Chapter Nine

LABORATORY MODELS FOR THE BIOGENESIS OF INDOLE ALKALOIDS

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INTRODUCTION

In an earlier discussion, we described some useful techniques for the study of indole alkaloid biosynthesis based mainly on relatively short term incubations (5 min - 7 days)[1]. The problems presented by strychnine biosynthesis in *Strychnos nux-vomica* called for the adoption of another variant in the methodology of reaching the site of biosynthesis in a higher plant, i.e. long-term incubation. Since this approach might be useful in other studies we begin our discussion with a description of these experiments. The delineation of the major pathway to the indole alkaloids having reached a fairly advanced stage, we have undertaken a systematic investigation of the biogenetic-type synthesis

of all of the major classes of alkaloid, starting with and as far as possible retaining Nature's own intermediates throughout. The following account of our researches and complementary studies by other workers in the field indicates the state of the art of this endeavor and at the same time hopefully points the way to further biochemical experiments. We also comment on the relevance of these studies for chemotaxonomy and for the enzymology of alkaloid biosynthesis.

BIOSYNTHESIS OF STRYCHNINE IN *STRYCHNOS NUX-VOMICA*

In contrast to the modest but measurable incorporation of the larger intermediate substrates in *Catharanthus roseus*, species such as *Rauwolfia serpentina* and *Strychnos nux-vomica* have, from the outset, displayed most disappointing properties as far as precursor uptake has been concerned. Indeed if these had been the only plants available for study it might be suggested that our knowledge of the intricate mechanisms whereby mevalonate and tryptophan are co-metabolized to the complex indole alkaloids of the major structural types (for example, the *Strychnos* alkaloids) would still be in its infancy. Thus the administration of geissochizine (a known precursor of the "*Strychnos*" alkaloid akuammicine in *V. rosea*) to *S. nux-vomica* under "normal" feeding conditions (5 to 7 days) led to insignificant ($<2 \times 10^{-3}$ percent) incorporations into strychnine. More surprisingly, the Wieland-Gumlich aldehyde and diaboline, which seem almost certain candidates for intermediacy (near the end of the pathway), failed to incorporate over the same time scale. Reinvestigation of the problem involved the technique of replanting young seedlings after 5 day's incubation with 2-, 15-, 16-, and 17-labeled intermediates (^3H, aryl group). After a further 100 days, strychnine was isolated from each feeding, crystallized to constant radioactivity, and (in the case of positive incorporation) degraded to locate the position of the tritium label. Reference to Figure 1 shows that indeed geissoschizine fulfills the same pivotal role in *S. nux-vomica* as it does in *C. roseus* but that the result would have been in doubt without resource to the "prolonged contact" technique. Similarly the anticipated but hitherto unrealized conversion of Wieland-Gumlich aldehyde to strychnine (1.60 percent) could be demonstrated. The nonincorporation of diaboline after 70 days suggests strongly that the seventh ring of the complex strychnine molecule is built in

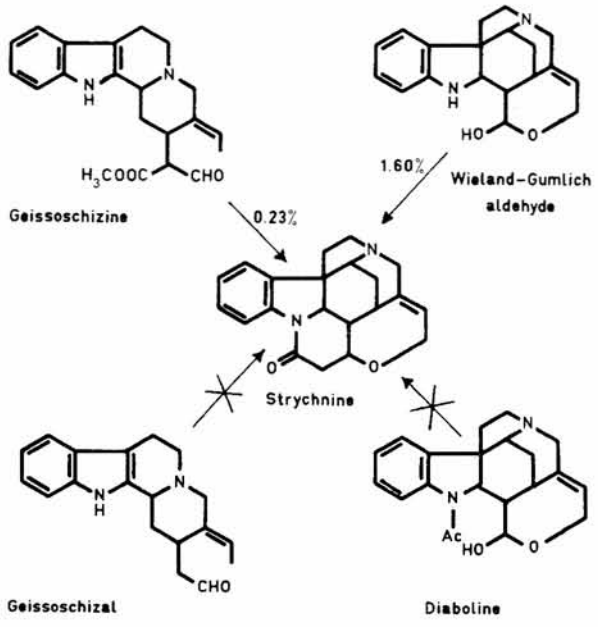

Figure 1. Interrelationships between strychnine and potential precursors in *Strychnos nux-vomica* revealed by prolonged feeding.

a clockwise carbon-to-carbon addition of the acetic acid unit, rather than from the nitrogen-carbon condensation which would have required diaboline as an intermediate. The nonincorporation of geissoschizal reveals that, by a hitherto undisclosed mechanism, a carbon atom is lost somewhere between geissoschizine and Wieland-Gumlich aldehyde but not by a simple decarbomethoxylation reaction.

These experiments do not begin to answer the question of genetic control and evolution in a plant such as *S. nux-vomica*, which, although producing quite massive concentrations of strychnine and brucine by a process now shown to emanate from geissoschizine, does not elaborate the variety of rearranged structures of the *Aspidosperma* and *Iboga* families engendered by *V. roseus* from the latter precursor, as discussed below.

BIOGENETIC-TYPE SYNTHESIS OF THE INDOLE ALKALOIDS

The family of alkaloids derived from tryptophan now boasts over 1000 members[2,3] thus accounting for almost 10% of known natural product structures[4]. It therefore seemed particularly appropriate to apply the principles of biogenetic analysis and biogenetic-type synthesis* to this group, many of whose members and indeed whole sub-classes are endowed with interesting biological and chemo-therapeutic properties. To this end several laboratories have, over the years, devoted considerable effort towards the laboratory construction of tryptophan-derived alkaloids based on authenticated or presumptive biochemistry. Much of the early work in this field has been reviewed in depth[3,6,7] and a seminal essay on the progress and philosophy of biogenetic-type synthesis[8] sets the background for the present description of more recent work, largely but by no means exclusively selected from the author's own published and unpublished experiments which have been directed towards solutions to the many and diverse problems in the intermediary metabolism of tryptophan in higher plants. Several detailed accounts of the nontryptophan-derived segment of the major classes of indole alkaloid have appeared[9,10,11,12] and we shall therefore only refer briefly to the major incorporation results in this area.

The Tryptophan - Corynanthe Sequence

The observation[10,13] that the *Corynanthe* alkaloids geissoschizine (1), corynantheine (2) and ajmalicine (3) are laid down as the earliest recognisible members of the indole family in *Catharanthus roseus* could be easily reconciled with the recognition of vincoside (4) as the "primordial" alkaloid of the series. Thus the structure and stereochemistry enjoyed by 4 represents the first encounter of tryptophan (or tryptamine) with the "C_{10}" unit contained

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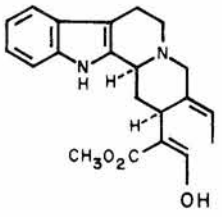
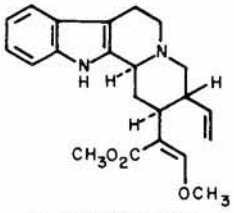
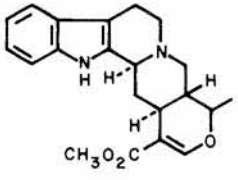
*For definitions of biogenetic-type synthesis see Van Tamelen[5]. The experiments described in this review fall into the class of "amphosynthesis" whereby the *in vitro* chemistry of a recognized of presumptive intermediate is used to carry out a partial synthesis of a complex natural product.



