Families.



(67)

Fundamental to Wenkert's postulate was the transannular cyclization step for conversion of the tetracyclic nine membered ring systems into the pentacyclic ring systems of the aspidosperma and iboga alkaloids. In an effort to test this transformation, initial experiments determined it to be a facile *in vitro* process[89-93] as exemplified by the conversion of quebrachamine (68) and 16-carbomethoxycleavamine (15) (via their N(b) iminium salts) to their pentacyclic analogs aspidospermidine (69) and catharanthine (11).

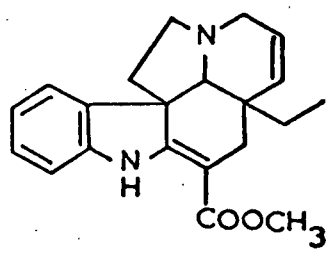


(15) (11)

However, all subsequent labelling experiments to test the parallel *in vivo* conversion to either the pentacyclic aspidosperma or iboga alkaloids failed,[94-5] no incorporation was found into any of the alkaloids studied.

These results strongly suggested that the latter portion of Wenkert's postulate was incorrect and that if a pivotal intermediate such as the seco C_3-C_7 piperidiene is involved in the biosynthesis of the aspidosperma and iboga bases, then a rational whereby it could account for the natural tetracyclic and pentacyclic structures would have to be determined.

Support was given the intermediacy of a pivotal seco C_3-C_7 precursor by the observation that tabersonine (70) was efficiently incorporated into both the aspidosperma alkaloid vindoline (10) and the iboga alkaloid catharanthine (11) in V. rosea plants[94] and seedlings.[77] This interconversion not only involves a considerable rearrangement of the aspidosperma skeleton of tabersoninine (70) including a reversal of the transannular cyclization, but it further demonstrates the sequence corynanthe-strychnos → aspidosperma → iboga.



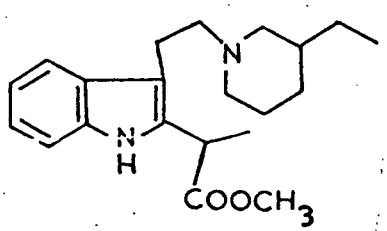
(70)

It is pertinent to point out that the reverse process, the conversion of catharanthine (11) to tabersonine (70), was found not to occur[77] suggesting that equilibration with a pivotal intermediate lies only to the side of the aspidosperma representative tabersonine (70).

Observations by Scott based upon the in vitro interconversions of selective

alkaloids in hot acetic acid led to the proposal that the acrylic ester 72 available by requisite rearrangement of stemmadenine (13) may be involved as the sought after pivotal intermediate in the biosynthesis of the aspidosperma and iboga bases.[96-7] Through the transformations outlined in figure 13 the tetracyclic and pentacyclic ring systems can be generated without having to invoke the transannular cyclization process.

Evidence for the occurrence of a seco intermediate such as the acrylic ester 72 was obtained by the isolation of the dimeric indole compounds, the secamines, from Rhazya species.[98-9] Of particular significance was the presence of the monomeric secodines 73, 74 and 75.[100-1]



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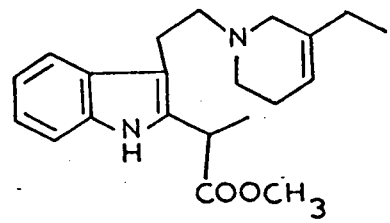

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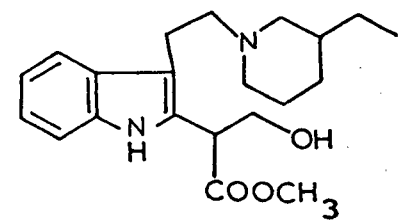


(75)

Due to the inherent instability of the dihydropyridinium system in the acrylic ester 72 since named dehydrosecodine, the synthesis of labelled forms of this compound for the testing of the Scott proposal has not to date been possible. In view of this, the more stable reduced form of the acrylic ester 72, secodine (76) and the hydroxy ester 77 both available by synthesis[102-3] were considered as useful intermediates in the biosynthetic investigations.

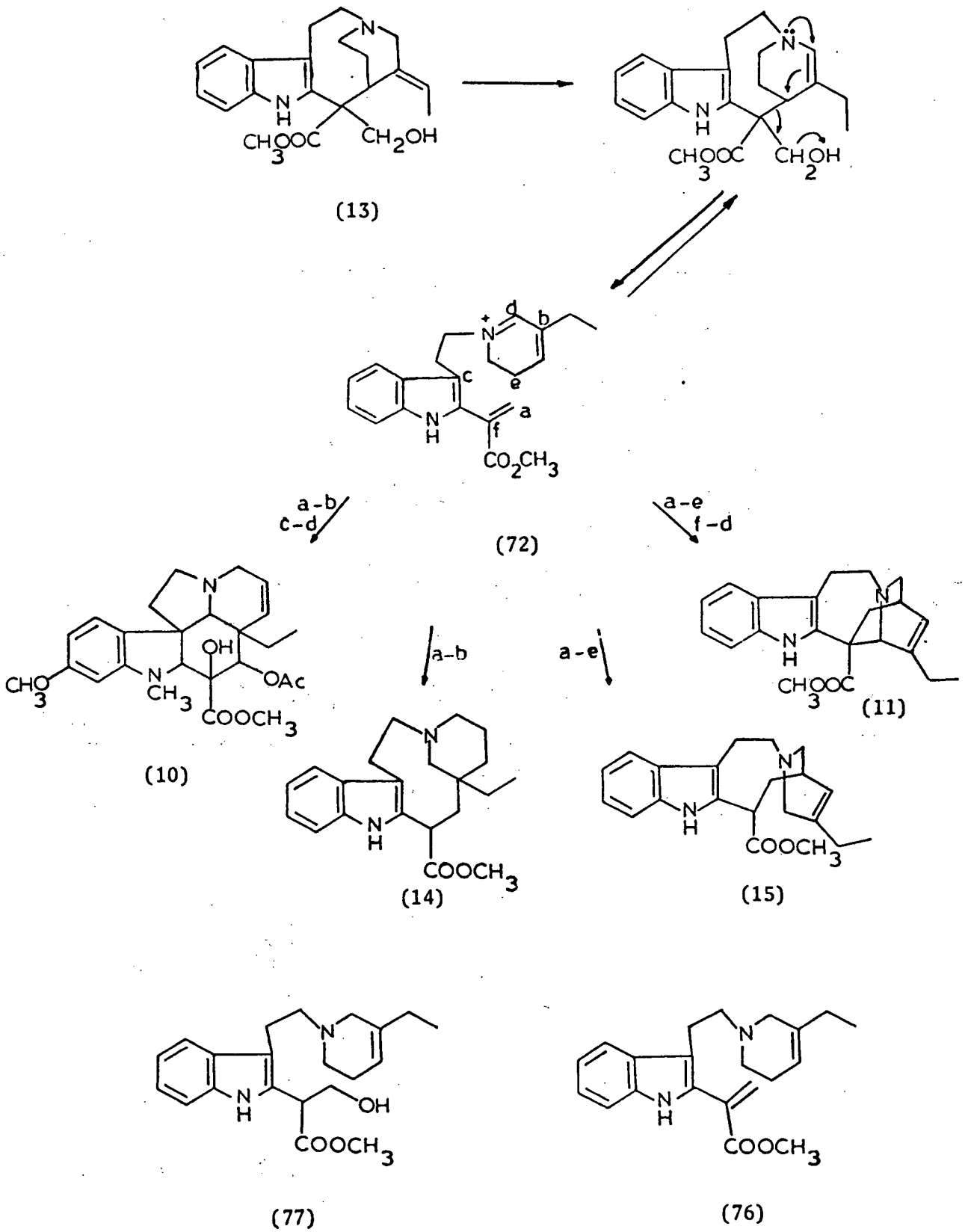


Figure 13. The Acrylic Ester Postulate for the Biosyntheses of the Aspidosperma and Iboga Families.

This view received support when Battersby proposed, on the basis of isotopic dilution experiments that 16,17-dihydrosecodin-17-ol (77) occurs naturally in V. rosea and R. orientalis[104] (figure 13, bottom).

Administration of 16,17-dihydrosecodin-17-ol-(Ar-^{3}H) (77) into V. rosea[105-8], V. minor,[106,109] and Aspidosperma pyricollum[106-8,110] resulted in each case in deterioration of the plants with no detectable incorporation into the alkaloids studied. Subsequent experiments with (Ar-^{3}H)-Secodine (76)[103,105-6,109] have been more successful, however the results must remain tentative as only very low but constant incorporations of this precursor have been obtained into Vindoline (10) and catharanthine (11) in V. rosea, and the alkaloids of V. minor.

Further studies using various forms of doubly labelled secodine (76) coupled with degradation of the alkaloids isolated lent stronger support to the conviction that the secodine skeleton is incorporated intact into the aspidosperma and iboga alkaloids.[105-112]

It is evident from the secodine results that the inherent difficulties of low incorporation and the necessity of feeding a precursor of the wrong oxidation state will have to be overcome before the acrylic ester, dehydrosecodine (72) can be firmly accepted as the pivotal precursor of the aspidosperma and iboga alkaloids.

The abnormally bridged indole alkaloids uleine (18), apparicine (19), olivacine (16) and ellipticine (17) (as well as the reduced froms of the latter) are presently believed to be derived from late stage intermediates in the biosynthetic pathway. Very little is presently known regarding their biosynthesis and as a consequence a number of different postulates have been put

forward to rationalize their presence in the Aspidosperma and Ochrosia plant systems.

The anomolous structures of these compounds initially led investigators to believe however, that by contrast to the normal β-ethylamine bridged compounds they were not tryptophan derived. In conjunction with the proposal of the SPF unit 33 as the progenitor of the monoterpene portion of the alkaloids, Wenkert[28] proposed that the biosynthetic pathway to uleine (18), olivacine (16) and ellipticine (17) involved reaction of the SPF unit 33 with glycosylideneanthranilic acid (78) a precursor of tryptophan (27). Condensation of the requisite carbonyl groups of the SPF unit with a nitrogen source (such as methylamine) and reaction at the C-3 of indole with loss of the glycosyl function leads to the alkaloids (Figure 14).

With the structure elucidation of apparicine (19) in 1965, Djerassi[113] proposed that Wenkert's intermediate 79 could serve as a precursor in its biosynthesis. Isomerization to the exocyclic iminium species 80 followed by cyclization with the indole nucleus as shown in figure 15 would yield apparicine (19).

Wenkert's postulate[28] invokes a separate biosynthetic pathway to these alkaloids not involving tryptophan (27) so as to extrude the two carbon chain, and as such it is in discord with the opinion that these indole alkaloids are tryptophan (27) derived via the pathway elucidated for the form alkaloidal families.

In order to test the basic assumption behind this proposal, tryptophan - (Ar-^{3}H) (27) was administered to A.pyricollum, a plant system reported to

contain apparicine (19) and uleine (19)[9]. Significant incorporation was obtained into apparicine (19)[110] which indicates it to be tryptophan derived, contrary to Djerassi's proposal.[113]

Insufficient quantities of uleine were isolated however to permit a determination of any incorporation into it. Despite subsequent attempts to radiolabel this molecule in A. australe,[119,120] no biosynthetic data is presently available for it or for the pyridocarbazoles olivacine (16), ellipticine (17), N-methyltetrahydroellipticine (26) and guatambuine (25).

With tryptophan (27) as the precursor of the indole portion of apparicine (19), it is necessary to determine the mechanism whereby one or both of the carbons of the β-ethylamine side chain are extruded. Some insight was gained when (Ar-^{3}H) and either C-2 or C-3-^{14}C doubly labelled tryptophan (27) was fed to A. pyricollum. It was found that C-3 of tryptophan (27) is incorporated into apparicine (19) with retention of the ^{3}H/^{14}C ratio whereas over 97% of the label at C-2 was shown to be lost.[108]

Extrapolating the knowledge that tryptophan is the likely precursor of apparicine (19) to include uleine (18), Kim and Erickson[114] studied a mechanism proposed by Joule et al[115] whereby the two carbon bridge of uleine (18) is extruded. Their experimental model for uleine (18) biosynthesis was based upon the co-occurrence and close similarity between uleine (18) and alkaloids of the condylocarpine type such as 24. They attempted without success to accomplish the in vitro expulsion of the two carbon unit from the oxidation produce of a condylocarpine system 81 (figure 16).

This mechanism is probably of little biosynthetic significance however as it is specific to uleine (18) only and cannot readily be adapted to account for

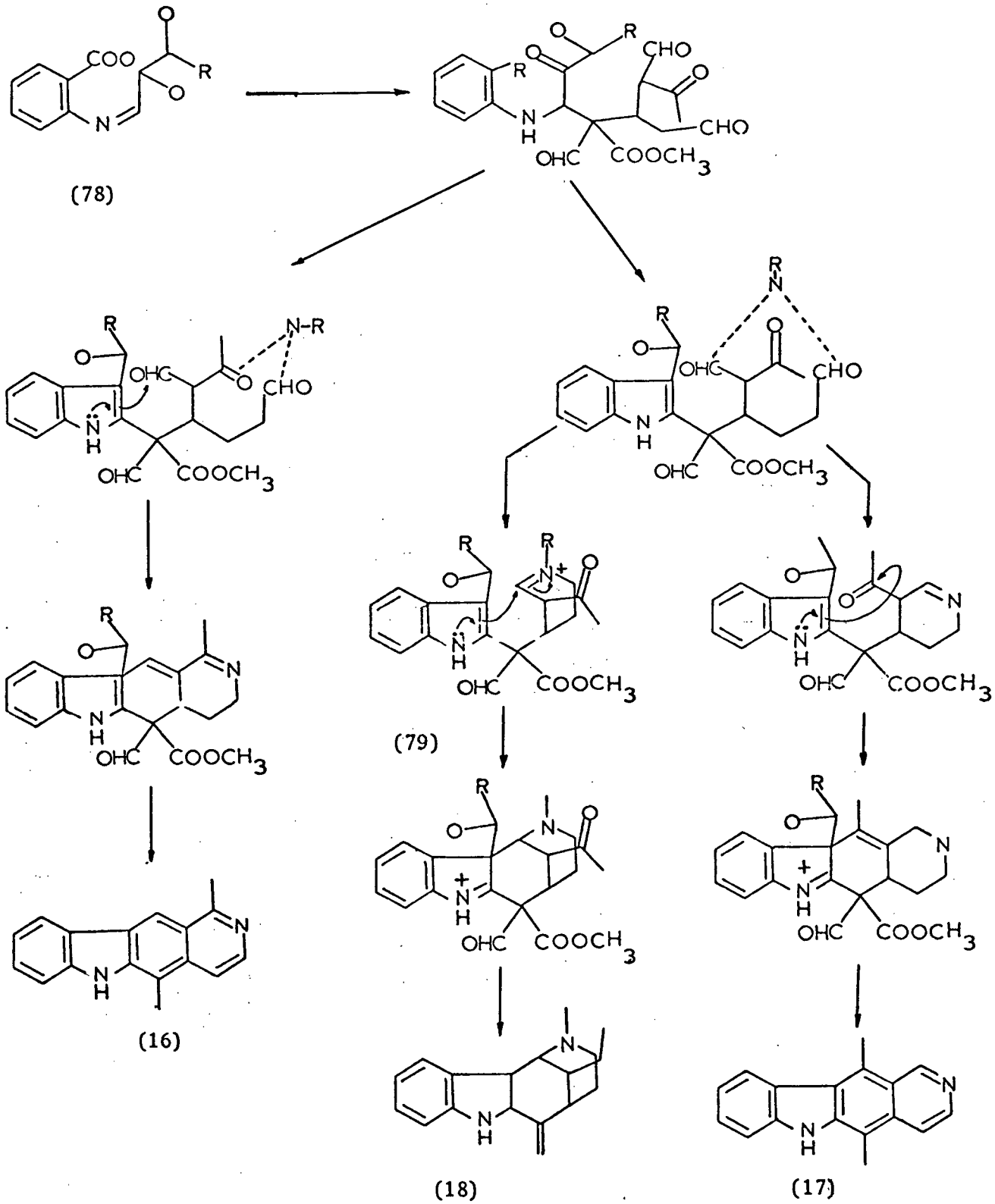


Figure 14. The Wenkert Postulate for the Biosyntheses of Olivacine (16), Ellipticine (17) and Uleine (18).

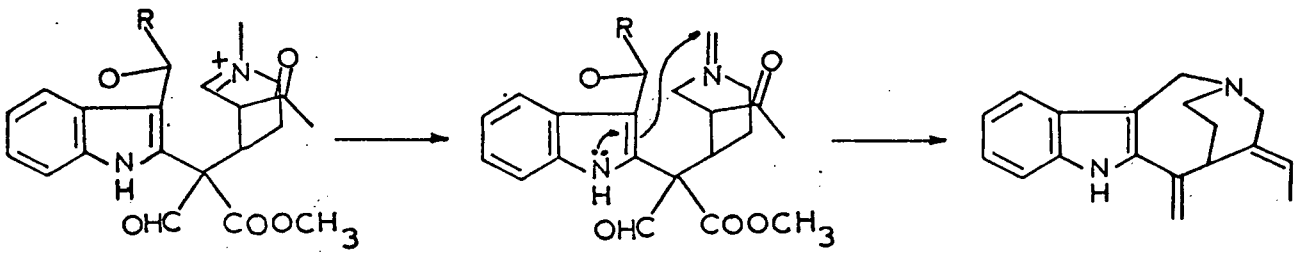


Figure 15. The Djerassi Postulate for the Biosynthesis of Apparicine (19).

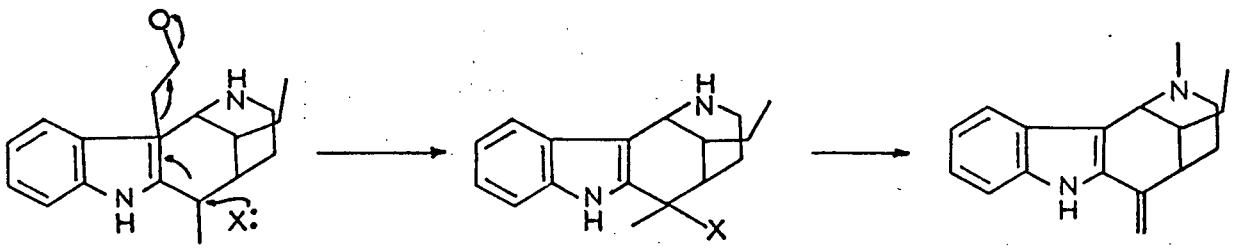


Figure 16. Kim and Erickson's <u>in vitro</u> Study of Uleine Biosynthesis.

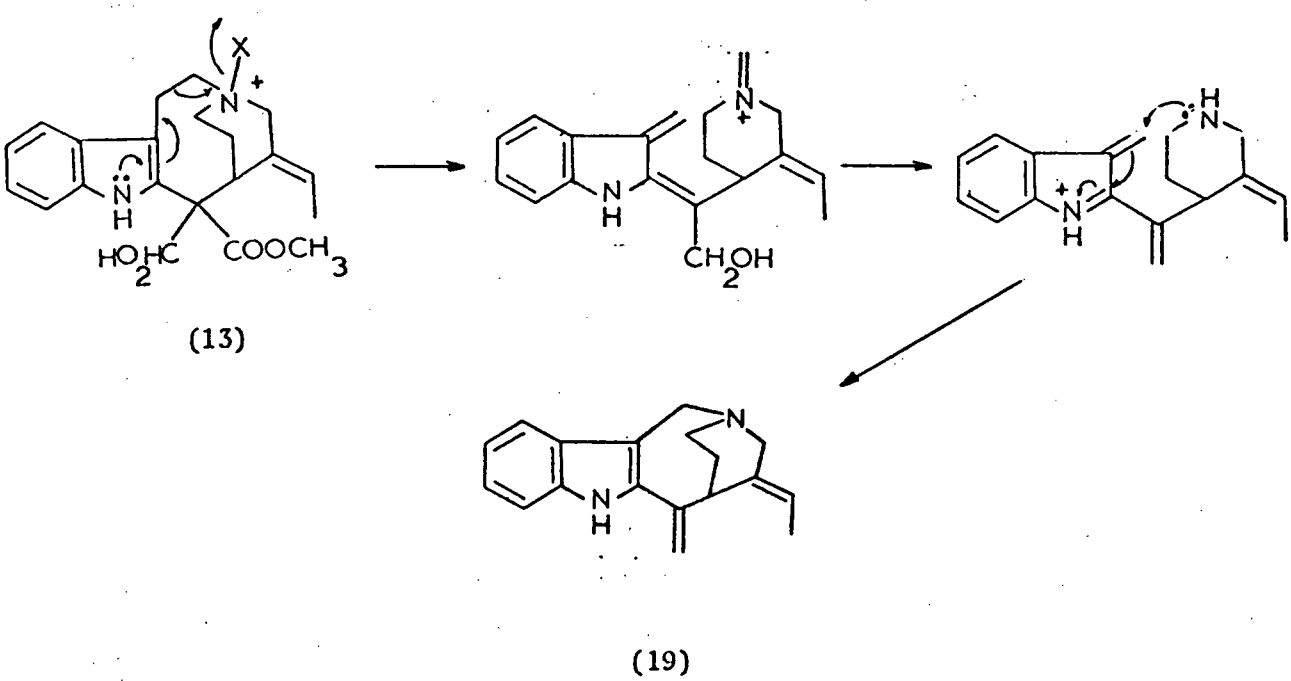


Figure 17. Postulated Synthesis of Apparicine (19) <u>via</u> Fragmentation of the Tryptamine Bridge.

the lack of the β-ethylamine bridge in the closely related apparicine (19) structure.

Stemmadenine (13) known to co-occur with apparicine (19) and uleine (18) in A. pyricollum[116] was found to be efficiently incorporated into apparicine (19).[108] This important result places apparicine (19) and very likely uleine (18) and the pyridocarbazole alkaloids of the olivacine (16) and ellipticine (17) at a late stage in the biosynthetic scheme.

The subsequent low incorporation of vallesamine (20)[108] an alkaloid closely reminiscent of Stemmadenine (13) but having only a methylene bridge suggests but does not prove that the extrusion of the carbon from the tryptophan bridge and the necessary modification to the exocyclic methylene at C-3 of stemmadenine may occur as a concerted process.

Incorporation of various forms of secodine (76) in A. pyricollum[106-8,110] coupled with degradation data places the rearrangement processes which lead to the abnormal skeleton of apparicine (19) at even a later stage in the biosynthetic scheme. It is not known at the present time which if either of these two precursors secodine (76) or stemmadenine (13) is the immediate precursor of the apparicine system for it is known that an equilibrium exists between stemmadenine (13), dehydrosecodine (72) and the aspidosperma alkaloids.

A more enlightened rationale for the biosynthesis of these non-tryptamine bridged alkaloids was deduced from a study by a French group[117] of the in vitro fragmentation of the tryptamine bridge under modified Polonovski reaction conditions (i.e. reaction of the N-oxide with trifluoroacetic acid). It was suggested that a similar fragmentation mechanism could be involved in the extrusion of the one carbon unit during the biosynthesis of apparicine (19) (figure 17).

This proposal encompases a large portion of the known incorporation data, including the involvement of stemmadenine (13) as a crucial precursor and the extrusion of the C-2 and not C-3 of tryptophan in the formation of apparicine (19).[108] A minor discrepancy exists however, for it has been shown that the hydroxymethylene and not the carbomethoxy functionality at C-16 is lost during the rearrangement process.

Subsequently this mechanistic proposal has been extended[118] to account for biosynthesis of uleine (18) and the oxidized and reduced members of the olivacine (16) and ellipticine (17) series (figure 18). As such, it represents the abnormal alkaloids to be derived from the convergent indole biosynthetic pathway and is thus a very viable alternative to the Wenkert[28] and Djerassi[113] postulates.

Also, the generation of uleine (18), apparicine (19) and the pyridocarbazole alkaloids 16, 17 25 and 26 from common intermediates suggesting the involvement of similar enzymic processes in the biosynthetic pathway is in accord with the observed frequent co-occurrence of these compounds in Aspidosperma and Ochrosia plants.

As mentioned however, no biosynthetic data exists at the moment to prove that the unified hypothesis as proposed by the French group[118] for olivacine (16), ellipticine (17), uleine (18), guatambuine (25), or N-methyltetrahydro-ellipticine (26) is correct (figure 18), or whether the Wenkert postulate[28] can be substantiated (figure 14).

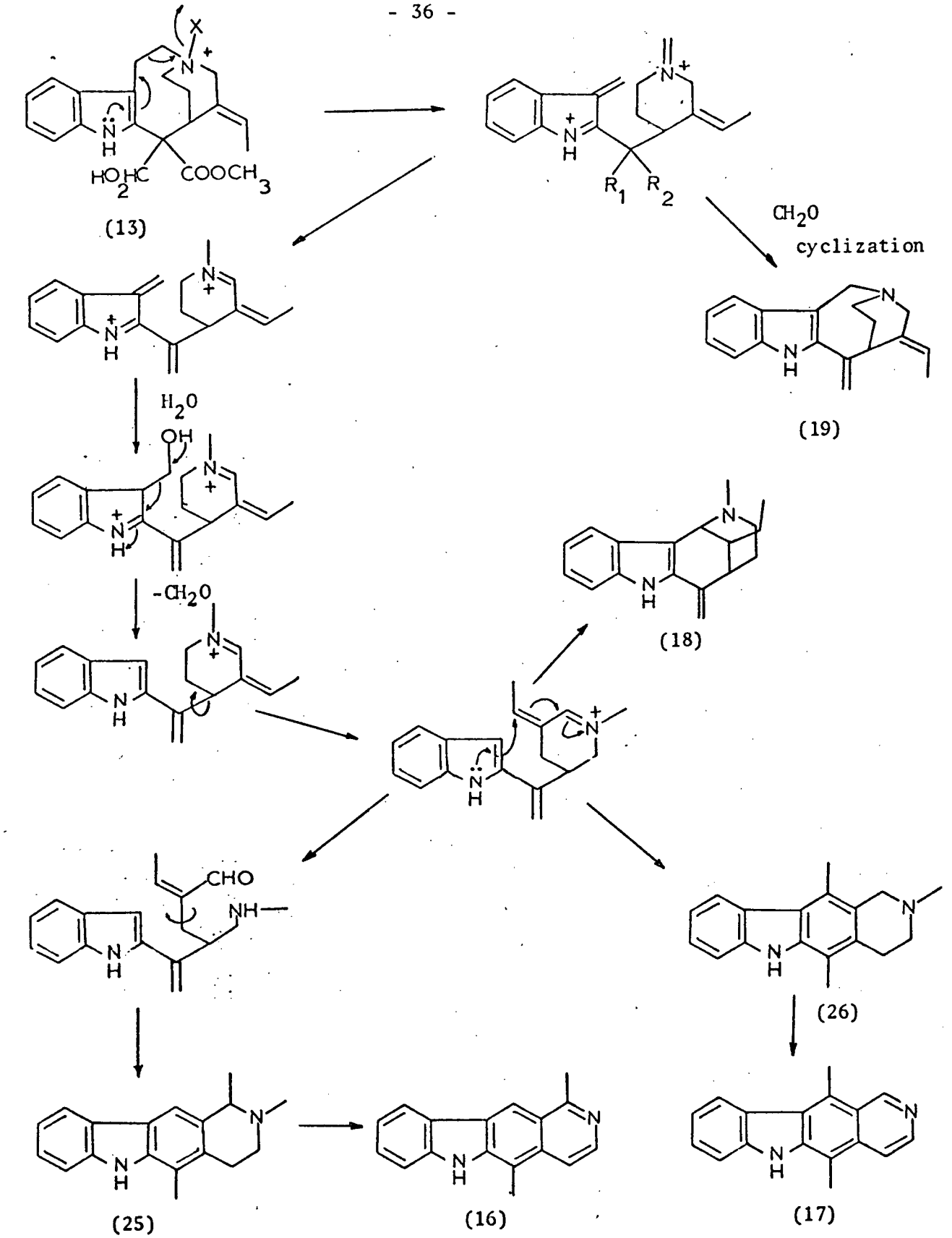


Figure 18. The Potier-Janot Postulate for the Biosynthesis of the Non-Tryptamine Bridged Alkaloids.

DISCUSSION - PROLOGUE

The work presented in this thesis represents part of a long-range program in our laboratory for the investigation of the biosynthesis of representative alkaloids of the uleine (18), apparicine (19), and pyridocarbazole systems in a variety of *Aspidosperma* plants. Previous work on this project has concerned itself primarily with the biosynthesis of apparicine (19) and uleine (18) in *A. pyricollum*.[106, 108, 110, 119] Some preliminary feeding experiments have also been conducted on the biosynthesis of uleine (18), olivacine (16) and guatambuine (25) in *A. australe*.[119, 120] The emphasis in the present study however has been on developing the background work necessary for a detailed study of the biosynthesis of the pyridocarbazole systems represented by olivacine (16), guatambuine (25), ellipticine (17) and N-methyltetrahydroellipticine (26).

Four basic requirements were considered initially to be essential to this detailed biosynthetic study:

1. a suitable plant source that contains the compounds of interest and is actively biosynthesizing them.
2. a synthetic scheme for the specific labelling of proposed precursors,
3. an efficient degradation scheme for the isolation of specific radiolabelled atoms.
4. an adequate source of unlabelled compounds for purposes of comparison, for dilution studies, and for chemical transformation.

Different aspects of these requirements were developed for biosynthetic work in two plant systems A. australe, and A. vargasii as described in the three parts of this thesis. Part 1 is concerned with plant extractions so as to obtain the required alkaloids from a crude A. australe extract, and to determine the feasibility of plant feeding experiments in A. vargasii. Part 11 describes the development of a degradation scheme for both the olivacine (16) and ellipticine (17) series, and Part 111 describes the synthesis of olivacine (16) and guatambuine (25) for the continuation of future biosynthetic work.

DISCUSSION - PART 1

Section A, Plant Extractions:

Aspidosperma Australe (Mull. Argov.)

A. australe has been reported by Ondetti and Deulofeu[121-3] to contain as their principle alkaloidal components olivacine (16) and both enantiomers of guatambuine (25). The availability of approximately one hundred grams of a crude methanolic extract from a large scale extraction of *A. australe*[124] therefore prompted the development of an isolation procedure whereby convenient but limited supplies of these two alkaloids could be obtained for degradation work.

A general alkaloid extraction procedure described by Gilbert[10] was used. This method involved treatment of the methanolic extract with 15% acetic acid and extraction with petroleum ether to remove neutral and acidic plant material. By subsequent basification and extraction with chloroform the basic alkaloidal fraction was isolated.

Thin layer chromatography (TLC) on this extract showed the presence of two major components, a visible bright yellow spot which fluoresced under UV and sprayed brown with ceric sulphate which corresponded to olivacine (16) and a dark blue spot under UV which sprayed turquoise with ceric sulphate corresponding to guatambuine (25).

For their isolation, advantage was taken of the observation on TLC that both compounds possessed a similar retention time on alumina whereas they exhibited very different Rf values on silica gel. On silica chromatoplates

guatambuine (25) occurred at very low Rf whereas olivacine (16) moved with the solvent front, this behavior reflecting the considerable differences in their known basicities.[125] This characteristic difference made it possible to first separate both components from the small amounts of other materials present in the extract by column chromatography on alumina and subsequently to separate them from each other by chromatography on silica gel.

Considerable difficulties were encountered, however, in the application of the alkaloidal extract onto the column. Olivacine (16) and guatambuine (25) are soluble only in polarsolvents, for example methanol, acetonitrile and pyridine. It was found, however, that a chloroform solution containing minimum amounts of methanol and pyridine would solubilize the extract without interfering with the separations.

Upon recrystallization from methanol, it was determined that olivacine (16) and (-) - guatambuine (25) were present in the crude extract to the extent of 1.8 and 1.6% respectively.

Olivacine (16), obtained crystalline as yellow needles was characterized by its N.M.R. and mass spectra, as well as the superimposability of its UV. and IR. spectra with the reported spectra.[126] The N.M.R. spectrum exhibited two singlets at $\delta 2.80$ and $\delta 3.15$ for the C-5 and C-1 methyl hydrogens respectively. The mass spectrum exhibited a parent peak at m/e = 246 and very little fragmentation due to the aromatic nature of the molecule.

(-) - guatambuine (25), obtained as cream coloured cubes was also characterized by its spectral data.[126] The mass spectrum exhibited a parent peak at

m/e = 264 and characteristic fragments at m/e = 249, 233, 221-218, 204. The N.M.R. spectrum is shown in figure 19 because it possesses several important features which will be alluded to repeatedly in subsequent discussions. Of note is the presence of a doublet at 1.52 (J = 7 Hz) for the C-1 methyl group and the presence of a quartet at 3.90 (J = 7 Hz) for the methine hydrogen at the same position. The chemical shift of the methine hydrogen is very characteristic as it is influenced by the fact that it is both benzylic and adjacent to the basic nitrogen atom. Also present in the N.M.R. spectrum is a singlet at 2.40 ($N-CH_3$) and a singlet at 2.54 ($C-5,CH_3$).

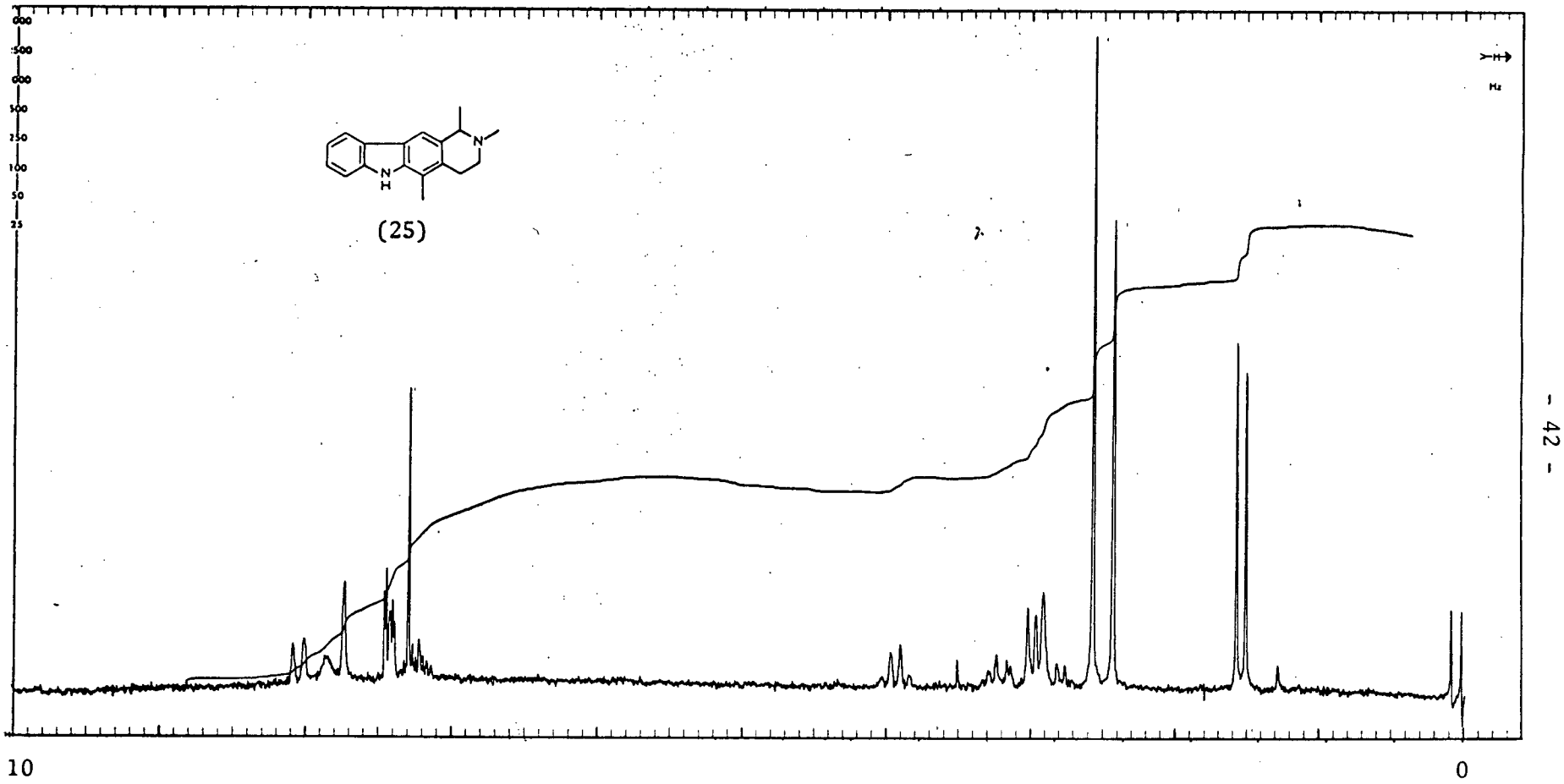
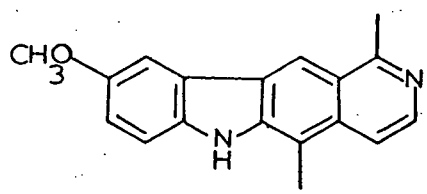


Figure 19. The N.M.R. Spectrum of Guatambuine (25)

Aspidosperma vargasii (A.DC.)

A. vargasii plants when mature are trees standing up to twenty meters in height with relatively slender branches and a close thin bark.[127] They are indigenous to rocky arid slopes and transition forest in Venezuala, and adjacent Columbia and Guiana. The bark of A. vargasii has been studied previously by Burnell and Della Casa (1967)[128] and has been shown to contain as its principle constituents three pyridocarbazole alkaloids, guatambuine (25), N-methyltetrahydroellipticine (26) and 9-methoxyolivacine (82).



(82)

A. vargasii represents, therefore, an ideal plant in which to further extend our biosynthetic program for the two components 9-methoxyolivacine (82) and N-methyltetrahydroellipticine (26) are not present in the previously studied plants A. pyricollum and A. australe.

Prior to any radioactive feeding experiments the suitability of A. vargasii as a plant source for biosynthetic study had to be determined. This involved firstly the determination of the presence of these constituents in the live plants in quantities that could be isolated and secondly the development of a feasible isolation scheme whereby the desired constituents could be obtained in pure crystalline form for radioactive counting.

A typical masceration-extraction procedure was utilized to obtain a crude methanolic extract of the plant. Utilizing the observation by Burnell and Della Casa[128] that guatambuine (25) was extractable from aqueous acidic solution with chloroform an extraction procedure was devised to partition the alkaloids present in the crude methanolic extract into acidic-chloroform extractable and basic-chloroform extractable fractions. Suspending the crude methanolic extract in 10% hydrochloric acid and extracting first with petroleum ether to remove non-polar material and then with chloroform (acidic-chloroform extract) afforded a mixture of three major alkaloidal components. One of these components provided an identical retention time and colour development with either guatambuine (25) or N-methyltetrahydroellipticine (26), TLC characteristics of these two alkaloids being very similar. Subsequent basification of the aqueous phase and extraction with chloroform (basic-chloroform extract) yielded two further major components and one minor component the least polar of which corresponded to an olivacine-like compound by comparison of its Rf value and fluorescent response to UV with authentic olivacine (16).

By means of thin layer chromatography it appeared as though the partitioning was highly efficient as very little of either of the basic-chloroform extract components could be detected in the acid-chloroform extract, and similarly only small amounts of the guatambuine-like (25) component could be detected in the basic-chloroform extract.

Of particular significance in the above extraction was the detection of three previously unreported components in relatively large amounts.

The separation of the various components in each extract was accomplished by column chromatography on alumina. Table 1 provides a summary of the results obtained during this separation.

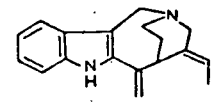
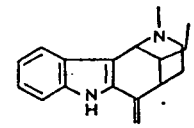
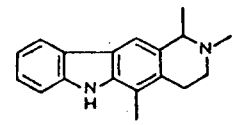
In most instances the column chromatography had to be repeated on the various combined fractions in order to obtain sufficiently pure material for recrystallization. This was particularly true for the separation of Uleine (18), guatambuine (25) and the minor "uleine like" component. In each case the chromatography fractions were monitored by TLC, UV. and, where necessary, Fourier transform N.M.R. spectroscopy.

The UV. spectrum was a particularly important diagnostic tool in revealing the presence of the minor "uleine like" component on chromatography of the combined guatambuine (25) fractions. This component was present in very small quantities and could be detected only on TLC as a light blue colouration in the tail of the turquoise-green spot for guatambuine (25). On the basis of the UV. spectrum, this component in the extract mixture was shown to possess the chromophoric system characteristic of Uleine (18) and apparicine (19). No subsequent data was obtained to further determine its structure.

The Fourier transform N.M.R. spectrum of the guatambuine (25) fraction demonstrated it to be homogeneous and not containing any N-methyltetrahydroellipticine (26) which possesses almost identical properties. Although this latter component was anticipated to be present in the plant extract,[128] no trace of it was ever detected in any of the several plant extraction experiments conducted

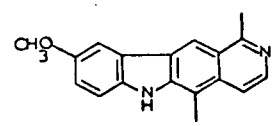
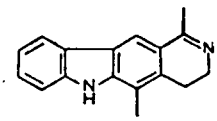
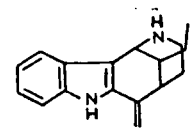
Table 1 - Column Chromatography Results on Extracts from A. vargasii

A) Acid Chloroform Extract:

Frac. No.	Solvent	Wt.(mg.)	x 10^2 % of Total Plant	Structure
1 - 8	$CHCl_3$	105	3.88	 apparicine (19)
10 - 20	$CHCl_3$:EtOAc 20:60%	33	1.22	 uleine (18)
21 - 44	$CHCl_3$:EtOAc EtOAc:MeOH 8%	47	1.74	 guatambuine (25)

* From rechromatography of fracs. 21 - 44 "Uleine like component"

B) Base Chloroform Extract:

1 - 15	$CHCl_3$ to $CHCl_3$:EtOAc 20%	100	3.70	 9-Methoxyolivacine (82)
17 - 20	$CHCl_3$:EtOAc 40%	-	-	 dihydroolivacine (141)
21 - 35	$CHCl_3$:EtOAc 40% to EtOAc: MeOH 5%	46	1.73	 Desmethyluleine (84)

The absence of N-methyltetrahydroellipticine was disappointing since it would have been desirable to evaluate the biosynthesis of the whole series of these alkaloids in the same plant species. On the other hand, its presence would have complicated the isolation procedure greatly, for even trial separations of a mixture of pure guatambuine (25) and N-methyltetrahydro-ellipticine (26) proved to be very difficult on a small scale.

The identities of apparicine (19), uleine (18) and desmethyluleine (84) were determined by a comparison of mass spectral, N.M.R. and UV. data with that already reported.[129,113] A characteristic feature in the N.M.R. spectra of all three compounds is a pair of singlets in the olefinic region δ4-5 for the two protons of the exocyclic methylene group. The presence of a doublet at δ4.11 in the spectrum of the uleine compounds and assigned to the C-1H (see table 1) which is both benzylic and adjacent to the basic nitrogen, also provided a facile differentiation from apparicine (19) since the latter possesses geminal hydrogens at the C-1 position and exhibits an AB quartet in the same region. Of particular note also was the absence of the N-methyl signal in the spectrum of desmethyluleine (84).

The structure of guatambuine (25) was based upon the superimposability of both N.M.R. and IR spectra with those of an authentic sample.

The structure of 9-methoxyolivacine (82) was determined from its N.M.R. spectrum which exhibited two singlets at δ2.80 and 3.16 for the aromatic methyl protons and a singlet at δ4.00 for the methoxy group. It is noteworthy that the position of these absorptions is close in value to those obtained for olivacine (16), and markedly different to the position of the singlet peaks in

ellipticine (17). The UV. spectrum was almost identical to that reported for 9-methoxyolivacine (82) and distinctly different than for olivacine (16). The mass spectrum possessed a parent peak at m/e = 276 consistent with the required molecular formula and like olivacine (16) it possessed an almost negligible fragmentation pattern. The minor component in the basic chloroform extract (fractions 17 - 20, Table 1) of which only several milligrams were isolated was tentatively determined to be 3,4-dihydroolivacine (83) on the basis of the TLC and UV comparisons with 3,4-dihydroolivacine (83) available by synthesis (See sequence A, page 190).

It is clearly evident from the isolation results that A. vargasii is a very suitable plant source for biosynthetic investigations. Not only does it contain representatives of uleine (18), apparicine (19), and the olivacine series of compounds, but each of the five major components are present in 30-100 mg. quantities and can readily be purified by either crystallization or sublimation techniques.

It was observed however that the quantities of uleine (18) obtained depended markedly upon the size (i.e. age) of the plant used in the experiment. Only large and thick woody stemmed plants yielded relatively large quantities of uleine (18) (230 mg.). Smaller plants yielded considerably less of this valuable alkaloid (5-10 mg.).

It is of interest to note that the A. vargasii results further demonstrate the frequent co-occurrence of the different types of non-tryptamine alkaloids with each other in Aspidosperma plants.

Section B, Incorporation Experiments in A. vargasii (A.DC.)

Only a preliminary investigation of the incorporation of radioactivity into the alkaloids of A. vargasii has been conducted at this time. The objective behind the experiments was to determine whether active biosynthesis was occurring in the plant and not necessarily to determine any information regarding the rearrangements that take place on conversion of precursor molecules into the alkaloids.

With regards to the present knowledge of indole alkaloid biosynthesis as outlined in the Introduction, the precursors that were logically chosen for feeding experiments were aromatic tritium labelled tryptophan (27) and stemmadenine (13).

The (Ar-3H)-tryptophan (27) experiment was of primary importance for it would demonstrate that olivacine (16), guatambuine (25), and uleine (18) are derived from the elucidated indole alkaloid pathway, thus invalidating the Wenkert[28] and Djerassi[113] proposals. The incorporation of (Ar-3H)-Stemmadenine (13) was also of major importance because by the Potier-Janot postulate[118] it represents the immediate precursor of the whole series of non-tryptamine bridged alkaloids.

Both precursors were labelled with tritium in the aromatic ring for reasons that the tritium exchange synthesis of the precursors is relatively simple and there is no significant loss of label by transformations occurring during biosynthesis. Also until synthetic stemmadenine (13) becomes available in our laboratory,[119, 120, 130] Stemmadenine (13) labelled in the aromatic ring is the only radioactive form of this precursor available.

The aromatically labelled precursors were prepared by an exchange reaction in tritiated trifluoroacetic acid, crystallized to constant activity and incorporated in 1-3 mg. quantities to approximately five year old A. vargasii plants. The precursors were administered by the cotton wick technique and the biosynthesis was allowed to proceed for a one-week period. The plants were then mascerated and the alkaloidal components isolated in an identical manner to that described in section A for A. vargasii. The compounds studied were recrystallized to constant activity meaning that the difference between successive countings was less than 5%. The effects of colour quenching[134] on the efficiency of scintillation counting of methoxyolivacine were standardized by preparing counting samples of identical concentration after each recrystallization. A summary of the incorporation results is presented in Table II.

The results show that there has been a definite incorporation of both tryptophan (27) and stemmadenine (13) into methoxyolivacine (82) and that tryptophan has also been incorporated into guatambuine (25). Both precursors however, were not incorporated into uleine (18). The radioactive incorporation of these two compounds into the other constituents of A. vargasii was not determined during these initial experiments.

The absence of any incorporation into uleine (18) was frustrating because similar results have been obtained for this molecule during incorporation experiments in other plant systems. Nothing is presently known concerning the nature of the enzymic processes that produce these compounds within the plant or what conditions are necessary to stimulate

Table II. Results of Incorporation of Tryptophan (27) and Stemmadenine (13) into *A. vargasii*.

Compound Isolated	Activity fed (DPM)	Specific Activity isolated (DPM/mmole)	Weight isolated (mg.)	% incorporation
Experiment I (Ar-3H)-Tryptophan (27). (2.50×10^{11} DPM/mmole)				
9-Methoxyolivacine (82)	5.82×10^{11}	3.17×10^{5}	91	0.0049
Guatambuine (25)	5.82×10^{11}	1.71×10^{5}	40	0.0018
Uleine (18)	5.82×10^{11}	0.00	6	--
Experiment II (Ar-3H)-Stemmadenine (13). (3.21×10^{10} DPM/mmole)				
9-Methoxyolivacine (82)	6.29×10^{10}	5.74×10^{4}	85	0.0077
Uleine (18)	6.29×10^{10}	0.00	10	--

these processes. It is only possible therefore to put forward speculations as to the nature of the difficulties associated with uleine (18) biosynthesis. It could be that at the time of year at which the feeding experiments were conducted that uleine (18) was not being actively biosynthesized, or that some environmental or external condition was not being met which would stimulate its production. It could also be that the time scale of the experiments was too long or of insufficient length for its biosynthesis to be detected.

The problem of membrane transport can also be suggested to account in part for the low incorporation of tryptophan (27) and stemmadenine (13) into the alkaloids which did possess activity. It is undoubtedly probable that the precursors especially tryptophan (27) were also utilized for purposes other than alkaloid production which would also contribute to the low activities obtained. Due to the nature of the feeding technique however, it was highly probable that the proposed precursors were absorbed into the plant before significant external decomposition could take place (absorption time 2-5 hr.).

The only significance that can be attributed to these preliminary incorporation results is that both stemmadenine (13) and tryptophan (27) have been utilized by the plant system to produce methoxyolivacine (82) and guatambuine (25). This does not imply in the absence of enzymic studies that these two compounds are necessarily the precursors existing naturally within the plant. It also cannot be said that because stemmadenine (13) was incorporated into methoxyolivacine (82) that the

transformations that occurred proceeded according to the Potier-Janot postulate.[118]

The biosynthetic results presented here are however the first to be obtained for the pyridocarbazole alkaloid system and do give some indication as to intermediacy of tryptophan (27) and stemmadenine (13) in their biosynthesis. Further biosynthetic investigations are currently being conducted in our laboratory to elucidate the pathway leading to this class of indole alkaloids.

EXPERIMENTAL - PART I

Melting points were determined on a Kofler block and are uncorrected. All ultraviolet (UV.) spectra were recorded in methanol using a Cary 15 recording spectrophotometer. The infrared (IR.) spectra were recorded with a Perkin-Elmer Model 457 spectrophotometer either in chloroform solution (cavity cells 0.5 mm.) or as a nujol mull between sodium chloride plates as indicated. All measurements were made in cm^{-1} and calibration of the spectra was achieved using the 1604 cm^{-1} absorption band of polystyrene. Nuclear magnetic resonance spectra (N.M.R.) were obtained in deuterochloroform solutions (unless otherwise indicated) at 100 MHz on a Varian HA-100 or a Varian XL-100 nuclear magnetic resonance spectrometer. All N.M.R. spectra obtained via the Fourier Transform technique (F.T.) will be so noted and were obtained with the Varian XL-100 instrument. Chemical shifts were given in (ppm) with reference to tetramethylsilane as the internal standard. The multiplicity, integrated areas, and proton assignments are given in parentheses. Mass spectra were determined on an AEI-MS-902 or an Atlas CH-4B mass spectrometer, with high resolution mass spectra determined with the former. Woelm neutral alumina and EM Reagents GF254 silica gel were used for thin and preparative layer chromatography. In part I, unless otherwise specified, ethyl acetate was used as the solvent for development of the chromatoplates. Woelm neutral alumina (Act. III) and Merck silica gel (Act. 2-3) were used for column chromatography.

Radioactivity was measured with a Nuclear-Chicago Mark II liquid scintillation counter in counts per minute (cpm). The radioactivity of the sample

in disintegrations per minute (dpm) was subsequently determined by the external standard technique[133a,b] using a built-in Barium-133 source of gamma radiation. Scintillation cocktails used were either a prepared solution of toluene and a Nuclear-Chicago PPO-POPOP concentrate or a premixed solution of Nuclear-Chicago PCS cocktail. A scintillation counting sample consisted of a solution of the sample dissolved in 1 ml. of methanol and 14 ml. of the appropriate cocktail (total volume 15 ml.). For each sample the background of the vial was predetermined and subsequently subtracted from the measured dpm's for the sample in subsequent calculations. Each sample was counted for a time period long enough for the total counts for the sample, less the total counts for the background to exceed ten thousand counts.

The A. vargasii plants used in this study were grown in the Horticulture Department greenhouse, the University of British Columbia.

Extraction of A. australe (Mull. Argov.)

The Crude methanolic extract[124] (20 gm.) was suspended in 15% acetic acid (350 ml.) and stirred for 1.5 hr. The aqueous suspension was then extracted with petroleum ether (3 x 200 ml.) and insoluble particles that remained in the aqueous phase were removed by suction filtration. The aqueous phase was basified with 10% sodium hydroxide solution to pH 10 and extracted with chloroform (3 x 200 ml.). The chloroform layer was dried and concentrated to give a solid alkaloid extract (1.26 gm.). The very polar components were removed by rapid

filtration through an alumina column (Act III). The olivacine-guatambuine fraction obtained from the filtration was dissolved in chloroform containing a small amount of methanol and pyridine (1 ml.) and then applied to a silica-gel column (100 gm., Act 2-3). Initial, elution with chloroform, followed by ethyl:acetate and ethyl acetate-methanol (10%) gave olivacine (16) (360 mg., 1.8%). Subsequent elution with methanol yielded guatambuine (25) (320 mgs., 1.6%).

The olivacine (16) was recrystallized from methanol to give yellow needles, m.p. 318-325° (lit., m.p. 318-324°).[126] UV.; λmax (log ε): 374 (3.56), 325 (3.76), 291 (4.84), 284 (4.88), 275 (4.72), 265 (4.57), 235 (4.33), 222 (4.40). N.M.R. (FT.): 3.15 (singlet, 3H, C-1 $\underline{CH_3}$), 2.80 (singlet, 3H, C-5 $\underline{CH_3}$). Mass spectrum: M^+, m/e = 246.

Guatambuine (25) was obtained as cream coloured cubes by recrystallization from methanol, m.p. 242-250° (lit., 248-250°).[126] UV.; λmax (log ε): 341 (3.48), 327 (3.63), 297 (4.26), 288(sh) (4.08), 262 (4.36), 250 (4.50), 240 (4.64). N.M.R. (FT.) (Figure 19): 3.90 (quartet, 1H, J = 7 Hz, C-1 H), 2.54 (singlet, 3H, C-5$\underline{CH_3}$), 2.40 (singlet, 3H, N-CH_3), 1.52 (doublet, 3H, J = 7 Hz, C-1 $\underline{CH_3}$). Mass spectrum: M^+, m/e = 264; main peaks: 249, 233, 221-218, 204.

Extraction of A. Vargasii (A.DC.)

Two woody A. vargasii plants (270 gm.) (approximately 5 years old, and containing an extensive root system) were ground up in a Wiley mill (not predried). The collected pulp was suspended in methanol (3 x 300 ml.) and

further mascerated in a Waring blendor (x3). After each masceration the solid plant residues were separated by suction filtration and washed liberally with methanol. The combined methanol filtrates were concentrated to dryness.

The crude methanol extract was suspended in 10% hydrochloric acid (500 ml.) and stirred at room temperature for 0.5 hr. It was then extracted successively with petroleum ether 65-110° (3 x 250 ml.) and chloroform (3 x 250 ml.). The petroleum ether extract was discarded while the chloroform extract (Acid chloroform extract) was dried over sodium sulphate, and concentrated to give a solid mixture (500 mg.)

The aqueous phase was then basified to pH 11 with concentrated ammonium hydroxide and extracted with chloroform (3 x 250 ml.). The chloroform solution (Base chloroform extract) was dried over sodium sulphate and concentrated to give a yellow solid mixture (300 mg.).

The acid chloroform extract was column chromatographed on alumina (30 gm.). The extract was dissolved in hot chloroform and applied immediately to the column. The column was eluted (5 ml. fractions) by gradient elution starting with chloroform followed by chloroform:ethyl acetate mixtures (10% increment increases of ethyl acetate) and finally ethyl acetate-methanol (9:1) (See Table 1).

Fractions 1-8 eluted with chloroform contained apparicine (19) which after recrystallization from acetone yielded colourless needles (105 mg., 3.88 x 10^{-2} %), m.p. 192-194° (lit., m.p. 192-194°), $[\alpha]_D^{26}$ = -165.2^{o} (C. 1.0 in $CHCl_3$), (lit., $[\alpha]_D^{27}$ = ± 177° (C. 2.16 in $CHCl_3$).[113] UV.; λmax

(log ε): 303 (4.50); λmin (log ε): 265 (3.70). N.M.R.: 7.90 (broad singlet, 1H, N-H), 5.42, 5.28 (two singlets, 1H each, $=CH_2$), 5.30 (multiplet, 1H, $=\underline{CH}-CH_3$, partly obscurred by exocyclic methylene singlet), 4.28, 4.58 (AB quartet, 2H, J = 18 Hz, C-1 $\underline{CH_2}$), 1.47 (split doublet, 3H, J = 8 Hz, J = 1.5 Hz, $=CH-\underline{CH_3}$), mass spectrum: M^+, m/e = 264; main peaks: 249, 235, 222, 208. Found: C, 81.51; H, 7.63; N, 10.45%. Calc. for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60%.

