SYNTHETIC APPROACHES TO AN INDOLE ALKALOID PRECURSOR

by

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G.R.I.C., Kingston College of Technology, 1969

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Supervisor: Professor Gerald A. Poulton

ABSTRACT

In Part 1 of this thesis, synthetic endeavours leading to the acrylic ester [56] are presented. This compound, resembling the precursor secodine [52] is postulated as having a resemblance to the dihydropyridine or dihydropyridinium intermediates implicated as being involved in the later stages in biosynthesis of the major indole alkaloid families. A suggestion is made that the new compound [56] would be a more appropriate precursor than secodine for experimental bio-evaluation.

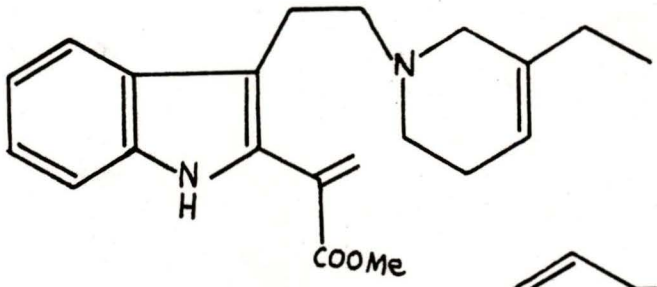
Synthesis was specifically directed at obtaining the alcohol [86], from which the expectedly unstable acrylic ester [56] could be generated in one step under carefully controlled conditions.

N-[β{3(2-Carbomethoxylmethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine [70] was prepared by condensation of 3-acetylpyridine with the appropriate tryptophyl derivative, followed by reduction. All attempts to alkylate in the ester side chain of [70] using methyl formate and subsequent reduction were without success.

Two basic alternative routes leading to [86] were followed. One involved prior elaboration of the ester side chain, to the alcohol [88]. The alcohol group was then protected by benzylation before attempting to introduce the ethyl bridge at C-3 of the indole nucleus, condense with 3-acetylpyridine, and catalytically reduce the pyridinium salt yielding [86]. In the other method, the alcohol group of methyl 3(β-hydroxyethyl)-indole-2-acetate was suitably protected, either as the tetrahydropyranyl or benzyl ether, and attempts made to alkylate in the ester side chain as with [70].

The second investigation concerns the generation of 1,4-dihydropyridines from the corresponding pyridinium compounds having the stabilising 3-acetyl function. This research is related in another direction again, to the implicated biointermediate(s).

Reduction of N-[β-(3-indolyl)ethyl]-3-acetylpyridinium bromide [102] with sodium hydrosulphite, gave the rather unstable N-[β(3-indolyl)ethyl]-3-acetyl-1,4-dihydropyridine [103]. Reaction of the bromide salt [102] with potassium cyanide in alcohol gave the relatively more stable cyano adduct [104]. These dihydropyridines were stable to base, but very labile to dilute acid, undergoing transformation to a mixture of products. The nature of these products in the case of the indole substituted compounds is not known with certainty.



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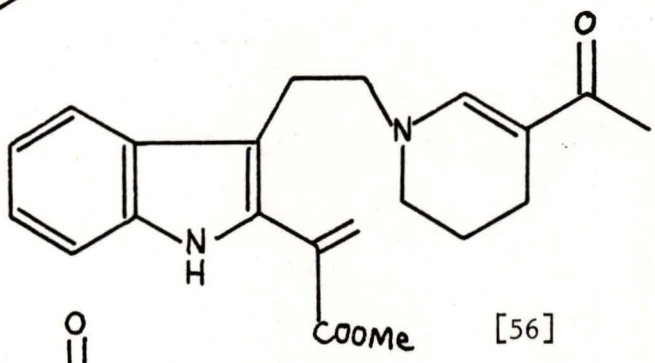

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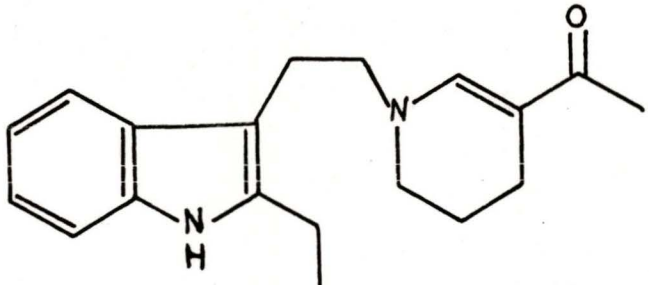

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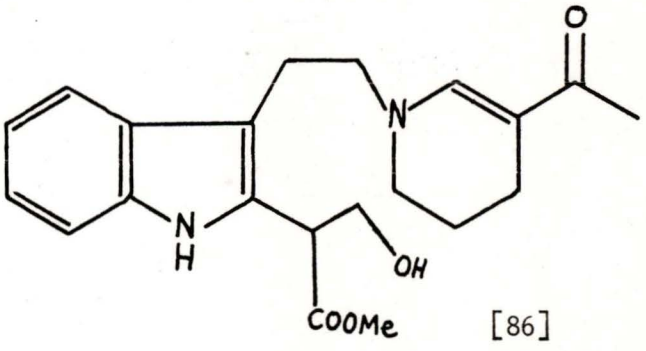

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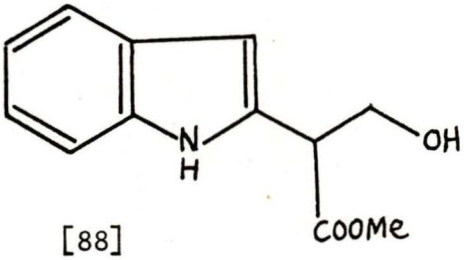

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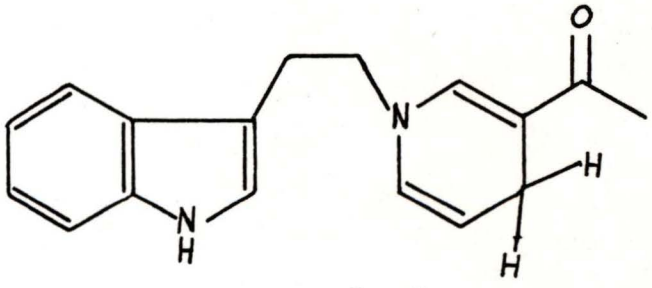

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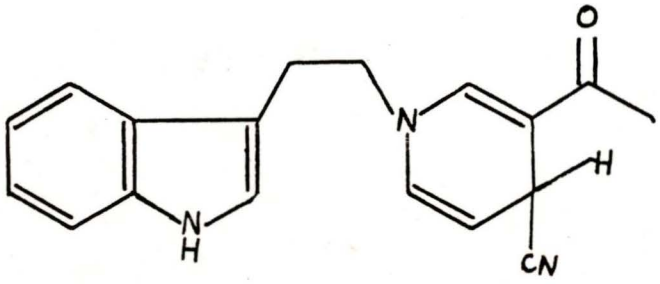

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ACKNOWLEDGEMENT

I wish to express my gratitude to my supervisor, Dr. G. A. Poulton for his excellent guidance throughout the course of my research.

My thanks go to Professor J. P. Kutney for his constant encouragement during the period in which part of my research was conducted at the University of British Columbia.

Financial assistance from the University of Victoria in the form of a Graduate Scholarship is gratefully acknowledged.

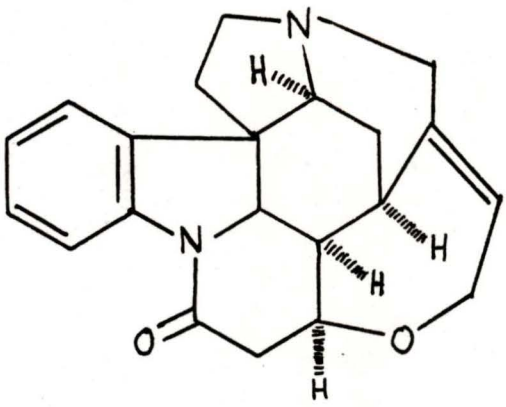
TO JULIE

INTRODUCTION

Within the vast array of natural products known to man, alkaloids have always been of considerable interest. Alkaloids are nitrogenous bases that usually occur in plants. The term was first coined by Meisner in the last century to describe such vegetable alkalies. It has been estimated that 10-20% of all plants produce alkaloids,[1] and of these, at least one-quarter contain indole alkaloids having either the indole or reduced indole nucleus.[2] The indole alkaloids therefore constitute a large class of compounds, and are among the earliest known. A recent compilation by Hesse lists some 500 of these bases derived from about 300 plants.[3]

Interest in the indole alkaloids has to a large extent been stimulated by their physiological potency and extensive use in medicine. Strychnine [1] is the well-known poison and is used as a heart-stimulant. Yohimbine [2] was employed as a veterinary aphrodisiac. Lysergic acid diethylamide, LSD, well-known for its hallucinogenic powers and ability to produce symptoms likened to schizophrenia, is a synthetic derivative of lysergic acid [3], one of the alkaloids of Ergot. The recently discovered anti-cancer agent, Vinblastine, VLB [4], and Vincristine, VCR [5], have complex structures seen to be dimeric forms of indole and dihydroindole.

Early studies in biosynthesis were based on the close resemblances between the natural compounds and proposed intermediates which could undergo reactions of biogenetic significance. The various hypotheses concerning the origin of indole alkaloids were therefore speculative and could not be supported experimentally. With the comparatively recent advent of radioactive isotopes becoming available in the 1950's, it became possible to test many



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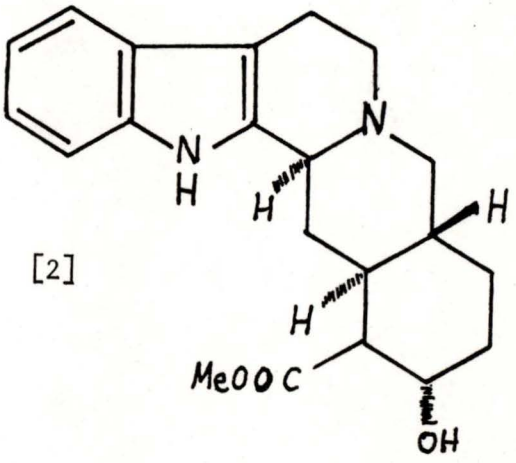

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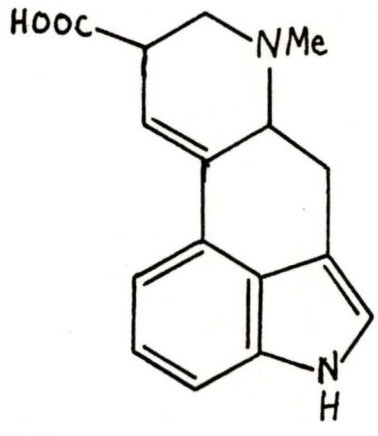

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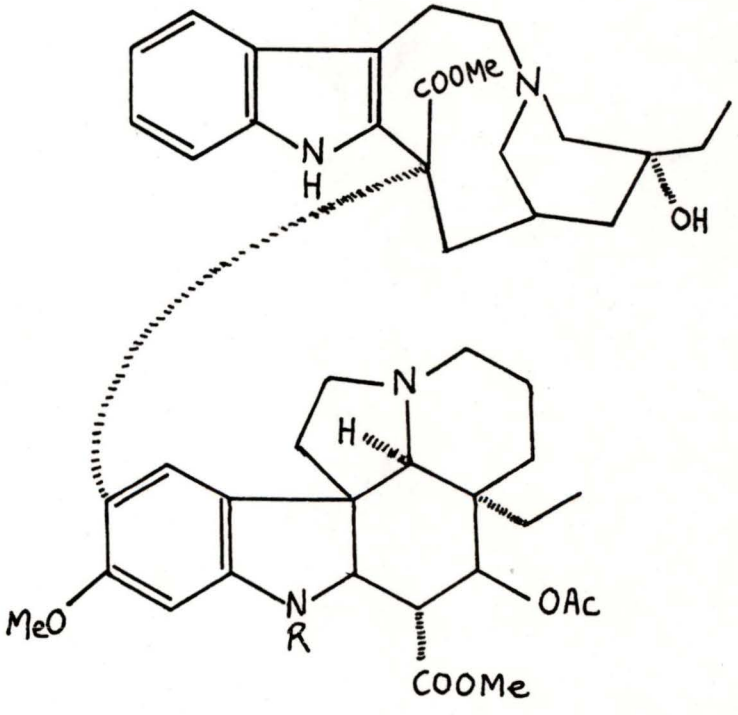


[4] R = CH_3
[5] R = CHO

Figure 1. Examples of Indole Alkaloids

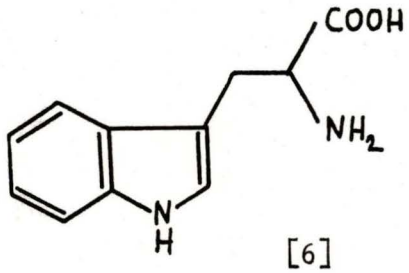
of these hypotheses. Postulated biological intermediates could be synthesised having radioactive isotopic labels. These could be administered to the growing system, and later the natural product isolated, which would be either appropriately labelled or non-labelled, as evidence to support various of the postulates.

The amino acid tryptophan [6] and tryptamine [7] both have the β-(2-aminoethyl)indole moiety recognised for many years as being a common structural feature of many indole alkaloids. These compounds were therefore reasoned to be progenitors of the indole portion of these alkaloids. Labelled tryptophan was indeed shown by various workers to be positively incorporated into such alkaloids as vindoline[4,5] [8], ajmaline[6] [9], reserpine[7] [10], vincamine [11], and catharanthine[5] [12], to mention only a few (Figure 2).

While the origin of the "tryptophan" portion of the indole alkaloids has never been questioned, the origin of the "non-tryptophan" portion was the subject of much controversy for a number of years. Many theories were advanced to account for the origin of this C_{9-10} unit, but it was not until the last decade that radioactive labelling techniques were applied to the problem.

The historical record of the various postulates and investigations is very adequately discussed in a doctoral thesis of Beck,[8] while a summary of the many experimental results obtained from the laboratories of prominent researchers in this area, particularly those of Arigoni, Battersby, Leete and Scott, is provided in a review by Scott.[9] Only the most important developments will be alluded-to here.

An early, longstanding hypothesis due to Barger[10] and Hahn,[11,12] expanded and modified by Robinson[13] and Woodward,[14,15] involved dihydroxy-



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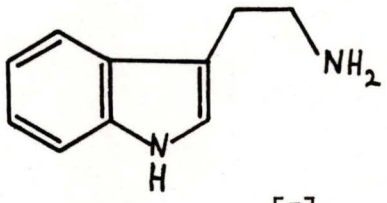

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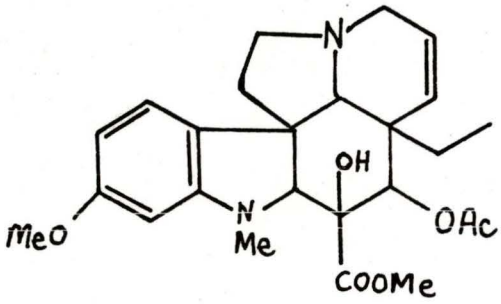

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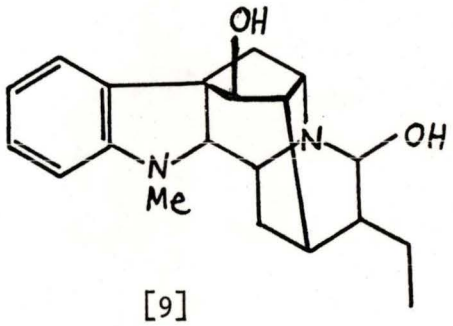

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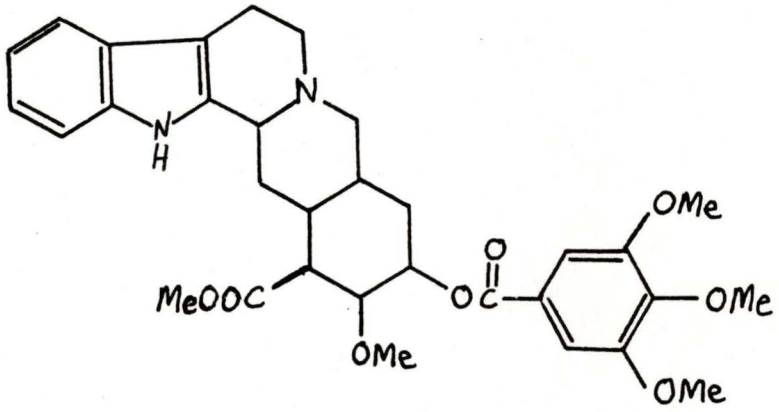

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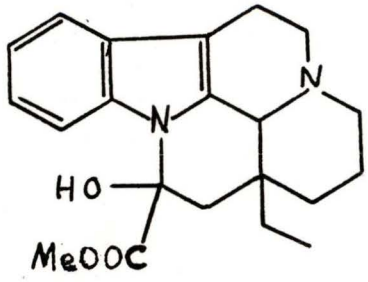

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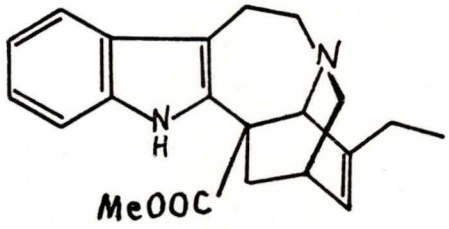


[12]

Figure 2. Examples of Indole Alkaloids.

phenylacetaldehyde [13] or an equivalent, plus two C_1 units. Mannich type condensation at either the ∝- or β- position of the indole system of tryptamine [7], gives intermediates which were envisaged as combining with the two C_1 units, accounting for either strychnine [1] or yohimbine [2] as illustrated in Figure 3.

The theory became subject to some basic criticisms, as pointed out by Wenkert and Bringi in 1959.[16] Wenkert, in an elegant alternative proposal, suggested that prephenic acid[17] [16] was the direct progenitor of the C_{9-10} unit via a crucial intermediate, the seco-prephenate formaldehyde (SPF) unit [17] which can be incorporated into yohimbine [2] and corynantheine [18], (Figure 4). Prephenic acid arises from carbohydrate metabolism via shikimic acid [14] and pyruvic acid [15] in the shikimate-chorismate pathway.

In another proposal, Wenkert,[17] and independently Thomas,[18] noted that certain monoterpenes resembled the seco-prephenate formaldehyde unit in structure, which led them to suggest a monoterpenoid origin for the C_{9-10} unit.

When his monoterpenoid hypothesis was advanced, Wenkert[17] also suggested a biosynthetic scheme for the *Aspidosperma* and *Iboga* families of indole alkaloids (Figure 5). This relates to the <u>later stages</u> in biosynthesis <u>after</u> the initial condensation of the SPF unit with tryptamine to form a "complex" such as [19]. Attention is drawn at this time to important features of the depicted sequence. This proposal will be relevant later on in connection with this thesis and there will be occasion to refer to it again. Both [21] and [22] are acrylic ester intermediates, and in addition, it can be seen that the proposal suggests that more than one family can arise from the same precursor, e.g. [20].

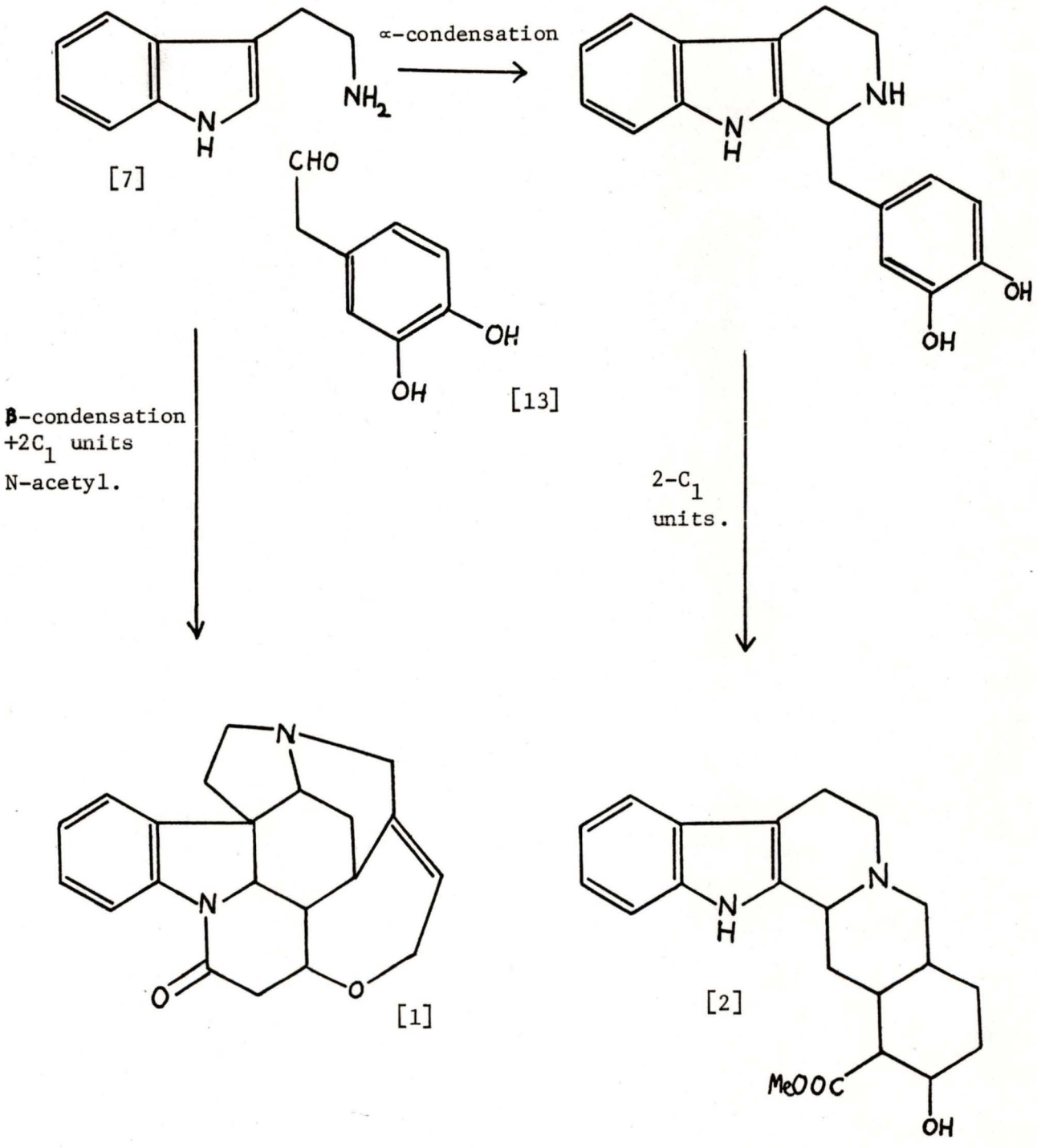


Figure 3. The Barger-Hahn-Robinson-Woodward Hypothesis.

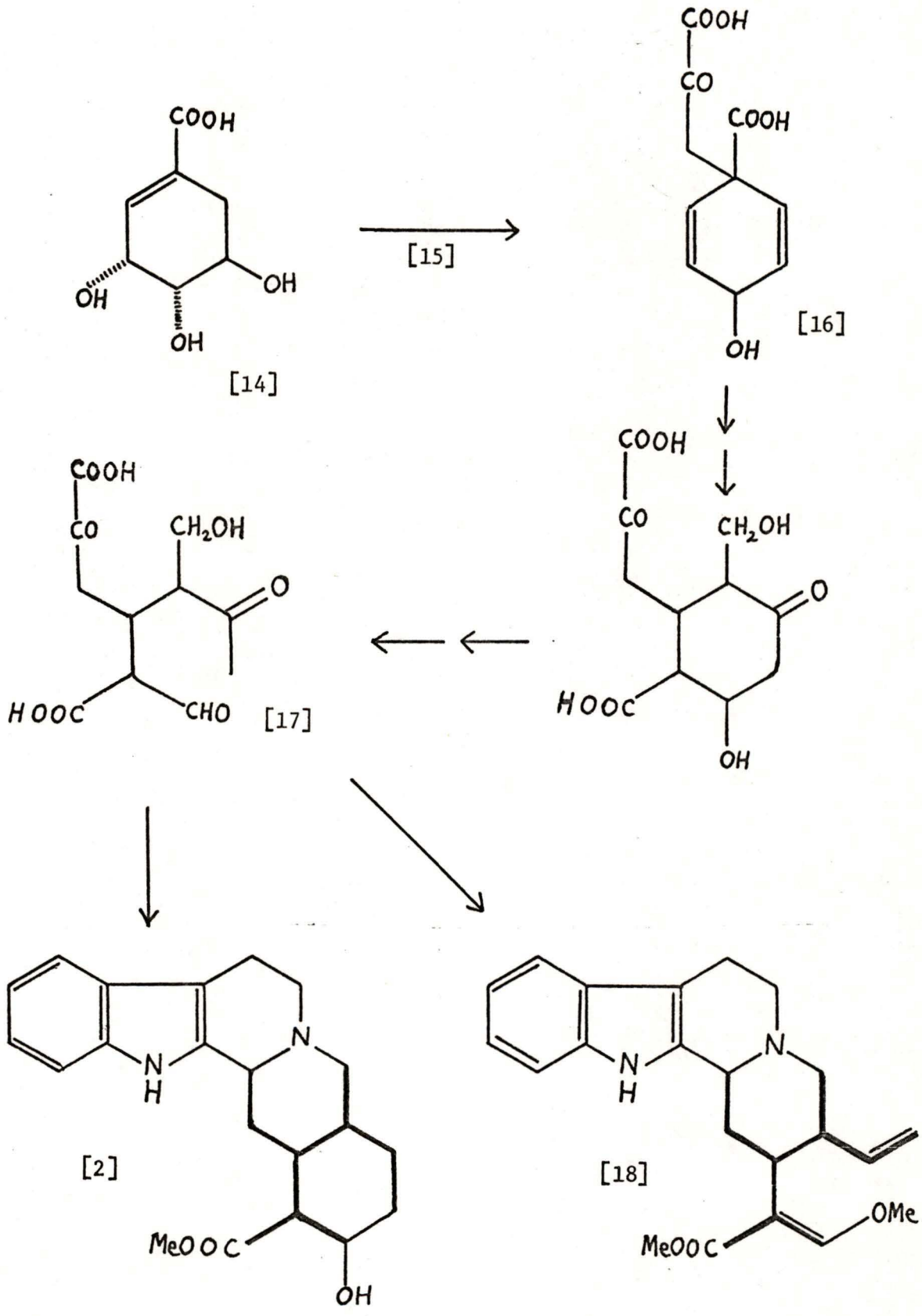


Figure 4. Wenkert Prephenate Postulate of Alkaloid Biosynthesis.

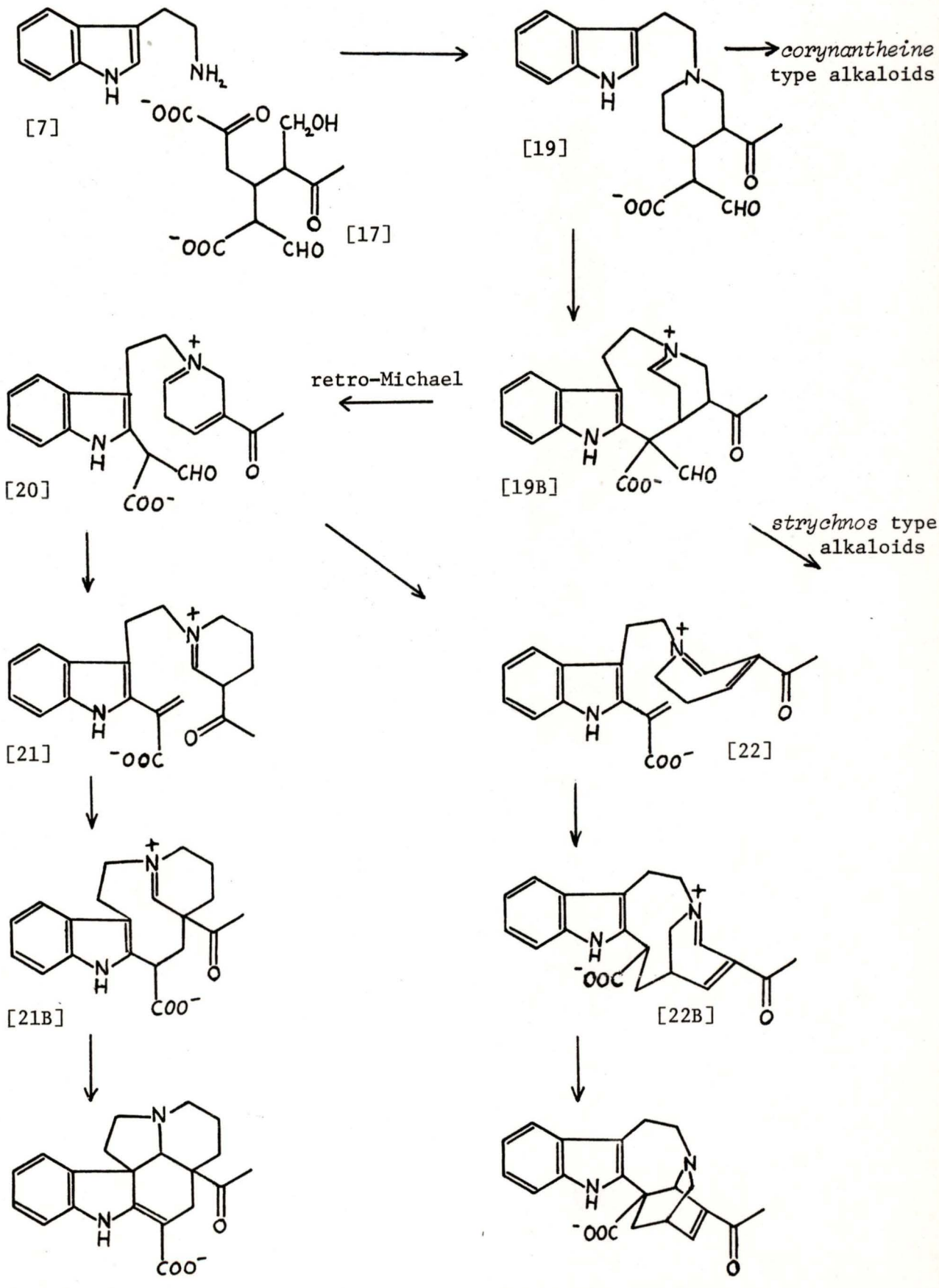


Figure 5. Wenkert's proposal for the biosynthesis of *Aspidosperma* and *Iboga* alkaloids.

The initial biosynthetic experiments, applied to the C_{9-10} unit problem using labelled precursors, notably those of Leete,[19] tended to disprove rather than support all of the prior held theories. As a result, yet another theory was advanced[19-22] in which the non-tryptophan portion had an acetate origin. Three acetyl-coenzymeA units, a malonyl-coenzymeA unit and a C_1 unit derived from formate would condense to give a C_{10} unit [23] having a nearly identical skeletal arrangement to Wenkert's SPF unit (Figure 6). Leete, reported initial results supporting this idea,[22] but researchers under the direction of Battersby repeated the work and disagreed with the original findings.[23,24] The hypothesis was later withdrawn. At the same time Battersby's group was able to obtain evidence which finally laid to rest the original postulate of Barger and Hahn.[24]

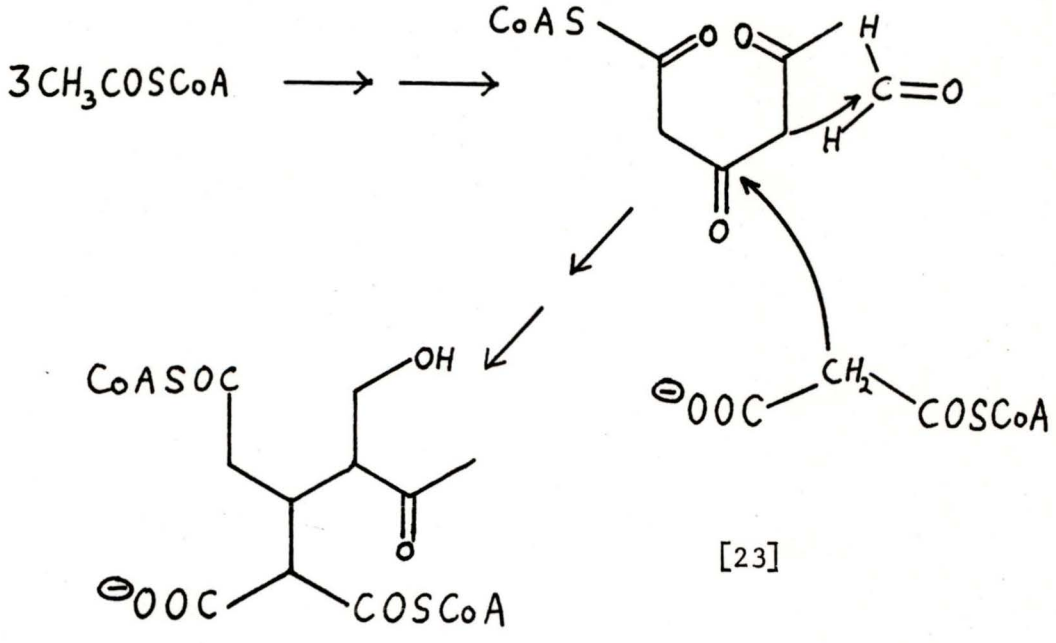


Figure 6. The Leete Acetate hypothesis.