1, Geissoschizine



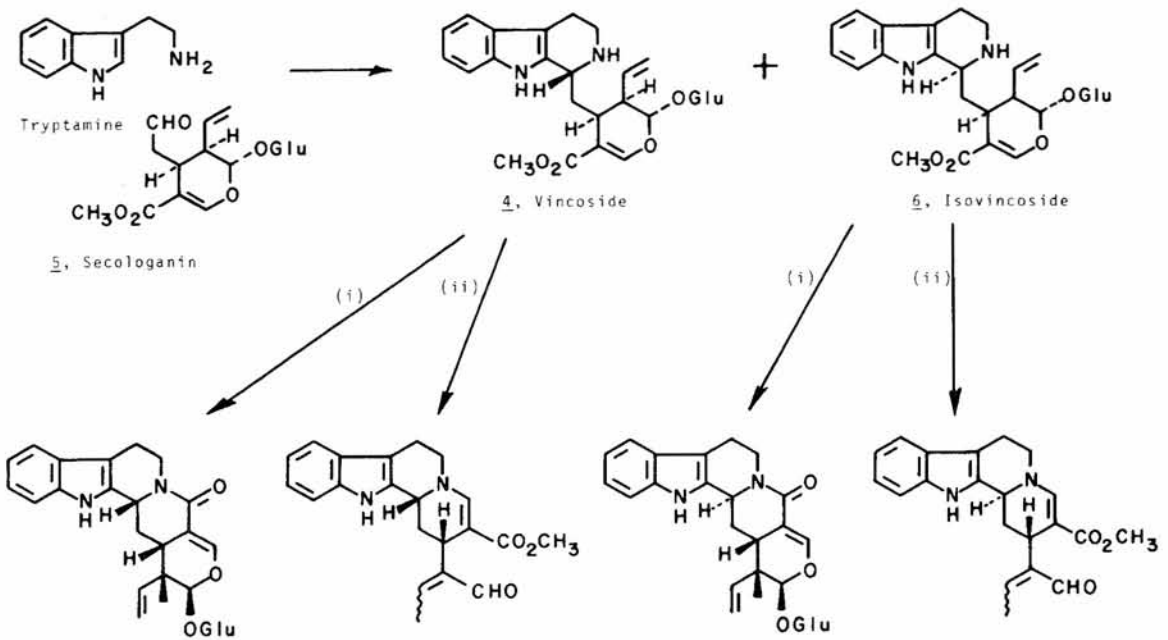
2, Corynantheine



3, Ajmalicine

in the proven monoterpenoid precursor secologanin (5). In order to maintain a logical rather than a historical account of this field, a most important reaction, viz the condensation of tryptamine and secologanin to a separate mixture of vincoside (4) and the C-3 epimer isovincoside (6) described by Battersby, can be regarded as the first biogenetic-type transformation. Both 4 and 6 occur as natural products (e.g. in *C. roseus*) but only the 3βH-diastereisomer, vincoside, serves as an efficient precursor of the *Corynanthe*, *Aspidosperma* and *Iboga* alkaloids *in vivo*[11-14-15-16-17]. Although until recently of extreme scarcity, secologanin has now become available in considerable abundance, thus paving the way for further experiments designed to transform vincoside (4) and/or its isomer (6) into the *Corynanthe* and other alkaloids. Earlier work in this field had been performed with vincoside to give the C_3 epimer of vallesiochotamine (7) and vincoside lactam (8) by appropriate condensations as shown in Scheme 1[16-17]. Similarly the isolactam (9) and vallesiochotamine (10) could be prepared from 6.

The effect of removing the glucoside function with β-glucosidase at pH 5.2 has been re-examined. In the case of vincoside (3βH) (4) the resultant product was again


Tryptamine
5, Secologanin
4, Vincoside
6, Isovincoside
(i)
(ii)
(i)
(ii)
8, Vincoside Lactam
7, Isovallesiachotamine
9, Isovincoside Lactam
10, Vallesiachotamine

Scheme 1

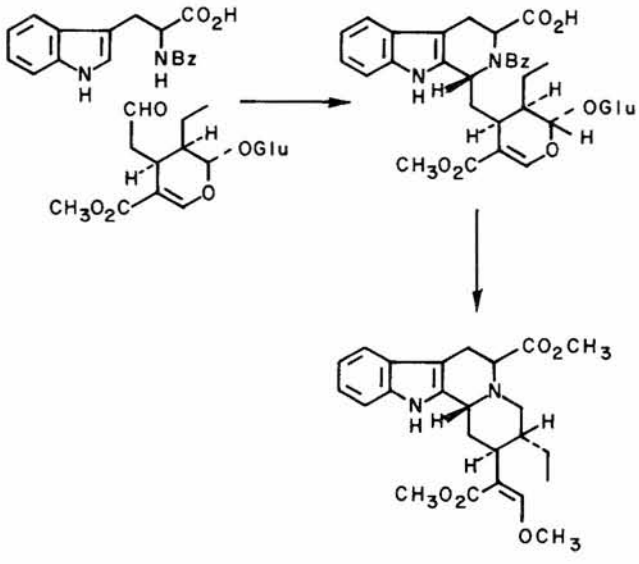
(see Scheme 1) mainly the C-3 epimer of vallesiachotamine (7). However, as portrayed in Scheme 2, when the same reaction was carried out using isovincoside (3αH) (6) as substrate there was isolated in addition to vallesiachotamine (10) a small but reproducible yield (2-3%) of an alkaloid $C_{21}H_{22}O_3N_2$ which has been assigned the structure of the dieneamine (11) corresponding to a "dehydro geissoschizine" (A.I. Scott *et al.*, unpublished). The gross structure of 11 was readily demonstrated (Scheme 2) by catalytic hydrogenation to the tetrahydro compound (12) identical with the reduction product (12) of geissoschizine (1). The structure of 11 is of more than passing interest since this formula was proposed for a very active biosynthetic intermediate uncovered in previous work with short term incubations of *C. roseus*[18]. Comparison of the autoradiographs of 11 from these latter studies and the behavior of the new dehydro *Corynanthe* alkaloid (11) showed complete identity (A.I. Scott *et al.*, unpublished). Thus the *Corynanthe* series has been reached in this simple experiment which however does not simulate the unusual switch of the stereochemistry at C-3 implicit in the biochemical conversion vincoside (3βH) to geissoschizine (3αH). In a recent, related investigation, Brown and Chapple describe the preparation of 13 by an ingenious variant on this theme in which tryptophan is condensed with dihydrosecologanin and the subsequent condensation is controlled via N-benzylation (Scheme 3)[19]. Obviously, the next refinement in this series requires the exercise of control in the generation of the reactive species such as 6a and 6b (Scheme 2).

With the successful conversion of secologanin and tryptophan to the *Corynanthe* and vallesiachotamine series the stage now appears to be set for the logical progression to the more complex alkaloids, e.g. *Strychnos*, *Aspidosperma*, and *Iboga*.

From Corynanthe to Strychnos - The missing link

In spite of many interesting theories and even more ingenious experiments it has not yet proved possible to emulate Nature in passing between structural classes (A) and (B). The pattern of the "C_{10}" unit is not disturbed in this process, the new result being the migration of bond marked "O" from the α- to the β-position of the indole nucleus, followed by formation of the new bond (*) from the activated

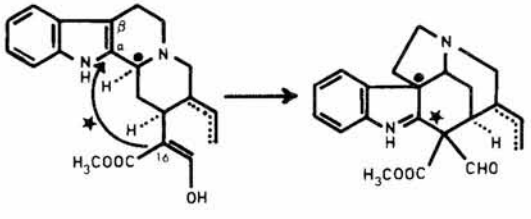
Scheme 2



13

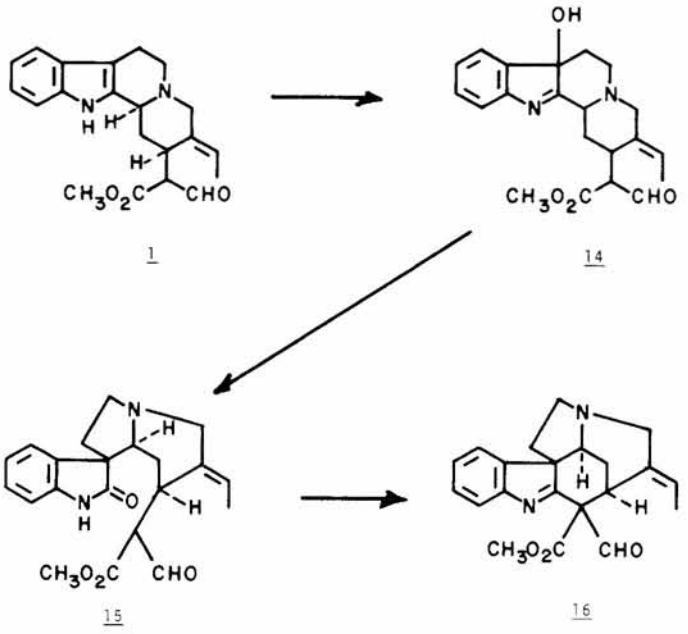
Scheme 3

C-16 position to the α-indole position. The sole working process for this theme is the special but very instructive case of Harley-Mason and Waterfield[20]. An earlier experi-



A B

ment with geissoschizine (refluxing acetic acid) in which conversion to type B was observed[21] suffers from extensive side reactions and variability in yield and obviously requires further definition. Other analogies involving β-oxidation (1 → 14) and oxindole chemistry (15 → 16) have been proposed[10], there being preliminary feeding evidence



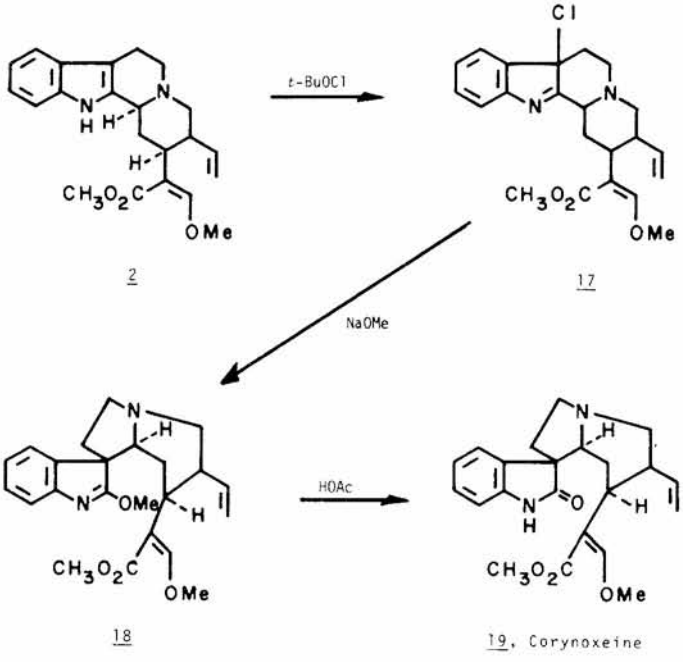
Scheme 4

to support the latter pathway *in vivo* (Scheme 4). However no satisfactory chemical operation on a *Corynanthe* alkaloid has yet been discovered to bridge this major gap.