Fractions 10-20 eluted with chloroform:ethyl acetate (20-60%) contained uleine (18) which after recrystallization from methanol yielded colourless cubes (33 mg., 1.22 x 10^{-2}%). UV.; λmax (log ε): 301 (4.50). N.M.R.[129]: 8.22 (broad singlet, 1H, N-H), 7.6-7.1 (multiplet, 4H, aromatic), 5.28, 5.00 (two singlets, 1H each, $=\underline{CH_2}$), 4.11 (doublet, 1H, J = 2 Hz, C-1 H), 2.30 (singlet, 3H, $N-\underline{CH_3}$). Mass spectrum: M^+, m/e = 266; main peaks: 251, 237, 223, 222, 209, 208, 207, 194, 180.

Fractions 21-44 eluted with chloroform:ethyl acetate 1:1 up to ethyl acetate:methanol 8% contained guatambuine (25) which after recrystallization from methanol yielded cream coloured cubes (47 mg., 1.74 x 10^{-2}%), m.p. 250-252°, (lit., 248-250°).[128]. UV.; λmax (log ε): 340 (3.50), 325 (3.63), 297 (4.25), 287(sh) (4.08), 260 (4.36), 248 (4.50), 239 (4.64). N.M.R. (FT.): 3.86 (quartet, 1H, J = 6 Hz, C-1 H), 2.46 (singlet, 3H, C-5 CH_3), 2.34 (singlet, 3H, $N-\underline{CH_3}$), 1.45 (doublet, 3H, J = 7 Hz, C-1 CH_3). Mass spectrum: M^+, m/e = 264; main peaks: 249, 233, 221-218, 204. Found: C, 55.85; H, 5.62; N, 6.73%. Calc. for $C_{19}H_{23}N_2I$: C, 56.17; H, 5.71; N, 6.89%.

By preparative layer chromatography (alumina, 1.0 mm., EtOAc) of a

small portion of the Fraction 21-44 material the blue colouration at the tail of the guatambuine (25) spot (turquoise, ceric sulphate) was isolated. UV.; λmax: 303; λmin: 265.

The base chloroform extract was chromatographed on alumina (20 gm.) in an identical fashion. Fractions 1-15 eluted with chloroform contained 9-methoxyolivacine (82) which after recrystallization from methanol yielded yellow needles (100 mg., 3.70 x 10^{-2}%), m.p. 290-292° (lit., m.p. 291-293°).[128] UV.; λmax (log ε): 330 (3.80), 295 (4.70), 269 (4.59), 239 (4.37), 222 (4.32). N.M.R. (FT.): 4.00 (singlet, 3H, $\underline{OCH_3}$), 3.16 (singlet, 3H, C-1 CH_3), 2.80 (singlet, 3H, C-5 CH_3). Mass spectrum: M^+, m/e = 276; main peaks: 261, 245. Found: C, 74.50; H, 6.35; N, 9.02. Calc. for $C_{18}H_{16}N_2O(CH_3OH)$: C, 74.02; H, 6.49; N, 9.09.

Fractions 17-20 eluted with chloroform:ethyl acetate (20-40%) contained a yellowish solid which was further purified by preparative layer chromatography on alumina (1.0 mm., EtOAc:MeOH 20%). A small undetermined amount of a yellow powder corresponding to 3,4-dihydroolivacine (141) by TLC (alumina, EtOAc) was obtained. UV.; λmax: 367, 310, 294, 280, 275, 235.

Fractions 21-35 eluted with chloroform:ethyl acetate (40%) to ethyl acetate:methanol (5%) contained desmethyluleine (84) as a yellow film (46 mg., 1.73 x 10^{-2}%). UV.; λmax: 303; λmin: 265. N.M.R. (FT.): 5.30, 5.00 (two singlets, 1H each, $=\underline{CH_2}$), 4.34 (multiplet, 1H, C-1 H). Mass spectrum: M^+, m/e = 252; main peaks: 235, 233, 209-8, 194-5, 180.

(Ar-3H)-Stemmadenine (13)

Tritiated trifluoroacetic acid was prepared by distillation of trifluoroacetic anhydride (0.80 ml., 5.55×10^{-3} mole) into tritiated water (100 µl., 5.55×10^{-3} mole, 100 mCi) using a vacuum transfer system. The tritiated trifluoroacetic acid was then combined with stemmadenine (13) (25 mg., 7.0×10^{-5} mole) by means of the vacuum transfer system and the resulting acid solution was maintained under a dry nitrogen atmosphere at room temperature for 48 hr. The tritiated trifluoroacetic acid was then distilled off and the residue was taken up in dilute ammonium hydroxide (75 ml.) and extracted with methylene chloride (3 x 30 ml.). The methylene chloride fractions were combined, dried over sodium sulphate, and concentrated to a colourless foam (17 mg., 68%). After six recrystallizations from methanol the measured radioactivity was constant (8.98×10^{7} DPM/mg., 3.21×10^{10} DPM/mmole.) (PPO-POPOP cocktail).

Plant Incorporation Experiment:

The Administration of (Ar-3H)-Tryptophan (27) to *A. vargasii*

(Ar-3H)-Tryptophan (27) (2.33 mg., 2.50×10^{11} DPM/mmole) was dissolved in a solution of methanol:water:acetic acid (1 ml.:4 ml.: 1 drop) and administered to an *A. vargasii* plant (∿130 gm.) by the cotton wick technique. This method required the threading of a cotton string through the stem of the growing plant at a point above the ground, but below the branching point. The intertwined ends of the wick were placed in a 18 x 75 mm. test

tube containing the precursor solution located at the base of the plant. For the remaining period of the incubation after the precursor had been absorbed (2-5 hr.) the test tube was kept full by repeated additions of distilled water. After 7 days under intermittent fluorescent lamp illumination the plants were extracted for their alkaloid content by the procedure previously described. Methoxyolivacine (82), guatambuine (25) and uleïne (18) were isolated by column chromatography on alumina and recrystallized to constant activity (PPO-POPOP cocktail). The quantities isolated, specific activities and percentage incorporations for these compounds are presented in Table II, page 51.

The Administration of (Ar-3H)-Stemmadenine (13) to *A. vargasii*

(Ar-3H)-Stemmadenine (13) (1.96 mg., 3.21 x 10^{10} DPM/mmole) was dissolved in methanol:water (1:4) (5 ml.) and administered to an *A. vargasii* plant (∿130 gm.) by the cotton wick technique. After 7 days under intermittent fluorescent lamp illumination the plants were extracted as previously described: Acid chloroform extract (312 mg. 5.13 x 10^7DPM); Base chloroform extract (247 mg. 1.07 x 10^7DPM). Methoxyolivacine (82), and Uleine (18) were isolated by column chromatography on alumina and recrystallized to constant activity (PPO-POPOP cocktail). The quantities isolated, specific activities and percentage incorporations for these compounds are presented in Table II, page 51.

INTRODUCTION - PART II

The elucidation of the transformations that take place in the biosynthetic pathway leading to the non-tryptamine bridged indole alkaloids by the radioactive labelling technique requires both the synthesis of specifically labelled molecules that are postulated to be their precursors and the development of a degradation scheme for determining the position of the incorporated radiolabels into the alkaloidal products.

To parallel, therefore, the biosynthetic feeding experiments that have been initiated in our laboratory[119] on olivacine (16) and guatambuine (25) in A. australe, a degradation scheme has been developed for these molecules. Further, as a consequence of the availability of large amounts of ellipticine (17)[131] and initially of only limited quantities of olivacine (16) and guatambuine (17) from natural sources, reactions studied in the ellipticine (17) series provided both a model system and an extension of the degradation scheme to include this series.

Initial experiments in A. australe involved feeding labelled forms of secodine (76), however, it is currently thought that secodine (76) occurs to far along the pathway to be a precursor of the non-tryptamine bridged alkaloids. Stemmadenine (13) is considered to be the direct progenetor of these compounds and it is also considered that the equilibrium between dehydrosecodine (72) and stemmadenine (13) is responsible for the observed low incorporations of secodine (76) into olivacine (16) and guatambuine (25). As a result, stemmadenine (13) is currently in the stages of being synthesized in our laboratory[119,120,130] with the objective in mind of being able to unambiguously introduce labelled

atoms into the positions indicated in figure 20.

In accord with the Potier-Janot postulate,[118] the labelled atoms in stemmadenine (13) would end up in the positions indicated in olivacine (16), guatambuine (25), ellipticine (17) and N-methyltetrahydroellipticine (26). The arrow connecting tryptophan (27) and stemmadenine (13) indicates where a label in the C-2 position of tryptophan (27) would occur in stemmadenine (13) and subsequently in the alkaloids. As can be seen, with the exception of the labelled carbomethoxy grouping which may be incorporated into the C-5 position in the "C" ring,[108] the labelled atoms all occur in the "D" ring.

The degradation scheme developed, therefore, was devised to permit the isolation of the C-1 methyl, C-2 methyl (N-methyl), and the C-3 carbon atom in the olivacine (16) series and similarly the C-2 methyl and the C-3 carbon atom in the ellipticine (17) series.

This scheme necessarily had to take into account that generally only small amounts of alkaloids (20-50 mg.) with low specific activities were available from plant incorporation experiments. The demands of having to work on a small scale without the option of diluting with unlabelled materials placed constraints upon the developed sequence. The reactions involved had to be simple i.e. not involving any complex work-ups, etc., very efficient and reproducible in terms of yields, and also, the overall sequence should involve a minimum number of reactions.

A further and important stipulation was that the end products had to be easily purified and in crystalline form either in their natural state, or as a suitable derivative for radioactive counting.

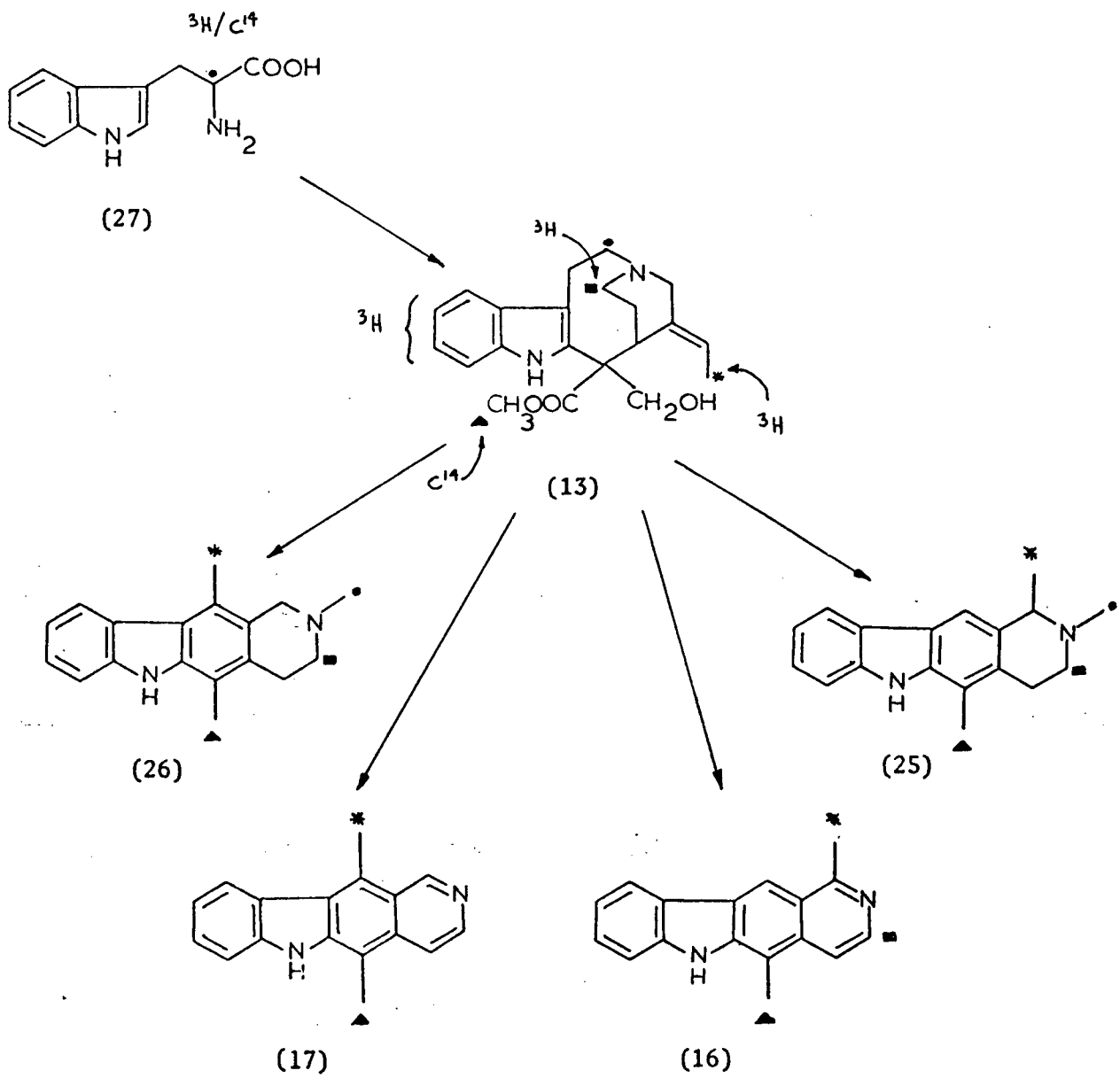


Figure 20. The Labelling Pattern for the Biosynthesis of the Olivacine (16) and Ellipticine (17) Series According to the Potier-Janot Postulate.

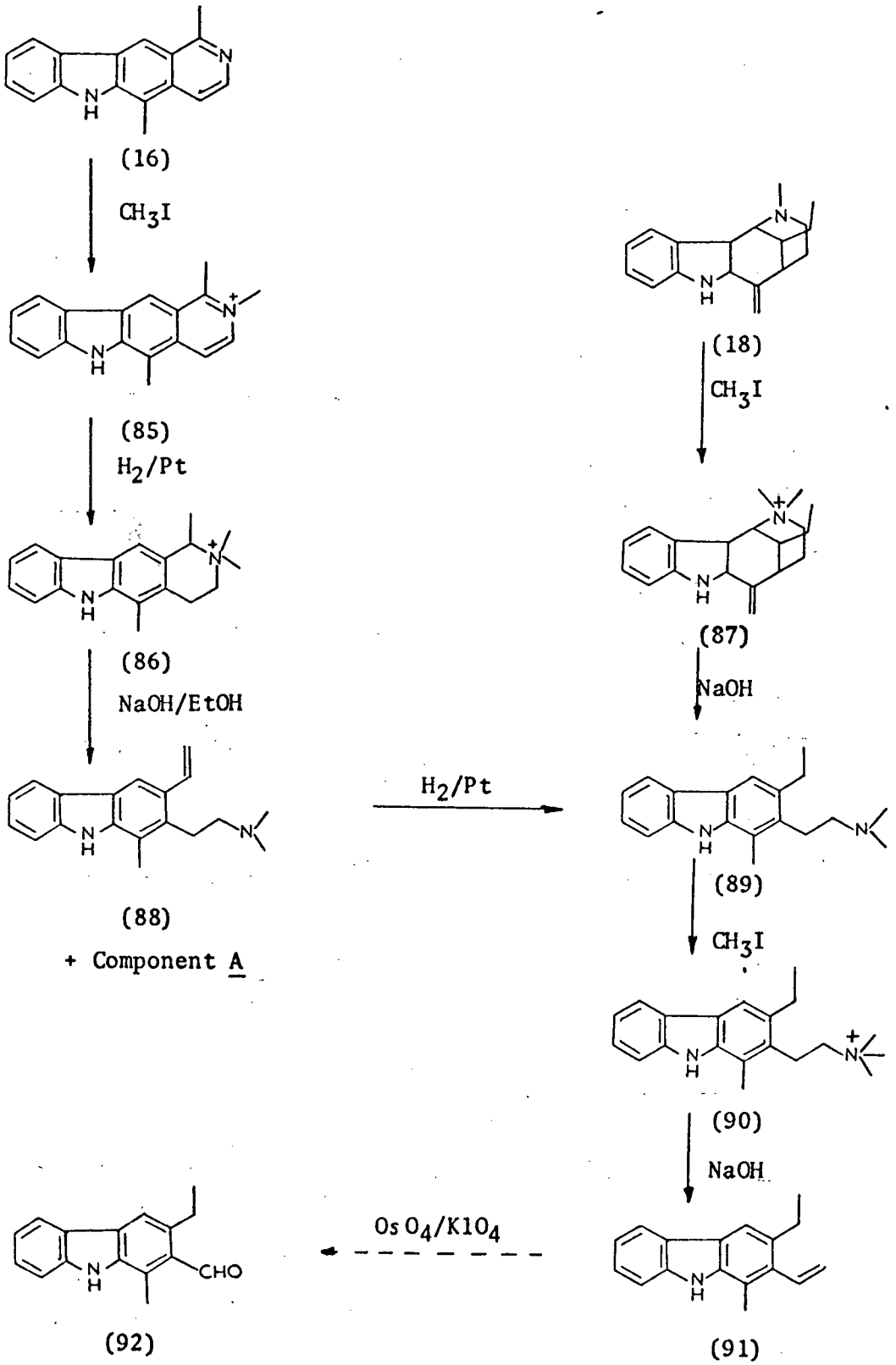


Figure 21. The Structure Elucidation of Olivacine (16) by Ondetti and Deulofeu (1961).

Very little chemistry of the olivacine (16)-guatambuine (25) system has been studied to date, however, the work by Ondetti and Deulofeu (1961)[121-3] was particularly applicable to the desired degradation of the olivacine (16) and ellipticine (17) "D" rings (Figure 21). These workers elucidated the structure of olivacine (16) by its conversion via its methiodide (85) to guatambuine (25) and a subsequent comparison of the Hofmann degradation products obtained from guatambuine methiodide (86) with those obtained from uleine (18) via its methiodide (87). It was found that guatambuine methiodide (86) undergoes ring opening under Hofmann conditions (NaOH/EtOH) to give two major products. The main component A (32%) was not characterized, but it was determined by lack of hydrogen uptake to be a non-olefinic material, and the microanalysis of its methiodide correlated with the composition $C_{20}H_{25}N_2I$. The second component (22%) after hydrogenation to 89 and conversion to its methiodide 90 was proposed to have the olefinic structure 88 by comparison of IR., UV., melting point, and mixed melting point with methiodide 90 shown by Schmutz et al.[132] to be obtained from uleine (18). Support for their assignments were further strengthened by a comparison of the IR., UV., and melting points of the second Hofmann product 91.

Buchi et al.[135] working concurrently in this area cleaved the styryl side chain of 91 under osmium tetroxide-periodate conditions to give the carbazole aldehyde 92.

The work of these authors was however incomplete in many respects. The correlation of the degradation products of the two alkaloids led to the correct assignment of the structure of olivacine (16) and guatambuine (25), however it is

difficult to indicate with certainty that the comparisons are valid based upon the IR., UV. and rudimentary physical data. No data were obtained for the alternate Hofmann product 97 that could arise from guatambuine (25), a compound whose spectral data, and quite possibly whose melting point would bear a close resemblance to compounds 88 and 89. Only through the application of N.M.R. and mass spectroscopic techniques (which were not available to these authors) could the structure of olefin 88 be unambiguously determined.

However, this sequence potentially permits the isolation of all three desired centers in olivacine (16) and guatambuine (25) and consequently it provided the basis on which the degradation of these two alkaloids as well as the ellipticines (17) and 26 was developed.

DISCUSSION - PART II

1. Interconversions in the Olivacine (16)-Guatambuine (25) and Ellipticine (17)-N-Methyltetrahydroellipticine (26) Series.

The conversion of olivacine (16) and ellipticine (17) to their corresponding N-Methyltetrahydro counterparts guatambuine (25) and N-methyltetrahydroellipticine (26) was necessary for the totally aromatic structures were not in a suitable form to undergo reactions that would effect scission of the pyrido "D" ring.

Ondetti and Deulofeu[121-3] have converted olivacine (16) to guatambuine (25) by formation of the methiodide of olivacine 85 followed by catalytic hydrogenation under low pressure (Figure 22). In a similar fashion Goodwin, Smith, and Horning[136] converted ellipticine (17) to N-Methyltetrahydroellipticine (26) by formation of its methiodide 93 and subsequent reduction of the pyridinium salt with sodium borohydride. Buchi *et al.*[137] developed the first portion of an alternate method of transforming the "D" ring of ellipticine (17). They found that ellipticine (17) underwent facile hydrogenation at atmospheric pressure to give the tetrahydroderivative 94. By subsequent simple methylation it would be possible to obtain the methiodide of N-methyltetrahydroellipticine (95).

The transformation of ellipticine (17) to its N-methyltetrahydro counterpart presented no difficulties. Both routes were investigated but it was found

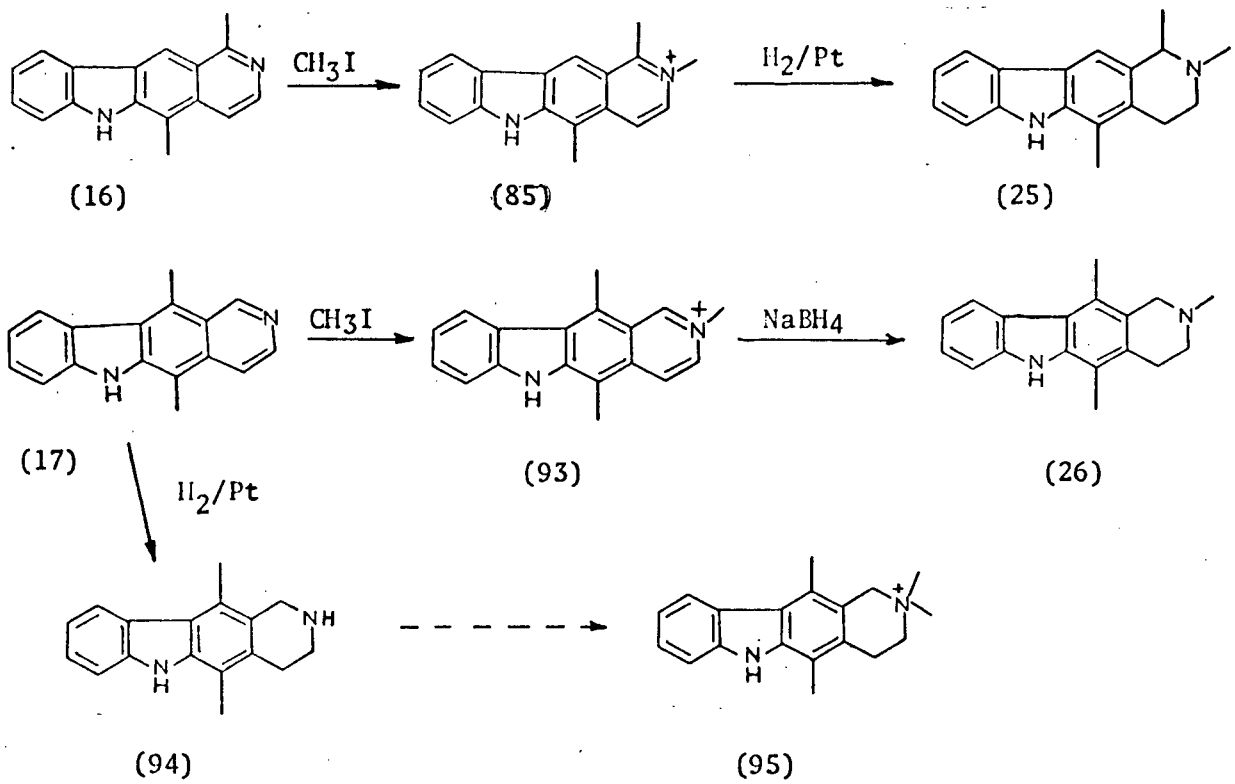


Figure 22. The Conversion of Olivacine (16) and Ellipticine to their N-Methyltetrahydro Forms.

that the sequence of Goodwin *et al.*[136] was the simplest to follow.

The formation of ellipticine methiodide 93 never went totally to completion despite the use of a great excess of methyl iodide. This necessitated the removal of the small amount of residual starting material before reduction because its presence made subsequent purification by either recrystallization or column chromatography difficult. This was most easily accomplished by a rapid column chromatography on alumina. The methiodide was efficiently eluted from the column with methanol:ammonium hydroxide (1%) to give bright orange crystals after concentration (77%).

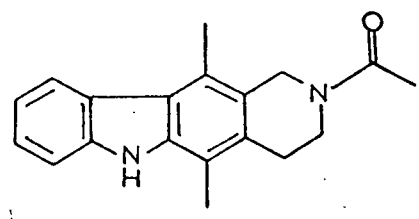
Reduction of the pyridinium system with sodium borohydride gave N-methyltetrahydroellipticine (26) as cream coloured crystals. It was observed that the crude reaction mixture began to yellow on standing, even in a vacuum dessicator. This decomposition was checked by recrystallization from methanol to give cream coloured needles (91%).

The spectral data (UV., N.M.R.) for 26 agreed closely with that previously reported.[126, 128] The N.M.R. spectrum possessed two singlets at δ2.56 and 2.70 for the aromatically substituted methyl groups at C-5 and C-11 respectively. Two other singlets were also observed, one for the N-methyl group (δ2.38), and the other for the isolated methylene group at C-1 (δ3.74). A triplet occurred at δ2.98 (J = 5Hz) for the C-4 methylene protons while the corresponding triplet for the C-3 methylene protons (δ2.76) was partly obscured by the singlet (δ2.70) for the C-11 methyl group.

The conversion of 26 to its methiodide 95, in preparation for Hofmann degradation, by reaction with methyl iodide in methanol proceeded in quantitative yield to give colourless needles.

The catalytic hydrogenation of ellipticine (17) with platinum oxide developed by Buchi *et al.*[137] was slightly modified by substituting acetic acid for ethanol as the solvent. It was found that with acetic acid a cleaner product was obtained. The hydrogenation was complete after several hours at atmospheric pressure to give a highly fluorescent pale blue solution which after work-up yielded a colourless crystalline product 94. As reported, the tetrahydro compound 94 was considerably sensitive to air, turning yellow on standing. For this reason a small portion of it was characterized as its

N-acetyl derivative 96 and the remainder was converted directly to N-methyl-tetrahydroellipticine methiodide 95 by reaction with methyl iodide.



(96)

The efficient conversion of olivacine (16) to guatambuine (25) unlike the transformation of ellipticine (17) was unexpectedly found to be very difficult. The major hurdle in the synthesis proved to be the methylation of olivacine (16) to its methiodide 85.

Reaction of olivacine (16) with methyl iodide in ethanol under a wide range of conditions from room temperature to a sealed tube at 150° resulted consistently in only a 30% conversion to its methiodide. A persistent problem with the reaction also was the presence of considerable quantities of unidentified dark coloured material which hampered work-up. The salt 85 was obtained in low yield as a yellow microcrystalline solid. For purposes of degradation the yield of the methiodide was increased to 80% by repeated recycling of reaction mother liquors which contained unreacted olivacine (16).

The problem of formation of methiodide 85 was eventually overcome by using methylfluorosulphonate as the alkylating agent[138] and acetonitrile as

the solvent medium. The reaction proceeded practically instantaneously with methofluorosulphate formation. The salt was not isolated (due to the presence of flurosulphuric acid as a high boiling contaminant) but reduced directly to give guatambuine (25) as described below.

In a similar fashion to methiodide formation it was found that catalytic hydrogenation of olivacine methiodide (85) with platinum oxide at low pressure (5 atm., 80°) proceeded in poor or more correctly inconsistent yield.

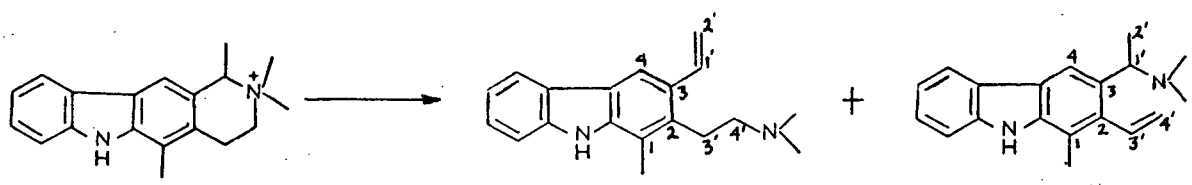
On the other hand reduction of the pyridinium ring of 85 (methiodide or fluorosulphate) proceeded readily and in excellent yield by reaction with sodium borohydride. In this manner guatambuine (25) was obtained as a pure cream coloured crystalline solid (93%). The spectral data for this compound was identical to that previously obtained (Part I).

The subsequent conversion of guatambuine (25) to its methiodide 86 proceeded in quantitative yield by reaction with methyl iodide.

A preliminary attempt was made to by-pass olivacine methiodide (85) formation by direct hydrogenation of olivacine (16) in an analogous fashion to that developed for ellipticine (17). Even after prolonged hydrogenation under pressure (2.5 atm.) considerable starting material remained and the product that was formed, as previously observed, proved to be unstable in air thereby adding complications to its isolation and purification. This approach was consequently abandoned.

2. Hofmann Elimination:

From a consideration of the mechanism of the Hofmann elimination reaction two structurally isomeric olefinic products 88 and 97 can be anticipated to be formed during the base induced (Hofmann conditions) ring opening of the methiodide of guatambuine (25).



(86) (88) (97)

In accord with the Hofmann rule[139] it could be anticipated that in an unsymmetrical cyclic amine like guatambuine (25) there would be a preferential formation of one olefinic product over the other. In the vast majority of cases the hydrogen removed during elimination derives from the least substituted carbon atom, this leading in turn to the least substituted olefin. In the guatambuine system elimination in either direction leads to an unsubstituted olefinic product, however it might be expected on the above consideration that elimination in the direction of the C-1 methyl group leading to the C-3 olefin 88 would be the preferred course.

However, an overriding influence to this general trend results from the

presence of a relatively acidic benzylic hydrogen on the C-4 carbon atom. In situations where a benzylic hydrogen can become involved in the β-elimination process, elimination in its direction tends to predominate. The predominant elimination product anticipated for guatambuine (25) would therefore be the C-2 olefin 97.

For purposes of degrading guatambuine (25) the production of both olefinic products by Hofmann elimination would be ideal for then a simple oxidative cleavage of both double bonds would complete the isolation of the two desired carbon atoms. In the situation where only one of the two possible olefins is isolated oxidative cleavage followed by a second Hofmann reaction again completes the sequence.

From the results of Ondetti and Deulofeu[121-3] it appeared feasible that the C-3 olefin 88 proposed by them to be the only unsaturated product formed by reaction with sodium hydroxide in refluxing ethanol could be isolated in quantities large enough to degrade. On repeating their reaction on a small scale (50 mg.) two products were again observed, however it was determined from the measured integration of the N.M.R. signals that the product ratio had altered from that reported (Figure 23), only 5-10% of the product mixture corresponded to olefinic material.

The major non-olefinic component isolated in 60% yield exhibited spectral data consistent with structure 98.

The N.M.R. spectrum had a quartet at δ4.85 (J = 6Hz) and a corresponding doublet at δ1.53 (J = 6Hz) for the methine hydrogen and the methyl group which are substituted at the C-1' carbon atom (see 97 for numbering system) and are

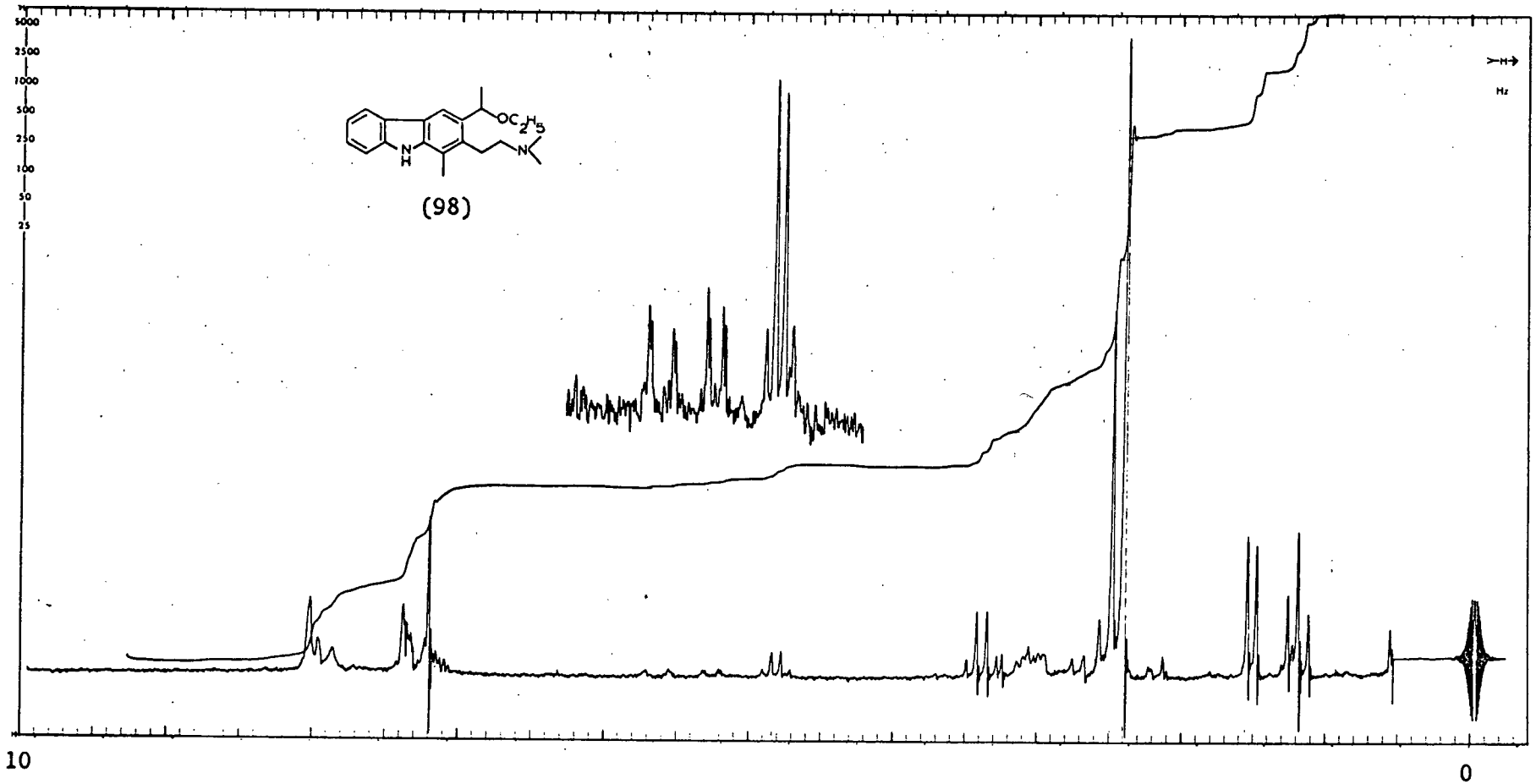
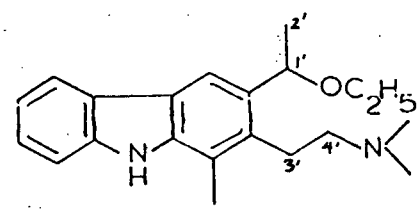


Figure 23. The N.M.R. Spectrum of the Crude Reaction Mixture (NaOH/EtOH).

adjacent to the oxygen atom. The increased deshielding effect of the oxygen compared to nitrogen resulted in the substantial downfield shift of the methine quartet with respect to the corresponding quartet in the spectrum for guatambuine (25) (Figure 19., Table III). Two singlets occurring at δ2.49 and 2.40 were attributed to the C-1 methyl group and the dimethylamino group respectively. A multiplet centered at δ3.04 was attributed to the C-3' methylene protons whereas the multiplet for the C-4' methylene protons was obscured by the singlets for the above mentioned methyl groups. The quartet (δ3.38, J = 7Hz) and triplet (δ1.18, J = 7Hz) absorptions were assigned to the methylene and methyl group respectively of the substituted ethoxy group.



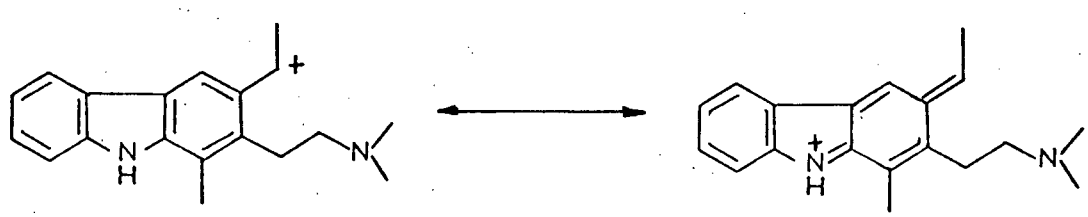
(98)

The mass spectrum possessed a parent peak at m/e = 324 and only two major fragmentations at m/e = 295, 278 for the α-cleavage of the ether linkage in both possible directions. The high resolution mass spectrum parent peak at m/e = 324.2207 was within acceptable limits of the value 324.2200 calculated for $C_{21}H_{28}N_2O$.

For purposes of comparison with the unassigned component A isolated by Ondetti and Deulofeu,[121-3] compound 98 also possesses a normal carbazole UV.

spectrum. It is highly probable that the same product has been isolated in both instances. This assumption is strongly supported by the observation that their microanalytical data is within experimental error for both the molecular composition $C_{20}H_{25}N_2I$ proposed by them for component A and the calculated values for the composition $C_{22}H_{31}N_2OI$ for the methiodide derivative of compound 98.

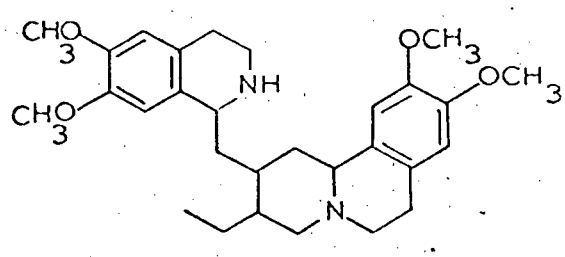
It is apparent that 98 is formed by substitution of the quaternary nitrogen of guatambuine methiodide by ethoxide ion generated in the reaction medium. Substitution reactions are always a competing process with elimination and as will become more evident in later discussion, the benzylic center (C-1) onto which the nitrogen is attached in guatambuine (25) as well as in uleine (18)[140] and apparicine (19)[113] is considerably activated towards substitution processes. This is to a major extent a consequence of stabilization of the benzylic carbonium ion that is formed during reaction by the lone pair of electrons on the indole nitrogen oriented para to it.



The intermediacy of such a stabilized benzylic carbonium ion implies that the substitution reaction occurs by a SN_1 type mechanism. This further implies that on formation of substitution products the optical activity of the asymmetric benzylic center in guatambuine (25) would be lost. Such has been found

to be the case when the (-)-methiodide 86 was treated under the strongly basic reaction conditions employed by Ondetti and Deulofeu.

It has been demonstrated also in closely related systems exemplified by the aromatically oxygenated tetrahydroisoquinoline alkaloids such as emetine (99)



(99)

that the cyclic six-membered ring structure does not undergo substitution reaction unless strong basic conditions normally associated with SN_2 reactions are employed.[139,141-3] The probable rationale for this requirement is that sufficient driving force must be provided to overcome the tendency of the nitrogenous leaving group to revert back to the quaternary amine by internal return.

Considerable effort was made to isolate the minor olefinic product from the crude mixture, however even by repetitive column chromatography of olefin enriched fractions it was not possible to adequately eliminate the presence of the substitution product 98 as contaminant. It was eventually possible though to determine with a good degree of confidence the identity of the olefin produced during the reaction. This assignment was inferred from the N.M.R. spectrum (Figure 23) by a comparison with the chemical shifts for the

components of the α- and β-dimethylaminoethyl, and vinyl side chains found in the olefinic and saturated forms of the ring-opened products of guatambuine (25) (Table III).

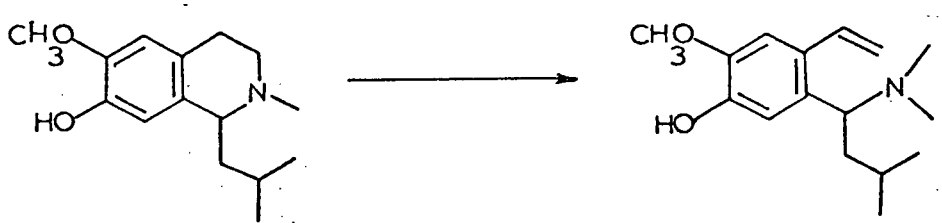
As will be alluded to again in subsequent discussion the two major distinguishing features between the two possible elimination products are: the relative positions of the singlet for the protons of the dimethylamino group as it occurs in the C-2 and C-3 side chains and ii) the chemical shifts for the cis and trans - coupled olefinic hydrogens.

It can be seen from Table III that the singlet for the dimethylamino group in the C-3 side chain of compounds 97, 103, and 98 occurs at higher field than does the singlet for the same group in the C-2 side chain of compounds 88 and 89. The absence therefore of a peak at ∿ δ2.25, for the C-3 side chain, integrating for 10% of the dimethylamino group singlet at δ2.40 would suggest that the elimination product possesses a β-dimethylaminoethyl side chain at C-2 and that it has structure 88. This assignment was confirmed as a result of the occurrence of the trans coupled hydrogen of the double bond to lowerfield (δ5.68, $J_{1',2'}$ = 17Hz) than the cis coupled hydrogen (δ5.29, $J_{1'2'}$ = 11Hz). This justaposition of the olefinic signals is characteristic of the C-3 vinyl substituent.

The N.M.R. data substantiated the production of the ring-opened olefin 88 under Hofmann conditions as predicted by Ondetti and Deulofeu.[121-3] It was somewhat surprising that it was the only unsaturated product produced by the reaction since the majority of structurally similar molecules such as the tetrahydroisoquinolines salsolidine (100)[144] and lophocerine (101)[145] ring open so

as to give the alternate Hofmann product which is predicted by the Hofmann rule to be the predominant product.

(100)



(101)

It would appear evident from the observed formation of the substitution product 98 with the olefin 88 as the minor side product that the benzylic carbonium ion mechanism plays a central role in the reactivity of guatambuine methiodide (86) under sodium hydroxide in ethanol conditions. The formation of the small amount of the elimination product 88 during the reaction can be envisaged to result from a competing neutralization of the carbonium ion by loss of a proton from the adjacent methyl center (E_1 elimination).

The lack of substantial quantities of the elimination product 88 in the reaction mixture necessitated the study of other reaction conditions to increase

its overall yield or that of the alternate elimination product 97. The substitution product formed in 60% yield wasn't, but could have been used for subsequent isolations of the N-methyl group (see Section 3.) because the ether functionality would be essentially inert to the basic reaction conditions used and its presence therefore would not lead to any complications.

To try and enhance the formation of olefinic product during the Hofmann reaction, potassium t-butoxide in t-butanol was selected as the base. The reason for this choice being that potassium t-butoxide is a stronger base and weaker nucleophile than sodium ethoxide and it would be less prone therefore to produce undesirable substitution products during reaction.

The N.M.R. spectrum for the crude reaction mixture did indicate that the product ratios had altered significantly. A small percentage of substitution product was produced as deduced from the presence of a singlet at δ 1.28 for the hydrogens of the t-butyl group, however the predominant portion of the reaction mixture consisted of a mixture of two olefinic products (Figure 24). From the measured integration of the two singlets for the dimethylamino groups the olefinic product ratio was determined to be 60:40 (compound 97:88).

During initial experiments (30 mg. scale) isolation of the components of the reaction mixture was accomplished by preparative layer chromatography. Only one product corresponding to the major spot on TLC could be isolated in yields (30%) which enabled spectral data to be obtained.

From the spectral data the identity of the olefinic compound was consistent with the structure 97 which is the expected product on the basis of the Hofmann rule, but which is opposite to that reported by Ondetti and Deulofeu.[121-3]

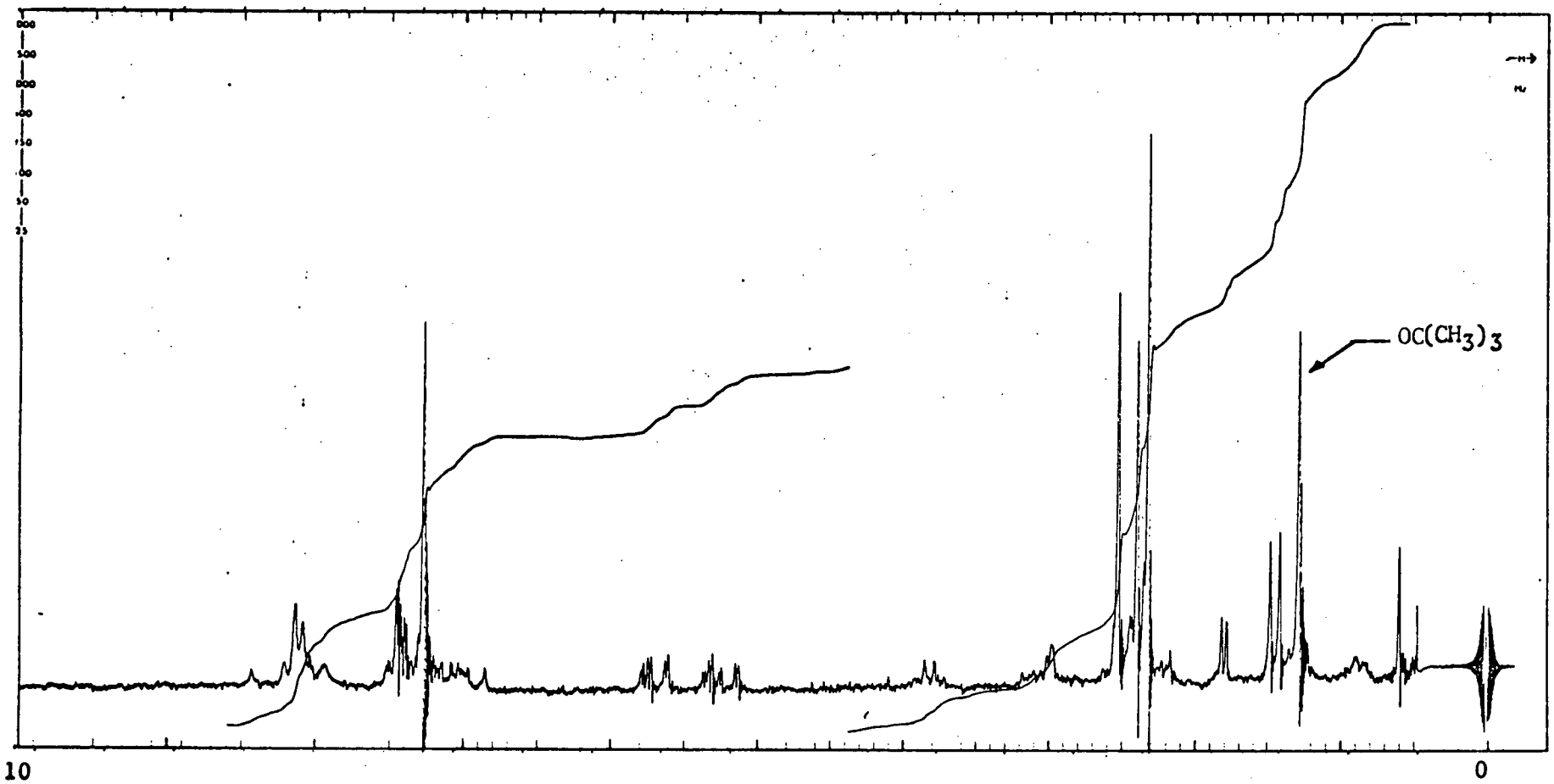
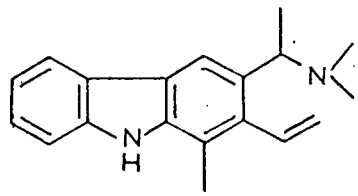


Figure 24. The N.M.R. Spectrum of the Crude Reaction Mixture (KOt-Bu/t-BuOH).



(97)

Identifying features in the N.M.R. were the doublet (δ1.39, J = 6Hz), quartet (δ3.75, J = 6Hz), and singlet (δ2.25 for the methyl group, methine hydrogen and dimethylamino group respectively for the side chain at C-3 of the carbazole system (Figure 25). A singlet was also present at δ2.50 for the protons of the C-1 methyl group. The signals for the three hydrogens of the vinyl side chain at C-2 portrayed a typical ABX system. A pair of doublet of doublets occurred in the olefinic region, one at δ5.20 ($J_{3',4'}$ = 17Hz, $J_{4',4'}$ = 2Hz) for the trans C-4' hydrogen, the other at 5.65 ($J_{3',4'}$ = 11Hz, $J_{4',4'}$ = 2Hz) for the cis C-4' hydrogen. A series of four lines was also observed downfield at δ6.98 ($J_{3',4'}$ = 17Hz, $J_{3',4'}$ = 11Hz) for the C-3' hydrogen of the double bond.

The difference in the magnitude of the coupling constant enabled the cis and trans hydrogens to be distinguished and it is of interest that the chemical shift positions of these resonances in olefin 97 are reversed from that observed for styrene. This characteristic feature may be a consequence of steric or electronic interactions of the N-dimethylamino group with the vinyl system which results in a reversal of the influence that the aromatic ring has on the two terminal hydrogen atoms.

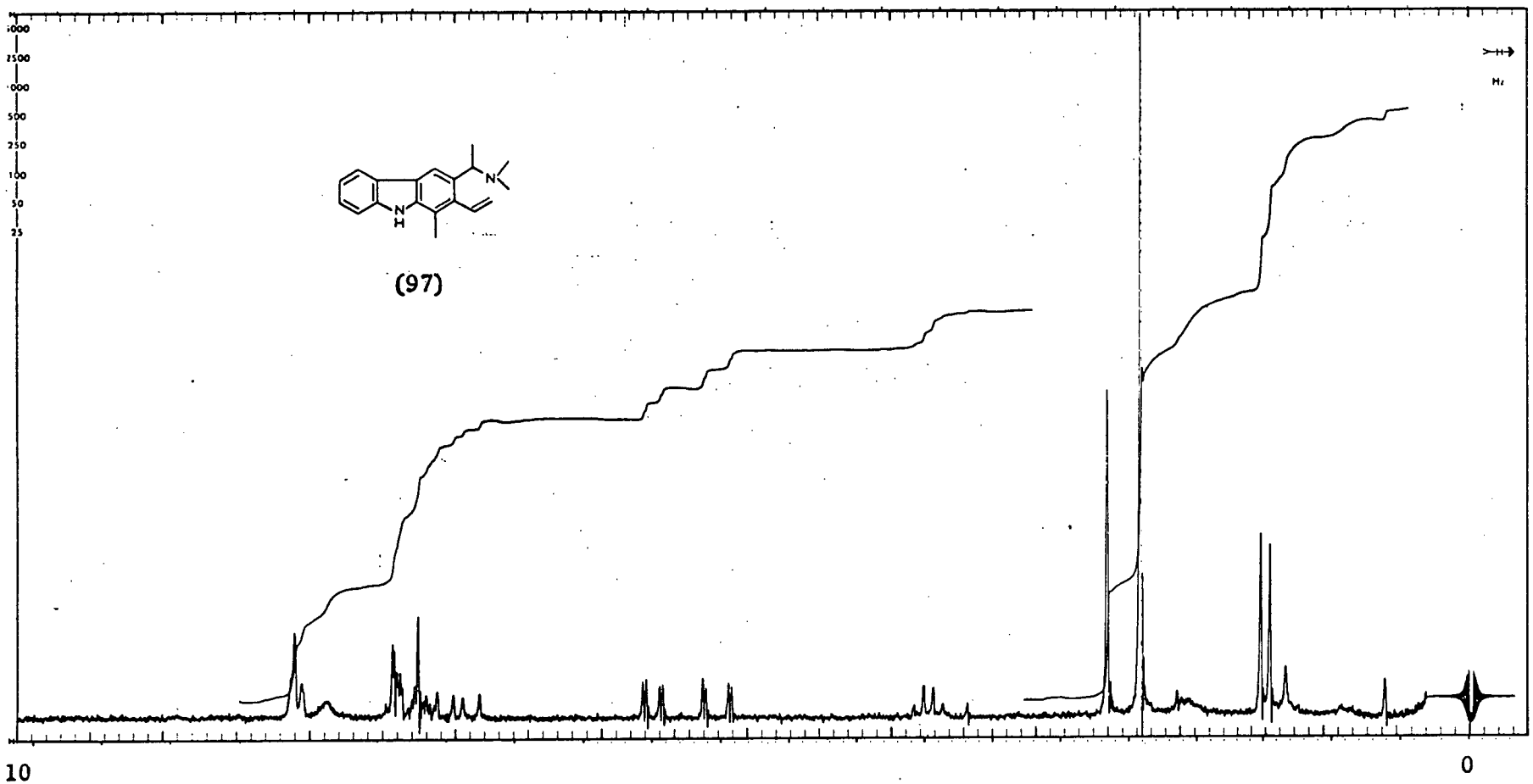


Figure 25. The N.M.R. Spectrum of 1-Methyl-2-vinyl-3-(α-dimethylaminoethyl)carbazole (97)

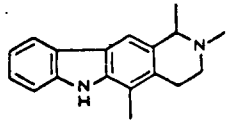
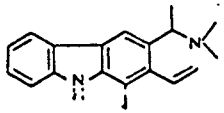
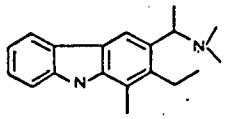
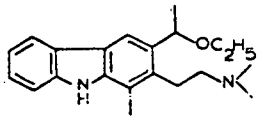
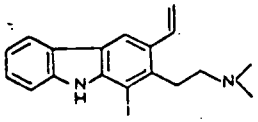
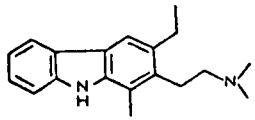
A parent peak was observed at m/e = 278 in the mass spectrum of 97, and major fragmentation peaks came at m/e = 263, 234, 233, 219, 218, 204. The fragmentation reactions that occur for the series of C-2 and C-3 unsaturated and saturated ring-opened products are very characteristic and for this reason a discussion of the mass spectrum will be reserved till later (Section 5). The parent peak at m/e = 278.1780 in the high resolution spectrum was within acceptable limits of the calculated value m/e = 278.1782 for the composition $C_{19}H_{22}N_2$.

The interaction of the vinyl system with the carbazole back bone caused only a slight modification of the normal carbazole UV. spectrum. There was a bathochromic shift of 10mm to λ_{max}247 for the predominant absorption. The spectrum obtained for olefin 97 bore no resemblance to the highly modified carbazole UV. spectrum reported[121-3] for olefin 88.

Prior to the eventual isolation of the second elimination product from the reaction by column chromatography its identity was also deduced by inference from the N.M.R. spectrum of the crude reaction (Figure 24). As previously discussed, it has been shown from the data presented in Table III that the two different nitrogen containing side chains for the ring-opened products could be distinguished by the position of the chemical shift for the respective dimethylamino group.

Two singlets for the dimethylamino group of both side chains are present in the spectrum of the crude reaction mixture. The singlet at δ2.32 corresponded to the C-3 side chain of the major olefinic product 97 isolated from

Table III: Characteristic N.M.R. Chemical Shifts for the Degradation Products of Guatambuine (25)

Structure:	Chemical Shift: δC-1 CH_3	δN$(CH_3)_2$	δC-1'H[1a]	δC-1'CH_3[1b]	δ = CH_2(trans)[1c]	δ = CH_2(cis)[1d]
 (25)	2.54[f]	2.40	3.90	1.52	-	-
 (97)	2.50	2.25	3.75	1.39	5.20	5.65
 (103)	2.49	2.29	3.62	1.42	-	-
 (98)	2.49	2.40	4.85	1.53	-	-
 (88)	2.50	2.38	-	-	5.68	5.29
 (89)	2.64[1e]	2.54	-	-	-	-
(89) from LAH on (25)	2.52	2.46	-	-	-	-
(89) from (18) degradation	2.48	2.38	-	-	-	-

1a. (q,J = 6Hz), b. (d,J = 6Hz), c. (J_{XB}= 17 Hz,J_{BC} = -2.0Hz) d. (J_{XC} = 11 Hz)
e. (FT)N.M.R., very low concentration. f in guatambuine This carbon is C-5

the reaction mixture. It was very probable therefore from the correlations that the remaining singlet peak (δ2.40) corresponded to the C-2 side chain present in olefin 88. A pair of doublet of doublets in the olefinic region (δ5.70 (J = 17Hz, 2Hz); δ5.32 (J = 11Hz, 2Hz)) could be distinguished from these attributed to olefin 97. From the measured coupling constants it was determined that the chemical shifts of the cis and trans-oriented terminal hydrogens coincided with those for styrene but were reversed with respect to the resonances for the elimination product 97. This would be expected for compound 88 for no steric or electronic interaction would be anticipated between the C-3 vinyl group and the dimethylamino group.

It was not possible to conclusively prove the structure of the unisolated olefin from the N.M.R. spectrum of the crude mixture, however the data are consistent with the postulated structure 88.

During the initial experiments it was thought that only the predominant Hofmann product 97 could be isolated in adequate yield and that it would not be possible to isolate the C-3 olefinic product 88 directly by the Hofmann degradation approach. It became necessary in order to determine any activity at the C-1 methyl of guatambuine (25) in subsequent incorporation experiments to find an alternate method which would generate the C-3 vinyl side chain in a ring-opened compound.