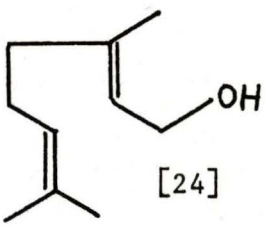
Wenkert's prephenic acid proposal was definitively tested with experiments using ^{14}C-labelled shikimic acid.[25] Administration to *Catharanthus roseus* plants gave good incorporations of label into the plants Vindoline [8] and Catharanthine [12], but subsequent degradative studies revealed that all activity resided only in the indole portion of the molecule. The postulate was therefore discounted.

Only the monoterpene postulate remained for serious appraisal. In 1965 Scott and co-workers[26] reported the first successful incorporations of mevalonate into the C_{9-10} unit. Mevalonic acid is the established precursor of terpenes. Progress then became rapid.

Specifically labelled mevalonic acid was shown by several research groups[27-30] to be incorporated into the alkaloids in a manner consistent with the monoterpenoid hypothesis. Within a year the Wenkert-Thomas postulate was fully substantiated. Subsequent efforts to find other monoterpene precursors yielded fruitful results. Geraniol [24] was shown to be incorporated,[31-34] and loganin [25] was found to be specifically incorporated as an intact unit into the alkaloids of *Vinca rosea*, *Rauwolfia serpentina* and *Cephaelis ipecacuanha*.[35-38] Furthermore, this precursor was demonstrated to be formed from mevalonate and geraniol.[35,37] Secologanin [26], the cleaved monoterpene derivative of loganin, was isolated from *V. rosea* plants.[39] Both [26] and the "secolactone" sweroside [27] were shown[40] to be further along the biosynthetic pathway than loganin itself, and their close structural relationship to the functionalised form of the Wenkert-Thomas SPF unit[39] [28] was evident.

These labelling studies fully resolved the question of the origin and biosynthesis of the C_{9-10} portion of the indole alkaloids. A summary

of all this work is provided in Figure 7.



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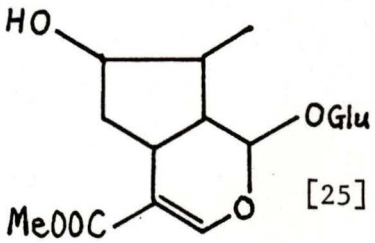

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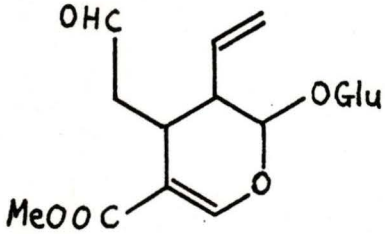

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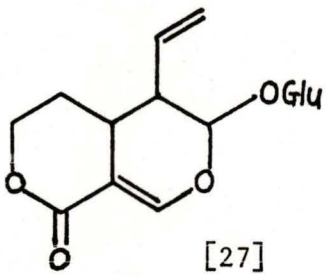
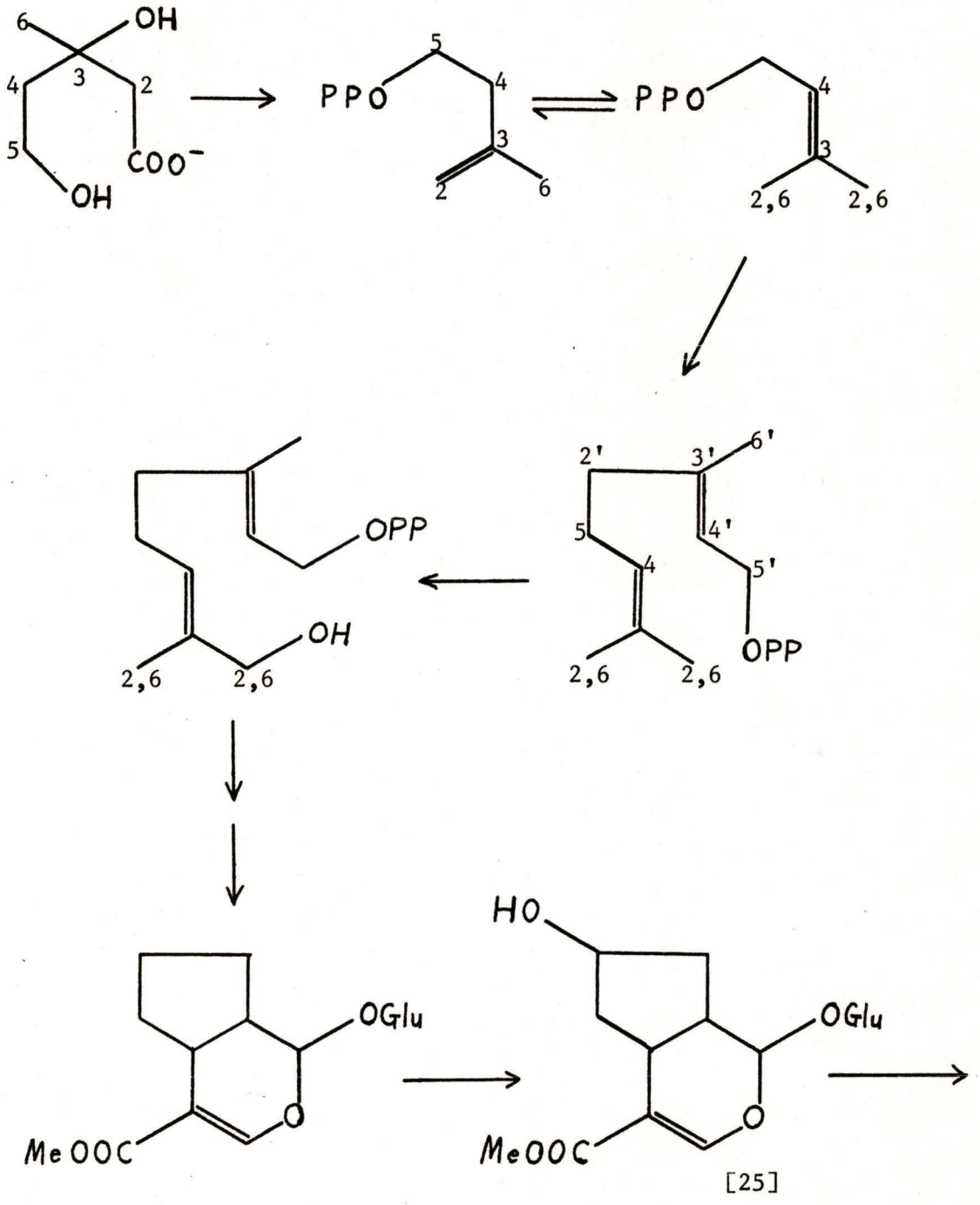


[27]

The numbering system indicates the exact location of the mevalonate carbon atoms as they are, both in the unrearranged alkaloid system of corynantheine [18] and the rearranged *Iboga* and *Aspidosperma* systems, typified by vindoline [8] and catharanthine [12].

With origin of the C_{9-10} unit established, research interest was, and still is, actively concerned with the later stages in biogenesis of the various alkaloids. Investigators have adopted several different methods of approach to the problem, as will become apparent.

The discovery and isolation of strictosidine [29] in *Rhazya* species,[40] and vincoside [30] from *V. rosea*,[41] indicated that combination of the C_{9-10} unit with tryptamine occured at the secologanin stage. The combination with secologanin *in vitro*[41] yields two bases vincoside [30] and isovincoside [31] having the same gross structure as strictosidine, however, only vincoside, which is specifically incorporated into the main families (*Aspidosperma, Iboga, Corynanthe* and *Strychnos*) of alkaloids,[41,42]



[25]

Figure 7. Biosynthesis of the C_{9-10} unit.

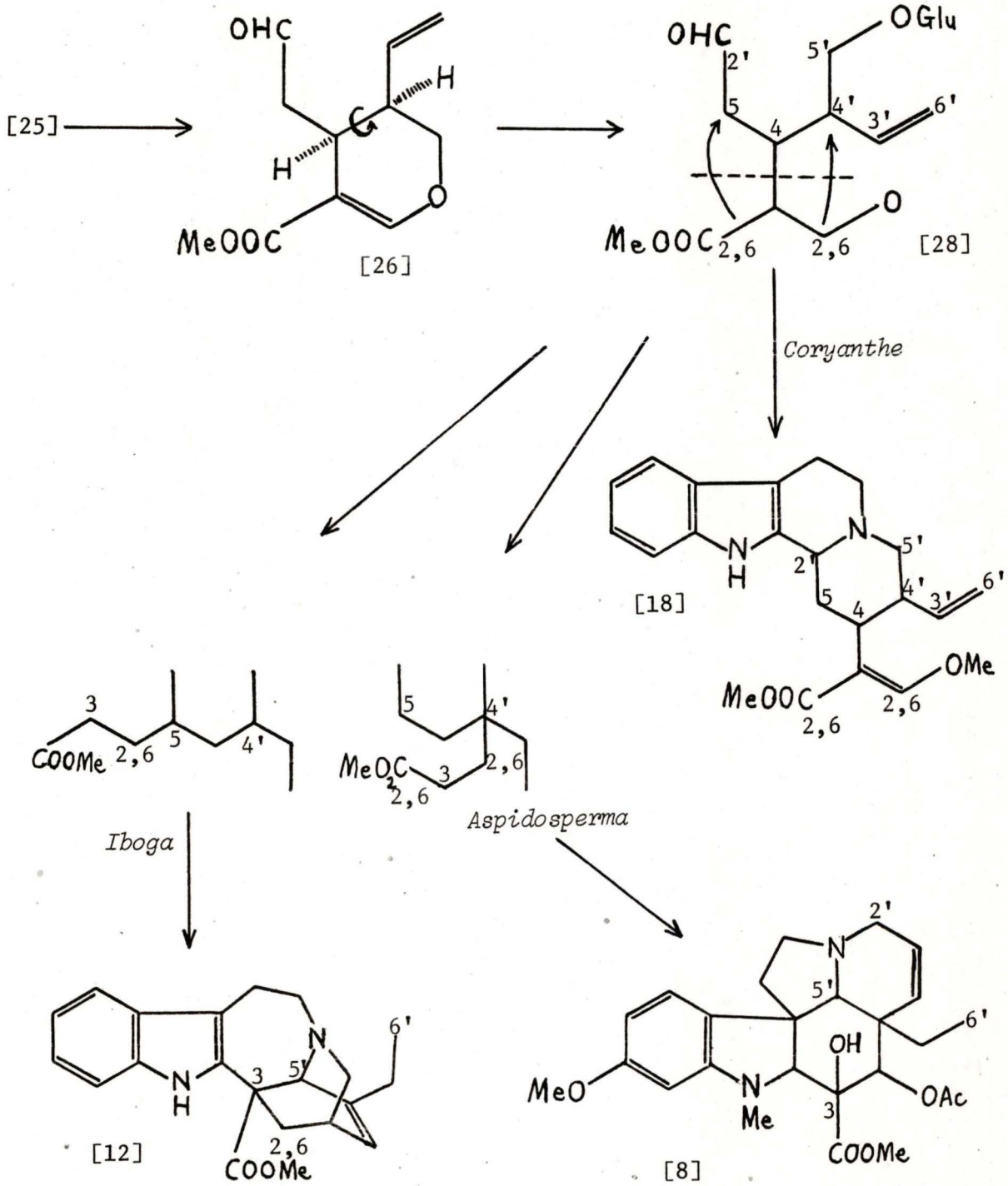
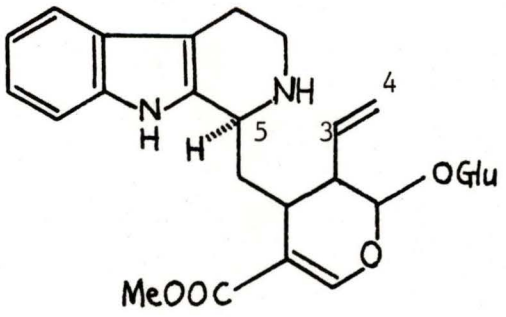


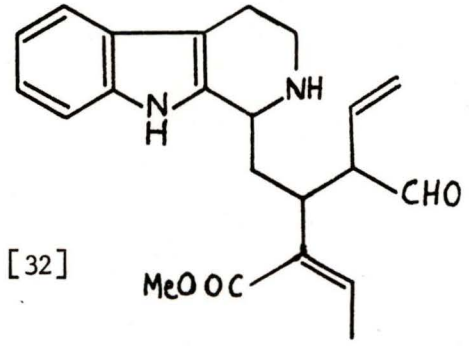
Figure 7. Biosynthesis of the C_{9-10} unit.

is involved in biosynthesis.

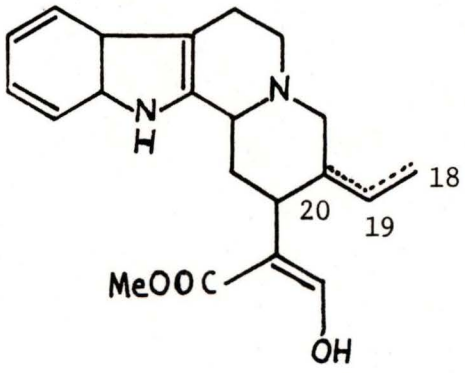


[29] Strictosidine
[30] $C_5H\alpha$ vincoside
[31] $C_5H\beta$ isovincoside

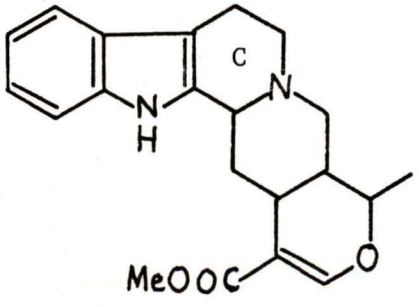
Bio-conversion of vincoside to the various alkaloid families necessitates skeletal rearrangement. Enzymic hydrolysis of the glucosidic residue, followed by reductive condensation via Schiff base formation from the aldehyde [32] and appropriate reduction could lead to the *Corynanthe* alkaloids, such as corynantheine aldehyde [33], geissoschizine [34], ajmalicine [35], and serpentine [36].



[32]



[33] 18(19)-ene
[34] 19(20)-ene



[35]
[36] C-ring aromatized

No incorporation of corynantheine aldehyde into the alkaloids obtained from *V. rosea* plants was reported,[43,44] but geissoschizine was shown both to be present in *V. rosea*,[42,45] and to be specifically incorporated into ajmalicine and serpentine, as well as members of the *Strychnos*, *Iboga* and *Aspidosperma* families.[42] The higher order alkaloids would therefore appear to be derivable from the structurally simpler types, such as geissoschizine. This rationale was indeed invoked by Wenkert in 1962, to explain the possible route from the *Corynanthe* to the *Strychnos* family alkaloids.[17]

To arrive at the structurally more complex *Iboga* and *Aspidosperma* systems, rearrangements of the C_{9-10} unit, as it occurs in e.g. corynantheine [18], are required. Wenkert[17] put forward the first proposal to account for these rearrangements of his SPF unit, as illustrated in Figure 5. The acrylic esters [21] and [22] are both derivable from the imine [19B] via a retro-Michael reaction, reduction of the dihydropyridine ring, and dehydration. These intermediates then undergo intramolecular Michael and Mannich condensations, giving the nine-membered ring systems [21B] and [22B], which on transannular cyclisation lead to the *Aspidosperma* and *Iboga* type skeletons respectively. The viability of such a proposal has received consideration in the laboratories of several research groups, notably those of Scott,[43] and of Kutney at the University of British Columbia. The research concern of the latter is to be discussed more fully later, since it had a more direct bearing on the derivation of my own research interest. Briefly, the initial objectives of the Kutney team involved examination of the transannular cyclisation required by part of the Wenkert postulate, both *in vitro*,[46-50] and *in vivo*.[5,51]

The cyclisation was found to be both facile and stereospecific *in vitro*, but when the appropriate experiments were performed no evidence for an *in vivo* cyclisation was found.[5] The same result was also obtained for the reverse ring opening process.[51]

Scott's group evaluated the postulate for rearrangement of the *Strychnos* skeleton by feeding labelled stemmadenine [37] to germinating seeds of *V. rosea*. A high specific incorporation of this alkaloid into vindoline and catharanthine, representative of the higher order *Aspidosperma* and *Iboga* families in this plant system, was observed.[43] The close structural similarity of stemmadenine [37] to Wenkert's precursor [19B] is readily apparent, and the result verified the primary aspect of Wenkert's postulate for the derivation of the *Aspidosperma* and *Iboga* alkaloids from the *Strychnos* series. Labelled tabersonine [38]† was also specifically incorporated into vindoline[43] [8] in the same plant system. This result was not unexpected since the basic skeletal arrangements of the two structures are the same. All that is involved in the transformation of [38] into [8] is the introduction of relatively minor functionalities. These minor changes must therefore occur after completion of the major skeletal rearrangements, as Wenkert also suggested.[17] However, incorporation of tabersonine into the *Iboga* alkaloid, catharanthine [12], was also observed. This entirely unexpected result could only be rationalised in terms of the Wenkert postulate if one assumes a facile reversible reaction at the branching point [20] of the sequence shown in Figure 5. An alternative rationale is now made possible as a consequence of the investigations of Kutney and co-workers, later referred to.

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†Structure shown on page 19.

Labelled catharanthine administered to *V. rosea* seeds failed to be incorporated into any of the other alkaloids present.[43] This suggested catharanthine was merely the irreversible end-product of the biosynthetic branch leading to the *Iboga* alkaloids.

An alternative approach was adopted by Scott, other than incorporations of possible intermediates to examine the later steps of alkaloid genesis. This involved a sequential alkaloid study using germinating *V. rosea* seeds.[9,43,45] The seeds themselves were virtually devoid of alkaloid content, thus after germination it was possible to observe the sequential formation, with time, of alkaloidal material. Vincoside [30] appeared at an early stage, some 26 hrs. after germination. Examination of the 35 hr. seedling fraction afforded a separable mixture of four corynanthe type alkaloids, - corynantheine [18], corynantheine aldehyde [33], giessoschizine [34], and ajmalicine [35]. The next alkaloids isolated (40-50 hrs.) were preakuammicine [39], stemmadenine [37] of the *Corynanthe-Strychnos* type, and akuammicine [40] of the *Strychnos* series.

An oxidation of the *Corynanthe* series is required in order to reach the *Strychnos* level. Alternative proposals for this have been suggested by Wenkert and others.[17,52,53,54] After 72 hrs. tabersonine [38], an *Aspidosperma* alkaloid appeared, but not so catharanthine [12], the principal *Iboga* alkaloid of *Vinca* until a full 100 hrs. had elapsed. The observed sequence, therefore, coupled with the additional incorporation results of Scott[43] and supported by Battersby,[42] suggested, but did not prove the order: *Corynanthe* → *Strychnos* → *Aspidosperma* → *Iboga*, as originally postulated by Wenkert, was correct. These experimental findings are summarised in Figure 8.

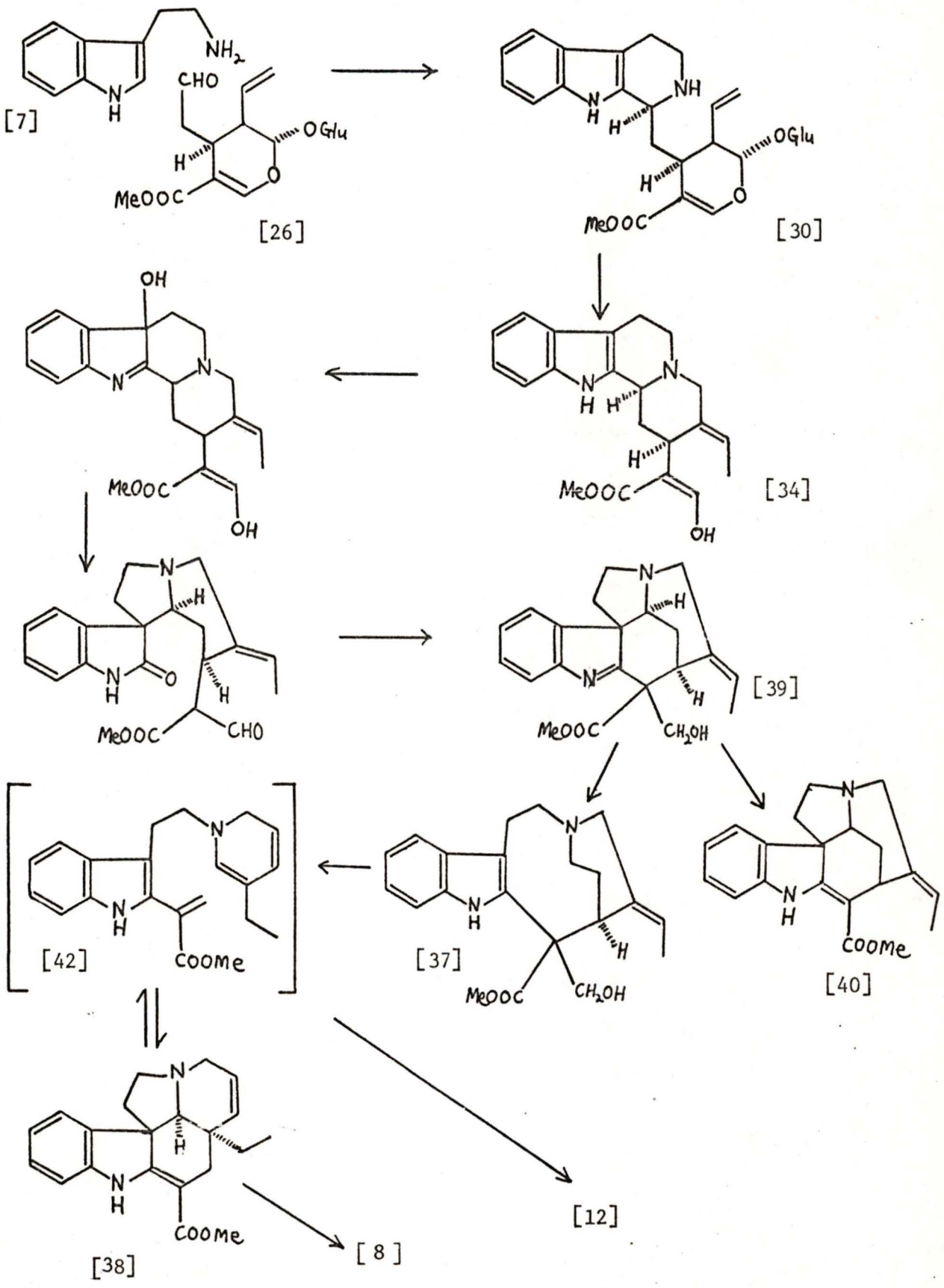


Figure 8. Sequential study of Indole Alkaloid Biosynthesis in *Vinca rosea*.

Scott, in 1968, advanced a most attractive mechanism linking stemmadenine [37], with tabersonine [38], and catharanthine [12], through the achiral intermediate [42] which can be generated from stemmadenine by dehydration[55,56] (Figure 9). The dehydration process, likely involves iso-stemmadenine [41], formed by migration of the exocyclic double bond of stemmadenine. Iso-stemmadenine can, with the aid of the nitrogen lone pair electrons, expel the hydroxide group, giving the dihydropyridine acrylic ester [42]. This acrylic ester, now named dehydrosecodine,[57] rather closely resembles the acrylic ester intermediates [21] and [22] which appear in the Wenkert postulate for the *Aspidosperma* and *Iboga* alkaloids.[17] The only major difference is the presence of a 3-ethyl, rather than a 3-acetyl, group as the piperideine ring substituent. [42B] is the imonium form of dehydrosecodine, which can by loss of a proton, and cyclisation by mode A give tabersonine [38]; it is also possible that [42B] may lose a proton without cyclisation, giving [42]. Dehydrosecodine may then cyclise in a Diels-Alder fashion to give tabersonine or, by another Diels-Alder reaction involving both double-bonds of the bent ring, yield catharanthine by mode B. In yet a third mode, C, the acryclic ester [42] can explain the genesis of vincadine [43] and like alkaloids. This idea places stemmadenine in a key position between *Strychnos*, and other alkaloid families, and also accounts for the occurance of racemic *Aspidosperma* alkaloids, such as (±) - vincadifformine [44] mediated by the achiral acrylic ester (Figure 9).

Concurrently with the above hypothesis being advanced, a large body of biochemical and structural evidence has accumulated from the laboratories of Kutney and Battersby, which supports the utilisation of a dihydropyri-

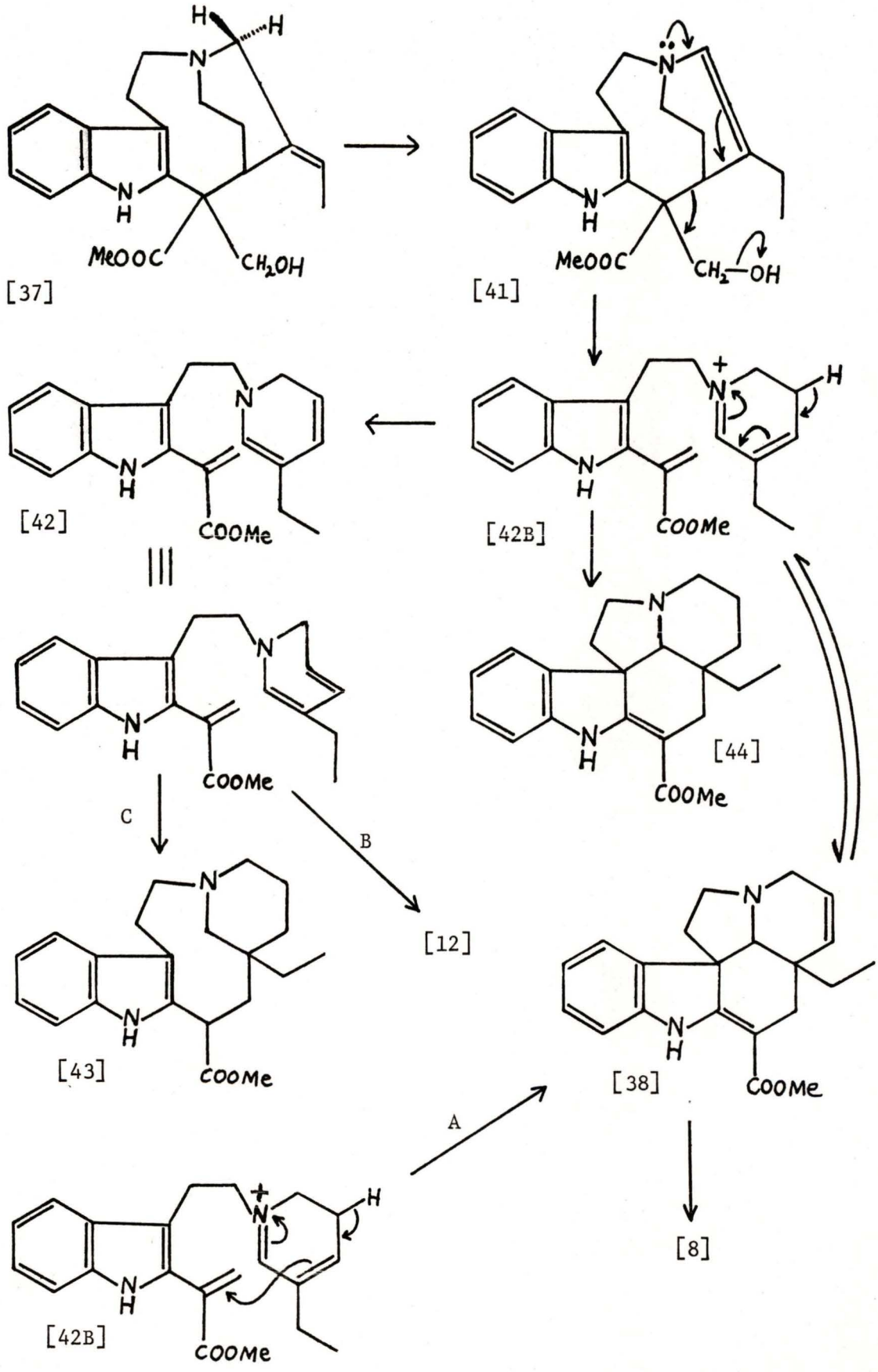


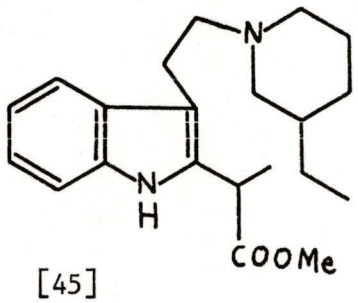
Figure 9. Possible rationale for the conversion of dehydrosecodine and its imine form to the *Aspidosperma* and *Iboga* families.

dine acrylic ester as a highly reactive common intermediate for the generation of all of the alkaloid families considered. The results obtained by Kutney's group will now be more specifically discussed.

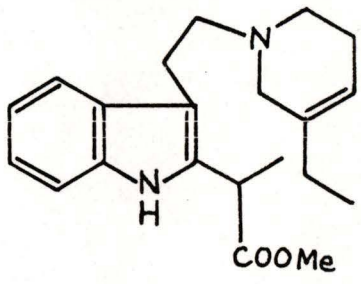
Interest in the investigation of the later stages of biosynthesis by the team at the University of British Columbia, came forth from the early work of J. P. Kutney, which showed the transannular cyclisation possibility of Wenkert to be non-operative in biosynthesis.[5,46-51] This being the case, it was inferred that some alternative pathway to the generation of the *Iboga* and *Aspidosperma* bases must be involved in the biosynthetic process. All the results obtained, including time sequence studies using DL-tryptophan-3-^{14}C and *V. minor* plants,[51] served to suggest the existence of a common intermediate. Consideration of the functionality such an intermediate might have, taking into account the results of Scott with regard to the incorporation of stemmadenine and the time-sequence studies with germinating *V. rosea* seedlings[43,45] (Figure 8), led Kutney to identify the common intermediate with the acrylic ester structure [42B], as proposed independently by Scott. Various bond-making processes can lead to all of the 9-membered and pentacyclic ring systems in the *Iboga* and *Aspidosperma* series, as already indicated (Figure 9).

There was now an obvious requirement of experimental support for the acrylic esters, [42] or the imine form [42B]. Not surprisingly, only derivatives of these compounds have been detected in biological systems. These take the form of monomeric derivatives, such as tetrahydrosecodine [45] and dihydrosecodine [46], which have been detected specroscopically in *Rhazya stricta*,[57] tetrahydrosecodin-17-ol [51] detected in *R. orientalis*,[57] and dihydrosecodin-17-ol [48] in *V. rosea* plants.[58] Also dimeric

alkaloid derivatives have been isolated: prescamine [49] from the leaves of *R. stricta* and tetrahydropresecamine [50] from *R. orientalis* roots.[59] In referring to this topic of natural occurance, it should be said that no such acrylic ester derivatives bearing a 3-acetyl function in the piperidine ring, rather than the 3-ethyl group, have been reported. Mention of this is made at this time in connection with my own research interest, concerning the acrylic ester [56].