At this juncture we should note that several *Corynanthe* alkaloids have been used as substrates for some interesting interconversions to related families and some of these branch-lines are now explored.

Oxindoles. The use of *t*-butyl hypochorite has provided entry into the β-chloroindolenine series (17) which in turn serves as a useful substrate for the oxindole, corynoxeine (19), and imino ether formation (18) (Scheme 5). The problems of the stereochemical control of these reactions and the equilibration at C-3 and C-7 have been reviewed in full detail[22].

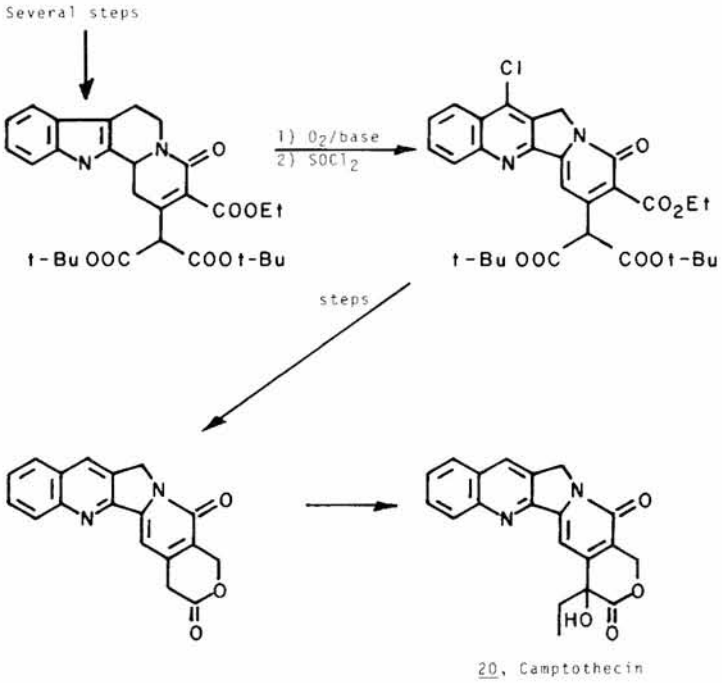
Camptothecin (20). An ingenious partial synthesis (based on biogenetic analogy) of the camptothecin system has been described by Winterfeldt and his colleagues[23-24] as summarized in Scheme 6.



Scheme 5

Ajmaline (22). A most plausible and interesting model for the formation of ajmaline from the aldehydocarboxylic acid (21) [Scheme 7] has been presented[25], in which generation of the immonium species (21a) leads to the desired bond formation (C-16 → C-8) and thence to the relay substance (21b). This is a particularly important model for the timing of the biochemical decarboxylation of the tryptophan moiety of the *Corynanthe* series.

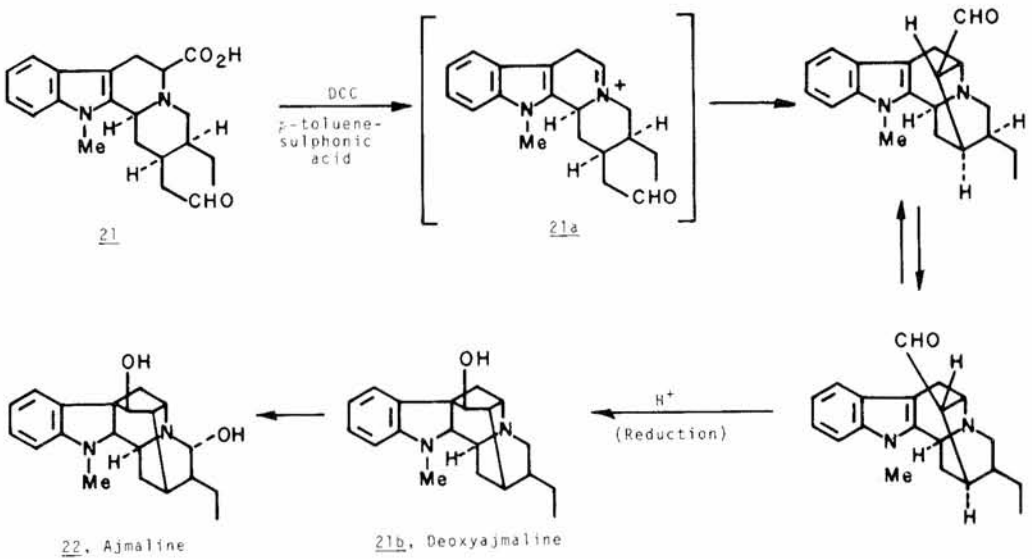
Flavoperierine and Related Alkaloids. A simple model for the generation of the flavoperierine system (24,25) has been found in the reverse Mannich chemistry of the enamine (23) (Scheme 8) which can be formed by mercuric acetate oxidation of geissoschizine (1) (A.I. Scott *et al.*, unpublished results). Although the appropriate biochemical correlation has not been made, the above experiment suggests that loss of the "C_3" segment may well arise by such a process which occurs with facility *in vitro*.



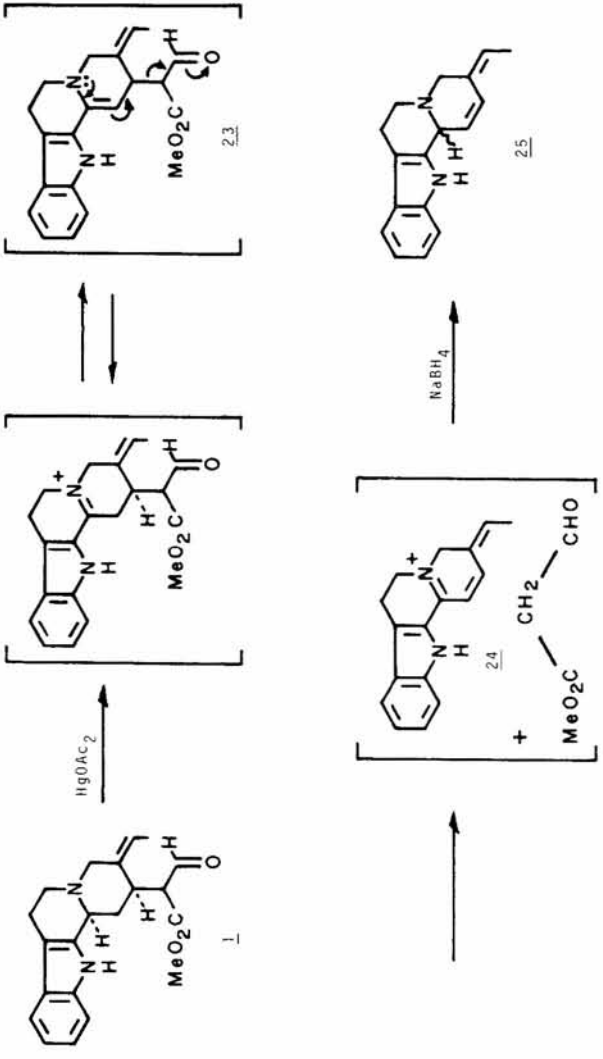
Scheme 6

Related reactions of the *Picralima* alkaloids. A close analogy for *Strychnos* alkaloid biosynthesis from a *Corynanthe* precursor concerns the manipulation[26,27] of the indolenine system of 26 (Scheme 9) in the presence of potassium *t*-butoxide in a remarkable rearrangement to norfluorocurarine (27). Successful conversion of a *Corynanthe* alkaloid (e.g. 1 to 26) would complete this intriguing system but, with the exception of some work by Dolby on models for echitamine synthesis, a satisfactory method for the vital bond-making process from C-16 has yet to be described. In fact all attempts to utilize the anionic or radical-forming propensity of C-16 have been uniformly unsuccessful, indicating the necessity for finding a new approach to the chemistry of this apparently reactive center.

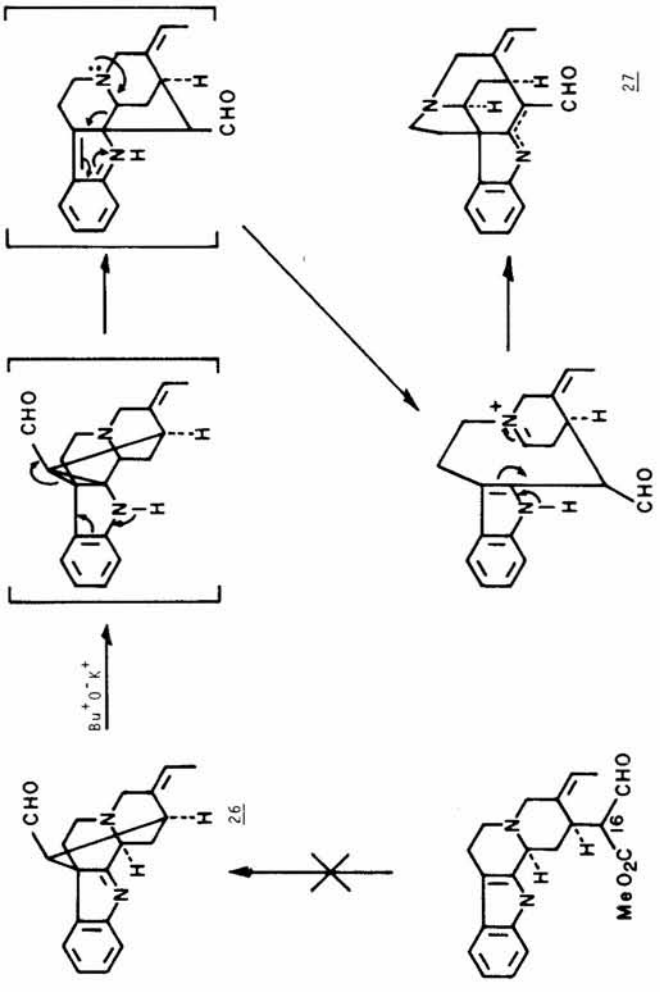
With the key link (*Corynanthe* → *Strychnos*) still missing from the chain of events in the biogenetic synthesis of the major classes we now consider some of the successful