To this end the C-2 olefin 97 became of particular importance for not only could it be oxidatively degraded so as to isolate the C-3 carbon atom of guatambuine (25), but its potential second Hofmann elimination product would contain the desired C-3 (carbazole numbering) vinyl side chain. The

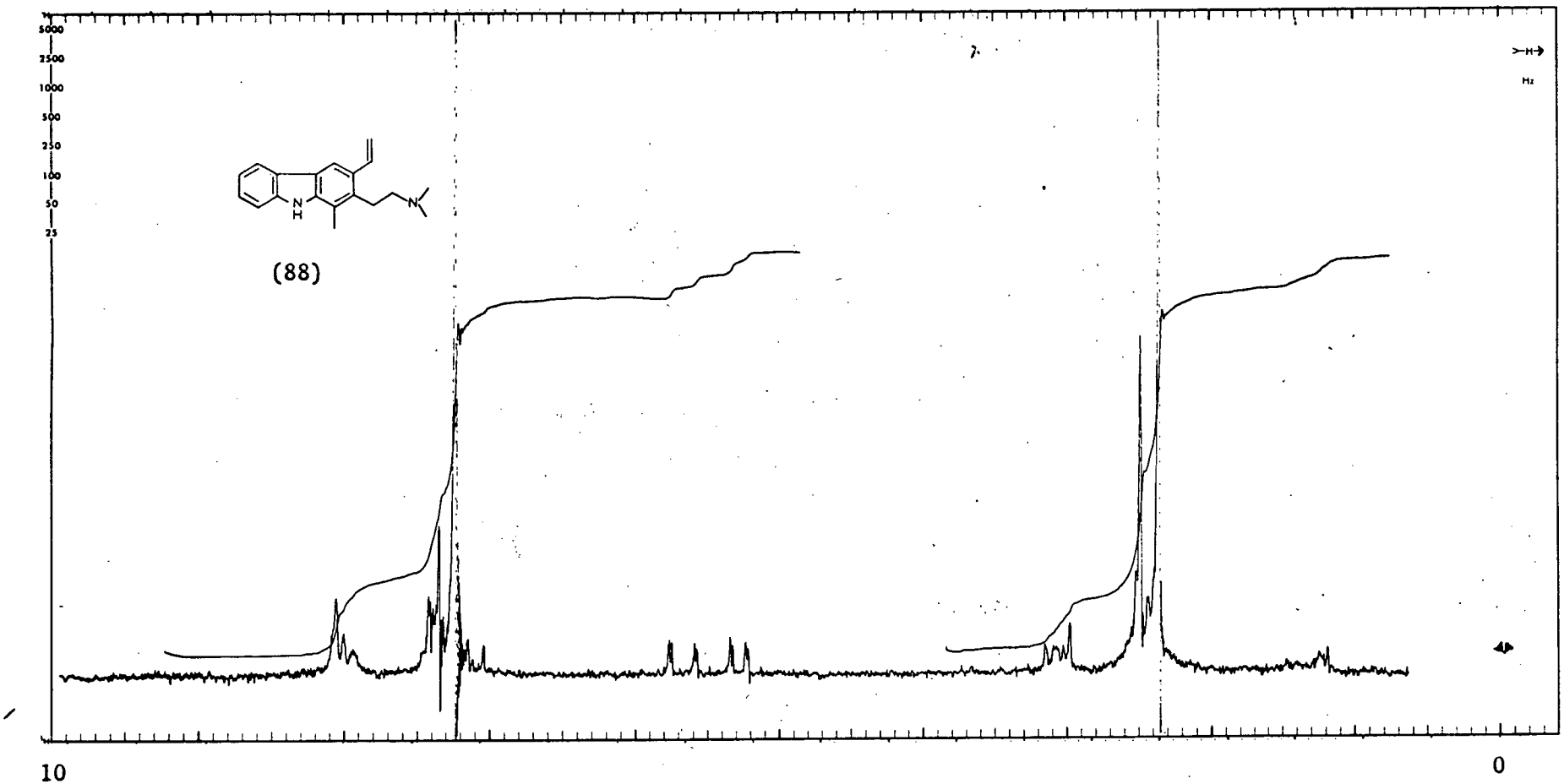
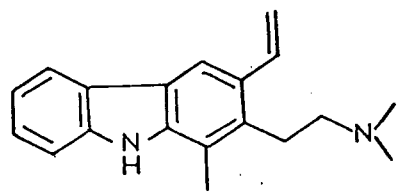


Figure 26. The N.M.R. Spectrum of 1-Methyl-2-(β-dimethylaminoethyl)-3-vinylcarbazole (88).

development of this latter chemistry and the study of a number of alternate methods aimed at producing a ring-opened compound with the C-3 vinyl side chain comprises a considerable portion of the work on the degradation sequence described in subsequent sections.

It has been found however from further work that by purifying the crude reaction mixture by column chromatography on alumina that it became possible to isolate the C-3 olefin 88 in 18% yield (olefin 97 was isolated in 36% yield).



(88)

The N.M.R. spectrum (Figure 26.) as anticipated possessed a singlet at $\delta 2.38$ which was in close proximity ($\Delta\delta 0.12$) to the singlet for the C-1 methyl group ($\delta 2.50$). A multiplet centered at $\delta 3.08$ integrated for the benzylic methylene protons of the β-dimethylamino side chain while the multiplet for the remaining two protons was buried beneath the two singlet absorptions for the respective methyl groups. Two pairs of multiplets (doublets of doublets) occurred in the olefinic region for the terminal C-2' hydrogen atoms. The shift of the <u>trans</u> coupled C-2' hydrogen $\delta 5.68$ ($J_{1',2'}$ = 17Hz, $J_{2',2'}$ = 2.0 Hz) to lower field with respect to the one which is coupled in a <u>cis</u> fashion $\delta 5.29$ ($J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 2.0 Hz) was shown to be characteristic for the C-3 olefinic side chain as suspected from the N.M.R. spectrum of the crude

reaction mixture.

The mass spectrum of 88 possessed a parent peak at m/e = 278 with major fragmentations found at m/e = 263, 249, 234, 233, 220, 219-8, 204 (see section 5 for a detailed discussion). The high resolution spectrum possessed a parent peak at 278.1780 which was consistent with the composition $C_{19}H_{22}N_2$ for compound 88.

The UV. spectrum of 88 bore no resemblance to either the normal carbazole spectrum or the spectrum obtained for the C-2 olefin 97 (Figure 27). The gross dissimilarities in the UV. spectra of 88 and 97 and the close coincidence of the values obtained in the spectrum of 88 with the reported values by Ondetti and Deulofeu[121-3] proved conclusively that they had proposed the correct structure for the elimination product isolated in their study.

It would have been difficult to have predicted beforehand either the shape of the absorption patterns for the two elimination products 88 and 97 or the fact that the difference in C-2, C-3 substitution patterns would result in completely different spectra for the two compounds. Now that these data are available it is possible to consider the interactions of the vinyl groups in the two positions with respect to the carbazole ring system and postulate that the differences may be due to the different resonance contributions in the excited states of 88 and 97 (as shown).

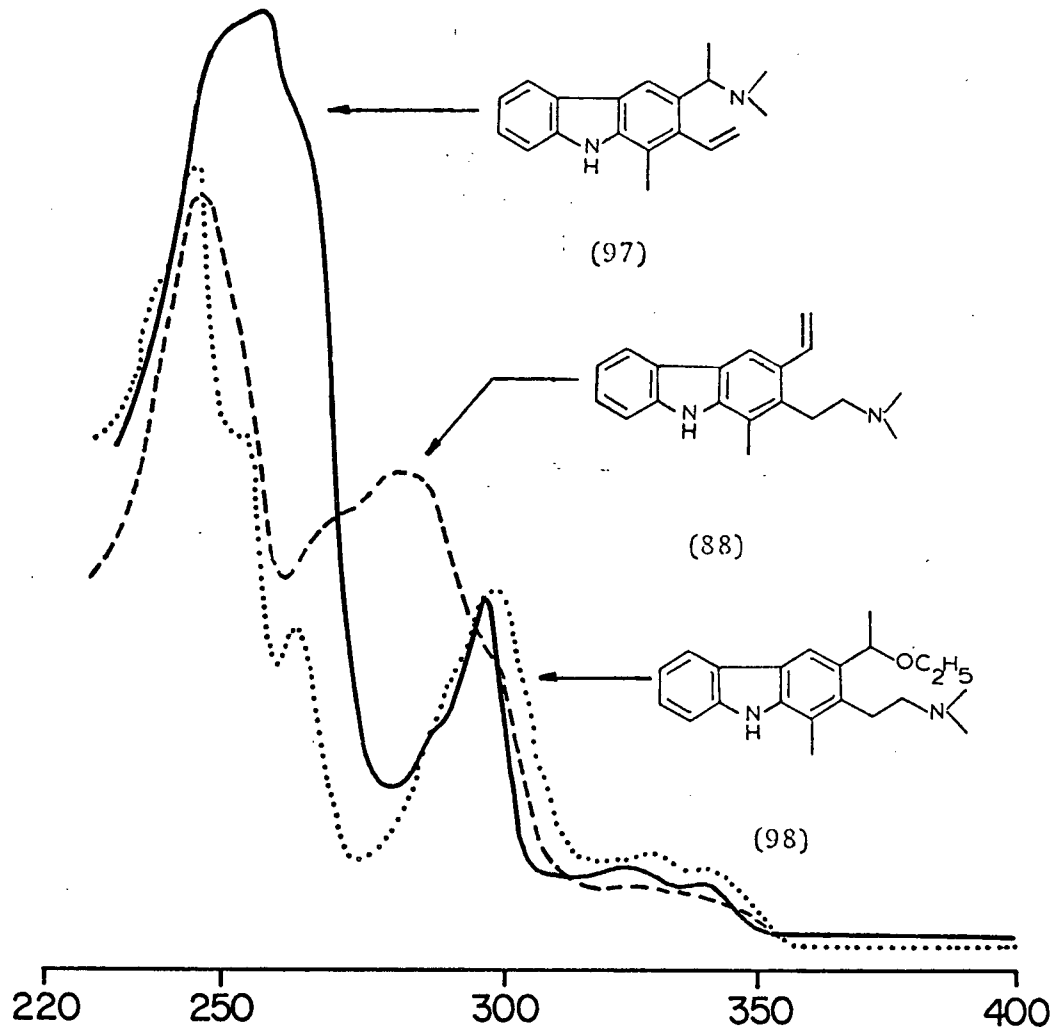
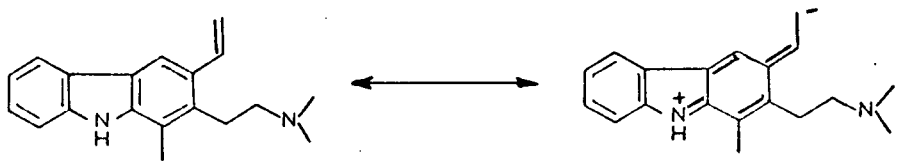


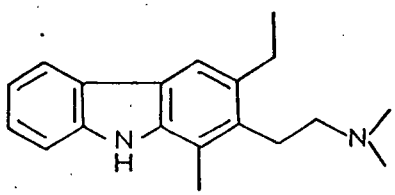
Figure 27. A Comparison of the UV. Spectra for the Olefins 88 and 97 with the Normal Carbazole Spectrum of 98.



(88)

(97)

Further confirmation for the production of olefin 88 was provided by reducing it to its saturated derivative 89, and comparing its spectral data with 89 obtained both by uleine degradation and lithium aluminum hydride ring opening of guatambuine methiodide (86) (Section 3).



(89)

The product distribution (2:1 by weight) for the ring opening of guatambuine methiodide (86) using potassium t-butoxide in t-butanol reflects the enhancement of the E_2 elimination mechanism over the E_1 mechanism observed with the previous conditions.

The isolation of the C-3 olefin 88 directly from the Hofmann reaction was an important result because as discussed in later sections it is questionable whether or not it would be feasible to isolate the C-1 methyl group of guatambuine (25) by the alternate routes developed. However, even the

yield of 88 obtained under these conditions (18%) becomes of marginal practicality when the reaction is repeated on a projected plant isolation scale (30-40 mg.).

To see if the product distribution could be shifted or the overall yield of the two elimination products could be increased, hot aqueous hydroxide was studied as the basic medium. It was found however that the product ratio did not alter significantly (N.M. R. integration) and that the overall reaction yield was lower (60%).

The use of sodium hydride as the base in hot dimethylformamide was considered in the hope that by using a very powerful base the relative acidities of the β-hydrogens in guatambuine might become the sole determining factor in the reaction and as a consequence the product ratio might be shifted completely towards the C-2 olefin 97.

It was found quite unexpectedly after reaction, however, that exactly the opposite had occurred. The C-3 olefin 88 obtained in 70% yield was the only reaction product isolated. The spectral data was identical to that obtained for 88 above using potassium t-butoxide as the base.

In terms of the mechanistic rationale it was not entirely clear why olefin 88 was the exclusive reaction product, however, the practical aspect of this result was that the C-3 olefin 88 became available in adequate quantities such that subsequent oxidative cleavage (see Section 3) enabled the C-1 methyl groups of guatambuine to be unambiguously isolated.

The application of the Hofmann elimination reaction to the degradation of N-methyltetrahydroellipticine methiodide (95) was straight forward for there is only one direction in which β-elimination can occur.

(95) (102)

To reduce the occurrence of the competing substitution reaction the reaction was conducted using potassium t-butoxide in refluxing t-butanol. A single product was formed during the reaction (TLC) in 96% yield and the spectral data was consistent with the olefinic product 102.

The N.M.R. possessed two singlets at δ2.50 and 2.90 for the protons of the aromatically substituted methyl groups at C-1 and C-4 respectively. A third singlet occurred at δ2.24 for the dimethylamino group. As in the guatambuine (25) series the proximity of this signal reflected the presence of the dimethylamino group on the C-3 position. Another singlet (δ3.64) was attributed to the methylene protons both benzylic (C-1) and adjacent to the nitrogen atom. Two pairs of multiplets (doublets of doublets) came at δ5.20 ($J_{2',3'}$ = 17Hz, $J_{3',3'}$ = 2Hz) and δ5.64 ($J_{2',3'}$ = 11 Hz, $J_{3',3'}$ = 2 Hz) and once again the upfield position of the <u>trans</u> coupled hydrogen with respect to the <u>cis</u> hydrogen was characteristic of the C-2 vinyl side chain.

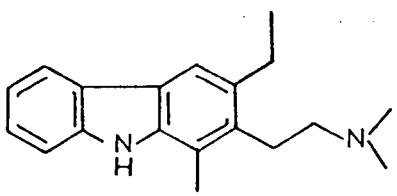
The mass spectrum possessed a parent peak at m/e = 278 and major fragmentations at 263, 234, 233, 219, 218, 204. The high resolution spectrum possessed a parent peak at 278.1780 which was within acceptable limits of the value 278.1782 calculated for the composition $C_{19}H_{22}N_2$.

The UV . spectrum bore a distinct resemblance to that obtained for carbazole, this behavior again being characteristic of the presence of the C-2 vinyl side chain in the elimination product.

The oxidative degradation of the Hofmann product is discussed in section 3.

3. N-Methyl, C-3 Methylene and C-1 Methyl Group Isolations.

Due to the scale of the biosynthetic experiments, it was accepted that for the guatambuine system (25) all three carbon centers could not be isolated from the same experiment. With the Hofmann elimination reaction developed to the point where the ring-opened olefins 88 and 97 from guatambuine (25) and 102 from N-methyltetrahydroellipticine (26) were available in adequate yields, the two carbon centers (C-1 and C-3) could then be isolated individually by ozonolytic cleavage. Determination of the N-methyl group would then be a separate experiment involving a Hofmann reaction from the methine methiodides of any of the above olefins or from their saturated derivatives 89, 103 or 104.



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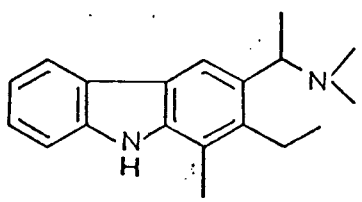
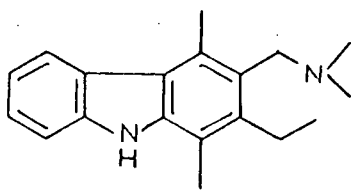


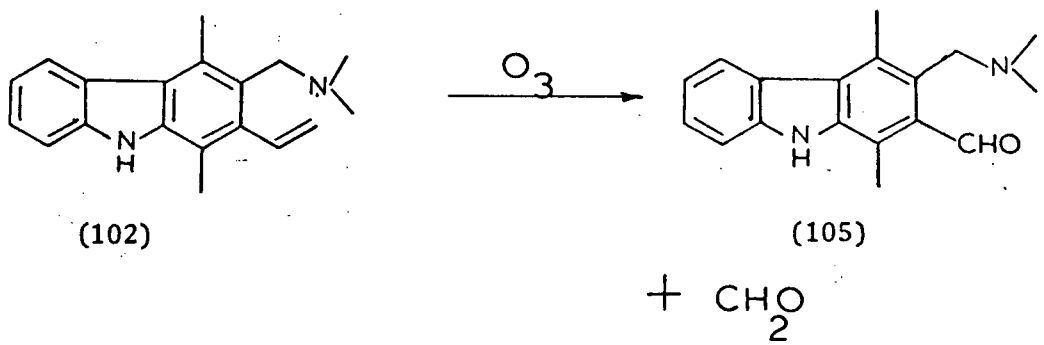
(103)



(104)

To gain the most information from the biosynthetic experiments utilizing the Hofmann reaction chemistry, the isolation experiments were divided into two catagories. In the first, the C-1 methyl group was unique to guatambuine (25) and its isolation via the ozonolytic cleavage of C-3 olefin 88 was considered as a separate experiment. In the second, the N-methyl and C-3 methylene groups were common to both 25 and N-methyltetrahydroellipticine (26) and efforts were therefore directed at developing a combined experiment whereby both centers could be isolated sequentially.

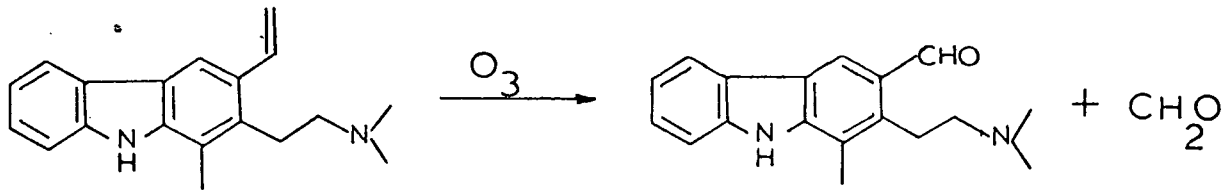
Before attempting the ozonolysis of the valuable C-3 olefin 88, the reaction conditions were worked out for compound 102 derived from N-methyltetrahydroellipticine (26). The procedure utilized by Battersby and Harper[146] was adopted, being modified only in that methylene chloride was used as the solvent. The formaldehyde produced (C-3 carbon) on reductive work-up was isolated as the bisdimedone derivative[210] by steam distillation of the reaction mixture into a saturated dimedone solution.



(102) (105)

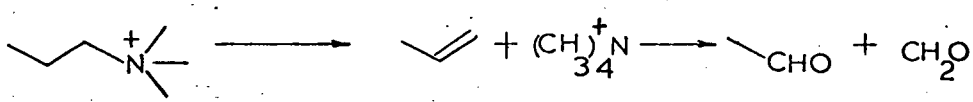
No attempt was made to isolate the carbazole aldehyde residue 105 and it was thus not known whether ozone also attacked the benzylic dimethylamino group. The formaldehyde dimedone was isolated as long crystalline needles in 87% yield. The ease with which the C-3 carbon could be isolated by ozonolysis indicated that the technique would be generally applicable within the series of C-2 and C-3 vinyl carbazole derivatives.

C-3 vinyl system of Olefin 88 was similarly readily cleaved on ozonolysis. The formaldehyde dimedone (C-1 methyl) was isolated as long needles in 60% yield. Again, no attempt was made to isolate the aldehyde 106 also proposed to be formed during the reaction. This then completed the degradation of guatambuine so as to isolate the C-1 methyl group.



(88) (106)

For the isolation of the remaining two centers in 25 and 26, it was considered that the most efficient order in which the degradation could be conducted would be by sequential Hofmann reaction which would eliminate the N-methyl group and produce the C-2 vinyl system that would subsequently be cleaved oxidatively.

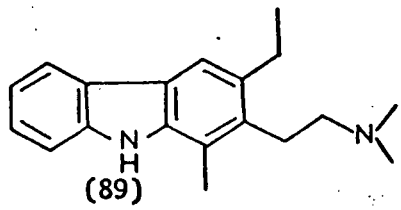


By conducting the reaction in the reverse sequence i.e. oxidative cleavage, first it was thought that problems would arise, for it was known that in general, the techniques used for isolating the one carbon unit as the dimedone derivative[145-6] lead to poor recovery of the remainder of the molecule.

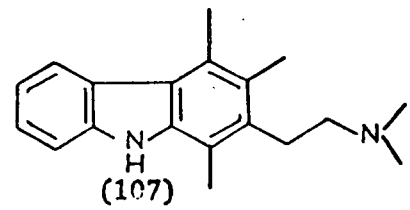
It was necessary to utilize the β-dimethylaminoethyl side chain for this degradation because as will become clearer in Section 4, considerable difficulties arise during methiodide formation of compound 103 containing the benzylic dimethylamino group.

To produce a suitable derivative in both the guatambuine (25) and N-Methyltetrahydroellipticine (26) advantage was taken of the reactivity of the C-1 benzylic center towards nucleophiles. It has been shown that in both uleine[140] and apparicine[113] that reaction of the quaternary salts with lithium aluminum hydride

effected displacement of the nitrogen center by hydride. In a similar manner it was found that the methiodides of 25 and 26 were attacked by lithium aluminum hydride to give in good yields compound 89 and 107.



(89)



(107)

Compound 89 was prepared also for the purpose of comparing its physical and spectral properties with those for the degradation product of uleine[132] and with the hydrogenated Hofmann product of Olefin 88 prepared both in the present work and previously[121-3] (Table III).

The N.M.R. spectrum of compound 89 possessed a multiplet δ3.00, and a triplet (δ1.36, J = 7 Hz) for the newly formed ethyl side chain. Present also were the singlets at δ2.52 and 2.46 for the C-1 methyl and dimethylamino groups respectively. As mentioned previously the chemical shift for the latter group was characteristic for the presence of the β-dimethylaminoethyl side chain at the C-2 position.

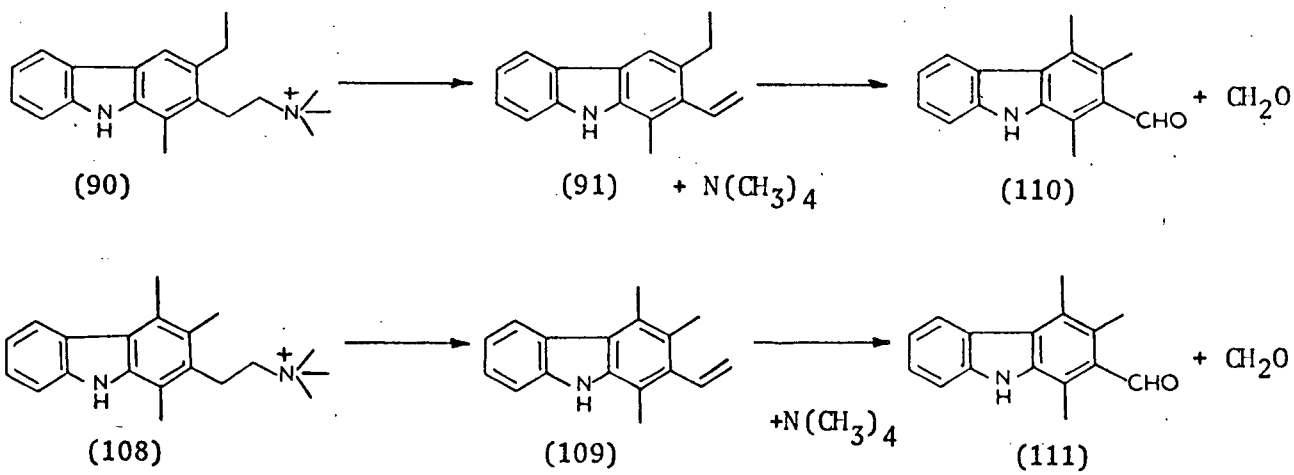
The mass spectrum possessed a parent peak at m/e = 280 and major fragments at m/e = 266, 236, 222, 207, 206, 204 (Section 5). The high resolution mass spectrum parent peak at m/e = 280.1975 was consistent with that calculated (m/e = 280.1938) for the composition $C_{19}H_{24}N_2$.

For further comparison and proof of structure 89 the methiodide of uleine 87 was ring-opened under Hofmann conditions[120,132] to give the compound 89. The spectral data for 89 prepared by the two different routes were almost identical. An added feature to conversion of 87 → 89 was that the subsequent isolation of the N-methyl and C-3 methylene groups from compound 89 constituted a degradation of

both uleine (18) and guatambuine (25).

The N.M.R. spectrum of compound 107 exhibited three singlets at δ2.75, 2.42, and 2.36 for the C-4, C-1 and C-3 methyl groups respectively. A fourth singlet was present at δ2.32 for the dimethylamino group. The mass spectrum possessed a parent peak at m/e = 280 with major fragments at m/e = 266, 249, 236, 222, 207-6, 204, 191 (Section 5). The parent peak in the high resolution mass spectrum m/e = 280.1903 was again consistent with the calculated value m/e = 280.1938 for $C_{19}H_{24}N_2$.

Both compounds 89 and 107 were converted to their methiodides 90 and 108 in quantitative yields in preparation for the Hofmann reaction. The procedure used for isolating the N-methyl group will be described below, at the present time attention will be focussed on the formation of the olefins 91 and 109.



The N-methyltetrahydroellipticine derived methiodide 108 was reacted with potassium t-butoxide in t-butanol in the same manner as described in Section 2. The desired olefin 109 was obtained as a brown oil in 85% yield.

The N.M.R. spectrum for the product showed a doublet of doublets at δ5.68 ($J_{3',4'}$ = 12 Hz, $J_{4',4'}$ = 2 Hz) for the <u>cis</u> C-4H' and a complimentary doublet

of doublets at δ5.24 ($J_{3',4'}$= 18 Hz, $J_{4',4'}$ = 2 Hz) for the trans C-4' H. The singlets for the three methyl groups occurred at δ2.84 (C-4), 2.52 (C-1), and 2.40 (C-3). The mass spectrum possessed a parent peak at m/e = 235 with main peaks at m/e = 220, and 205 (Section 5). The high resolution spectrum possessed a parent peak at m/e = 235.1325 which was within acceptable limits for that calculated (m/e = 235.1360) for $C_{17}H_{17}N$.

The reaction of the methiodide 90 derived from the guatambuine system with potassium t-butoxide in t-butanol did not proceed as well as anticipated. a dark brown product containing considerable quantities of decomposition material was obtained. The olefin 91 was obtained as a brown oil in 57% yield after purification by preparative layer chromatography on alumina.

A cleaner product was obtained by reaction of the methiodide 90 with sodium hydride in dimethylformamide. However in this instance as well, the product yield was low (40%).

The N.M.R. spectrum for this product (of poor quality) showed the pair of doublets of doublets, δ5.65 ($J_{3',4'}$ = 12 Hz, $J_{4',4'}$ = 2 Hz) for the cis C-4' H and δ5.29 ($J_{3',4'}$ = 18 Hz, $J_{4',4'}$ = 2 Hz) for the trans C-4' H. The singlet for the C-1 methyl group was readily discerned at δ2.54 but the signals for ethyl groups occurred as complex multiplets due to the presence of impurities.

The mass spectrum for 91 possessed a parent peak at m/e = 235 and major fragments at m/e = 220 and 205. The high resolution spectrum possessed a parent peak at m/e = 235.1384 within acceptable limits of the value 235.1360 calculated for $C_{17}H_{17}N$.

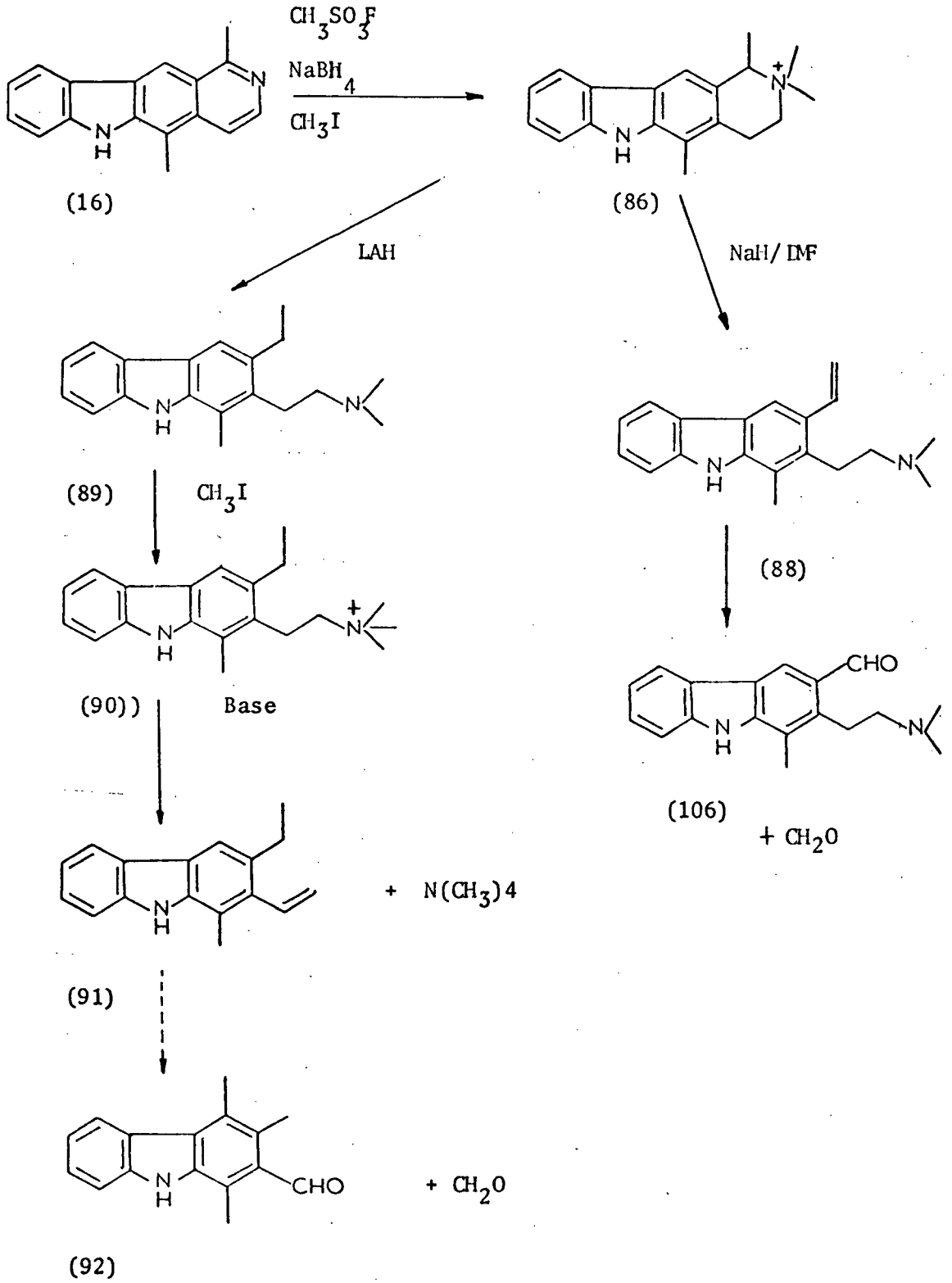
The N-methyl group of guatambuine (25) and N-methyltetrahydroellipticine (26) was isolated during the Hofmann reactions by converting the trimethylamine that was formed to its tetramethylammonium iodide derivative. This was

done by passing the gases produced during the reaction through a solution of methyl iodide.

To determine the efficiency of the trapping technique, a mock biosynthetic experiment was conducted where (N-C^{14} methyl) guatambuine (25) and N-Methyltetrahydroellipticine (26) were converted to their ring-opened methiodides 90 and 108, and subjected to the Hofmann reaction. A total of 59 % and 72 % of the activity present in 25 and 26 was recovered as the tetramethylammonium iodide salt. These results demonstrated that the N-methyl group could be readily isolated and that the isolation technique would be suitable to future biosynthetic experiments.

The subsequent ozonolyses of olefins 91 and 109 so as to isolate the C-3 methylene group of 25 and 26 was unfortunately not investigated at this time. In order for the ozonolysis reaction to be feasible in the guatambuine series however, it would be first necessary to further develop the Hofmann reaction of 108 so as to obtain a higher yield of the olefin 109. Considering the success of the ozonolysis reaction in the cleavage of the C-3 vinyl side chain of olefins 88 and 102, it would be anticipated that no major difficulties would be encountered during ozonolysis of 91 and 109.

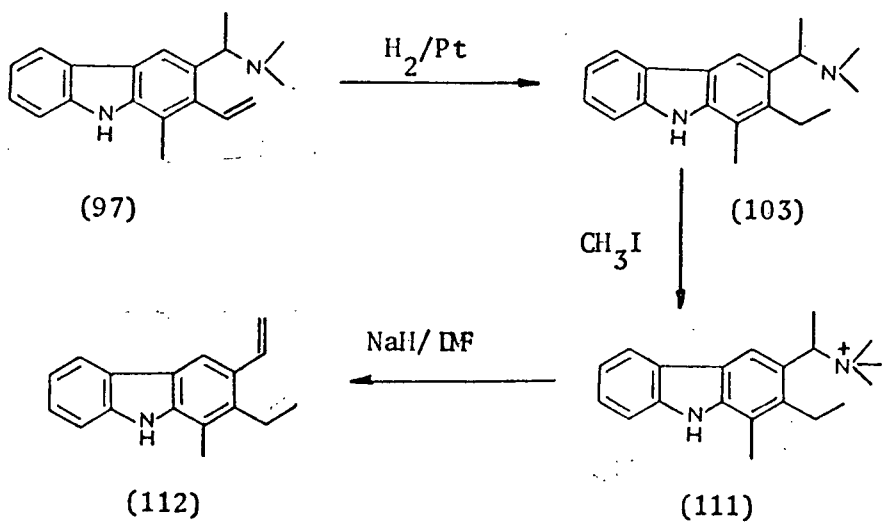
An overall view of the degradation scheme for the olivacine (16) system involving the Hofmann reaction followed by ozonolytic cleavage reactions is presented in below. Considerable work was also done to develop alternate routes whereby the N-methyl, C-1 methyl and C-3 methylene groups could be isolated. This work is presented in subsequent sections.


CH_3SO_3F
$NaBH_4$
CH_3I
(16)
(86)
LAH
NaH/ DMF
(89)
CH_3I
(88)
(90))
Base
CHO
(106)
+ CH_2O
+ $N(CH_3)4$
(91)
CHO
+ CH_2O
(92)

4. <u>The Synthesis of 1-Methyl-2-ethyl-3 vinylcarbazole (114) from the C-2 Olefin 97.</u>

To reiterate for a moment, the synthetic schemes described in the following sections were aimed at devising a feasible alternative to the direct Hofmann reaction of guatambuine methiodide (86) for creating a C-3 vinyl carbazole derivative for the subsequent degradative isolation of the C-1 methyl carbon of guatambuine (25). This work was prompted by the initial belief that the C-3 olefin 88 would be unavailable from the potassium t-butoxide-t-butanol reactions.

Two different approaches were developed, an alternate ring opening procedure (Section 6) and the second Hofmann reaction approach starting from the relatively abundant C-2 olefin 97. This latter approach to be discussed in this section is illustrated below.



In preparation for the second Hofmann reaction the C-2 vinyl side chain of 97 was reduced to compound 103 in near quantitative yield by

catalytic hydrogenation over platinum oxide. This compound like its saturated Hofmann counterpart 89 was also prepared as an aid for interpreting the N.M.R. of the crude Hofmann elimination reaction mixtures (Table III, section 2.).

The N.M.R. spectrum for the reduced compound 103 showed a triplet (δ1.20, J = 6 Hz) and a quartet (δ2.94, J = 6 Hz) for the methyl and methylene hydrogens respectively of the newly formed C-2 ethyl side chain. Present also was a doublet (δ1.42, J = 6 Hz) and a quartet (δ3.62, J = 6 Hz) for the C-1' methyl group and the methine hydrogen substituted both benzylic and adjacent to nitrogen in the C-3 side chain. Two singlets occurred at δ1.48 and 1.28 for the C-1 methyl and dimethylamino group, the position of the later absorption being characteristic for the C-3 nitrogen bearing side chain.

The mass spectrum possessed a parent peak at m/e = 280 and major fragmentations occurred at m/e = 265, 236, 235, 220, 207-5, 204 (see discussion section 5).

Compound 103 exhibited a normal carbazole UV. spectrum as expected. Little information was obtained from the I.R. spectrum other than it resembled very closely the spectrum obtained for the saturated component 89.

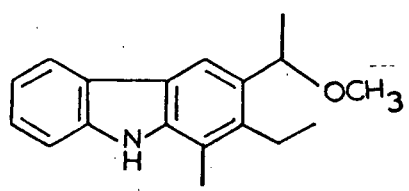
The subsequent formation of the methiodide 111 in pure form on a small scale proved to be extremely difficult. Reaction of 103 with methyl iodide as a neat solution or in a variety of solvents such as ether, chloroform, and methanol resulted in the co-formation of several non-polar products. This was a consequence of the instability of the newly formed quaternary nitrogen

with respect to nucleophilic displacement, which is inherent to its gramine like structure.[147]

Purification of the methiodide 111 by column chromatography was not attempted as it was thought that the molecule would decompose. Recrystallization from polar solvents like methanol was unsuccessful since TLC showed that with increasing time in methanol the concentration of non-polar side product increased.

The methiodide was sufficiently purified for subsequent reaction by simply washing the concentrated reaction product with chloroform which removed the soluble non-polar materials and left behind the insoluble methiodide 111.

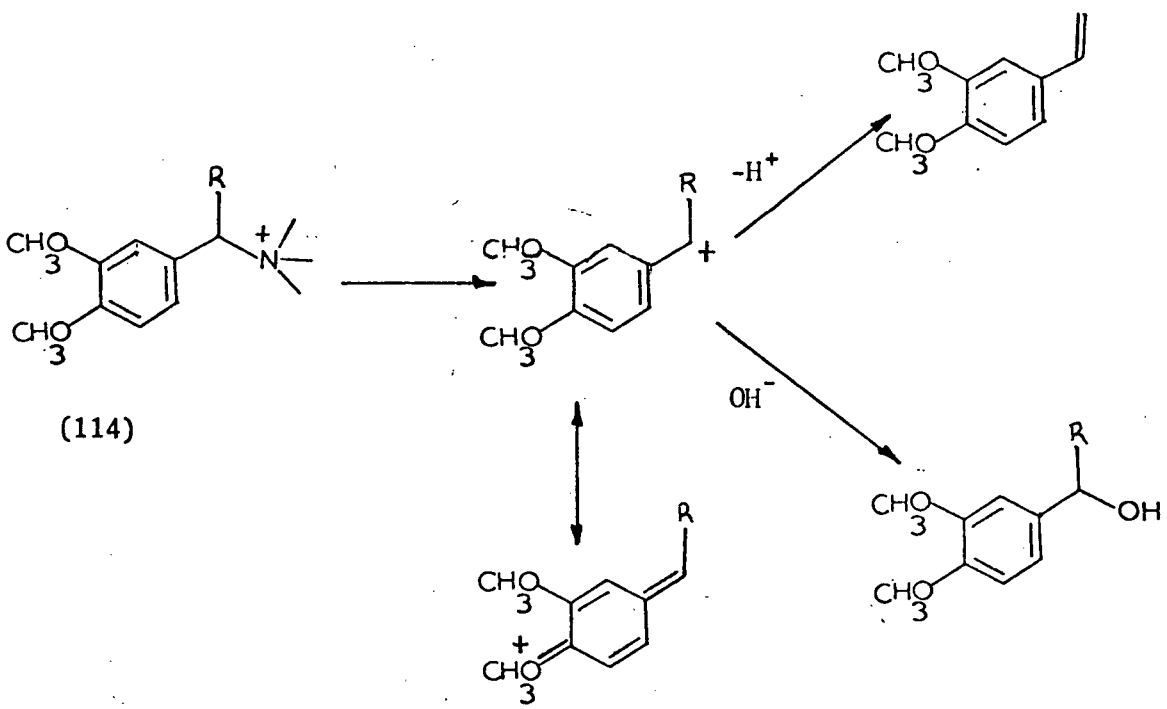
In the case where the methiodide 111 was formed by reaction with methyl iodide in methanol it was shown by N.M.R. that the major non-polar component corresponded as anticipated to the O-methyl ether 113, formed by displacement.[143, 147]



(113)

Unfortunately the inability to obtain 111 in pure form made it impossible to compare its physical properties with those previously reported.[121-3]

The choice of reaction conditions for the subsequent elimination reaction to 112 had to take into account the labile nature of the quaternary nitrogen group, substituted at the benzylic C-1' center, towards displacement reaction. It has been shown by Norcross and Openshaw[143] in a study of the cyclic and acyclic methiodides of model compounds related to the emitene system (99) that in the acyclic case exemplified by 114, the benzylically substituted quaternary amino group, which has an electron donating group oriented para to it, possesses an exceptional reactivity towards nucleophilic substitution. This reactivity was attributed to the ability of a stabilized carbonium ion to be formed in the transition state of the reaction (SN_1 mechanism).

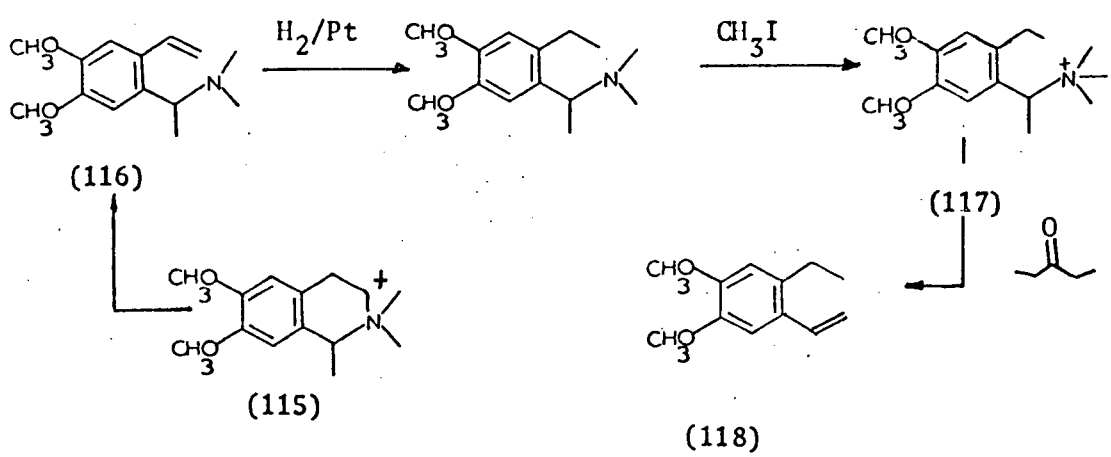


(114)

The close parallel between these systems and the methiodide 111 derived from guatambuine (25) was self evident. It was apparent from this and other studies[148] that the basic aqueous or alcoholic conditions normally employed for the Hofmann degradation would fail to produce the desired olefinic product 112.

To circumvent this same problem in the emetine (99) series, Openshaw *et al.*[142-3] found that pyrolysis of the acyclic methiodides in an aprotic solvent like diethyl ketone resulted in olefin formation. It was also observed, however, that in accord with the carbonium ion mechanism, the yield of the reaction depended markedly on the mesomeric influence of the alkyl substituent (R) on the side chain. As the size of the R group decreased, so did the yield and it could be anticipated from this trend that the side chain where R = H indiginous to 111 may result in a poor yield of olefin 112.

With this possibility in mind, it was decided to study the feasibility of the pyrolytic elimination reaction on a model compound (117) derived from salsolidine methiodide (115) instead of using valuable quantities of 111.

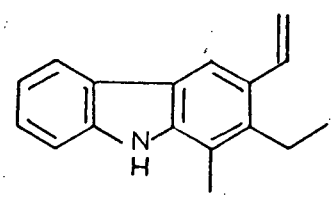


It was found that prolonged heating of 117 in diethyl ketone resulted only in the isolation of small amounts of unidentified materials. None of the desired olefin 118 was detected, and this approach was consequently abandoned.

The C-3 olefin 112 was eventually obtained in 70% yield from 111 by reaction with sodium hydride as the base in dimethylformamide. This reaction had previously proven successful in ring opening guatambuine methiodide (86) to the analogous olefin 88.

To avoid confusion at this point it must be mentioned that the successful completion of the sequence to the C-3 vinylcarbazole 112 (the compound that was supposed to take the place of 88 in the degradation sequence) came after the conditions were found to obtain 88 directly.

The spectral data for the olefinic product was consistent with its structure 112 . The presence of the vinyl grouping at C-3 was readily



(112)

determined by its characteristic UV . spectrum and the position of the olefinic absorptions (5.69 ($J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2 Hz), δ5.28 ($J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 2 Hz)) in the N.M.R. spectrum.

The mass spectrum possessed a parent peak at m/e = 235 and major fragmentations at m/e = 220 and 205. The parent peak in the high resolution spectrum 235.1380 was consistent with the composition $C_{17}H_{17}N$.

The considerable difficulty in preparing the methiodide 113 makes the sequence to compound 112 unreliable and low yielding in terms of the quantities of olefin obtained. It would be doubtful if sufficient quantities of formaldehyde (as its dimedone derivative) would be obtained from the oxidation of 112 to substantiate any incorporation experiments by this route. It was for this reason that the work presented in section 6 was initiated.

5. Mass Spectral Correlations of Ring "D" Opened Derivatives of Guatambuine (25) and Ellipticine (17).

A discussion of the mass spectra of the olefinic (88, 97, 102) and reduced (89, 103, 107) ring-opened products of guatambuine (25) and ellipticine (17) as well as their second Hofmann products (91, 112, 109) has been reserved for a separate section because close similarities existed in the fragmentation patterns of these derivatives. It appears as though the occurrence of several characteristic fragments in the spectra is internally consistent with their tri- and tetra- substituted carbazole structures.

Characteristic features in the spectra of the ring opened products are a substantial parent peak either at m/e = 278 (unsaturated) or m/e = 280 (saturated) and a fragmentation pattern consisting of successive losses of 14 and 15 mass units which corresponds to the formal loss of successive methylene and methyl groups (Figure 28 & 29). These characteristic fragments occur at m/e = (M^+:280), 265, 236, 235, 222, 220, 207, 205 and m/e =(M^+:278), 263, 249, 234, 233, 220, 219, 218, 205, 204.

Weaker fragments are also observed in the region m/e = 191-193, 180, and 167 for further losses of 13-15 mass units. The m/e = 167 fragment presumably represents the carbazole ion.

Except in the spectra of the olefinic compounds the peak at m/e = 249 corresponding to loss of 29 mass units occurred only to a minor extent, and with the exception of the compounds 97 and 103 with the α-dimethylaminoethyl side chain the M^+-15 peak was also not intense. In the latter instance, the presence of a substantial M^+-15 is probably a consequence of a facile cleavage

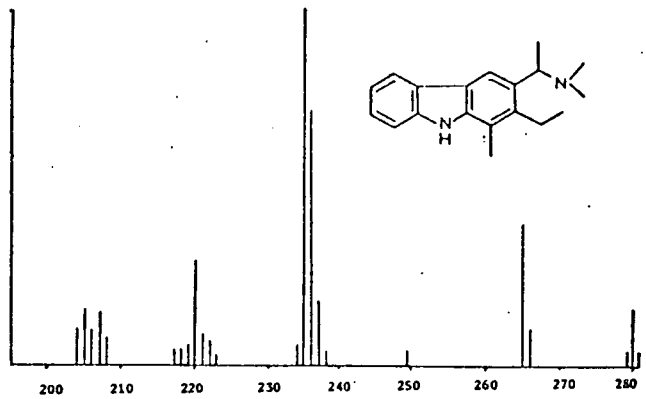


Figure 28. Mass Spectrum of Carbazole Compound (103).

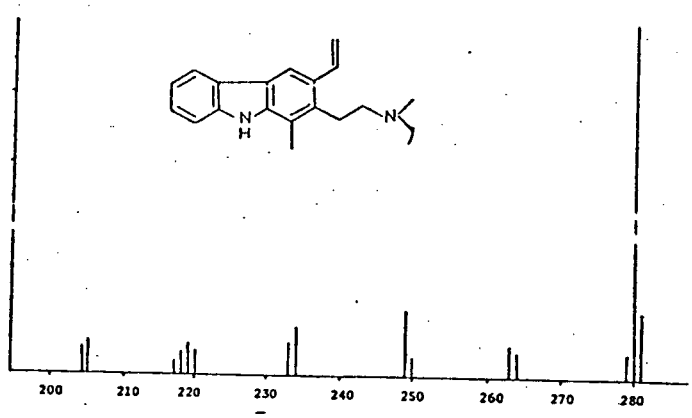


Figure 29. Mass Spectrum of Carbazole Compound (88).

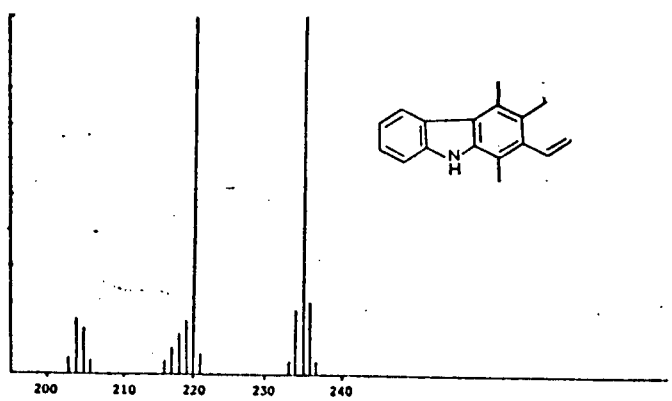


Figure 30. Mass Spectrum of Carbazole Compound (109).

of the C-1' methyl group so as to form an even electron system (Figure 28). This same cleavage is an important fragment in the mass spectrum of guatambuine (25) and is only of minor occurrence in the spectrum of N-methyltetrahydroellipticine (26).

The first major fragment after the parent occurs at m/e = 266, 265 (264, 263) for loss of 44 and 45 mass units which corresponds to the cleavage of the dimethylamino group plus one hydrogen (equivalent to three methyl groups).

Plausible fragmentation pathways for compounds 97 and 88 in the guatambuine series which are consistent with the presence of the observed fragments and the compositions for these fragments determined from the high resolution spectrum are presented in figures 31 & 32. Pathways involving similar processes can be formulated for their isomeric ring-opened counterparts 103 and 89. It should be emphasized however that in the absence of any selective labelling and other experiments to validate the structures of the fragment ions the proposed structures and fragmentation patterns are purely hypothetical.

Several comments can be made concerning the postulated breakdown of compound 103 ($M^{+\cdot}$, m/e = 280). Only a single metastable at m/e = 206 corresponding to the fragmentation 235 → 220 was evident in its spectrum. Considerably more information was obtained from the spectrum of its unsaturated analog 97 ($M^{+\cdot}$, m/e = 278) where metastables were present for the analogous fragmentations:

$M^{+\cdot}$, m/e =	234 for m/e =	263 → 248
	205	234 → 219
	191	218 → 204
	184	263 → 220

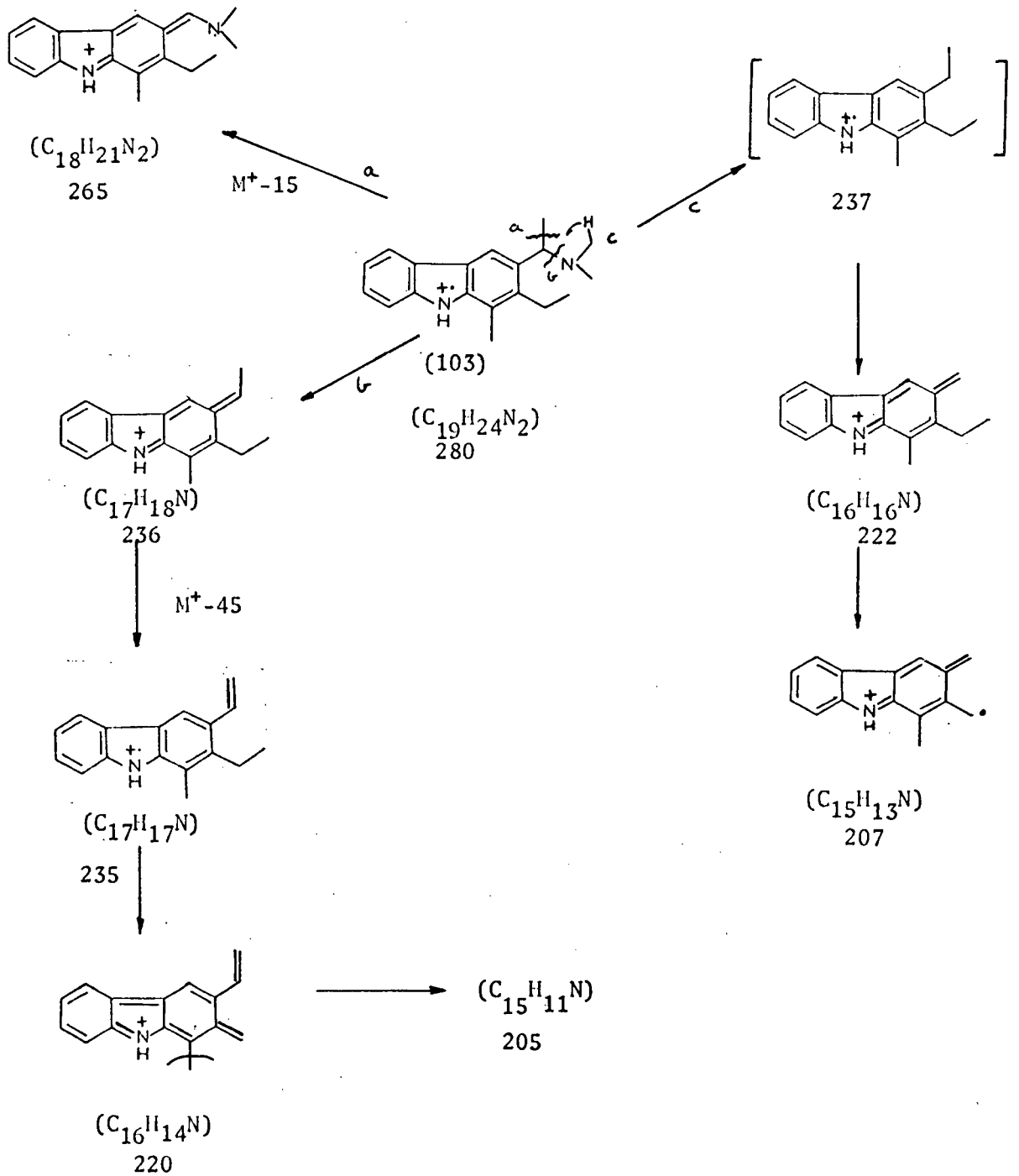


Figure 31. Plausible Mass Spectral Fragmentation Pattern for Compound 103.

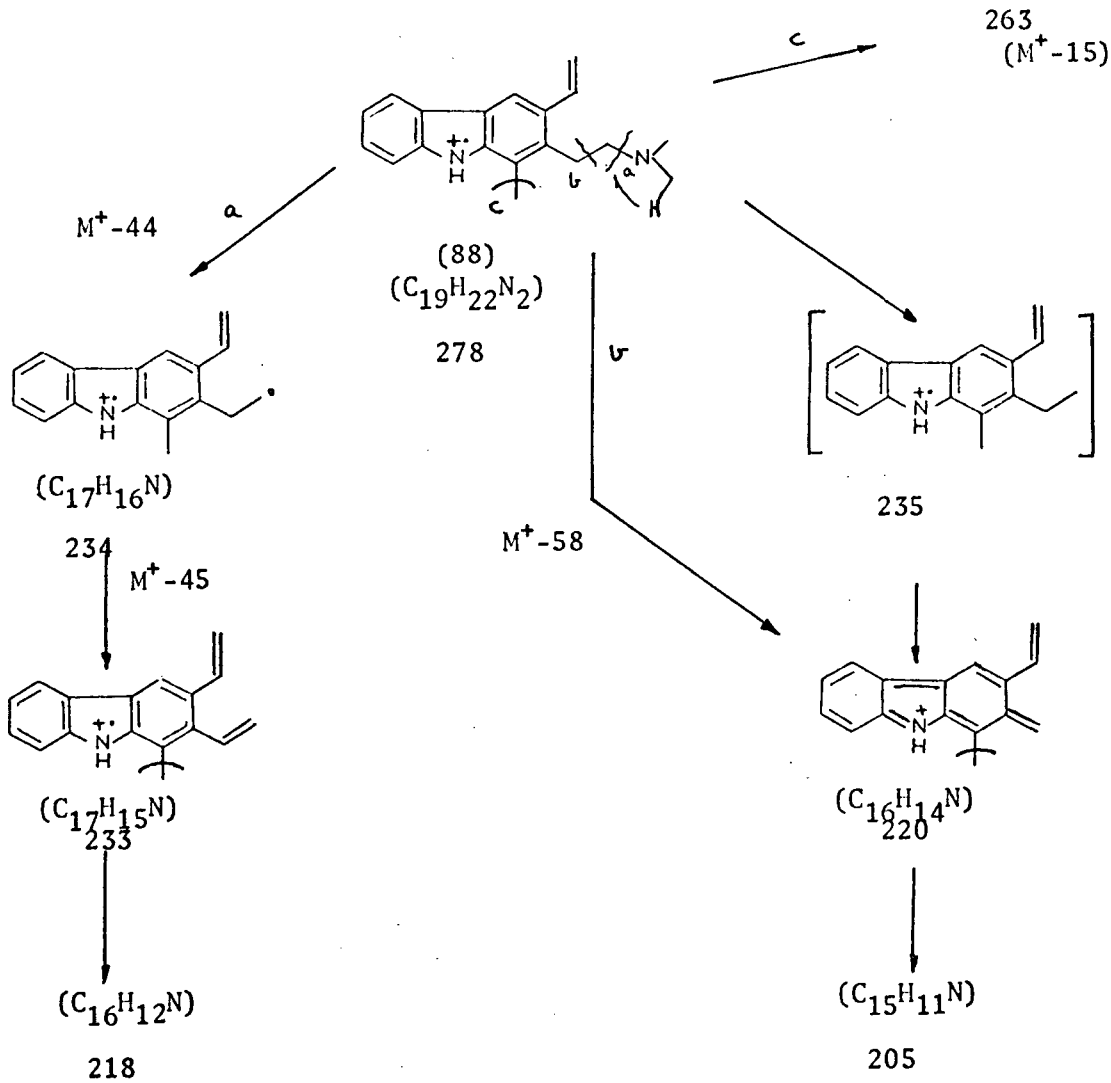


Figure 32. Plausable Mass Spectral Fragmentation Pattern for Compound 88.