[45]



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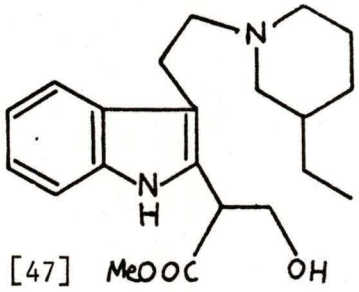
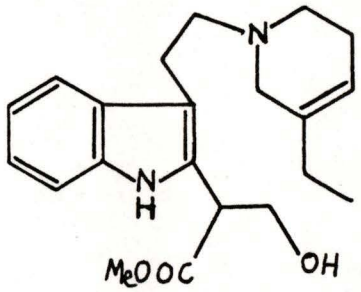
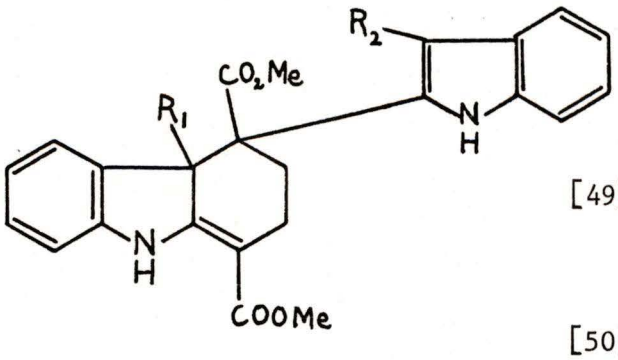
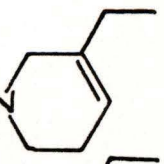


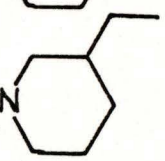
[47]



[51]



[49] $R_1 = R_2 =$ 

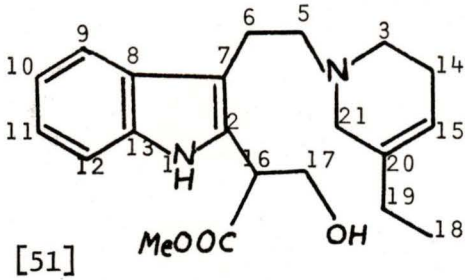
[50] $R_1 = R_2 =$ 

It was predictable from the outset that the synthesis of a dihydropyridine such as [42] or [42B] would present considerable difficulty.

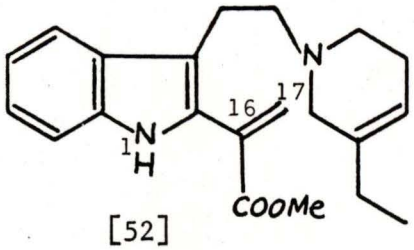
The instability of dihydropyridines in general, is well known. This point will be elaborated further in the discussion section, and again in Part II of this thesis dealing with my own research involvement in the dihydropyridine field. In addition to the dihydropyridine problem, acrylic esters are well known for their reactivity. The Kutney research group therefore undertook the synthesis of more stable analogues of dehydrosecodine. These were 16, 17-dihydrosecodin-17-ol[51], and secodine [52], both being tetrahydropyridines.

Details of the synthetic sequence they developed and executed, from which my own synthetic work was derived, are given in the discussion section of this thesis, for comparison purposes. Their synthesis was planned such as to allow introduction of tritium and/or carbon-14 at various positions in the molecule, as desired. Secodine [52], as expected, turned out to be much less stable than 16,17-dihydrosecodin-17-ol[51], but could be generated from [51] under carefully-controlled conditions and administered to the various plants chosen. It was experimentally proven, that with the appropriate feeding method, the plants could take up secodine as such, into the system, before extensive decomposition due to the very facile dimerisation reaction became a problem.[8] Biological evaluation of labelled [51] and [52] was carried out using three different plant systems. Studies with *V. rosea* were to investigate the *Iboga* (catharanthine) and *Aspidosperma* (vindoline) alkaloid biosynthesis. Those on *V. minor* were to provide evaluation of the eburnamine-vincamine (vincamine [53]) group, for which no experimental data was up till that time available. Finally the alkaloids of *Aspidosperma pyricollum* (aparicine [54] and uleine [55]) were of interest since they lacked the normal tryptophan side-chain. Various postulates for these groups of alkaloids

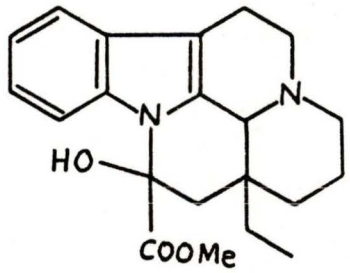
required evaluation.



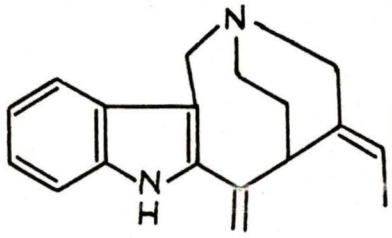
[51]



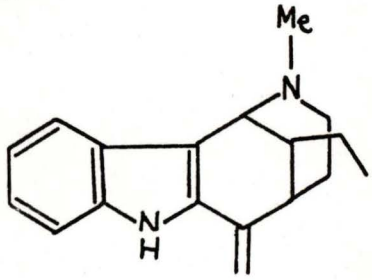
[52]



[53]



[54]



[55]

There was no incorporation (<0.001%) of the alcohol [51] into any of the alkaloids isolated from these plants; in fact there was evidence of toxicity to the plants.[60] The secodine [52] results were much more encouraging: no plant deterioration was observed and low but positive incorporations into several alkaloid families was evident. Hence it was apparent that the plant enzyme systems were incapable of performing the necessary dehydration step on the alcohol [51] to secodine. Experiments were conducted using both singly [ar ^{3}H] and doubly labelled [ar ^{3}H, $^{14}COOCH_3$] secodine.[60] The amount of incorporation into the various alkaloids is shown in Figure 10. The double labelling results showed that significant exchange or loss of tritium in the indole portion of the molecule, did not occur in biosynthesis. Subsequent degration studies with vindoline [8], established that the indole portion was incorporated into [8] with little or no alteration. Further work with tritium label in the piperidine unit of secodine [$^{14}COOCH_3$, 3,14,15,21-^{3}H] using

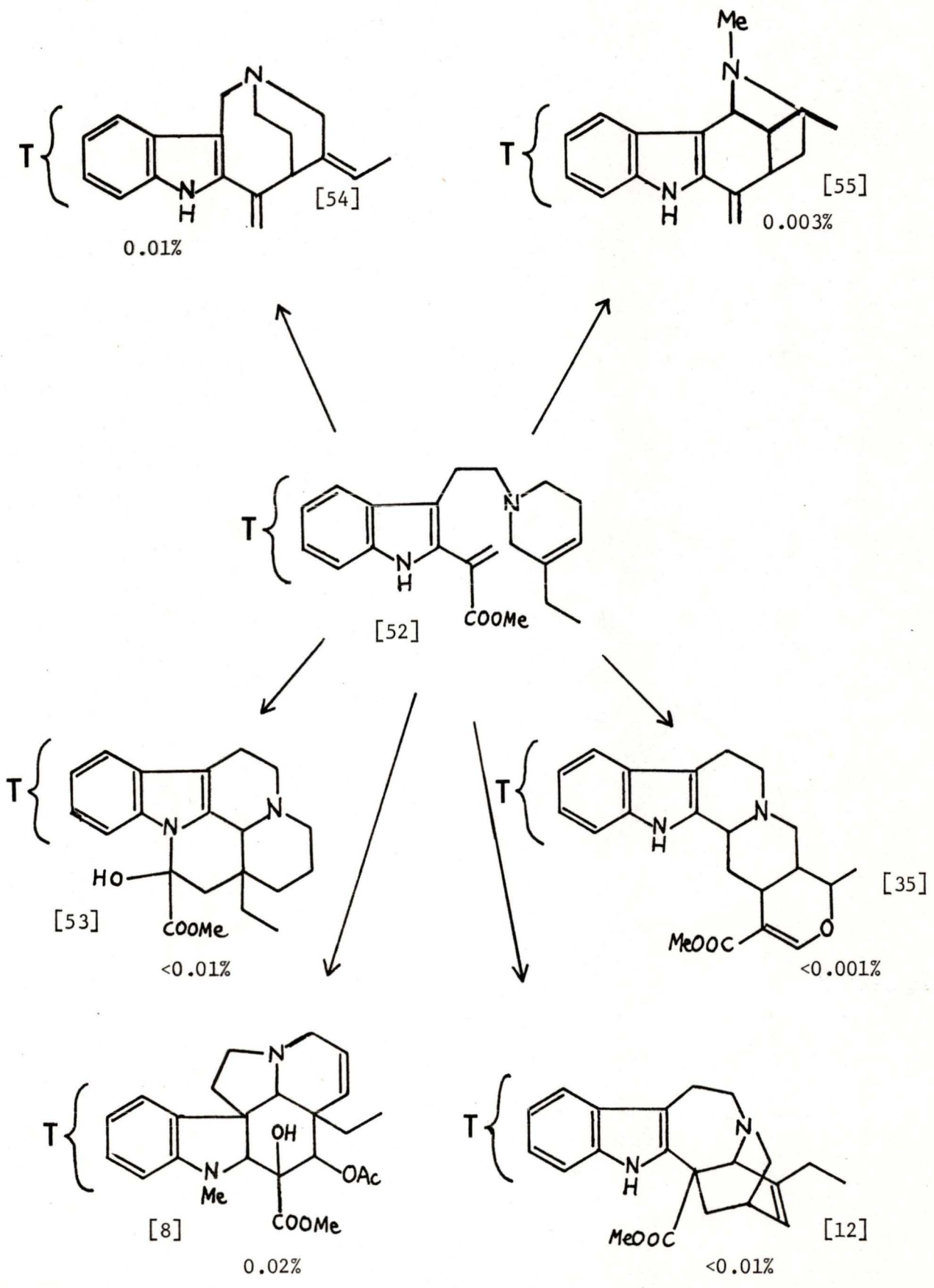


Figure 10. Incorporations of [ar-^{3}H] Secodine into Various Alkaloids[60]

V. rosea and *A. pyricollum* plants, supported the conclusion that [52] was specifically incorporated intact into the various alkaloids.[60] The measurable loss in tritium label observed was then equated with the loss of hydrogen from the piperidine unit during the necessary bond-making process implicit in the biosynthetic elaboration of secodine, and deductions made from this.

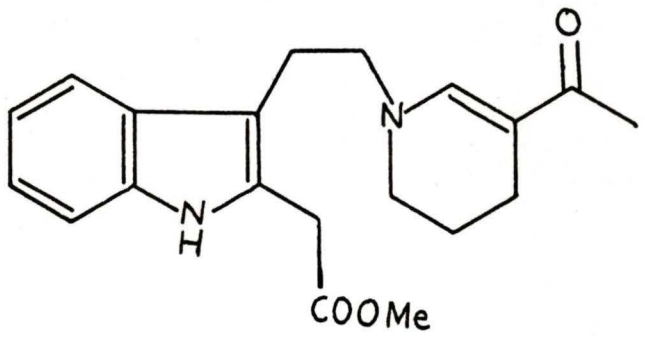
The section of their researches described above demonstrated that the plant *V. rosea*, can utilise the acrylic ester secodine in its biosynthesis of the *Aspidosperma* alkaloids. The actual biological intermediate is very probably very similar in structure to secodine. Furthermore the Scott-Kutney postulate that the critical, "central-roll" intermediate in the biosynthesis was the dihydropyridine [42] or dihydropyridinium [42B] acrylic ester was in accord with their own findings.

It should be said that the levels of incorporation into the different alkaloids, while significant, are all quite low (see Figure 10). A number of explanations for very low relative incorporations can be advanced, for example if the turnover rate of alkaloids leading to vindoline is slow, compared to the possible rapid incorporation of secodine into the biosynthetic pathway. Vindoline is quite highly functionalised compared to catharanthine, and in addition Scott's sequential studies with *V. rosea* seedlings[9,43,45] revealed that whereas catharanthine appeared after about 100 hrs. germination, vindoline was not detected before 200 hrs. had elapsed. An alternative explanation could be at the enzymic level. If there exists two enzymic binding sites in secodine to catalyse the conversions to the *Iboga* and *Aspidosperma* alkaloids, complex formation at the binding site leading to the *Iboga* bases could be less efficient, etc. However even considering these possibilities, the incorporation

of secodine into vindoline itself was not very good.

In view of the low incorporations with secodine it is clearly desirable to carry out further investigations with precursors resembling the dihydropyridine acrylic esters [42] or [42B] even more closely than secodine.

Consideration was therefore given to an alternative acrylic ester, differing in the position of the double bond in the piperidine ring, and having the 3-acetyl, rather than 3-ethyl gp. ring substituent. Possible explanations for the new compound [56], being a more appropriate precursor than secodine [52] for biosynthetic evaluation are presented in the discussion section to follow.



[56]

DISCUSSION

PART 1

As the results of the Kutney research group at U.B.C. have shown, the precursor secodine [52] with its low, but nevertheless positive, incorporations into all the various alkaloids of concern has provided evidence in support of the involvement of the dihydropyridine [42] or dihydropyridinium [42B] acrylic ester as a common intermediate in the later stages of biosynthesis. Consequently additional biosynthetic work with an alternative precursor(s) was obviously desirable from the standpoint of providing even better support for the common intermediate.

For various reasons, synthesis and bioevaluation of the vinylogous amide-acrylic ester compound [56] was chosen as the primary objective for our own research entry into this field. The new acrylic ester, differs from secodine essentially in being a 1,4,5,6-tetrahydropyridine, rather than the 1,2,5,6-isomer and bearing the 3-acetyl function rather than the 3-ethyl group, i.e. containing now the vinylogous amide chromophore (>N-C=C-C=O, also variously known in the literature as an enamide, β-amino-α, β-unsaturated ketone, or enamino ketone). This compound was thought to have, in a sense, even closer chemical resemblances to the dihydropyridine "true" biointermediate than secodine,

although both compounds, being tetrahydropyridines give a less desirable oxidation level to the ring.

The great instability of the dihydropyridine ring as a generalization is well known and was an obvious curb to any attempts at a direct synthesis of the dihydropyridine bio-intermediates [42] or [42B] themselves. Dihydropyridines are very prone to oxidation, and even contact with atmospheric oxygen may be sufficient to effect conversion to the corresponding pyridine.[61] Another type of reaction they are noted to undergo is disproportionation, either by heat, light, acids, bases or palladium. Mixtures of pyridines and reduced pyridines are the products, but whether the reduced portion of the product is a tetrahydropyridine or a piperidine has not been rigorously proven in many cases.[61] Isomerisation of dihydropyridines readily occurs and has even led to uncertainty concerning their structures.[61] The major product usually isolated, is the most stable one for a particular set of ring substituents, in the case of the substituted derivatives.

Dihydropyridines having substituents capable of conjugation with the unsaturated system have long been known to exert a stabilising influence on the molecule. The 3-acetyl group of the vinylogous amide, appropriately chosen for our work, exerts such a stabilising effect. Further mention of this is made in Part II of the discussion.

Another reason for choosing [56] as the new precursor, is that a number of electrophilic and nucleophilic reactive centres are introduced into the pyridine part of the molecule with the vinylogous amide system. This functionality may thus lend itself to promoting many of the possible alternative modes of cyclisation of the acrylic ester, leading to the different alkaloid skeletons. Incorporation could therefore be assisted or even increased. Wenkert[17] interestingly included the 3-acetyl function in the various imonium ion structures undergoing the intramolecular Michael and Mannich reactions embodied in his postulate for the *Aspidosperma* and *Iboga* alkaloids, illustrated earlier (Figure 5). The postulate was proven incorrect, at least with regard to the transannular cyclisation; also the carbonyl side chain he implied was derived ultimately from prephenic acid, again incorrect from what is now known concerning the origin of the C_{10} unit.

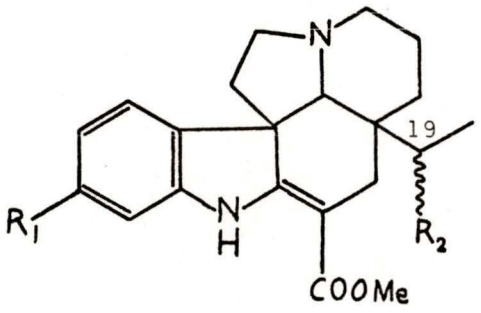
Justification for the new precursor also requires some consideration be given to the possible biochemical origin and metabolism of the acetyl function, since it is not found as such in any of the alkaloids of direct interest. Of particular concern would be the possibility of enzymic interconversion with the reduced products, attaining the oxidation level of either the ethyl side chain, as found for example in vindoline [8], or possibly the vinylic group found in such alkaloids as stemmadenine [37] and preakuammicine [39]. These alkaloids were implicated in biosynthesis <u>immediately prior</u> to the common intermediate.[55,56]

Generation of the vinylic residue might well occur biochemically in a stepwise manner. Such a process could involve a reduction, followed by phosphorylation with, for example, ATP, and finally, elimination of phosphate in the presence of a phosphatase enzyme. The reduced form of DPN, which in a well-known analogy reduces pyruvate to lactate, could serve as a good hydrogen donor for reduction of the acetyl group to a secondary alcohol. The alcohol once formed, may also eliminate directly by loss of a molecule of water. Further reduction from the formed vinylic group, to the ethyl side chain may then occur, with, for example, a suitable enzyme dehydrogenase containing reduced flavine adenine dinucleotide ($FADH_2$).

In choosing a precursor like [56] containing the 3-acetyl function, it is pertinent to consider whether any *Vinca* or *Aspidosperma* derived alkaloids, contain a carbonyl or like group at the same position as the ethyl side chain in their skeleton, while retaining all of the other important features, in particular, the carbomethoxy group. It must be admitted that only a handful of such alkaloids have so far been identified. Three alkaloids of interest accompanying vincadifformine [44], having the same skeleton, but possessing 19- oxygenated functions, have been isolated from *V. minor*.[62] These are minovincine [60], minovicinine [61], and 11-methoxy-minovincine (minoricein) [62]. Physical data for vincaminine [63] and vincinine [64], two eburnamine alkaloids recently isolated from *V. minor*,[63] also is compatible with the structures shown for these. With the occurence of so few of the oxygenated

derivatives compared to those bearing the ethyl side chain, it may well be that the carbonyl or alcohol chain is superficially involved as a late functionalisation step in the biosynthesis of these alkaloids, rather than having any direct relationship with the common intermediate.

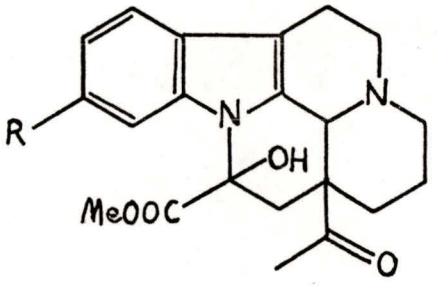
The various synthetic endeavours leading to the chosen precursor [56] will be discussed.



[60] R_1 = H; R_2 = O

[61] R_1 = H; R_2 = OH

[62] R_1 = OMe; R_2 = O



[63] R = H

[64] R = OMe

An important prediction conerning the stability of the required compound can be made, in view of the experiences of the Kutney workers and others with the very labile acrylic ester, secodine [52]. Acrylic esters are generally known to undergo polymerisation under the influence

of heat, light, oxygen or peroxides, and secodine was no exception. Ready dimerisation of the monomer occurred, presumably by an intermolecular Diels-Alder reaction to give the dimeric alkaloid presecamine [49], which has recently also been isolated from plant material.[59] This facile dimerisation was largely avoided by developing a technique such that the acrylic group was generated by the dehydration of secodinol [51] only at the last step in the synthesis employing a mild freeze-drying procedure. Secodine suffered only partial dimerisation at the plant administration stage.[8]

These results suggested it may have been easier to plan a synthetic route not too different to that developed by the U.B.C. researchers for secodine, allowing us to synthesise the relatively stable hydrated form of the acrylic ester corresponding to secodinol. The essence of scheme developed for secodinol [51] is portrayed in Figure 11.[8,60] 2-Carboethoxy-3-(β-chloroethyl)indole, [65] conveniently prepared by a Fischer indole synthesis was condensed with 3-ethylpyridine, and the resulting pyridinium salt reduced with sodium borohydride to give the 1,2,5,6-tetrahydropyridine. Subsequent elaboration of the ester side chain in six steps gave secodinol [51]. Successful tritium labelling of the ester [66] was carried out prior to the alkylation reaction.

In attempting the parallel procedure,[64,65] [65] was condensed with 3-acetylpyridine to give N-[β{3(2-carboethoxyindolyl)}-ethyl]-3-acetylpyridinium chloride. This compound upon borohydride reduction, gave the required vinylogous amide, but,

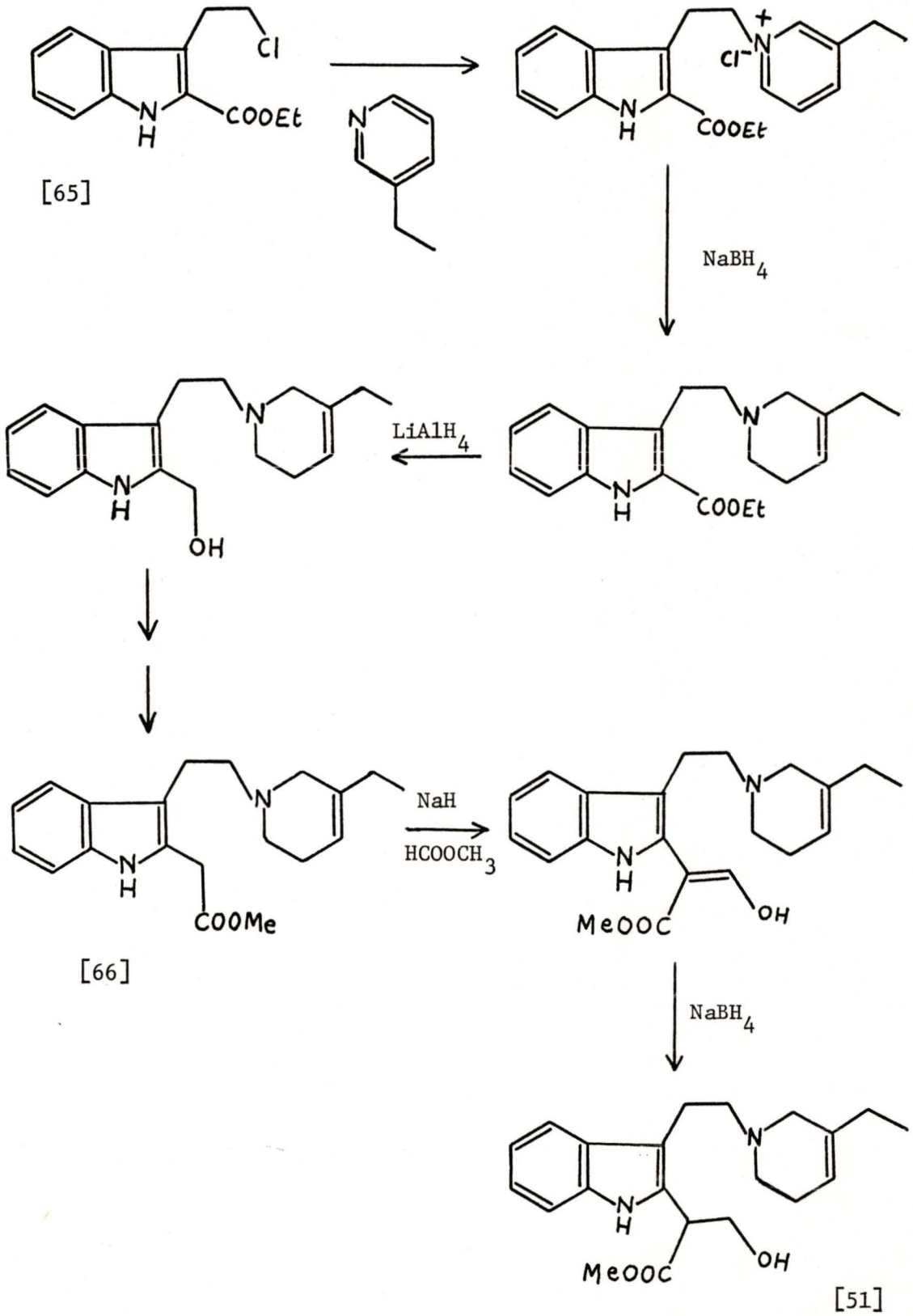


Figure 11: The Synthesis of 16,17-Dihydrosecodin-17-ol.[8,60]

however, selective reduction of the ester group as the next step to the primary alcohol function necessary for homologation of the chain, could not be carried out without destroying the amide system. In another scheme[65] (illustrated in Figure 12), condensation of [65] with 3-acetylpyridine ethylene ketal, and borohydride reduction of the pyridinium salt, gave the 1,2,5,6-tetrahydropyridine ethylene ketal [67]. The ester side chain could then be successfully elaborated as shown, but isomerisation of the piperideine [68] to the vinylogous amide system, via oxidation to the pyridinium salt and catalytic reduction, failed. In yet another alternative scheme,[64] the carbonyl group of the piperideine ring of [68] was reduced with borohydride to give the allylic alcohol, and alkylation then gave the diol [69]. Elimination of the primary alcoholic function would yield the acrylic ester, while elimination of the secondary alcohol would yield the potentially useful vinyl side chain alternative to the acetyl group. Unfortunately dehydration of the diol gave no useful products.

Because of the barriers encountered in the previous schemes, we devised a method which would permit a partial elaboration of the indole C-2 side chain prior to linkage of the 3-acetylpyridine ring and formation of the vinylogous amide [70]. The sequence followed is illustrated in Figure 13. Wenkert et al.[66] have also prepared the enamide [70] via condensation of 3-acetylpyridine with methyl 3-(β-chloroethyl)-2-indoleacetate [79], synthesised by two alternative multistep procedures. Neither of these appeared

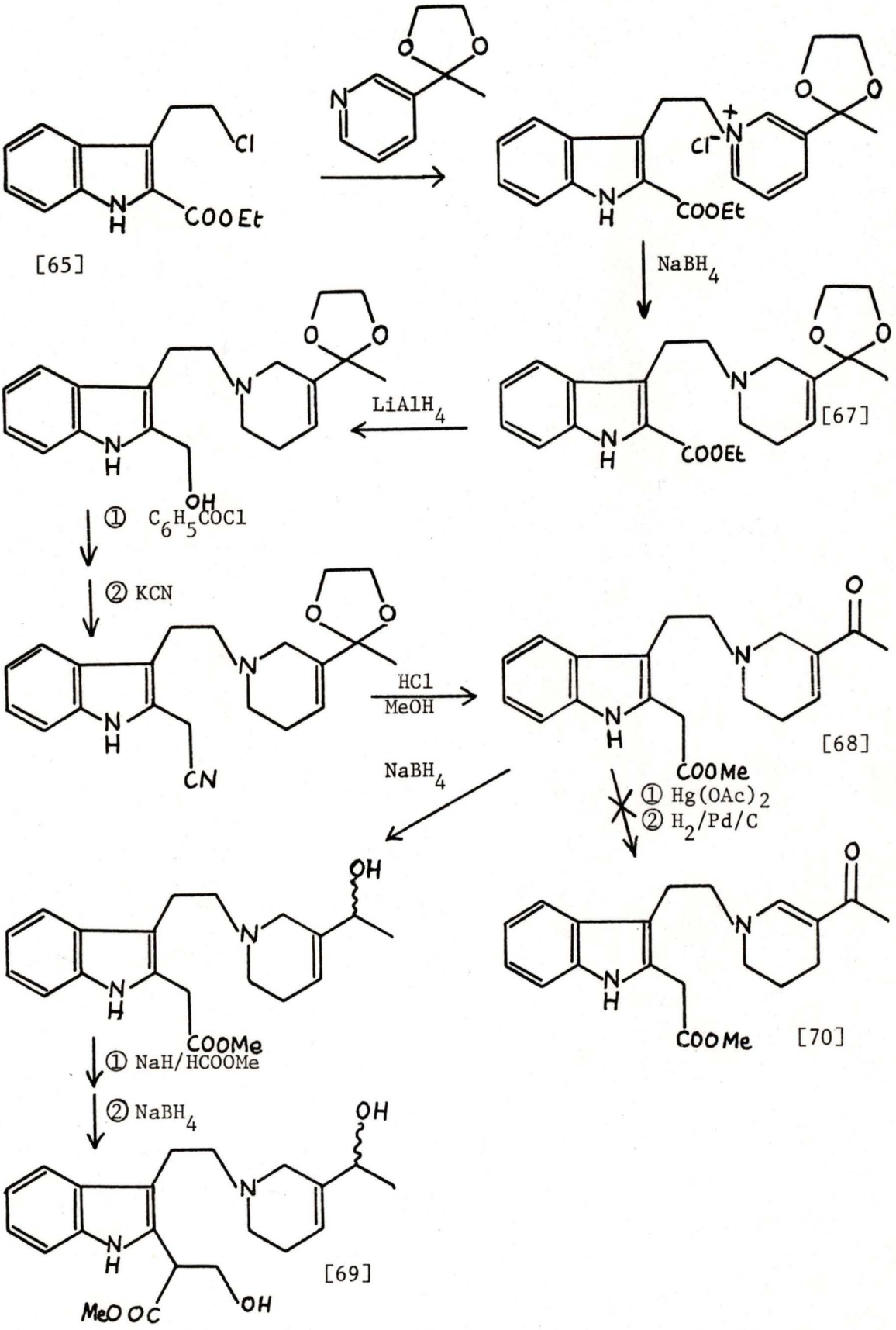


Figure 12. Alternative synthetic schemes using 3-acetylpyridine ethylene ketal.[64,65]

to hold any advantage over our route with respect to product yield and time expended.

Ethyl indole-2-carboxylate [71] was reduced with lithium aluminium hydride in tetrahydrofuran to give 2-hydroxymethylindole [72], obtained in 90% yield after crystallisation. Benzoylation of the alcohol with benzoyl chloride gave the 2-benzoxymethylindole derivative [73] in high yield (86%) as a white solid after crystallisation. Displacement of the benzoate group with cyanide ion furnished the nitrile [74]. Improved conversions were made possible in this reaction by using dry dimethylformamide as the solvent, with a large excess of potassium cyanide and heating at 80°C for a much longer period (24 hours) rather than the shorter times used formerly. The nitrile was obtained as pale-yellow crystals after chromatography and crystallisation, in 85% yield.