CO2H
DCC
p-toluene-sulphonic acid
CHO
21
21a
H+
(Reduction)
OH
21b, Deoxyajmaline
22, Ajmaline

Scheme 7



Scheme 8

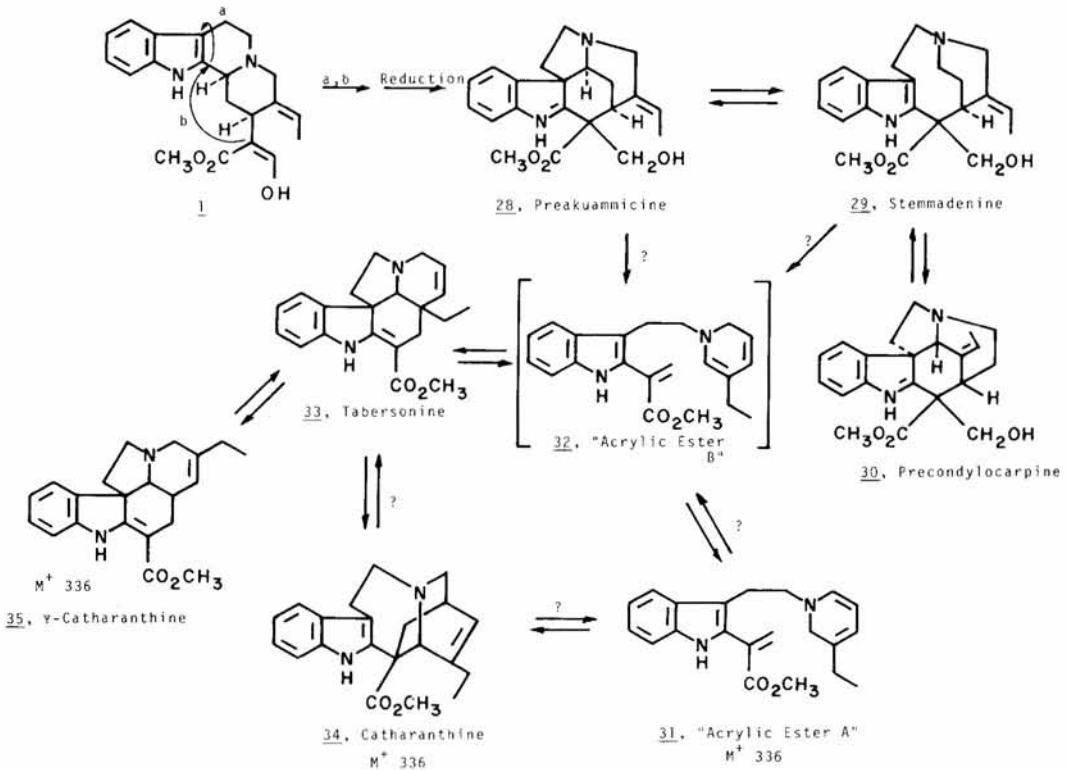


Scheme 9

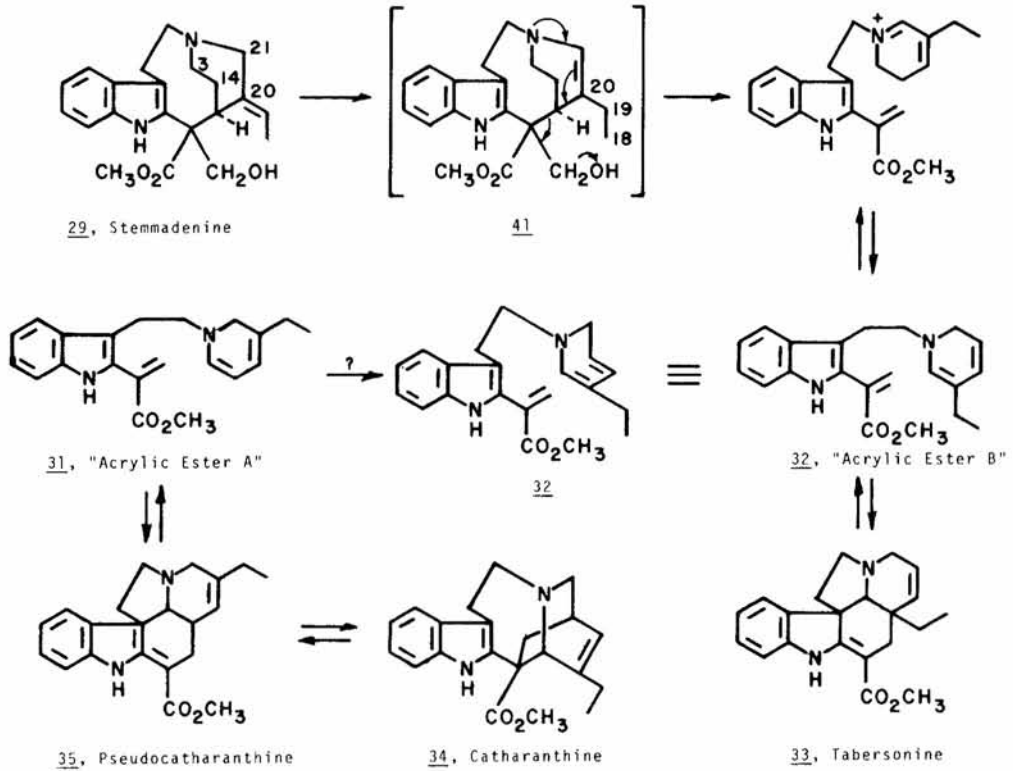
conversions wrought upon the *Strychnos* and related systems in the quest for further analogies in the biochemical pathway. The overall scheme is shown again for the sake of clarity in Scheme 10 which emphasizes the importance of the acrylic ester or secodine intermediates (31 and 32). The latter have proved valuable touchstones in considering the deep-seated rearrangements which must convert *Corynanthe/Strychnos* alkaloids to the typical members of *Aspidosperma* and *Iboga* series.

From Stemmadenine to Aspidosperma and Iboga Alkaloids - The "336" Concept

As soon as the discovery and intermediacy of stemmadenine (29) and tabersonine (33) in the main pathway of indole alkaloid biosynthesis were established it was suggested[10-13] that the formal dehydration of stemmadenine (M.W. 354) to the species with M.W. 336 introduced, as an intriguing consequence, the cycloaddition or extended Mannich chemistry of the hypothetical achiral reactive intermediate (32). Thus it was predicted that the dihydropyridine acrylic ester (32) or its isomer (31) could account for the skeletal rearrangements in going from the *Corynanthe* pattern (1) and preakuammicine (28) or stemmadenine (29) to either the *Aspidosperma* or *Iboga* families. An especially attractive facet of this theory which represents a modification and simplification of the earlier ideas of Wenkert[28-29] is its ability to explain the occurrence of pentacyclic *Aspidosperma* alkaloids in racemic and antipodal versions. Yet another interesting consequence of this speculation (which was originally conceived as a result of the mass spectral behavior of tabersonine!) involves the various recombination characteristics of the hypothetical intermediate (32) which not only serves as a highly plausible model for the observed biochemical conversions of stemmadenine (29) to tabersonine (33) but also forges a mechanistic link between (33) (*Aspidosperma* series) and the isomeric catharanthine (34) (*Iboga* series) as shown in Scheme 11. In its simplest form this isomerization process operates at the oxidation level of M.W. 336. In fact our first experiments[30] with the rare alkaloid stemmadenine provided a welcome model for these concepts which, as the subsequent biochemical experiments and the structures of new isolates of Apocynaceae were to show, constituted not only a working laboratory analogy for the labyrinth of inter-connecting pathways between families, but allowed the prediction of hitherto