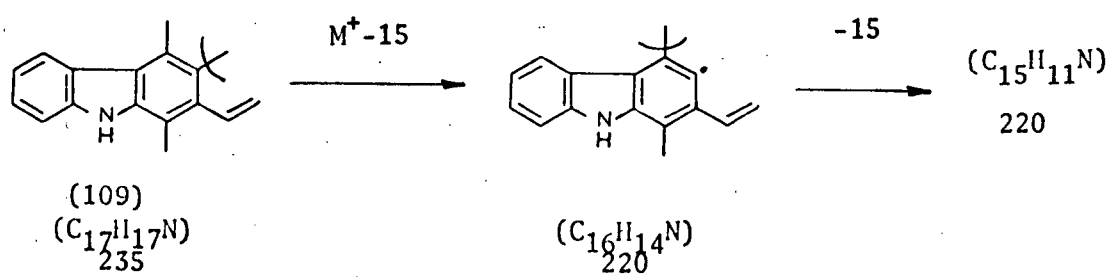
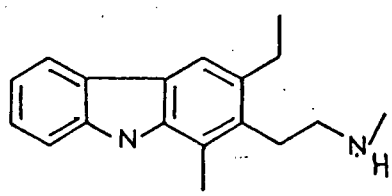


Figure 33. Plausable Mass Spectral Fragmentation Pattern for Compound 109.

The fragmentation m/e = 220 → 205 involved cleavage of the C-1 methyl from the aromatic ring. Such processes are well known and it is currently believed that the species obtained is not a phenyl type radical but a ring opened species containing an acetylenic linkage.[149] Cleavage of the vinyl side chain to liberate an acetylenic system was not postulated since its involvement in the fragmentation processes would lead to a more complex spectrum than was observed.

Precedent for the formulation of the structures of a number of the fragments was available from a detailed study of the mass spectrum of uleine (18) and its derivatives.[140] Uleine fragments in an expected manner to the ring-opened trisubstituted carbazole derivative 119 which is very similar to compound 89. The mass spectrum of this synthesized compound exhibits many of the fragments observed for the guatambuine (25) derived components.



(119)

The fragmentation processes postulated for the elimination product 88 are straightforward, being governed by the α- and β- cleavage of the C-2 side chain. On the other hand the fragmentation processes occurring for the

N-methyltetrahydroellipticine derived compounds 102 and 107 are more difficult to postulate structures for because the fragmentation process involves successive losses of methyl groups from the carbazole ring.

The second Hofmann products (91 , 112 and 109) possess structures which potentially correspond to the postulated fragments at m/e = 235 for the first Hofmann products. It is not too surprising, therefore, that their spectra also possesses dominant fragments at M^+-15 (m/e = 220) and M^+-30 (m/e = 205) (Figure 30). This does not necessitate however that the fragmentation processes are the same in both cases.

In the spectrum of 109 metastables are present at m/e = 206 for the fragmentation m/e = 235 → 220 and m/e = 191 for the process m/e = 220 → 205. The second metastable is very weak and the later fragmentation process may not be of major importance (Figure 33).

The general features of the mass spectra of these compounds are also found in the spectra of many of the other C-2 and C-3 substituted 1-methylcarbazole derivatives discussed in this thesis.

6. Alternate Ring "D' Opening Reactions of Guatambuine (25).

This section is concerned with the work that was directed toward ring-opening guatambuine (25) in a manner which would specifically cleave the C-1 carbon-nitrogen bond and enable the C-1 methyl group to be isolated. Two approaches to the problem were considered : i) the direct opening of the "D" ring by pyrolytic elimination to give the C-3 vinyl system, and ii) the development of substitution and oxidation reactions which would introduce an oxygen bearing functionality into the C-1' position (carbazole numbering). This functionality could subsequently be elaborated into a derivative suitable for cleavage by one of three routes depicted in figure 34.

Of these different routes the dehydration-ozonolysis was the most suitable because it would be anticipated from the Potier-Janot postulate[118] that radioactive stemmadenine (13) would label the C-1 methyl group with tritium only. The Baeyer-Villiger-acetate hydrolysis approach would permit the isolation of the total

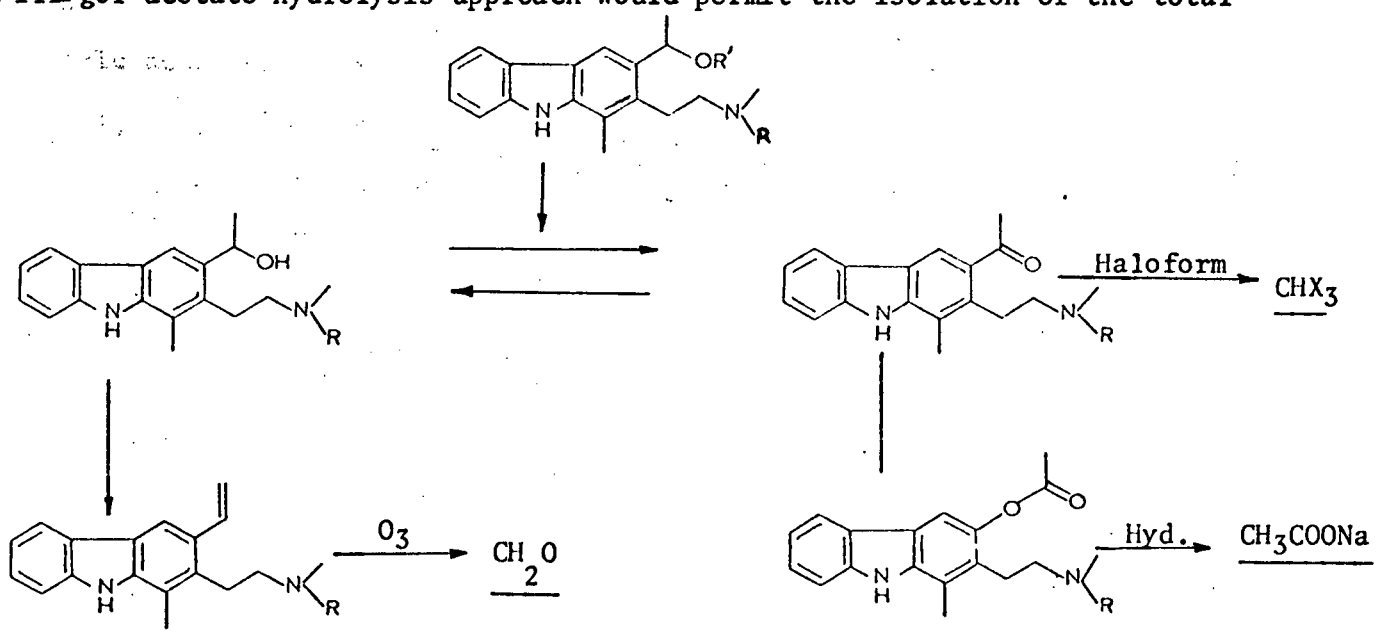
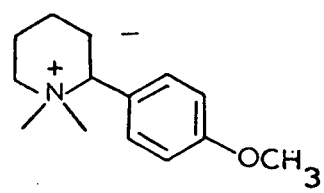


Figure 34. Approaches to the Isolation of the C-1 Methyl Group Involving a C-1' Oxygen Functionality.

specific activity, however, considerable difficulties would be associated with isolating the acetate salt. The Haloform reaction would be the least useful since all three of the radioactive hydrogens would be lost.

A. Pyrolytic Elimination :

The pyrolysis of guatambuine methiodide (86) as with the base induced Hofmann reaction can lead to formation of either/or both olefins 88 and 97. It was hoped, however, that pyrolytic cleavage would lead to a preference for the production of the C-3 olefin 88. It was found that pyrolysis of guatambuine methiodide (86) in diethyl ketone at 100° did not induce cleavage of the quaternized tetrahydropyridine ring system. This result parrallels the observation by Norcross and Openshaw [143] that the 2-phenylpiperidinium ring system of 120 also failed to open under these conditions. This they attributed to the stability of the six membered ring since the anologeous ring-opened compound 114 cleaved readily on pyrolysis in diethyl ketone.



(120)

Pyrolysis of the methiodide 86 in the absence of solvent at 200° also failed to induce olefin formation. It was determined from the N.M.R. spectrum that instead of ring-opening under these more drastic conditions, the nitrogen dequaternized with reformation of guatambuine (25). This was not a totally unexpected result since some dequaternization generally occurs during Hofmann

elimination,[139] and dequaternization of methoacetates by pyrolysis is a known synthetically applicable procedure.[150]

The pyrolysis of the quaternary ammonium hydroxide (classical Hofmann reaction) was not tried although it was probably that elimination would have occurred under these conditions. This was due to technical difficulties that were encountered during the pyrolysis of the methiodide 86. Very little product was formed and it was always contaminated by considerable quantities of decomposition products.

Total selectivity towards formation of the C-3 olefin could have been achieved by pyrolysis of the amine oxide of guatambuine (25).[151] This reaction would have been limited to elimination in the direction of the C-1 methyl group because unlike the β-hydrogens in the six membered ring, the hydrogens of the methyl group are able to adopt the cis-coplanar orientation in the transition state, a condition which is necessary for elimination. The reaction has the major drawback however, in that elimination can occur from the cis (a,e) configuration only, this limits the maximum yield to 50%. For this reason and due to the difficulties previously encountered in the pyrolysis technique the amine oxide reaction was not attempted and the pyrolysis approach to the ring-opening of guatambuine was abandoned.

Acetate Substitution Ring-Opening:

The susceptibility of the C-1 benzylic center of guatambuine towards attack by a nucleophile provided an opportunity to introduce an oxygen functionality into this position. From a consideration of the possible degradation routes

presented in figure 34 the introduction of a hydroxyl group to give the alcohol derivative became the goal.

This could not be achieved directly because it was known that reaction of guatambuine methiodide (86) with aqueous sodium hydroxide resulted in elimination rather than substitution, and solvolysis with water failed to effect reaction of the six membered ring. It has been shown, however, that reaction of the closely related gramine system under acetylation conditions resulted in a facile displacement of the tertiary nitrogen by acetate.[147] Application of this reaction to the cleavage of guatambuine (25) followed by subsequent hydrolysis of the acetate presented an entirely feasible route to the formation of the desired α-hydroxyethyl side chain.

Due to the limited resources of guatambuine, this sequence and the subsequent dehydration were developed beforehand using N-methyltetrahydroellipticine (26) and 1-methyl-3-(α-acetoxyethyl)-9-benzylcarbazole (223) (available from the synthesis presented in sequence B, part III) as model systems (Figure 35). The acetylation ring-opening conditions were worked out using compound 26 and the conditions necessary for the hydrolysis were developed using both compounds 26 and 223. The dehydration reaction was developed using the carbazole alcohol 222.

Joule and Djerassi[140] observed that when uleine was treated with acetic anhydride in pyridine, it was pyridine and not acetate that displaced the initially formed N^+(b)-acyl ion. This somewhat surprising result was probably unique to the uleine system because treatment of N-methyltetrahydroellipticine

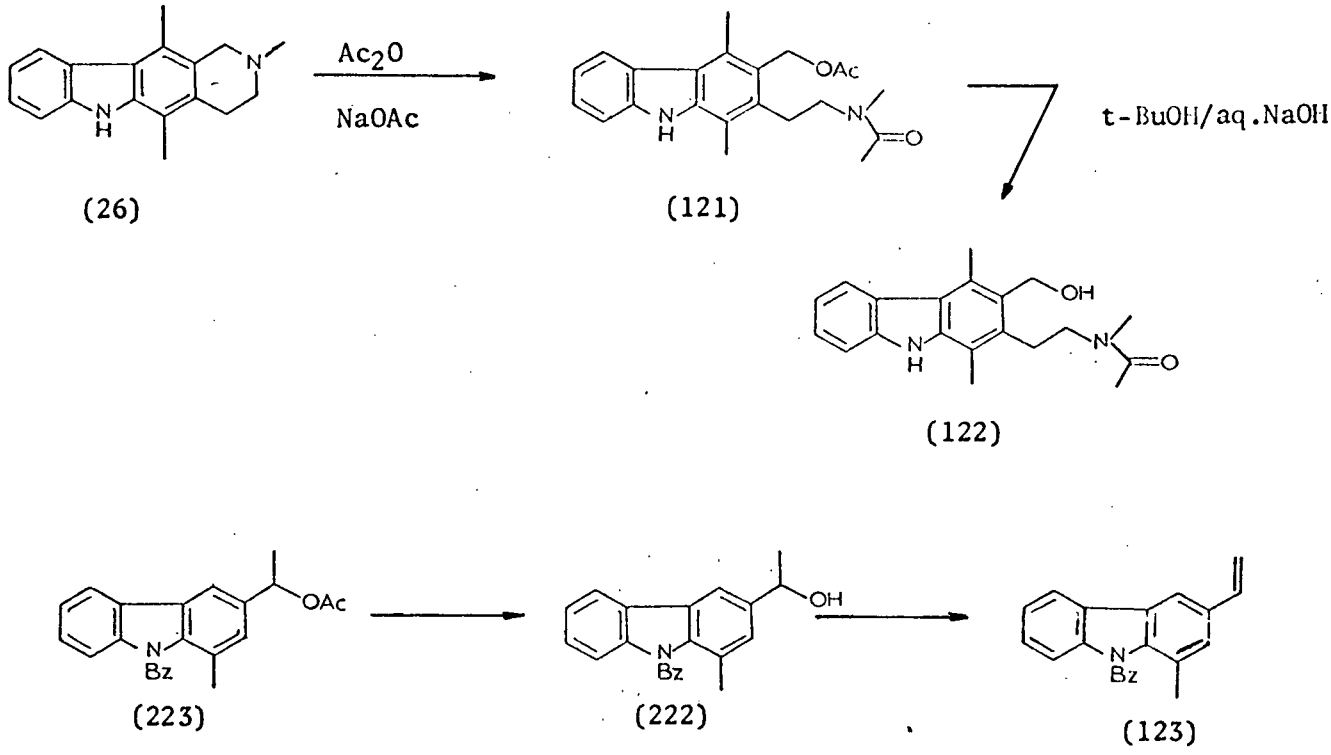


Figure 35. Acetate Substitution Approach Studied on Model Compounds.

under the same conditions led to the formation of the expected N-acetyl acetate 121. Singlets in the N.M.R. spectrum for the acetyl methyl groups were readily discernable in the region of δ2.1 as was the characteristic downfield position of the C-1' methylene singlet.

The crude product mixture was dark in colour which was typical for acetylation reaction in refluxing pyridine. A cleaner product mixture which spontaneously crystallized in benzene was obtained by reaction with acetic anhydride and sodium acetate (76% yield). The spectral data for the acetate formed under these conditions was identical to that obtained by reaction in pyridine.

The N.M.R. spectrum of 121 was considerably more complex than anticipated (Figure 36). A mixture of two separate conformers of 121 (a,b) (about 2:1 by

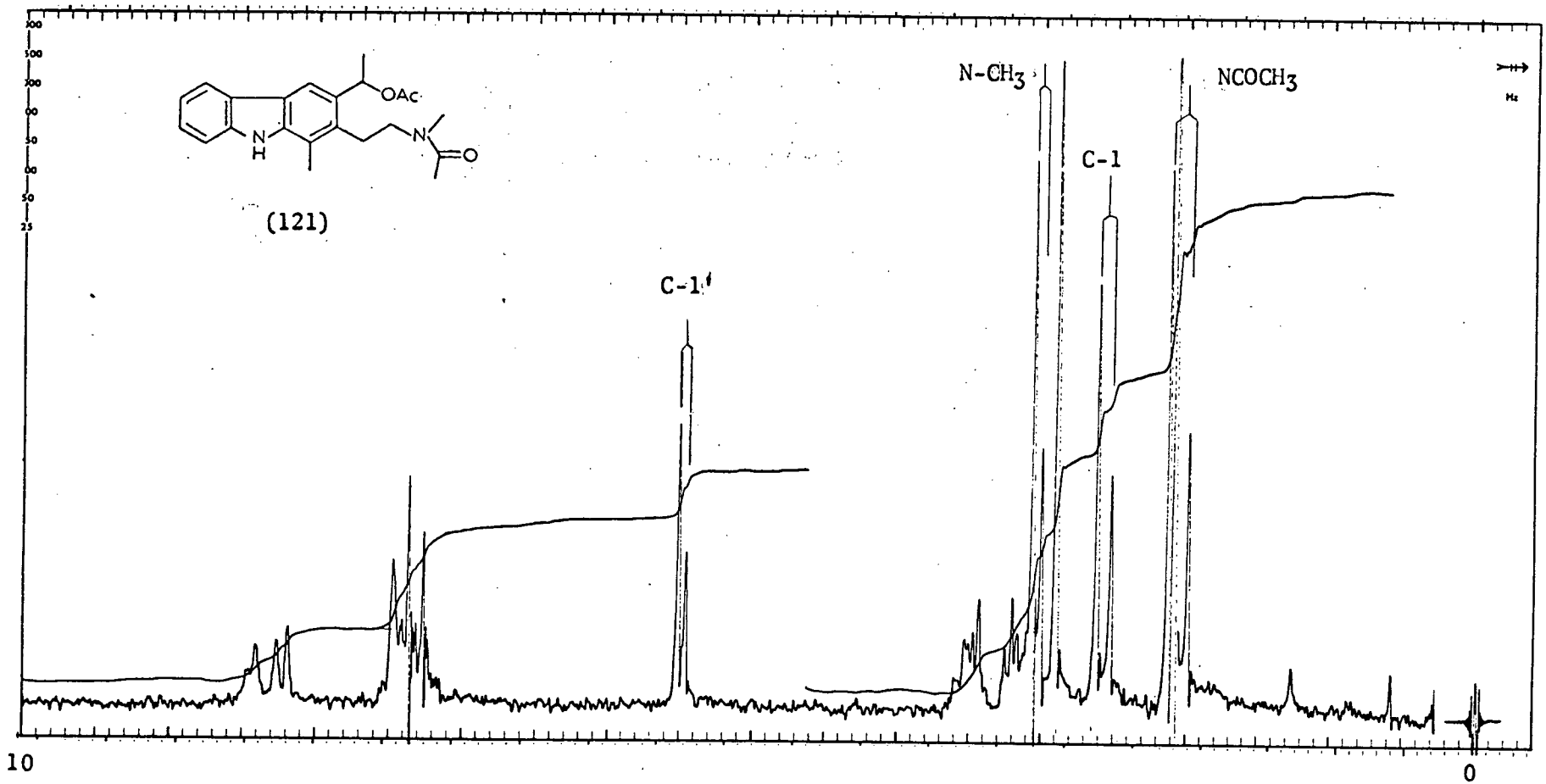
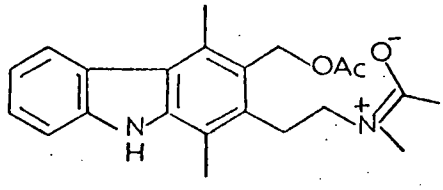
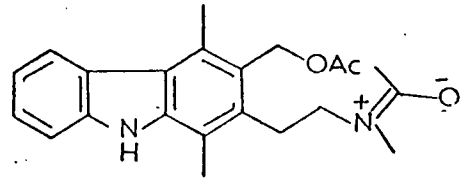


Figure 36. N.M.R. Spectrum of 1,4- Dimethyl-2-(β-(N,N-methylacetylamino)ethyl)-3-acetoxymethylcarbazole (121).

integration) could be distinguished which most probably arose from a combination of hindered internal rotation about the amide linkage[152-4] and steric crowding of the C-3 side chain by the bulky N-acetyl group. These two conformers influenced the environments of the C-1 methyl (δ2.60, 2.52) and N-methyl groups (δ3.05, 3.00) as well as the N-acetyl methyl (δ2.08, 1.98)



(121a)



(121b)

and the C-1' methylene group (δ5.50, 5.46). This resulted in twin sets of peaks being present for each of these groups. Singlet resonances occurred at δ2.88 and 2.03 for the C-4 methyl and the acetate methyl groups, respectively. These groups were not influenced by the existence of the molecule in two distinct conformation.

The IR. spectrum possessed absorptions at 1730 cm^{-1} and 1635 cm^{-1} for the carbonyls of the acetate and N-acetyl groups, respectively. A normal carbazole spectrum was obtained in the UV. of compound 121. The mass spectrum possessed a parent peak at m/e = 366 with major fragmentations at m/e = 306 (M^+ - HOAc), 280 (M^+ - C_4H_8NO), 263, 233, 222-1. The parent peak at 366.1956 was within acceptable limits of the calculated value 366.1943 for the composition $C_{22}H_{26}N_2O_3$).

The hydrolysis of the acetate group of 121 to the corresponding alcohol 122 proved to be more difficult than expected. The N-acetyl group was completely inert to the basic media whereas the acetate group was readily displaced by alkoxide when either aqueous methanol or ethanol was used as solvent. A two phase system of t-butanol and aqueous sodium hydroxide was found to effect hydrolysis without any competing nucleophilic substitution by t-butoxide ion. The reaction proceeded slowly at room temperature. After 24 hr. unreacted starting material was detected. When the temperature was raised to 100°, the acetate group of 121 was completely hydrolyzed after 1 hr.

Only a single peak at 1635 cm^{-1} for the carbonyl of the N-acetyl group was present in the I.R. spectrum which was consistent with the hydrolysis to the alcohol 122. The UV. spectrum remained essentially unchanged as expected. The mass spectrum possessed a weak parent peak at m/e = 324 and a major fragmentation at m/e = 308 for loss of 16 mass units. The parent peak at m/e = 324.1814 in the high resolution mass spectrum was consistent with the composition $C_{20}H_{24}N_2O_2$.

The N.M.R. spectrum for 122 was too complex to properly analyze, again as a result of the presence of conformers. The complexity of the spectrum may also have been increased by the ability of the hydroxyl group to hydrogen bond to the amide carbonyl oxygen. A pair of multiplets were discernable at δ6.20 and 5.00 for the methylene hydrogens at C-1'. The remaining singlets for the various methyl groups were split in a complex manner which made their assignment ambiguous.

The hydrolysis of the 3-(α-acetoxyethyl) carbazole derivative 223 using the same two phase basic media also proceeded to completion after 1 hr. at 100° (isolated yield 79%). All the spectral and analytical data for the product alcohol 222 was identical to that obtained for the same compound 222 synthesized in sequence B, part III (page 225). Pertinent at this point was the loss of the acetate carbonyl peak at 1720 cm^{-1} in the IR spectrum.

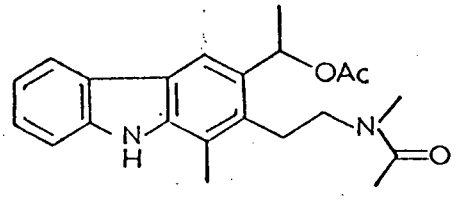
Basic media was required for the subsequent dehydration of the alcohol 222 to the olefin 123 because acidic media (for example 20% H_2SO_4) would result in concomitant hydrolysis of the amide group. The dehydration was effected by taking advantage of the known instability of benzylic tosylates with respect to elimination. By refluxing the alcohol 222 in pyridine containing p-toluenesulphonyl chloride the olefin 123 was obtained in 60% yield.[155]

The N.M.R. spectrum possessed a doublet of doublets at δ5.78 ($J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 1.5 Hz) for the trans C-2' Hydrogen and a second doublet of doublets at δ5.21 ($J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 1.5 Hz) for the cis C-2' hydrogen. The corresponding doublet of doublets for the C-1' hydrogen was partly obscured by the aromatic signals. The chemical shifts for the cis and trans C-2' hydrogens coincided closely with those found for olefin 88 which also possesses the C-3 vinyl side chain. Singlets occurred at δ5.72 and 2.60 respectively for the methylene and C-1 methyl group.

The UV. spectrum also bore a close resemblance to that obtained for olefin 88 as expected. The mass spectrum possessed a parent peak at m/e = 297 and

almost no fragment peaks. The parent peak at 297.1472 in the high resolution spectrum was within acceptable limits of the calculated value for the composition $C_{22}H_{19}N$.

Having worked out the conditions necessary for formation of the ring-opened acetate, its hydrolysis, and dehydration of the alcohol for the model compounds, attention was directed towards applying these reactions to guatambuine (25). The acetylation reaction was initially conducted using acetic anhydride in pyridine. Under these conditions the reaction proceeded as anticipated to give the N-acetyl acetate 124.



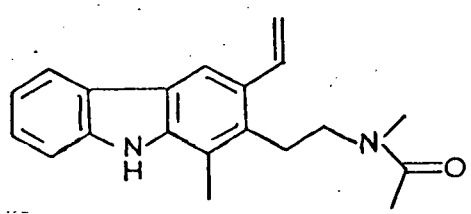
(124)

The presence of two conformers of 124 was again observed in the N.M.R. The N-acetyl and C-1 methyl groups and the C-1' methine hydrogen were influenced the most by the two separate conformations of the molecule. Shoulders were observed on the singlet peak for the N-methyl group (δ3.00) and on the doublet peak for the C-1' methyl group (δ1.64, J = 6 Hz). Also, a multiplet instead of the anticipated quartet was obtained for the C-1' methine hydrogen (δ6.34). The resonances for the C-1 methyl (δ2.48, 2.40) and N-acetyl methyl groups (δ2.08, 1.98) were present as a distinctly separated pair of singlets. The singlet for

the acetate methyl group (δ2.08) was superimposed upon the singlet for N-acetyl methyl group of the major conformer of 124. A single peak was thus obtained for both groups.

The mass spectrum possessed a weak parent peak at m/e = 366 and a major fragment at m/e = 306 for loss of the elements of acetic acid. Two peaks were present at 1710 and 1620 cm^{-1} in the IR. for the carbonyls of the acetate and N-acetyl groups respectively. A carbazole UV. spectrum was obtained for 124 as expected.

When sodium acetate in acetic anhydride was used for the acetylation ring-opening, it was observed that considerable quantities of olefinic material was present in the reaction mixture. By conducting the reaction at reflux temperature for 10 hr. the olefin 125 was obtained as the major product in 64% yield.



(125)

The spectral data for 125 was consistent with its assigned structure. The occurrence of two distinct conformers of 125 resulting from hindered rotation about the N-methylamide linkage was again observed in the N.M.R. spectrum (Figure 37). Twin sets of peaks were observed for the N-methyl (δ3.00, 2.92), C-1 methyl (δ2.58, 2.52) and N-acetyl methyl groups (δ2.10, 1.96), and the C-2' cis oriented vinyl proton (δ5.37, 5.34). A doublet of doublets was present at δ5.72 for the trans C-2' hydrogen. This proton was far enough removed from the

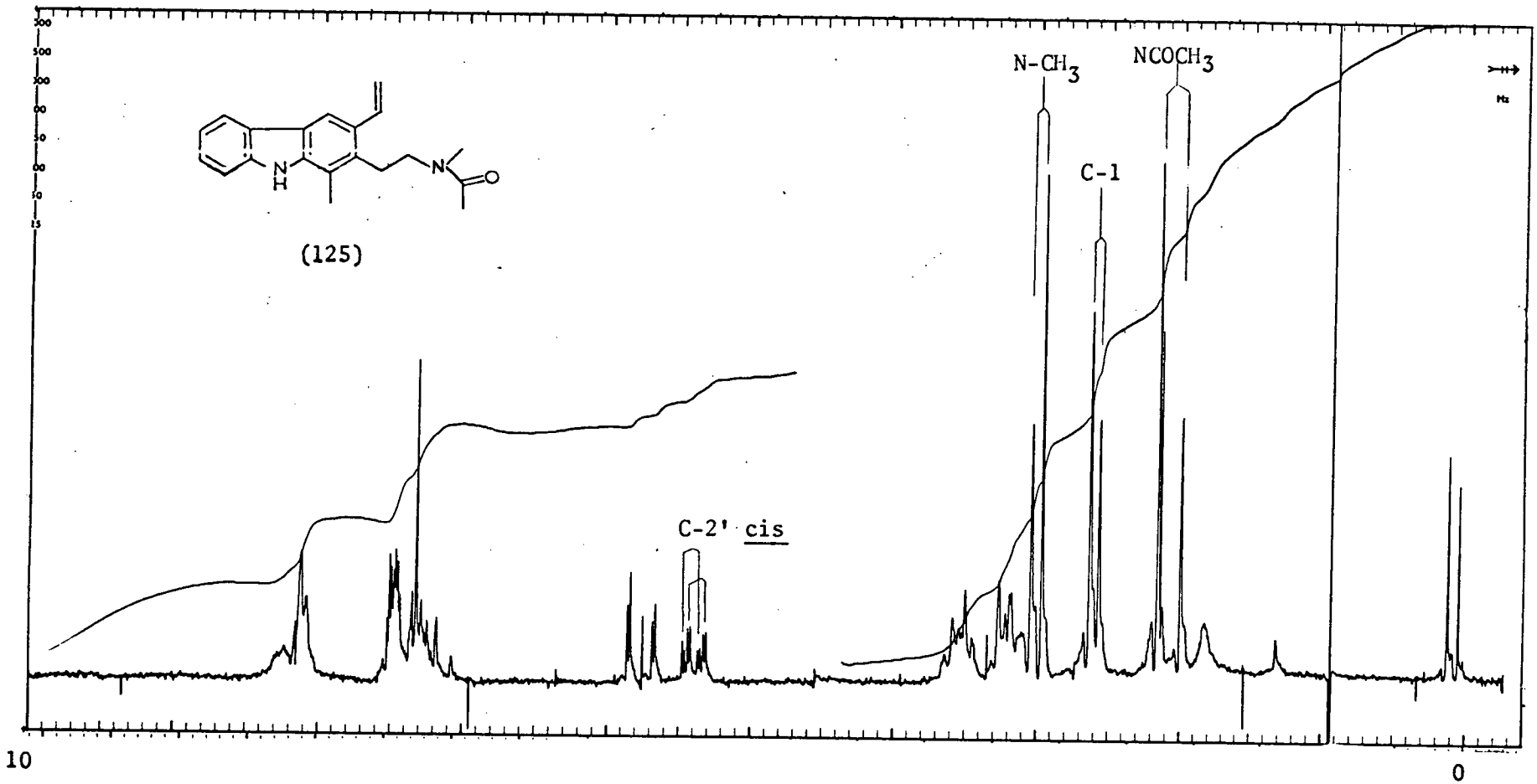


Figure 37. N.M.R. Spectrum of 1-Methyl-2-(β′-(N,N-methylacetylamino)ethyl-3-vinylcarbazole (125).

amide group to be uninfluenced by it.

The mass spectrum possessed a parent peak at m/e = 306 and major fragments at 234, 220 and 204. The parent peak in the high resolution spectrum at m/e = 306.1736 was within acceptable limits of the calculated value m/e = 306.1732 for $C_{20}H_{22}N_2O$. A single carbonyl absorption at 1660 cm^{-1} was present in the IR. spectrum for the N-acetyl group. The UV. spectrum for compound 125 was similar to that for the C-3 olefin 88 in that the absorption maxima consisted of two broad humps in the region of 280 mm. and 240 mm.

It was apparent from these results that reaction with acetic anhydride in sodium acetate induced the pyrolytic elimination of the initially formed acetate to occur. Identical reactivity has been observed for α-arylalkyl quaternary ammonium salts on pyrolysis.[143] This property was of considerable value since it meant that guatambuine (25) could be transformed in one step and in good yield to the desired C-3 vinyl derivative.

In terms of the development of the degradation sequence for the isolation of the C-1 methyl group either the acetate ring-opening reaction or the Hofmann reaction using sodium hydride in dimethylformamide could be used. The only advantage that the Hofmann reaction offered was that the yield was somewhat higher and the reaction time was considerably shorter.

C. Oxidation Reactions

This part of the discussion is concerned with a partial review of the possible application of several different types of oxidation-hydrolysis reactions towards the isolation of the C-1 methyl group of guatambuine (25). Very little work was done in this area however, due to the successes achieved with the other approaches.

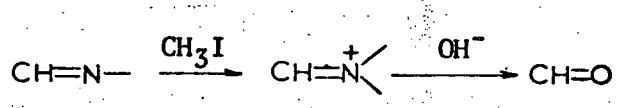
One area that was not studied at all was the possibility of the direct oxidative cleavage of the C-1 methyl from the parent aromatic compound olivacine (16). The pyridine nitrogen imparts a reactivity to the α-substituted C-1 methyl which is not possessed by the C-5 methyl group. It should be possible therefore through the choice of the proper oxidizing conditions to selectively oxidize the C-1 methyl group preferentially to the C-5 methyl group.

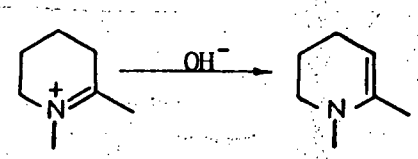
Both Ce^{IV} and Ag^{II} oxidizing reagents[156-8] have been shown to selectively oxidize a single alkyl group of a polyalkyl benzene system. It has been further shown that it was possible to conduct a stepwise oxidation of that methyl group from the alcohol to the benzoic acid derivative. The control over the oxidation process exhibited by these compounds makes their application to the oxidation of the C-1 methyl of olivacine (16) entirely possible. Permanganate ion would also be a selective oxidizing agent since it is known that 2-methylpyridine is readily oxidized to pyridine-2-carboxylic acid.

A number of methods including the use of permanganate ion,[159] manganese dioxide,[160] and Ag^{II} salts[161-2] are available for the oxidation of amines to the corresponding carbonyl compound. They are generally applicable only to primary or secondary amines however since the intermediate imines which are formed are hydrolytically unstable with respect to the aldehydes or ketones. Dimethyl sulphoxide[163] has been shown to be a relatively good oxidizing agent for tertiary amine hydrochlorides and quaternary ammonium salts. However, the oxidation of 1-phenylethylamine salts which were closely related to guatambuine (25) failed due to the instability of the ketonic products formed in the reaction media. These types of reactions were therefore not attempted on the guatambuine system.

The majority of reactions for oxidizing amines to the carbonyl compounds

involve formation of the imine or imminium cation as the starting material or as the reaction intermediate. Mercuric acetate[164] has been utilized successfully for the oxidation of tertiary amines to their carbonyl counterparts. The reaction involves prior formation of the iminium cation which readily hydrolyzes in aqueous base. The application of this reaction to cyclic systems such as guatambuine (25) however does not result in ketone formation, basification results in the formation of the α,β-enamine. The subject of enamine formation will be returned to shortly.



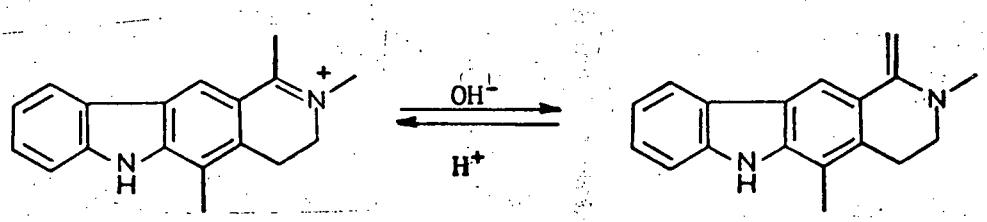


Ozonolysis[165] has been used to cleave the carbon-nitrogen double bond of Schiffs bases and nitrones to the corresponding aldehydes or ketones. Oxaziranes and amides are generally formed also during the reaction and in comparable yields to the carbonyl component. This would be a major drawback to its application to small scale degradation work.

A method which has been developed specifically for the oxidation of tertiary amines involves treatment of the amine or the iminium salt with warm buffered permanganate followed by rapid product removal.[166] It would be necessary

however for the degradation of 142 to modify the isolation procedure for the reaction to accommodate working on a small scale with non-volitile carbonyl compounds.

Some preliminary experiments that were tried involved an attempt to hydrolyze the iminium salt of guatambuine 142 in acidic and basic media. The iminium salt 142 was available as a stable crystalline solid from the synthesis presented in sequence A, part III. Ketone formation could not be detected under either conditions. In aqueous base however, an intense yellow coloured solution was produced and an amorphous yellow precipitate developed. The formation of this intense colouration has been ascribed in related systems to the formation of the α,β-enamine (anhydro base).[136,167-8] In comparison to the iminium cation 142 only minor shifts in the UV. spectrum were observed to accompany the formation of the enamine 126 (Figure 38).



(142) (126)

Attempts to isolate the enamine 126 (anhydro base) by extraction into ether failed. This was not a totally unexpected result since they are known to be unstable with respect to the quaternary ion.

A ring-opening of the β-carboline ring system has been developed by Gupta and Spenser[167-8] during their investigation of the methylation of the anhydro bases of Harman derivatives (Figure 39).

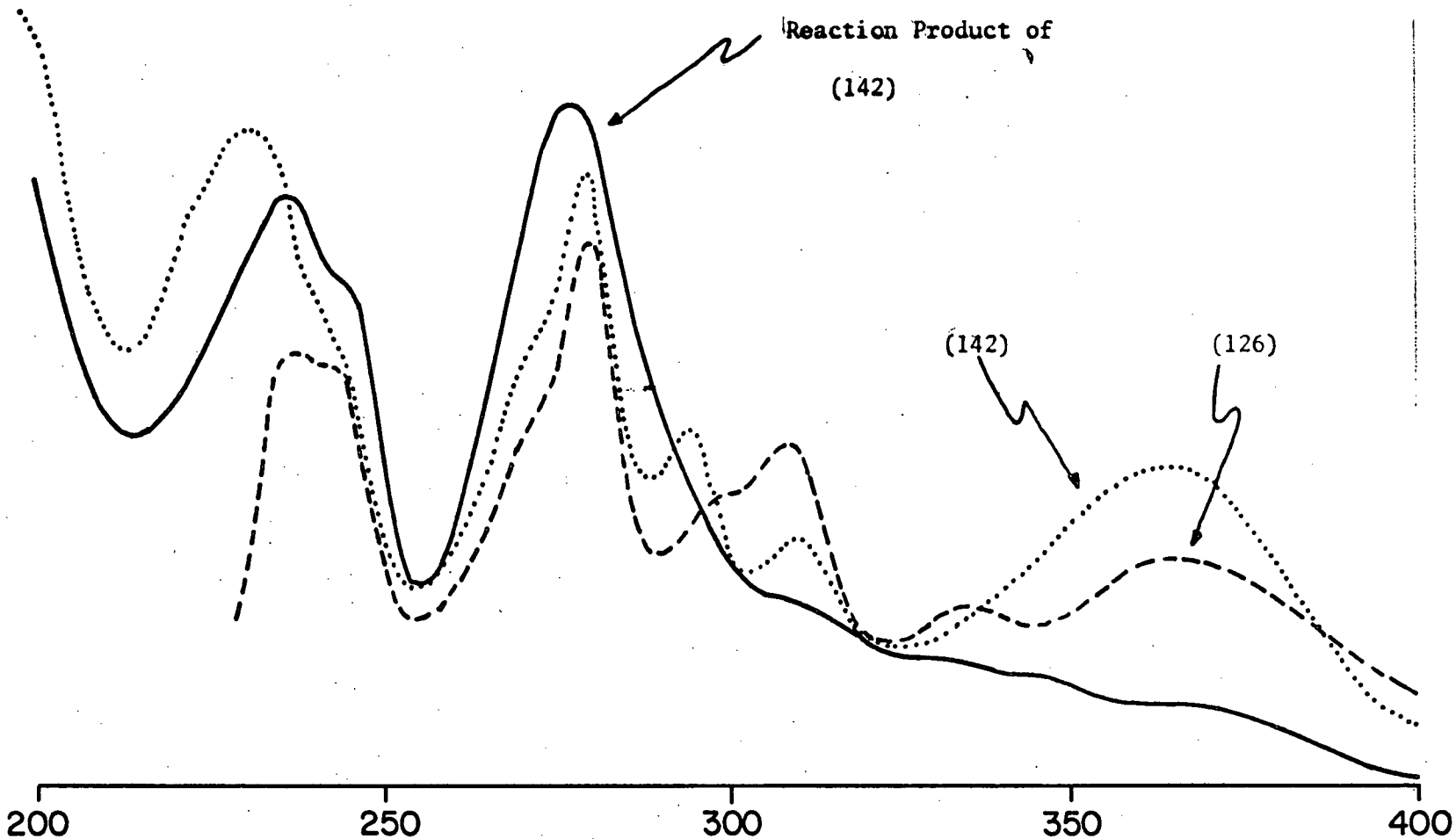


Figure 38. The UV. Spectrum of the Iminium Cation (142) Enamine (126), and Reaction Product of 142 Treated with Dimethylsulphate in Strong Base.

They found that the enamine of Harmaline 128 could be isolated after base treatment of the iminium salt 127. When the enamine was subsequently treated with methyl iodide, the quaternary ammonium iodide produced was displaced by hydroxide to give the ring opened ketone 129. A Hofmann elimination of the amine function of the C-3 side chain led to the C-3 vinyl product 130. This same process has also been shown to occur for the 1-methyl-3,4 dihydroisoquinoline systems by treatment with dimethylsulphate in concentrated base.[169]

It is entirely feasible that this sequence of reactions could be carried out on guatambuine (25). The enamine 126 could be made available by mercuric acetate oxidation of guatambuine (25).[164] The reaction with dimethyl sulphate in 20% aqueous sodium hydroxide has been conducted on a small scale. Within 5 min. after heating the reaction mixture at 100° the yellow colouration for the enamine 126 disappeared. At the present time, however, only the UV data was available (Figure 38). The absorption curve for the reaction mixture was quite reminiscent of both the imine (141)[125] and the C-3 olefin 88 (Figure 27) which gives some indication at least that the starting material 142 alters in the reaction media and that perhaps the keto-olefin (131) was produced.

If the chemistry of this ring-opening reaction could be developed to the point where good yields were obtained, then it could serve as a feasible alternative to the Hofmann or acetate substitution approaches previously discussed. By a proper choice of reactions, the selective isolation of the C-1 methyl, N-methyl and C-3 carbons of guatambuine could be achieved, i.e. borohydrive reduction of the ketone to the alcohol, then ozonolysis of the double bond followed by a Haloform reaction on the secondary alcohol.[170-171]

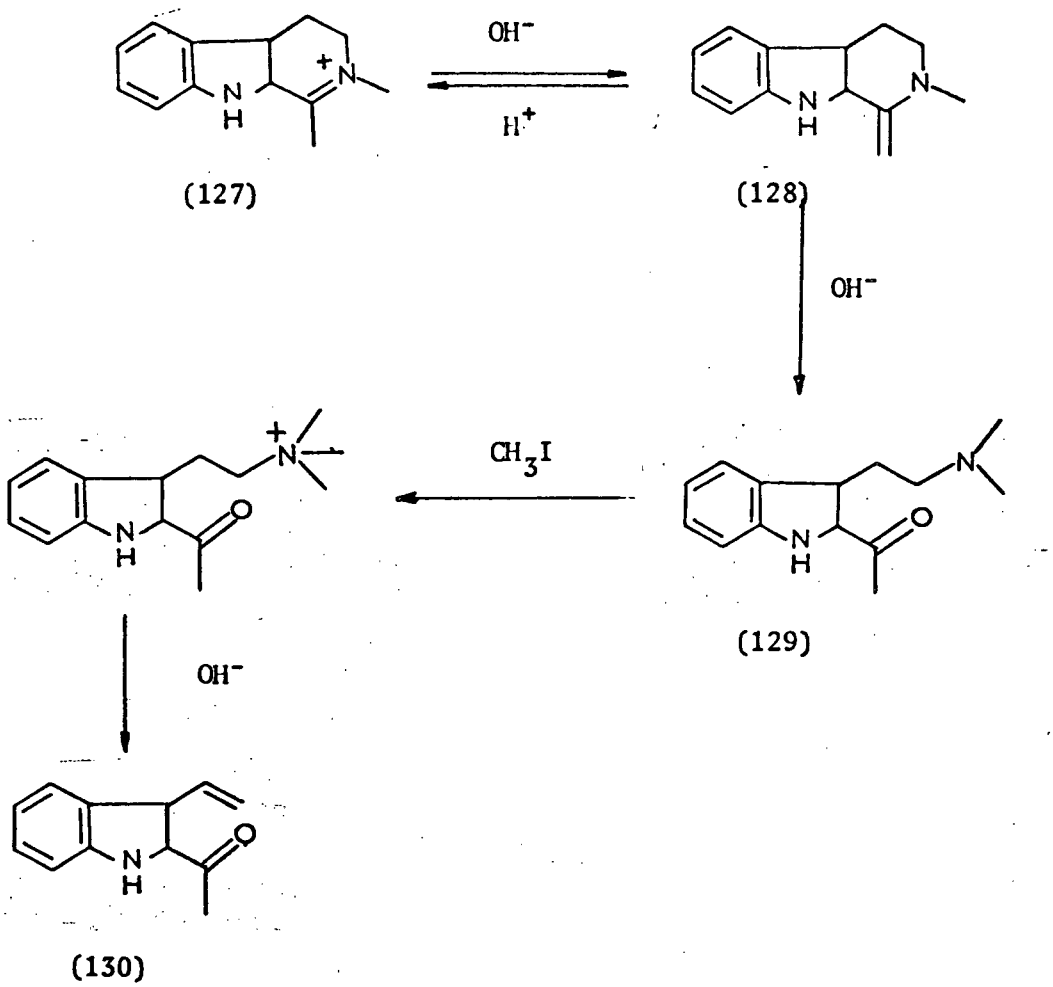


Figure 39. Ring Opening of the β-Carboline Ring System by Methylation of the Anhydro Base.

EXPERIMENTAL - PART II

For a description of the general experimental information, see Experimental part 1.

All T.L.C. plates were developed in chloroform ($CHCl_3$) or ethyl acetate unless otherwise indicated, and the alumina for column chromatography was deactivated to Activity III by the addition of water (6%).

N-Methyl-1,2,3,4-tetrahydroellipticine (26)

A solution of ellipticine (17) (500 mg., 2.03×10^{-3} mole) dissolved in methanol (400 ml.) containing an excess of methyl iodide (5 ml.) was stirred at room temperature for 24 hr. The reaction mixture was then concentrated to dryness, taken up in methanol (200 ml.) and preadsorbed onto alumina (10 gm.), and applied to the top of an alumina column (40 gm.). Traces of unreacted starting material were eluted with ethyl acetate:methanol 5% and the desired methiodide product 93 was subsequently eluted with methanol: ammonium hydroxide 1%. The methiodide 93 was obtained as a bright orange crystalline solid (630 mg., 77%).

The methiodide 93 (500 mg. 1.24×10^{-3} mole) was dissolved in aqueous

ethanol (250 ml.) and reacted with an excess of sodium borohydride at room temperature for 15 hr. The reaction mixture was then concentrated, taken up in water (150 ml.) and extracted with chloroform (3 x 50 ml.). The combined chloroform fractions were dried over sodium sulphate and concentrated to give 26 as a pure colourless crystalline solid (300 mg., 91%) which turned yellow on prolonged standing. The product 26 was stabilized by recrystallization from methanol to give cream coloured needles, m.p. 217-220° (lit., m.p. 215-220°).[126] UV.; $_{max}$ (log ε): 340 (3.49), 326 (3.62), 296 (4.25), 286 (sh)(4.02), 260 (4.49), 248 (4.60), 238 (4.74). N.M.R. (F.T.): 8.20 (doublet, J = 7Hz, C-10H), 7.84 (broad singlet, N-H), 3.76 (singlet, C-1 $\underline{CH_2}$), 3.00 (triplet, J = 5Hz, C-4 $\underline{CH_2}$), 2.76 (triplet, J = 5Hz, (partly obscured by a singlet at 3.70), C-3 $\underline{CH_2}$), 3.70 (singlet, C-11 $\underline{CH_3}$), 2.56 (singlet, C-5 $\underline{CH_3}$), 2.38 (singlet, N-CH_3). Mass spectrum: M^+; m/e = 264; main peaks: 263 (base peak), 249, 233, 221, 204-5. Found: C, 81.67; H, 7.64; N, 10.30. Calc. for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60.

N-Methyl-1,2,3,4-tetrahydroellipticine methiodide (95).

A solution of 26 (265 mg. 1.00 x 10^{-3} mole) in methanol (25 ml.) containing an excess of methyl iodide was allowed to stand at 0° for 24 hr. The colourless crystals of 95 that precipitated were collected by suction filtration, washed with methanol and dried under vacuum (396 mg. 98%), m.p. 307 - 308° Found: C, 55.91; H, 5.87; N, 6.50. Calc. for $C_{19}H_{23}N_2I$: C, 56.29; H, 5.69; N, 6.91.

1,2,3,4-Tetrahydroellipticine (94).

Ellipticine (17) (100 mg., 4.06 x 10^{-4} mole) was dissolved in glacial acetic acid (20 ml.) and the solution was hydrogenated at room temperature and atmospheric pressure over Adams catalyst (PtO_2, 85 mg.) for 12 hr. The mixture was filtered to remove the catalyst which was carefully washed with additional acetic acid (10 ml.). The combined filtrates were concentrated, taken up in water (75 ml.), basified with 10% sodium hydroxide and the resulting white suspension was extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to give 94 as colourless crystals (95 mg.,93 %), which rapidly turned yellow on standing. UV.; λ_{max}: 340, 324, 293, 284, 261, 250, 241 (log (ε) values were not obtained, however, the relative extinction coefficients for the absorption maxima were virtually identical to those observed for N-methyl-tetrahydroellipticine (17)). Compound 94 was converted to its N-acetyl derivative for further characterization.

The preparation of the methiodide 95 of compound 94 was carried out in an identical manner to the reaction of N-methyltetrahydroellipticine (17). The melting point and microanalytical data agreed satisfactorily with the values previously obtained.

N-Acetyl-1,2,3,4-tetrahydroellipticine (96).[137]

Compound 94 (35 mg. 1.40 x 10^{-4} mole) was dissolved in a mixture of acetic anhydride (3 ml.) and pyridine (3 ml.), and stirred at 70° for 3 hr.

The reaction mixture was then concentrated, taken up in water (75 ml.), basified with 10% sodium hydroxide solution, and extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were washed with water, dried over sodium sulphate, and concentrated to give crude 96 (40 mg., 97%). Several recrystallizations from methanol afforded 96 as colourless plates, m.p. 262-265° (lit. m.p. 272.5-273°). Found: C, 78.01; H, 7.02; N, 9.30. Calc. for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 5.47.

Olivacine methofluorosulphate (85) and its reduction to Guatambuine (25).

Olivacine (16) (20mg., 8.13 x 10^{-5} mole) dissolved in acetonitrile (25 ml.) was treated with methylfluorosulphate (30µl, 3 equiv.) (freshly distilled) at room temperature. A yellow precipitate formed within minutes and after 15 min. the reaction mixture was concentrated to give the fluorosulphate salt 85 as a yellow paste.

The crude product 85 was suspended in aqueous ethanol (100 ml.) and reacted with an excess of sodium borohydride at room temperature, for 15 hr. The yellow colouration for the olivacine salt disappeared almost instantaneously on addition of the reducing agent. The reaction mixture was then concentrated to a paste, taken up in water (75 ml.), and extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to give guatambuine (25) as a cream coloured solid (20 mg., 93%). Recrystallization from methanol gave colourless needles, m.p. 248° (lit., m.p. 248-250°). The UV. and N.M.R. and low resolution mass spectra for the reaction product was consistent with that previously obtained for guatambuine (25). High resolution mass spectrum: Calc. for $C_{18}H_{20}N_2$: 264.1626. Found: 264.1663.

Guatambuine methiodide (86).

The methiodide 86 was prepared in quantitative yield by reaction of 25 with an excess of methyl iodide at 0° for 24 hr. Colourless needles were obtained which were recrystallized from methanol, m.p. 299° (lit., 299-301°)[121-3]. N.M.R. (DMSO-d6): 8.10 (doublet, 1H, J = 8Hz, C-10 H), 2.90 (singlet, 1H, C-11 H), 4.96 (quartet, 1 H, J = 6Hz, C-1 H), 3.80 (broad multiplet, 2 H, C-4$\underline{CH_2}$), 3.30 (multiplet, obscured by singlet absorption at 3.18, C-3 CH_2), 3.18, 3.12 (two singlets, 3 H, $N(CH_3)_2$), 1.74 (doublet, 3H, J = 6Hz, C-1 $\underline{CH_3}$). Found: C, 56.34; H, 5.69; N, 6.63. Calc. for $C_{19}H_{23}N_2I$: C, 56.29; H, 5.68; N, 6.80.

Olivacine methiodide (85) and its Reduction to Guatambuine (25).

Olivacine (16) (15 mg., 6.09 x 10^{-5} mole) dissolved in ethanol (25 ml.) was treated with an excess of methyl iodide and refluxed for 0.5 hr. The crystalling precipitate was collected and the mother liquors were concentrated, taken up in a minimum amount of methanol and treated again with methyliodide. The second crop of crystalline material was collected and the mother liquors were again concentrated and rereacted with methyl iodide. By recycling the mother liquors in this fashion four times, an overall 80% yield of impure olivacine methiodide (85) (18.4 mg.) was obtained.

The crude product 85 (14 mg., 3.49 x 10^{-5} mole) was reduced with sodium borohydride in an identical manner to the methofluorosulphate salt 85 described in the previous experiment. The spectral data was consistent

with that previously obtained for guatambuine (25).

Hofmann Degradation of (-)-Guatambuine Methiodide (86).

i) Preparation of 1-Methyl-2-(β-dimethylaminoethyl)-3-(α-ethoxyethyl) carbazole (98) and C-3 olefin 88.[121-3]

(-)-guatambuine methiodide (86) (40.2 mg., 9.92 x 10^{-5} mole) was suspended in a 10% sodium hydroxide in 95% ethanol solution (60 ml.) and refluxed for 2.5 hr. The reaction mixture was then concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a light brown oil containing a mixture of compounds 98 and 88 (40 mg., >100%) N.M.R.: (See Figure 23).

Compound 98 was isolated as a transparent oil by successive preparative layer chromatography on alumina (1 mm., EtOAc) (24 mg., 75%) and silica gel (19 mg., 60%). An analytical sample was obtained by distillation of 98 at 160-180° at 0.07-0.01 mm. $(\alpha_D)^{25} = 0°$ (before: $(\alpha_D)^{25} = -112°$). UV.; λ_{max}: 340, 326, 297, 287 (sh), 261, 250, 240. N.M.R. (Figure 23): 4.85 (quartet, 1 H, J = 6Hz, C-1'H), 3.42 (quartet, 2 H, J = 7Hz, $O\underline{CH_2}CH_3$), 3.05 (multiplet, 2 H, C-3' $\underline{CH_2}$), 2.49 (singlet, 3 H, C-1 CH_3), 2.40 (singlet, 6 H, $N(\underline{CH_3})_2$), 1.53 (doublet, 3 H, J = 6Hz, C-1' $\underline{CH_3}$), 1.20 (triplet, 3 H, J = 7Hz, $O\text{-}CH_2\text{-}\underline{CH_3}$). Mass spectrum: M^+, m/e = 324; main peaks: 295, 278. High resolution mass spectrum: Calc. for $C_{21}H_{28}N_2O$: 324.2200. Found: 324.2207. Found: C, 56.85; H, 6.60; N,

5.75. Calc. for $C_{22}H_{31}N_2IO$: C, 56.80; H, 6.67; N, 6.0.

ii) Preparation of 1-Methyl-2-vinyl-3-(α-dimethylaminoethyl)carbazole (97), and 1-Methyl-2-(α-dimethylaminoethyl)-3-vinylcarbazole (88).

A) Guatambuine methiodide (86) (20 mg., 4.93 x 10^{-5} mole) was suspended in t-butanol (12 ml.) containing potassium t-butoxide (30 mg.) and heated at reflux for 1.5 hr. The solution was then concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a light brown oil containing a mixture of compound 97 and 88 (10 mg., 72%). N.M.R. (Figure 24): 5.70 (distorted doublet of doublets, 1 H, $J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2 Hz, C-2' H trans (88) overlapped with C-4' H cis (97)) 5.68 (doublet of doublets, 1 H, $J_{3',4'}$ = 11 Hz, $J_{4',4'}$ = 2 Hz, C-4' H cis (97)), 5.23 (doublet of doublets, 1 H, $J_{3',4'}$ = 17 Hz, $J_{4',4'}$= 2 Hz, C-4' trans (97)), 5.32 (doublet of doublets, 1 H, $J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 2Hz, C-2' H cis (88)), 3.82 (quartet, J = 6Hz, C-1' H), 2.52 (singlet, C-1$\underline{CH_3}$), 2.40 (singlet, N($\underline{CH_3}$)$_2$), 2.32 (singlet, N($\underline{CH_3}$)$_2$), 1.44 (doublet, J = 6 Hz, C-1' CH_3), 1.28 (singlet, 0($\underline{CH_3}$)$_3$).