Methanolysis of the nitrile [74] in 99% v/v methanol saturated with hydrogen chloride gas, gave the carbomethoxy ester [75]. Again, experience derived from several repeated preparations, allowed the reaction conditions to be optimised such that it became possible to obtain yields of nearly 80% after purification by chromatography. The best yields were obtained by allowing the heat of absorption of the hydrogen chloride in the methanolic solution to raise its temperature to about 50°C, rather than carrying out this process at 0°. In the improved procedure, the rate of hydrolysis was also significantly increased

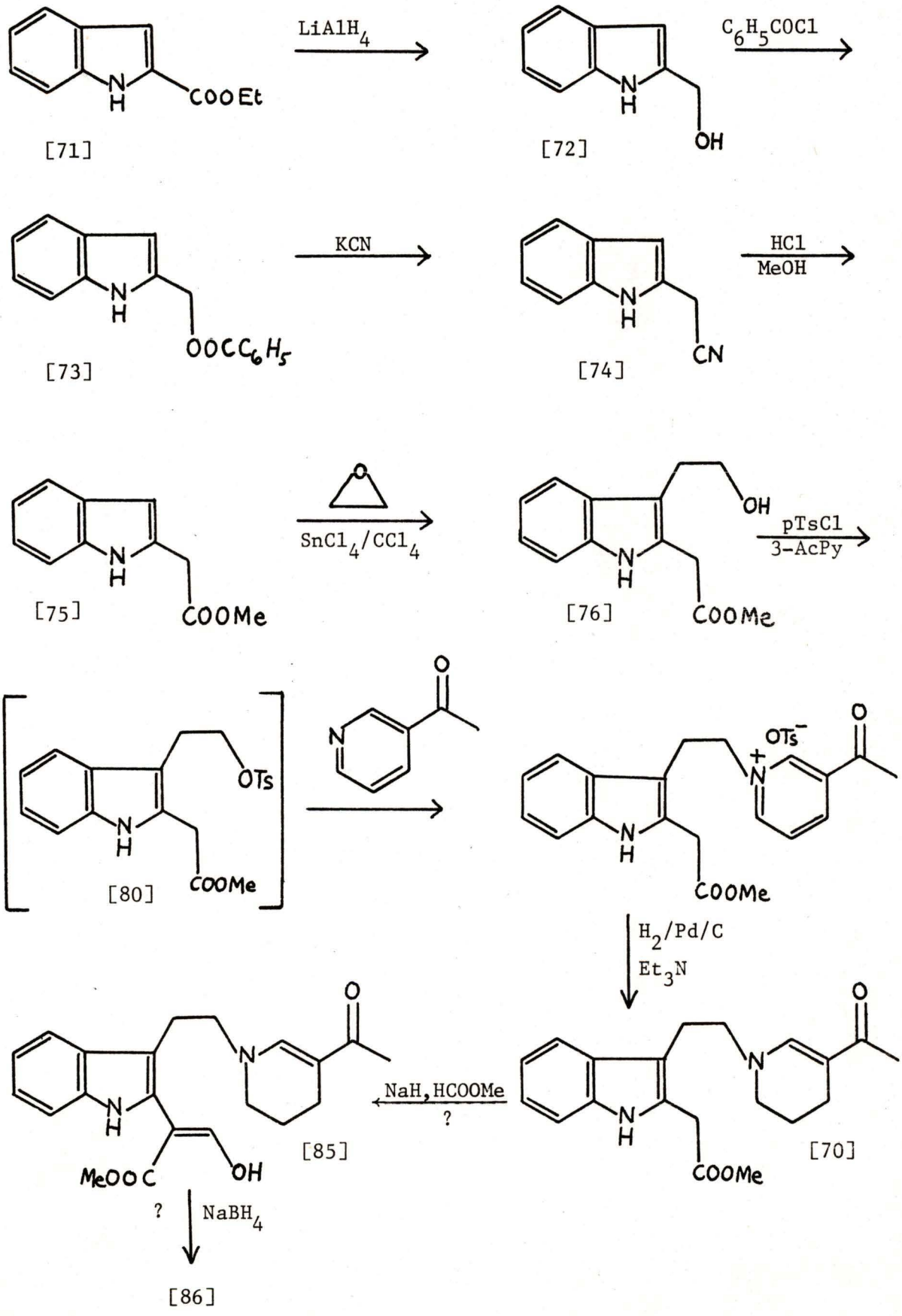


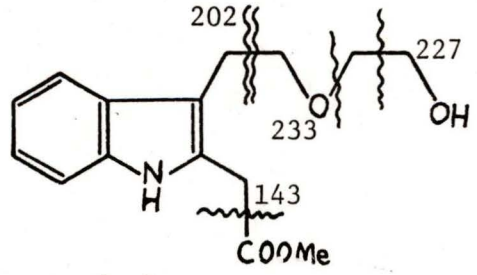
Figure 13: Synthesis of vinylogous amide [70].

such that the reaction was essentially complete in 6-8 hours. A small amount (2-3%) of 2-methylindole was observed as a by-product of this reaction. The compound was identified by its mp., mass, and pmr spectrum, which showed signals due to the indole C-3 proton at τ3.88 and methyl group at τ7.75. 2-methylindole presumably arises from acid decarboxylation of any carboxylic acid also formed in the hydrolysis.

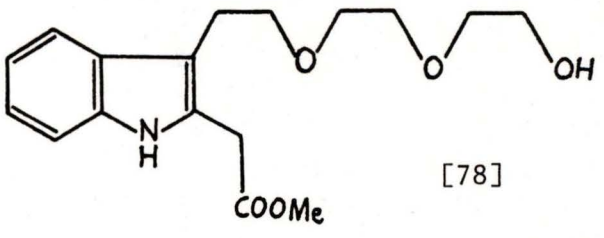
The next step was the introduction of the two carbon bridge at C-3 of the indole nucleus, which could be inserted under relatively mild conditions using a modified Friedel-Crafts alkylation reaction with ethylene oxide and stannic chloride.[67] The yield of methyl 3(β-hydroxyethyl) indole-2-acetate [76] obtained in this reaction was variable, but consistantly poor, the best conversion obtained being only 25% overall. This may be compared with tryptophol itself (having no indole C-2 substituent) prepared by this procedure in a yield of 50%.[67] On most occasions some starting material [75] could be recovered after chromatography and reused. Other reaction products were always observed (by tlc), and sometimes little if any alcohol [76] was isolated. In the latter case, large amounts (up to 20%) of the hydroxyethyl derivative [77] of the alcohol, or even higher molecular weight material suggestive of compound [78], were often isolated. The presence and amount of these bothersome side-products did not appear to be directly correlated

with the reaction conditions chosen, but generally substitution of the carbon tetrachloride with another solvent, e.g. chloroform, concentrated solutions, or use of large excesses of ethylene oxide (>2 equivalents), seemed to give reduced yields of the desired alcohol. The tryptophol [76] was isolated as an oil, which showed, in the nmr spectrum, pairs of triplets at τ6.22 and 7.08 assigned to the methylene groups of the hydroxyethyl side chain. Significantly the closely coupled doublet at the shift of τ3.68 in the carbomethoxy ester [75], assigned to the indole C-3 proton, was absent suggesting that substitution at C-3 had occurred as indicated.

One reaction, in which gaseous rather than liquid ethylene oxide (2 equivalents) was used in an effort to avoid the ether formation, gave just the opposite result, giving [77] in 20% yield and none of the tryptophol. The hydroxyethyl derivative [77] was a stable solid, crystallisable from benzene, and showed additional splittings at τ6.70 in the nmr spectrum, due to the polyoxyethylene-type protons. The mass spectrum showed an abundant molecular ion peak at m/e 277 and a fragmentation pattern consistent with that indicated:



[77]



[78]

Another type of polar reaction product, sometimes contributing

up to 50% by weight of the starting material, remained strongly adsorbed to the alumina used for product separation. It was possible to extract this with sodium bicarbonate solution, and carbon dioxide was evolved. Acidification and ether extraction furnished a red coloured tar which deepened in colour rapidly upon exposure to air. The infrared and nmr spectra suggested it to be a mixture of C-2-, C-3-substituted indoles containing a large amount of carboxylic acid. Thus the strong OH absorption in the ir, disappeared upon methylation with methanol and hydrochloric acid, as also did the broad singlet at τ-1.50 due to the carboxyl proton in the nmr spectrum. It is suggested that the material largely results from partial hydrolysis of the carbomethoxy ester [75] in the presence of the stannic chloride used for the alkylation reaction, under conditions not completely anhydrous.

Several attempts at ether cleavage of [77] were made in an effort to scavenge more of the required alcohol, all without any success. The reagents used were all appropriate to the cleavage of aliphatic ethers under relatively mild conditions, notably acetyl p-toluenesulphonate,[68] boron tribromide, phosphorous tribromide, lithium bromide and aluminium chloride. p-Toluenesulphonyl chloride gave the same negative result.

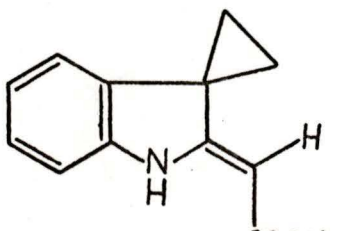
The next step in the reaction sequence involved conversion of the tryptophol to a derivative having a suitable leaving group for displacement by the 3-acetylpyridine. The resulting pyridinium salt had then to be reduced to the enamide [70]. Some preliminary model experiments relating to this part of the sequence were undertaken

using tryptophyl chloride† [79] and tosylate [80] derivatives, in condensation with 3-ethylpyridine, in order to determine whether any optimisation of product yield was possible. It was soon established that the conditions appropriate to the condensation of 2-carboethoxy-3-(β-chloroethyl)indole [65] with 3-ethylpyridine in the secodine sequence, by the Kutney researchers,[65] could not be applied to our synthesis. Condensation in their scheme was achieved by heating the two reactants in a Carius tube at 120°C for 18 hours. When 3(β-chloroethyl)indole-2-acetate [79] was condensed in this way, and the resulting piperideine after borohydride reduction examined, it was found to have lost the carbomethoxy ester group. This result suggested that either the reaction temperature was too high leading to decarboxylation, or alternatively the methylene group of the indole C-2 side chain was made sufficiently active for possible carbanion formation and subsequent reactions. Direct condensations of the tryptophyl derivatives with excess 3-acetylpyridine at temperatures of 50-70° were sufficiently mild to allow isolation of the piperideine with the intact ester group, however, the yields were invariably low (<20%). The same, or better results were obtained in our sequence using an *in situ* procedure shortly to be discussed, in which the tryptophol [76] was effectively converted to the enamide [70] in two steps without actually isolating the tryptophyl derivatives. The tryptophyl chloride, bromide, and tosylate were, however, all sufficiently stable to be isolated and characterised.

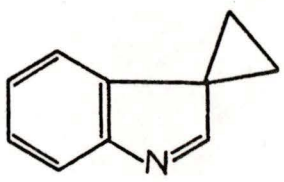
†Sample kindly provided by Mr. N. Eggers of the Chemistry Department, University of British Columbia.

The tosylate [80] was obtained as a yellow oil in 55% yield by reacting [76] and p-toluenesulphonyl chloride in pyridine at low temperature ($-30^{o}C$). Careful work-up was necessary to separate it from the chloride [79] also produced. The nmr spectrum showed two somewhat distorted doublets in the aromatic region, due to A_2B_2 type coupling of the p-disubstituted benzene ring protons of the toluenesulphonate moity, also a singlet at τ7.57 assigned to the methyl hydrogens in the *para* position.

A rather interesting side-reaction was observed when the preparation of [80] was carried out at a somewhat higher temperature, and the product exposed to basic conditions in its work-up. This led to the isolation of a small quantity of the spiro compound [81]. This compound was a crystalline solid which gave signals in the nmr spectrum at τ5.65 (singlet) and τ8.50 (A_2B_2 multiplet) assigned to the olefinic and cyclopropyl ring protons respectively.



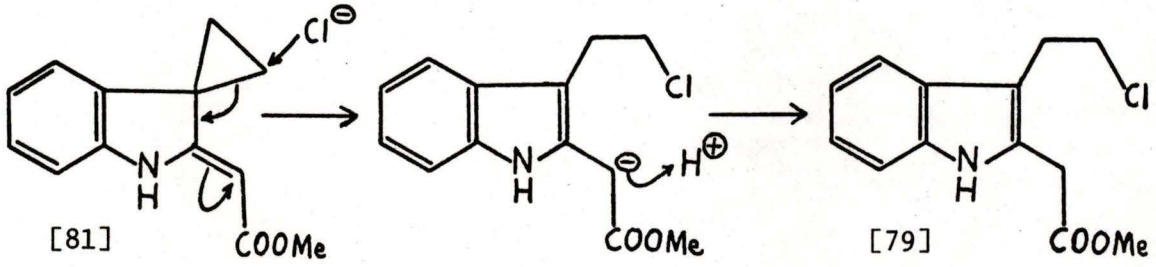
[81]



[82]

The 3,3-dimethyleneindoline [82], analogous to [81] is reported in the literature[69] to result from anchimeric participation of the indole nucleus in the potassium t-butoxide solvolysis of tryptophyl tosylate itself. Usually it was only possible to obtain minor amounts of

[81], presumably because of the facile cyclopropyl ring opening possibility in the presence of chloride ion and aqueous work-up of the tosylate. This may be rationalised in terms of the mechanism shown:

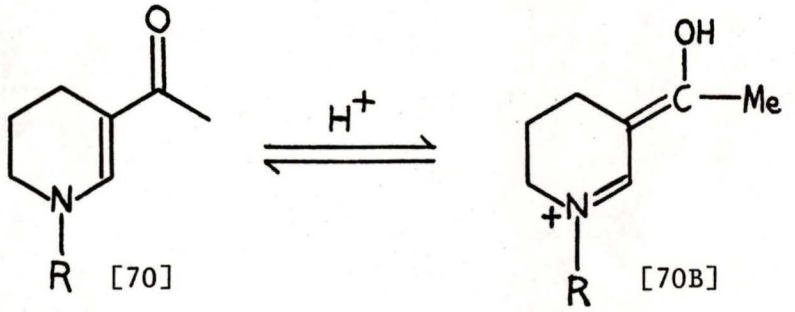


Synthesis of the vinylogous amide [70] was achieved by two types of procedure involving the *in situ* conditions. The tryptophol [76] was either reacted with phosphorous tribromide or p-toluenesulphonyl chloride, in excess 3-acetylpyridine at low temperature to achieve conversion to the bromide or tosylate derivatives respectively. Condensation was then effected by heating at 75-80°C and the crude pyridinium salt, isolated with minimal handling, was subjected to catalytic hydrogenation over palladium. After various extraction and purification steps to allow separation from the other products of the hydrogenation, the enamide [70] was isolated as a yellow crystalline solid, identical in every respect with the compound prepared from the alternative reaction sequence of Wenkert.[66]

Yields were rather better by the tosylate method (*Ca* 25%), than via the bromide derivative. Pressure hydrogenation did not improve the yield.

Certain spectral characteristics of the enamide (Figure 14) were noted for later comparisons with the products obtained further on in

the synthetic work. The ir spectrum showed absorption due to the ester carbonyl (1735 cm^{-1}) and for the vinylogous amide moity, an intense band assigned to carbon-carbon double bond stretching (1560 cm^{-1}) with a weaker higher frequency absorption (1615 cm^{-1}), assigned to the α,β-unsaturated carbonyl absorption. The hypsochromic shift and decrease in extinction coefficient upon acidification in the uv spectrum of [70] was in direct accord with the generation of the stabilised O-protonated species [70B].

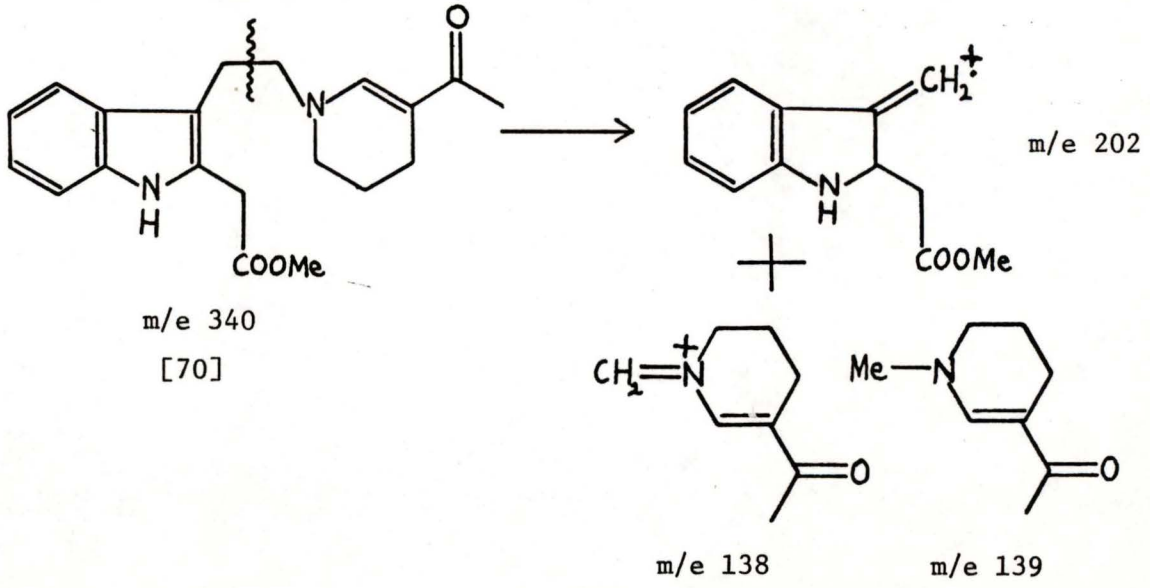


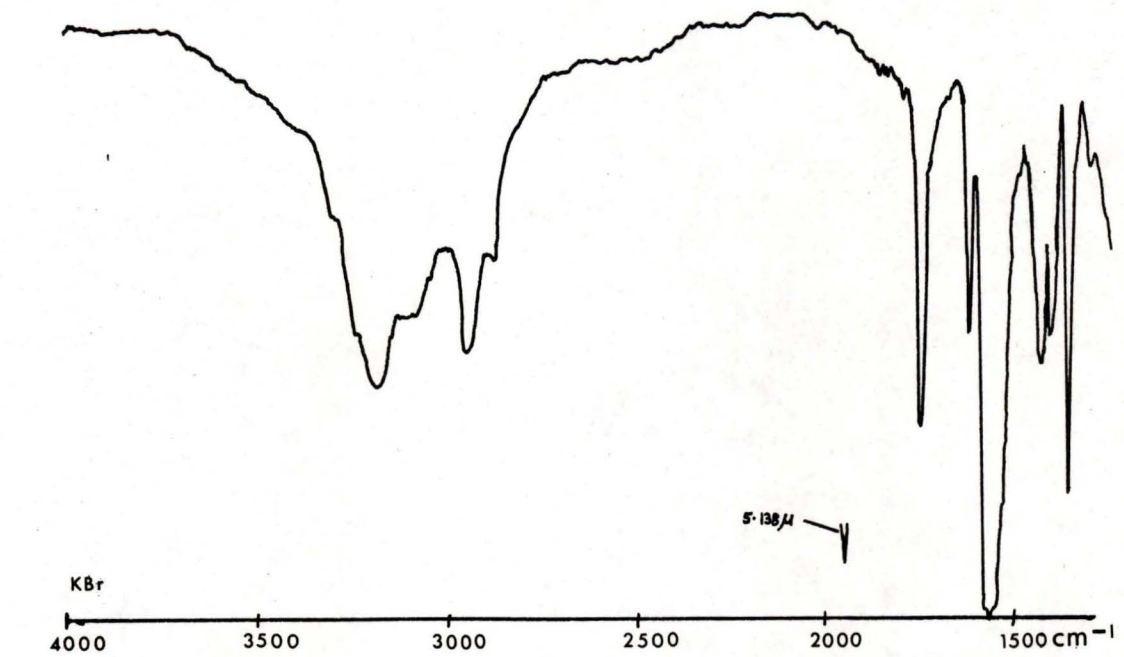
λ_{max} 316
Logε 4.60

λ_{max} 302
Logε 4.51

R = β{3(2-carbomethoxymethylindolyl)}ethyl

The mass spectrum of [70] showed the molecular ion at m/e 340, and abundant ion peaks at m/e 138, 139, and 202 associated with the primary fragmentation of [70] .





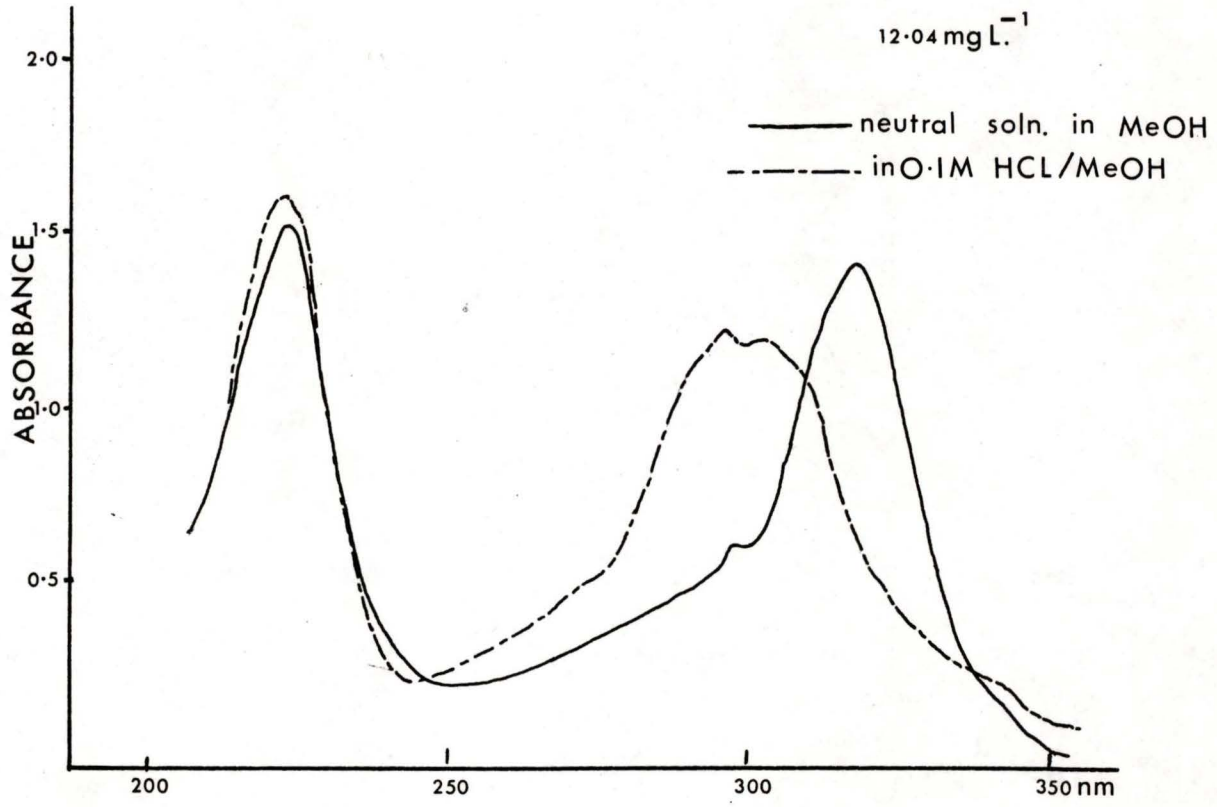
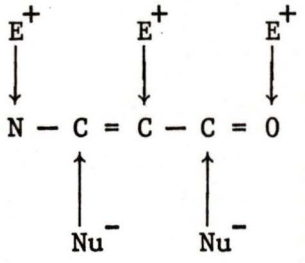


Figure 14. Infrared and ultraviolet spectrum of the vinylogous amide [70].

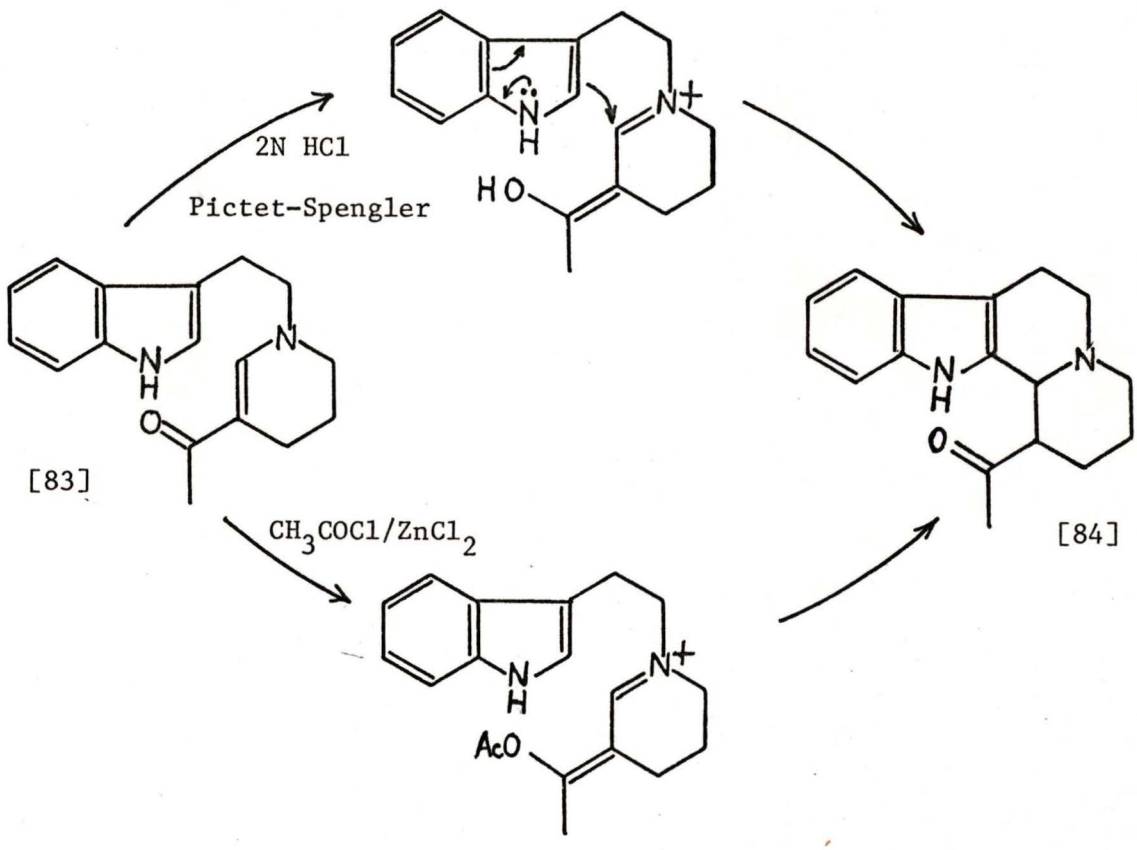
In the synthesis of secodinol[8,60] [51], the alkylation step [66] → [51], (Figure 11) was achieved via formylation with methyl formate and sodium hydride, then with borohydride reduction of the enolate. The question inevitably arose whether this reaction sequence could be applied to the enamide [70] with equal facility. This led to consideration being given to the reactivity of the vinylogous amide system.

A survey of the available literature rather surprisingly revealed that comparatively little systematic chemistry of enamides had been attempted. It was however, generally known that all the atoms comprising the system present possible centres for a reaction. There are in fact three nucleophilic sites available for attack by electrophiles (E^+), and two electrophilic sites where nucleophilic addition (Nu^-) could occur, as shown:



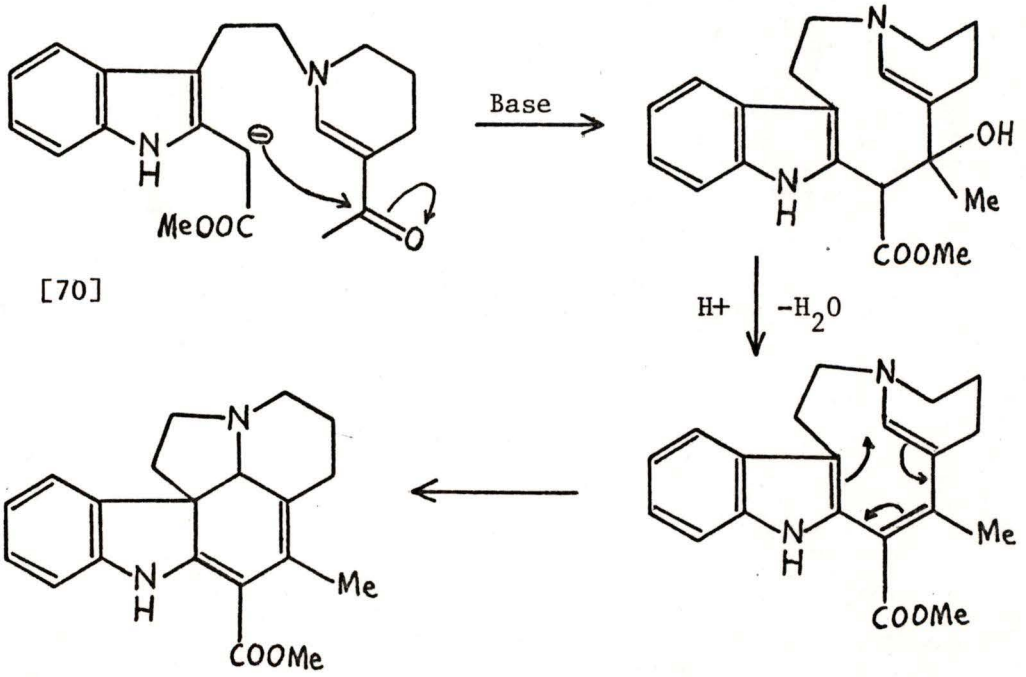
The type and preference for substitution depends on whether the vinylogous amide is cyclic or acyclic, and whether the carbonyl group is *cis* or *trans* to the nitrogen atom. The structure [70] constitutes a cyclic transoid case. Such a system as this appears to be nucleophilic only at the O-atom, producing only O-alkylated or acylated derivatives.[71]

The enamide can however, under such a circumstance, become activated at some other centre allowing subsequent reactions to occur. A good illustration of this was a reaction observed by chance in our laboratory, in connection with independent synthetic routes to the acrylic ester [56]. This reaction, analogous to the Pictet-Spengler cyclisation of [83] noted by Wenkert,[52] involved an attempted condensation of [83] with acetyl chloride or methyl pyruvate to introduce a side chain at C-2 of the indole. Rather than any substitution occurring, all that was isolated was the tetracycle [84]. Clearly nucleophilic attack from the α-position of the indole, on C-2 of the activated enamide (imonium salt) occurred after O-acylation, as indicated:



With regard to nucleophilic substitutions, of more relevance to the proposed alkylation of [70], any detailed investigations with regard to monocyclic *trans* vinylogous amides have yet to be reported. Other members of this laboratory[64] had already noted that such systems did not add phenylhydrazine or form an oxime; however, with lithium aluminium hydride, reduction of the double bond occurred.[64] It also has been reported that certain active methylene containing compounds will condense with vinylogous amides under certain conditions.[72]

In the light of all these results it still appeared worthwhile to go ahead with the attempted alkylation of [70] in the same way as the secodine sequence, but mindful of the possibility of complications introduced through the enamide system. Product analysis to detect the presence of the intact enamide was an obvious necessity. Just one of the possibilities which could be conceived, with the generation of a reactive carbanion in the ester chain of [70] is illustrated:



[70]

A prior experiment designed to assess the stability of the enamide moity of [70] to nucleophilic bases in the formylating medium was devised, using N-hydroxyethyl-3-acetyl-1,4,5,6-tetrahydropyridine† as the "model" compound. When a mixture of this compound, sodium hydride, methanolic sodium methoxide, and methyl formate in benzene-tetrahydrofuran was heated at 35°C, the compound was recovered unaltered. Evidently the vinylogous amide was resistant to these reagents.

Alkylation of [70] was first attempted using as nearly as possible the same conditions appropriate for the piperideine [66] with excess methyl formate and sodium hydride as the base in benzene at 35°C. Although very carefully dried equipment and reagents were used throughout, no reaction occurred and only starting compound was recovered. More forcing conditions, using higher temperatures (up to 60°C) and prolonged reaction times gave the same disappointing result. In a control experiment using NaH/benzene and a D_2O work-up, deuterium substitution at the methylene adjacent to the carbomethoxy ester group of [70] was verified from the pmr and mass spectrum. The result clearly indicated that, although for some reason no formyl group had been introduced in the above reaction, at least the first necessary step of carbanion formation had occurred.

Another formylation of [70] was attempted in which a catalytic amount of sodium methoxide in methanol was added to the hydride. The

†Sample kindly provided by Mr. B. Herten of this laboratory.

result initially appeared much more encouraging. Solid material, taken to be the salt of the enolate [85] separated from the reaction mixture. Pmr spectral examination of the whole product after work-up (suggested to be two components by tlc), notably revealed a broad singlet at a shift of τ-3.10. This was assigned unequivocally to the enol proton of the tautomer [85], since only enol protons absorb at such an inordinately low field. A singlet at τ0.10 was assigned to an aldehydic proton. Without further characterisation, the crude material was immediately subjected to borohydride reduction to obtain the sought-for alcohol [86]. Reduction in methanol at -20°C afforded a yellow solid which no longer showedsignals for the enol and aldehyde protons in the pmr spectrum. Chromatography cleanly separated two components, but only the one present in major amount appeared to have retained the carbomethoxy ester group intact, therefore meriting further attention. All the spectral features of this compound were in accord with preservation of the piperideine and indole portion of the molecule, by spectral comparison with [70]. In the pmr spectrum the signal corresponding to the carbomethoxy ester appeared as an apparent singlet at τ6.35, overlapping with a one proton signal, giving an integral total of 4 protons, compared to the rest of the spectrum. Significantly, the peak at τ6.31, assigned to the methylene group of the ester side chain of [70] was absent from the spectrum; instead an additional singlet corresponding to two protons was observed at τ6.00. A hydroxymethyl substituent in the ester side chain might be

expected to show its presence, in the form of additional spectral features associated with spin-coupling of methylene and methine protons. No such evidence was in fact observed; however, a similar lack of coupling has also been noted for the alcohol [88]† which resembles [86], except for having no indole C-3 substituent. [88] rather interestingly, also showed an apparent singlet corresponding to two protons, very close in shift to the τ6.00 peak observed for the reduced product.