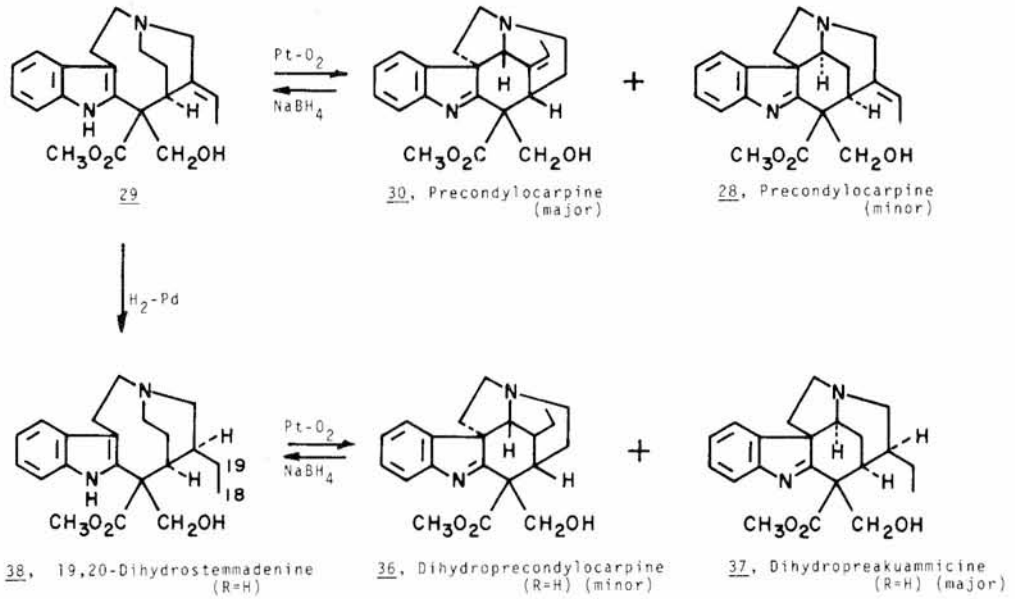

a
b
a,b
Reduction
1
28, Preakuammicine
29, Stemmadenine
30, Precondylocarpine
33, Tabersonine
32, "Acrylic Ester β"
35, Y-Catharanthine
M+ 336
34, Catharanthine
M+ 336
31, "Acrylic Ester A"
M+ 336

Scheme 10


29, Stemmadenine
41
31, "Acrylic Ester A"
32
32, "Acrylic Ester B"
35, Pseudocatharanthine
34, Catharanthine
33, Tabersonine

Scheme 11

undiscovered structural types. The logical evolution of this area of bioorganic chemistry was however not to prove facile and trenchant criticism[31-32-33] of the first communication[30] on this topic required some reinvestigation in order to define extremely critical reaction conditions. Due to the scarcity of stemmadenine a series of expeditions to the state of Vera Cruz (Mexico) was made to identify and collect the fruits of *Stemmadenia Donelli-Smithii* in early November 1971[34]. Harvesting and extraction of this plant at other times of the year appear to be particularly unpropitious, the yield of 29 falling from 0.5% to less than 0.001%. With a fresh supply of the vital *Corynanthe-Strychnos* alkaloid in hand, the early experiments were examined with a most satisfactory outcome: the achievement of both regiospecific and stereospecific control in the transformation of stemmadenine to both *Aspidosperma* and *Iboga* alkaloids as described below. In addition to the *in vivo* conversion indicated it could be shown that platinum-catalyzed oxidation of 29 gave the pentacyclic system corresponding to oxygenation or dehydrogenation of the carbon adjacent to N (b) in stemmadenine. These reactions could be reversed by treatment of 28 and 30 with sodium borohydride (Scheme 12). Thus the facile redox equilibria depicted in Scheme 12 may indicate the branch point at which the stemmadenine molecule is drawn enzymatically into either the *Aspidosperma* or *Iboga* series. In this first set of preliminary experiments, stemmadenine was heated under vigorous reflux conditions in acetic acid over silica boiling stones at high (~200°) external bath temperatures. Great variability in composition and yield of the products was encountered from such reactions which were frequently maintained for 1-2 days with concomitant loss of solvent. However, it was shown by analytical and preparative tlc techniques that stemmadenine was converted first to the O-acetate (a reaction that can be effected very simply by reflux at 150° bath temperature). The latter undergoes a deep-seated rearrangement to give products of the M.W. 336 set, i.e. tabersonine (33) and the structural isomer of catharanthine, pseudocatharanthine (35). A small amount of catharanthine could also be detected. Thus although the reactions are extremely sensitive to concentration, surface and/or thermal conditions, the discovery of the formal dehydration products of stemmadenine in racemic form - as would be required by any mechanism involving 32 - lends credence to a most rewarding concept. As originally described[30], this biogenetic-type interconversion turned out


Pt-O2
NaBH4
H2-Pd
CH3O2C
CH2OH
29
30, Precondylocarpine (major)
28, Precondylocarpine (minor)
38, 19,20-Dihydrostemmadenine (R=H)
36, Dihydroprecondylocarpine (R=H) (minor)
37, Dihydropreakuammicine (R=H) (major)

Scheme 12

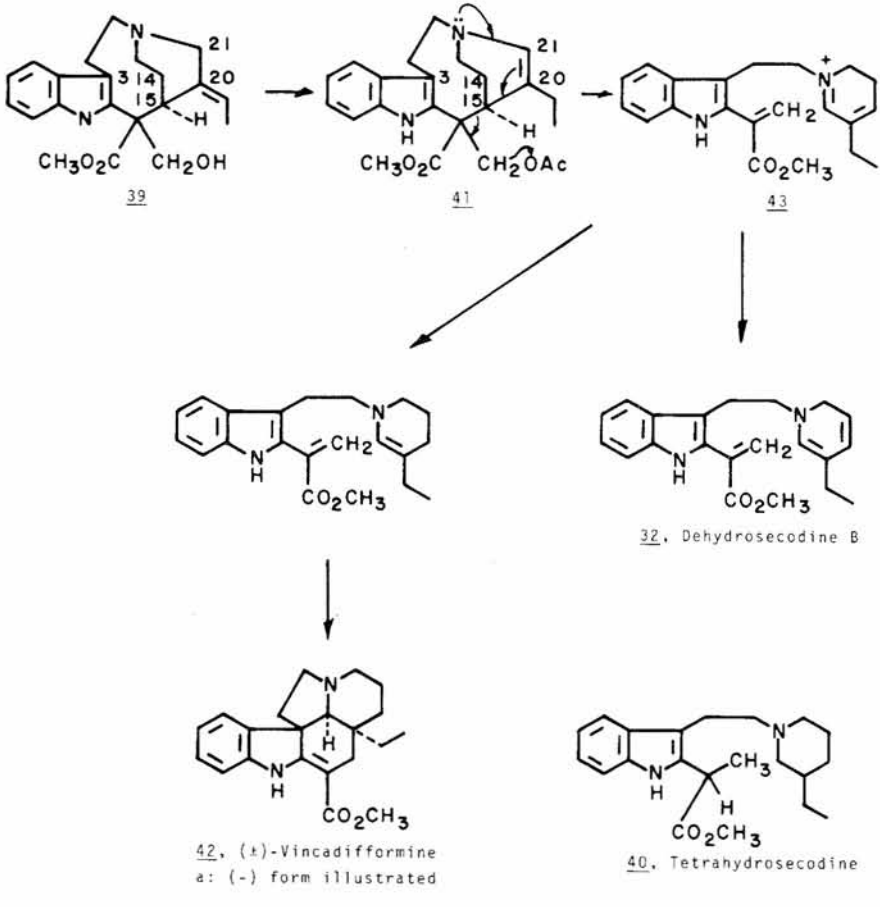
to have severe limitations as a practical and reproducible route to the more complex alkaloids of the indole family. A complete account of this phase of the study is outside the scope of our review and the interested reader is referred elsewhere for a discussion of the problem and its eventual solution[30-32-33].

EVOLUTION OF A REGIO- AND STEREOSPECIFIC MODEL

In order to rationalize the promising but erratic results of the early (acetic acid) experiment the new source of stemmadenine was used to explore and define the conditions necessary, not only for the generation of the seco ester (32), but also its recombination to the known isomers of the "336" series in a more rigorous manner.

The Corynanthe-Aspidosperma Relationship

The reverse Mannich chemistry discussed above (Schemes 10, 11) for *Aspidosperma* biosynthesis via dehydrosecodine B (32) differs in regio-specificity from the *Iboga* model described below in that the formation of the secodine system B can take place via C-20, C-21 rather than C-3, C-14. The introduction of unsaturation at C-21 could, in principle, be realized by two methods. First it was hoped that the simple isomerization process (Scheme 11) previously adduced for reaction of stemmadenine (acetate) in hot acetic acid solution, might be capable of more rigorous control and thus lead only to the *Aspidosperma* framework by formation and recombination of dehydrosecodine B (32). A further spur towards a search for isomerization conditions was the discovery that, whereas stemmadenine (29) is hydrogenated unexceptionally to the 19, 20-dihydro derivative (38) in the presence of platinum catalyst (Scheme 12), the behavior of stemmadenine acetate (39) is quite different towards reduction. Thus, at atmospheric pressure, hydrogenation of 39 over platinum in ethanol solution leads to (±)-tetrahydrosecodine (40) (Scheme 13) in 75% yield. The facile cleavage of the 15, 16 sigma bond in 39, but not in 29 is suggestive that the primary acetoxyl at C-17 aids the irreversible loss of acetate from the Δ20,21 isomer (41) which is in equilibrium with the Δ18,19 exocyclic olefinic group of the starting material. This high yielding partial synthesis[36] of the naturally occurring member of the secodine family could be