The major compound 97 was isolated by preparative layer chromotography on alumina (1 mm., ethyl acetate) (4 mg., 30%). UV. (figure 27); λ_{max}: 340, 326, 297, 248. N.M.R. (Figure 25): 8.08 (multiplet, 214, C-4, C-5 H), 6.98 (doublet of doublets, 1 H, $J_{3',4'}$ = 11 Hz, $J_{3',4'}$ = 17 Hz, C-3'H), 5.65 (doublet of doublets, 1 H, $J_{3',4'}$ = 11 Hz, $J_{4',4'}$ = 2 Hz, C-4' H cis),

5.20 (doublet of doublets, 1 H, $J_{3',4'}$ = 17 Hz, $J_{4',4'}$ = 2 Hz, C-4' H trans), 3.75 (quartet, 1 H, J = 6Hz, C-1' H), 2.50 (singlet, 3 H, C-1 CH_3), 2.25 (singlet, 6 H, $N(CH_3)_2$), 1.39 (doublet, 3 H, J = 6 Hz, C-1'CH_3). Mass spectrum: M^+, m/e = 278 (base peak); main peaks: 263, 249, 235-3, 220-217, 205, 204. High resolution mass spectrum: Calc. for $C_{19}H_{22}N_2$: 278.1782. Found: 278.1780. Found: C, 56.92; H, 5.71; N, 6.63. Calc. for $C_{20}H_{25}N_2I$: C, 57.27; N, 5.96; N, 6.68.

B) In a second experiment, guatambuine methiodide (86) (80 mg., 1.97 x 10^{-4} mole) was suspended in t-butanol (30 ml.) containing potassium t-butoxide (80 mg.) and heated at reflux for 2.5 hr. On work-up a mixture of olefins 88 and 97 was obtained (40 mg., 72%). The mixture was separated by column chromatography on alumina (5 gm.). By elution with benzene: chloroform 1:1 olefin 97 was obtained (20 mg., 36%). The spectral data for 97 was identical to that obtained in experiment A. By increasing the solvent polarity to benzene:chloroform 70%, olefin 88 was eluted to give a light brown oil (10 mg., 18%). UV. (Figure 27); λ_{max}: 325 (broad hump), 295 (sh), 280, 267 (sh), 241. N.M.R. (Figure 26): 8.00 (multiplet, 3 H, C-4, C-5 H, N-H), approximately 7.2 (doublet of doublets, 1 H, C-1'H (obscured by $CHCl_3$ peak)), 5.68 (doublet of doublets, 1 H, $J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2 Hz, C-2'H trans), 5.29 (doublet of doublets, 1 H, $J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 2 Hz, C-2'H cis), 3.10 (multiplet, 2 H, C-3'CH_2), 2.50 (singlet, 3 H, C-1CH_3), 2.38 (singlet, 6 H, $N(CH_3)_2$). Mass spectrum: M^+, m/e = 278 (base peak); main peaks; 263, 249, 234, 233,

220, 219-19, 204. High resolution mass spectrum: Calc. for $C_{19}H_{22}N_2$: 278.1782. Found: 278.1780. Found: C, 56.86; H, 6.27; N, 6.22. Calc. for C H N I: C,57.15; H, 5.99; N, 6.66, For the methiodide of 88. m.p. 280-282°. UV.;λ_{max} (logε): 330(3.25), 296(sh)(3.98), 278(4.23), 240(4.41).

iii) Preparation of Olefins 88 and 97.

Guatambuine methiodide (86) (15 mg., 3.70 x 10^{-5} mole) was suspended in a 40% aqueous sodium hydroxide solution (20 ml.) and heated at reflux temperature for 3 hr. The cooled reaction mixture was diluted with water (50 ml.) and extracted with chloroform (3 x 30 ml.). The combined chloroform extracts were washed with water, dried over sodium sulphate, and concentrated to give a brown oil (6mg., 60%). The spectral data for the crude reaction mixture was identical to that using potassium t-butoxide as the base in t-butanol (reaction ii).

iv) Preparation of 1-Methyl-2-(β-dimethylaminoethyl)-3-vinylcarbazole (88).

Guatambuine methiodide (86) (76 mg., 2.42 x 10^{-4} mole) and sodium hydride (50 mg.) in dry dimethylformamide was heated at 100° for 2 min. after which time the excess hydride was destroyed by the careful addition of water. The reaction mixture was then diluted with water (75 ml.) and the resultant white suspension was extracted with ether (4 x 30 ml.). The combined ether layers were washed with water (3 x 30 ml.), dried over sodium sulphate and concentrated to give olefin 88 as a colourless oil which slowly solidifies to an amorphous white solid under vacuum (37 mg., 70%). UV. (Figure 27); λ_{max}: 325 (broad hump), 295 (sh), 280, 267 (sh),

(Figure 26)
241. N.M.R.: 8.00 (multiplet, 3 H, C-4, C-5, N-H), 5.68 (doublet of doublets, 1 H, $J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2 Hz, C-2'H trans), 5.29 (doublet of doublets, 1 H, $J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 2Hz, C-2'H cis), 3.10 (multiplet, 2 H, C-3'CH_2), 2.50 (singlet, 3 H, C-1CH_3), 2.38 (singlet, 6 H, N(CH_3)$_2$).

Hofmann Degradation of N-Methyltetrahydroellipticine methodide (95).

Preparation of 1,4-Dimethyl-2-vinyl-3-dimethylaminomethylcarbazole (102).

N-Methyltetrahydroellipticine methiodide (95) (50 mg., 1.23 x 10^{-4} mole) was suspended in t-butanol (25 ml.) containing potassium t-butoxide (40 mg.) and heated at reflux for 4 hr. The solution was concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give 102 as a tan coloured oil (33 mg., 96%). UV.: λ_{max}: 335, 322, 293, 250. N.M.R.: 8.25 (broad doublet, 1 H, J = 6 Hz, C-10 H), 8.02 (broad hump, 1 H, N-H), 7.46-6.96 (multiplet, 5 H, 4 aromatic H, C-3'H), 5.64 (doublet of doublets, 1 H, $J_{3',4'}$ = 11 Hz, $J_{4',4'}$ = 2 Hz, C-4'H, cis), 5.20 (doublet of doublets, 1 H, $J_{3',4'}$ = 17 Hz, $J_{4',4'}$ = 2 Hz, C-4'H, trans), 3.64 (singlet, 2 H, C-1'CH_2), 2.90 (singlet, 3 H, C-4 CH_3), 2.50 (singlet, 3 H, C-1 CH_3), 2.24 (singlet, 6 H, N(CH_3)$_2$). Mass spectrum: M^+, m/e = 278; main peaks: 263, 249, 248, 234, 233 (base peak), 219, 218, 204. High resolution spectrum: Calc. for $C_{19}H_{22}N_2$: 278.1782. Found: 278.1780.

1-Methyl-2-(β-dimethylaminoethyl)-3-ethylcarbazole (89) from (88).

Olefin 88 (2 mg., 7.19 x 10^{-6} mole) dissolved in methanol (3 ml.) was hydrogenated at room temperature and atmospheric pressure over Adams catalyst (PtO_2, 5 mg.) for 0.5 hr. The mixture was filtered to remove the catalyst which was carefully washed with additional methanol (10 ml.). The combined filtrates were concentrated under vacuum to give 89 as a colourless oil (2 mg.). The spectral data for the reaction product 89 was consistent with that for the same product 89 obtained through reaction of guatambuine methiodide (86) with lithium aluminum hydride and through Hofmann degradation of Uleine (18) (to be subsequently described). N.M.R. (FT): 2.64 (singlet, 3H, C-1 CH_3), 2.54 (singlet, 6 H, $N(\underline{CH_3})_2$).

1-Methyl-2-(β-dimethylaminoethyl)-3-ethylcarbazole (89) from (86).

Guatambuine methiodide (86) (50 mg., 1.23 x 10^{-4} mole) and lithium aluminum hydride (50 mg.) suspended in dry tetrahydrofuran (25 ml.) was heated at reflux temperature for 4 hr. The excess hydride reagent was destroyed by the successive addition of water (1 ml.), 10% aqueous sodium hydroxide (2 ml.), and water (1 ml.). The resultant precipitate was removed by suction filtration, and washed with methanol. The combined filtrates were concentrated, taken up in ether (75 ml.) and washed with water (3 x 30 ml.). The ether layer was then dried over sodium sulphate, and concentrated to give 89 as a transparent oil (30 mg., 88%). UV: λ_{max}: 340, 326, 296, 285 (sh), 260, 247, 239. N.M.R.: 8.03 (doublet, 1 H, J = 7 Hz, C-5 H), 7.78 (singlet, 1 H, C-4H), 3.2-2.6 (multiplet, 6 H, Ar-$\underline{CH_2}$-CH_3, CH_2-CH_2-$N(CH_3)_2$), 2.52 (singlet, 3 H, C-1 CH_3), 2.46 (singlet, 6 H,

$N(\underline{CH_3})_2$), 1.36 (triplet, 3 H, J = 7 Hz, Ar-CH_2-$\underline{CH_3}$). Mass spectrum: M^+, m/e = 280 (base peak); main peaks: 266, 236, 222, 207, 206, 204. High resolution mass spectrum: Calc. for $C_{19}H_{24}N_2$: 280.1938. Found: 280.1975

Compound 89 obtained from guatambuine methiodide (86) was further characterized as its methiodide salt 113 which was formed in quantitative yield by reaction with an excess of methyl iodide in methanol at 0°C for 24 hr. m.p. 291-293° (lit., m.p. 287-8)[121-3]. Found: C, 56.72; H, 6.40; N, 6.42. Calc. for $C_{20}H_{27}N_2I$: C, 56.88; H, 6.44; N, 6.63. UV.; λ_{max} (logε) 340 (3.32), 325 (3.40), 297 (4.12), 285 (3.88), 260 (4.09), 239 (4.52).

1-Methyl-2-(β-dimethylaminoethyl)-3-ethylcarbazole (89) from the Hofmann Degradation of Uleine Methiodide (87).

Uleine methiodide (87) was prepared according to Schmutz *et al.*[132] and a modification of their procedure[120] was used to degrade this compound.

Uleine methiodide (87) (60 mg., 1.47 x 10^{-4} mole) was suspended in a solution of 5% sodium hydroxide in 95% ethanol (25 ml.) and refluxed for 2 hr. The reaction mixture was then concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a brown oil. Polar contaminants were removed by column chromatography on alumina (5 gm.). Elution with chloroform yielded 89 as a light brown oil (31 mg. 75%) UV; λ_{max}: 337, 324, 296, 285 (sh), 260, 245, 237. N.M.R.: 7.74 (singlet, 1H, C-4H), 3.2-2.7 (multiplet, 6 H, Ar-$\underline{CH_2}CH_3$, -$\underline{CH_2}$-$\underline{CH_2}$-$N(CH_3)_2$), 2.48 (singlet 3H, C-1 $\underline{CH_3}$), 2.36 (singlet, 6 H, $N(\underline{CH_3})_2$), 1.30 (triplet, 3H, J = 7 Hz, Ar-$CH_2\underline{CH_3}$). Mass spectrum: M^+, m/e = 280 (base peak); main peaks: 266, 236, 222, 207, 206, 204. High resolution mass spectrum: calc. for $C_{19}H_{24}N_2$: 280.1938. Found: 280.1915.

Compound 89 obtained from uleine methiodide (87) was further characterized as its methiodide salt 113, which was formed in quantitative yield by reaction with an excess of methyl iodide in methanol at 0^oC for 24 hr., m.p. 285° (lit. m.p. 287-8°).[121-3] Found: C, 56.89; N, 6.47; N, 6.70. Calc. for $C_{20}H_{27}N_2I$: C, 56.88; H, 6.44; N, 6.63.

1,3,4-Trimethyl-2-(β-dimethylaminoethyl)carbazole (107).

N-Methyltetrahydroellipticine methiodide (95) (50 mg., 1.23 x 10^{-4} mole) and lithium aluminum hydride (50 mg.) suspended in dry tetrahydrofuran (25 ml.) was heated at reflux temperature for 4 hr. The excess hydride reagent was destroyed by the successive addition of water (1 ml.), 10% aqueous sodium hydroxide (3 ml.), and water (1 ml.). The resultant precipitate was removed by suction filtration, and washed with methanol. The combined filtrates were concentrated, taken up in ether (75 ml.) and washed with water (3 x 30 ml.). The ether layer was then dried over sodium sulphate, and concentrated to give 107 as an opaque solid (30 mg., 88%). Recrystallization from chloroform yielded107 as colourless crystals, m.p. 302-304°. UV.; λ_{max} : 340, 326, 297, 284 (sh), 262, 241. N.M.R. : 8.18 (doublet, 1 H, J = 7Hz, C-5 H), 7.90 (broad hump, 1 H, N-H), 3.04 (multiplet, 4 H, $\underline{CH_2}$-$\underline{CH_2}$-$N(CH_3)_2$), 2.75 (singlet, 3 H, C-4 $\underline{CH_3}$), 2.42 (singlet, 3 H, C-1 $\underline{CH_3}$), 2.36 (singlet, 3 H, C-3 $\underline{CH_3}$), 2.32 (singlet, 6 H, $N(CH_3)_2$). Mass spectrum : M^+, m/e = 280 (base peak); main peaks : 266, 249, 236, 222, 207, 206, 204, 191. High resolution mass spectrum : Calc. for $C_{19}H_{24}N_2$: 280.1938. Found : 280.1903

Compound 107 obtained from N-methyltetrahydroellipticine methiodide (95) was further characterized as its methiodide salt 108, which was formed in quantitative yield by reaction with an excess of methyl iodide in methanol at 0° for 24 hr. Found : C, 56.93; H, 6.36; N, 6.36. Calc. for $C_{20}H_{27}N_2I$: C, 56.88; H, 6.44; N, 6.63.

1,3,4-Trimethyl-2-vinylcarbazole (109).

Methiodide 108 (25 mg., 5.93 x 10^{-5} mole) was suspended in t-butanol (20 ml.) containing potassium t-butoxide (30 mg.) and heated at reflux for 2.5 hr. The reaction mixture was concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a dark coloured oil. Polar contaminants were removed from the crude product mixture by column chromatography on alumina. By elution with chloroform the desired olefin 109. was obtained as a tan coloured oil (12 mg., 85%). UV.; λ_{max}: 335, 322, 293, 250. N.M.R.: 8.26 (doublet, 1 H, J = 7 Hz, C-5H), 6.94 (doublet of doublets, 1 H, $J_{3',4'}$ = 18 Hz, $J_{3',4'}$ = 12 Hz, C-3'H), 5.68 (doublet of doublets, 1 H, $J_{3',4'}$ = 12 Hz, $J_{4',4'}$ = 2 Hz, C-4'H, cis), 5.24 (doublet of doublets, 1 H, $J_{3',4'}$ = 18 Hz, $J_{4',4'}$ = 2 Hz, C-4'H, trans), 2.84 (singlet, 3 H, C-4 CH_3), 2.52 (singlet, 3 H, C-1 CH_3), 2.40 (singlet, 3 H, C-3 CH_3). Mass spectrum: M^+, m/e = 235; main peaks: 220, 205. High resolution spectrum: Calc. for $C_{17}H_{17}N$: 235.1360. Found: 235.1325.

1-Methyl-2-vinyl-3-ethylcarbazole (91).

A. Methiodide 90 (25 mg., 5.93 x 10^{-5} mole) was suspended in t-butanol (15 ml.) containing potassium t-butoxide (40 mg.) and heated at reflux for 2.5 hr. The reaction mixture was then concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a dark coloured oil (17 mg.). Column chromatography on alumina (3 gm.) (elution with chloroform)

removed a portion of the dark coloured contaminants (14 mg.). The crude product was further purified by preparative layer chromatography on alumina (1 mm., Bz:$CHCl_3$ 1:1). Compound 91 was obtained as a dark brown oil (about 8 mg., 57%). UV.: Indicated the presence of contaminants. N.M.R.: 5.65 (doublet of doublets, 1 H, $J_{3',4'}$ = 12 Hz, $J_{4',4'}$ = 2 Hz, C-4'H cis), 5.29 (doublet of doublets, 1 H, $J_{3',4'}$ = 18 Hz, $J_{4',4'}$ = 2 Hz, C-4'H trans), 2.54 (singlet, 3 H, C-1 CH_3). Mass spectrum: M^+, m/e = 235; main peaks: 220, 205. High resolution mass spectrum: Calc. for $C_{17}H_{17}N$: 235.1360. Found: 235.1384.

B. Methiodide 90 (40 mg., 9.50 x 10^{-5} mole) was dissolved in dimethylformamide (5 ml.) containing sodium hydride (10 mg.) and heated at 100° for 2 min. The reaction mixture was then diluted carefully with water (70 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were washed with water (3 x 30 ml.), dried over sodium hydride, and concentrated to give 91 as an opaque oil (9 mg., 40%). The spectral data for compound 91 was described in experiment A.

(N-^{14}C methyl) Guatambuine methiodide (86).

Guatambuine (5 mg., 1.89 x 10^{-5} mole) dissolved in methanol (2 ml.) was reacted with ^{14}C-methyl iodide (1 ml., 1.0 x 10^9 DPM/ml.) for 15 hr. at room temperature. The solvent was removed and the crude methiodide 86 was diluted with unlabelled 86 (50 mg.) and recrystallized from methanol (36 mg.), m.p. 299° (1.29 x 10^4 DPM/m mole).

(N-^{14}C methyl) N-methyltetrahydroellipticine methiodide (95).

N-methyltetrahydroellipticine (5 mg., 1.89 x 10^{-5} mole) dissolved in methanol (2 ml.) was reacted with ^{14}C-methyliodide (1 ml., 1.0 x 10^{8} DPM/ml.) for 15 hr. at room temperature. The solvent was removed and the crude methiodide 95 was diluted with unlabelled 95 (50 mg.) and recrystallized from methanol (47 mg.), (8.87 x 10^{3} DPM/m mole).

Lithium Aluminum Hydride Ring-Opening of (86), Hofmann Degradation of (90), and Isolation of the N-Methyl Group of (25).

Methiodide 86 (36 mg., .4.6 x 10^{5} DPM) was suspended in tetrahydrofuran (25 ml.) and reacted with lithium aluminum hydride (50 mg.) at reflux temperature for 4 hr. The product 89 was isolated and converted to its methiodide 90 (19 mg., 51%) as previously described.

The methiodide 90 (19 mg., 4.51 x 10^{-5} mole) was reacted with potassium t-butoxide (30 mg.) in t-butanol (20 ml.) at reflux temperature for 2.5 hr. A slow stream of nitrogen gas was passed through the solution and the effluent gas containing the trimethylamine was passed through methyl iodide solution. The methyl iodide solution was concentrated and the (N-^{14}C methyl) tetramethylammonium iodide was recrystallized from methanol (2.5 mg., 2.69 x 10^{5} DPM) PCS cocktail). Isolation Efficiency: 59 %.

Lithium Aluminum Hydride Ring-Opening of (95), Hofmann Degradation of (108), and Isolation of the N-Methyl Group of (26).

Methiodide 95 (47 mg., 4 .16 x 10^5 DPM) was suspended in tetrahydrofuran (25 ml.) and reacted with lithium aluminum hydride (50 mg.) at reflux temperature for 4 hr. The product 107 was isolated and converted to its methiodide 108 (25 mg., 51%) as previously described.

The methiodide 108 (25 mg., 5.93 x 10^{-5} mole) was reacted with potassium t-butoxide (30 mg.) in t-butanol (20 ml.) at reflux temperature for 2.5 hr. A slow stream of nitrogen gas was passed through the solution and the effluent gas containing the trimethylamine was passed through methyl iodide solution This methyl iodide solution was concentrated and the (N-^{14}C methyl)tetramethylammonium iodide was recrystallized from methanol (3 mg. 3.10 x 10^5 DPM) (PCS cocktail). Isolation Efficiency: 72 %.

Ozonolysis of (102), Isolation of the C-3 Methylene Group of (26).

The procedure followed was essentially that by Battersby and Harper.[146]

Ozonized oxygen (200 bubbles per minute) was passed through a solution of olefin 102 (44 mg., 1.58 x 10^{-4} mole) in methylene chloride (10 ml.) at -78° and then through acidified potassium iodide solution. The gas was passed for two times the interval required to produce the first colour of iodine in the potassium iodide solution (3-4 min.). After the methylene chloride had been evaporated the ozonide was decomposed by being heated under reflux for 0.5 hr. with water (20 ml.), zinc dust (200 mg.), and silver nitrate (10 mg.). Half the water was

then distilled at atmospheric pressure into a solution of dimedone (300 mg.) in water (20 ml.), and ethanol (8 ml.), water (10 ml.) was added to the distilling flask and the distillation to half volume was repeated into the same dimedone solution. Microcrystalline needles of the dimedone derivative began to separate out during distillation. After 15 hr. at 0° the crystals were collected by centrifugation (27 mg., 87%) and recrystallized from 50% aqueous ethanol to affort the pure derivative,

Ozonolysis of (88), Isolation of the C-1 Methyl Group of (25).

Olefin 88 (50 mg., 1.18×10^{-4} mole) was ozonolyzed in an identical manner to compound 102. Microcrystalline needles of the dimedone derivative began to separate out during distillation. After 15 hr. at 0° the crystals were collected by centrifugation (20 mg., 60%) and recrystallized from 50% aqueous ethanol to afford the pure derivative,

1-Methyl-2-ethyl-3-(α-dimethylaminoethyl)carbazole (103).

Olefin 97 (18 mg., 6.47 x 10^{-5} mole) dissolved in methanol (10 ml.) was hydrogenated at room temperature and atmospheric pressure over Adams catalyst (PtO_2, 10 mg.) for 0.5 hr. The mixture was filtered to remove the catalyst which was carefully washed with additional methanol (10 ml.). The combined filtrates were concentrated under vacuum to give 103 as a colourless film (18 mg., 99%). UV.; λ_{max}: 337, 324, 297, 286 (sh), 261, 248, 239. N.M.R.: 3.62 (quartet, 1 H, J = 6 Hz, C-1'H), 2.94 (quartet, 2 H, J = 7 Hz, C-3'$\underline{CH_2}$), 2.50 (singlet, 3 H, C-1 CH_3), 2.38 (singlet, 6 H, N($\underline{CH_3}$)$_2$), 1.34 (doublet, 3 H, J = 6 Hz, C-1'$\underline{CH_3}$), 1.20 (triplet, 3 H, J = 7 Hz, C-4'$\underline{CH_3}$). Mass spectrum: M^+, m/e = 280; main peaks: 265, 236, 235 (base peak), 220, 207-204. High resolution mass spectrum: Calc. for $C_{19}H_{24}N_2$: 280.1938. Found: 280.1923. Attempts were made to further characterize compound 103 as its methiodide 113, (see following experiment).

1-Methyl-2-ethyl-3-(α-trimethylammonioethyl)carbazole iodide (111).

The hydrogenated compound 103 (18 mg., 6.4 x 10^{-5} mole) dissolved in chloroform methanol 1:1 was reacted with an excess of methyl iodide at 0° for 12 hr. The reaction mixture was concentrated to give a granular oil (27 mg.) consisting of three products. The desired methiodide 111 was obtained in partially pure form by washing the crude product mixture with chloroform (2 x 10 ml.). The partually purified methiodide 111 was obtained as an opaque solid (15 mg., 55%). No physical data was obtained for this compound.

The chloroform washings were combined and concentrated to a yellow oil. The major non-polar component 113 was isolated (12 mg.) by elution of an alumina column (5 gm.) with chloroform. N.M.R. (60 MHz) : 3.72 (quartet, 1 H, J = 6 Hz, C-1'H), 3.26 (singlet, 3 H, $O\underline{CH_3}$), 2.50 (singlet, 3 H, C-1 $\underline{CH_3}$), 1.52 (doublet, 3 H, J = 6 Hz, C-1'CH_3).

1-Methyl-2-ethyl-3-vinylcarbazole (112).

Methiodide 111 (15 mg., 3.60 x 10^{-5} mole) and sodium hydride (15 mg.) in dry dimethylformamide was heated at 100° for 2 min. after which time the excess hydride was destroyed by the careful addition of water. The reaction mixture was then diluted with water (75 ml.) and the resultant white suspension was extracted with ether (4 x 30 ml.). The combined ether layers were washed with water (3 x 30 ml.), dried over sodium sulphate, and concentrated to give olefin 112 as a brown oil (7 mg.). The crude product was purified by chromatography on alumina. By elution with benzene 112 was obtained as a light brown oil (4 mg.). UV.; λ_{max}: 325, 295(sh), 280, 267(sh), 241. N.M.R. : 5.69($J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2Hz, C-2' H trans), 5.28($J_{1',2'}$= 11 Hz, $J_{2',2'}$= 2 Hz, C-2' H cis), 2.46(singlet, C-1 CH_3). Mass spectrum: M^+, m/e = 235; main peaks : 220, 204. High resolution mass spectrum: Calc. for $C_{17}H_{17}N$: 235.1360. Found : 235.1380.

Salsolidine methiodide (115).

A mixture of salsolidine (100)[144] (3.0 gm., 1.71 x 10^{-2} mole), methyl iodide (28 gm.), and aqueous sodium carbonate (6 gm. in 40 ml.) was warmed under gentle reflux overnight. The reaction mixture was then concentrated to dryness and taken up in water (50 ml.) whereupon tan coloured crystals immediately precipitated (2.3 gm.). The aqueous mixture was basified with 5% sodium hydroxide and extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to an amber coloured foam. By recrystallization from methanol-ether beige crystals of 115 were obtained (2.20 gm., 35%), m.p. 205° (lit., m.p. 229-231° d.). Found: C, 46.13; H, 6.10; N, 3.59. Calc. for $C_{14}H_{22}O_2NI$: C, 46.41; H, 6.07; N, 3.87.

1-(α-dimethylaminoethyl)-2-vinyl-4,5-dimethoxybenzene (116).

Salsolidine methiodide (115) (50 mg., 1.38 x 10^{-4} mole) was suspended in a 10% sodium hydroxide in 95% ethanol solution (30 ml.) and refluxed for 1.5 hr. The reaction mixture was then concentrated to a paste, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether fractions were dried over sodium sulphate, and concentrated to give a light brown oil (20 mg., 61%). UV.; λ_{max} : 310 (sh), 290, 262, N.M.R.: 7.23 (doublet of doublets, 1 H, $J_{3',4'}$ = 16 Hz, $J_{3',4'}$ = 10 Hz, C-3'H), 7.02, 6.94 (2 singlets, 1 H each, C-3, C-6 H), 5.50 (doublet of doublets, 1 H, $J_{3',4'}$ = 16 Hz, $J_{4',4'}$ = 2 Hz, C-4'H), 5.16 (doublet of doublets, 1 H, $J_{3',4'}$ = 10 Hz, $J_{4',4'}$ = 2 Hz, C-4'H), 3.96 (singlet, 6 H, OCH_3), 3.50 (quartet,

1 H, J = 7 Hz, C-1'H), 2.33 (singlet, 6 H, $N(\underline{CH_3})_2$), 1.35 (doublet, 3 H, J = 6 Hz, C-1' $\underline{CH_3}$).

Reduction of (116) to 1-(α-dimethylaminoethyl)-2-ethyl-4,5-dimethoxybenzene and formation of methiodide 117.

Olefin 116 (64 mg., 2.72 x 10^{-4} mole) dissolved in methanol (10 ml.) was hydrogenated at room temperature and atmospheric pressure over Adams catalyst (PtO_2, 10 mg.) for 0.5 hr. The mixture was filtered to remove the catalyst which was carefully washed with additional methanol (10 ml.). The combined filtrates were concentrated under vacuum to give 117 as a colourless oil (64 mg., 98%). UV; λ_{max} : 280. N.M.R. : 7.10 (singlet, 1 H, C-6 H), 6.64 (singlet, 1 H, C-3 H), 3.90 (split singlet, 6 H, $O\underline{CH_3}$), 3.43 (quartet, 1 H, J = 7 Hz, C-1'H), 2.65 (quartet, 2 H, J = 7 Hz, Ar-$\underline{CH_2}CH_3$), 2.33 (singlet, 6 H, $N(\underline{CH_3})_2$), 1.40 (doublet, 3 H, J = 6 Hz, C-1'$\underline{CH_3}$ (overlapped by triplet at 1.13)), 1.13 (triplet, 3 H, J = 6 Hz, $ArCH_2\underline{CH_3}$ (overlapped by doublet at 1.40)).

The hydrogenated compound (64 ml.) was converted to its corresponding methiodide 117 by reaction with an excess of methyl iodide in methanol. Recrystallization from methanol, m.p. 151-153°. Found : C, 47.97; H, 6.20; N, 3.29. Calc. for $C_{15}H_{26}NO_2I$: C, 47.59; H, 6.88; N, 3.70 (analysis unsatisfactory).

Attempted formation of 1-Vinyl-2-ethyl-4,5-dimethoxybenzene (118).

The methiodide (30 mg., 8.24 x 10^{-5} mole) was refluxed in diethyl ketone (10 ml.) for 2 hr. The solvent was then removed under vacuum to give

an amber oil. The crude product was distilled at 120-180° at 0.05 mm.

A yellow film was obtained which was dissolved in ether (25 ml.) and washed with 10% sodium thiosulphate solution to give a colourless film of product (yield undetermined). From separate experiments the N.M.R. spectrum for the crude and purified reaction product showed that the starting material had totally decomposed.

Thermal Pyrolysis of Guatambuine Methiodide (86).

Guatambuine methiodide (about 10 mg.) was heated under vacuum for 12 hr. at 0.05 mm. and 200°C. A small yield of yellowish coloured crystals sublimed during this period, m.p. 200-210°. The N.M.R. spectrum was identical to that obtained for guatambuine (25).

1-Methyl-3-(α-hydroxyethyl)-9-benzylcarbazole (222).

Carbazole acetate 223 (100 mg., 2.80 x 10^{-4} mole) was partitioned between a two phase medium consisting of t-butanol (5 ml.) and 20% aqueous sodium hydroxide (5 ml.), and heated at 100° with vigorous stirring for 1 hr. The reaction mixture was then concentrated to remove the t-butanol, diluted with water (75 ml.) and extracted with ether (3 x 30 ml.). The combined ether layers were washed with water, dried over sodium sulphate and concentrated to give a colourless foam. The crude product was purified by column chromatography on alumina (10 gm.). Trace amounts of unreacted carbazole acetate 223 and other non polar contaminants were removed by elution with benzene. By subsequent elution with chloroform and concentration, the desired alcohol 222 was obtained as a colourless foam (70 mg., 79%). UV.; λ_{max}: 342, 327, 293, 287, 283, 263, 237. N.M.R.: 8.08 (doublet, 1 H, J = 2 Hz, C-5H), 7.98 (multiplet, 1 H, C-4H), 7.2, 6.95 (2 multiplets, 9 H, aromatic), 5.66 (singlet, 2 H, N-$\underline{CH_2}$-C_6H_5), 5.00 (quartet, 1 H, J = 6 Hz, Ar$\underline{CH}$(OH)CH_3), 2.54 (singlet, 3 H, C-1 $\underline{CH_3}$), 1.97 (singlet, 1 H, OH), 1.54 (doublet, 3 H, ArCH(OH)$\underline{CH_3}$). Mass spectrum: M, m/e = 315 (base peak); main peaks: 300, 297. High resolution mass spectrum: calc. for $C_{22}H_{21}NO$: 315.1623. Found: 315.1632.

1-Methyl-3-vinyl-9-benzylcarbazole (123).

Carbazole alcohol 222 (75 mg., 2.38 x 10^{-4} mole) and toluenesulphonyl chloride (58 mg., 1.3 equiv.) in pyridine (8 ml.) was heated at reflux temperature for 6 hr. The pyridine was then removed under vacuum and the

residue was dissolved in chloroform (75 ml.) and washed successively with dilute hydrochloric acid, 5% sodium hydroxide and water. The chloroform layer was dried over sodium sulphate, and concentrated to give a dark coloured oil (54 mg.). The crude product was purified by filtration through a column of alumina (3 gm.). By elution with chloroform the olefin 123 was obtained as an amber coloured oil (42 mg., 60%). UV.; λ_{max}: 350, 336, 279, 270 (sh), 241. N.M.R.: 8.14 (multiplet, 1 H, C-5H), 8.05 (multiplet (split singlet), 1 H, C-4 H), 6.90 (doublet of doublets, C-1'H, obscured by aromatic signals), 5.78 (doublet of doublets, 1 H, $J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 1.5 Hz, C-2'H <u>trans</u> (partly obscured by 5.72 singlet), 5.72 (singlet, 2 H, Ar-CH_2-N), 5.21 (doublet of doublets, 1 H, $J_{1',2'}$ = 11 Hz, $J_{2',2'}$ = 1.5 Hz, C-2'H <u>cis</u>), 2.60 (singlet, 3 H, C-1 CH_3).

1,4-Dimethyl-2-(β-(N,N-methylacetylamino)ethyl)-3-acetoxymethylcarbazole (121)

N-Methyltetrahydroellipticine (26) (50 mg. 1.89 x 10^{-4} mole) and anhydrous sodium acetate (20 mg.) in acetic anhydride (3 ml.) was heated at reflux temperature for 8 hr. The cooled reaction mixture was then diluted with 5% aqueous sodium hydroxide solution (75 ml.) and stirred for 15 min. after which time the resultant suspension was extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to a tan coloured glass which readily crystallized from benzene to give colourless crystals of 121 (53 mg., 76%). UV.; λ_{max}: 335 (3.50), 321 (3.56), 293 (4.13), 285 (3.95), 263 (4.49), 250 (4.70), 243 (4.74). IR. ($CHCl_3$): 1730, 1635 cm^{-1}. N.M.R.: 5.50, 5.46 (2 x singlet, 2 H, Ar$\underline{CH_2}$OAc), 3.50 (multiplet, 2 H, C-2'$\underline{CH_2}$), 3.05, 3.00 (2 x singlet, 3 H, NCH_3), 2.88 (singlet, 3 H, C-4CH_3), 2.60, 2.52 (2 x singlet, 3 H, C-1 CH_3), 2.08, 1.98 (singlet, 3 H, C-1' NCO$\underline{CH_3}$), 2.03 (singlet, 3 H, NCO$\underline{CH_3}$). Mass spectrum: M^+, m/e = 366; main peaks: 306, 280, 263, 233, 222-1. High resolution mass spectrum: Calc. for $C_{22}H_{26}N_2O_3$: 366.1943. Found: 366.1956. Found: C, 72.20; H, 7.11; N, 7.20. Calc. for $C_{22}H_{26}N_2O_3$: C, 72.11; H, 7.18; N, 7.64.

1,4-Dimethyl-2-(β-(N,N-methylacetylamino)ethyl)-3-hydroxymethylcarbazole (122)

Carbazole acetate 121 (50 mg., 1.36 x 10^{-4} mole) was partitioned between a two phase medium consisting of t-butanol (5 ml.) and 20% aqueous sodium hydroxide (5 ml.), and heated at 100°C with vigorous stirring for 1 hr. The reaction mixture was then concentrated to remove the t-butanol, diluted with water (75 ml.), and extracted with ether (3 x 30 ml.). The combined ether fractions were washed with water, dried over sodium sulphate and concentrated to give 122 as a colourless solid. The crude product was purified by preparative layer chromatography

on alumina (1 mm., EtOAc:MeOH 20%). UV.; λ_{max}: 335, 321, 293, 285, 263, 250, 243. IR.: 1635 cm^{-1}, N.M.R.: 6.20, 5.00 (multiplets, 2 H, $-\underline{CH_2}OH$). Mass spectrum: M^+, m/e = 324; main peak: 308. High resolution mass spectrum: Calc. for $C_{20}H_{24}N_2O_2$: 324.1837 . Found: 324.1814.

1-Methyl-2-(β-(N,N-methylacetylamino)ethyl)-3-(α-acetoxyethyl)carbazole(124).

Guatambuine (60 mg., 2.27 x 10^{-4} mole) was dissolved in pyridine (4 ml.) containing acetic anhydride (2 ml.) and heated at reflux temperature for 2 hr. The solvent was then removed under high vacuum to give a dark red oil. The crude product was partially purified by column chromatography on alumina (3 gm.). By elution with chloroform 124 was obtained as an amber coloured oil (58 mg., 70%). UV.; λ_{max}: 335, 323, 295, 285, 260, 247, 239. IR.: 1710, 1620. N.M.R.: 6.34 (multiplet, 1 H, C-1'H), 3.50 (multiplet, 2 H, C-3' $\underline{CH_2}$), 3.00 (singlet, 3 H, $N-CH_3$), 2.48, 2.40 (2 x singlet, 3 H, C-1 CH_3), 2.08 (Singlet, 3 H, $OCO\underline{CH_3}$), 2.08, 1.98 (2 x singlet, 3 H, $NCO\underline{CH_3}$). Mass spectrum: M^+, m/e = 366, main peaks: 306 234, 233, 221, 220 (base peak), 205,204.

1-Methyl-2-(β-(N,N-methylacetylamino)ethyl)-3-vinylcarbazole (125).

Guatambuine (25) (60 mg., 2.27 x 10^{-4} mole) and anhydrous sodium acetate in acetic anhydride was heated at reflux temperature for 10 hr. The cooled reaction mixture was then diluted with 5% aqueous sodium hydroxide solution (75 ml.) and stirred for 15 min. after which time the resultant suspension was extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to a light brown oil. The crude reaction mixture was purified by column chromatography on alumina (5 gm.). By elution with

chloroform the olefin 125 was obtained as a faintly coloured oil (45 mg., 64%). UV; λ_{max}: 325 (broad hump), 295 (sh), 285-275 (broad peak), 262, 240. IR.: 1630 cm^{-1}. N.M.R.: 5.72 (doublet of doublets, 1 H, $J_{1',2'}$ = 17 Hz, $J_{2',2'}$ = 2 Hz, C-2'H trans.), 5.37, 5.34 (2 x doublet of doublets, 1 H, $J_{1',2'}$ = 11 Hz $J_{2',2'}$ = 2 Hz, C-2' H cis), 2.50 (multiplet, 2 H, C-3' CH_2), 2.20 (multiplet, 2 H, C-4' CH_2), 3.00, 2.92 (2 x singlet, 3 H, NCH_3), 2.58, 2.52 (2 x singlet, 3 H, C-1 CH_3), 2.10, 1.96 (doublet(J = 2 Hz) and singlet, 3 H, $NCOCH_3$). Mass spectrum: M^+, m/e = 306; main peaks: 234, 220 (base peak), 204. High resolution mass spectrum: Calc. for $C_{20}H_{22}N_2O$: 306.1732. Found: 306.1736

Formation of the Enamine (126), and Reaction with Dimethylsulphate.

In a small scale experiment the enamine 126 was found to be precipitated as an amorphous yellow solid after the addition of 20% aq. sodium hydroxide solution (10 ml.) to a water solution (10 ml.) of the iminium cation (about 3 mg.). UV. (20% aq. NaOH) (Figure 38); λ_{max}: 365, 335, 310, 294, 280, 270 (sh), 243, 237.

Addition of dimethylsulphate (1 ml.) to the basic solution of the enamine and heating at 100 for 10 min. resulted in the total disappearance of the yellow colouration. UV. (Figure 38); λ_{max}: 278, 245 (sh), 235.

INTRODUCTION: (PART III)

During the course of experiments on the biosynthesis and degradation of the pyridocarbazole alkaloids olivacine (16) and guatambuine (25), it became apparent that for the continuation of the work that larger quantities of these compounds would be required than were available from plant extracts of *Aspidosperma australe*.[124] Attention was, therefore, directed toward the development of an efficient synthesis of these two alkaloids adaptable to large scale preparation (1 - 10 gm.).

Both the olivacine (16) and ellipticine (17) systems have been previously synthesized as a consequence of two separate interests. In the early sixties synthetic corroboration[172-6] was necessary following their isolation from various *Aspidosperma* and *Ochrosia* plants, and the elucidation of their novel tetracyclic structure. The subsequent determination that olivacine (16), ellipticine (17) and their analogs exhibited anti-tumor activity, led again to the development of syntheses of these alkaloids.[177-180]

The approach adopted in the present synthesis of olivacine (16) and guatambuine (25) was designed in view of what was known about the chemistry of these molecules. It is, therefore, necessary to briefly review the relevant details of the established syntheses.

For purposes of structure elucidation Schmutz and Wittwer (1960)[172] were the first to publish a synthesis of olivacine (16). They attacked the problem of construction of the complex tetracyclic ring system by dividing the synthesis into two distinct stages. The first stage was the synthesis of

an appropriately substituted tricyclic carbazole intermediate containing the "A, B, and C" rings of olivacine (16). The second stage was the construction of the fourth or (4,3-b) fused pyrido "D" ring.[11]

The first stage carbazole intermediate required a methyl group in the 1-position and a versatile group in either the 2- or 3- position which could subsequently be elaborated to give the remaining "D" ring. Bearing in mind the proposed design of the "D" ring synthetic efforts were aimed at producing as the intermediate, 1-methylcarbazole-2-carboxylic acid chloride (135).

This compound was prepared in six steps from 2-methyl-3-aminobenzonitile (132) (Figure 40). By either of two well known condensation reactions, the modified Bischler or the Borsche synthesis, the starting material 132 was converted to 7-cyano-8-methyl-1,2,3,4-tetrahydrocarbazole (133). Hydrolysis of the cyano group and subsequent esterification, followed by dehydrogenation of the saturated ring gave the carbazole ester 134. By reaction with thionyl chloride the carbazole ester 134 was transformed into the desired acid chloride 135.

Wolff rearrangement of the diazoketone 136 formed by reaction of the acid chloride 135 with diazomethane produced the corresponding homologous amide 137. This amide 137 was transformed into the N-acetyl derivative 140 by dehydration to the nitrile 138 with phosphorus oxychloride followed by reduction with Raney nickel to the amine 139 and subsequent acetylation with acetic anhydride in pyridine.

Bischler cyclization of the amide 140 to the imine, 3,4-dihydro olivacine (141), completed the synthesis of the tetracyclic olivacine skeleton. The imine 141 was easily converted to guatambuine (25) by formation of the methiodide salt 142 and reduction over platinum oxide. Alternatively the imine 141 was

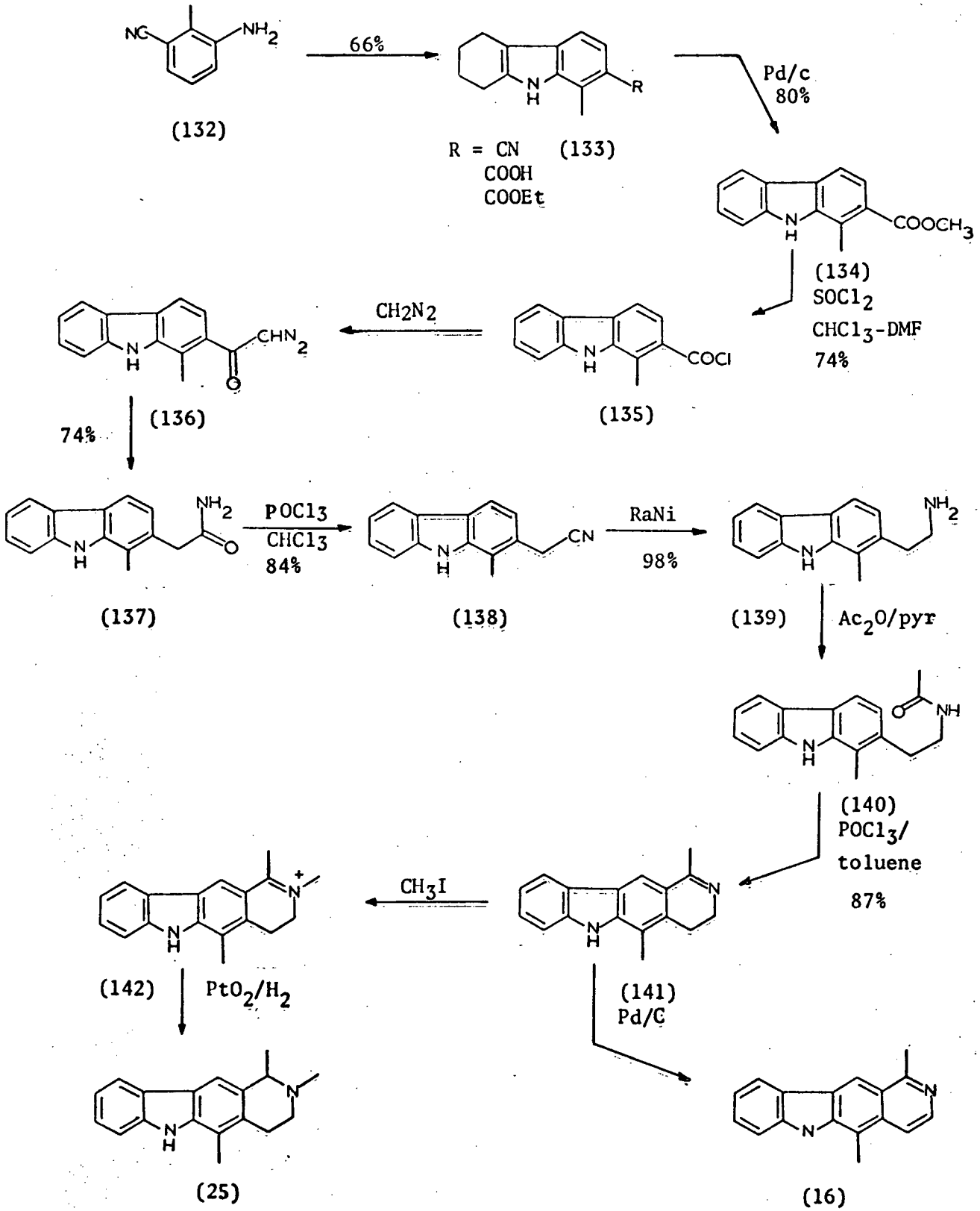


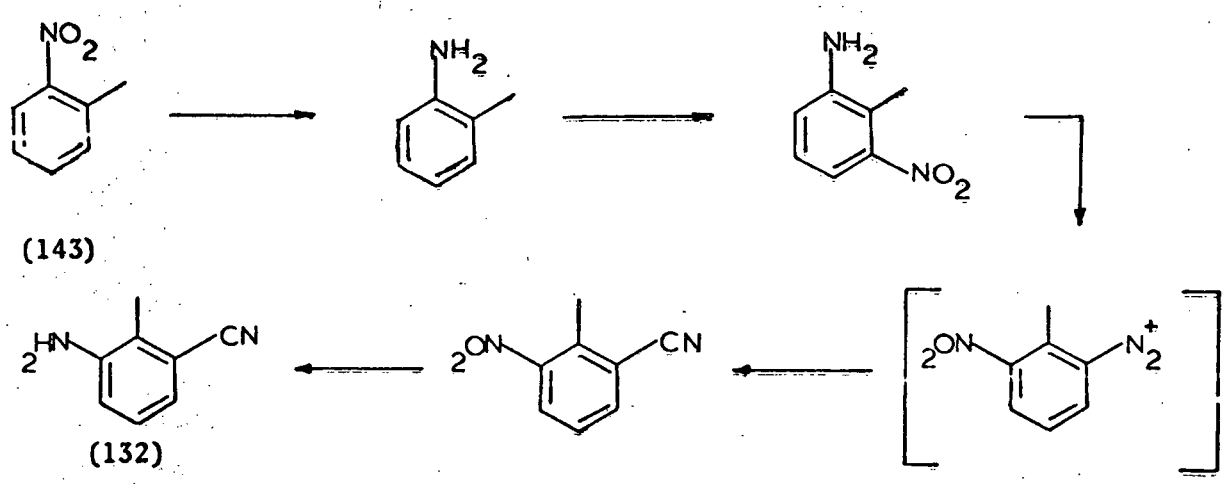
Figure 40. Synthesis of Olivacine (16) and Guatambuine (25) by Schmutz and Wittwer (1960).

readily dehydrogenated with palladium on charcoal to give olivacine (16).

The cyclization of 140 was aided by the electronic influence of the indole nitrogen para to the C-3 position in the "C" ring. A stable resonance contributing structure can be drawn depicting increased electron density centered at the C-3 position which enhances the attack on the carbonyl carbon of the amide.

(140)

The synthesis has the advantage that it can for the most part be adapted to large scale preparation of either guatambuine (25) or olivacine (16). The construction of the "D" ring however suffered from several drawbacks, it was both indirect and necessitated the use of large quantities of diazomethane. The major drawback to the overall sequence was however, its excessive length, seventeen steps in total are involved when it is considered that it was necessary to first synthesize the starting material 132 from o-toluidine (143).[181]



A second synthesis of olivacine (16) was published by Wenkert and Dave (1962)[173] shortly after the work by the Swiss group. As with their predecessors they aimed at the synthesis of an intermediate carbazole 152 which possessed an aldehyde functionality at the C-2 position required for the elaboration of the "D" ring. A considerably different approach for the synthesis of this intermediate was chosen however, utilizing 1-ketotetrahydrocarbazole (144) as the starting material, this compound being readily available by Fisher indole synthesis. The ketone group of this molecule served the dual role of directing the course of functionalization onto the C-2 position and subsequently the preferential methylation onto the C-1 position. The two schemes that were developed are presented in figure 41.

Considering Scheme A, sodium ethoxide induced acylation of 1-ketotetrahydrocarbazole (144) with ethyl oxalate gave the ethoxalyl derivative 145 (R=H), which on acetylation yielded the diester 145 (R=OAc). Zinc and acetic acid reduction of either the diester 145 (R=OAc) or the α,β-unsaturated keto-ester 146 (obtained by partial hydrogenation of the diester) produced the saturated keto-ester 147. Reaction of the keto-ester 147 with methyllithium gave two products 148 and 149, which on palladium on charcoal dehydrogenation led to the corresponding carbazoles 150 and 151. Ozonolysis of the olefinic side chain of compound 150 gave the carbazole aldehyde 152, the vital intermediate for the total synthesis. In the study of the haloform reaction on the acetone side chain of carbazole 151 it was hoped that the presence of the C-methyl group would provide sufficient steric hindrance to prevent reaction at the benzylic carbon over reaction at the methyl carbon thus giving rise to the

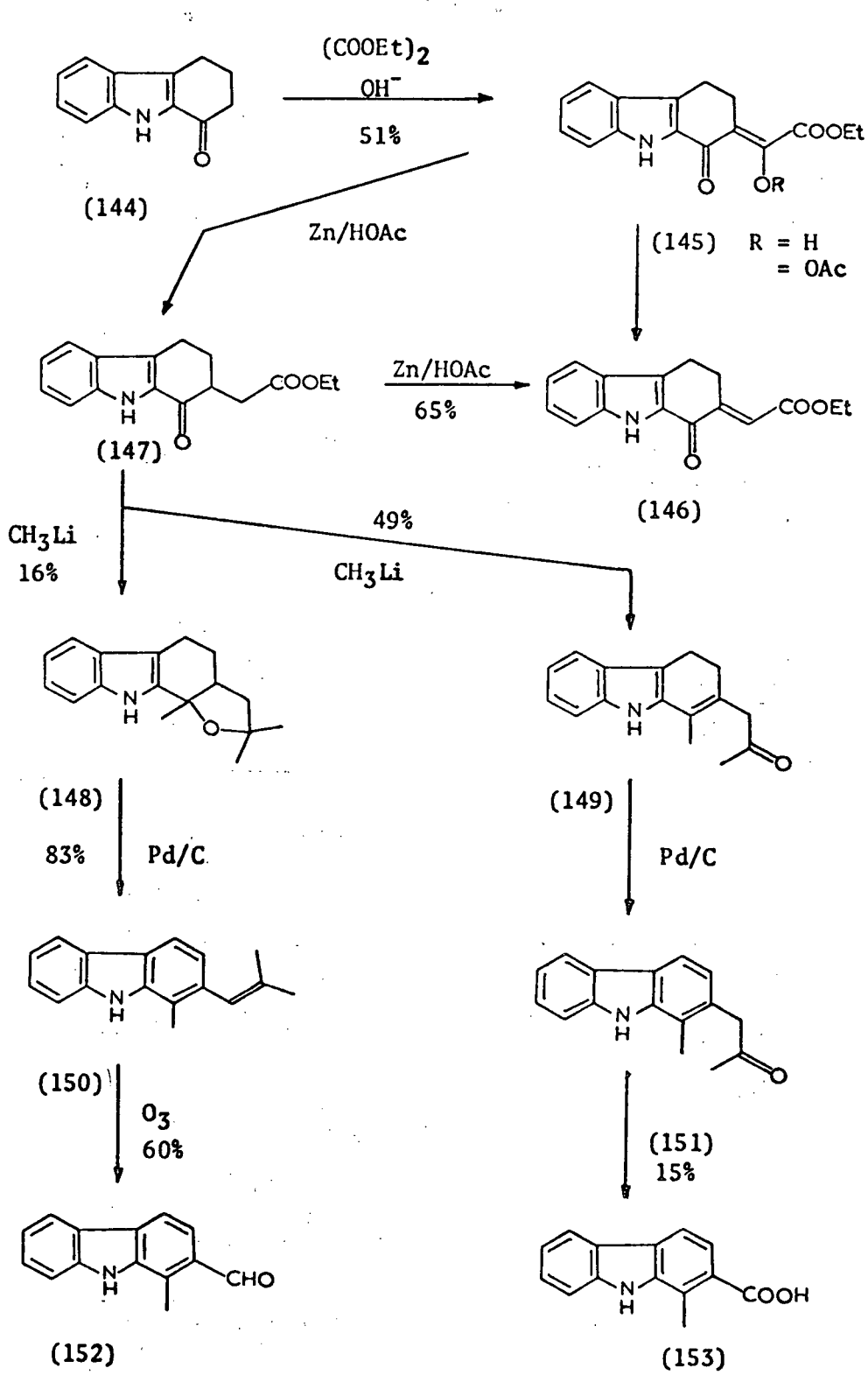


Figure 41. Scheme A, Synthesis of Olivacine (16) According to Wenkert and Dave (1962).

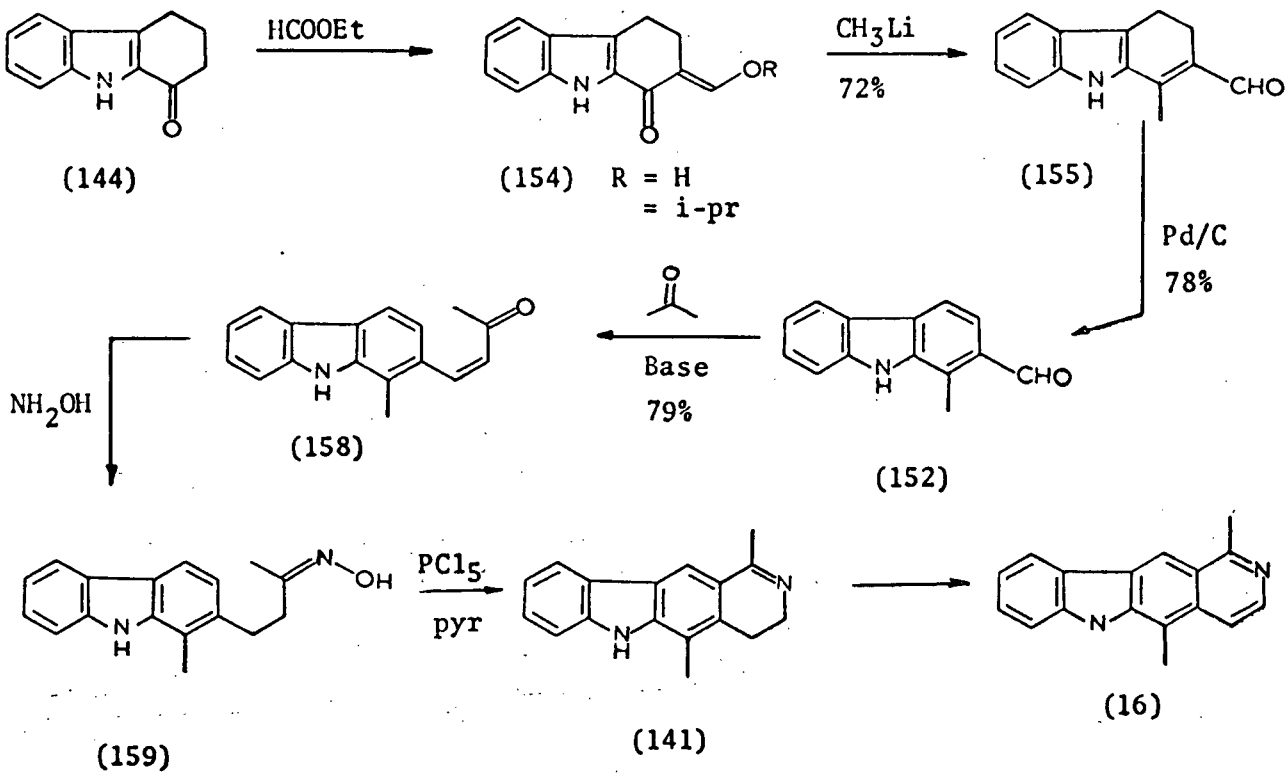
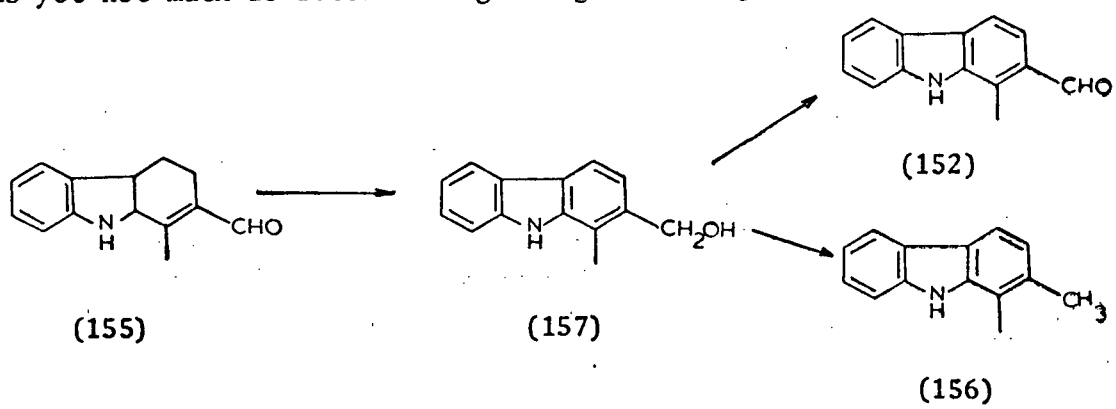


Figure 41. Scheme B, Synthesis of Olivacine According to Wenkert & Dave (1962)

acetic acid derivative. However in analogy with phenylacetone the corresponding carbazole carbocylic acid 153 was obtained. In view of the fact that carbazoles with only one carbon side chains were available from the above sequence, it was decided to study the same reaction with a one-carbon acylating agent, Scheme B.