A broad, intense absorption in the ir spectrum of the solid could not be held as definitive evidence of alcohol OH-stretching, since a similar intense absorption has also been noted for the indole NH of the vinylogous amide [70]. Finally, but by no means of lesser importance, was the mass spectral finding that undeniably provided convincing evidence against the product of reduction corresponding to [86]. The peak at highest mass in the spectrum, corresponding to m/e 368 was two units less than the parent ion for [86]. This result alone was of no great significance since many primary and secondary alcohols often show only very small molecular ion peaks and can give rise to M-2 and M-3 ions; however, absolutely no fragmentation corresponding either to the loss of H_2O, OH, or CH_2OH, an obvious expectation for an alcohol corresponding to [86], was detected. All of these essential fragmentations have been recognised in the case of the "model" alcohol [88].

Unfortunately insufficient material was available for any further examination, so another reaction was carried out. The reaction conditions of the previous experiment were reproduced as nearly as possible,

†Structure shown on page 55.

but the outcome was totally different. A bright-red coloured oil was obtained which lacked the pmr signals corresponding to the enolate [85]. A mixture of two compounds was again indicated by tlc, but after separation, only the major component, a red solid, was shown to have retained both the carbomethoxy ester and enamide moities, therefore warranting further investigation. Although not the required enolate, this compound was of some interest on account of additional features not found in the pmr spectrum of [70]. Notably the broad singlet associated with the indole NH proton in [70] was absent from the spectrum, and a new sharp one proton singlet was apparent at a somewhat unusual shift of τ1.74 which was not removed with D_2O. An additional three proton singlet was also present at τ6.70. A speculative interpretation for the lowest field peak, whose position correlated quite well with quoted shifts[73] for a formyl proton bound to a heteroatom [HCO-O, τ1.8-2.0, or HCO-N<, τ1.9-2.1] was that preferential formylation at the indole nitrogen had occurred after base removal of the N-H proton to form the nucleophilic indolyl anion. The more acidic indoles can form salts when treated with bases such as potassium t-butoxide and sodium methoxide. While carboxyl and carbonyl substituents on N-1 are particularly prone to rearrange to C-3 of the indole, the latter position was already substituted. The compound appeared to be resistant to amide hydrolysis with concentrated hydrochloric acid. The three proton singlet could fit equally well with an $>N-CH_3$ or $O-CH_3$ group. In any event the molecular weight of this compound, 386 from vapour

pressure measurements, and possibly 420 or 438 from the mass spectrum, was far in excess of that calculated for [85], (i.e. 368).

When the alkylation attempt was repeated using sodium methoxide alone as the base, no material corresponding to the enolate [85] was obtained, and it appeared that at least partial decomposition of [70] had occurred to give other products of no interest. Potassium t-butoxide, a stronger base than sodium methoxide, also gave no products corresponding to [85], but at least the starting compound was largely recovered.

A factor associated with the reluctance of [70] to undergo formylation, could have been the low solubility of bases such as sodium hydride, in the reaction solvents (benzene and/or tetrahydrofuran). With this in mind, further formylation attempts using the benzene soluble bases lithium diethylamide and 1,8-bis(dimethylamino)naphthalene ("proton-sponge") were carried out. Both are notable as strong bases, with the latter having the remarkable feature of also being non-nucleophilic. The results, however, were again very disappointing. Lithium diethylamide (prepared from phenyl lithium and diethylamine[74]) gave no enolate, and a large amount of "base-line"† material indicating destruction of the starting compound, which was recovered only in small amount. Surprisingly, the other base was totally without effect, and a quantitative amount of [70] was recovered unchanged.

Another strong base, trityl sodium, while less preferred, has been used as a substitute for sodium hydride in the alkylation of the

†As defined on page 83-84.

piperideine [66] of the secodine sequence. Apart from the experimental difficulties attendent upon the handling of very small quantities of this very labile intermediate, the base was found to be totally ineffective with the enamide [70].

With several of the base variants having been explored without success, one final possibility was that the alkylation could be promoted in a solvent medium of higher solvolytic power. Dimethylformamide has been shown to considerably enhance the rate of alkylation of enolate anions.[75] Again, inclusion of dimethylformamide in the reactant medium with sodium hydride and [70] did not result in formylation.

The failure to achieve the necessary alkylation of [70] was obviously a major impediment to continued progress in the synthetic sequence as we originally conceived it. For the various synthetic alternatives worthy of consideration, two choices presented themselves, either to modify the existing route in some way making the best use of some of the intermediates already prepared, or opt for a completely independent synthetic approach to [86]. I chose the former.

Unlike the enamide [70], methyl indole-2-acetate [75] could be formylated readily, and in high yield (90%), using just the same conditions as for the piperideine [66] in the secodine sequence. This result suggested the obvious possibility of a synthetic route in which the indole C-2 side chain of the required alcohol [86] could be fully elaborated, then suitably protected prior to alkylation at C-3 and attachment of the pyridine ring, as illustrated in Figure 15.

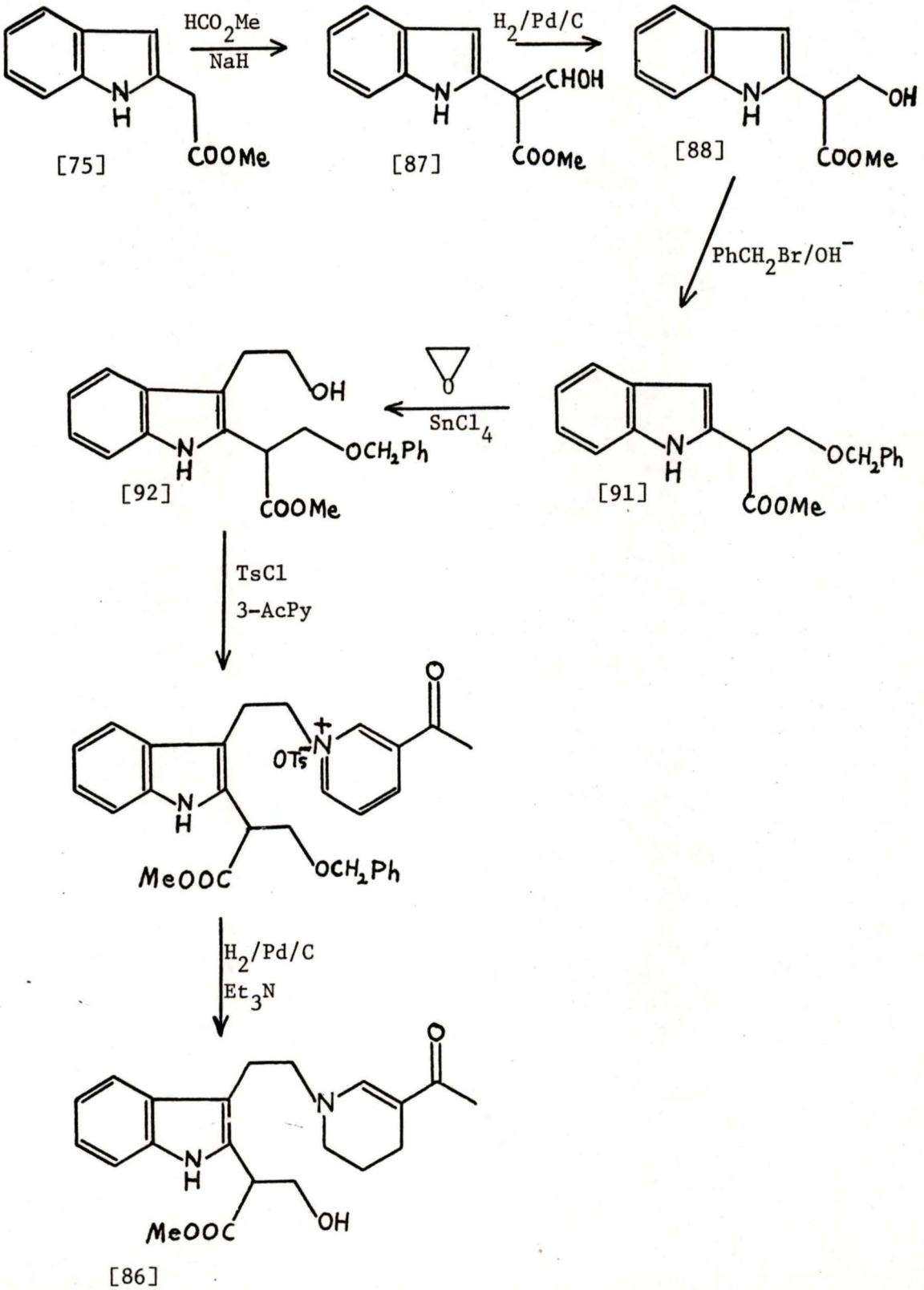
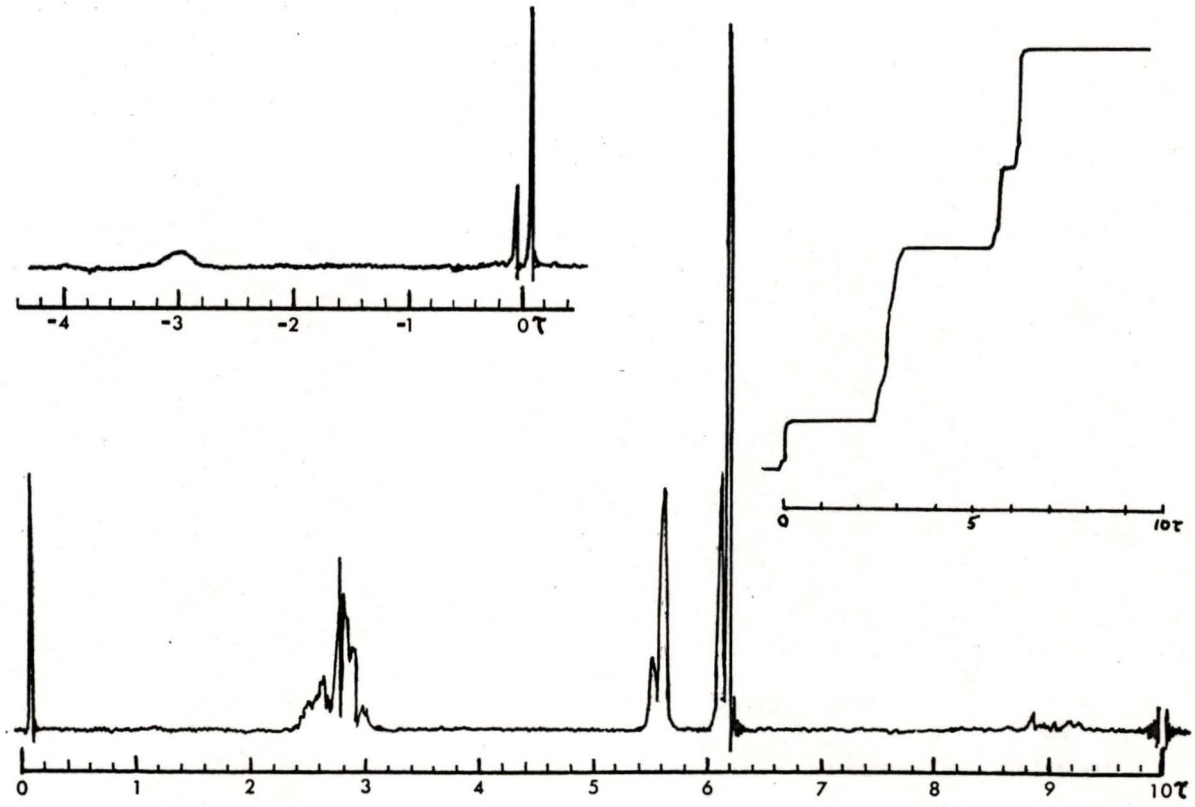


Figure 15. Alternative synthetic scheme for [86] allowing prior elaboration of indole C-2 side chain.

Before going ahead with this new sequence, some rather intriguing results obtained in the characterisation of the formylated compound [87], required clarification. The pmr spectrum (Figure 16) in particular appeared very perplexing. Defined signals expected for the indole N-H, and C-3 protons were both absent from the spectrum of this compound. Whereas the integrated peak area for the protons in the aromatic region, did suggest the indole NH could be included in their multiplet, the C-3 proton normally always appeared as a well-defined singlet in the region τ3.6-3.8 as in, e. g. methyl indole-2-acetate. Various other features in the spectrum appeared strange, including the small peaks apparent at τ-0.05 and τ5.53. In addition, the spectrum appeared remarkably simple for that expected for the enolate [87] where splittings corresponding to the coupling of two methine protons implicit in the enol tautomer, would be predicted. The notable absence of any consistancy of peak separation over the entire spectrum suggested that there was indeed no coupling between any of the protons. This conjecture was also supported by both spin decoupling, with irradiations at the resonant frequency corresponding to the aldehyde proton (595 Hz), and the singlet at τ5.62 (263 Hz), also from the addition of a shift reagent [$Eu(FOD)_3$]. That any of the features observed in the spectrum could be associated with impurities in the compound seemed unlikely bearing in mind that the material was a well-defined, crystalline solid with a sharp melting point and tlc characteristics of one component. Dimerisation of the indole ring through C-3, could adequately account



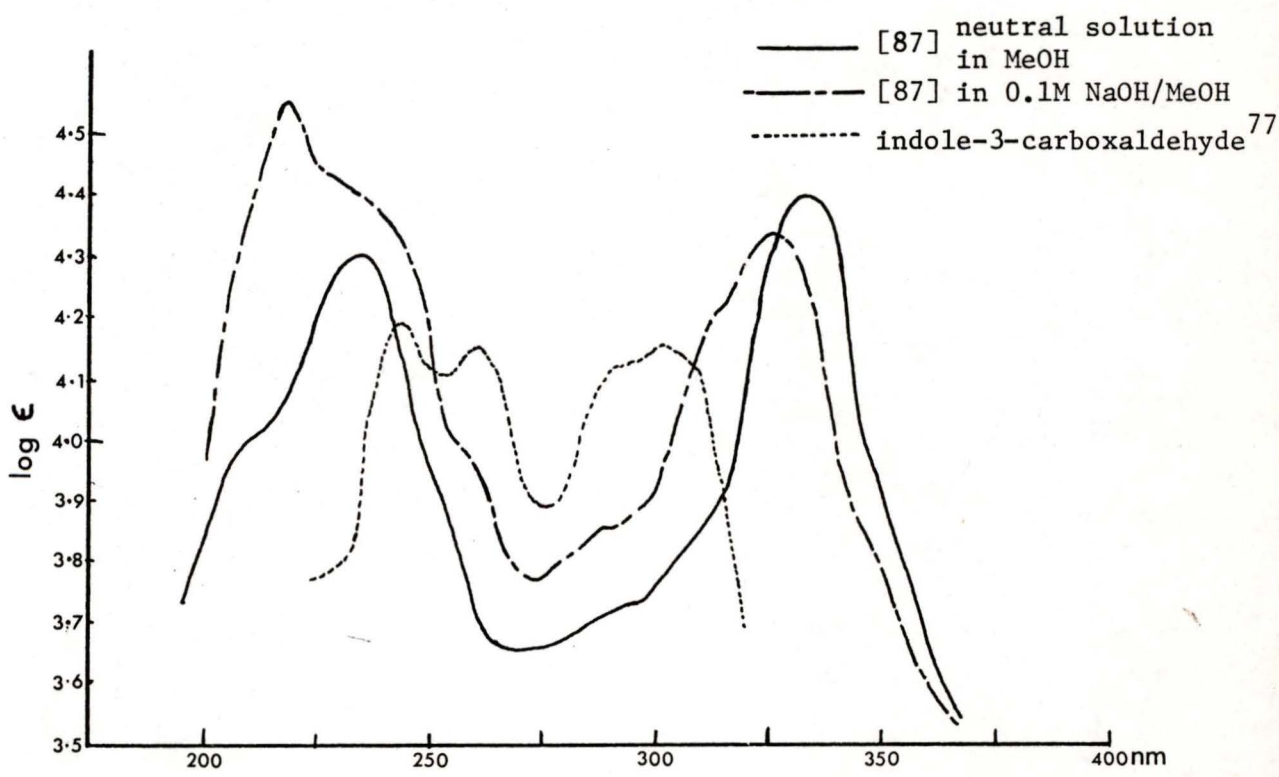
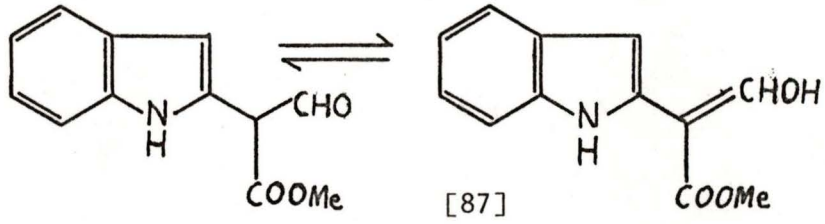


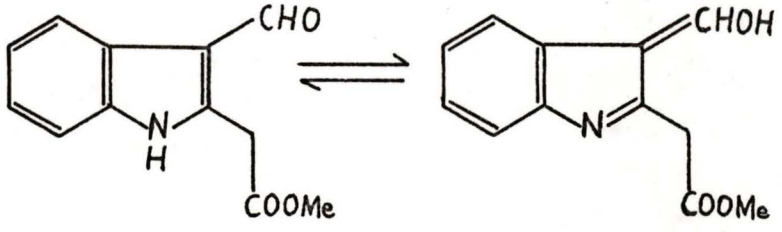
Figure 16: Pmr and uv spectrum of the formyl compound [87].

for the observed absence of the C-3 proton. This possibility was excluded on the basis of the mass spectrum, which showed an abundant molecular ion peak corresponding to [87], and from the molecular weight obtained by vapour pressure osmometry (228 ± 3%, c.f. calculated 217.2).

These results suggested the possibility of one valid alternative structure [87B] for the compound. Both [87], and [87B] are capable as existance as tautomers, and [87B] could nicely account for the absence of the C-3 signal in the pmr spectrum, while still displaying the observed enol and aldehyde proton peaks.



[87]



[87B]

A conjecture for the formation of [87B] would be that formylation at the indole nitrogen had occurred as an initial step in the reaction, followed by subsequent rearrangement of the formyl group to C-3. This mechanism was mentioned previously. That the compound isolated was not

N-1 formylated was readily apparent from the pmr results. Chemically, both alternatives could have broadly similar properties. The compound had a notable acidity, resembling indole-3-carboxaldehyde in this respect, and was soluble in dilute aqueous sodium hydroxide. The rather high melting point observed (173-4°C), attributed to the high polarity and effective hydrogen bonding in the solid, would also not be in conflict with a C-3 formyl substituent.

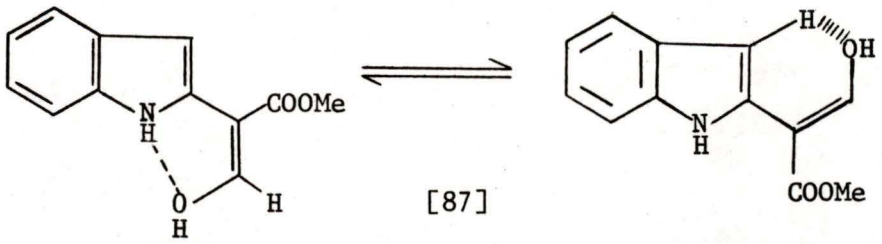
The infrared spectrum of the compound conveyed some information, if anything tending to support the alternative structure [87B]. Thus the spectrum showed a split carbonyl band at 1630 and 1610 cm^{-1}. This may be compared with a similar band in indole-3-carboxaldehyde (1634 and 1618 cm^{-1}).[76] Another feature was the presence of definitive indole NH stretching absorption (at 3700 cm^{-1}), showing conclusively that there was no substitution at N-1. The mass spectral result could do little to resolve the structural ambiguity, the two possibilities [87] and [87B] having the same molecular weight and giving predictable fragmentations consistant with what was observed.

That [87] <u>was</u> in fact the correct structure, only emerged after uv spectral comparisons and the use of chemical methods. The uv spectrum (Figure 15) was totally unlike that of indole-3-carboxaldehyde.[77] In addition, according to Wenkert,[77] the 3-formylindole would be expected to show a bathochromic shift in alkali due to anion formation. With [87] on the other hand, a hypsochromic shift of the highest wavelength band was observed.

Conclusive evidence supporting [87] came forth from two types of experiments. In the first ethyl indole-2-carboxylate [71] and 2-methylindole were separately subjected to the standard formylating conditions, using excess sodium hydride and methyl formate. In no case was any substitution of a formyl group at C-3 of the indole ring detected. 2-Methylindole gave an almost quantitative recovery of starting compound, whereas [71] gave essentially complete conversion to methyl indole-2-carboxylate. The latter result was easily interpreted as an ester exchange to the methyl ester with the methyl formate present in large excess under basic conditions. Any tendency for deactivation of the indole to formylation at C-3 due to the directly linked ethyl ester, was certainly not present when the C-2 of the indole nucleus was substituted by a methyl group. The other reaction involved acid decarboxylation of [87] using 6M hydrochloric acid. Any C-3 formyl substitutuent might be expected to be lost in strongly basic medium, but much less readily so in dilute acid. The major product of the decarboxylation was 2-methylindole. No trace of any material still retaining the enol/aldehyde function was found after reaction, hence it was implied that the material reacted could not have been [87B].

Finally, evidence was also obtained from the reduction of [87], the next step in the new sequence. Several of the resulting products including the required alcohol [88], showed the signal corresponding to the indole C-3 proton in the pmr spectrum, unlike [87] itself. The seemingly great reluctance of [87] to undergo the necessary reduction to [88], as indicated below, coupled with the absence of discrete indole

NH and C-3 proton signals in the pmr spectrum could possibly be accounted for in terms of a loose association or intramolecular hydrogen bonding between the indole NH group, and enol form of [87] as represented:

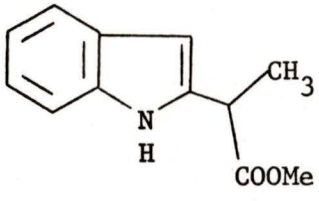


It should be said that the phenomenon of the NH proton not appearing as a separate signal in the pmr spectrum but being shifted upfield and combined into the aromatic multiplet, is by no means unique to [87]. Some other indoles including 2-methylindole have been observed to show similar behaviour in deuterochloroform.

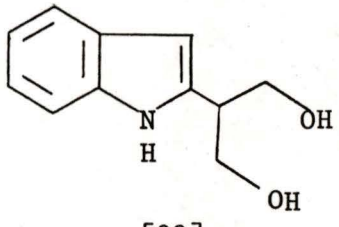
Having established the true identity of [87], the sequence next required reduction to the alcohol [88]. Sodium borohydride in methanol at -25°C, which satisfactorily reduced the enolate to secodinol [51] in the secodine sequence,[65] gave no reduction whatever. Only under very forcing conditions, using more than a thirtyfold excess of borohydride at room temperature, was it possible to achieve any reduction at all with this compound. The product mixture so formed contained only a very small amount (10%) of the required alcohol, together with unaltered [87], and material devoid of the carbomethoxy ester group. [88] gave a pmr spectrum which clearly showed a broad singlet due to the D_2O exchangeable indole NH proton at τ1.15, and a singlet at τ3.62 assigned to the C-3 proton. Significantly the peaks at τ5.53 and 5.62 in the spectrum of [87] were absent, instead a new apparent singlet was observed at τ5.99, assigned to the methylene protons of the

hydroxymethyl substituent. A multiplet centred at τ6.40 could be assigned to the methine hydrogen coupling with them. The mass spectrum (at 40 eV) was fully in accord with the structure of [88] in showing an abundant molecular ion peak at m/e 219, and major fragment ion peaks corresponding to the loss of H_2O, CH_2O, CH_2OH or CH_3O.

Borohydride in refluxing isopropanol has been reported[78] to smoothly reduce 2-methylindole-3-carboxaldehyde to the corresponding alcohol in high yield. Only 30% of [87] so treated underwent any reduction, and of this material, nothing corresponding to the alcohol [88] was detected. The major products were the hydrogenolysis compound [89] and material lacking the ester group, possibly the polar diol [90].



[89]



[90]

In the pmr spectrum, [89] showed a characteristic three proton doublet at τ8.41 (J = 7.3 Hz), and a one proton quartet at τ6.20 (J = 7.3 Hz) assigned to coupling of the methyl and methine hydrogens, while the carbomethoxy ester protons gave rise to the usual singlet at τ6.35. The mass spectrum was equally consistant for the hydrogenolysis compound.

Diborane, which normally smoothly reduced all aliphatic aldehydes to the corresponding alcohols, was not at all appropriate for a similar reduction of [87]. Instead, a complex mixture of products was produced, including a large amount of indole itself, and the hydrogenolysis compound [89]. Catalytic hydrogenation over platinum oxide gave essentially

the same type of result. Recourse to the available literature then suggested that all these disappointing results could be attributed to the abnormally high reactivity of enol-aldehydes compared to other types. This was exemplified in the ready hydrogenolysis of [87].

Phenylacetaldehyde has been successfully reduced by hydrogenation over palladium on charcoal, in acetone solution.[79] Similar reduction of [87] at atmospheric pressure resulted in a slow uptake of hydrogen. The reaction appeared to be much cleaner, with complete reduction to give two products corresponding to [89], and the required alcohol [88] in 20% yield. A better conversion than this might well be possible once experience has established the best experimental conditions. Other reducing agents of the newer, more "selective" variety appropriate to the reduction only of the aldehyde group, notably lithium tri-t-butoxy-aluminium hydride,[80] and disiamylborane,[81] either gave no reduction of the enolate, or else reaction but no useful material.

The alcohol [88] was next benzylated using benzyl bromide with silver oxide to give the benzyl ether [91] in 78% yield. Although the pmr spectrum suggested this material to be largely the O-benzylated compound, some contamination with the O-, N-disubstituted derivative was also indicated. Also in accord with this, the mass spectrum of the mixture showed the highest mass peak to be at m/e 399, appropriate for the molecular ion of a dibenzyl derivative.

The next step in the synthesis involved electrophilic alkylation at C-3 of the indole, as for the tryptophol [76]. It was envisaged that a potential problem could be introduced here as a result of the use of stannic chloride in the presence of the benzyl ether

protecting group. Lewis acids characteristically complex with the ether oxygen, while certain of the stronger types are effective cleaving agents for ethers. Benzyl ethyl ether, a model compound for [91] in the form of a concentrated solution in carbon tetrachloride was found at least to complex strongly with anhydrous stannic chloride at ambient temperature, as indicated by the signal shifts in the pmr spectrum. Using a dilute solution and under the conditions suitable to the preparation of [76], the pmr spectrum of the product after work-up verified that no cleavage to alcohol had occurred. It therefore appeared reasonable to proceed with the alkylation of [91] itself. Attempted reaction with *Ca* 2equivalents of ethylene oxide gave only unreacted starting compound, and nothing corresponding to [92].

The sequence [75] → [86] had seemed initially attractive, but the problems arising quite early in the synthesis with the poor conversion to[88] and the failure of the above alkylation step, led to yet another alternative route to [86] being considered (Figure 17). In essence, the newest proposal involved protection of the alcohol group of the tryptophol [76], followed by alkylation in the C-2 side chain of the indole and subsequent full elaboration of the C-2 and C-3 substituents. To a large measure, the success of this method would depend upon the relative stability of the protecting groups used, under the various reaction conditions. In principle either the benzyl or pyranyl ether protecting groups could be utilised. The pyranyl ether is known to be stable in the presence of bases and reducing agents, but not to acids.

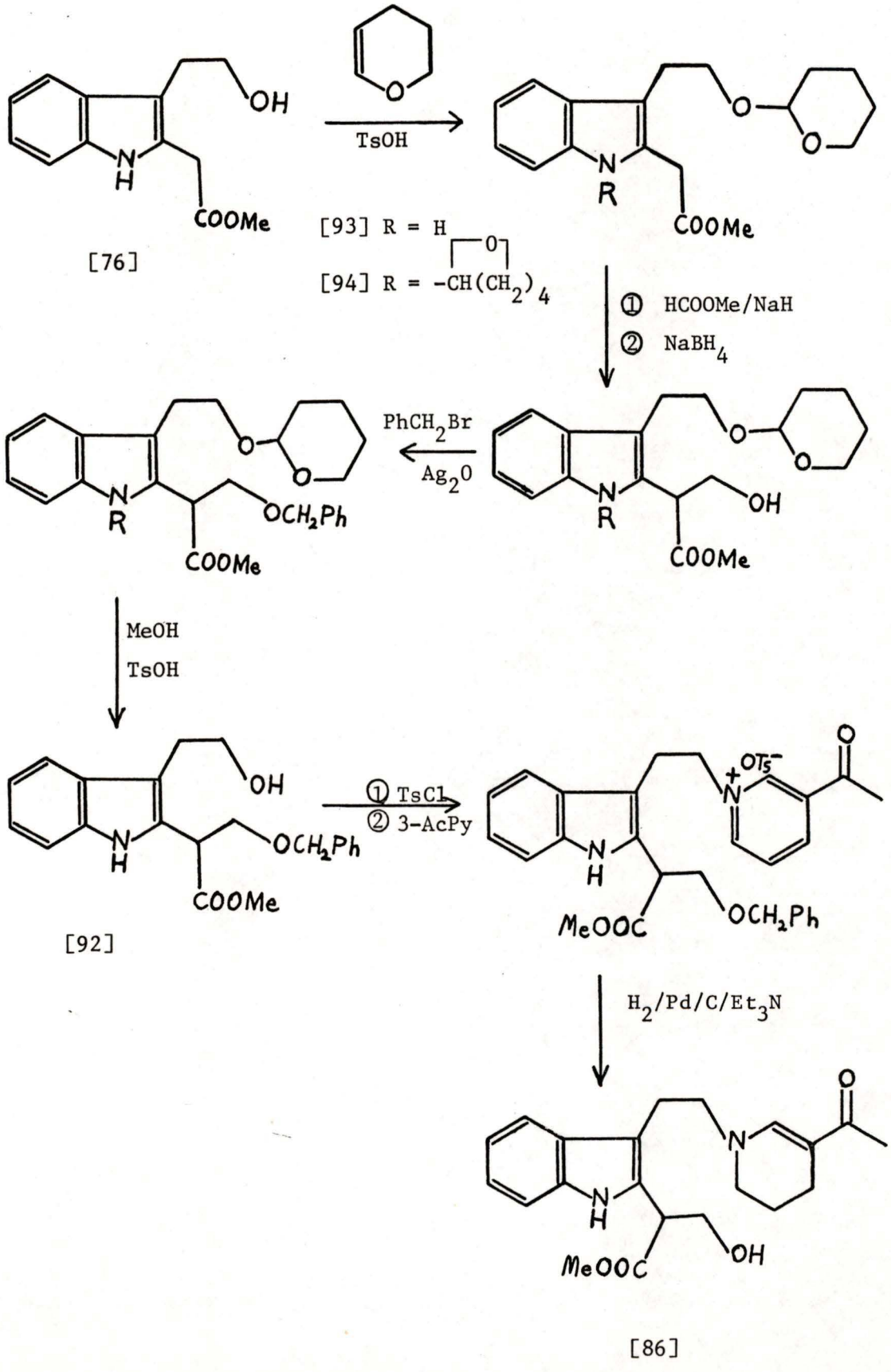
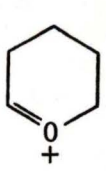


Figure 17. Alternative Synthetic Scheme for [86] Using Two Protecting Groups.

The benzyl ether, however, is said to be stable to all these types of reagents, but can be readily cleaved upon catalytic hydrogenation to regenerate the alcohol. For these reasons it appeared rather advantageous, although not a necessity to obtain first, the pyranyl, rather than the benzyl ether of [76]. This would permit easy removal of the protecting group with, e.g. p-toluenesulphonic acid in methanol. The resulting alcohol [92] could then be converted through to [86] by the standard procedure.