Scheme 13

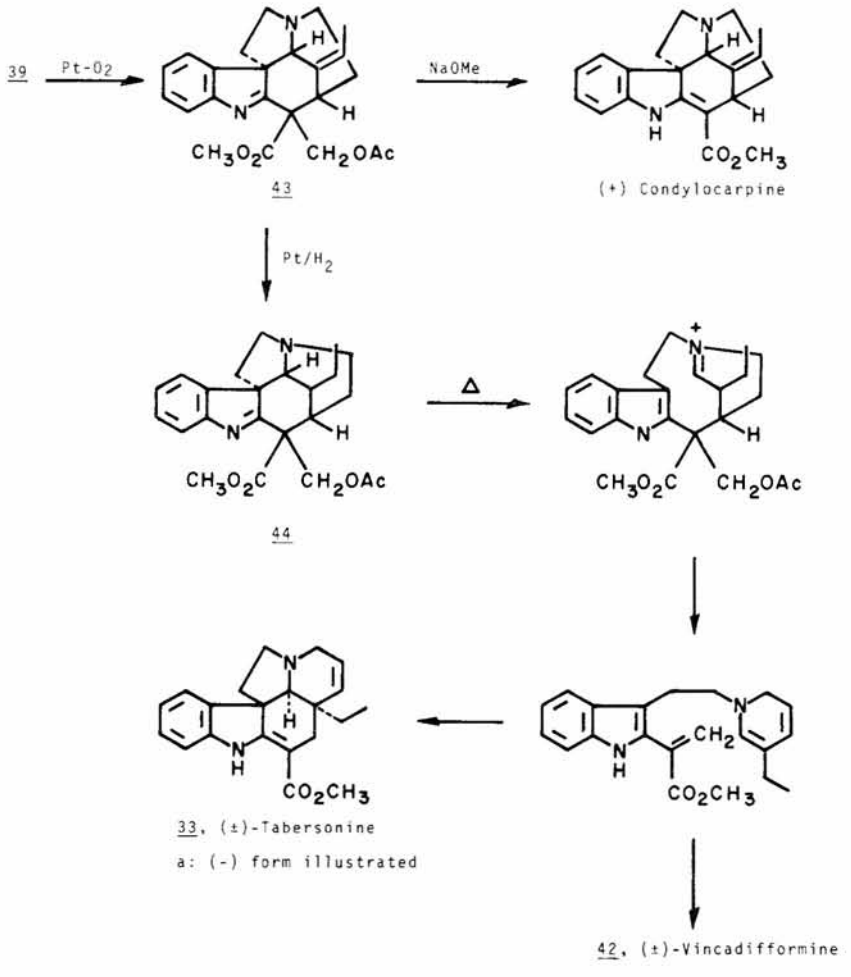
regarded as a model for the biosynthesis of these alkaloids from stemmadenine.

Experiments designed to capture the fugitive dihydropyridine (32) in the cyclized form corresponding to the *Aspidosperma* alkaloids were carried out as follows. Firstly, in a reaction designed to separate the *Aspidosperma* pathway of the "early" experiments, conversion of (39) to (±)-vincadifformine (42) was achieved in low yield (0.15 - 0.20%) by thermolysis at 150° on a silica gel surface for 25 minutes.

The analytical and preparative procedures used for this and the succeeding experiments left no doubt that tabersonine was not a product of the reaction. A plausible reaction pathway via 41 is shown in Scheme 13, which suggests that the intermediate immonium species (43) suffers reduction by disproportionative release of hydride to dehydrosecodine B (32) which then cyclizes to (±)-vincadifformine and that this sequence is favored over the more direct rearrangement (without reduction) to (±)-tabersonine. An alternative method was then studied, which takes into account the ready aerial oxidation (which must have occurred under the vigorous conditions of the "early" experiment) in acetic acid solution of 39 to the acetate (43) of the naturally occurring pentacyclic alkaloid, precondylocarpine (30)[37], a step which can be achieved more efficiently using platinum catalyzed oxidation (Scheme 14)[38]. Catalytic reduction of the ethylidene group of 43 affords the dihydroacetate (44) which is now formally capable of regiospecific collapse to dehydrosecodine B as shown in Scheme 14. When the acetate was subjected to thermolysis (150°, silica gel, 25 min) a separable mixture of (±)-tabersonine (33) (0.2%) and (±)-vincadifformine (42) (0.2%) was produced, while no trace of pseudocatharanthine (35) could be detected. It was concluded from these results that the generation of the acrylic ester B takes place without rearrangement to the A isomer according to Scheme 11. Although the yields in these models for "*Aspidosperma* synthetase" are extremely low, the reactions provide a working hypothesis for the complex series of rearrangements which accompany the biochemical conversion of the *Corynanthe* to the *Aspidosperma* and Secodine families. At the same time, it is believed that the multi-step reaction sequence which was concealed in the earlier model experiments can now be understood in terms of the operation of indiscriminate oxidative and reductive attack on stemmadenine acetate, the first formed product of the reaction between acetic acid and the original substrate, stemmadenine. Depending on the timing of the oxidative and reductive steps, the acetate is converted to both the preakuammicine and precondylocarpine skeletons which at the appropriate oxidation level collapse to *Iboga* and *Aspidosperma* alkaloids respectively.

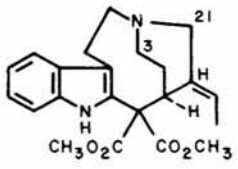
The Corynanthe - Iboga Relationship[39]

The thermolysis of 19,20-dihydrostemmadenine was next



Scheme 14

examined. This work took the reductive factor of the "early" experiment into account, for it is well known that systems such as 45, 46 readily disproportionate to the pyridinium salt (47) and the tetrahydropyridine (48). Allowance was also made for the regiospecific (aerial) oxidation of 38 at C-3 to dihydropreakuammicine (37). In order to complete the analogy with the earlier work using acetic acid (which has been shown to form the primary acetate),



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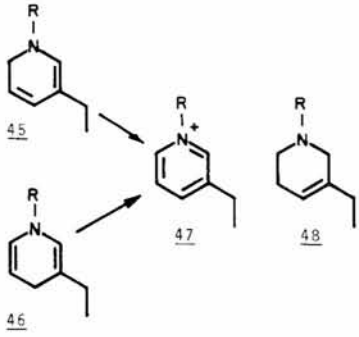
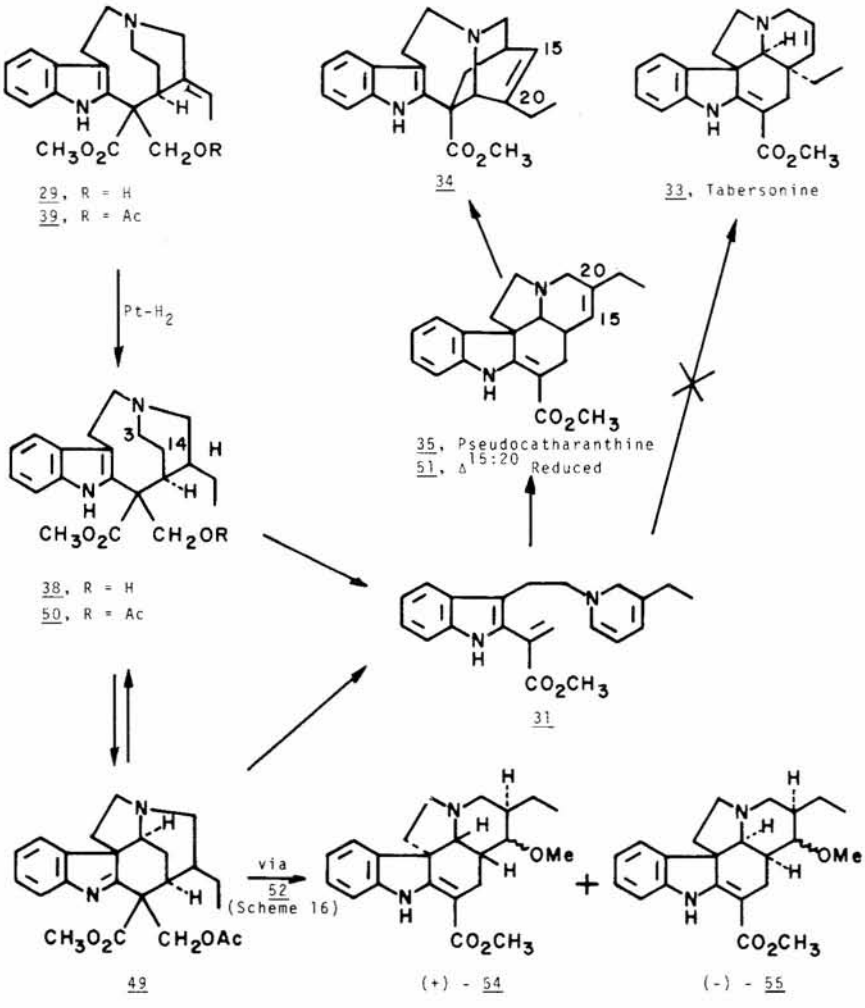


dihydrostemmadenine was converted to the acetate (50). When the latter compound was heated on a silica gel tlc plate at 150° for 45 min the resultant mixture afforded (±)-pseudocatharanthine (35) (1%) - and its dihydro derivative (51) (0.5%) (Scheme 15). No trace of tabersonine (33) was detectable in this experiment. In order to rationalize this result it is necessary to generate a double bond at C-3,C-14 (50a) so that the extended reverse Mannich chemistry shown in Scheme 16 can operate. The further reaction of the iminium salt (52) is seen as a rearrangement to 3,14-dehydrosecodine (31), which we have designated dehydrosecodine A (See Scheme 16).