Condensation of 1-ketotetrahydrocarbazole (144) with ethyl formate gave the formyl derivative 154 (=H). Reaction with i-propyl iodide produced the i-propyl ether 154 (R=i-pr), which on treatment with methyllithium yielded the 1,2-dihydrocarbazole aldehyde 155 . Upon dehydrogenation of this aldehyde 155, it was surprising to observe that the major isolated product was 1,2-dimethylcarbazole (156). Equally surprising was the extreme ease with which the dehydrogenation could be carried out, it was found that by merely heating a benzene solution

of the 1,2-dihydrocarbazole aldehyde 155 in the presence of palladium on charcoal for one-half hour gave the desired carbazole aldehyde 152. However, even in this extremely mild case, small amounts of the 1,2-dimethylcarbazole (156) was produced. It is probable that the facility of the reaction was due to a rapid disproportionation of the 1,2-dihydrocarbazole aldehyde 155 to the carbazole alcohol 157 prior to its reduction to the 1,2-dimethyl compound 156 or reoxidation to the carbazole aldehyde 152 (isolated in small quantities). Such disproportionation reactions have been observed previously[182] although as yet not much is recorded regarding the energetics of the process.



The "D" ring was constructed by base catalyzed condensation of the intermediate aldehyde 152 with acetone to give the chalcone 158. Hydrogenation of the latter and reaction with hydroxylamine gave the oxime of the dihydrochalcone 159. Beckmann rearrangement of the oxime 28 occurred with the concomitant cyclization to give the imine, 3,4-dihydroolivacine (141). Dehydrogenation of the imine 141 yielded olivacine (16).

The synthesis developed by Wenkert's group was efficient in that olivacine (16) could be obtained in only nine steps from starting material. However, it was adapted to only small scale preparations.

A further synthesis of olivacine and its analogs has been developed by Mosher et al (1966)[177] as part of a program on cancer chemotherapy. This synthesis represents a refinement of the latter stages of the scheme developed by Schmutz and Wittwer (1960)[172]. Instead of utilizing the Wolff rearrangement

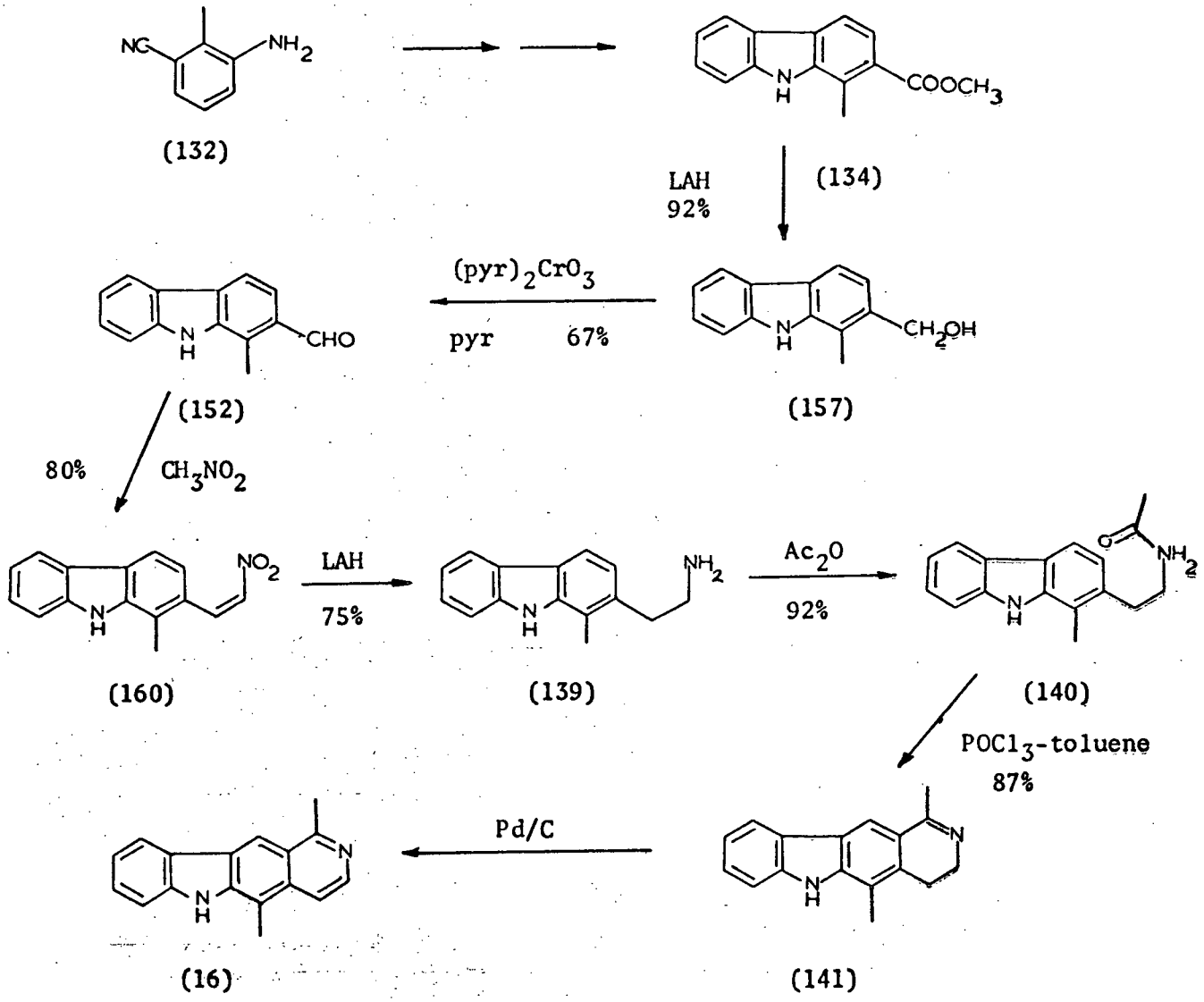


Figure 42. Synthesis of Olivacine (16) by Mosher *et al.* (1966).

they used the nitromethane condensation, developed by Govindachari *et al*[176] in the ellipticine (17) series as a more direct means of constructing the "D" ring. In essence the synthesis represents the better halves of two other syntheses united together to give the first synthesis of olivacine (16) adaptable to large scale preparation (Figure 42).

The intermediate carbazole aldehyde 152 was obtained by reduction of the ester 134 followed by Sarett oxidation of the alcohol 157. Condensation

of the aldehyde 152 with nitromethane led to the nitrostyrene 160 in high yield, which on lithium aluminum hydride reduction produced the amine 139, again in high yield. Conversion of the corresponding amide 140 to the imine 141 was again carried out according to the scheme of Schmutz and Wittwer.[172] Olivacine (16) was immediately available from this imine .

Recently some exploratory work on the synthesis of the pyridocarbazole skeleton has been published by T. Kametani et al (1974)[184-5]. For several years they have been utilizing benzocyclobutene derivatives as precursors for the synthesis of isoquinoline alkaloids[183], and are at present attempting to expand the scope of their benzocyclobutene approach to include the synthesis of indole alkaloids of the olivacine (16) and ellipticine (17) type (Figure 43). It has been found[184] that on intermolecular cycloaddition of indole (161) with the benzocyclobutene analog 4,5-dibromomethyl-3- hydroxy-2-methylpyridine hydrobromide (162) in dimethylformamide for four hours, followed by acetylation gave the expected dihydropyridocarbazole derivative 163 in 4% yield and its structural isomer 164 in 15% yield. Subsequent dehydrogenation produced the olivacine type compound 165 and its corresponding isomer 166.

The various syntheses that have been described represent the current state of the synthesis of the olivacine (16) skeleton. The design of the present synthesis of this system was not however derived solely from the chemistry of olivacine (16) but to a considerable extent from a study of the synthesis of the closely related pyridocarbazole, ellipticine (17). It is relevant, therefore, to discuss the chemistry of the ellipticine (17) series since the difference in the position of one methyl group has resulted in a number of quite different approaches to the synthesis of this system.

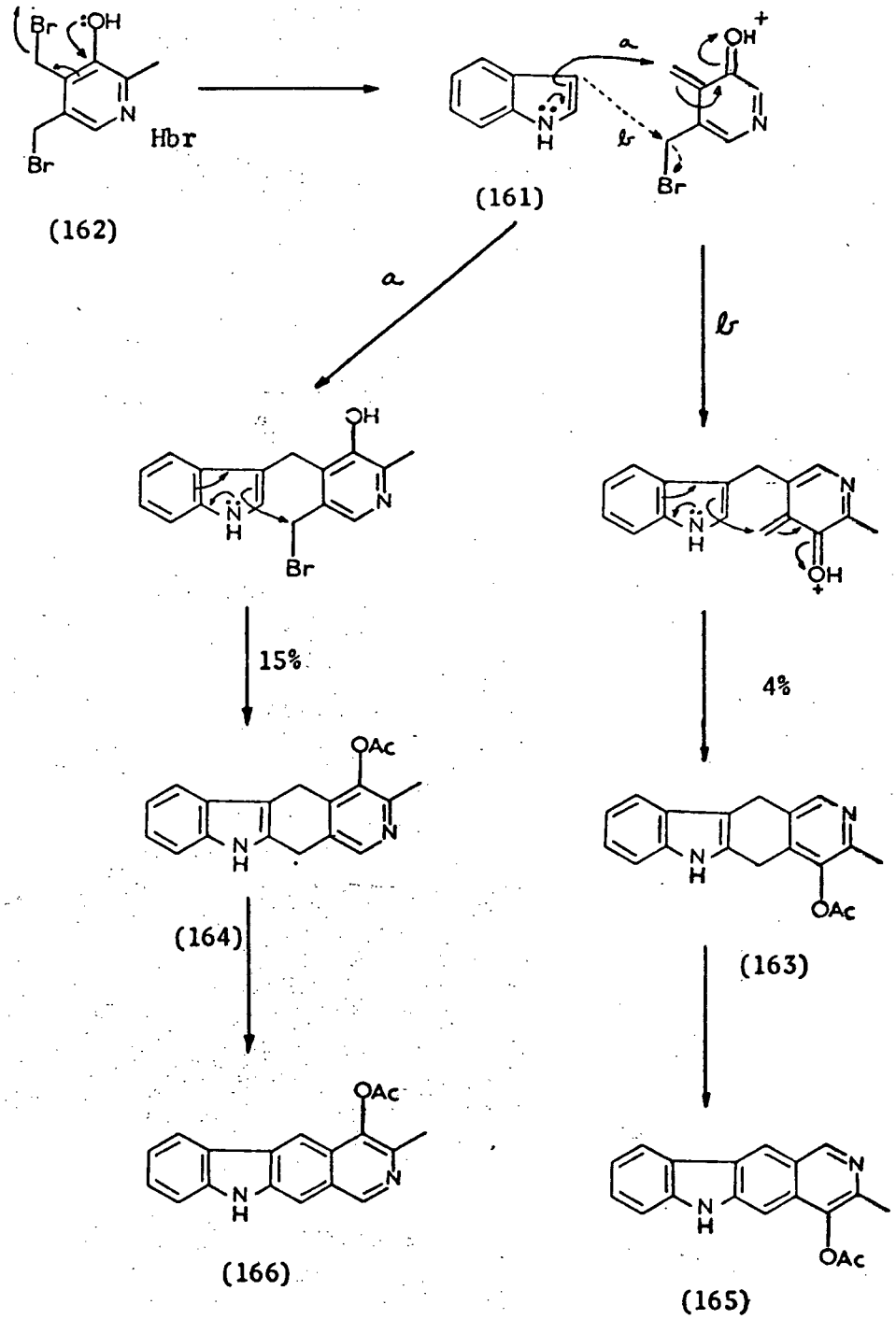


Figure 43. The Kametani Benzocyclobutene Analog Approach (1975)

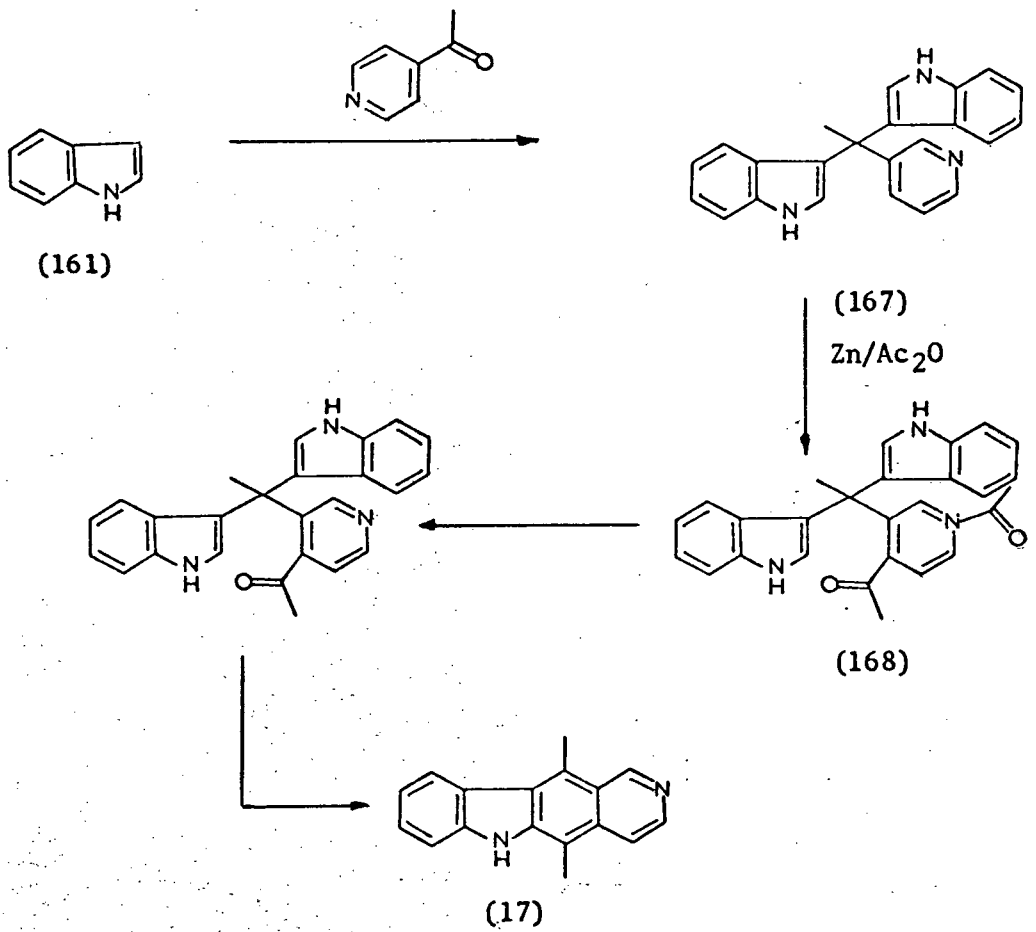


Figure 44. Woodward (1959) Synthesis of Ellipticine (17).

The first synthesis of ellipticine (17) was achieved in a somewhat unusual and elegant manner by Woodward and co-workers (1959)[174] as part of structure elucidation work on Aspidosperma alkaloids. They were able to incorporate both the desired methyl functionality in the C-5 position of the "C" ring and the complete pyrido - "D" ring in one step through the condensation of indole (161) with 3-acetyl pyridine to give 1,1-bis-(3_indolyl)1-1 (3-pyridyl)ethane (167) as illustrated in figure 44. The pyridine ring of this dimeric product was reduced with zinc in acetic anhydride to the

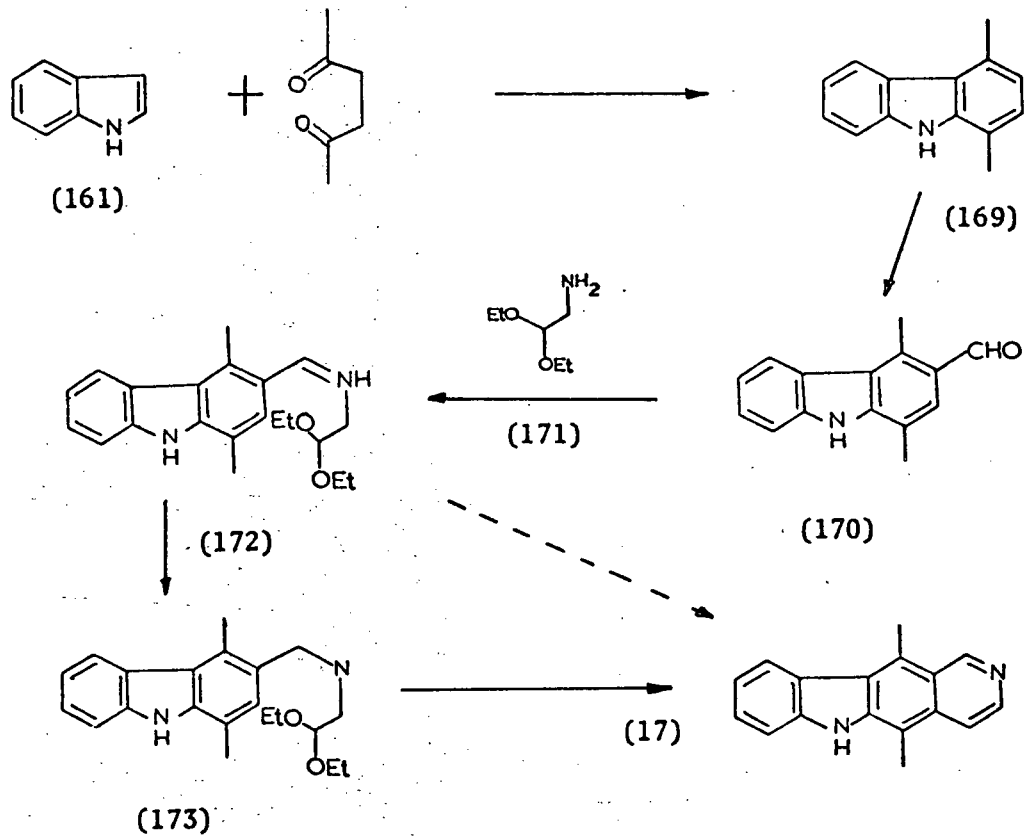


Figure 45. The Synthesis of Ellipticine (17) by Cranwell and Saxton (1962)

1,4-diacetylpyridine derivative 168 the 4-acetyl group of which was ideally set-up for condensation with the electron rich 2-position of the indole molecule in a manner identical to the initial condensation with 3-acetyl pyridine. The condensation was effected by pyrolysis under vacuum at 200°C. Unfortunately however, even though the process was intramolecular, ellipticine (17) was isolated in only a 2% yield. This approach was, therefore, incompatible with any scheme for large scale preparation of the alkaloid.

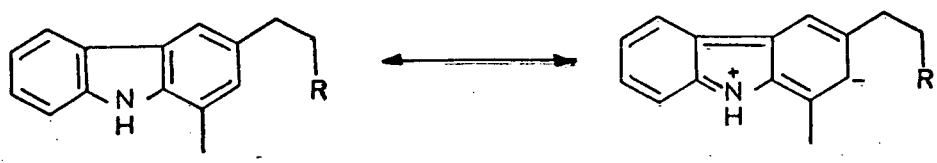
A different and more elementary approach was devised several years later by Cranwell and Saxton (1962)[175]. Following the theme developed for the synthesis of olivacine (16) they considered a two stage synthesis of ellipticine (17) to avoid the necessity of a lengthy Fisher indole synthesis. By

reaction of indole (161) with hexane-2,5-dione in ethanol-HCl 1,4-dimethyl-carbazole (169) was obtained in 36% yield. Subsequent formylation under controlled conditions gave the desired 1,4-dimethyl-3- formyl carbazole (170) as the predominant product. The direction of formylation was influenced by the presence of the methyl groups in the "C" ring (Figure 45).

The construction of the "D" ring from the aldehyde 170 required the direct condensation of nitrogen onto the carbonyl functionality with subsequent provision for a ring closure. Such requirements form the basis of a well known Pomeranz-Fritsch reaction between an aldehyde and an amino acetal. Reaction of the aldehyde 170 with aminoacetaldehyde diethylacetal (171) yielded in 85% yield the requisite imine acetal 172. It was found however, that reaction of this imine acetal 172 under any of the normally employed acidic conditions, i.e. sulphuric acid, polyphosphoric acid, boron trifluoride etherate, or arsenic pentoxide failed to effect cyclization. Cyclization under the above conditions was also tried without success on the amino acetal 173 and on the corresponding acid derived from condensation of the aldehyde 170 with glycine. Cyclization was eventually achieved in low yield on the amino acetal 173 by refluxing in dry ethanol - HCl. The proposed product obtained 3,4-dihydroellipticine (191) was not purified but immediately dehydrogenated with palladium on charcoal to give ellipticine (17) in very low yield.

The failure of the cyclization process to proceed in a facile manner can be rationalized to be a consequence of the poor nucleophilic character

of the C-2 position of the carbazole skeleton. It may be seen that resonance structures invoking participation of the indole nitrogen in the build up of election density at C-2 are unfavourable due to the required disruption of the aromatic resonance of the "A" ring,



On the basis of its design this synthesis was very practical, however the difficulties encountered in the cyclization step totally deplete its value.

Almost simultaneously with the publication of the above work, Govindachari, Rajappa and Sudarsanam (1963) [176] reported a new synthesis of ellipticine (17). They utilized the classical Fisher indole approach for the synthesis of appropriate carbazole intermediates. Difficulties were encountered during two key stages of their sequence requiring in each instance complete redesign of the approach. The three sequences are presented in figure 46, schemes A to C.

In scheme A, 2,5-dimethyl-4-nitrobenzoic acid (174) was converted to the tetrahydrocarbazole 175 which on dehydrogenation yielded the desired 1,4-dimethyl carbazole-3-ester (176). The subsequent objective was to convert the ester 176 into the corresponding aldehyde 178 by lithium aluminum hydride reduction and oxidation of the alcohol 177. However, reaction with lithium aluminum hydride resulted unexpectedly in total reduction of the ester 176 to 1,3,4-trimethylcarbazole (179). Similar total reduction has been observed on

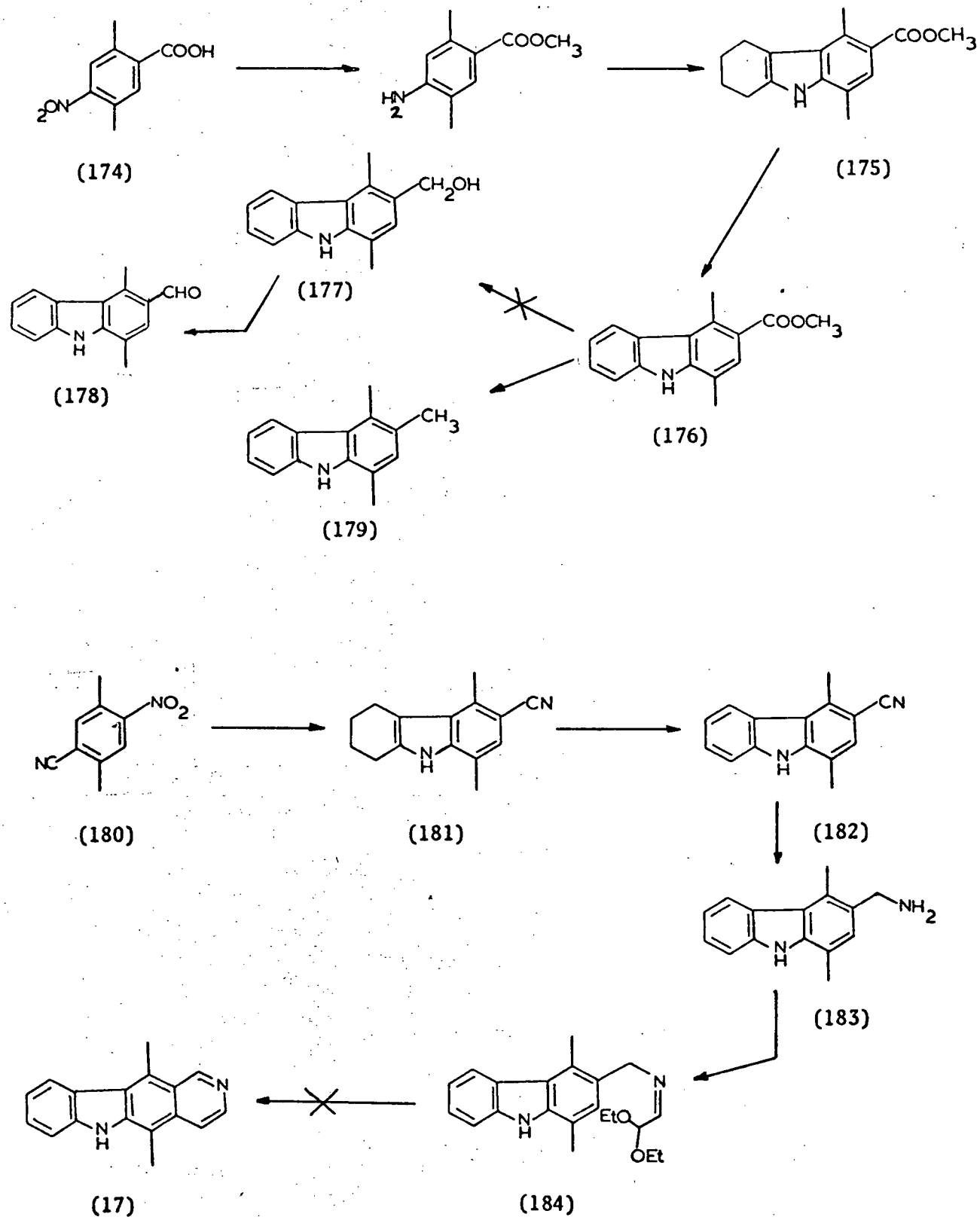


Figure 46. Attempted Syntheses of Ellipticine (17) by Govindachari *et al*. (1963), Schemes A & B.

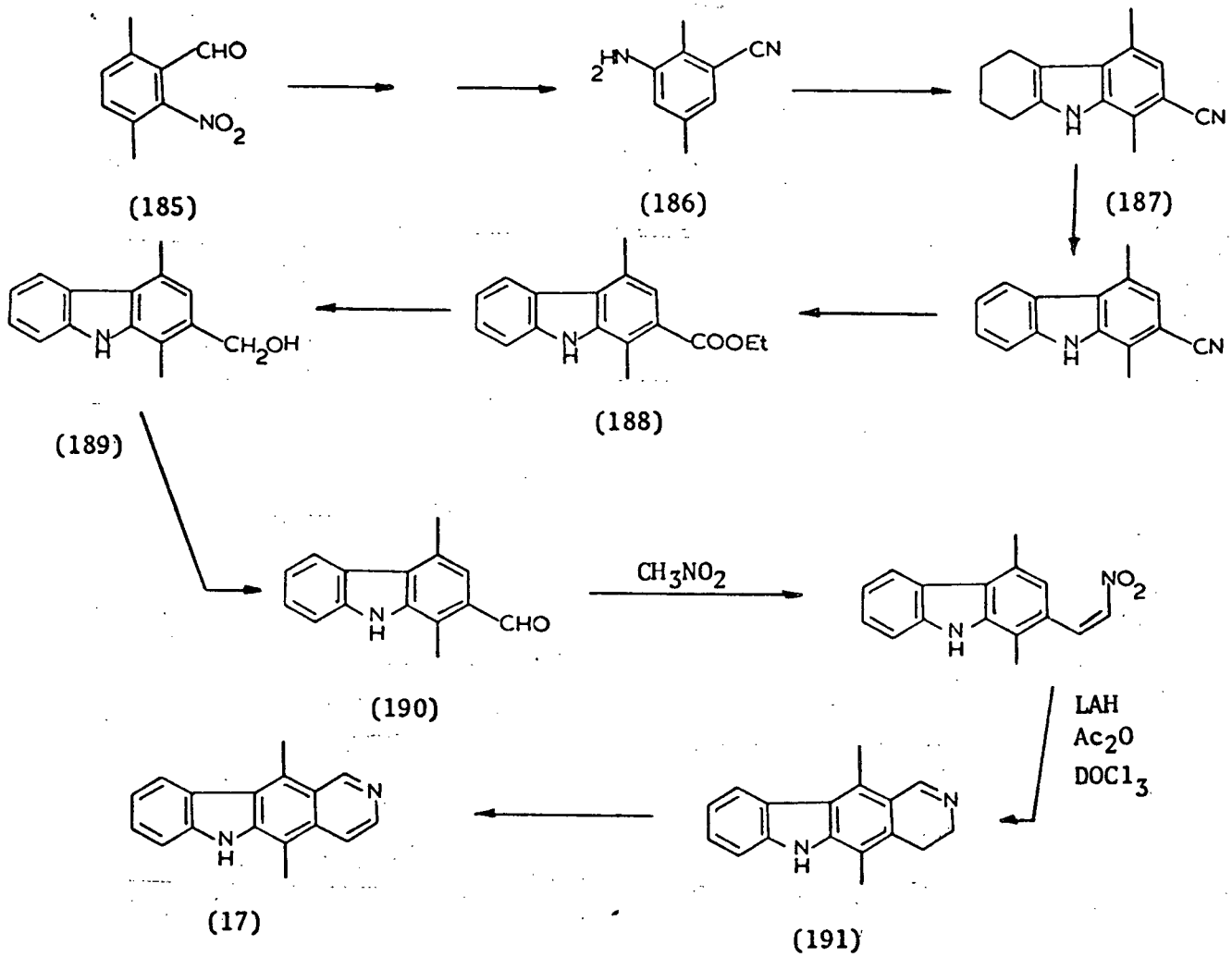
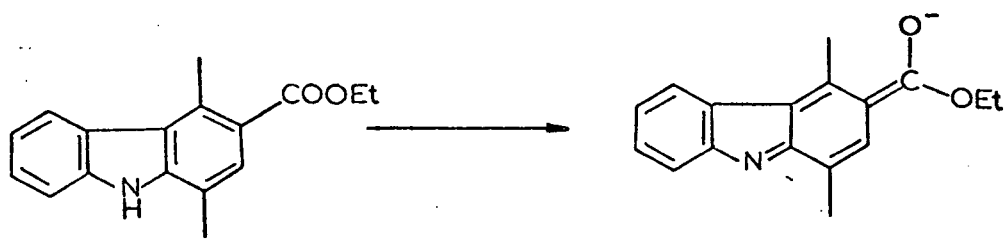


Figure 46. Synthesis of Ellipticine (17) by Govindachari *et al.* (1963) Scheme C.

reaction of 3-acetyl and 3-formylindole with lithium aluminum hydride.[186] This result can be rationalized by proposing that in the basic solution it is the conjugate base that is the reactive species, this intermediate being hydrogenolyzed.



(176)

Attempting to modify the synthesis, in order to alleviate the necessity of having to reduce a C-3 ester, the Scheme B was undertaken. In this approach 5-cyano-2-nitro-p-xylene (180) was converted via the appropriate arylhydrazine to 6-cyano-1,2,3,4-tetrahydro-5,8-dimethylcarbazole (181). Dehydrogenation of this compound and subsequent Raney nickel reduction of the nitrile 182 gave the amine 183. It was thought that condensation of the amine 183 with glyoxyl diethoxyacetal followed by Pomeranz-Fritsch cyclization of 184 would then yield ellipticine (17) directly. However the same difficulties were encountered with this conversion as were experienced by Cranwell and Saxton[175] and the approach was abandoned.

Scheme C involves the conversion of 3,6-dimethyl-2-nitrobenzaldehyde (185) to an intermediate, 6-amino-2-cyano-p-xylene (186) very reminiscent of the starting material utilized by Schmutz & Wittwer (1960)[172]. (Figure 40) Fisher indolization to the tetrahydrocarbazole 187, hydrolysis of the cyano group, and subsequent dehydrogenation afforded 1,4-dimethyl-2-carbomethoxycarbazole (188). It was found that this C-2 ester could easily be reduced to

the corresponding alcohol 189 without the complication experienced in Scheme A, this again probably being a result of low electron density at C-2. Sarett oxidation of the alcohol 189 yielded the aldehyde intermediate 190.

The ω-nitrostyryl approach to the synthesis of the "D" ring developed by these workers has been discussed previously in conjunction with Mosher's synthesis of olivacine (16)[177]. The dehydrogenation of the imine, 3,4-dihydroellipticine (191) obtained in four steps from the aldehyde provided ellipticine (17).

The first stage of the work portrayed in Scheme C parallels closely that developed by Schmutz & Wittwer (1960),[172] and consequently it suffers from the same drawback, its excessive length. The nitromethane condensation reaction however as Mosher[177] realized was a very direct and highly efficient method for construction of the "D" ring.

Subsequently Dalton and co-workers (1967)[178] were able to overcome the problem of the cyclization of the imine acetal 172 to produce ellipticine (17). The synthetic scheme of Cranwell and Saxton's[175] was adopted (Figure 45) with improvement of the initial condensation step between indole (161) and hexane-2, 5-dione. By substituting ρ-toluenesulphonic acid-ethanol for this condensation it was found that the yield could be increased to 51%, compared with the 36% previously obtained. It was found that the crucial cyclization of the imine acetal 172 could then be effected in 33-40% yields using o-phosphoric acid.

Contrary to the results of Cranwell and Saxton[175] who claimed that ethanol - HCl cyclization of the amino acetal 173 produced 3,4-dihydroellipticine (191) (not characterized) it was found that when the amino acetal 173 was heated

in o-phosphoric acid that ellipticine (17) was isolated directly. It is known that 1,2-dihydroisoquinolines produced by similar cyclizations undergo disproportionation[187] and oxidation to isoquinolines, so that the previous authors may have produced ellipticine (17) directly and their dehydrogenation step was probably unnecessary.

This synthesis proved to be of considerable value for large scale preparation of ellipticine (17) due to its brevity although the yields were uniformly low.

Recently there has been a revival of interest in the chemistry and biological activity of ellipticine (17). Two new syntheses have been reported and both adopt the approach, first devised by Woodward (1959)[174], involving the condensation of an appropriate unit containing a preformed pyrido ring onto the indole nucleus. The general view taken with this strategy is that ring closure of the "C" ring would require fewer conversions than are involved in the build up of the pyridine ring.

Kilminster & Sainsbury (1972)[179] reinvestigated Woodward's condensation of 3-acetylpyridine onto indole (161). They preferred however to study the condensation into respective indolin-2 and -3-ones 192 and 193. It was found that although initial condensation products would form with ease the subsequent ring closure reaction could not be induced, presumably due to the inert character of the carbonyl groups. From these observations they were, however, successful in synthesizing ellipticine (17) through condensation of a substituted 3-acetylpyridine with 1-acetylindol-3-yl acetate (196) (Figure 47).

The diacetyldihydropyridine 194 was prepared, oxidized to the acetyl

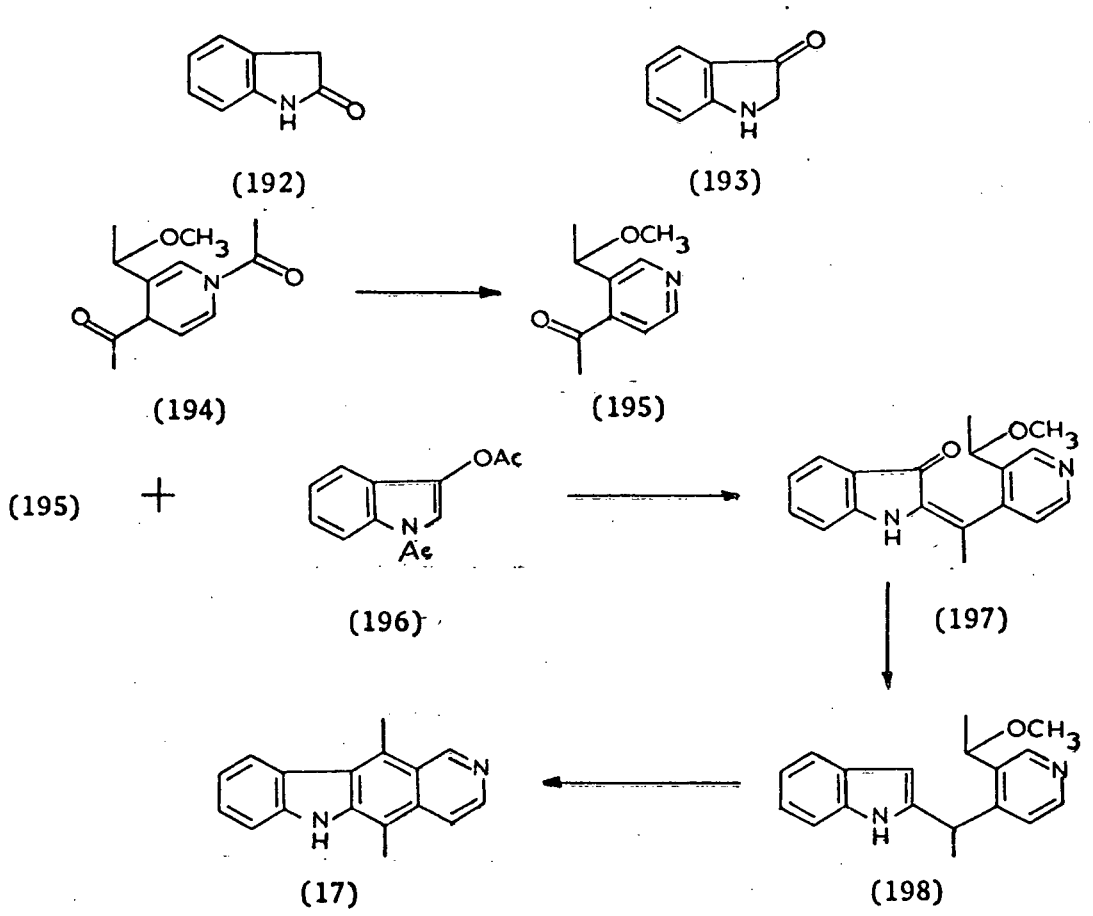


Figure 47 . Kilminster and Sainsbury (1972) Synthesis of Ellipticine.

pyridine 195, and condensed with the indole 196 affording a mixture of both geometrical isomers 197. Reduction of either isomer with sodium borohydride followed by acidification gave the compound 198 .

This material when heated in aqueous hydrogen bromide, followed by neutralization and absorptions onto silica gel afforded ellipticine (17) in 40% yield. This synthesis offers both a short and efficient route to ellipticine (17).

In the second synthesis, Le Goffic, Gouyette, and Ahond (1973)[180], proposed to unite the preformed "D" ring through a piperidone enamine condensation with an appropriately substituted gramine molecule.

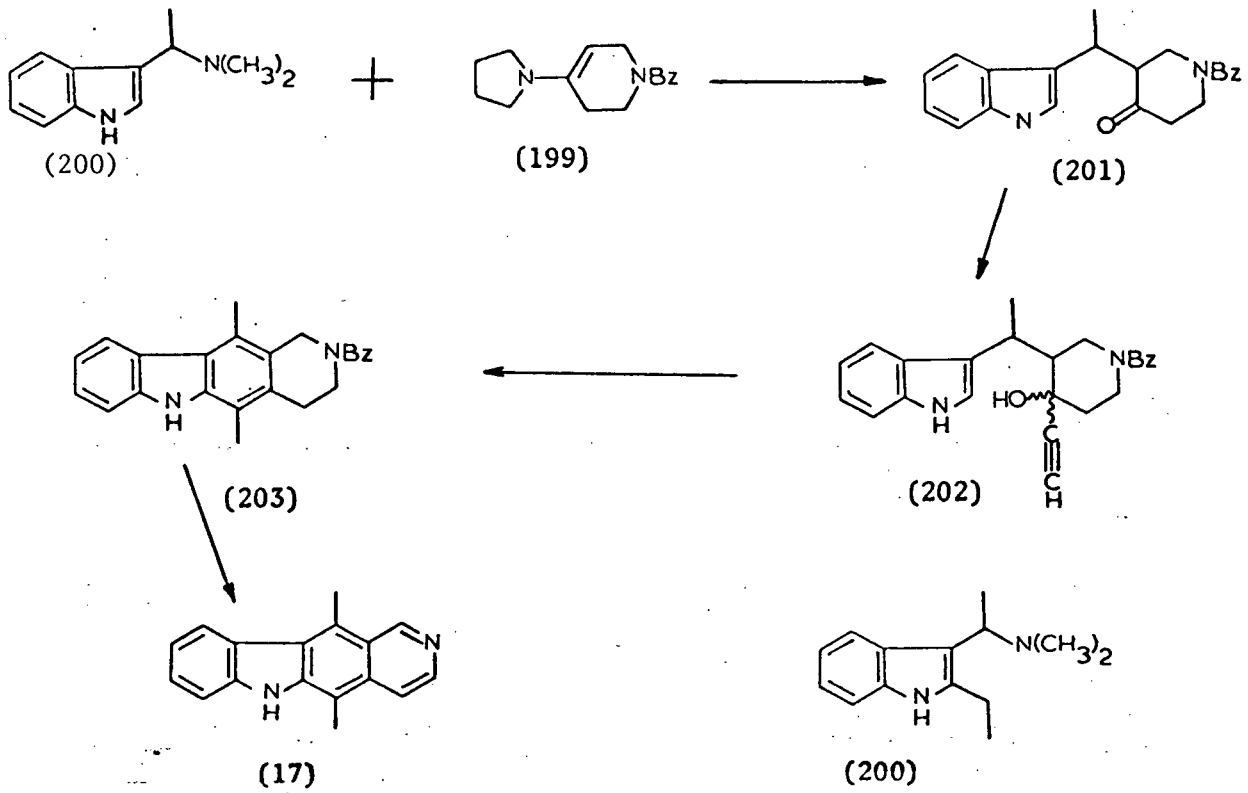


Figure 48. The Synthesis of Ellipticine (17) by Le Goffic, Goyette, and Ahond (1973).

Consideration was initially given to the condensation between the pyrrolidine enamine of 1-benzyl-4-piperidone (199) and 2-ethyl gramine (200) (Figure 48). This would ideally set the stage for a two-step synthesis of ellipticine (17). However it was found that the attempted condensation reaction gave only dimeric products and none of the desired material.

To circumvent this problem the ethyl group was incorporated onto the ring "D" instead. This was accomplished by reaction of the condensation product 201 with sodium acetylide, epimeric tertiary alcohols 202 being isolated. The alcohols were reacted in the presence of formic anhydride to

give the desired tetracyclic carbazole product 203 possessing the proper orientation of the two methyl groups. Debenzylation & dehydrogenation over palladium on charcoal produced ellipticine (17) in 24% overall yield from indole (161).

The excellent work done by the French group represents the culmination of the work to date in the field of the synthesis of the pyridocarbazoles olivacine (16) and ellipticine (17).

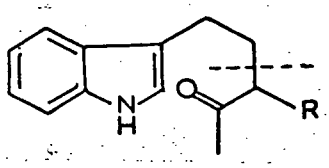
DISCUSSION - PART III

Two distinct synthetic themes presided in the many syntheses of olivacine (16) and ellipticine (17) presented in the preceding introduction, the two-stage approach where a suitable tricyclic carbazole intermediate was first formed and subsequently elaborated into the tetracyclic pyridocarbazole system, and the approach involving the condensation of a preformed "D" ring onto the indole nucleus, followed by ring closure to give the "C" ring and the complete tetracyclic structure. The former, two-stage approach was adopted in the design of the present syntheses of olivacine (16) and guatambuine (25). In this design consideration was taken of the fact that the ω-nitrostyryl approach utilized by Mosher[177] provided an efficient means of constructing the pyrido "D" ring of olivacine (16), whereas The Pomeranz-Fritsch approach adopted in the ellipticine (17) series provided a considerably shorter but lower yielding method of pyrido ring construction. An efficient synthesis of these two pyridocarbazole alkaloids could be obtained, therefore, if a short & high yielding synthesis of either of the two formylcarbazole compounds 1-methyl-2-formylcarbazole (152) or 1-methyl-3-formylcarbazole (219) could be devised.

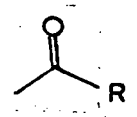
These considerations formed the basis behind the developed syntheses of olivacine (16) and guatambuine (25) presented in Sequences A & B.

Sequence A:

By analogy with Cranwell and Saxton's[175] synthesis of ellipticine (17) the condensation reaction between a methyl ketone functionality and indole was adopted for the synthesis of the carbazole-2-aldehyde 152. Unlike ellipticine (17) however, the absence of a symmetrical methyl group substitution pattern in olivacine (16) necessitated the prior construction of an appropriately functionalized compound 204 where a subsequent methyl ketone condensation with C-2 of the indole moeity would comprise the second step in the construction of the carbazole skeleton.



(204)



(205)

R = CHO
= $COOCH_3$

An inspection of the structure of compound 204 immediately indicated that it could be synthesized by the alkylation reaction of an appropriate 1,3-dicarbonyl with a 3-substituted indole derivative like tryptophyl bromide (207). Methyl acetoacetate (205, R = $COOCH_3$) was chosen as the 1,3-dicarbonyl component instead of the corresponding aldehydo compound (205, R = CHO) for reasons that the carbonyl of the carbomethoxy group is less reactive towards nucleophilic attack than the carbonyl of the ketone, thus eliminating the competition between these two centers during the subsequent condensation reaction. Also, the transformation of the carbomethoxygroup into the desired aldehyde functionality could be effected *via* a published procedure.

The synthesis of compound (204, R = $COOCH_3$) from commerically available tryptophol (206) and its cyclization with subsequent dehydrogenation to the carbazole ester 134 or its cyclization in the presence of a hydrogen acceptor reagent to give the carbazole ester 134 directly is presented in figure 49. The reduction of the ester 134 to the carbazole alcohol 157 and its subsequent oxidation to the desired 1-methyl-2-formylcarbazole (152) is also depicted. Elaboration of this aldehyde 152 into both olivacine (16) and guatambuine (25) via the ω-nitrostyryl approach proceeded in an anologous fashion to that reported by Mosher et al (1966)[177].

The present synthesis of the carbazole ester 134 in three high yielding steps from the readily available starting material tryptophol (206) represents a considerable improvement in efficiency over the alternate nine-step Fisher indolization approach previously employed. The overall synthesis consisting of ten steps to olivacine (16) is thus more efficient than those previously reported[172-3,177] and is easily adaptable to the large-scale preparation of both olivacine (16) and guatambuine (25).[188]

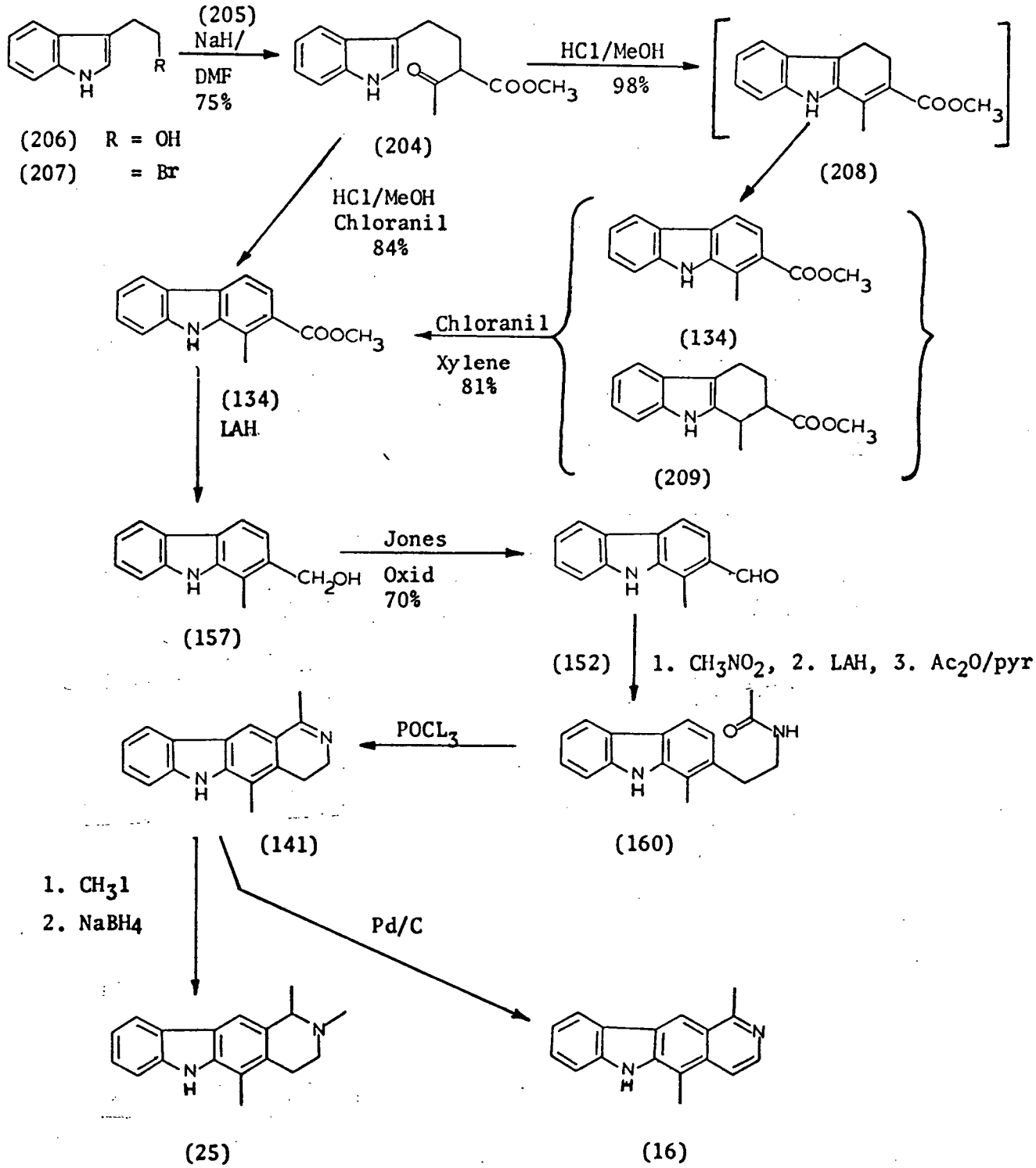


Figure 49. An Improved Synthesis of Olivacine (16) and Guatambuine (25), Sequence A.

Tryptophol (206)

Tryptophol (206) was obtained both commercially and via a simple synthesis. Before entering into a discussion of the synthesis of olivacine (16) from this starting material, it is perhaps advantageous to briefly discuss its synthesis. Several methods are reported,[189,190-1] but for the purposes of the present work the reaction of indole (161) with oxalyl chloride (210) was considered to be the most adaptable to large-scale preparation (Figure 50).

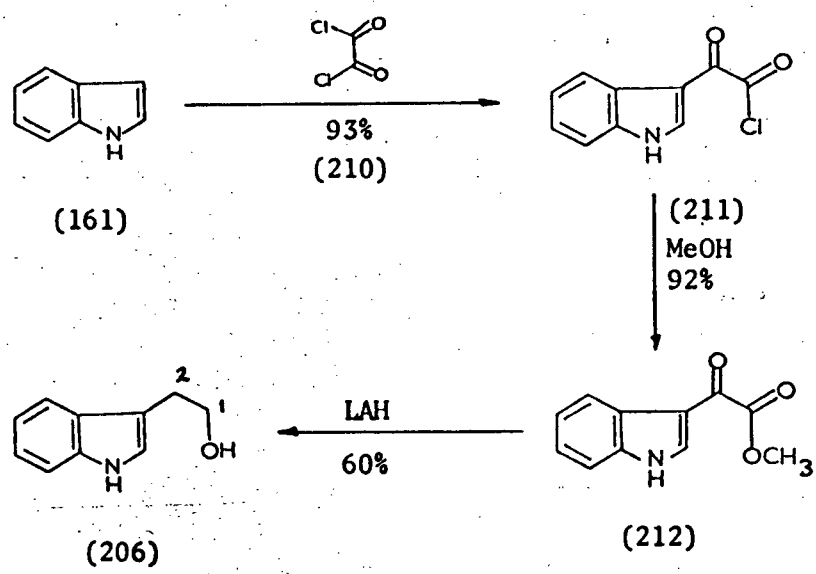


Figure 50. Oxalyl Chloride Route to Tryptophol (206).

This synthesis follows quite closely in experimental detail with the parallel synthesis of tryptamines by the same method[190]. The glyoxyl chloride 211 was obtained as easily isolated bright yellow crystals in 93% yield. Recrystallization proved unnecessary since the microanalysis was in agreement with the anticipated structure. The product proved to be moderately stable, not reacting perceptibly with water, and discolouring on standing in air only

after a period of several days.

Reaction of the glyoxyl chloride 211 with methanol gave the corresponding ester 212 in 92% yield. Again, the microanalytical data supported the structural assignment. Reduction of the ester 212 in the presence of an excess of lithium aluminum hydride gave tryptophol (206) in 60% yield. Column chromatography on alumina was sufficient to remove base line contaminants present in the crude product mixture. Tryptophol (206) was identified by its characteristic N.M.R. spectrum consisting of a pair of well separated triplets centered at δ2.96 (J = 6Hz) and 3.86 (J = 6Hz) for the C-1 (CH_2OH) and C-2 (CH_2) protons respectively. A sharp singlet was present at δ1.53 for the hydroxyl proton.

Tryptophol (206) could readily be converted to the bromide 207 by reaction with phosphorus tribromide.[192] Purification by recrystallization proved to be more difficult than expected, and the product was subsequently purified by column chromatography on alumina. The mass spectrum exhibited the expected doublet for the parent ion at m/e = 223 and 225 due to the equal abundance of the two bromine isotopes. The N.M.R. spectrum possessed a single complex multiplet centered at δ3.6 for the four methylene hydrogens. The triplet at δ 3.0 for the methylene protons adjacent to the oxygen atom in tryptophol (206) has shifted upfield in the spectrum of the bromide.

<u>3-Carbomethoxy-5-(3-indolyl) -2-pentanone (204)</u>

Several different reaction conditions were studied for the alkylation reaction in order to obtain a reproducible result. A 2:1 ratio of the sodium

salt of methyl acetoacetate (205) to tryptophol bromide (207) was normally employed to compensate for any neutralization of the anion that might occur as a result of removal of the indolic hydrogen atom.

Conducting the condensation in refluxing tetrahydrofuran proved to be generally unsuccessful. Varying periods of reflux from three to fifteen hours were tried in an effort to complete the reaction, however, the N.M.R. and mass spectral data indicated that normally only a mixture of starting materials was obtained.

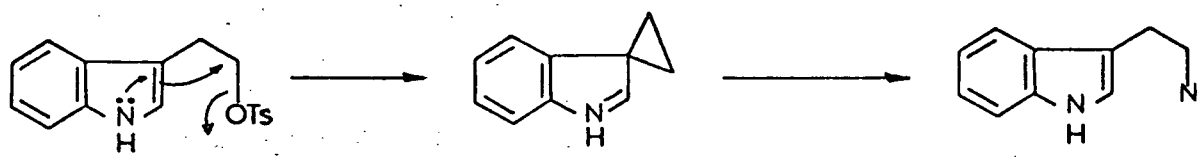
Zaugg et al (1960)[193] indicated dimethylformamide to be a superior solvent for the alkylation of the anion of ethyl acetoacetate with a series of primary and secondary aliphatic halides. Usual yields of about 70% were obtained when the reaction was conducted at 100° for three to four hours.

In a preliminary experiment with tryptophol bromide (207) using dimethylformamide the reaction was permitted to remain at 100° for fifteen hours. The N.M.R. spectrum of the isolated product showed several expected absorptions; a singlet due to the ketone methyl at δ2.20 and a group of distorted triplets at δ2.3 and 2.8 due to the methylene hydrogen atoms. However, concomitant with these observations was the absence of a singlet for the methyl hydrogens of the carbomethoxy group, indicating that decarboxylation had accompanied the cyclization process. The mass spectrum supported this interpretation, having a parent peak at m/e = 201 consistent with an alkylation product lacking the ester functionality. This phenomenon was thought to be a result of excessive reaction time and that by shortening the reaction period the undesirable side reaction might be eliminated.