Reaction of [76] with excess 2,3-dihydropyran in the presence of a catalytic amount of tosic acid, gave an oily product, corresponding to a mixture of O-, and O,N-disubstituted pyranyl ethers. The monosubstituted compound [93] was the minor component (*Ca* 20% of total mixture), and, being a solid and less soluble than [94], was separable by a precipitation technique. Both compounds showed a complex multiplet of overlapping peaks corresponding to the C_2-C_4 pyranyl protons, centred at τ8.40 in their pmr spectra. [93] was distinguishable, however, by having a signal at τ1.00 associated with the indole NH proton, also giving rise to an infrared absorption band at 3400 cm^{-1}. The mass spectra of the pyranyl ethers showed the expected fragmentation pattern, and the most abundant peak in both their spectra (m/e 85) was associated with the relatively stable oxonium ion species:-



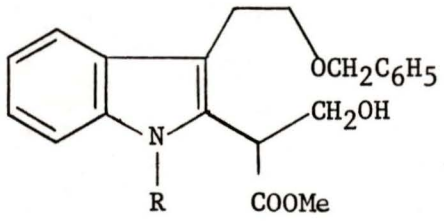
m/e 85

Attempted alkylation of [94] under the standard conditions (as for experiment (a), p. 93) but avoiding use of mineral acid in the

product work-up, gave only unchanged [94]. Substitution of sodium hydride for the stronger base trityl sodium, gave the same negative result. The lack of success with this reaction was certainly surprising, but steric complications may have been important. Unfortunately insufficient of the monosubstituted compound [93] had become available for it to have been worthwhile repeating this reaction.

Attention was next turned to the possibility of alkylation with the benzyl ether of [76], since it was assumed to be rather less likely that much N-benzylated product would be formed. Benzylation using benzyl bromide, and silver oxide (silver oxide giving better yields than potassium carbonate as the base[82]) gave in fact, a mixture containing about equal amounts of the O-, and O,N-dibenzyl ethers, with some unreacted [76]. Chromatography could only effect a partial separation of the dibenzylated compound, but a sample of the solid O-benzyl derivative was obtained in reasonably pure form, having a satisfactory pmr and mass spectrum. The mixed benzyl ethers which were used for the alkylation attempt, failed to react using methyl formate and trityl sodium, so a new method was tried. Reaction of the mixed ethers in the presence of excess formaldehyde and sodium hydride in tetrahydrofuran, gave two types of material differing in ether solubility. Only the ether-soluble fraction was of any interest. Preparative tlc on this mixture furnished a small amount (5 mg) of material having a mass spectrum strongly suggestive of the sought after hydroxymethyl compound(s), [95], [95B]. At 20ev the highest peak in the spectrum (m/e 443)

correlated with the parent ion expected for the O,N-disubstituted compound [95]. Several major fragmentation peaks near the highest molecular weight end of the spectrum were predictably derived from it, including fragments associated with the loss of H_2O, CH_2O and C_7H_7. Also observed in the spectrum was a peak at m/e 353 which could be associated with either the loss of a benzyl group from [95], or more likely, the molecular ion corresponding to the O-benzylated derivative [95B]. Evidence for the latter was also supported by the position of the base peak in the spectrum (at m/e 335) corresponding to the elimination of water from [95B].



[95] R = $-CH_2C_6H_5$

[95B] R = H

Some additional evidence for the alkylated material came from the infrared spectrum which showed, both in the solid phase and in solution, a broad intense absorption band at 3450 cm^{-1}. Allowing for any absorption due to indole NH stretching which is usually somewhat weaker than what was observed, this band could be tentatively assigned to the OH stretching mode for the alcohol. Although a note of optimism was raised by the result of the last experiment, a similar treatment of methyl indole-2-acetate [75] with formaldehyde disappointingly failed to give anything corresponding to the previously isolated and characterised alcohol [88], or formyl derivative [87]. Further investigations using formaldehyde are underway.

In closing this part of the discussion it should be mentioned that while the research was continuing, another member of this laboratory has been concerned with independent synthetic routes to [56]. Despite some earlier frustrations, one reaction scheme at the moment appears to hold promise (Figure 18).

Acylation at the indole C-2 position of tryptophyl tosylate [96] with methyl glyoxyl chloride [97], in the presence of a catalytic amount of zinc chloride, gave [98] in 63% yield. Displacement of the tosylate group with 3-acetylpyridine, gave the salt [99] in a yield of 90%. Catalytic reduction should produce the vinylogous amide [100] from which complete elaboration of the C-2 side chain might then be achieved.

Once a viable route to the synthesis of the acrylic ester [56], has been demonstrated, biochemical evaluation of the new precursor can be initiated.

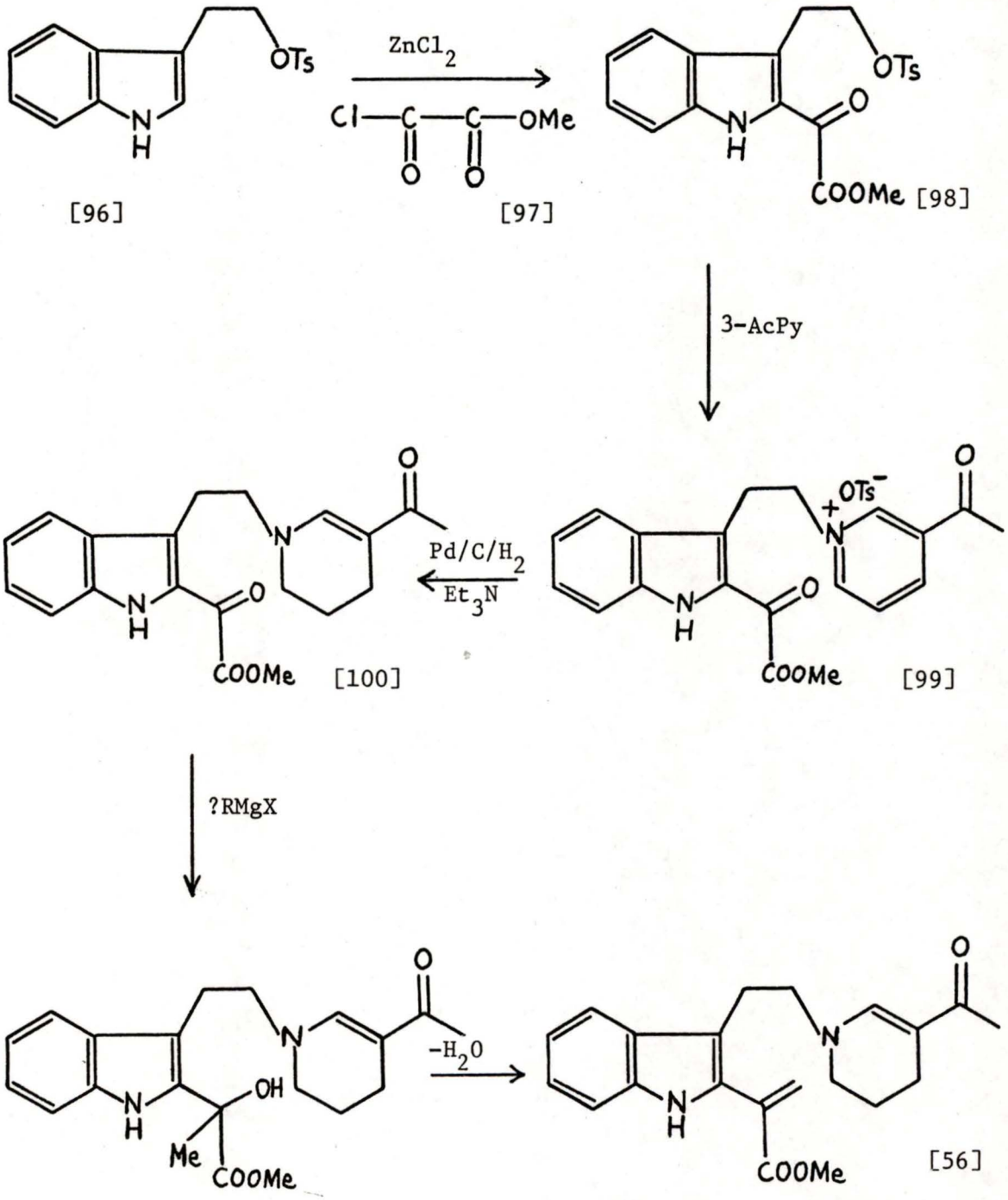
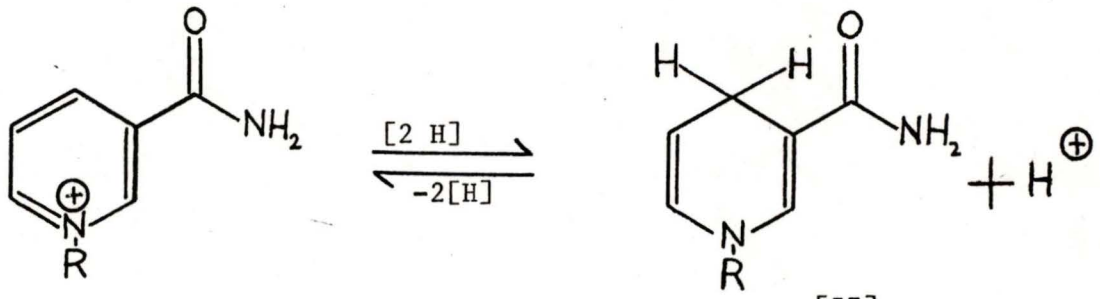


Figure 18. Independent scheme for [56].

DISCUSSION

PART II

Although it is well known that dihydropyridines in general are unstable entities,[60,61] the 1,4-dihydro isomer, substituted with an electron-attracting group at the 3- position, has been recognised as being relatively much more stable than any of the others. Such 1,4-dihydropyridines do not undergo oxidation as readily, and are the ones preferentially formed in laboratory syntheses involving condensations and cyclisations. They have also been observed in nature. Famous examples are the reduced forms of the coenzymes diphosphopyridine nucleotide, DPNH [57], and triphosphopyridine nucleotide, TPNH. These enzymes occur in all animal and plant tissue and are derived from either nicotinic acid or vitamine B6.

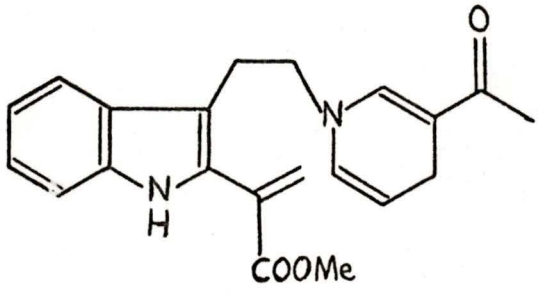


[57]

R = - ribose - phosphate$^{\ominus}$ - phosphate - ribose - adenine

Karrer, in the 1930's, showed that the stabilised 1,4-dihydro compounds which would serve as models of [57], could be generated by sodium hydrosulphite (dithionite) reduction of the corresponding pyridinium salts.[83a-e] He originally suggested that reduction occurred at the 2- position of the pyridine ring, to yield the 1,2-dihydropyridine rather than the 1,4-isomer. Deuterium labelling and other means has since proven that the former supposition was incorrect.[84,85]

Since the implicated biointermediate(s) [42], [42B] are thought to be dihydropyridines, the relevance of the foregoing to the present research activity assumes special importance. When and if a workable synthesis of the acrylic ester [56] materialises, it would be of considerable interest to also synthesis and biochemically evaluate the corresponding dihydropyridine compound [101]



[101]

While this precursor being a 1,4- rather than the 1,2-isomer, would not be an ideal model for the true biointermediate(s) it is unlikely for the reasons already considered (Part 1 of thesis discussion) that these intermediates themselves could ever be prepared and evaluated.

Attendent upon any proposed synthesis of [101] would be the necessity to consider the stability of the dihydropyridine moiety to the various types of reagent used in the preparation. Ideally it is envisaged that the reactive dihydropyridine system would only be generated at the latest step in the sequence, but in practice this might not be possible.

With this in mind, some investigations of a preliminary nature were undertaken to show how two such 1,4-dihydropyridines could be produced, (Figure 19) and some assessment of their stability made. One of these compounds [103], bearing an N-β-(3-indolyl)ethyl substituent would serve as a reasonable model for the precursor [101].

The formation and disappearance of the dihydropyridine system in any type of reaction could be very easily and conveniently monitored by changes in the highest wavelength band in the uv spectrum. The dihydropyridine in conjugation with the 3-acetyl function characteristically gives rise to an absorption band at *Ca* 370 nm, while the highest wavelength band of the corresponding pyridinium salt is only of the order of 260 nm.[83,86] Karrer surprisingly made no mention of the reduction of a pyridinium salt having the 3-acetyl function, although he looked at many other substituents. Some more-recent work[86,87] relates to the reduction of 3-acetylpyridinium methiodide but no compound corresponding to [103] had hitherto been prepared.

The limited solubility of N-[β-(3-indolyl)ethyl]-3-acetylpyridinium bromide [102] in most solvents presented some early practical problems

with the hydrosulphite reduction (Figure 19) mainly on account of the need to also have the inorganic salts ($Na_2S_2O_4$, Na_2CO_3, or $NaHCO_3$) in solution. A heterogeneous reaction using aqueous dimethyl sulphoxide, gave only a partial reduction of the salt [102].† A stirred interfacial technique using chloroform and water gave much improved results, and a virtually quantitative conversion to [103] was achieved. The progress of the reaction could be followed by observing the gradual dissolution of the salt and the appearance and intensification of the uv absorption at 374 nm. The crude product, an orange-yellow coloured solid, could be stored under a dry nitrogen atmosphere for short periods, but it showed noticeable decomposition in air after about a day. In solution (e.g. chloroform), the compound appeared to be stable for much longer periods. All attempts to purify the crude substance were fruitless, and merely resulted in accelerated decomposition as revealed by the loss of the 374 nm band. For this reason a spectral characterisation was carried out on the crude material, soon after its preparation. Details of the ir, uv, and pmr spectra are reproduced in Figure 20. The ir spectrum showed characteristic absorptions at 1610, 1560, and 1675 cm^{-1} associated with the diene and carbonyl groups respectively. In the pmr spectrum, signals arising from the vinylic proton at C-2 of the pyridine ring (at τ3.51), and from couplings of the C4-6 protons (at τ7.03, 5.18, and 4.34) were observed. Coupling of the C-5 proton, with those at C4 and C6, interestingly gave rise to a doublet of triplets (at τ5.18), with only five of the peaks resolved.

†Sample kindly provided by Mr. B. Herten of this laboratory.

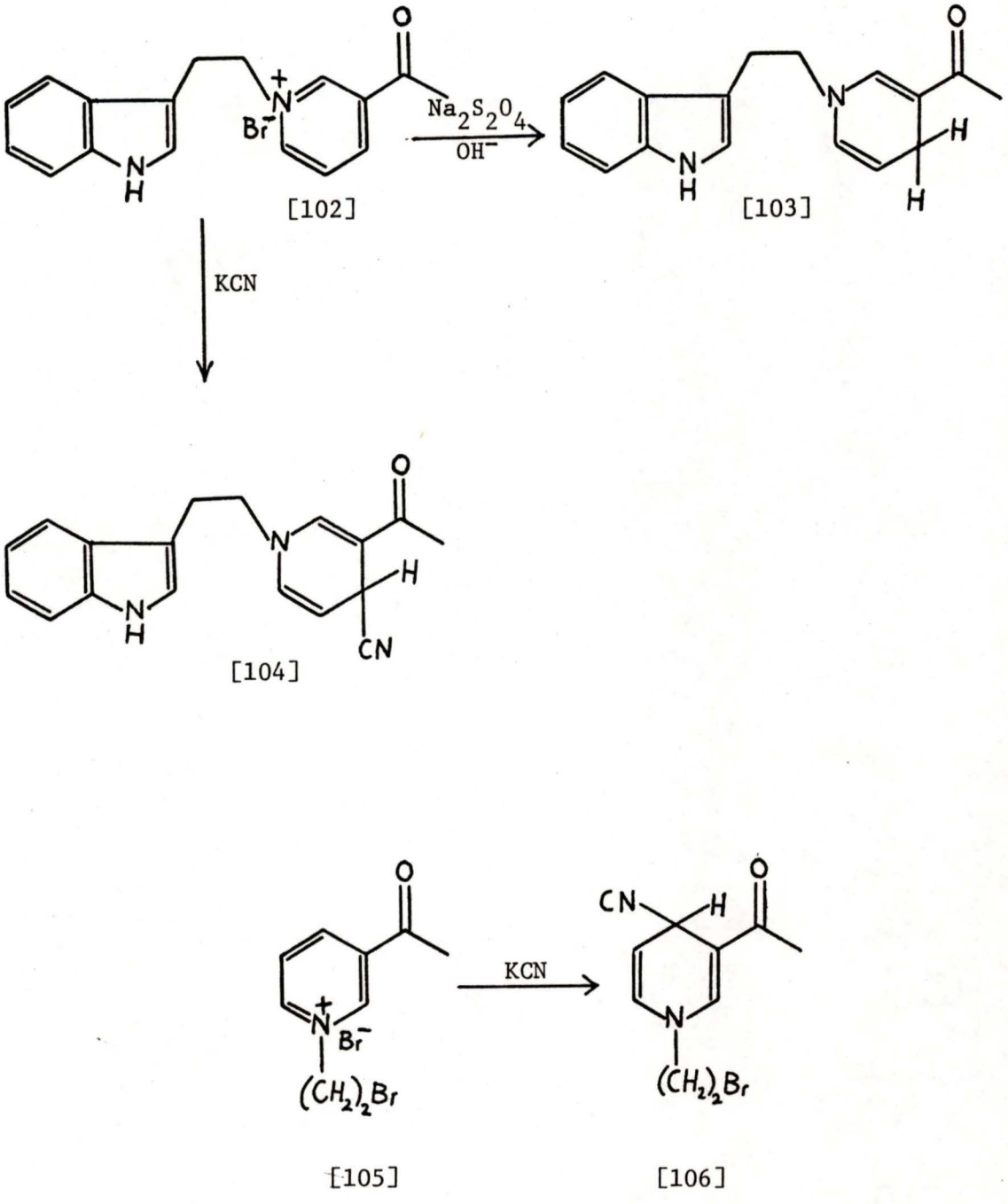


Figure 19. Generation of 1,4-dihydropyridines.

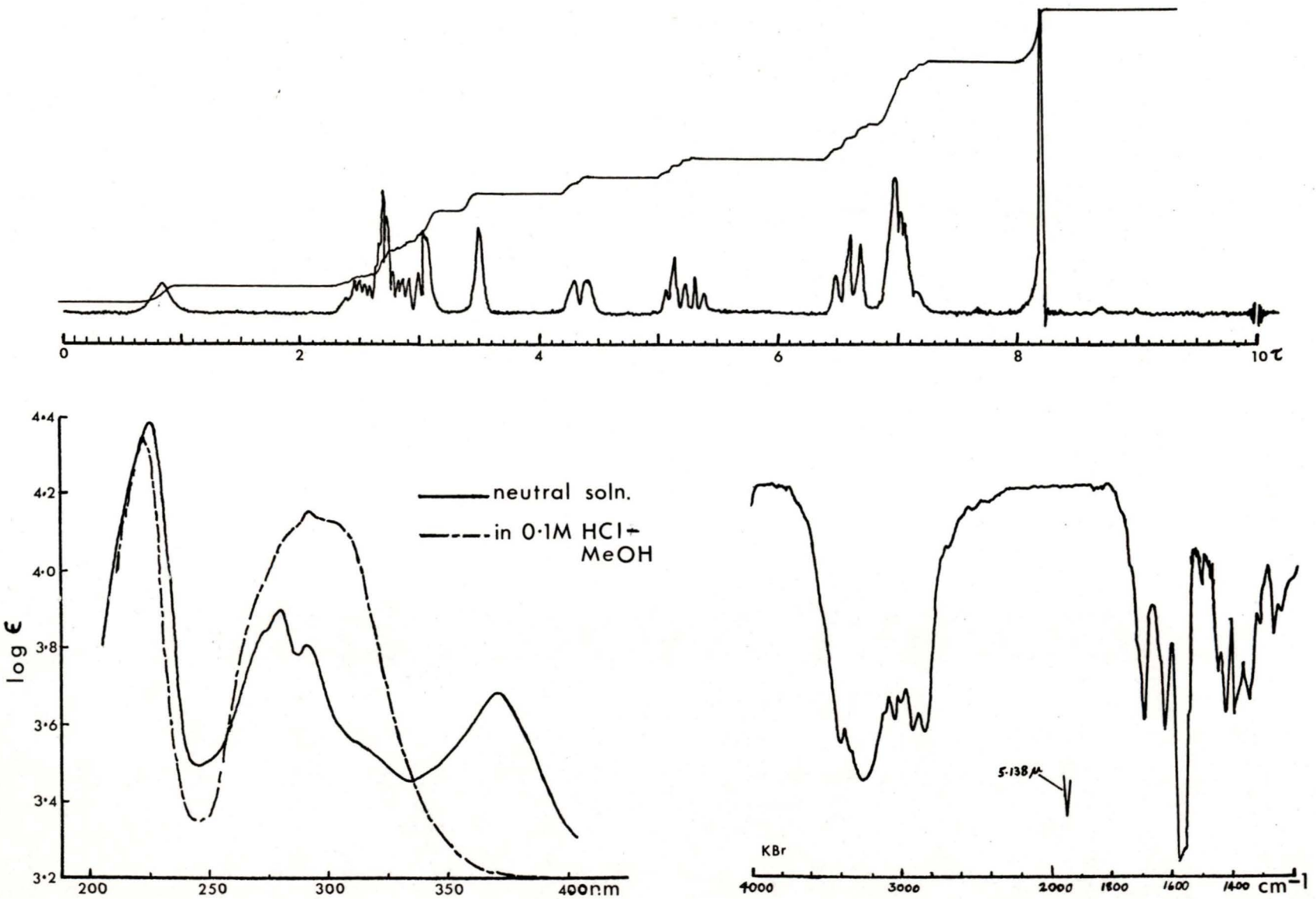


Figure 20. Ultraviolet, infrared and nuclear magnetic resonance spectrum of the dihydropyridine [103].

Information concerning the acid/base stability of [103] was obtained from the uv spectrum. While the spectral features were hardly altered in dilute alkali, the addition of hydroxhloric acid resulted in rapid disappearance of the 374 nm spectral band, while a new absorption at 302 nm appeared (Figure 20). Evidently [103] was very susceptible to mineral acid, a result which was not unexpected. Further mention of this is made later in this discussion.

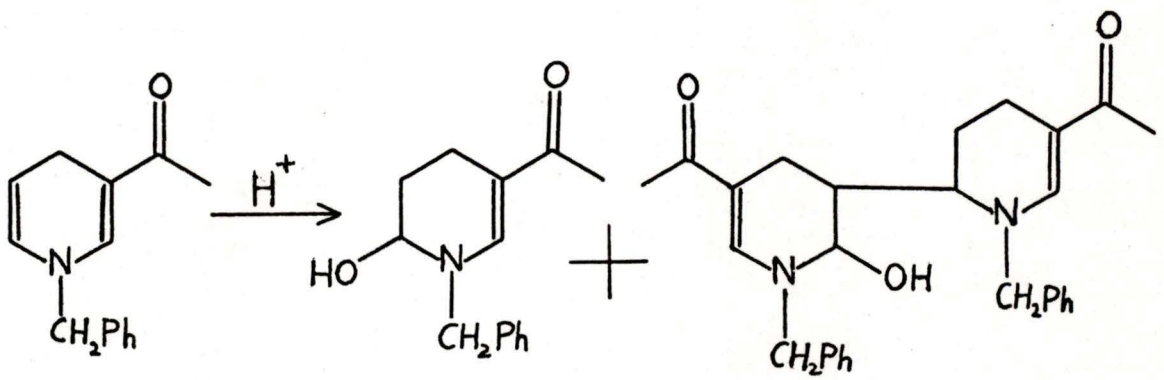
The later steps involved in the synthesis of the precursor [101] might necessarily require the employment of a base such as sodium hydride. For this reason the effect of such a base, if any on [103] was worth investigating. The insolubility of [103] in solvents such as benzene or tetrahydrofuran and its apparent decomposition in dimethylformamide (loss of the 374 nm absorption) precluded a stability test using sodium hydride. The reasonable solubility of [103] in methanol, however, was good reason for using sodium methoxide for this experiment. Despite a prolonged contact period (1 day) using a large excess of the base, all of the compound, other than that which had undergone decomposition on standing over this time, was recovered unchanged. There was no evidence of methoxide substitution. Evidently, the compound was resistant to bases of the sodium methoxide type.

With the report that 3-acetylpyridinium methiodide could be successfully reduced using aqueous or alcoholic potassium cyanide to form the cyano adduct of the corresponding dihydropyridine, it also appeared worthwhile trying this reaction with [102]. Substitution in the pyridine ring of [103] with a nitrile group might be expected to further aid in stabilisation of the molecule. Excess potassium cyanide in

methanol rapidly reduced the pyridinium bromide giving the cyano adduct [104] in 80% yield. The product, a pale-yellow solid, was indeed found to be relatively more stable than the unsubstituted compound [103] and could be freely manipulated in air without any marked decomposition. Like [103], the compound could not be recrystallised, but tlc of the crude substance again revealed it to be essentially all one compound. [104] had expectedly a behaviour similar to [103] towards acid and base, and was easily oxidised by Tollens reagent, presumably back to the pyridium salt. The ir spectrum, in addition to the carbonyl and diene absorptions, also showed a weak C ≡ N stretch at 2240 cm^{-1}. Cyanide reduction of the N-(β-bromoethyl) substituted salt [105] furnished the cyano adduct [106] only as an oil which would not solidify. This product was found to be almost as stable as [104] and amenable to spectral characterisation also.

There have been few speculations in the literature relating to the transformations involved in the reactions with mineral acid, to which all the prepared dihydro compounds were highly susceptible. Karrer,[83b,c] working with N-alkyl-dihydronicotinamides suggested that the product(s) arose from electrophilic addition of the acid to one of the double bonds in the ring, however, the use of groups other than carboxamido in the 3-position had not been reported. In some kinetic studies with N-benzyl substituted pyridines[88] including N-benzyl-3-acetyl-1,4-dihydropyridine [107], the results were consistent with a mechanism involving an initial rapid protonation equilibrium

probably at C-5 of the pyridine, followed by a slow product forming step. Two products absorbing near 300 nm were isolated from the so-called primary acid reaction of [107] only after very extensive isolation and purification procedures. They were assigned the structures [108] and [109] on the basis of their chemical properties, their spectra, and the kinetic data.



[107] [108] [109]

Treatment of a cold methanolic solution of the cyano adducts [104] or [105] with dilute hydrochloric acid, resulted in evolution of HCN, and darkening of the solution with the loss of the dihydropyridine absorption at 355 nm. With anhydrous methanol and HCl gas, no HCN was detected.

Concerning the products of the acid attack on the dihydro compounds, it was difficult to arrive at any definite conclusions. All of the prepared dihydropyridines gave a strong absorption close to 300 nm upon

acidification, as noted above, but whether this consistancy could be correlated with the formation of one particular type of product, or with a mixture of compounds, could not be readily deduced. Tlc results suggested the product(s) were in fact mixtures, which would not be entirely unexpected bearing in mind the diversity of possible reactions dihydropyridines can undergo under acidic conditions, other than conversion to compounds such as [108] and [109]. These include disproportionations, isomerisations and ring contractions. Positive identification of the reaction product(s) was made almost impossible, largely as a result of difficulties with their separation and isolation. In many cases it appeared that the materials actually decomposed upon work-up and attempted separation yielding insoluble, intractable secondary products of no interest.

Only one type of experiment conveyed any meaningful information. This was an attempt to monitor the reaction by observing changes in the pmr spectrum after acidification. For this study the cyano compound [104] in deuteromethanol was used. Addition of hydrochloric acid, and heating to 50°C resulted in a complete loss of the dihydropyridine system within minutes. The spectrum revealed new signals, but none at the low field shifts associated with pyridinium protons, so that at least the disproportionation or oxidation reactions could be ruled out. Reported spectral data for the tetrahydropyridine [83],[52] of the tetracyclic ketone [84] resulting from a Pictet-Spengler cyclisation of [83], also could not be correlated with the spectrum observed. The cyclisation

was envisaged as a potential complication in studies with [103] or [104] owing to the unsubstituted C-2 position of the indole[52] (see Part 1 of discussion, p. 47).

Preparative tlc separation of a major component of the reaction mixture provided a substance having uv and pmr spectral characteristics similar to the acidified methanolic solution used in the original pmr study. A neutral methanolic solution had an absorption band at 303 nm, which was hypsochromically shifted only slightly to 300 nm in 0.1M methanolic HCl. The decrease in absorbance upon acidification, however, was quite marked (12%). Although a spectral shift of more than just a couple of nm upon O-protonation of a vinylogous amide system would normally be expected,[70] the uv behaviour pattern did provide some support for the product containing such a system. The ir spectrum also provided what could be interpreted as some positive evidence for a vinylogous amide. A series of bands in the carbonyl region were visible: weak absorptions at 1640 and 1630 cm^{-1} were close to a slightly lower frequency, intense absorption band (at 1580 cm^{-1}). If the latter two bands were assignable to (C = O) and (C = C) stretching respectively, they would correlate quite well with the vinylogous amide moiety. In spite of the uv and ir result, the pmr and mass spectral interpretation was in total disagreement with any compound resembling [108] or [109] or indeed with any vinylogous amide system.

Another feature of interest in the infrared spectrum was a strong absorption at 1738 cm^{-1} which in the absence of the nitrile absorption

of [104], would not be out of agreement with an ester C = O stretch. A three proton singlet included in the multiplet centred at τ6.35 in the pmr spectrum, might also be associated with a methyl ester. Such an ester group could easily arise from methanolysis of the cyano group of [104], in the presence of acid. Finally, the existance of a hydroxyl group demanded by a product corresponding to [108] or [109] was not inferred from any of the spectra examined.

In summary the research has shown that the 1,4-dihydropyridine system can be generated quite easily under relatively mild conditions. In particular an N-β-(3-indolyl)ethyl substituted compound could be prepared in the absence of acid. Whether these results could find useful application in the projected synthesis of the dihydropyridine-acrylic acid precursor [101] remains to be seen.

EXPERIMENTAL

PART 1

Melting points were determined using a Monoscop VS apparatus, and are corrected. The ultraviolet (uv) spectra were measured in methanol solution using a Cary 17 or Unicam SP800B recording spectrophotometer. The infrared (ir) spectra were taken on either a Perkin Elmer 337, Beckman IR.20 or Unicam SP1000 spectrophotometer. Proton magnetic resonance (pmr) spectra were recorded in deuterochloroform solution (unless otherwise indicated) at 60 MHz, (unless otherwise noted) using a Varian HA-60 or a Perkin Elmer R12A instrument. Chemical shifts are given in Tiers τ scale with reference to tetramethylsilane as the internal standard. Chemical shifts for multiplets are reported with reference to the apparent centre of the signals. Mass spectra were obtained with a Hitachi-Perkin Elmer RMU-7 mass spectrometer using an ionising energy of 70ev (unless otherwise indicated). Molecular weight measurements were made using a Hitachi-Perkin Elmer 115 vapour pressure osmometer. Throughout the work, Camag neutral alumina or Macherey, Nagel Silica Gel G/UV_{254}, were used as adsorbent for thin layer chromatography (tlc). For preparative layer chromatography, plates 0.3 mm in thickness were used. Chromoplates were developed using the spray reagent antimony pentachloride - carbon tetrachloride (1:2). Components that did not

elute off of the original sample spot applied to the plate will be designated as "base-line" material. Column chromatography was usually carried out on Woelm neutral alumina (Brockmann activity III - unless otherwise indicated).

2-hydroxymethylindole [72]

In a typical preparation, ethyl indole-2-carboxylate [71], (ex Aldrich Chem. Co.), 50 g (0.264 mole) in anhydrous tetrahydrofuran (250 ml) was added dropwise to a stirred suspension of lithium aluminium hydride, 23 g (0.610 mole) in tetrahydrofuran (500 ml) at $0^{o}C$. After addition, the mixture was refluxed for 3 hours, cooled, and the excess hydride decomposed by addition of 15% sodium hydroxide solution (50 ml) and ice cold water (100 ml). The lithium salts were removed by filtration and washed with dichloromethane. The organic solution was washed with brine, dried over sodium sulphate, and evaporated. Drying *in vacuo* gave the crude alcohol [72] as a pale yellow solid, 38.6 g (quantitative). Crystallisation from benzene-hexane gave colourless crystals (34.6 g, 90% recovery), mp 75-6^{o}, (Lit[65] mp 73-$4^{o}C$). ν_{max}^{KBr} 3380 (OH) cm^{-1}; λ_{max}(logε): 228(3.80), 271(3.98), 281(3.97), and 291(3.86)nm; pmr: τ1.51 (1H, broad singlet, NH), 3.76(1H, doublet J = 3Hz, C_3H), 5.46(2H, singlet, CH_2OH), 7.15(1H, singlet, O<u>H</u>).