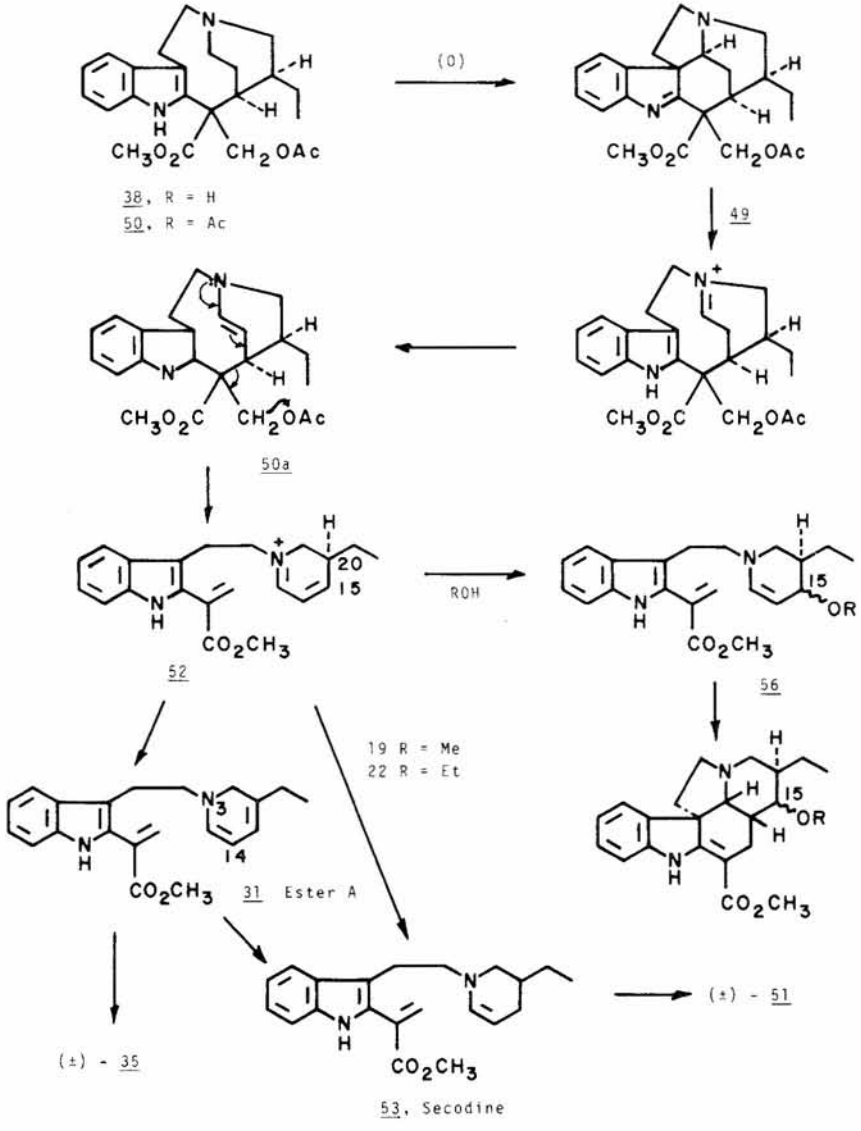
Recombination of the achiral ester (31) affords (±)-pseudocatharanthine (35)[40] whereas conjugate reduction of 52 leads to the secodine (53) whose cyclization to (±)-dihydropseudocatharanthine (51) is unexceptional. A more efficient way of demonstrating this regio- and stereospecific rearrangement was found by preparing 19,20-dihydropreakuammicine acetate (49), a known autoxidation product of 50 by platinum/oxygen oxidation of 50. Thermolysis of 49 at 150° for 20 min afforded (±)-35 in yields which, although by no means optimized, average 5%. The known equilibrium between 35 and catharanthine (34) completes the partial synthesis of the latter member of the *Iboga* family in racemic form.

Further insight into the mechanism of this remarkable reaction was gained by methanolysis of the acetate (49) at room temperature for 4 hr, or at 80° for 15 min. The products of this reaction were now optically active and were separated to afford a dextrorotatory 15-methoxydihydropseudocatharanthine (54) and the levorotatory diastereomer



Scheme 15

(55) in the ratio 9:1. The combined overall yield of this reaction from dihydrostemmadenine (38) was 3.5%. This result is in full accord with the postulate that the immonium species (52) is an intermediate in the rearrangement process and that conjugate addition of methanol affords the diastereomeric mixture 54 & 55 via 56. The stereospecific preference for the absolute configuration 54 over 55 may


(O)
38, R = H
50, R = Ac
49
50a
ROH
52
56
19 R = Me
22 R = Et
31 Ester A
(±) - 35
(±) - 51
53, Secodine

Scheme 16

be dictated by the configuration of the methoxyl group at C-15 which controls the observed stereochemistry of the cyclization process. This particular model is also illustrative of the remarkable variation[41] in absolute configuration of the pentacyclic *Aspidosperma* alkaloids within the same plant, which could be mediated by conjugate addition of the appropriate prosthetic group of the synthesizing enzyme to such an immonium species. Another pertinent example is the co-occurrence of (-)-coronaridine (57) and (+)-catharanthine (34) within the same species (*Catharanthus roseus*)(A.I. Scott *et al.* unpublished results). This duality of absolute stereochemistry for such complex alkaloids again can be viewed as the result of the timing of the reduction step of the immonium ion (52) which can either be reduced and cyclized to give (-)-coronaridine or pass through the achiral intermediate (31) with protropic loss of C-20 stereochemistry and thence by enzymic control to the antipodal series represented by catharanthine (34). In this connection it is of interest to note that the *in vitro* conversion described above in which 52 is suggested as the first formed *chano* intermediate, the thermal reaction serves to epimerise this center so that the products of the reactions are (±) pseudocatharanthine and the corresponding racemic dihydro compound (51). The milder conditions used in the methanolysis experiment however give products which, on the basis of molecular rotation differences with other memebers of this series, indicate an optical purity of approximately 70-80%. The configuration of the methoxyl group at C-15 in these diastereoisomers is at present unknown. A similar reaction was observed when dihydropreakuammicine acetate was heated in ethanol at 80° for 15 min to yield a separable mixture of the dextrorotatory and levorotatory diastereomers of 15-ethoxy-15,20-dihydropseudocatharanthine in the ratio 10:1, in 10% (combined) yield.

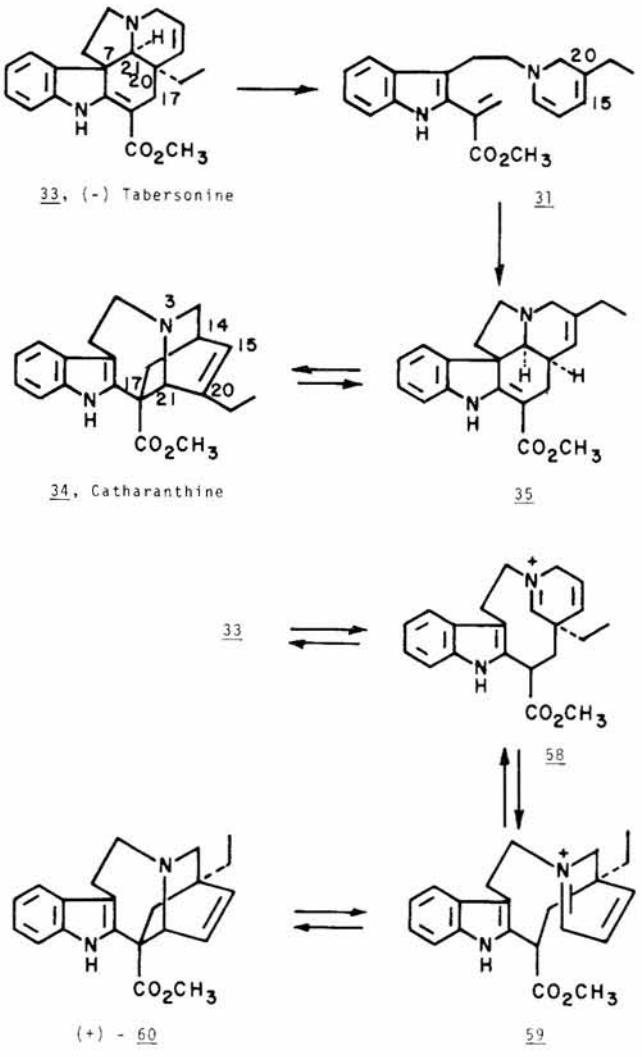
The above experiments indicate that an "*Iboga* synthetase" model in the form of dehydrosecodine A has been demonstrated to operate and that not only the regio specific generation and recombination of 31 mediated by the collapse of reduced preakuammicine (49), but the stereospecificity of the recyclization process leads to the *Iboga* isomer, (±)-pseudocatharanthine, which in turn is convertible to (±)-catharanthine. Although the yields in this reaction are not yet of preparative value, it is our view that the synthesis and reactivity of the dehydrosecodine system is worthy of more detailed study as a new method of preparing quite complex pentacyclic

alkaloids from simple starting materials, with full stereospecific control.