Thus, it was found that conducting the reaction in dimethylformamide at 100° for three to four hours gave one predominant product as depicted from T.L.C. By column chromatography on alumina the desired product was isolated as a pure yellow oil. The yields obtained were found to be very dependent on the scale of the chromatography, the yield varying from 75% to 50% on proceeding from a small scale (1-2 gm.) to large scale (10-20 gm.) purification.

No attempts were made to further optimize the aklylation reaction in terms of time or temperature of reaction, although it was known that the reactivity of the corresponding tryptophyl tosylate (213) towards nucleophilic displacement is considerably greater than that of simple primary aliphatic tosylates.[194]

This rate enhancement has been demonstrated to be the result of a unique ability of the 3-position of the indole ring to participate in the displacement of the leaving group through formation of the spiro-indolenine 214.



(213) (214)

Subsequent solvolysis or reaction of the cyclopropyl intermediate with a nucleophile being a facile step, thereby providing the substituted tryptophol derivative.

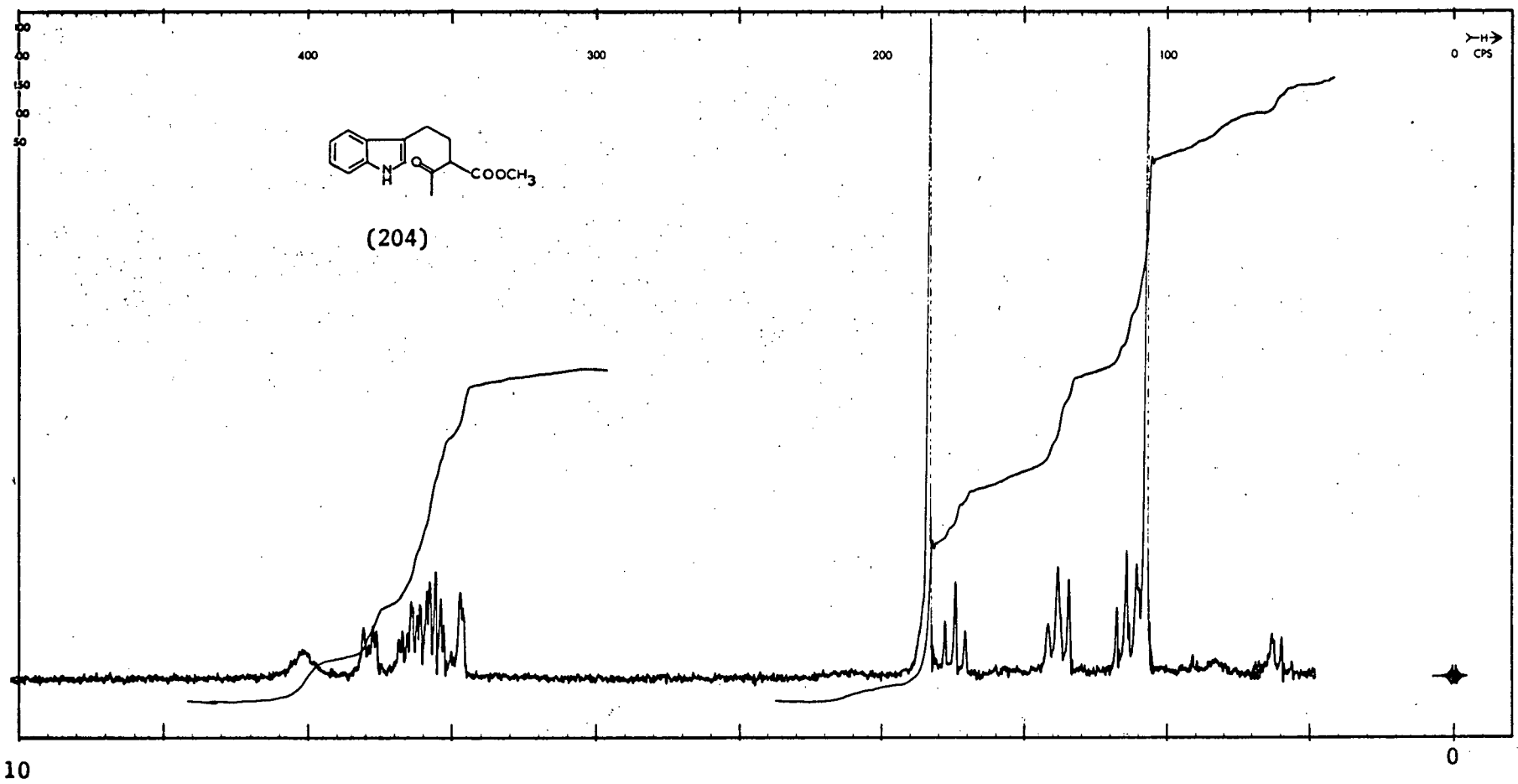


Figure 51. N.M.R. Spectrum of 3-Carbomethoxy-5-(3-indolyl)-2-pentanone (204)

Under reaction conditions where an appropriate nucleophile is either very weak or absent, the spiro indolenine 214 has been isolated.[111] The reaction conditions employed for the present alkylation were such, however, that its presence would not be detected as a reaction product.

Conclusive identification of the alkylation product 204 was made on the basis of its spectral data. The N.M.R. spectrum (Figure 51) was very characteristic. Two singlets for methyl protons were readily assigned, one at δ 2.12 for the ketone methyl, the other at δ 3.66 for the carbomethoxy methyl group. Both signals were shifted slightly upfield from those obtained for the corresponding groups (δ 2.20 and 3.76) in methyl acetoacetate. Three distinct sets of distorted triplets were assignable, one for each of the two methylene groups δ 2.28, (C-4) and δ 2.76 (J = 7Hz,C-5), and the third for the methine hydrogen at δ 3.48 (J = 7Hz,C-3). Of particular note was the presence of a spike at δ 6.94, integrating for the one hydrogen atom at the C-2 position of the indole ring. This signal indicated that under the reaction condition the alkylation product had remained in tact without subsequent cyclization.

Further confirmation that cyclization had <u>not</u> occurred was derived from the U.V. spectrum which showed a typical indole absorption. The I.R. spectrum contained two carbonyl absorptions at 1750 and 1725 cm^{-1} for the ester and ketone carbonyls respectively. In the low resolution mass spectrum the parent peak at m/e = 259 was relatively intense (25%). Important fragmentations were seen at m/e = 228, 186, 184, 144, 143, and 130. A possible mode of fragmentation is presented in Figure 52. The high resolution spectrum was within accepted limits and corroborates the parent peak at m/e = 259.1212 (calc. 259.1207) for $C_{15}H_{17}O_3N$.

It further corroborates the formulation of the fragment peaks giving the expected compositions for each fragment.

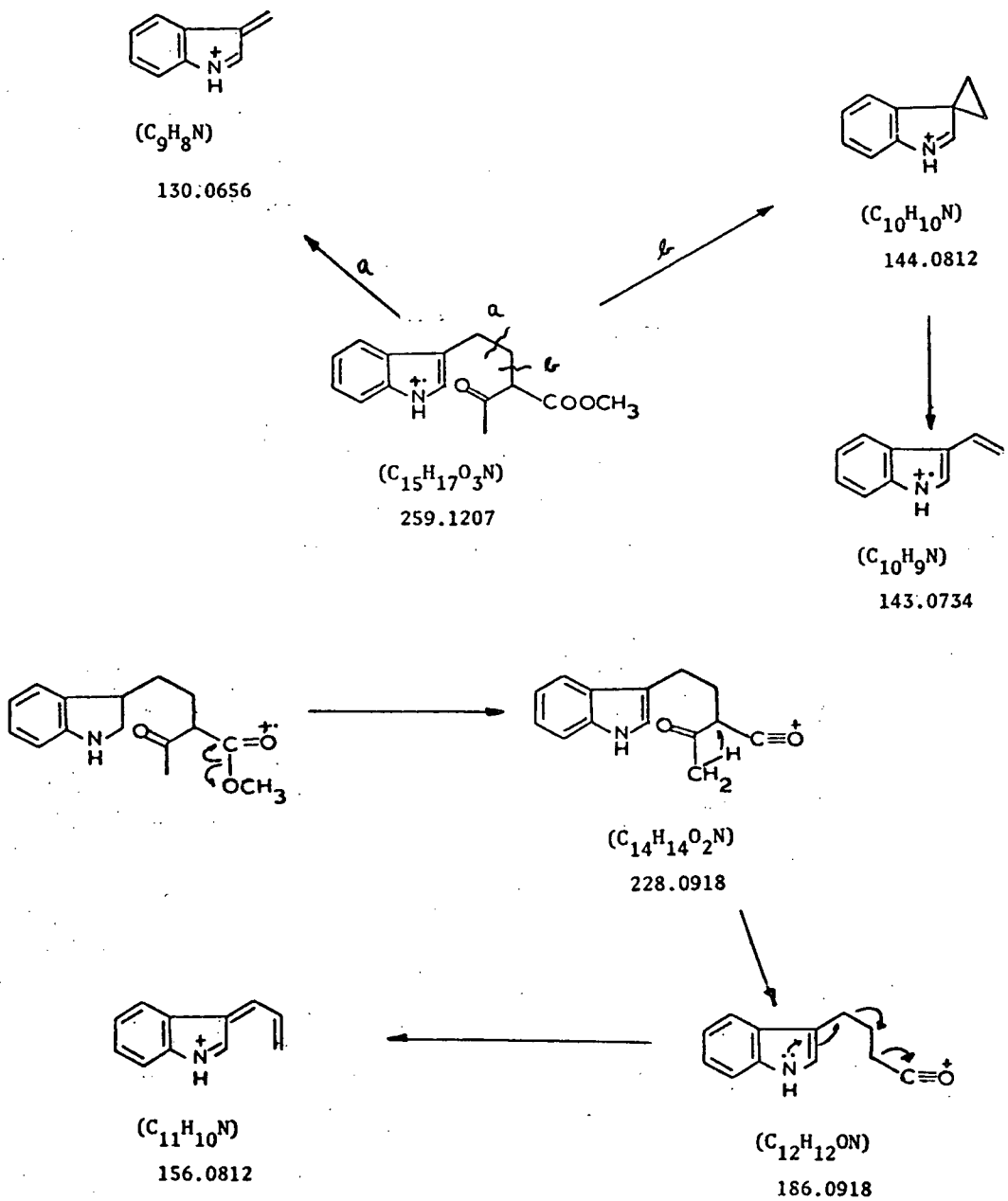


Figure 52. Plausable Mass Spectral Fragmentation Pattern for the Alkylation Product 204.

Cyclization Reaction

The conditions employed by Dalton *et al*[178] in the similar condensation of a methyl ketone center with the C-2 position of indole presented an obvious first choice for the cyclization of the alkylation product 204. It was consequently found that a reaction did occur with complete consumption of the starting material when the alkylation product 204 was treated with toluene sulfonic acid in refluxing ethanol. T.L.C., however, indicated the presence of an equal mixture of two products. These results were unexpected in that the spectral data for the products were inconsistent with the anticipated product, 1-methyl-2-carbomethoxy-3,4-dihydrocarbazole (208).

Alternate conditions were sought in an effort to control the course of the cyclization so as to produce the desired compound 208 as the exclusive product. Bischler-Napieralski cyclization conditions, phosphorous oxychloride in toluene were thus studied. Reaction conditions ranging from refluxing in toluene for one hour to stirring at room temperature for fifteen minutes were all found to effect quantitative conversion of the alkylation product 204. T.L.C. and N.M.R. indicated that the product composition obtained was identical with that observed using Dalton's conditions.

Methanol-HCl, conditions utilized by Cranwell and Saxton[175] resulted once again in a quantitative conversion to a mixture of the same two products. T.L.C. demonstrated that the reaction had occurred instantaneously on mixing the reactants. It was evident from these results that the cyclization under a variety of acidic conditions leads to the formation of a mixture of two products.

A detailed examination of the spectral data revealed the nature of the processes that occurred. The N.M.R. spectrum, (Figure 53) portrayed the presence of two carbomethoxy methyl group singlets at δ 3.84 and 3.68 as well as a pair of doublets at δ 1.00 and 1.15 (J = 7Hz) indicative of two different methyl groups attached to a saturated carbon atom bearing a methine hydrogen. These two methyl doublets integrated for a total of three hydrogen atoms thereby suggesting that they are due to the presence of epimers. The methylene hydrogens were spread out over the region δ 1.5 to 3.5 and the integration of these signals was difficult to ascertain with any degree of accuracy. The presence of a singlet at δ 2.67, integrating for three hydrogens was indicative of a methyl attached to an unsaturated center. The multiplet in the aromatic region centered at δ 7.2 integrated for ten hydrogens, two more than twice the number of hydrogens expected in the desired substance. This fact, coupled with the presence of two signals for carbomethoxy methyl and methyl groups suggested that the product mixture consisted of two components each containing the indole moeity, a methyl group and a carbomethoxy group. The absence of a peak at δ 6.94 for the indole C-2 hydrogen was important in that it meant that cyclization onto the C-2 position had occurred as expected.

Based on a comparison of the relative intensities of the carbomethoxy signals it was deduced that the reaction mixture consisted of a 1:1 mixture of the two products. The identity of the two components could not be deduced however, from the N.M.R. spectrum alone, but with the added support of the mass spectral data, their structures were determined.

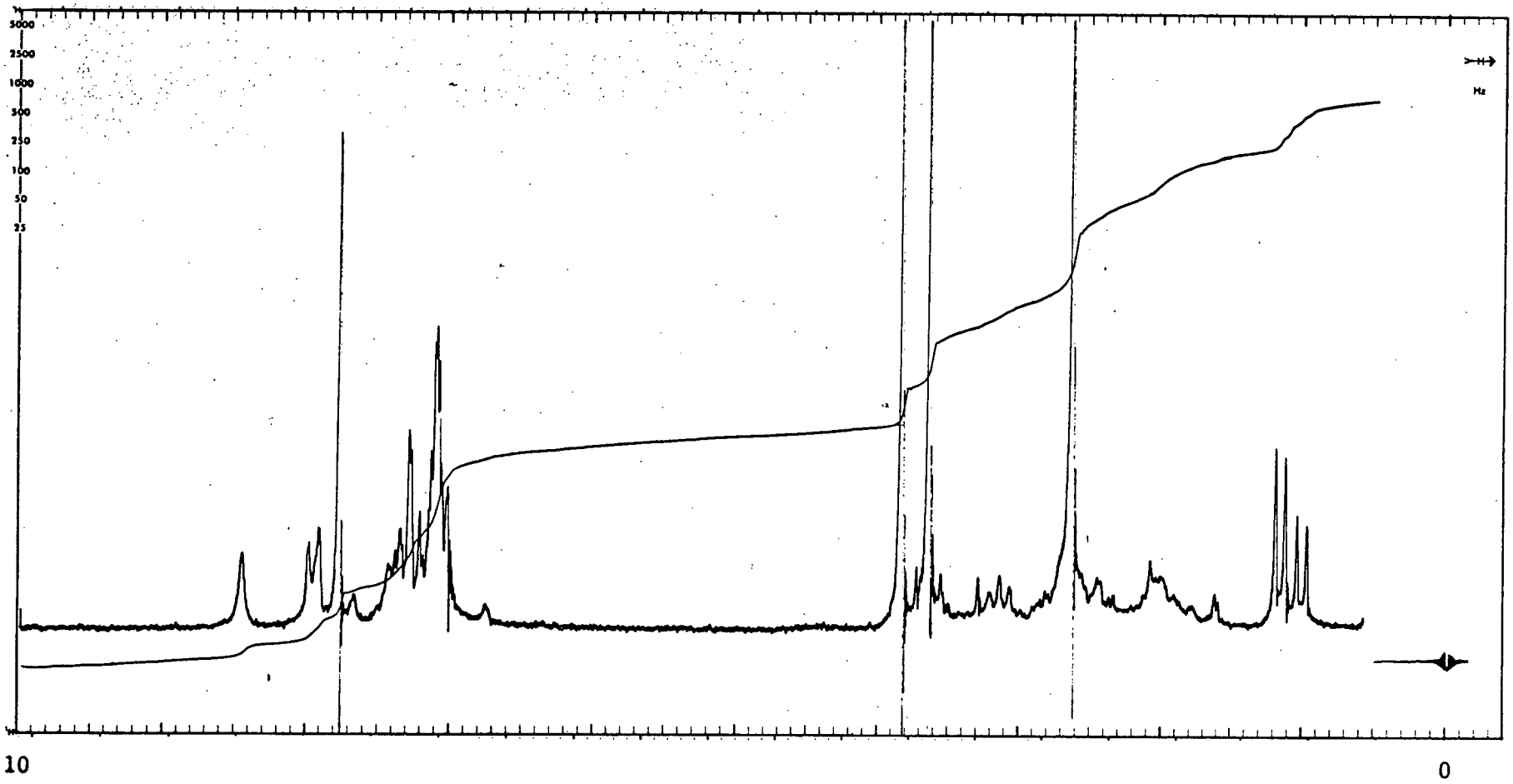


Figure 53. N.M.R. Spectrum of the Cyclization Reaction Mixture.

The region of the mass spectrum corresponding to the expected molecular ion was characterized by the presence of two intense peaks at m/e = 239 (100%) and m/e = 243 (50%), and an almost negligible peak at m/e = 241, the expected molecular ion for the proposed 3,4-dihydrocarbazole 208. These two peaks were considered to be the parent peaks for each of the two isolated components. That the m/e = 239 peak did not arise via fragmentation of the m/e = 243 peak was strongly supported by the presence of fragmentations for loss of CH_3, OCH_3, and $COOCH_3$ from each of these parent peaks, figure 54 illustrates the characteristic fragmentations observed in the low resolution spectrum. Of great importance was the presence of fragmentation peaks at m/e = 169, 168, 157, 143, and 130 which could only arise through disruptions of a saturated carbazole C" ring. Such an appropriately methyl and carbomethoxy substituted tetrahydro carbazole would give rise to the observed m/e = 243 peak. The m/e = 239 peak can thus be considered to arise from loss of four hydrogens in the former to give the fully aromatized carbazole compound.

To determine whether or not the species observed in the mass spectrum arose from a disproportionation of the 3,4-dihydrocarbazole 208 during cyclizatio or was simply an event in the spectrometer, experiments were conducted where low ionizing voltages of 53eV and low temperatures for volatilization were employed. In all cases the spectra were identical to that obtained under normal operating conditions (i.e. 70eV)

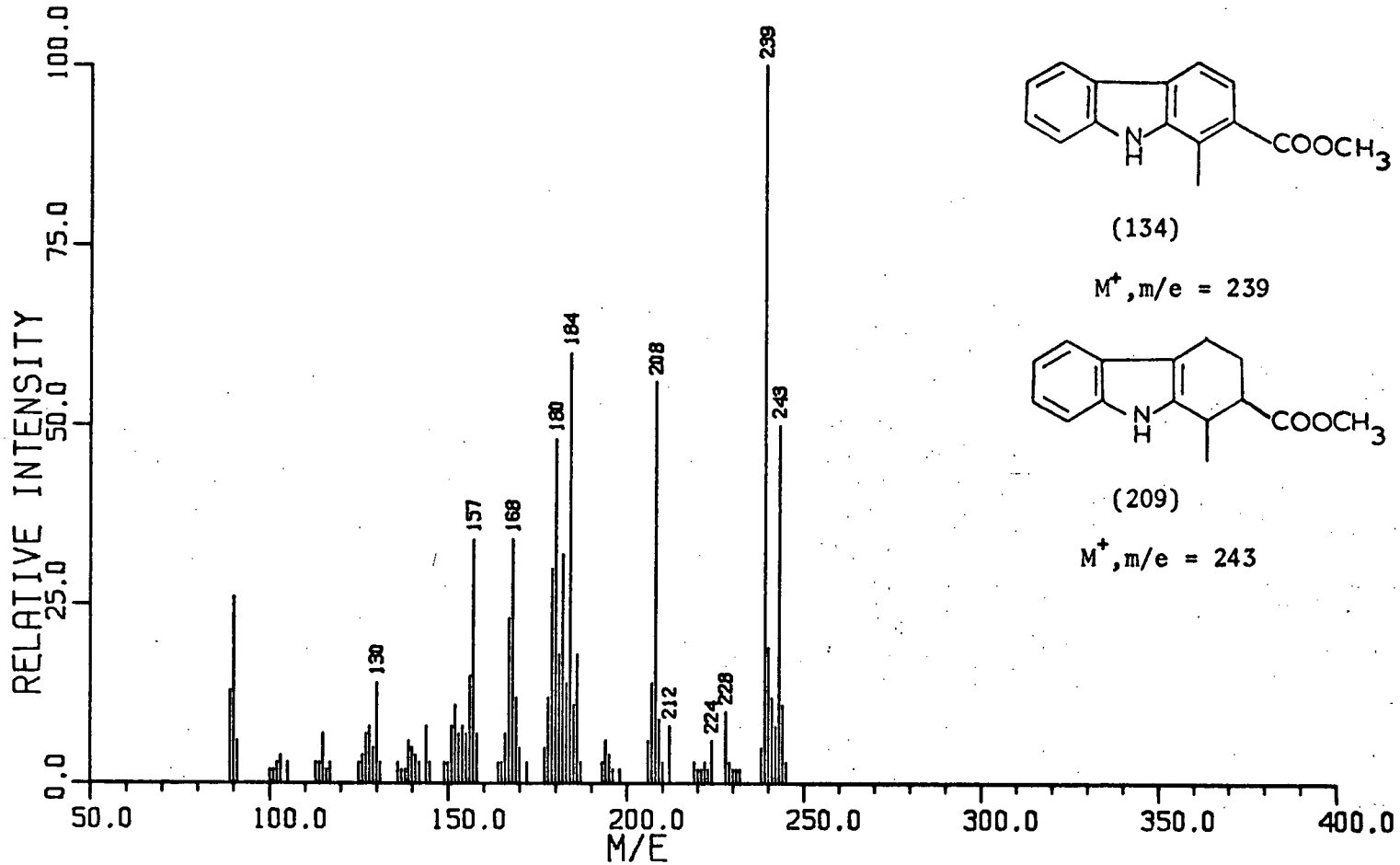
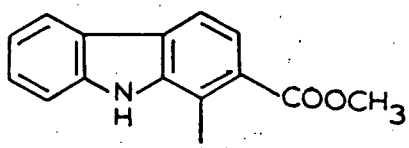


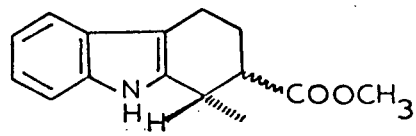
Figure 54. Mass Spectral Fragmentation Patterns for Cimponents 134 and 209 of the Cyclization Reaction Mixture.

On the basis of the N.M.R. and mass spectral analysis it was proposed that the cyclization reaction produced the fully unsaturated compound, 1-methyl-2-carbomethoxy carbazole (134) and its fully saturated counterpart 1-methyl-2-carbomethoxy-1,2,3,4-tetrahydrocarbazole (209).

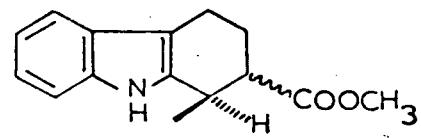
To accommodate the existence of the saturated methyl group in two separate environments, two configurations of the methyl with respect to the carbomethoxy groups were inferred.



(134)



(209a)



(209b)

Conclusive proof of the above assignments was not possible however without separation of the two components and provision of appropriate characterization data. Considering that the carbomethoxy functionality is essentially neutral, it was not surprising to find that the T.L.C. characteristics for the cyclization product mixture were identical to those obtained for an equal mixture of carbazole and tetrahydrocarbazole. In both cases two closely overlapping spots were obtained giving identical colour reactions when sprayed with ceric sulphate.

Separation of the two reaction components proved to be very difficult. On column chromatography an enrichment of the impure tetrahydro compound was obtained, whereas, pure carbazole component 134 was obtained in good yield (about one half of the material applied). This latter material crystallized spontaneously from highly concentrated chloroform solution to give colourless

crystals, M.P. = 138-140°. Very poor weight recovery of the tetrahydrocarbazole 209 from the column was achieved. This situation also prevailed when this material was subsequently further purified by preparative layer chromatography. After several purifications small amounts of carbazole 134 were still detected.

Complete analytical and spectral data were obtained for the crystalline carbazole 134 and these were in compliance with its structure. The N.M.R. spectrum exhibited the anticipated singlets at δ3.84 and 2.70 for the hydrogens of the two methyl groups, while signals for methylene protons observed in the above mixture were now totally absent.

The U.V. spectrum was surprising in that it was identical in shape to that for the crude reaction mixture. The measured extinction coefficients compared favourably with those for the corresponding ethyl ester prepared by Schmutz and Wittwer (1960)[172], however a discrepancy arose as to the position of the maximum. The maximum for the methyl ester (measured in methanol) was reported at 303 nm and that for the ethyl ester (measured in ethanol) is observed at 308 nm.

The fragmentation pattern in the low resolution mas spectrum corresponded to one half of the observed fragments in the composite spectrum for the crude reaction mixture. The parent peak in the high resolution spectrum m/e = 239.0946 was found to be consistent with the correct molecular composition $C_{15}H_{13}O_2N$, as were the compositions for the fragment ions corresponding to the loss of CH_3, OCH_3 and $COOCH_3$.

The spectral data determined for the tetrahydrocarbazole 209 was poor in that inadequate quantities of the compound could be obtained in pure form. Crystallization could not be induced, consequently it was studied as a viscous oil. The U.V. spectrum exhibited a typical indole absorption having maxima at 290, 282, and 275 nm, this being expected for a tetrahydrocarbazole. The extinction coefficients for the indole chromophore are low, compared to a carbazole chromophore, and they may represent only a small contribution to the composite spectrum which may possibly explain the similarity of the spectra of the carbazole 134 and the crude mixture.

Fourier transform N.M.R. portrayed the presence of the carbomethoxy functionality and the saturated methyl as a singlet at δ3.68 and a pair of high field doublets respectively (the latter partly obscured by impurities). Particularly significant were the small set of multiplets centered at δ3.38 and 3.96 attributed to the methine protons in their two respective configurations (209 a and b). The absorption for the methine hydrogen geminal with the carbomethoxy group was masked by the singlet peak at δ3.68 for this group.

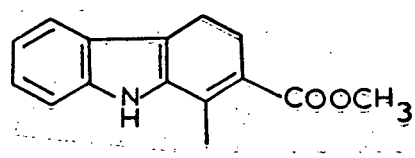
The parent peak in the mass spectrum at m/e = 243 (85%) was clearly evident, as were the fragments corresponding to loss of CH_3, OCH_3 and $COOCH_3$ respectively. Those fragments corresponding to the disruption of the saturated "C" ring were also evident.

Thus the cyclization of the alkylation product 204 did not give the expected 3,4-dihydrocarbazole 208, but proceeded further to yield the carbazole 134 and tetrahydrocarbazoles (209 a and b). Their formation can be mechanistically

envisaged to result from a total disproportionation of the initially formed 3,4-dihydrocarbazole 208 under the reaction conditions employed (figure 49), (204) → |(208)| → (134) + (209).

Such a disproportionation reflects the inherent instability of the dihydrosystem with respect to its anomatic counterpart.

Other examples of this occurrence exist, particularly in cases where potential dihydropyridine systems are involved such as the 3,4-dihydroisoquinolines[195] and 3,4-dihydro-β-carbolines[168]. It is of interest to note that the 3,4-dihydrocarbazole aldehyde 155 synthesized by Wenkert and Dave (1962)[173] did not undergo disproportionation on its formation. Whether this reflects the difference in the method of preparation of this compound or its



(155)

stability is not known. The aldehyde 155 did disproportionate (see page 172) however even on mild dehydrogenation treatment with palladium on charcoal.

Methanol - HCl (2%) conditions were employed for all large scale cyclizations due to the simplicity of the reaction and the quantitative yields of the two products. Column chromatography was not used to separate the two components during preparative work because subsequent dehydrogenation gave pure carbazole 134. The overall yield for the subsequent dehydrogenation could be increased by prior selective recrystallization of the carbazole

component 134 from a highly concentrated chloroform solution of the mixture. Dehydrogenation was, therefore, conducted only on the mother liquors from the cuptallization of 134.

1-Methyl-2-carbomethoxycarbazole (134).

The use of palladium on charcoal for the dehydrogenation of tetrahydrocarbazole and other dihydro compounds has considerable precedence particularly for the systems dealt with in the present work (see introduction part III).

Palladium on charcoal was, therefore, the obvious choice for the conversion of the cyclization reaction mixture into the carbazole 134 . Under typical reaction conditions, however, only partial dehydrogenation was observed.

Changing to platinum oxide as the catalyst enabled complete dehydrogenation in all experiments conducted. However, the large quantities necessary made its use prohibitive on a preparative scale.

Attention was thus turned towards the use of quinone dehydrogenating agents. Quinones, especially chloranil and dicyanodichlorobenzoquinone (DDQ) have been utilized with great success in the dehydrogenation of a wide range of carbazole compounds.[196-7] With chloranil a homogeneous carbazole ester 134 reaction product mixture was obtained. Traces of coloured impurities were removed from the crude product either by recrystallization from chloroform or by column chromatography. The product was eluted with benzene as a yellow oil which subsequently crystallized to yield colourless crystals. The yields were quite variable ranging normally between 70-80% although occasionally dropping as low as 60%.

To prevent manipulative difficulties during chromatography due to the non-polar nature of both the carbazole compound and the coloured contaminants

purification was normally postponed until after reduction to the corresponding alcohol 157 . The reduction was found not to be affected by the presence of these impurities and the alcohol 157 being more polar was more easily separated from them.

The spectral and analytical data obtained for the dehydrogenation product were identical to that obtained for the carbazole component 134 of the cyclization mixture, thus confirming the above conversion.

Cyclization - Dehydrogenation

Advantage was taken of the disproportionation reaction to achieve the direct conversion of the alkylation product 204 into the carbazole 134 in one step by conducting the cyclization reaction in the presence of an excess of chloranil as the hydrogen acceptor. The rationale behind this reaction was that the 3,4-dihydrocarbazole 208 would act as a poor hydrogen acceptor compared with chloranil. On this basis the alkylation product 204 dissolved in benzene containing excess chloranil was treated with methanol - HCl (2%). The isolated product, formed in 84% yield, was indeed the desired carbazole 134 . Thus the necessity for a distinct dehydrogenation step was eliminated.

1-Methyl-2-hydroxymethylcarbazole (157).

The conversion of the ester group of the carbazole 134 to the corresponding alcohol 157 was readily accomplished by making use of lithium aluminum hydride reduction conditions developed by Schmutz and Wittwer[172] . Reaction of pure carbazole ester 134 gave the desired carbazole alcohol 157 as a colourless crystalline solid in 95% yield.

The corresponding reduction of impure carbazole ester 134 obtained via

chloranil dehydrogenation was conducted in an identical manner. Chromatographic purification of the product provided an overall 60% yield of the alcohol 157.*

The spectral data were consistent with the structure 157. The mass spectrum showed a parent peak at m/e = 211 as well as an M^+-17 for loss of hydroxyl. The absence of the carbonyl absorption at 1710 cm^{-1} in the I.R. spectrum was noted. The U.V. spectrum exhibited a typical carbazole absorption as expected due to loss of conjugation with the carbomethoxy functionality. The singlet at δ 3.84 in the N.M.R. for the ester methyl group was absent, having been replaced by a singlet at δ4.88 (CH_2OH) and a singlet at δ 1.50 (OH).

1-Methyl-2-formylcarbazole (152)

The conversion of the benzylic alcohol 157 to the aldehyde 152 proved to be a low yielding step. Mosher *et al*[177] used bispyridine-chromium trioxide ($CrO_3 \cdot pyr_2$) in pyridine, i.e. Sarett conditions to oxidize this alcohol 157, reporting a yield of about 70% for a large scale reaction (16 gm). Govindachari *et al* report a higher yield of 84% for a small scale (1 gm) Sarett oxidation of the closely related alcohol 189 in the ellipticine (17) synthesis.[176]

It was found during the present work, however, that consistently lower yields (60%) were obtained for the Sarett oxidation of the alcohol 157. In an attempt to improve upon this yield a considerable number of oxidation conditions were studied during the present synthesis. A tabulation of the various oxidation

*footnote: For ease of manipulation the entire sequence could be conducted without purification at each step. In terms of overall yield starting from tryptophyl bromide, a comparison of both procedures showed there to be no advantage in not purifying each product (see experimental).

methods including the Sarett, the conditions under which the reactions were conducted and yields or comments on the reaction outcome is presented in Table III.

From this study it was determined that an efficient oxidation procedure depended upon two criteria. i) The solubility of the alcohol in the reaction media and ii) An efficient uncomplicated work-up.

Collins conditions[198] were an obvious alternative to the Sarett reaction with yields of 90% being reported for the related conversion of benzyl alcohol to benzaldehyde. Similarly high yields are obtained from Silver$^{(II)}$ oxide[156] or Ce$^{(IV)}$ oxidation[199] of the benzylic alcohols. However in all three cases poor solubility of the alcohol 157 in the reaction solvent hindered oxidation and under the conditions quoted for the reaction no detectible formation of aldehyde 152 was observed.

Considerable difficulties during work-up resulting from mutual solubilities of the extracting solvents and reaction reagents were encountered for the pyridine-based oxidation methods and this was one of the major drawbacks to the Sarett oxidation. The influence of the work-up on product yield was particularly drastic in the case of the HOAc/CrO_3·pyr_2 oxidation[200] where a drop from 85% to 35% yield accompanied the scaling of the reaction to larger quantities.

The pyridine-SO_3/DMSO-Et_3N oxidation method developed by Doering and co-workers[201] for work in the steroid field gave a mixture of three products on work-up . Separation of the aldehyde component from this mixture by either

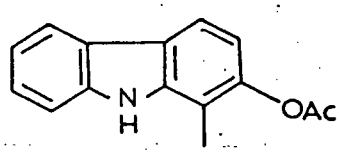
Table IV. Results for Oxidation of Alcohol (157)

Method	Conditions	Yield or Comment
Sarett	$CrO_3 \cdot pyr_2$ in pyr 24 hrs., RT.	60-65%[d]
$HOAc/CrO_3.pyr$	in HOAc -5 to +16° 15 min.	85%, 35%[d]
Collins	$CrO_3 \cdot pyr_2$ in CH_2Cl_2 RT., 0.5 hr.	no product detected[a]
$Ag^{II}O$	1 M H_3PO_4, 25° 3-4 hr.	no reaction[a,b]
Ce^{IV}	$HOAc/H_2O$ 90° 12 hr.	no reaction[a]
$Pyr \cdot SO_3/DMSO$ Et_3N	RT., 24 hr.	inseparable mixture[d]
$Pb(OAc)_4/pyr$	RT., 24 hr	50-60%[d]
Jones	CrO_3, H_2SO_4(aq.) in Acetone 1 min., RT.	70-75%

a. TLC detection (Al_2O_3, CHCl), b. UV detection, c. small scale $\leq$ 300 mg., d. large scale $\geq$ 2.0 gm.

alumina or silica chromatography was unsuccessful as was attempted recrystallization from chloroform.

The problems associated with the presence of pyridine during extraction were eliminated by using the lead tetraacetate in pyridine conditions[202]. At the completion of reaction the solvent was first removed in vacuo before extraction of the product. The aldehyde 152 was obtained as the major product i.e. (50-60% yield) after column chromatography. Several minor by-products were detected in this reaction. The R_f of a non-polar component coinciding on T.L.C. with authentic 1-methylcarbazole-2-acetate (215), this material being an expected by-product.



(215)

Jones oxidation[203] proved to be a viable alternative to the Sarett reaction yielding the aldehyde 152 as the sole product. This reaction often leads directly to the carboxylic acid , but in this instance the reaction stopped at the aldehyde stage. The reaction did not appear to be dependent on either the time span or the amount of oxidant present. Attempted further oxidation of the aldehyde 152 resulted in the re-isolation of starting material.

Although a single reaction product was produced the yields were not exceptionally high (about 70%), this again reflecting the inefficiency of product extraction during chromium (VI) oxidations. Despite the only moderate, if any, improvement in yields, the Jones conditions were considerably more

efficient than the Sarett in terms of reaction time and ease of work-up.

Proof of the aldehyde structure 152 was derived from a comparison of the melting point and quantitative U.V. spectrum with that reported by Mosher et al,[177] as well as from the other spectral and analytical data. The N.M.R. spectrum exhibited two singlets, one at δ2.87 due to the aromatic methyl, and the other at δ10.39 for the aldehydic hydrogen. The I.R. spectrum indicated the expected aldehyde carbonyl band (1665 cm^{-1}) and in the mass spectrum parent peak was at m/e = 209. High resolution mass measurement provided the value m/e = 209.083] which established the desired molecular formula ($C_{14}H_{11}NO$ requires:209.0842).

"D" Ring Synthesis

The construction of the "D" ring of olivacine through formation of the imine, 3,4-dihydro olivacine (141) from the aldehyde intermediate 152 was accomplished by following the procedure published by Mosher et al.[177] No unexpected complications were encountered during the interconversions and all analytical and spectral data were in accord with the proposed structures and compared within acceptable limits with literature values where quoted.

Condensation of the aldehyde 152 with nitromethane in the presence of ammonium acetate produced the nitrostyrene 160 as the sole reaction product in 94% yield. Recrystallization to crimson needles was possible from large volumes of ethanol. The nitrostyrene 160 was reduced to the amine 139 by lithium aluminum hydride reduction in tetrahydrofuran at room temperature. The product isolated as a white crystalline solid was obtained in 95% yield. The amine 139 was converted without purification to the N-Acetylamine by reaction

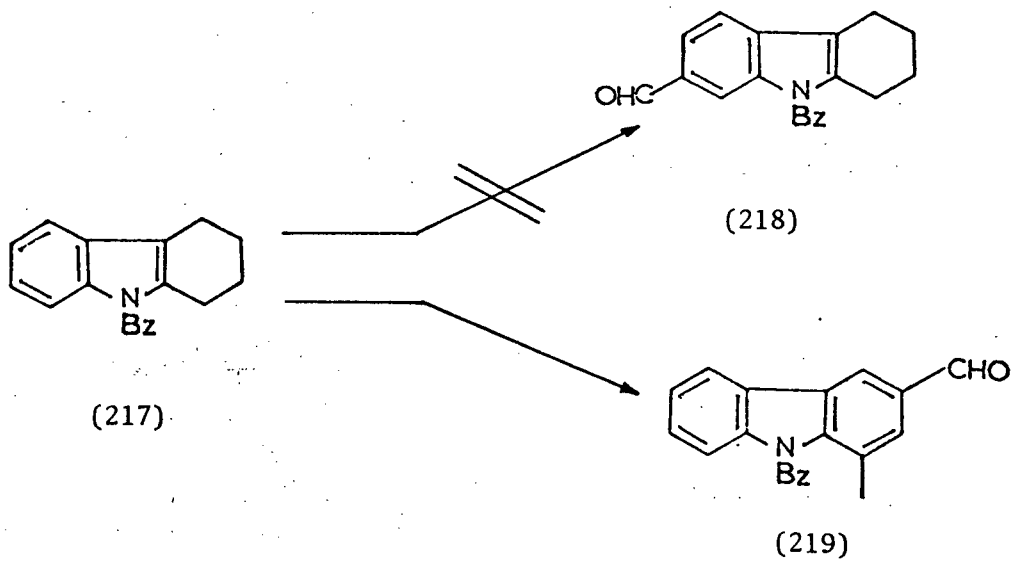
with acetic anhydride in pyridine. The product was a white crystalline solid obtained in 95% yield. The Bischler-Napieralski cyclization of the latter to 3,4-dihydroolivacine 141 was accomplished by refluxing the amide 140 with phosphorous oxychloride in toluene. The ultraviolet spectrum of 141 run in water and dilute hydrochloric acid showed the expected bathochromic shift[125] accompanying the transformation $C = N \rightarrow C = NH^+$.

Reaction of the imine 141 with methyl iodide in methanol produced the methiodide salt 142 in quantitative yield. The shifts observed in the UV. spectrum of this iminium cation in base reflected its rearrangement to its corresponding enamine (anhydro base)[167-8] (Section 6., part II). Reduction of the methiodide [142] either by sodium borohydride in aqueous ethanol or by catalytic hydrogenation over platinum oxide gave in high yield the tetracyclic alkaloid (±)-guatambuine (25). Proof of the structure of guatambuine (25) was derived from the superimposibility of the UV. and IR. spectra with the reported spectra[126] and by the close comparison of the N.M.R. and mass spectrum with that obtained for guatambuine (25) isolated from A. australe (Figure 20).

Dehydrogenation of the imine 141 by reaction over palladium on charcoal in refluxing decalin produced the tetracyclic alkaloid olivacine (16) in 70% yield. Total characterization of this product was also derived from a comparison of the N.M.R. and mass spectral data with that obtained for olivacine isolated from A. australe. The N.M.R. spectrum displayed a pair of singlets at δ2.82 and 3.16 for the C-1 and C-5 methyl groups respectively. The mass spectrum possessed a parent peak at m/e = 246 corroborated by the high resolution spectrum parent peak at m/e = 246.1165 ($C_{17}H_{14}N_2$ requires m/e = 246.1156).

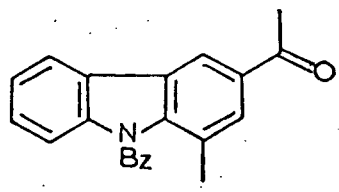
Sequence B

A short route to the synthesis of the carbazole-3-aldehyde 219 was provided from the reported observation[204] that the Vilsmeier-Haack formylation of 9-benzyl-1,2,3,4-tetrahydrocarbazole (217) instead of leading to the expected 7-formyl-9-benzyl-1,2,3,4-tetrahydrocarbazole[205] (218) gives 1-methyl-3- formyl-9-benzylcarbazole (219). The yield of about 50% for this conversion was acceptable since this provided a one step synthesis of this desired intermediate aldehyde from a readily available starting material (217).



In the application of the Pomeranz-Fritsch reaction for the synthesis of the pyrido ring the necessity of incorporating a methyl group at C-1 of the olivacine system (16) created complications. It is known that the condensation of amino acetals with ketones is far less efficient[206] than the

corresponding reaction with aldehydes. Hence the most direct route to olivacine (16) by reaction of aminoacetaldehyde diethylacetal (171) with the 3-acetylcarbazole 220 had little chance of being successful.



(220)

Two alternatives to this condensation were available. On the one hand the 1-methyl-3-formylcarbazole intermediate (219) was first condensed with aminoacetaldehyde diethylacetal (171) and the resulting iminomethyl acetal 221 was then methylated with methylmagnesium chloride to give the aminoethyl acetal 224. Alternatively, the 3-formylcarbazole compound 219 was reacted with methylmagnesium chloride to give the α-carbazolyl ethanol system 222 which was converted to the corresponding acetate 223. Substitution of the acetate functionality by the aminogroup of aminoacetaldehyde diethylacetal (171) again produced the desired aminoethyl acetal 224 (Figure 55).

Cyclization of this aminoethyl acetal 224 to 6-benzoolivacine (225) followed by debenzylation to olivacine (16) was not attempted. However, the conditions necessary for the cyclization have been worked out for the synthesis of the closely related molecule, ellipticine (17).

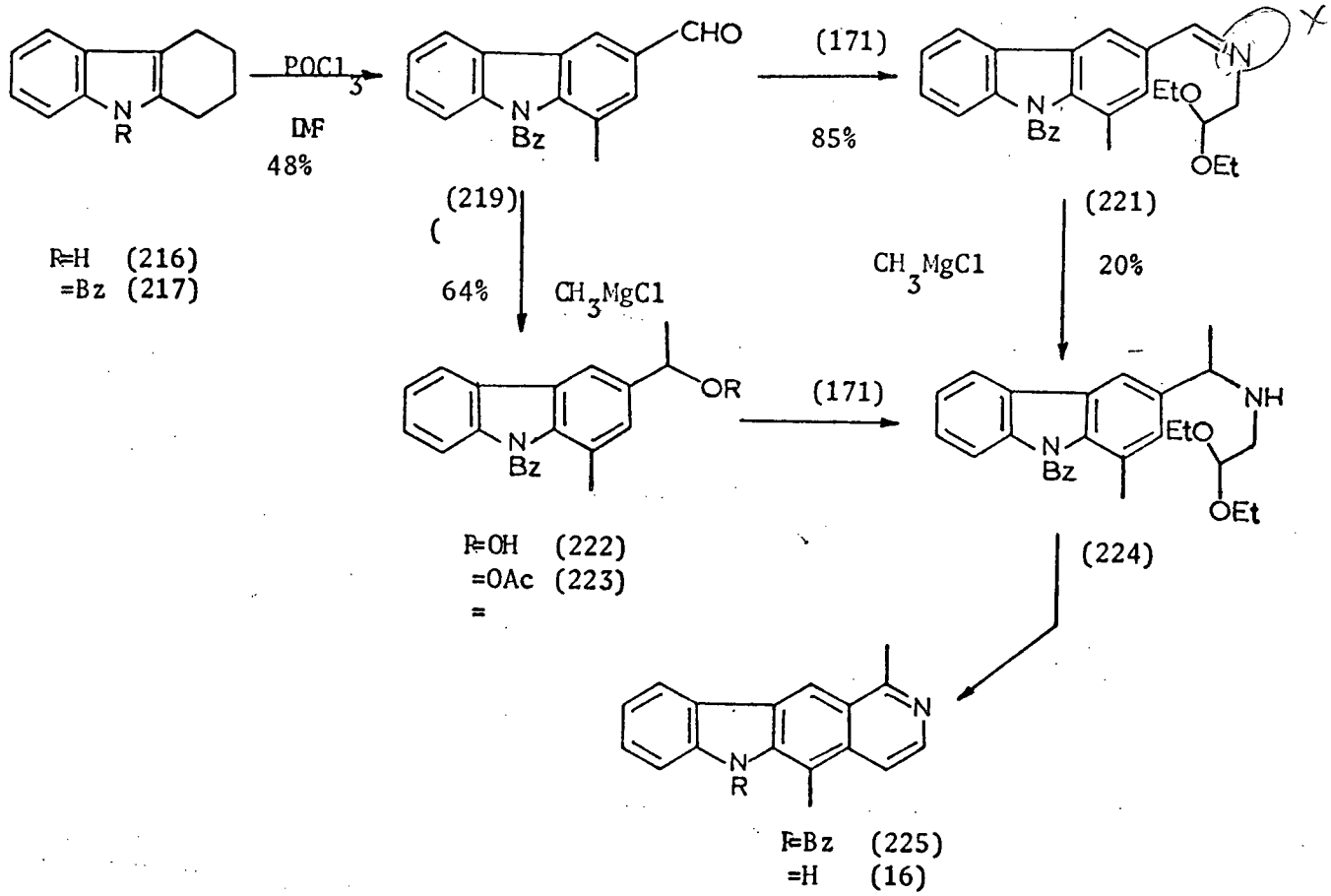


Figure 55. Synthesis of Olivacine (16) from Tetrahydrocarbazole (216).

This synthesis as it presently stands is generally low yielding in each of the individual steps, however, it is potentially a short and simple route to olivacine (16) starting from readily available starting material. It could easily be adapted to large scale preparation and towards the preparation of various forms of radioactive olivacine (16) (particularly the C-1 CH_3 group) which may be required in future biosynthetic experiments.

9-Benzyltetrahydrocarbazole (217)

Tetrahydrocarbazole (216) was readily available in large quantities as a starting material through the condensation of phenylhydrazine with cyclohexanone in the presence of acid.[207] The indole nitrogen (Na) of tetrahydrocarbazole (216) was converted to its benzyl derivative 217 by reaction of benzyl bromide and sodium hydride. A colourless oil obtained in 77% yield possessed the expected spectral characteristics. Thus the mass spectrum showed the expected parent peak at m/e = 261 while the N.M.R. spectrum exhibited the presence of the methylene of the benzyl group (δ 5.10, singlet) and the methylenes of the carbocyclic ring system (pair of multiplets centered at δ 2.60 and 1.86). The absence of the N-H stretch signal in the region of 3500 cm^{-1} in the IR spectrum was also indicative of reaction at the indole nitrogen.

The 9-benzyl derivative 217 was formed for three reasons. Firstly, the subsequent Vilsmeier-Haack formylation to be carried out has been shown to proceed to the desired 1-methyl-3-formylcarbazole compound 219 only when the indole nitrogen is protected by an alkyl group i.e. methyl, benzyl, etc.

Secondly, having the indole nitrogen protected eliminates any undesirable side reactions that may occur during methylation of the formyl or iminomethyl group under Grignard conditions. Thirdly, the benzyl group is practically the only alkyl protecting group which can be effectively removed in the last step to yield olivacine (16).

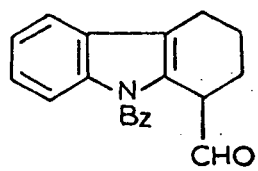
1-Methyl-3-formyl-9-benzylcarbazole (219).

Reaction of 9-benzyltetrahydrocarbazole (217) with 1.3 equivalents of phosphorus oxychloride in dimethylformamide (Vilsmeier-Haack conditions) proceeded as reported[204] to give the desired 1-methyl-3-formyl-9benzylcarbazole (219). The reaction mixture was purified by column chromatography on alumina. Whereupon the aldehyde 219 was recovered in 48% yield as a pure yellow oil which was subsequently crystallized to give pale yellow rosettes.

The N.M.R. spectrum of the product 219 possessed a singlet at δ 10.00 for the aldehyde proton as well as singlets at δ 5.72 and 2.60 for the methylene of the benzyl group and the C-1 methyl groups respectively. The correct justaposition of the methyl (C-1) and formyl (C-3) groups to each other was clear from the presence of two distorted singlets (meta coupling not resolved) at δ8.46 and 7.66 for the C-4 and C-2 hydrogens respectively. The absence of any multiplet absorptions for the methylene protons characteristic of a tetrahydrocarbazole system confirmed the aromatic nature of the molecule.

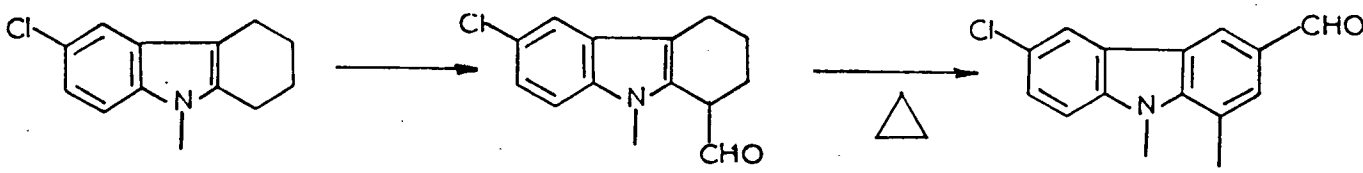
The UV. spectrum was altered considerably from the normal carbazole spectrum as a consequence of the extension of the carbazole chromophore by the formyl group. The IR spectrum possessed a carbonyl absorption at 1670 cm^{-1} for the aldehyde carbonyl in conjugation with the aromatic ring. The mass spectrum displayed a very dominant parent peak at m/e = 299 (100%) and very little fragmentation. The position of the parent peak was corroborated by the high resolution spectrum which had a peak at m/e = 299.1309 which was within accepted limits of the calculated mass at m/e = 299.1305 for $C_{21}H_{17}NO$.

A slightly less polar component eluted during column chromatography was the only detectable side product of the reaction. Attempts to separate it completely from the carbazole aldehyde 219 failed, however its N.M.R. spectrum gave every indication that it corresponded to 1-formyl-9- benzyl-tetrahydrocarbazole (226). Thus present in the N.M.R. spectrum was a singlet at 5.13 for the benzyl methylene and two multiplets at δ 2.60 and 1.80 for the methylenes of the tetrahydro ring. The aldehyde proton absorption occurred at δ 9.83 slightly up field from the aldehyde absorption in 219.



(226)

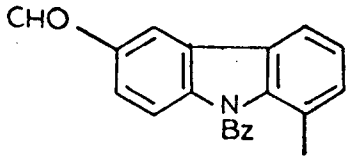
The presence of the formyl tetrahydro compound 226 was of considerable significance for although little is presently known about the mechanism of the alkylation-formylation-dehydration process, it has been shown[208] that Vilsmeier-Haack formylation of 6-chloro-9-methyltetrahydrocarbazole (227) under mild conditions produces as the reaction product 1-formyl-6-chloro-9-methyltetrahydrocarbazole (228) and that subsequent further reaction of this compound 228 at elevated temperature resulted in conversion to the 1-methyl-3-formyl carbazole 229.



(226) (227) (228)

It is apparent from this observation that in the present reaction of 9-benzyltetrahydrocarbazole (217) that the C-1 methyl of the product aldehyde 219 is also derived via an initial C-1 formylation of 217.

On mechanistic grounds it was anticipated that 1-methyl-6-formyl-9-benzylcarbazole (230) would also be produced during the formylation reaction. However a detailed examination of the aromatic region of the N.M.R. spectra of respective column fractions gave no indication of its presence.



(230)

In light of these results a plausible mechanism can be put forward which suggests that both alkylation and formylation takes place before aromatization of the tetrahydro ring (Figure 56). If aromatization occurred first, then a mixture of the 3- and 6-formyl carbazole compounds would have been observed.

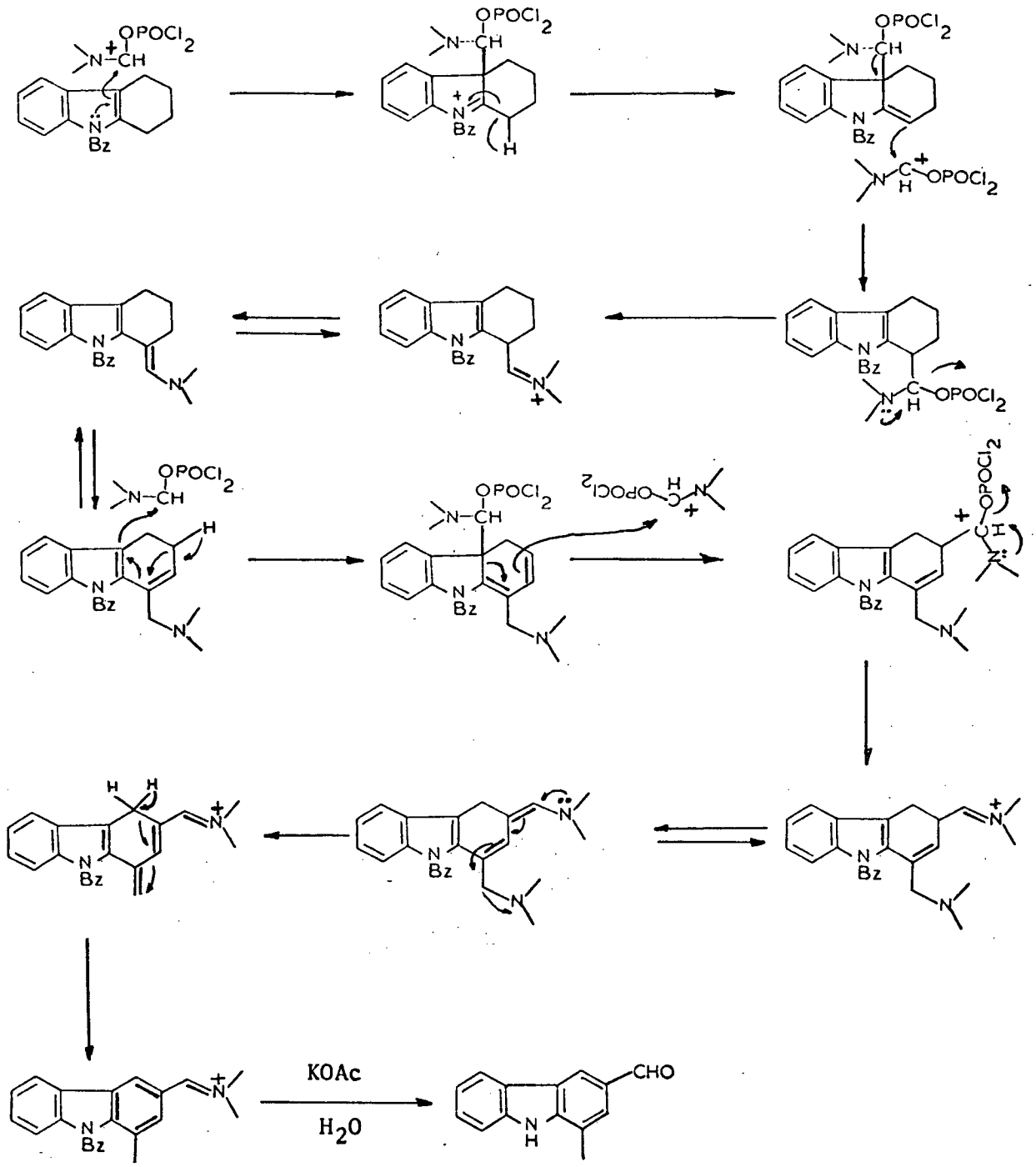


Figure 56. Proposed Mechanism for the Vilsmeier Haack Formylation of 9-Benzyltetrahydrocarbazole (216).