2-benzoxymethylindole [73]

In a typical preparation, the alcohol [72], 6.70 g (45.5 mmole) was dissolved in tetrahydrofuran (130 ml) and triethylamine, 10.3 ml (2 equivs) was added. After cooling to $0^{o}C$, benzoyl chloride, 7.75 g (55.2 mmole) was added dropwise with stirring. The benzoate ester

separated as a white precipitate. Stirring was continued for 3 hours at 0°, then saturated potassium carbonate solution was added followed by dichloromethane. The aqueous layer was separated and extracted with one portion of dichloromethane. The organic layers were combined, dried over sodium sulphate and evaporated to yield the crude benzoate [73], (10.9 g). Crystallisation from benzene, gave a white solid, 9.8 g (86%), mp 126-7° (Lit[65] mp 128-9°C): ν_{max}^{Nujol} 3360(NH), 1705 (ester C = O) cm^{-1}; λ_{max}(logε): 233(4.81), 272(4.18), 282(4.14), and 293(3.87) nm; pmr: τ1.25(1H, broad singlet, NH), 1.92(2H, quartet, ar-H), 2.65 (7H, multiplet, ar-H), 3.36(1H, doublet, J = 3Hz, C_3H), 4.51(2H, singlet, $\underline{CH}_2O$).

2-cyanomethylindole [74]

Optimal conversions to 2-cyanomethylindole were achieved using the following type of procedure: The benzoate [73], 36.0 g (143 mmole) was dissolved in anhydrous N,N-dimethylformamide (600 ml). Potassium cyanide, 50 g (770 mmole) was added. The mixture was stirred and heated under nitrogen atmosphere to 80°C over 1 hour, then maintained at that temperature for a further 24 hours. Tlc showed one major spot corresponding to the nitrile [74]. After cooling to room temperature, dichloromethane and water were added. The layers were separated, and the aqueous layer extracted with portions of fresh dichloromethane. The organic layers were combined, washed with water, dried over sodium sulphate and evaporated. Residual dimethylformamide was removed by freeze drying, and the solid residue chromatographed on alumina. One major fraction eluted with benzene, from which the product [74] was isolated as a dark-brown solid, 22 g (98%). Crystallisation from

benzene - light petroleum gave yellow needles (19 g) mp 103-4° (Lit[65] 102-3°C). $\nu_{max}^{CHCl_3}$ 2240 (CN)cm^{-1}; pmr: τ1.80(1H, broad singlet, NH), 2.63(4H, multiplet, ar-H), 3.50(1H, doublet, C_3H), 6.10(2H, singlet $C\underline{H}_2CN$).

Methyl indole-2-acetate [75]

A technique which gave a good conversion from the nitrile [74] to this acetate ester is described.

The nitrile [74], 9.9 g (63 mmole) was dissolved in 99% $^v/_v$ methanol (600 ml) at room temperature, and the solution saturated with hydrogen chloride gas. The temperature was then allowed to increase to a maximum of 50° while the gas was being absorbed. The reaction mixture was left to stand for 12 hrs. at room temperature, when tlc showed two spots, the major one corresponding to the desired compound [75]. After evaporation of the methanol, and neutralisation of the residue with sodium bicarbonate solution, the crude product was extracted with chloroform. The combined extracts were washed with water, dried over sodium sulphate and the chloroform evaporated. Chromatography on alumina (150 g), and elution with benzene gave the ester [75], 9.4 g (78%). Crystallisation from cyclohexane gave a 90% recovery of beige coloured crystals mp 72-73°C (Lit[65] mp 71-72°C). ν_{max}^{KBr} 3370(NH), 1720(ester C=O)cm^{-1}; λ_{max}(logε): 229(4.00), 273(4.04), 279(4.02), 282(4.01), 290(3.88)nm; pmr: τ1.70 (1H, broad singlet, NH), 2.65(4H, multiplet, ar-H), 3.64(1H, doublet C_3H), 6.19(2H, singlet $C\underline{H}_2COO$), and 6.29(3H, singlet $COOC\underline{H}_3$).

Methyl 3(β-hydroxyethyl) indole-2-acetate [76]

Product yields in this reaction were never very satisfactory, as mentioned in the discussion. In particular, varying amounts of the hydroxy-

ethyl derivative [77] of the tryptophol [76] were obtained. Physical characterisation data for [77] is included below. A typical preparation giving the best possible yield of the tryptophol [76] is described.

The acetate ester [75], 1.56 g (8.3 mmole), was dissolved in carbon tetrachloride (150 ml) and the solution cooled to -15°C under nitrogen. 630 µl(12.6 mmole) of twice distilled ethylene oxide was added, and the mixture further cooled to -20°C. Next, anhydrous stannic chloride, 1020 µl(9.1 mmole) in carbon tetrachloride (20 ml) was added dropwise with rapid stirring. After the addition was complete, stirring was continued for ½ hour, allowing the temperature to increase to -5°C. Tlc indicated several components, one corresponding to the desired tryptophol. Chloroform (50 ml) and saturated sodium carbonate solution (25 ml) were added rapidly, keeping the temperature below 10°C. The organic layer was separated, and the aqueous layer extracted with chloroform. The combined organic solutions were dried over anhyd. potassium carbonate and the solvent removed. The resulting oil (2.14 g) was chromatographed on alumina (70 g). Elution with benzene-dichloromethane gave unaltered acetate [75] 460 mg and elution with chloroform the tryptophol [76], 441 mg (23% overall yield) as a brown oil. Later chloroform fractions contained the hydroxyethyl compound (50 mg). $\nu_{max}^{Liq.\ film}$ 3600-3200 (NH + OH), 1735 (ester C = 0) cm^{-1}; λ_{max}(logε): 224sh, 233(4.32), 276sh, 283(4.18), and 292(3.91)nm; nmr: τ1.35(1H, broad singlet, NH), 2.70(4H, multiplet, ar-H), 6.22(2H, triplet, J = 6.7Hz, $\underline{CH_2}OH$), 6.26(2H, singlet, $\underline{CH_2}COO$), 6.37(3H, singlet, $COOCH_3$), 7.08(2H, triplet, J = 6.7Hz, $-\underline{CH_2}-CH_2-O$), and 7.45(1H, broad singlet, OH).

The <u>hydroxyethyl derivative [77]</u> of the tryptophol was initially isolable as an oil which crystallised on standing. Recrystallisation

from benzene gave colourless needles, mp 145-6°: λ_{max}(logε): 224(4.06), 232sh, 297(4.22), and 319(4.33)nm; pmr: τ0.56(1H, broad singlet, NH), 2.95(4H, multiplet, ar-H), 6.20(4H, multiplet, $\underline{CH_2}COO + \underline{CH_2}OH$), 6.29 (3H, singlet, $COO\underline{CH_3}$), 6.70(4H, multiplet $-\underline{CH_2}-O-\underline{CH_2}-$), 7.81(2H, triplet, indole $-CH_2-$), 8.03 (1H, singlet, OH); mass spectrum (intensity): m/e 277(30), 246(14), 233(50), 202(100), 170(35), 144(16), 143(21), 78(38): high resolution 277.1235, $C_{15}H_{19}NO_4$ requires 277.1314.

Attempted ether cleavage of hydroxyethyl derivative [77]

I. The compound [77] 321 mg (1.16 mmole) was dissolved in acetonitrile (4 ml). Acetyl p-toluenesulphonate 274 mg (1.28 mmole) was added, and the resultant solution stirred and heated at 70°C for 15 hours. The bulk of the solvent was removed by freeze drying, and the brown oily residue extracted with dichloromethane. The organic solution was washed with water, followed by saturated sodium carbonate solution, then dried over sodium sulphate. After evaporation of the solvent, the product was chromatographed on alumina (8 g). Elution with a range of solvents, benzene to methanol removed only material wholly aliphatic in nature, as indicated by the pmr spectrum.

II. [77] 150 mg (0.54 mmole) was dissolved in dichloromethane (2 ml) and cooled to 0°C. Boron tribromide 20 µl, (0.21 mmole) was added, and the resulting solution heated to reflux. A dark brown solid separated. Saturated sodium carbonate solution (5 ml) was added, and the solids filtered. The solid, and supernatent were separately extracted with ether. Tlc on both extracts revealed largely only "base-line" material and nothing corresponding to the alcohol [76].

Attempted ether cleavage of [77] with *in situ* conversion to the tetrahydropyridine [70]

III. [77] 150 mg, (0.54 mmole) was dissolved in a mixture of 3-acetylpyridine (2 ml) and dichloromethane (3 ml) and the solution cooled to 0°C. 20 µl, (0.21 mmole) of boron tribromide was added, the mixture heated to 80°C under nitrogen, and maintained there for 6 hours. Tlc then indicated only base-line material, possibly indicative of pyridinium salt formation. The excess 3-acetylpyridine was removed by freeze drying and the residue triturated with ether, to give a brown solid (400 mg). This material was catalytically hydrogenated and worked-up according to the procedure for the tetrahydropyridine [70] (see page 92). No compound corresponding to [70] was obtained, as indicated by the tlc and nmr comparison with an authentic sample.

IV. [77], 539 mg (1.95 mmole) was dissolved in 3-acetylpyridine (2 ml) and dichloromethane (5 ml), and cooled to -10°C. Freshly recrystallised p-toluenesulphonyl chloride 400 mg (2.10 mmole) was added in small quantities over 15 minutes. The product was allowed to stand at -20° for 48 hours, then heated to 80° under nitrogen and maintained there for 24 hours. Tlc indicated that largely base-line material was present. The excess 3-acetylpyridine was removed and the solids treated as for experiment III. After chromatography of the hydrogenated product, no fraction corresponding to the vinylogous amide [70] as indicated by tlc or pmr, was obtained.

Methyl 3(β-tosylethyl)indole-2-acetate [80]

200 mg, (0.86 mmole) of the tryptophol [76] was dissolved in dry pyridine (1.5 ml) and cooled to -30° under nitrogen. p-Toluenesul-

phonyl chloride 180 mg, (0.95 mmole) in pyridine (1 ml) was added slowly, dropwise with stirring. When addition was complete, the temperature was raised to -20° and stirring continued for 3 hours. Tlc showed a major spot corresponding to the tosylate [80] derivative, and another spot corresponding to some unreacted starting material. Ice-cold 2N sulphuric acid (10 ml) was added, together with dichloromethane (10 ml). The layers were separated, and the acid layer rapidly run off and discarded. The organic solution was extracted with a further portion of cold dilute acid to remove nearly all the excess pyridine. After washing with water, the organic solution was dried over sodium sulphate and the solvent evaporated. Drying *in vacuo* gave a yellow oil (240 mg which was immediately chromatographed on activity IV alumina (15 g). The fraction eluting with benzene (198 mg) was shown by tlc to be very largely tryptophyl tosylate [80], contaminated with a small amount of the tryptophyl chloride [79]. Rechromatography on alumina (5 g) and elution with benzene allowed separation of the pure tosylate compound as a yellow oil, 183 mg (55%); pmr: τ1.60(1H, broad singlet, NH), 2.58 [8H, multiplet (4H, tos ar-H, 2.19 doublet, 2.69 doublet, J = 8Hz) + (4H, indole ar-H)], 5.88(2H, triplet, J = 4.5Hz, $C\underline{H}_2O$), 6.42(7H, multiplet, $COOC\underline{H}_3$ + $C\underline{H}_2COO$ + indole-$C\underline{H}_2$-), 7.57(3H, singlet, tos $C\underline{H}_3$); mass spectrum (intensity): m/e M^+, (0), 371(2), 252(80), 203(100), 171(32), 155(78), 91(82).

Methyl-3(cyclopropyl)indole-2-acetate [81]

This compound was obtained from the tryptophol [76], 450 mg, under rather similar conditions to those given above, but with two significant changes. The reaction was carried out at -10°C, and the product allowed to stand at 0°C for 24 hours before work-up. In addition, saturated sodium

carbonate solution was used to wash the dichloromethane solvent after the acid extraction. Under these modified conditions, benzene elution of the product mixture gave two principal fractions. The first, (406 mg) corresponded to the pure tosylate (54%), as before. From the other fraction, was obtained a pale-yellow crystalline solid, mp 53-55°C (10 mg). This product had the characteristic nmr spectrum of the spiro derivative [81]. The mass, and infrared spectrum of a recrystallised sample has been examined at U.B.C. The mass spectral data is included below. pmr: τ1.42 (1H, broad singlet, NH), 2.80(4H, multiplet ar-H), 5.65(1H, singlet, = C<u>H</u>COO), 6.29 (3H, singlet COOC<u>H</u>$_3$), 8.50(4H, A_2B_2 multiplet, cyclopropyl H); mass spectrum (intensity): ^{m}/e 215(28), 183(21), 174(44), 169(49), 156(44), 119(74), 100(21), 91(17), 69(100), 77(13); high resolution 215.0947 $C_{13}H_{13}NO_2$ requires 215.0946.

<u>N-[β{3(2-Carbomethoxymethylindolyl)}ethyl]-3-acetyl-1,4,5,6-tetrahydropyridine [70]</u>

(a) To the alcohol [76], 200 mg (0.86 mmole), in excess dry 3-acetylpyridine (2 ml), was added dropwise a solution of 196 mg (1.03 mmole) p-toluenesulphonyl chloride in dichloromethane (2ml) at -15° C. The reaction mixture was transferred to the freezer and left to stand at -10°C for 3 days, by which time good conversion to the tosylate derivative [80] was indicated by the tlc and separation of crystalline 3-acetylpyridinium chloride. The mixture was then stirred and heated under nitrogen to 80°C and maintained at that temperature for 24 hours. Tlc then showed virtually only one polar base-line spot corresponding to pyridinium salt formation. After cooling and freeze drying to remove the excess 3-acetyl pyridine, the product was triturated with ether to give a brown solid

(600 mg). This solid was dissolved in ethanol (100 ml) and filtered. Triethylamine (1.0 ml) and 10% palladium on charcoal (100 mg) were added and the product hydrogenated at atmospheric pressure until uptake of hydrogen ceased (72 hours). The catalyst was filtered off and the ethanol removed. Extraction of the residue with refluxing benzene (200 ml) to remove the bulk of the triethylamine hydrochloride, furnished a yellow solid after solvent evaporation. The solid was taken up in chloroform, extracted with pH3(3 x 50 ml) and pH2(3 x 50 ml) phosphate buffers, and the extracts discarded. The chloroform solution was dried over sodium sulphate and the solvent evaporated to leave a solid (270 mg) containing the vinylogous amide [70]. Chromatography on activity IV alumina (8 g) and elution with chloroform gave the crude vinylogous amide (124 mg), from which two recrystallisations from benzene-ligroin gave the pure compound, 70 mg (24%), mp 167-8°, (Lit[66] mp 166-7°C). ν_{max}^{KBr} 3180 (NH), 1735 (ester C = 0), 1615 (enamide C = 0), 1560 (enamide C = C)cm^{-1}; λ_{max} (logε): 223 (4.63), 293 (4.27), and 315 (4.60)nm; $\lambda_{max}^{0.1M\ HCl}$ (logε): 223 (4.64), 291 (4.52), 302(4.51)nm; pmr(100MHz): τ1.13(1H, broad singlet, NH), 2.70(4H, multiplet, ar-H), 3.08(1H, singlet, H-C =C), 6.31(2H, singlet, CH_2COO), 6.33 (3H, singlet, $COOCH_3$), 6.55(2H, triplet, J = 4Hz, $-CH_2N<$), 6.95(4H, multiplet C_6 piperideine + indole-CH_2), 7.78(2H, triplet, C_4 piperdeine), 8.18 (3H, singlet, $COCH_3$) and 8.27(2H, multiplet, C_5 piperideine); mass spectrum (intensity): m/e 340(25), 202(30), 142(10), 139(68), 138(100), 78 (20), 43(10); high resolution 340.1733, $C_{20}H_{24}N_2O_3$ requires 340.1787.

(b) The tryptophol [76], 100 mg (0.43 mmole) was dissolved in 3-acetylpyridine (1 ml) and the solution cooled to 0°C under nitrogen. Phosphorus tribromide, 18μl(0.19 mmole) was added, and after addition the temp-

erature was raised to 85°C and maintained there for 6 hours. Tlc then indicated substantial conversion to the pyridinium bromide salt. The crude salt, isolated by precipitation into ether, was dissolved in ethanol (50 ml), 10% palladium on carbon catalyst added (80 mg) together with triethylamine (0.5 ml) and the product hydrogenated at 3 atmospheres pressure in a Parr apparatus for 72 hours. The catalyst was removed and the tetrahydropyridine isolated, as above. After recrystallisation only 22 mg (15%) of [70] was obtained, identical in every respect to that obtained by method (a).

Attempted alkylation at the ester side chain of the tetrahydropyridine [70]

(a) Special precautions were necessary to prevent as far as possible any inclusion of moisture in the equipment or reagents. The apparatus used for this reaction was pre-dried by heating in a stream of dry nitrogen. 55 mg of a 57% oil dispersion of sodium hydride (*Ca* 1.3 mmole NaH) was stirred with dry benzene (2 ml) under nitrogen. The hydride was allowed to settle and the benzene drawn off and replaced with a fresh quantity. After washing with three separate portions in this way, the largely oil-free hydride was suspended in a further 2 ml of benzene. Methyl formate which hadbeen pre-dried over anhydrous potassium carbonate and distilled twice from phosphorus pentoxide, was distilled again from P_2O_5 directly into the hydride suspension (*Ca* 1 ml). Next, 52 mg (0.15 mmole) of the vinylogous amide [70] in benzene (pre-dried by azeotropic distillation of the benzene solution to a final volume of 4 ml), was added dropwise over 15 minutes and at room temperature. The reaction mixture was then warmed to 35°C and held there for up to 2.5 hours. At that time there was no

significant change in the tlc characteristics of the reaction mixture compared to the starting compound, except for the presence of an increased amount of material not eluted off of the base-line spot. The reaction mixture was worked up as for the enolate compound prepared by the Kutney researchers at U.B.C.[65] The product was cooled to 0°C and a few drops of methanol added to destroy the excess hydride. Crushed ice and 2N hydrochloric acid was added, followed by saturated sodium bicarbonate solution to neutralise the excess acid. The organic layer was separated and the aqueous phase washed with chloroform. The combined organic solution was dried over sodium sulphate and the solvent evaporated to yield 42 mg of a brown solid. This solid had a pmr spectrum consistent with largely unreacted starting compound. In particular no signals at shifts corresponding to an enol or aldehyde proton was detected. The material was however, subjected to sodium borohydride reduction in methanol at -25°C. After work-up and isolation of the solid material (37 mg) there was again no change in either the pmr spectra or the appearance as compared to [70].

(b) To gain further insight into why the above reaction failed to occur, the ability for carbanion formation to occur at the active methylene of the indole α-side chain was assessed.

The vinylogous amide, 67 mg (0.20 mmole) in dry benzene (5 ml) was added at room temperature to a suspension of oil-free sodium hydride (from 80 mg 57% paraffin oil suspension) in benzene (2 ml) under nitrogen. The mixture was heated at 40°C for 2 hours, then cooled in ice and treated with deuterium oxide (1 ml). 2N hydrochloric acid was added, followed by sodium bicarbonate to neutralise the excess acid, and the

heterogeneous mixture extracted with chloroform. Only 49 mg of [70] was recovered, all other material remained insoluble in chloroform. The product pmr spectra was found to correspond to the vinylogous amide [70] in every way, except that the peak intensity corresponding to the methylene protons of the ester side chain compared to the carbomethoxy methyl protons, was reduced by an amount corresponding to a change in ratio of 2:3 to 1:3. Verification of replacement by a deuterium atom was shown in the mass spectrum. An increase of one mass number unit over the molecular ion of [70] was found. Carbanion formation was not quantitative, however, since the extent of deuterium substitution was determined to be only 30%.

(c) Experiment (a) was repeated under more forcing conditions. The reaction mixture, when stirred for 5 hours while heating at 35-40°C, gave only recoverable starting material. Using a very large excess of methyl formate (*Ca* 5 ml), at vigorous reflux temperature (60°C), for 2 hours, gave the same result.

(d) Experiment (a) was repeated once more, but this time after observing that no reaction had occured, after 2.5 hours at 35°C, two drops of a 25% solution of sodium methoxide in absolute methanol was added to the hydride suspension. A rapid reaction immediately ensued resulting in a darkening, separation of solid material and evolution of hydrogen. The reaction mixture was stirred a further 0.5 hour at 35°, then worked-up as in experiment (a). Solvent evaporation and drying *in vacuo* gave a brown solid (37 mg). This material was shown to contain two major components by tlc. The pmr spectrum of the crude product was consistent with the presence of the required enolate [85] with intact preservation of the vinylogousamide moity. Pmr: τ - 3.10(broad singlet, enolic OH), 0.10

(sharp singlet, CHO), 1.20(broad singlet, D_2O exchanged, NH), 2.70(multiplet, ar-H), 3.12(singlet, N-CH = C), 6.22(singlet), 6.32(singlet, $COOCH_3$), 8.25(singlet, $COCH_3$); Integral ratio τ6.32/6.22 peaks 3:1.

Without attempting any separation or purification of the supposed enolate, the crude product was dissolved in methanol (5 ml) and the solution cooled to -20°C. Sodium borohydride, 100 mg (2.6 mmole) was added in small amounts over 45 minutes. After addition was complete, the mixture was stirred a further 15 minutes, then the excess borohydride was destroyed by careful addition of 2N hydrochloric acid (2-3 drops). The mixture was diluted with water and the methanol evaporated under reduced pressure. The remaining mixture was made acid with 2N HCl then neutralised with sodium bicarbonate solution and extracted with chloroform. The organic phase, after drying and evaporation, gave a yellow solid (30 mg). The pmr spectrum of this material notably showed absence of the signals corresponding to the enol and aldehyde protons. Chromatography on activity IV alumina (4 g) gave two principal fractions. Elution with chloroform gave a yellow oil (7 mg) having a pmr spectrum comparable with the vinylogous amide [70], but with a reduced intensity of the signal corresponding to the carbomethoxy ester protons compared to the other peaks. An informative mass spectrum of this compound could not be obtained owing to difficulties with sample vapour pressure. However, this material was clearly of no interest as far as the sought-for alcohol [86] was concerned, and was not investigated further. The more polar second fraction which was eluted off of the column using methanol-chloroform mixture gave, after solvent removal, a solid (13 mg), which initially appeared quite promising. Tlc indicated this material was largely one component. Although it was not possible to crystallise this material from a range of solvents,

for the purposes of mass spectral analysis it could be dissolved in dichloromethane and precipitated into benzene or ether. A spectral investigation was undertaken as indicated below, however as was mentioned in the discussion these results were not sufficiently unambiguous to conclude this material was the alcohol [86] or not. ν_{max}^{KBr} 3400(broad band, NH + OH?), 1710(ester C = O), 1650(C = O), 1615 and 1560(very weak absorption) cm^{-1}; λ_{max}(logε, assuming MW370): 225(4.38), 286sh, 293(3.96), 314(4.03); $\lambda_{max}^{0.1M\ HCl}$ 224(4.39), 292(4.04), 305(3.99)nm; pmr: τ0.90(1H, broad singlet, NH), D_2O exchanged, 2.65(4H, multiplet, ar-H), 3.05(1H, singlet, H-C = C), 6.00(2H, singlet), 6.35(4H, singlet, $COOCH_3$ + -CH?), 6.84(6H, multiplet, C_6 piperideine H + $-CH_2$ $-CH_2-$), 7.85(2H, triplet, C_4 piperideineH), 8.18(3H, singlet, $COCH_3$); mass spectrum (intensity) m/e: 368(1), 340(2), 325(2), 324(2), 202(3), 139(4), 138(10), 85(16), 83(16), 78(33), 57(88), 56(100), 44(84); molecular weight of alcohol [86], $C_{21}H_{26}N_2O_4$ = 370.2.

(e) The vinylogous amide [70], 55 mg (0.16 mmole) in benzene (10 ml) was added to a stirred suspension of freshly prepared sodium methoxide, 100 mg (1.85 mmole) in dry methyl formate (2 ml). The reaction mixture was heated to 35°C and maintained at that temperature for 2 hours. Tlc then revealed total disappearance of the starting compound, and the presence of a large amount of base-line material. Work-up of the reaction product, as before, gave a brown solid (40 mg). This material was shown by nmr not to have undergone formylation. Signals expected for the enol and aldehyde protons were absent in the spectrum. Attempted chromatography on activity IV alumina resulted in strong adsorption of the bulk of the material. Elution with chloroform-methanol removed only 10 mg of material, which tlc suggested contained two components. No attempt was made to separate these. The pmr spectrum bore only some similarity to the start-

ing amide, and none at all to that expected for the enolate. Notably the carbomethoxy ester signal was reduced in intensity compared to the rest of the spectrum, and this was supported by only a very weak ester carbonyl absorption in the infrared spectrum. A new singlet at 7.95τ, coupled with the appearance of another carbonyl absorption at 1695cm^{-1} in the infrared, suggested one of the components could have undergone conversion from a piperideine to a piperidine. However absorptions due to the vinylogous amide system were observed in all the spectra, pmr, infrared and uv, while the highest peak in the mass spectrum at $^{m}/e$ 340 was as for the parent ion of the vinylogous amide. No further investigations of the material were made therefore. The material strongly adsorbed onto the alumina was removed only by 10% sodium carbonate solution. A yellow solid (22 mg) was recovered from the chloroform extract of the alkali solution. This solid was insoluble in sodium bicarbonate solution and in dilute mineral acid, and showed no signals corresponding to the ester protons in the pmr spectrum. This material was also not further examined.

(f) This experiment was an attempt at repeating experiment (b), using 70 mg (0.21 mmole) of [70], 114 mg of 57% sodium hydride dispersion (2.06 mmole), methyl formate (*Ca* 1 ml) and 25% sodium methoxide in methanol (1 drop). Addition of the methoxide gave a different reaction to that noted before. A precipitate appeared as before, but rather than any darkening being observed, the supernatent liquid developed an orange-red colour which progressively intensified. After stirring for 15 minutes at room temperature, the reaction mixture was heated to 40^{o}C and held there for 30 minutes. Tlc then showed a spot having the same Rf value as [70] but giving different colour reactions after spraying with

developer. The usual work-up gave a red coloured oil (72 mg), which, unlike the product from experiment (b), gave no signals at chemical shifts in the pmr spectrum corresponding to the sought-for enolate [85]. Chromatography on alumina (activity IV, 6 g), cleanly separated two single components. Elution with chloroform provided a yellow solid (14 mg) after solvent removal. This compound had a pmr spectrum again showing no signals corresponding to the enolate. In addition no signals due to the indolic NH proton or to the C-2 vinylic hydrogen of the piperideine ring system were observed. This compound was clearly of no relevance in the attempted synthesis of the enolate and was not further investigated.

Column elution with chloroform-methanol removed the major product which gave a deep red coloured solid (44 mg). Although spectral analysis of this material proved it not to be the required enolate, the data collected (summarised below) was rather intriguing, and mention of these results was made in the discussion section. ν_{max}^{KBr} 3700-3200 (OH or NH?; not sharpened in $CHCl_3$), 1725(ester C = O), 1615(enamide C = O), 1560 (enamide C = C)cm^{-1}; λ_{max}(logε, if MW 370): 218(4.23), 274(4.42), 308 (4.49)nm; $\lambda_{max}^{0.1M\ HCl}$ (logε): 218(4.19), 274(4.44), and 302(4.42)nm; pmr: τ1.74(1H, sharp singlet, no D_2O exchange, HCO-N?), 2.50(5H, multiplet ar-H + NH?), 3.15(1H, singlet, H-C = C-), 6.17(4H, singlet, $COOCH_3$ + -CH), 6.30(4H, multiplet), 6.70(3H, singlet, O-CH_3?), 7.15(6H, multiplet, C_6 piperideine H + CH_2CH_2N), 7.90(4H?, multiplet, C_4 piperideine + CH_2?), 8.20(3H, singlet, $COCH_3$), 8.32(2H, multiplet, C_6 piperideine); mass

spectrum (intensity): m/e 438(1), 436(1), 420(4), 321(2), 283(5), 282(20), 281(5), 139(5), 138(15), 112(15), 78(81), 70(67), 44(100). Indicated molecular weight by vapour pressure osmometry 386 calculated molecular weight of enolate [85] $C_{21}H_{24}N_2O_4$ = 368.2.

(g) The inertness of the transoid enamide system to nucleophilic bases and methyl formate was assessed using sodium methoxide as follows: To a suspension of oil-free sodium hydride, prepared from a 57% paraffin oil dispersion, 250 mg (*Ca* 5.9 mmole), in dry 1:1 tetrahydrofuron-benzene (5 ml), was added 0.5 ml of a 25% solution of sodium methoxide in methanol (*Ca* 2.3 mmole NaOMe). This was followed by methyl formate (5 ml) and N-hydroxyethyl-3-acetyl-1,4,5,6-tetrahydropyridine 200 mg (1.2 mmole) in dry benzene (10 ml). After stirring for 2 hours at 35°C, and use of the standard work-up conditions a yellow oil (98 mg) was obtained. The material was identical in every respect to the starting compound. The low recovery was simply attributed to the high solubility of the vinylogous amide in water, rather than in the chloroform extractant.

(h) 43 mg (0.13 mmole) of [70] was dissolved in dry benzene (2 ml), transferred to the reaction vessel and an excess (2 ml) of the methyl formate distilled into it. 112µl (*Ca* 10 mg, 0.13 mmole) of an ether solution of lithium diethylamide[74] was added at room temperature. After stirring for 2 hours, no apparent change compared to the starting compound was indicated by tlc, so the temperature was raised to 40°C where it was maintained for a further 3 hours. Tlc revealed only an increased amount of base-line material. The usual work-up gave an oil (60 mg) which showed no signals corresponding to the required enolate in the pmr spectrum. Preparative tlc on alumina gave only a greatly reduced

recovery of the starting compound (9 mg), while the polar base-line fraction showed nothing of interest on spectral examination.

(i) In a similar experiment to (h) using proton-sponge [1,8-bis-(dimethylamino)-naphthalene] as the base, 20 mg (0.09 mmole) of it in benzene (2 ml) was added to a solution of the vinylogous amide, 30 mg (0.09 mmole) and methyl formate (*Ca* 0.5 ml) in benzene (3 ml). Stirring at room temperature and at 50°C for a day resulted in no reaction as indicated by tlc. Removal of solvent and chromatography of the residual solid on activity IV alumina (5 g) gave only recovered base and [70], and nothing corresponding to the enolate.

(j) In a like experiment, the vinylogous amide, 73 mg (0.21 mmole) in benzene (3 ml) was added to a stirred suspension of potassium t-butoxide, 48 mg (0.43 mmole) in dry tetrahydrofuran (2 ml) and methyl formate (2 ml). After heating for 18 hours at 45-50°C there was no change in the tlc compared to the starting compound, and on work-up the product (55 mg) had the same spectra as [70].

(k) To a solution of the vinylogous amide 60 mg (0.18 mmole) in dry tetrahydrofuran (3 ml), was added dropwise an ether solution of trityl sodium (3.5 ml, *Ca* 0.14M, 0.49 mmole) under nitrogen. The bulk of the red trityl sodium solution decolourised rapidly as it was added, with separation of a yellow-brown precipitate, however the colour persisted for 2-3 minutes when all of it had been added and before the first few drops of methyl formate (*Ca* 2 ml) were distilled into the reaction mixture. When all of the methyl formate had been added and stirring at room temperature continued for 2 hours, tlc showed the presence of a large amount of base-line material and triphenylmethane derived from the base used.

The solution was evaporated to dryness under vacuum and the product obtained as for experiment (a). The solid (200 mg) was chromatographed on activity IV alumina (10 g) to separate the indolic material from the large excess of triphenylmethane present. Of the former, only a few milligrams of the starting compound was recovered, and no compound corresponding to the enolate was detected.