1-Methyl-3-(α-hydroxyethyl) carbazole (222)

To explore the route whereby the methyl group in the potential C-1 position of olivacine (16) is introduced before reaction with aminoacetaldehyde diethylacetal (171) the 3-formyl group of 219 was methylated with methylmagnesium chloride in tetrahydrofuran. The reaction proceeded to completion (TLC) within minutes at room temperature to yield the alcohol 222 as the predominant product. Purification by column chromatography provided a 64% yield of the desired product.

The methylation reaction was also accomplished using methyllithium instead of the Grignard reagent, however it offered no advantages in terms of reaction time, yields, or ease of handling so it was not employed routinely.

Minor impurities were removed from the crude reaction mixture by rapid chromatography on alumina. The alcohol 222 was obtained as a colourless foam which subsequently proved to be extremely difficult to recrystallize. It was eventually necessary to convert the alcohol to its corresponding acetate in order to obtain a satisfactory microanalysis.

The N.M.R. spectrum of this product exhibited a doublet at δ 1.55 (J = 6Hz) for the benzylic methyl group and a corresponding quartet at δ 5.03 (J = 6Hz) for the methine proton which is both benzylic and adjacent to the oxygen of the hydroxyl group. Three singlets were also evident at δ 1.83 (O<u>H</u>), 2.60 (C-1<u>CH_3</u>), and 5.70 (N-<u>CH_2</u>-C_6H_5) respectively. The mass spectrum possessed a parent peak at m/e = 315 (100%) and only two major fragments at m/e = 300 for (M^+-CH_3) and m/e = 297 for (M^+-H_2O). The high resolution mass spectrum parent

peak at 315.1632 was within acceptable limits of the calculated mass of 315.1623 for $C_{22}H_{21}NO$.

The absence of a carbonyl absorption at 1670 cm^{-1} in the IR. spectrum was consistent with the proposed structure as was the UV. spectrum which exhibited a typical carbazole absorption.

1-Methyl-3-(α-acetoxyethyl) carbazole (223)

The acetate derivative 223 was prepared by reaction of the alcohol 222 with acetic anhydride in pyridine. It was obtained as a colourless crystalline solid in high yield after careful recrystallization from methanol. The acetate group proved to be somewhat labile however, as an overexposure to hot methanol resulted in a substantial conversion of the acetate to the methyl ether by solvent displacement of the acetoxy group. It was also observed (N.M.R.) that small amounts of olefinic products accompanied formation and subsequent heating of the carbazole acetate (section 6, part II).

The spectral data was very characteristic for the acetate derivative 223. The N.M.R. spectrum showed a characteristic downfield shift in the position of the benzylic methine quarter to δ 6.00 (J = 7Hz) due to the added deshielding influence of the carbonyl group. The corresponding benzylic methyl group removed from the influence of the acetate function absorbed at δ 1.56 (J = 7Hz). Singlets were observed at δ 1.98 and 2.52 for the protons of the acetate methyl and the methyl group at C-1 respectively. A singlet was also observed at δ 5.68 for the methylene of the benzyl group substituted at the indole nitrogen. The change in the election density of the "C" ring in the conversion of the

conjugated aldehyde 219 to the non conjugated acetate 223 reflected itself in the position of the absorptions for the C-2 and C-4 hydrogens of the "C" ring. Both of these signals shifted upfield, the C-4 proton signal to δ7.98 while that of the C-2 hydrogen had shifted to the region occupied by the large aromatic multiplet (centered at δ7.10).

The mass spectrum possessed a strong parent peak at m/e = 357 (100%) and a major fragment at m/e = 298 for loss of the acetate group. This fragmentation was accompanied by the presence of a metastable at m/e = 249 corresponding to m/e = 357 $\rightarrow$ m/e = 298. The high resolution spectrum parent peak at 357.1676 was within accepted limits of the value 357.1729 calculated for $C_{24}H_{23}NO_2$.

The UV spectrum portrayed a typical carbazole absorption as anticipated and the IR spectrum has a carbonyl absorption for the acetate group at 1720 cm^{-1}.

1-Methyl-3-(α-(N-2',2'-diethoxyethylamino)ethyl)carbazole (224) from acetate 223

In an attempt to synthesize the aminoethyl acetal 224 from the carbazole acetate 223 it was determined that high temperature and prolonged heating times were necessary in order to induce displacement of the acetate function with the amino group of aminoacetaldehyde diethylacetal (171). Even after fifteen hours at 160° considerable starting material was present in the reaction mixture which by this time had turned very dark.

By column chromatography on alumina a small quantity of material was

isolated whose spectral properties corresponded with the anticipated aminoethyl acetal 224 (see conversion (221) ⟶ (224) for a description of spectral data).

It was concluded in view of the drastic reaction conditions that were necessary to provide a small percent conversion to product that the acetate functionality was not a sufficiently good leaving group for this purpose. However, instead of exploring the properties of the corresponding bromide as a leaving group, it was decided to study the methylation of the iminomethyl acetal 221 as a means of obtaining the same aminoethyl acetal 224.

1-Methyl-3-(β,β-diethoxyethyliminomethyl)carbazole (221)

The aldehyde condensation reaction had already been developed in the synthesis of ellipticine (17)[175] and its application to the 3-formyl carbazole intermediate 219 lacking one methyl group at the C-4 position presented no difficulties. Reaction of the aldehyde 219 with an equimolar quantity of aminoacetaldehyde diethylacetal (171) with the azeotropic removal of the water formed gave the iminomethyl acetal in 85% yield.

The product was purified by recrystallization from a petroleum ether-benzene combination. An attempt to purify the product by column chromatography on alumina resulted in hydrolysis on the column of the imine bond to give back the starting aldehyde.

The N.M.R. spectrum (Figure 57) of the crystalline product 221 showed a triplet at δ 4.81 (J = 5Hz) for the hydrogen of the acetal carbon ($C\underline{H}(OEt)_2$), a second triplet at δ 1.14 (J = 7Hz) for the two methyls of the diethylacetal and a complex multiplet centered at δ 3.70 for the methylenes of the acetal and for

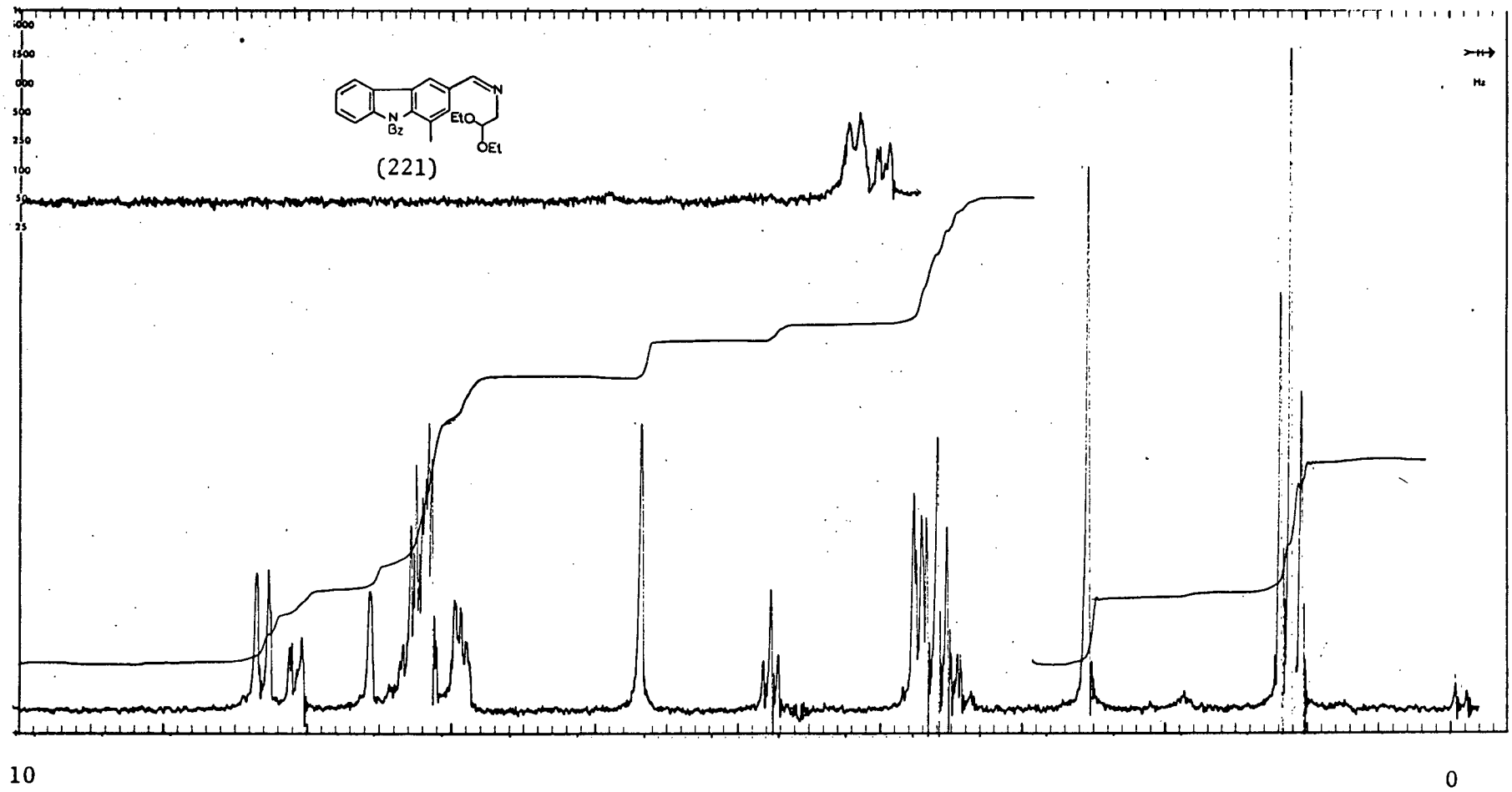


Figure 57. N.M.R. spectrum of 1-Methyl-3-(β,β-diethoxyethylaminomethyl) carbazole (221).

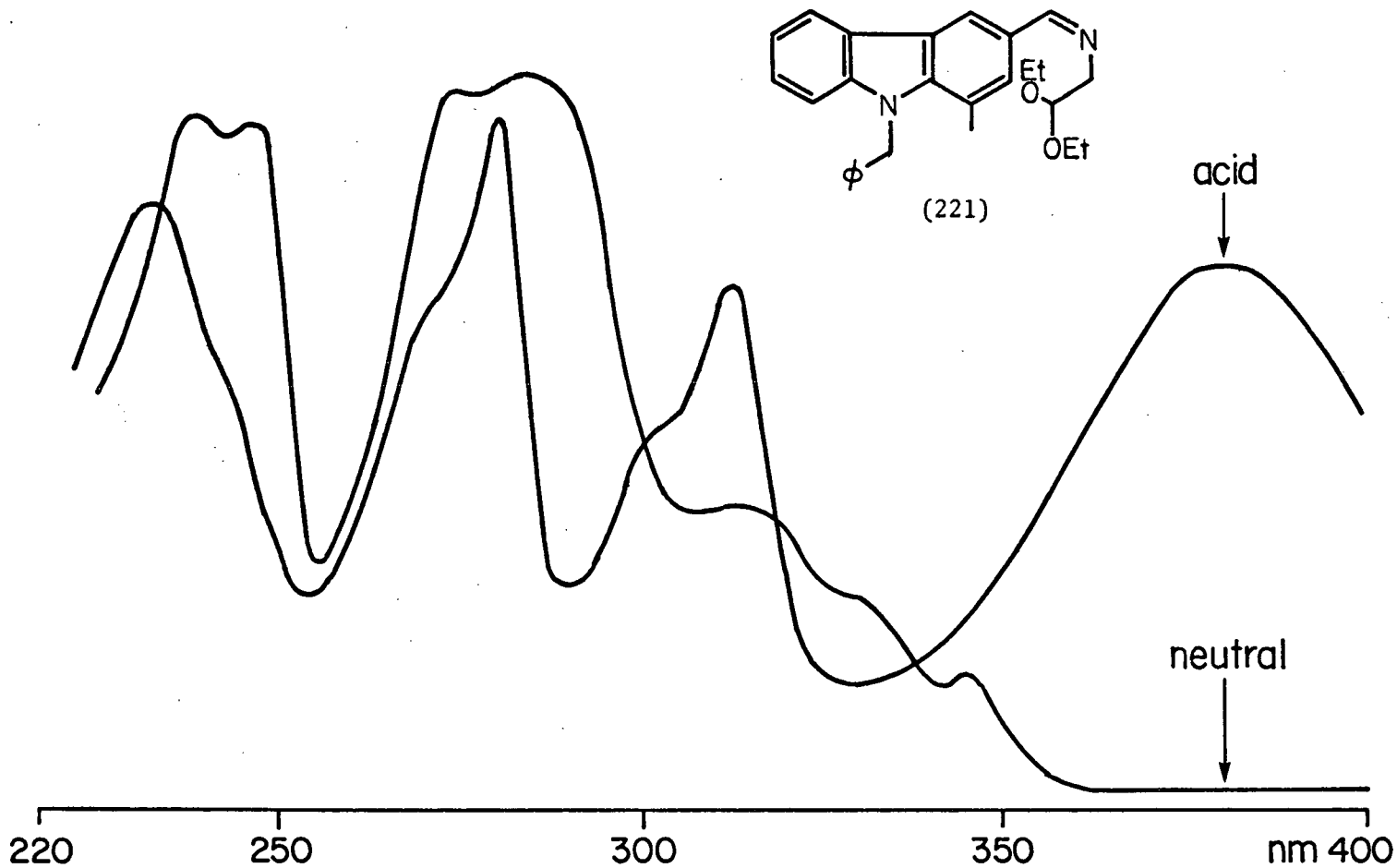


Figure 58. UV. Spectrum of Compound 221 in Water and in Dilute Hydrochloric Acid.

the methylene adjacent to the imine nitrogen. Two singlets were also present at δ5.72 and 2.58 for the benzyl methylene and the C-1 methyl group respectively. A distorted singlet at δ8.32 was attributed to the proton on the imine carbon while the singlets at δ8.40 and 7.60 having moved downfield with respect to the acetate 223 were assigned to the C-4 and C-2 hydrogens respectively.

The mass spectrum exhibited a parent peak at m/e = 414 and important fragmentations at m/e = 369, 339, 311, 298, 284. Metastable peaks were observed for the major fragmentation processes, m/e = 414 → m/e = 369 and m/e = 311 → m/e = 284. The high resolution mass spectrum corroborated the parent peak at 414.2250 and the molecular compositions for the postulated fragments.

The presence of the conjugated imine system was detected by an absorption at 1690 cm^{-1} in the IR. spectrum. The ultraviolet spectrum of 221 run in water and dilute hydrochloric acid showed the expected bathochromic shift accompanying the transformation $C = N \rightarrow C = NH^+$.

1-Methyl-3-(α-(N-β,β-diethoxyethylamino)ethyl)carbazole (224).

Methylation of the imine 221 as with the formyl compound 219 could be effected through reaction with methylmagnesium chloride in tetrahydrofuran. The less reactive nature of the imine system however, necessitated reaction at reflux temperature for twenty-four hours. Even after prolonged heating some starting material remained. Three major products were observed on TLC with the amino acetal 224 being the most polar. The three components were efficiently separated from each other by column chromatography on alumina.

The least polar component (bright orange spot on TLC) eluted with benzene remains uncharacterized. The N.M.R. spectrum possessed a quartet (δ3.93, J = 6 Hz) and doublet (δ1.36, J = 6 Hz) for the C-1' methine and C-1' methyl group which indicated that the molecule was a 3-(α-substituted ethyl)carbazole derivative. The nature of the substituted group could not be discerned from the N.M.R. or the IR spectrum. The mass spectrum possessed a parent peak at m/e = 368 with a major fragment at m/e = 299 for the loss of the unknown substituent.

It was not known which functionality was substituted onto the C-1' position of the unknown carbazole component with a mass of 69 and no characteristic absorptions in the N.M.R.

The second component isolated by elution with benzene corresponded to the aldehyde 219. The isolation of this compound resulted from the presence of unreacted starting material in the crude reaction mixture which hydrolyzed during the column chromatography.

The desired amino-acetal 224 was isolated in 20% yield by elution with benzene:chloroform mixtures and chloroform. The N.M.R. spectrum for 224 possessed a multiplet at δ8.14 for the C=5H and a singlet at δ7.98 for the C-4 H. The singlet for the C-2 H had moved upfield into the region of the aromatic multiplet compared to the position of the C-2 H in the imino acetal 221. A quartet was present at δ3.95 (J = 6 Hz) for the methine hydrogen on the alkylated C-1' carbon.

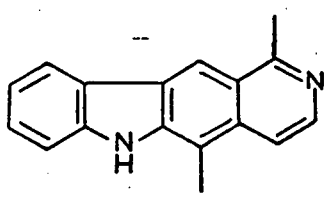
The doublet for the corresponding C-1' methyl group occurred at δ1.47 (J = 6 Hz). The doublet (δ2.69, J = 6 Hz) for the methylene group of the diethoxyethylamino group in 224 had shifted upfield from its position under the multiplet for the methylenes of the O-ethyl groups in compound 221. The signals for the

two methyls of the O-ethyl groups were very complex compared to the triplet for these groups in the imino acetal 221.

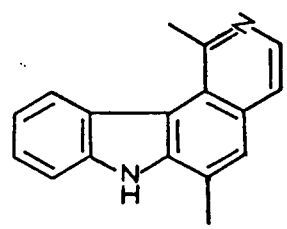
The mass spectrum possessed a parent peak at m/e = 430 with major fragmentation peaks at m/e = 415, 369, 298, 288. The high resolution mass spectrum possessed a parent peak at m/e = 430.2633 which was within acceptable limits of the value m/e = 430.2620 calculated for $C_{28}H_{34}N_2O_2$.

No attempt was made to improve the reaction yield either by changing the solvent, or the Grignard reagent or by conducting the reaction for a longer period of time.

The cyclization of the amino acetal 224 to 6-benzoolivacine (225) was not attempted at this time. It was known however that o-phosphoric acid effected the analogous cyclization in the synthesis of ellipticine (30% yield). It would be anticipated therefore that the cyclization reaction would also work for the olivacine system (16). There is the possibility however, which does not exist for ellipticine, that cyclization can occur in either of two directions to give compounds 225 or 231. Whether or not this will be a problem remains to be determined. The subsequent debenzylation of 225 to olivacine (16) would be a trivial step.



(16)



(231)

This synthesis has the potential of being a short and simple route to olivacine (16) which could readily be adapted to a large scale preparation of this material for future biosynthetic work. By minor modifications it would be possible to obtain quantities of guatambuine (25) also.

EXPERIMENTAL - PART III

For a description of the general experimental information, see Experimental part 1.

All T.L.C. plates were developed in chloroform ($CHCl_3$) unless otherwise indicated, and the alumina for column chromatography was deactivated to Activity III by the addition of water (6%).

Sequence A

Indole -3-glyoxylic acid chloride (211)

To a stirred solution of Indole (25 gm., 2.14×10^{-1} mole) in anhydrous ether (400 ml) at 0° was added an ether solution of oxalyl chloride (210) (31.2 ml, 3.64×10^{1} mole) over a 0.5 hr period. A bright yellow precipitate was produced almost immediately, however the reaction was allowed to continue for 0.5 hr after addition was completed. The product was collected by vacuum filtration, and washed liberally with dry ether to give the acid chloride as yellow crystals (41 gm. 93%), m.p.. 123-126° Found: C,58.13; H,3.10,N,6.38%. Calc. for $C_{10}H_6O_2NCl$: C,58.40; H,2.92; N,6.81%

Methyl indole-3-glyoxylate (212)

To the acid chloride 211 (39gm.,1.88×10^{-1} mole) dissolved in dry tetrahydrofuran (500 ml) and stirred at room temperature was added a large excess of dry methanol. A beigh precipitate formed almost immediately, however the reaction

was allowed to stir for 0.5 hr. The precipitate was collected by suction filtration (29 gm.) and washed liberally with tetrahydrofuran. The mother liquors were concentrated and the bright pink solid obtained (6 gm.) was washed also with tetrahydrofuran. The solid products were combined and recrystallized from methanol-tetrahydrofuran to give the ester 212 as beige coloured crystals (35 gm., 92% yield), m.p. 227-8°. Found: C,64.76; p H,4.44; N,6.62%. Calc. for $C_{11}H_9NO_3$: C,65.02; H,4.46; N.6.89%.

Indole-3-ethanol (Tryptophol) (206)[190]

The glyoxylate ester 212 (20.7 gm., 1.0 x 10^{-1} moles) was dissolved in dry tetrahydrofuran (600 ml) under gentle reflux and under a nitrogen atmosphere (mechanical stirrer). Lithium aluminum hydride (7.6 gm., 2.0 x 10^{-1} moles) was added to the refluxing solution in small portions either as a powder or as a slurry in tetrahydrofuran. After addition was completed, a further quantity of tetrahydrofuran (200 ml) was added, final volume (800 ml). The reaction mixture was refluxed gently for 15 hr. after which time the reaction vessel was cooled in ice and the excess lithium aluminum hydride destroyed by the sequential addition of water (8 ml), 15% sodium hydroxide (8 ml), and water (24 ml). The resultant granular precipitate was removed by suction filtration and washed twice with tetrahydrofuran (2 x 200 ml). The combined tetrahydrofuran solutions were concentrated to 50 ml and taken up in water (600 ml). The aqueous mixture was extracted repeatedly with ether (200 ml portions). The ether fractions were combined, washed with water, dried over

sodium sulphate, and concentrated to dryness (benzene azeotrope) to give a colourless crystalline product (9.6 gm., 60%). The crude product was purified by column chromatography on alumina (100 gm.). The product was eluted with chloroform to give colourless crystals, m.p. = ^{o} (lit ^{o}). N.M.R. (60 MHz): 3.86 (triplet, J = 6Hz,2H,C-2, CH_2), 2.96 (triplet, J = 6Hz2H,C-1, CH_2), 1.56 (Singlet, 1H, OH)

Indole-3-(2-ethylbromide) (Tryptophyl Bromide) (207)[192]

A solution of phosphorous tribromide (2ml,2.07 x 10^{-2} mole) in ether (25 ml.) was added dropwise over 0.5 hr. to a solution of tryptophol (206) (10 gm. 6.22 x 10^{-2} mole) in ether (200 ml.) at 0°. The reaction mixture was stirred at 0°C for 15 hr. after which time the supernatant was decanted, the red syrupy residue was washed with several portions of ether (100 ml), and the combined ether extracts were washed with saturated sodium bicarbonate and water. The ether layer was dried over sodium sulphate and concentrated (benzene azeotrope) to give a colourless crude crystalline product (12.5 gm., 91%). The crude product was purified by either recrystallization from benzene:hexane or column chromatography on alumina (200 gm.), eluting with benzene. The bromide was obtained as colourless crystals, m.p. = ^{o} (lit. ^{o}). N.M.R. (60 MHz): 3.40 (multiplet,4H,C-1 and $2CH_2$). Mass spectrum: $M,^{+}m/e$ = 223,225.

3-Carbomethoxy-5-(3-indolyl)-2-pentanone (204)

Methyl acetoacetate (205) (12.66 ml, 1.18 x 10^{-1} mole) was added in four portions to a suspension of sodium hydride (2.88 gm., 1.20 x 10^{-1} mole)

(prepared by washing a 57% oil dispersion (5.05 gm.) with benzene) in dimethylformamide (500 ml). The mixture was stirred at room temperature until gas evolution had ceased and a clear solution was obtained (about 1 hr.). Tryptophyl bromide (207) (12.3 gm., 5.5 x 10^{-2} moles) was then added in one portion and the reaction was stirred at 100°C for 3.5 hours under a nitrogen atmosphere. A white precipitate was formed during the course of the reaction. The cooled reaction mixture was diluted with water (700 ml) and extracted repeatedly with ether. The combined ether extracts were washed with water, dried over sodium sulfate and concentrated to give an amber coloured viscous oil (11.5 gm. 80%). The crude product was either cyclized directly or purified by column chromatography on Alumina (500 gm.). Elution of the column with benzene removed all contaminants and subsequent elution with benzene:chloroform 1:1 and finally with chloroform yield microanalytically pure 204 as a yellow oil (7.90 gm., 50% from tryptophyl bromide (207)), b.p. = 135±10° at 0.05 m.m. From column chromatography on a small scale (1-2 gm) typical yields were 70-75%. IR. ($CHCl_3$): 3500, 1750, 1725. UV. λ_{max}(log $\bar{\varepsilon}$): 290 (4.30), 280(4.37), 272(4,34). N.M.R. (see Figure 51): 8.04 (broad singlet, 1H, N-H), 7.6-7.0 (2 multiplets, 4H, aromatic), 6.94 (Spike, 1H, C-2H), 3.66 (Singlet, 3H, $COOCH_3$), 3.48 (triplet, J = 7Hz, 1H, C-3 CH), 2.76 (triplet, J = 7Hz, 2H, C-5 CH_2), 2.28 (multiplet, 2H, C-4 CH_2), 2.12 (Singlet, 3H, $COCH_3$). Mass spectrum: M^+, m/e = 259; main peaks: 228, 186, 184, 144,143 (base peak), 130. High resolution mass spectrum: calc. for $C_{15}H_{17}O_3N$: 259.1207. Found: 259.1212. Found: C, 69.36; H,6.52; N,5.20%. Calc for

$C_{15}H_{17}O_3N$: C, 69.48; H,6.61; N, 5.40%

Cyclization Reaction to (134) and (209)

A To a solution of the alkylation product 204 (7.90 gm., 3.05 x 10^{-2} mole) in methanol (100 ml.) at room temperature was added an equal volume of methanol - HCL (5%), and the reaction mixture was allowed to stir for one minute (final HCl concentration 2.5%). On mixing, the reaction was exothermic and accompanied by a change to a darker colour. The solvent was then removed to give a dark foam (7.15 gm., 98%) consisting of an equal mixture of 1-methyl-2-carbomethoxy-carbazole (134) and 1-methyl-2-carbomethoxy-1,2,3,4-tetrahydrocarbazole (209). IR. ($CHCl_3$): 3480, 1740-1680. UV; λ_{max}: 340, 303, 295 (shoulder), 248. N.M.R. (see Figure 53): 8.44 (broad singlet, 1H, N-H), 8.0-7.0 (2 multiplets, 11H, aromatic protons), 3.84 (singlet, 3H, $COOCH_3$), 3.68 (singlet, 3H, $COOCH_3$), 2.67 (Singlet, 3H, CH_3), 1.00, 1.15 (2 doublets, J = 7Hz, 3H, CH_3). Mass spectrum: M^{+}, m/e = 243, 239 (base peak); main peaks: m/e = 228, 224, 212, 208, 184, 180, 169, 168, 157, 143, 130. High resolution mass spectrum: calc. for $C_{15}H_{17}O_2N$: 243.1258, and for $C_{15}H_{13}O_2N$: 239.0946. Found: 243.1248 and 239.0933.

B The identical procedure was followed for the cyclization of impure alkylation material. In this manner the alkylation product (11.5 gm., 4.4 x 10^{-2} mole) was reacted with methanol-HCl (2.5%) to give an equal mixture of compounds 134 and 209 (10.12 gm., 88%). The spectral data were identical to those quoted in part A.

Separation of Compounds 134 and 209

Recrystallization of the crude mixture of compounds 134 and 209 (7.15 gm.)

from concentrated chloroform solution afforded pure compound 134 as colourless crystals. Column chromatography of a small portion of the mixture (500 mg.) on alumina (20 gm.,) using benzene gave impure compound 209 in the first fraction and pure compound 134 in subsequent fractions (about 200 mgs., 40%). The latter component was recrystallized from chloroform to give colourless crystals of 1-methyl-2-carbomethoxycarbazole (134) with m.p. 138-140°, (lit., m.p. 126-127° for ethyl ester). IR. (nujol): 3425, 1710. UV. λ_{max} (log ε): 340(3.62), 303(4.38), 246(4.69). N.M.R. (F.T.): 3.84 (Singlet, $COOCH_3$), 2.70 (Singlet, CH_3). Mass spectrum: M^+, m/e = 239 (base peak); main peaks: m/e = 224, 208, 180. High resolution mass spectrum: calc. for $C_{15}H_{13}O_2N$: 239.0946. Found: 239.0948. Found: C,75.15; H,5.38; N,5.77%. Calc. for $C_{15}H_{13}O_2N$: C,75.30; H,5.48; N,5.85%.

By preparative layer chromatography using alumina plates (1 m.m.) (Benzene; chloroform 1:1) a small quantity of 1-methyl-2-carbomethoxy-1,2,3,4-tetrahydro-carbazole (209) was isolated as an oil in about 90% purity. UV.; λ_{max}: 290, 282, 275, N.M.R. (F.T.): 3.96 multiplet, C-1 CH), 3.68 (Singlet, $COOCH_3$), 3.38 (multiplet, C-1 CH). Mass spectrum: M^+, m/e = 243; main peaks: 228, 212, 184 (base peak), 169, 168, 157, 144, 143, 130.

1-Methyl-2-carbomethoxycarbazole (134)

The cyclization mixture of compounds 134 and 209 (7.15 gm., 2.98 x 10^{-2} mole) was dissolved in m-xylene (300 ml.) and chloranil (7.15 gm., 2.90 x 10^{-2} mole) was added. The resultant dark solution was refluxed for 24 hr. after which time it was diluted with water (300 ml) and washed repeatedly with 10% sodium hydroxide solution to remove dark coloured contaminants. The

organic layer was then washed with water (3 x 200 ml.), dried over sodium sulphate and concentrated to give a dark crystalline produce (6.5 gm.). Recrystallization from chloroform gave grey coloured crystals of ester (2.6 gm.). Column chromatography of the mother liquors on alumina (200 gm.) using benzene yielded the ester as a yellow oil which crystallized to colourless crystals on standing (2.7 gm.), m.p. 138-140°. An overall yield of (5.3 gm., 81%) was obtained. IR. (nujol): 3425, 1710. UV.; λ_{max} (log ε): 340 (3.62), 303 (4.38), 246 (4.69). N.M.R. (F.T.): 3.84 (Singlet, $COOCH_3$), 2.70 (Singlet, CH_3). Mass spectrum: M^+, m/e = 239 (base peak); main peaks: m/e = 224, 208, 180. Found: C, 75.53: H, 5.65; N, 5.48%, Calc. for $C_{15}H_{13}O_2N$: C, 75.30; H, 5.48; N, 5.85%.

Cyclization - Dehydrogenation of 204 to 1-Methyl-2-carbomethoxycarbazole (134).

A solution of methanolic-HCl (2.0%) (10 ml.) was added dropwise over several minutes to a stirred solution of the alkylation product 204 (1.0 gm., 3.86 x 10^{-3} mole) and chloranil (1.5 equiv.) in benzene (15 ml.). The reaction mixture was stirred at room temperature for an additional 5 min. after which time it was concentrated to dryness redissolved in chloroform (50 ml.) and washed successively with 10% aqueous sodium hydroxide (5 x 30 ml.) and water (3 x 30 ml.). The chloroform layer was dried over sodium sulphate and concentrated to give light brown

crystals. Recrystallization from concentrated chloroform solution yielded 204 as colourless crystals (774 mg., 84%), m.p. 138-140°. The spectral data obtained for 134 obtained from 204 was identical to that previously described.

1-Methyl-2-formylcarbazole (152)

Jones reagent[203] (5M) was titrated into a solution of the carbazole alcohol 157 (1.35 gm., 6.4 x 10^{-3} mole) dissolved in acetone at room temperature until a presistently red coloured reaction mixture was obtained (about 1.5 equiv.). The reaction was stirred for an additional five minutes after which time methanol was added until a green colouration was obtained. The reaction mixture was then suction filtered and the residue washed with copious amounts of methanol. The filtrate was concentrated, taken up in water and exhaustively extracted with ether. The ether extracts were dried over sodium sulphate, concentrated and applied to an alumina column (10 gm.). The product was eluted with chloroform to yield yellow crystalline needles (1.00 gm., 70%). Recrystallization from chloroform afforded an analytical sample, m.p. 165-166° (lit., m.p. 164-164.5).[8] IR. (nujol): 1665. UV.; λmax (log ε): 365 (3.52), 317 (4.42), 255 (4.63). N.M.R. (FT.): 10.39 (singlet, CHO), 2.87 (singlet, CH_3). Mass spectrum: M^+, m/e = 209 (base peak); main peaks: 180, 152. High resolution spectrum: Calc. for $C_{14}H_{11}NO$: 209.0840. Found: 209.0831. Found: C, 80.08; H, 5.30; N, 6.39%. Calc. for $C_{14}H_{11}NO$: C, 80.36; H, 5.30; N, 6.69%.

1-Methyl-2-hydroxymethylcarbazole (157)

A The Carbazole ester 134 (6.6 gm., 2.77 x 10^{-2} mole) dissolved in anhydrous ether (100 ml.) was added dropwise over a period of 0.5 hr. to a slurry of lithium aluminum hydride (2 gm., 5 x 10^{-2} mole) in anhydrous ether (250 ml.) at room temperature. Large aggregate lumps of lithium aluminum hydride formed during the addition. The reaction was stirred for an additional 0.5 hr. after which time excess hydride reagent was destroyed by the addition of water (2 ml.), 15% sodium hydroxide (2 ml.) and finally water (6 ml.). The resultant granular precipitate was removed by suction filtration and washed liberally with ether. The combined ether fraction was washed with water (3 x 100 ml.), dried over sodium sulphate, and concentrated to give a colourless crystalline solid (5.26 gm., 90%). Recrystallization from chloroform gave an analytical sample of the alcohol 157 with m.p. 184 - 186° (lit., m.p. 187 - 188°). IR. (nujol): 3420, 3400-3325. UV.; λmax (log ε): 335 (3.63), 324 (3.68), 294 (4.36), 285 (4.16), 257 (4.33), 247 (4.56), 238 (4.73). N.M.R. (FT.): 4.88 (singlet, Ar-$\underline{CH_2}$OH), 2.58 (singlet, CH_3), 1.50 (singlet, OH). Mass spectrum: M^+, m/e = 211 (base peak); main peaks: 194, 180, 167. High resolution mass spectrum: Calc. for $C_{14}H_{13}NO$; 211.0996. Found: 211.0982. Found: C, 79.63; H, 6.49; N, 6.37%. Calc. for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63%.

B Impure carbazole ester 134 (9.3 gm., 3.89 x 10^{-2} mole) was reduced to the alcohol 157 in an identical manner. The dark crystalline material (7.2 gm.)

that was obtained was purified by column chromatography on alumina (350 gm.). Elution with benzene:chloroform 1:1 followed by chloroform:methanol 5% yielded the alcohol 157 as colourless crystals (4.9 gm., 60%).

1-Methyl-2-(ω-nitrovinyl)carbazole (160)[209]

The aldehyde 152 (2.50 gm., 1.19 x 10^{-2} mole) was taken up in nitromethane (20 ml.), ammonium acetate (0.50 gm.) was added and the mixture was heated at 100°C for 0.5 hr. During the course of heating the starting material first dissolved then an orange precipitate of product precipitated out. The cooled reaction mixture was suction filtered and the product obtained was washed with cold ethanol and dried to give orange needles (2.62 gm.). Concentration of the mother liquors, dissolution in water and repeated extraction with chloroform yielded further quantities of orange crystalline product (0.230 gm., overall yield 95%). Recrystallization from ethanol (250 ml/gm.) gave long crimson needles, m.p. 242-244° (lit., m.p. 244-247°). IR. (nujol): 3340, 1360. UV.; λmax (log ε): 375 (4.39), 292 (3.87), 248 (4.50), 237 (4.46). N.M.R. (FT.): 8.51 (doublet, J = 14 Hz, -C<u>H</u>=CH-NO_2), 7.59 (doublet, J = 14 Hz, -CH=C<u>H</u>-NO_2), 2.64 (singlet, CH_3). Mass spectrum: M^+, m/e = 252; main peaks: 220, 205 (base peak), 180. High resolution spectrum: Calc. for $C_{15}H_{12}N_2O_2$: 252.0938. Found: 252.0905. Found: C, 71.70; H, 4.97; N, 10.82%. Calc. for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10%.

1-Methyl-2-(β-aminoethyl)carbazole (139)

This compound was prepared according to the procedure developed by Moster *et al.*[177]

A solution of the nitrostyrene 160 (2.85 gm., 1.13 x 10^{-2} mole) in tetrahydrofuran (50 ml.) was added dropwise over 0.5 hr. to a stirred suspension of lithium aluminum hydride (2 gm.) in tetrahydrofuran (250 ml.) at room temperature. Stirring was continued for an additional 20 min., and the hydride was then decomposed by the successive addition of water (2 ml.), 10% aqueous sodium hydroxide (6 ml.), water (2 ml.). The resultant granular precipitate was filtered and washed with tetrahydrofuran (200 ml.). The combined filtrates were concentrated to a paste, taken up in ether (75 ml.) and washed with water (3 x 30 ml.). The ether layer was dried over sodium sulphate and concentrated to give 139 as a slightly yellowed crystalline solid (2.52 gm., 95%). UV., λ_{max}: 335,320,293,284(sh) 257,245,236. N.M.R. (for HCl salt): 4.76 (singlet, 2H, NHz), 2.40 (singlet, 3H, C-1 CH_3). Mass spectrum: M^+, m/e = 224.

1-Methyl-2-(β-(N-acetylamino)ethyl)carbazole (140)

This compound was prepared following the procedure developed by Schmutz and Wittwer.[172]

The amine 139 (2.52 gm., 1.12 x 10^{-2} mole) was dissolved in a mixture of pyridine (30 ml.) and acetic anhydride (3 ml.) and stirred at 60° for 0.5 hr. The solvent was removed under vacuum and the residue was dissolved in ether (75 ml.) and washed with dilute hydrochloric acid (3 x 30 ml.) and water (3 x 30 ml.). The ether layer was then dried over sodium sulphate and concentrated to give 140 as a colourless crystalline solid (2.93 gm., 95%). UV.; λ_{max}: 335, 320, 294, 283 (sh), 257, 245, 238. IR. ($CHCl_3$): 1660 cm^{-1}. N.M.R: 5.56 (broad hump, 1 H, N<u>H</u>$COCH_3$); 3.46 (triplet, 2 H, J = 6 Hz, Ar<u>CH_2</u>CH N), 3.00 (multiplet, 2 H, Ar<u>CH_2</u>CH_2N), 2.46 (singlet, 3 H, C-1 CH_3), 1.93 (singlet, 3 H, CO<u>CH_3</u>). mass spectrum: M^+, m/e = 266; main peaks: 223, 220, 207 (base peak), 193, 180, 167.

3,4-Dihydro-1,5-dimethyl-6H-pyrido-(4,3-b)carbazole (141).

Phosphorous oxychloride (9 ml.) was added to a solution of the amide 140 (1.45gm., 5.45 x 10^{-3} mole) in hot toluene (250 ml.). The reaction mixture was refluxed for 1 hr. after which time the solvent was removed to give a green crystalline solid which was extracted with hot dilute hydrochloric acid (3 x 250 ml.) Insoluble residues were removed by suction filtration. The combined acid fractions were basified to pH 11 with concentrated ammonium hydroxide and extracted with chloroform (4 x 300 ml.). The combined chloroform extracts were dried over

sodium sulphate and concentrated to give brown crystalline material. The crude product was recrystallized from chloroform or chloroform:methanol mixtures to give 3,4-dihydroolivacine (141) as colourless crystals (0.85 gm., 63%), m.p. 310° (lit., m.p. 300-315°). UV. (H_2O & aq HCl); λ_{max} log (ε): 370 (3.93), 340 (3.71), 324 (3.84), 310 (4.15), 290 (sh) (4.35), 280 (4.56), 273 (4.54), 245 (4.36), 234 (4.44). UV. (aq. NaOH); λ_{max}: 283, 272, 245, 236. N.M.R.: 3.76 (triplet, 2 H, J = 6Hz, C-4 $\underline{CH_2}$), 2.90 (triplet, 2 H, C-3 $\underline{CH_2}$), 2.60 (distorted singlet, 3 H, C-1 CH_3), 2.50 (singlet, 3 H, C-5 CH_3). Mass spectrum: M^+, m/e = 248 (base peak); main peaks: 233, 220-217, 204, 191. Found: C, 82.14; H, 6.55; N, 10.99. Calc. for $C_{17}H_{16}N_2$: C, 82.22; H, 6.49; N, 11.28.

The imine 141 was converted to its methiodide 142 in quantitative yield by reaction with methyl iodide in methanol:chloroform solution at room temperature for 4-5 hr. The pale orange methiodide 142 was recrystallized from methanol, m.p. > 300° (lit., m.p. 320°). UV. (H_2O) (Figure 38); λ_{max}(log ε): 365 (4.33) 308 (4.32), 300 (4.17), 279 (4.61), 265 (sh) (4.37), 225 (4.48). UV. (aq. NaOH); λ_{max}: 365, 335, 310, 294, 280, 270 (sh), 243, 237. N.M.R.: 8.86 (singlet, 1 H, C-11 H), 3.98 (multiplet, 2 H, C-4 $\underline{CH_2}$), 3.68 (singlet, 3 H, N-CH_3), 3.20 (multiplet 2 H, C-3 $\underline{CH_2}$), 2.90 (singlet, 3 H, C-1 CH_3), 2.48 (singlet, 3 H, C-5 CH_3). Found: C, 55.00; H, 4.90; N, 7.18. Calc. for $C_{18}H_{18}N_2I$: C, 55.40; H, 4.87; N, 7.06.

1,5-dimethyl-1,2,3,4-tetrahydro-6H-pyrido-4(,3-b)carbazole (25)

(Guatambuine)

A. The methiodide 142 (100 mg., 2.64 x 10^{-4} mole) was dissolved in methanol (20 ml.) and the solution was hydrogenated at room temperature and atmospheric pressure over platinum oxide (PtO_2, 20 mg.) for 2 hr. The mixture was filtered to remove the catalyst which was carefully washed with additional methanol (10 ml.). The combined filtrates were concentrated to give a pale yellow solid. The product was obtained as a colourless crystalline solid by filtration through a small alumina column ($CHCl_3$:MeOH 5%). All analytical and spectral data is presented below.

B. The methiodide 142 (250 mg., 6.17 x 10^{-4} mole) was dissolved in aqueous ethanol (100 ml.) and reacted with an excess of sodium borohydride at room temperature for 2 hr. The reaction mixture was then concentrated, taken up in water (75 ml.) and extracted with chloroform (3 x 30 ml.). The combined chloroform fractions were dried over sodium sulphate, and concentrated to give colourless crystals (160 mg., 98%). The product was recrystallized from methanol to give 25 as cream coloured cubes m.p. 248-250° (lit., 248-250°).[128] UV.; λ_{max} (log ε): 341 (3.48), 327 (3.63), 297 (4.26), 288 (sh) (4.08), 262 (4.36), 250 (4.50), 240 (4.64). N.M.R. (FT.): 3.90 (quartet, 1 H, J = 7 Hz, C-1 H), 2.40 (singlet, 3 H, C-5 CH_3), (singlet, 3 H, N-CH_3), 1.52 (doublet, 3 H, J = 7 Hz, C-1 CH_3). Mass spectrum: M^+, m/e = 264; main peaks: 249, 233, 221-218, 204. High resolution mass spectrum: Calc. for $C_{18}H_{20}N_2$: 264.1626. Found: 264.1626. Found: 264.1656. Found: C, 81.55; H, 7173; N, 10.76. Calc. for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60.

1,5-dimethyl-6H-pyrido-(4,3-b)carbazole (16) (Olivacine)

Dihydroolivacine (83) (30 mg., 1.20 x 10^{-4} mole) was suspended in decalin (4 ml.) containing 5% palladium on charcoal (50 mg.) and refluxed for 1.5 hr. The reaction mixture was cooled to 0° and the solvent was removed by suction filtration. The residue containing the olivacine (16) was taken up in hot chloroform (200 ml.) and refiltered. The washing procedure was repeated (3 x 200 ml.) and the combined chloroform filtrates were concentrated to give an orange coloured solid. The crude product mixture was recrystallized from methanol to give olivacine (16) as bright yellow needles (20 mg., 70%), m.p. 318-324° (lit., m.p. 318-326°).[126] UV.; λ_{max}(log ε): 374 (3.56), 325 (3.76), 291 (4.84), 275 (4.72), 265 (4.57),235 (4.33), 222 (4.40). N.M.R. (FT.): 3.16 (singlet, 3 H, C-1 CH_3), 2.82 (singlet, 3 H, C-1 CH_3). Mass spectrum: M^+, m/e = 246. High resolution mass spectrum: Calc. for $C_{17}H_{14}N_2$: 246.1156. Found: 246.1165. Found: C, 82.46; H, 5.94; N, 11.20. Calc. for $C_{17}H_{14}N_2$: C, 82.90; H, 5.73; N, 11.37.

Sequence B.

1,2,3,4-tetrahydrocarbazole (216)

This compound was prepared according to the procedure of Rogers and Corson.[207] Cyclohexanone (98 gm., 1.0 mole) and phenylhydrazine (108 gm., 1.0 mole) were reacted in acetic acid (360 ml.) at reflux temperature for 1 hour. The reaction mixture was then cooled to about 5° and the crystalline mass which precipitated was collected by suction filtration. The filter cake was washed with water (100 ml.), then with 75% ethanol (100 ml.) and dried under vacuum. The crude product was crystallized from methanol (700 ml.) after treatment with activated charcoal to yield colourless crystals of tetrahydrocarbazole (216) (135 gm., 79%), m.p. 113-115° (lit., m.p. 115-116°).

9-Benzyl-1,2,3,4-tetrahydrocarbazole (217)

Tetrahydrocarbazole (216) (10 gm., 5.84 x 10^{-2} mole) and benzyl bromide (7.10 ml., 5.84 x 10^{-2} mole) were dissolved in dry dimethylformamide (100 ml.) and stirred at 0°C. Sodium hydride (1.44 gm., 6.00 x 10^{-2} mole) was added in four portions over one hour. The reaction mixture was stirred for an additional hour after which time it was carefully diluted with water (400 ml.) and extracted with ether (3 x 200 ml.). The ether fractions were combined and washed with water, dried over sodium sulphate and concentrated to give a colourless mobile oil. Small amounts of starting material were removed by column chromatography on alumina (400 gm., Act III). Addition of the crude reaction mixture to the column in benzene and elution with petroleum ether yielded the desired 9-benzylated tetrahydrocarbazole 217 as a transparent oil (11.6 gm. 77%).

N.M.R.: 5.10 (Singlet, 2H, $N\underline{CH_2}C_6H_5$), 2.60 (multiplet, 4H, C-2,3$\underline{CH_2}$), 1.86

(multiplet, 4H, C-1,4CH). mass spectrum: M^+, m/e = 261.

1-Methyl-3-formyl-9-benzylcarbazole (219)

A solution of 9-benzyltetrahydrocarbazole (217) (10 gm., 3.83 x 10^{-2} mole) in dry dimethylformamide (50 ml.) with phosphorous oxychloride (4.55 ml., 1.3 equiv.) was stirred at 100°C for eight hours. Hydrolysis of the reaction mixture was then effected by addition of 30% potassium acetate solution (20 ml.) with heating for an additional 20 minute period. The cooled mixture was then diluted with water (200 ml.) and extracted with ether (5 x 100 ml.). The combined ether fractions were washed with water, dried over sodium sulphate and concentrated to give a viscous dark oil. The crude product was purified initially by column chromatography on alumina (250 gm. Act III). The crude mixture was applied to the column in benzene and the desired carbazole aldehyde 219 was eluted with petroleum ether:benzene 1:1 to give an orange glass (5.47 gm. 48%). By subsequent recrystallization form petroleum ether-benzene colourless needles were obtained, m.p. 102-106°. UV.; $_{max}$ (log): 340 (sh) (4.22), 327 (4.30), 289 (4.60), 273 (4.72), 244 (4.60), 235 (4.66). IR. ($CHCl_3$): 1670. N.M.R. (100 Mhz): 10.00 (Singlet, 1H, CHO), 8.46 (distorted singlet, 1H, C-4H), 8-10 (multiplet, 1H, C-5H), 7.66 (distorted singlet, 1H, C-2H), 7.4-7.1(multiplet, 6H, aromatic) 6.9 (multiplet, 2H, aromatic), 5.72 (Singlet, 2H, benzyl CH_2), 2.60 (Singlet, 3H, C-1CH_3). Mass spectrum: M^+, m/e = 299 (base peak). High resolution mass spectrum: calc. for $C_{21}H_{17}NO$: 299.1305. Found: 299.1309. Found: C,84.05; H,5.62; N,4.57. Calc. for $C_{21}H_{17}NO$: C,84.25; H,5.72; N,4.68.

1-Methyl-3-(α-hydroxyethyl)-9-benzylcarbazole (222)

To the carbazole aldehyde 219 (112 gm., 3.74 x 10^{-2} mole) in dry tetrahydrofuran (100 ml.) was added in one portion an excess of methylmagnesium chloride - THF solution* (20 ml., 3.16M). The reaction was stirred at room temperature for 0.5 hr. after which time the excess Grignard reagent was destroyed by the careful addition of dilute hydrochloric acid. The mixture was concentrated until all the tetrahydrofuran was removed, diluted with water (250 ml.) and extracted with ether (3 x 100 ml.). The ether fractions were combined, washed with water, dried over sodium sulphate and then concentrated to give a pale yellow oil (11 gm.). The crude reaction mixture was purified by column chromatography on alumina (200 gm., Act III). By elution with chloroform and ethyl acetate the carbazole alcohol 222 was obtained as a colourless foam (7.5 gm., 64%). UV.; λ_{max}: 342, 327, 293, 287, 283, 263, 237. N.M.R.: 8.08 (doublet, 1H, J = 2Hz, C-5H), 7.98 (multiplet, 1H, C-4H), 7.2, 6.95 (2 multiplets, 9H, aromatic), 5.66 (singlet, 2H, N-$\underline{CH_2}$-C_6H_5), 5.00 (quartet, 1H, J = 6Hz, Ar$\underline{CH}$(OH)$\underline{CH_3}$), 2.54 (Singlet, 3H, C-1CH_3), 1.97 (Singlet, 1H, OH), 1.54 (doublet, 3H, ArCH(OH)$\underline{CH_3}$). Mass spectrum: M^+, m/e = 315 (base peak); main peaks: 300, 297. High resolution mass spectrum: calc. for $C_{22}H_{21}NO$: 315.1623. Found: 315.1632.

1-Methyl-3-(α-acetoxyethyl)-9-benzylcarbazole (223)

The Carbazole alcohol 222 (7 gm., 2.22 x 10^{-2} mole) was taken up in pyridine (75 ml.) and acetic anhydride (10 ml.) and gently heated at 60°C for one hour. The solvent was then removed and the resulting residue taken up in ether (350

* Fisher Reagent.

ml.) and washed with 5% sodium hydroxide solution and with 5% hydrochloric acid respectively. The ether layer was then dried over sodium sulphate and concentrated to give a tan coloured gum. The crude reaction product was filtered through a short alumina column and recrystallized from methanol, m.p. 137-140 . UV; λ_{max} (log ε): 341 (3.75), 327 (3.71), 293 (4.30), 283 (4.04), 263 (4.55), 238 (4.79), 230 (4.77). IR.: 1720. N.M.R.: 8.05 (multiplet, 1H, C-5H), 7.98 (distorted singlet, 1H, C-4H), 7.3-6.8 (two multiplets, 9H, aromatic), 6.00 (quartet, 1H, J = 7Hz, Ar-$\underline{CH}(CH_3)OA_c$), 5.68 (Singlet, 2H, benzyl CH_2), 2.52 (Singlet, 3H, C-1$\underline{CH_3}$) 1.98 (Singlet, 3H, -CO$\underline{CH_3}$), 1.56 (doublet, 3H, J = 7Hz, Ar-CH($\underline{CH_3}$)OAc). Mass spectrum: M^+, m/e = 357 (base peak); main peak: 298. High resolution mass spectrum: Calc. for $C_{24}H_{23}NO_2$: 357.1727. Found: 357.1676. Found: C,80.40; N, 6.38; N, 3.93. Calc. for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92.

1-Methyl-3-(α-(N-β,β-diethoxyethylamino)ethyl)-9-benzylcarbazole (224) from (223).

The carbazole acetate 223 (50 mg. 1.40 x 10^{-4} mole) was dissolved in aminoacetaldehyde diethylacetal (171) (1ml.) and heated at reflux temperature (160°) for 15 hr. after which time the excess amine 171 was removed under high vacuum. The dark orange oil which was obtained was column chromatographed on alumina (3 gm.). A small quantity of the desired aminoethyl acetal 224 was obtained by elution with benzene. N.M.R.: 8.14 (multiplet, 1H, C-5H), 7.98 (Singlet, 1H, C-4H), 7.2 (complex multiplet, aromatic), 5.72 (singlet, 2H, N-$\underline{CH_2}$ C_6H_5), 4.64 (triplet, 1H, J = 6Hz, $\underline{CH}(OE^+)_2$), 3.95 (quartet, 1H, J = 6Hz,

Ar-CH(CH$_3$)-N), 3.58 (complex multiplet, 4H, CH(OCH$_2$CH$_3$)$_2$), 2.69 (doublet, 2H, J = 6Hz, -N-CH$_2$-), 2.60 (Singlet, 3H, C-1CH$_3$), 2.0 (broad singlet, 1H, N(b)H), 1.47 (doublet, 3H, J = 6Hz, Ar-CH(CH$_3$)-N), 1.20 (complex multiplet,

1-Methyl-3-(β,β-diethoxyethyliminomethyl)-9-benzylcarbazole (221)

1-Methyl-3-formylcarbazole (219) (20 gm. 6.68 x 10^{-3} mole) and aminoacetaldehyde diethylacetal (171) (1 ml., 8.26 x 10^{3} mole) were heated at 100° for two hours, then benzene (15 ml.) was added and distilled off to remove water formed in the reaction. The residue crystallized from petroleum ether (65-110°) - benzene to give a colourless crystalline powder (2.34 gm., 85%), m.p. 92-94 . UV. (MeOH) λ_{max} (log ε): 345 (3.87), 330(sh) (4.07), 310 (4.23), 285 (4.62), 275 (4.61), 247 (4.59), 237 (4.60). (MeOH-HCl) λ_{max}: 380, 313, 305, 280, 270, 233. IR.: 1690. N.M.R.: 8.40 (Singlet, 1H, C-4H), 8.32 (distorted singlet, 1H, CH = N-), 8.10 (multiplet, 1H, C-5H), 7.60 (Singlet, 1H, C-2H), 7.4-7.1 (multiplet, 6H, aromatic), 7.0 (multiplet, 2H, aromatic), 5.72 (Singlet, 2H, benzyl H), 4.81 (triplet, 1H, J = 5Hz, CH(OEt)$_2$), 3.70 (complex multiplet, 6H, CH(OCH$_2$CH$_3$)$_2$), = N-CH$_2$-), 2.58 (Singlet, 3H, C-1CH$_3$), 1.14 (triplet, 6H, J = 7Hz, CH(OCH$_2$CH$_3$)). Mass spectrum: M^+, m/e = 414 (base peak); main peaks: 369, 339, 311, 298, 284. High resolution mass spectrum: Calc. for $C_{27}H_{30}N_2O_2$; 414.2307. Found: 414.2250. Found: C,77.95, H, 7.20; N, 6.56. Calc. for $C_{27}H_{30}N_2O_2$: C, 78.23; H, 7.29; N, 6.76.

1-Methyl-3-(α-(N-β,β-diethoxyethylamino)ethyl)-9-benzylcarbazole (224)

The iminomethyl acetal 221 (1.6 gm., 3.86 x 10^{-3} mole) in dry tetrahydrofuran (50 ml.) was treated with an excess of methylmagnesium chloride (4.8 ml., 3.16M) at reflux temperature for 24 hr. The excess Grignard reagent was then destroyed by the addition of water and the resultant precipitate was removed by suction filtration and washed liberally with ether. The filtrate was concentrated, taken up in water (250 ml.) and extracted with ether (3 x 100 ml.). The ether layer was washed with water, dried over sodium sulphate and concentrated to give an orange foam (1.52 gm.). The crude product mixture was column chromatographed on alumina (100 gm., Act III) and the desired aminoethyl acetal was eluted with benzene:chloroform (1:1) and chloroform to give a faintly coloured oil (330 mg. 20%). UV; λ_{max}: 341, 327, 293, 284, 263, 239, 231. N.M.R.: 8.14 (multiplet, 1H, C-5H), 7.98 (Singlet, 1H, C-4H), 7.2 (complex multiplet, aromatic ($CHCl_3$ contamination)), 5.72 (Singlet, 2H, N-$\underline{CH_2}$ C_6H_5), 4.64 (triplet, 1H, J = 6Hz, $CH(OEt)_2$), 3.95 (quartet, 1H, J = 6Hz, Ar-$\underline{CH}(CH_3)$-N), 3.58 (complex multiplet, 4H, $CH(O\underline{CH_2}CH_3)_2$), 2.69 (doublet, 2H, J = 6Hz, -N-$\underline{CH_2}$-), 2.60 (Singlet, 3H, C-1CH_3), 2.0 (broad singlet, 1H, N(b)$\underline{H}$), 1.47 (doublet, 3H, J = 6Hz, Ar-CH($\underline{CH_3}$)-N), 1.20 (complex multiplet, 6H, $CH(OCH_2\underline{CH_3})$). Mass spectrum: M^+, m/e = 430; main peaks: 415, 369, 298 (base peak), 288. High resolution mass spectrum: Calc. for $C_{28}N_{34}N_2O_2$: 430.2620. Found: 430.2633.

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