(1) Experiment (a) was repeated, but substituting the benzene solvent with dimethylformamide. Darkening occured as the vinylogous amide was added to the reaction mixture. The dark-brown solid isolated after work-up showed no signals corresponding to the required enolate in the pmr spectrum, and the tlc of it indicated a mixture of unreacted [70] and base-line material only. The same result was obtained when this experiment was repeated using only a few drops of dimethylformamide in benzene solution.

Methyl-2-formyl-(2-indolyl) acetate [87]

In a typical preparation, methyl indole-2-acetate [75], 3.00 g (15.9 mmole) in dry benzene (80 ml) was added dropwise under nitrogen to a stirred suspension of sodium hydride (3.34 g 57% mineral oil dispersion, *Ca* 79 mmole NaH) in benzene (30 ml). Dry methyl formate (*Ca* 20 ml) was distilled into the reaction vessel and the suspension stirred at room temperature. A green colour soon appeared, changing to brown with the separation of a bulky precipitate of the enolate sodium salt as the temperature was slowly raised to 35°C. Stirring was continued a further 2 hours at 35°C, then the mixture was worked up as for the vinylogous amide formylation (experiment (a), p. 93). The crude enolate [87] was obtained as a grey coloured crystalline solid after solvent removal

(3.5 g). This solid, found to have a low solubility in most solvents and not amenable to chromatography on alumina (decomposition) or silica-gel (no separation), was best purified by recrystallisation from a large volume of benzene. Colourless needles were obtained, mp 173-4°C, 3.20 g (93%). Compound gave greatly intensified yellow colour to $FeCl_3$ solution and reduced Tollens reagent. ν_{max}^{KBr} 3200(H-bonding indole NH), 2860 (ald C-H), 1695(ester C = O), 1630 and 1610(H-bonding ald C = O)cm^{-1}; λ_{max}(logε): 234(4.29), 292(sh), 332(4.40)nm; $\lambda_{max}^{0.1M\ NaOH}$ (logε): 216(4.54), 292(sh), 311(sh), 324(4.37)nm; pmr: τ-3.0 (1H, broad singlet, D_2O exchanged, enol-H), -0.05(intensity <0.5H, sharp singlet), 0.10(1H, sharp singlet, CHO), 2.71(5H, multiplet, ar-H + NH?), 5.53(intensity <1H, singlet), 5.62(2H, singlet, D_2O exchange intensity 1H, - CH + OH?), 6.13 (~1H, singlet), 6.19(3H, singlet, $COOCH_3$), no separate indole NH or C_3H signals observed. Mass spectrum (intensity): m/e 217(61), 186(20), 185 (100), 157(25), 130(20), 129(75), 102(15), 77(9); molecular weight by vapour pressure osmometry 228; calculated for $C_{12}H_{11}NO_3$ = 217.2.

Chemical characterisation of [87]

(a) Freshly distilled 2-methylindole (150 mg), was subjected to the standard formylating conditions as for experiment (a) (p. 93) using sodium hydride (150 mg) and methyl formate (*Ca* 2 ml). After work-up, the crystalline product (nearly quantitative recovery) had an identical pmr spectrum to, and the same tlc characteristics as the starting material. The melting point (60-61°C) was also in accordance with 2-methylindole (lit mp 61°).

(b) Ethyl indole-2-carboxylate [71] (105 mg), was treated as (a) above. A crystalline solid (100 mg), mp 150-1°C (cf. ethyl ester, mp 125°) was

obtained. The pmr spectrum and melting point were consistent with methyl indole-2-carboxylate (lit mp 151-2°).

(c) [86], 25 mg was refluxed with 6N hydrochloric acid (5 ml) for 1.5 hours. The decarboxylated product was extracted with chloroform, the chloroform layer washed with sodium bicarbonate solution, dried over sodium sulphate and the solvent evaporated. The residue (11 mg), containing a mixture of components, but no aldehyde by tlc, was subjected to preparative tlc on alumina. The major fraction (8 mg), mp 56-8°C, was identified as 2-methylindole by comparison with an authentic sample: mp and mixed mp 59-60° (lit 61°). λ_{max}(logε) 222(4.35), 269(3.79), 288 (3.69)nm; mass spectrum (intensity): m/e 131(98), 130(100), 103(20), 77(33), and 51(18).

Attempted reduction of the formyl compound [87] to the alcohol [88]

(a) Initially the same conditions as were used by the Kutney researchers[65] to reduce their enolate compound to secodinol [51], were applied. [87], 62 mg (0.29 mmole) was suspended in methanol (10 ml). Complete dissolution was not possible. The suspension was cooled to -25°C and a total of 90 mg (2.38 mmole) of sodium borohydride added in small quantities over an hour. Tlc indicated that no reduction at all had occurred. This was verified by the pmr spectrum of the unreacted material (55 mg) after isolation by the work-up procedure given for the enamide (experiment (d), page 95).

(b) When experiment (a) was repeated, with the reduction stage carried out at -10°C or 0°C, again no reduction occurred. Doubling the amount of borohydride gave the same negative result.

(c) Using more forcing conditions, the enolate 50 mg (0.23 mmole) was

suspended in absolute methanol (25 ml) at 25°C. Sodium borohydride (50 mg) was added portionwise. After stirring for 30 minutes at 25°C, tlc again indicated no change had occured. When a further quantity of borohydride was added (total 300 mg, 7.9 mmole), the undissolved compound went into solution. The mixture was left standing for 24 hours. Tlc then revealed two additional spots in addition to the enolate spot. Work up as for (a) gave a brown solid (30 mg) which on chromatography on alumina (2.5 g) gave only a low recovery from the column (total 18 mg), owing to some enolate decomposition. Several components were eluted, including methyl indole-2-acetate (5 mg), identified by melting point (70-1°; lit[65] mp 71-2°), and its mass spectrum, also material having lost the carbomethoxyester group (5 mg). One of the components however, had spectral features later identified (when more material subsequently became available) with the sought-for alcohol [88]. The yield from this reaction was very low, (*Ca* 4 mg 10% on [87]). pmr: τ1.15 (1H, broad singlet, D_2O exchanged, NH), 2.70(4H, multiplet, ar-H), 3.62 (1H, singlet, indole C-3H), 5.99(2H, apparent singlet-CH_2-O-), 6.31(3H, singlet, $COOCH_3$), 6.40(1H, multiplet, $\underline{HC}$ - CH_2), and 7.30(1H, broad singlet, D_2O exchanged, OH); mass spectrum at 40ev (intensity): m/e 219(86), 201 (7), 189(46), 188(81), 157(84), 156(26), 130(78), 118(100), 84(93).

(d) Under even more forcing conditions than experiment (c), sodium borohydride, 35 mg (0.93 mmole) was added in one portion to [87], 100 mg (0.46 mmole) in boiling isopropanol (30 ml). The solution was refluxed for 2 minutes then cooled. Tlc of the reaction mixture revealed several spots, together with that corresponding to the unreduced enolate [87]. The solvent was evaporated and the unreacted material recovered by extraction from ether using 2% sodium hydroxide and acidification of the base

extract to give [87], (66 mg). From the ether layer was isolated a colourless oil (12 mg). This was suggested to be a mixture of three components by tlc, none corresponding to the alcohol obtained in experiment (c). The pmr spectrum of the mixture strongly suggested at least one component was the hydrogenolysis product [89], isolated in experiment (e) (below). The signal corresponding to the carbomethoxy ester protons was considerably reduced in intensity, suggesting at least partial reduction of the ester group had occurred.

(e) [87], 5.0 g (23.1 mmole) was suspended in dry tetrahydrofuran (80 ml). At room temperature, diborane, generated externally from BF_3-etherate 5.36 g (37.8 mmole), and 1M sodium borohydride, 0.72 g (18.9 mmole) in diglyme, was passed into the suspension. The reaction mixture soon darkened, all of the enolate dissolved and the temperature increased to 32°C. The reaction was completed by refluxing the mixture for 10 minutes. After cooling to 0°C, the excess hydride was decomposed with methanol and water and the solvent removed. The residue was taken up in dichloromethane, washed with water and dried over sodium sulphate. Evaporation of the dichloromethane left a brown viscous oil (4.9 g). Tlc of the crude product indicated that the enolate had reduced to a host of different products. These were largely separable by chromatography on alumina (50 g). Of the nine components investigated, none had any spectral features correlated with the required alcohol [88]. Included with the compounds eluted with benzene, was indole itself (450 mg), and the hydrogenolysis product [89] (340 mg). The latter compound, a yellow crystalline solid, showed characteristic signals in the pmr spectrum, notably a doublet (3H, J = 7.3 Hz) centred at 8.41τ, and a quartet at 6.20τ(1H, J = 7.3 Hz) due to coupling of the methyl and methine protons

of the indole C-2 side chain. Mass spectrum of [89] (intensity): m/e 203(10), 145(42), 144(68), 71(36), 42(100).

(f) Catalytic hydrogenation of [87], 600 mg in ethanol (40 ml) tetrahydrofuran (40 ml) using Adams catalyst (PtO_2, 20 mg) and ferric chloride promotor (0.2 ml of 0.1M aq. solution) at 2 atmospheres for 18 hours, again gave complete reaction of the enolate. Tlc on the product indicated a whole variety of reduction products, as before. When chromatographed on alumina (20 g), again none of these had any pmr spectral features in common with the alcohol. Among the components, the hydrogenolysis compound [89] was recognised.

(g) Catalytic hydrogenation of [87], 200 mg in acetone (50 ml) using 30% palladium on charcoal (20 mg) at 1 atmosphere for 48 hours, followed by removal of the catalyst and removal of the solvent, gave a partly crystalline oil (200 mg). This was shown to contain no unreduced enolate but showed three other spots on a tlc plate. Chromatography on alumina (10 gm) cleanly separated the products. One of these was identified as the hydrogenolysis compound [89] (30 mg), but from the more polar fraction eluting with chloroform-methanol mixture, the required alcohol [88] was obtained in 20% yield (40 mg). Spectral assignments for this compound were as given in experiment (c).

(h) The enolate [87], 40 mg (0.18 mmole) in tetrahydrofuran (10 ml) was cooled to -78°C (solid CO_2/acetone) under nitrogen. 70 mg (0.275 mmole) of lithium tri-tert-butoxyaluminium hydride[80] in tetrahydrofuran (2 ml) was added dropwise. When addition was complete, the reaction mixture was allowed to warm to room temperature, then stirred a further 6 hours at 60°C. Tlc of the reaction mixture revealed that no reduction had occurred. After solvent removal, acidification, neutralisation and

extraction with ether, the material isolated (30 mg), was totally unreduced starting compound.

When the experiment was repeated, using inverse addition of the enolate, partial reduction did occur. Of the material reduced, several components were again indicated by tlc, but after chromatographic separation, none of them had spectral features in common with [88].

(i) [87], 43 mg (0.20 mmole) in anhydrous tetrahydrofuran (3 ml) was added dropwise to bis[3-methyl-2-butyl]borane reagent,[81] (0.20 ml suspension in diglyme, *Ca* 0.33 mmole) in tetrahydrofuran (1 ml) at 0°C under nitrogen. The mixture was stirred at 0°C for 15 minutes and room temperature for 2 hours. Disappearance of [87] was indicated by the tlc and no reduction of silver II on an $AgNO_3$-alumina plate. After addition of ice, dilute acid, neutralisation with sodium bicarbonate, removal of the THF and extraction with ether, evaporation of the solvent gave a brown oil (56 mg). Of the several components separated by chromatography on alumina (4 g), again nothing corresponding to the alcohol was identified.

Benzyl ether of methyl 3-hydroxy-2-(2'-indolyl)propionate [91]

The alcohol [88], 40 mg (0.18 mmole) was dissolved in benzene (2 ml), and silver oxide 42 mg (0.18 mmole) was added together with benzyl bromide, 32 mg (0.20 mmole). The mixture was stirred and heated at 70°C for 6 hours under nitrogen, then cooled. The silver salts were filtered off, washed with ether and the combined organic solution washed with water and dried over sodium sulphate. Evaporation of the solvent gave the crude benzyl ether [91] as a brown solid (56 mg). Chromatography on silica-gel (6 g) and elution with ether gave the benzyl ether,

44 mg (78%), mp 75-8°C. The pmr and mass spectrum of this product suggested it was very largely the O-benzylated compound, but contaminated with some of the N and O- disubstituted derivative. Since the amount of material available for further synthetic use was limited, no attempt was made to separate the mixture. $\nu_{max}^{CHCl_3}$ 3450(NH), 1745 (ester C = O)cm^{-1}; pmr τ2.90(multiplet ar-H + indole NH?), 3.60(singlet, indole C_3H), 4.88(singlet $-CH_2-N$?), 5.62(singlet $PhCH_2O-$), 6.05(multiplet $-CH_2-O-$?), 6.36(singlet $COOCH_2$), 6.60(multiplet $\underline{H}-\overset{'}{C}-CH_2$); 20ev mass spectrum (intensity): 399(0.5), 369(2), 309(1), 278(4), 218(2), 106(22), 92(30, 91(36), 85(64), 83(100), 78(29).

Attempted alkylation at C-3 of the benzyl ether [91]

Before attempting this synthesis it became necessary to establish whether or not the benzyl ether protecting group of [91] could be cleaved under the conditions appropriate to the synthesis of the tryptophol compound [76], which used stannic chloride as the Lewis acid catalyst. When benzyl ethyl ether, a model for [91] was subjected to these reaction conditions (see page 87), it was easily shown by a pmr comparison that no such cleavage occurred.

The benzyl ether [91], 40 mg (0.13 mmole) as monosubstituted compound) was reacted with ethylene oxide, 15μl(0.30 mmole), and stannic chloride, 20μl(0.17 mmole) using the procedure for preparation of the tryptophol [76]. Work-up gave a brown solid (53 mg) which was chromatographed on alumina (3 g). 34 mg of material was recovered, which appeared to be largely one compound by tlc, and was identified as the unreacted starting material by pmr and mass spectra. No trace of the required alkylated compound [92] was detected.

Pyranyl ether of methyl 3-(β-hydroxyethyl)indole-2-acetate [93, 94]

To the tryptophol [76], 150 mg (0.64 mmole) in dichloromethane (2.5 ml) was added 75µl (0.71 mmole) of 2,3-dihydropyran and a catalytic amount (12 mg, 0.07 mmole) of tosic acid. The solution was stirred for 6 hours at room temperature, then diluted with dichloromethane (10 ml), washed with sodium carbonate solution (2 ml) and water (2 ml) and the solvent evaporated. The product, an orange oil (236 mg) was shown to be a mixture of O, and O,N-disubstituted pyranyl ethers; predominantly the latter (weak NH absorption in the infrared spectra etc.). The less soluble monosubstituted compound [93] was separable by precipitation upon addition of light petroleum (bp 40-60°) to an ether solution of the mixture. A yellow solid was obtained, mp 75-8°C (29 mg). ν_{max}^{KBr} 3400 (NH), 1735(ester C = O)cm^{-1}; pmr: τ1.00(1H, broad singlet, NH), 2.75(4H, multiplet, ar-H), 4.70(1H, triplet-O-C_1 pyranyl H), 6.30(9H, complex multiplet, C_5 pyranyl H + $C\underline{H}_2COOC\underline{H}_3$ + $C\underline{H}_2$-O-), 7.08(2H, triplet, indole-CH_2), 8.40(6H, multiplet C_2 - C_4 pyranyl H); mass spectrum (intensity): m/e M^+ (O), 233(33), 215(20), 202(33), 201(20), 142(30), 115(23), 85(100), 67(33).

Chromatography of the petroleum soluble fraction on an alumina column (5 g) with benzene furnished the O,N-disubstituted material [94] as a yellow oil (170 mg). $\nu_{max}^{Liq.\ film}$, 1735(ester C = O), 1120(C-O-C)cm^{-1}; pmr: τ2.72(4H, multiplet, ar-H), 4.70(1H, triplet, O-C_1 pyranyl H), 5.51(1H, triplet, N-C_1 pyranyl H), 6.30(11H, complex multiplet, C_5 pyranyl H + $C\underline{H}_2COOC\underline{H}_3$ + $C\underline{H}_2O$), 7.10(2H, triplet, indole-CH_2), 8.39(12H, C_2-C_4 pyranyl H); mass spectrum (intensity): m/e 401(4), 317(2), 233(6), 205(5), 202(5), 201(4), 85(100), 84(20).

Attempted alkylation in the C-2 side chain of the pyranyl ether, [94]

(a) The O,N-disubstituted pyranyl ether, 114 mg (0.28 mmole) in benzene (7 ml), sodium hydride (60 mg, 57% oil dispersion), methyl formate (*Ca* 1 ml), in benzene (3 ml) and dimethylformamide (2 ml) were reacted as for experiment (a) of the vinylogous amide alkylation (p. 93). Work-up after 2.5 hours gave an oil (113 mg) which gave no signals at the expected shifts in the pmr spectrum corresponding to those of the formylated compound. Chromatography on alumina (5 g) gave only unreacted starting compound.

(b) 1.4 ml (0.226 N, 0.31 mmoles) of an ether solution of trityl sodium was added dropwise to a solution of the disubstituted pyranyl ether, 82 mg (0.20 mmole) in tetrahydrofuran (3 ml) under nitrogen. The red colour of the base persisted until methyl formate (*Ca* 1 ml) was distilled into the mixture. The product was worked-up as for experiment (k) in the vinylogous amide work (p. 101) but omitting addition of the mineral acid. After chromatographic separation of the excess triphenylmethane, all the remaining material (53 mg) was shown to be only the unreacted dipyranyl ether.

Benzyl ether of methyl-3-(β-hydroxyethyl)indole-2-acetate

[76], 58 mg (0.25 mmole), benzyl bromide 46 mg (0.27 mmole) and silver oxide, 60 mg (0.27 mmole) were reacted together in benzene (2 ml) following the procedure described previously for [88], (p. 108). The product obtained was a solid (77 mg). The tlc and pmr spectra of this material suggested it was a mixture of the O, and O,N-dibenzylated ethers, together with some unreacted tryptophol. The pmr spectrum showed two clearly resolved singlets at shifts of 4.76 and 5.10τ which were assigned

to the O-benzyl, and N-benzyl methylene protons respectively (integral ratio *Ca* 1:1). Low temperature (0-10°C) column chromatography on alumina (3 g) and elution with dichloromethane, resulted in separation of the benzylated products from the tryptophol, but incomplete separation of the benzyl ethers themselves. The first fraction eluted contained essentially all monosubstituted material (19 mg), while a later fraction (20 mg) also a solid, was suggestive of the O,N-disubstituted derivative containing some of the former. For further synthetic usage these materials were combined together. Spectral assignments for the pure O-benzylated compound (mp 55-7°C) were as follows: pmr: 0.50(1H, broad singlet, NH), 3.00(9H, multiplet, ar-H), 4.76(2H, singlet, $O\underline{CH}_2Ph$), 6.25(7H, multiplet, $\underline{CH}_2COO\underline{CH}_3$ + $\underline{CH}_2O$-), 7.08(2H, triplet, indole CH_2); mass spectrum (intensity): m/e 323(31), 292(20), 250(21), 232(86), 214(22), 202(15), 170(56), 115(26), 91(100), 85(25), 83(39); high resolution 323·14250, $C_{20}H_{21}NO_3$ requires 323·15214.

Attempted alkylation in the C-2 side chain of the benzyl ethers

(a) To the mixed benzyl ethers (69 mg) in dry tetrahydrofuran (2 ml) was added an ether solution of trityl sodium, 830µl (0.207N, 0.17 mmole) under nitrogen. The red colour of the base persisted until excess methyl formate (*Ca* 2 ml) was distilled into the reaction mixture. After heating at 35°C for 2 hours, the product was worked-up, as for the enamide experiment (k) (p. 101) and chromatographed on activity IV alumina (4 g). Only unreacted starting mixture was obtained.

(b) The mixed benzyl ethers (25 mg) in tetrahydrofuran (1 ml) was added to a stirred suspension of oil-free sodium hydride (30 mg, 57% oil dispersion) in tetrahydrofuran (1 ml). Excess formaldehyde gas, generated externally from paraformaldehyde, was bubbled through the suspension at

room temperature. A heavy yellow precipitate soon appeared, but redissolved on warming the mixture to 30°C and continuing to stir at the temperature for an hour. After destroying the excess hydride and removing the solvent, the product was extracted into ether. The ether solution was washed with water, dried (Na_2SO_4) and the ether evaporated to give a yellow solid (21 mg). In addition a further quantity of material, insoluble in ether was separately removed by chloroform extraction (9 mg). Preparative tlc on silica was applied separately to these materials. The ether insoluble material was largely polar base-line, and mass spectral examination suggested that it did not contain the required alkylated compounds (either the enolate or the methylol derivatives). It was not examined further. The ether soluble fraction separated into four bands, and one of these components (5 mg) gave a mass spectrum suggestive of the hydroxymethylene compounds [95], [95B]. Unfortunately insufficient material was available for critical examination. ν_{max}^{KBr} 3700-3200 (broad, strong), 1740(ester C = 0), *Ca* 1200 (very weak absorption)cm^{-1}; 20ev mass spectrum (intensity): m/e 443(2.5), 441(4), 425(11), 413(8), 381(8), 353(2), 351(9), 335(100), 325(20), 323(18), 244(21).

EXPERIMENTAL

PART II

N-[β-(3-indolyl)ethyl]-3-acetyl-1,4-dihydropyridine [103]

As mentioned in the discussion section of this thesis, in view of the problems associated with the low solubility of the pyridinium salt [102], the dihydropyridine was most appropriately prepared using a stirred interfacial technique. Thus N-[β-(3-indolyl)ethyl]-3-acetylpyridinium bromide† [102], 135 mg (0.39 mmole) was suspended in a solution of sodium bicarbonate (250 mg) in water (3.5 ml), then chloroform (4 ml) was added. Sodium hydrosulphite 270 mg (1.6 mmole) was next added in small portions to the vigorously stirred mixture under nitrogen. Carbon dioxide was evolved as the hydrosulphite was added. Continued stirring for 12 hours, resulted in disappearance of the yellow pyridinium salt from the aqueous layer, while the chloroform layer had developed an orange-yellow colour of the dihydropyridine. The uv spectrum of the reaction mixture likewise revealed the loss of the salt band at 268 nm and the appearance of a new absorption at 374 nm, characteristic of the dihydropyridine. The chloroform layer was separated and drawn off, then quickly washed with a couple of portions of water, dried over sodium sulphate and the solvent

†Sample kindly provided by Mr. B. Herten of this laboratory (mp 214-217° dec).

evaporated under nitrogen. The crude dihydropyridine was obtained as an orange-yellow solid. This was carefully stored under nitrogen in a desiccator, while the reaction mixture was stirred with a fresh amount of chloroform (4 ml), to complete the reduction and extract the remaining compound. Isolation as before, gave a combined yield of [103] of 107 mg (100%). The dihydropyridine was rather unstable in the solid state in air, undergoing partial decomposition in periods of 1-2 days. All attempts at purification of the crude material, which had a wide melting range (50-70°) were without success. It could not be crystallised from any solvent, or solvent pairs, likewise fractional precipitation techniques from either alcohol or acetone in which the compound was readily soluble, merely resulted in decomposition as shown by changes in the uv spectra of the precipitated products, notably in the reduction in absorbance or complete loss of the 374 nm band. For these instability reasons, spectral identification was made on the crude material as soon as possible after its isolation. The dihydropyridine rapidly reduced Tollen's reagent in the cold, with precipitation of silver. ν_{max}^{KBr} 3240(NH), 1675 (C = O), 1610, 1560(diene C = C)cm^{-1}; λ_{max}(logε): 222(4.39), 276(sh), 282(3.90), 290(3.85), 374(3.71)nm; $\lambda_{max}^{0.1M\ NaOH}$ (logε): 224(4.46), 276, 282, 290, 374 (no change in absorbance); $\lambda_{max}^{0.1M\ HCl}$(log$\varepsilon$, assuming MW unchanged): 221(4.35), 276sh, 284sh, 291(4.17), 302(4.16), 374(0.00)nm; pmr: τ0.86 (1H, broad singlet, D_2O exchanged, NH), 2.75(5H, multiplet, ar-H + C_2 indole), 3.51(1H, singlet, H-C_2 = C), 4.34(1H, doublet, J = 7Hz, N = CH_6 = C), 5.18(1H, doublet of triplets, J = 7, 3Hz, C = CH_5 = CH_2), 6.62(2H, triplet, J = 6Hz, $C\underline{H}_2N$), 7.03(4H, multiplet $C\text{-}\underset{H}{\overset{H}{C_4}}\text{-}C$ + indole-CH_2-), 8.21(3H, singlet, $COCH_3$).

Effect of sodium methoxide on the dihydropyridine [103]

30µl of a molar solution of sodium methoxide (0.10 mole NaOMe) in methanol, was added to [103], 74 mg (0.28 mmole) in absolute methanol (1 ml) under nitrogen, and the mixture stirred for an hour at ambient temperature. No change in the uv spectrum of the reaction mixture was indicative of the preservation intact of the dihydropyridine. A further quantity, 310µl (total 1.1 mmole) of methoxide solution was added, the temperature was raised to 40°C and the stirring continued for a day. In this time there was no change in the uv spectrum compared to the dihydropyridine itself, except for a very slow decrease in the intensity of the 374nm band which could be attributed to the slow 'background' decomposition of the compound, (occurring in the absence of the base). The methanol was evaporated under nitrogen, the residue extracted with cold water and dried over phosphorus pentoxide *in vacuo*. Of the total product (70 mg) which remained soluble in chloroform, the pmr spectrum was found to correspond to that of the dihydropyridine. No methoxide substitution was detected.

N-[β-(3-indolyl)ethyl]-3-acetyl-4-cyano-1,4-dihydropyridine [104]

In a typical preparation of the cyanide adduct of [103], the pyridinium bromide salt, 300 mg (0.87 mmole) was dissolved in hot methanol (20 ml) and excess potassium cyanide (1 g) in water (2 ml) was added. The clear solution was allowed to stand at room temperature for a day, then the methanol was evaporated, the residue triturated well with cold water, filtered, and the product dried over phosphorus pentoxide *in vacuo*. The product [104] so obtained was a pale yellow powder, 205 mg (80%). No special precautions to exclude air were deemed necessary during either

the isolation or manipulation of this compound. As with the unsubstituted compound, repeated attempts to recrystallise the derivative were unsuccessful. This material was however, suggested by tlc to be substantially one compound, and this conjecture was supported by its spectral characteristics (pmr integration etc.), although the melting point was not sharp. Like the unsubstituted compound, the adduct was found to stable to bases (NaOH), labile to mineral acids, and to reduce Tollen's reagent. ν_{max}^{KBr} 3350(NH), 2240 (weak C ≡ N), 1690(C = O), 1642, 1590 (diene C = C)cm^{-1}; λ_{max}(log ε): 221(4.40), 276(sh), 283(3.86), 290(3.83), and 355(3.81)nm; $\lambda_{max}^{0.1M\ HCl}$ 226, 286(sh), 292, 300(sh), 355(0.00)nm; pmr: τ1.26(1H, broad singlet, D_2O exchanged, NH), 2.62(4H, ar-H), 3.02(1H, singlet, indole C_2H), 3.62(1H, singlet, $N\text{-}C_2 = C$), 4.15(1H, doublet, J = 7Hz, $C_6 = C$), 5.10(1H, double doublet, J = 7, 4.5Hz , 5.60(1H, doublet, J = 4.5Hz, $H\text{-}C_4\text{-}CN$), 6.60(2H, triplet, J = 5.5Hz, CH_2N), 7.06(2H, triplet, J = 5.5Hz, indole-CH_2), 8.34(3H, singlet, $COCH_3$).

N-(β-bromoethyl)-3-acetyl-4-cyano-1,4-dihydropyridine [106]

N-(β-bromoethyl)-3-acetylpyridinium bromide† [105] 500 mg (1.6 mmole) was dissolved in water (2 ml) and excess potassium cyanide (0.5 g) in water (2 ml) added. The dihydropyridine immediately separated as a dark yellow-brown tar. The supernatent liquid was decanted, and the product triturated with cold water. Extraction with refluxing ether, gave after removal of the solvent, a yellow oil (470 mg), which would not solidify. As before, this compound could not be recrystallised from any solvent. Like the indole substituted compound, this dihydropyridine was

†Sample kindly provided by Mr. B. Herten of this laboratory.

relatively stable, and amenable to spectral characterisation. $\nu_{max}^{Liq.film}$ 2240(C ≡ N), 1690(C = O), 1646, 1600(diene C = C)cm^{-1}; λ_{max} 218, 268, 346 nm; $\lambda_{max}^{0.1M\ NaOH}$ 224, 300(intense), 268, and 346(0.00)nm; pmr: τ2.58 (1H, singlet, $N\text{-}C_2 = C$), 3.76(1H, doublet, J = 7Hz, $HC_6 = C$), 5.02(1H, doublet of doublets, J = 7Hz, 4.5Hz, $= C_5\text{-}C$), 5.48(1H, doublet, J = 4.5Hz, $H\text{-}C_4\text{-}CN$), 6.32(4H, multiplet, J = 3.5Hz, $Br\text{-}CH_2\text{-}CH_2\text{-}N$), 7.75(3H, singlet, $COCH_3$).

Effect of acid on the cyano adduct [104]

In view of the problems associated with the isolation and examination of the solid products obtained from the reaction of various dihydropyridines with mineral acid, some effort was made to first monitor changes in the pmr spectrum which occurred after addition of hydrochloric acid to a methanolic solution.

In such a study, the cyano compound [104], 70 mg was dissolved in deuteromethanol in an nmr tube and the spectrum recorded. Addition of 6M hydrochloric acid (5 drops of solution in D_2O) and heating to 50°C for only 5 minutes resulted in darkening of the solution, evolution of HCN, and complete destruction of the dihydropyridine, as shown by disappearance of all the signals at shifts corresponding to the dihydropyridine protons. New signals were observed at 1.20τ(sharp singlet), 4.10τ (singlet), and a series of poorly resolved multiplets at 5.05, 5.80, 6.20, and 7.05τ. The latter shift was correlated with the β-ethyl side chain protons of the indole (approximate shift of protons in dihydropyridine spectrum in CD_3OD). The singlet assigned to the 3-acetyl protons, appeared to have shifted to a lower field (7.45τ), than its position in the dihydropyridine (8.32τ). No signals correlated with pyridinium pro-

tons were detected, likewise for either the 1,4,5,6-tetrahydropyridine [83] or the tetracyclic ketone [84] known to result from Pictet-Spengler cyclisation of the enamide.[52] After 5 hours at 50°, no significant further change in the pmr spectrum was observed. After transferring the reaction mixture to a flask, the methanol was removed, and the residue neutralised with sodium bicarbonate solution, to give a dark-brown solid. This was triturated, first with cold water, then with ether (in which the solid was totally insoluble), and dried. The product was no longer soluble in chloroform, but did redissolve in deuteromethanol, in which another pmr spectrum was taken. In this spectrum, the sharp singlet at 1.20τ had disappeared and was replaced by a broad singlet at 2.20τ, which was exchanged by D_2O(NH?).

The peak associated with the 3-acetyl protons was at a new shift of τ7.88. Tlc of the reaction product suggested it to be a mixture of four components, one of them present in major amount. Preparative tlc on alumina of the material (25 mg) using a chloroform-methanol mixture, separated it into two bands, together with the substance which remained on the base-line. One of the bands, associated with the major component, gave a yellow solid (12 mg) upon extraction from the plate. The uv spectrum of this material was found to correspond reasonably closely with the spectrum of the acidified dihydropyridine solution: λmax 221, 283(sh), 291, and 303nm. When the spectral solution was acidified, the 303 nm absorption band, was slightly hypsochromically shifted, with a significant decrease in the absorbance (12%). $\lambda_{max}^{0.1MHCL}$ 221, 284sh, 300nm. Other spectral data is presented below:

ν_{max}^{KBr} 3300(NH), 1738(ester C = O?), 1640, 1630(weak absorption, C = O?),

1580 (C = C) cm^{-1}; pmr: τ0.58(1H, sharp singlet, D_2O exchanged, NH), 1.02(1H, broad singlet, D_2O exchanged, NH?) 2.74(*Ca* 8H, multiplet ar-H), 6.35(*Ca* 4H, multiplet), 7.16(*Ca* 4H, multiplet), and 7.80(3H, singlet); mass spectrum, intensity: m/e 86(66), 84(100), 78(42); high sensitivity mass spectrum, intensity: m/e 364(17), 336(50), 308(67), 280(50), 278(100).

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