Further isomerizations of the "336 series. The Aspidosperma-Iboga-Secodine Relationship

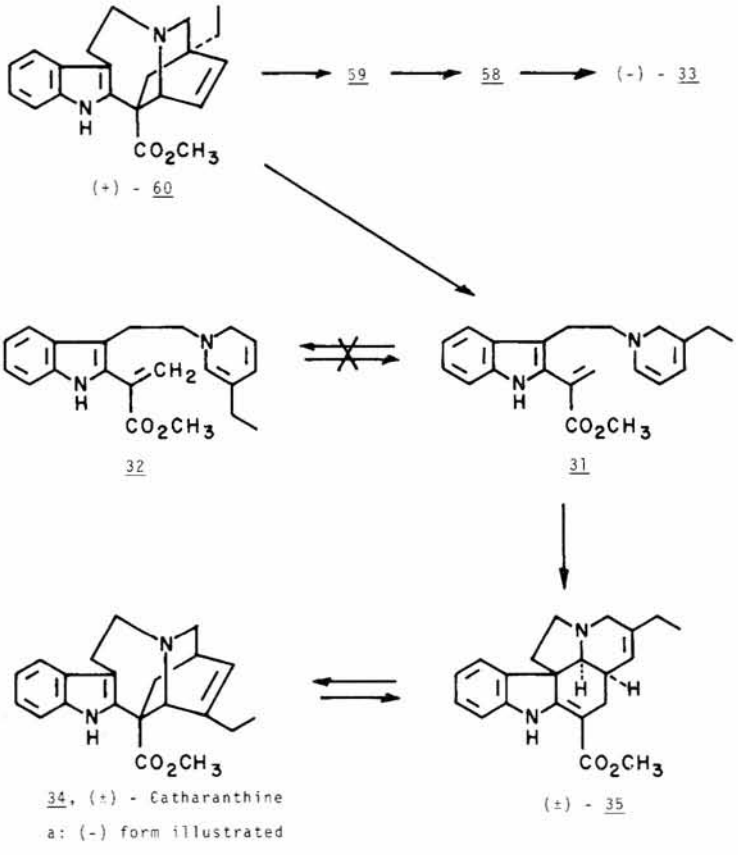
In 1968, a report[30] on the *in vivo* and *in vitro* transformations of the *Aspidosperma* alkaloid tabersonine (33) to the *Iboga* alkaloid catharanthine included the proposal for an intermediate (31) in this reaction which has since been found in a variety of stabilized versions. In 1969, Smith and his co-workers concluded[31] that this reaction, which involves rupture of both the 7-21 and 17-20 bonds of 33, proceeds only as far as cleavage of the 7-21 bond (Scheme 17). The resultant immonium species (58) then rearranges (59) and cyclizes to allocatharanthine (60), an optically active isomer of catharanthine (34) and pseudocatharanthine (35), the latter two *Iboga* structures being interconvertible (Scheme 17). Scott & Cherry stated, however, that both ionic and thermal requirements must be met in carrying out the full transformation[43] which, by passing through 60 affords the racemic products described earlier. In a reconfirmation and simplification of the 1968 report, the separation of the ionic and thermal components has been described recently[42].

(-)-Tabersonine (33) was heated in acetic acid for 15 hr (external bath temperature 140°). The resultant mixture was separated and (+)-allocatharanthine (60) (11%) isolated. A solution of 60 was applied to a silica gel tlc plate, the solvent removed, and the plate heated at 150° for 30 minutes. Elution and chromatography on $AgNO_3$-impregnated silica gel plates afforded two major products. These were (±)-pseudocatharanthine (35) (4%) and optically pure (-)-tabersonine (33) (4%). The absence of any racemization of tabersonine indicates reversal of the formation of allocatharanthine without cleavage at C-17, C-20 i.e. by the pathway 60 → 59 → 58 → 33 (Scheme 18). Thus not only is the *Aspidosperma* framework stable towards retro-Diels-Alder reaction but, in confirmation of the complete specificity noted in the previous section for the reaction of dehydro secodines A and B, only the racemic product, 35, of cyclization of dehydrosecodine A (31) is observed, there being no evidence for equilibration with the B isomer (32) which would have yielded (±)-tabersonine.



Scheme 17

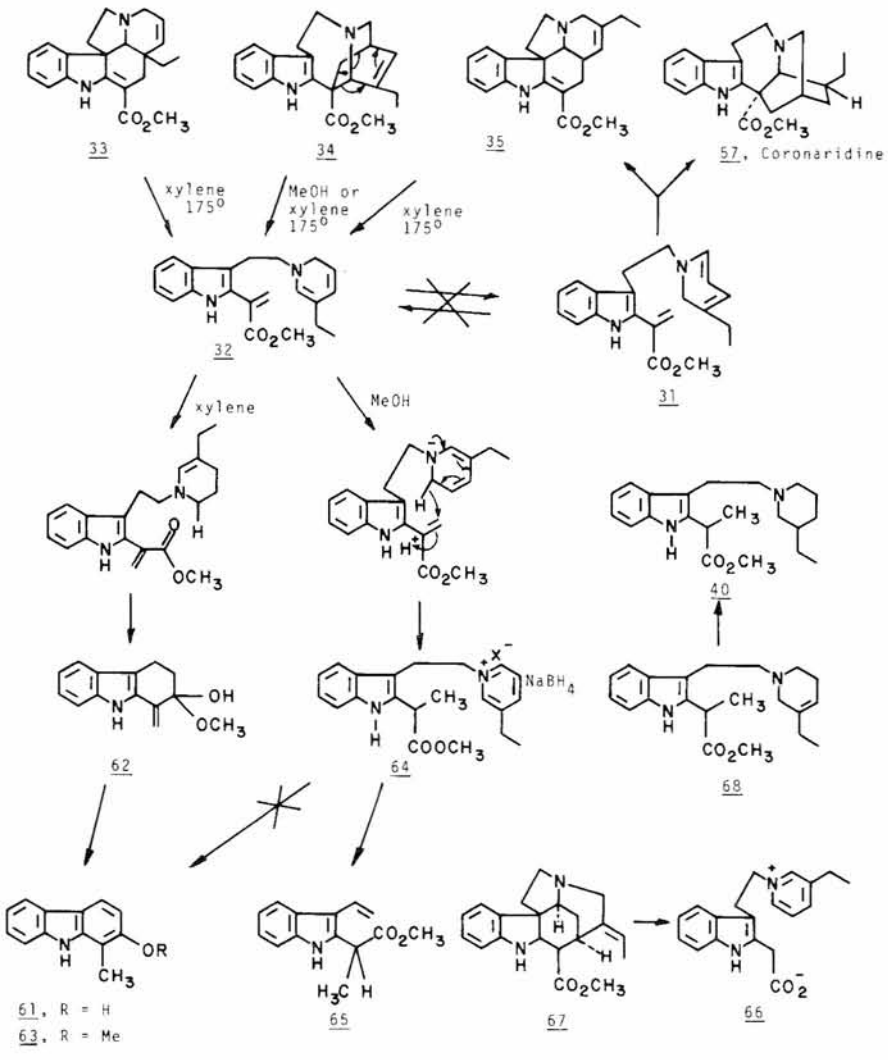
In order to gain further evidence regarding the intermediacy of the secodine esters (31 and 32) a study was made of the thermal reactions of the key members of this series. When xylene solutions of the isomeric alkaloids (-)-tabersonine (33), (+)-catharanthine (34), and (±)-pseudocatharanthine (35) were maintained in sealed tubes for 1.5 hr at the temperatures indicated (Scheme 19), in each case (but with



Scheme 18

a different energy requirement) the same products were isolated and characterized as 3-ethylpyridine and 1-methyl-2-hydroxycarbazole (61)[43].

We suggest that the formation of these products takes place by way of a retro-Diels-Alder reaction to afford the fugitive ester[39] followed by an intramolecular rearrangement and hydrogen transfer from the dihydropyridine to the acrylic ester function yielding the hemiketal (62) with loss of 3-ethylpyridine as indicated. Elimination of methanol and further rearrangements then give the carbazole (61). In support of the latter process, 1-methyl-2-methoxycarbazole



Scheme 19

(63) could be detected and characterized as a minor product of the reaction.

More direct evidence for the formation of 32 was obtained by the capture in 50% yield of the racemic salt 64 when catharanthine was heated in methanol at 140° for 2 hr[43].

In contrast to the intramolecular formation of the carbazole from the dihydropyridineacrylic ester in the aprotic solvent xylene, the availability of solvent protons in the latter case appears to divert the collapse of this intermediate in methanol via an ionic mechanism to the pyridinium salt. When the reaction is carried out in CH_3OD solution, the nmr spectrum of the salt no longer shows a signal at π 6.12 ($CD(CH_3)CO_2$-CH_3) and the doublet π 8.62 ($CH(CH_3)CO_2CH_3$) is replaced by a singlet (3 H) in accord with the mechanism 32 → 64 as shown in Scheme 19. This salt is stable in methanol at 175°, but on pyrolysis at this temperature affords the carbazole (61) presumably via elimination of ethylpyridine and cyclization of the resulting vinyl ester (65). The generation in methanol solution[39] could also be rationalized by an ionic mechanism which recalls the formation of the betaine (66) from akuammicine (67)[44]. Since the species 32 and 64 could be reached *in vivo* from stemmadenine, tabersonine and catharanthine, it will be of interest to test these three alkaloids as biochemical precursors for secodines and secamines and also to consider the system 32 → 64 as a labile but isolable biosynthetic entity in the *Aspidosperma* and *Iboga* metabolic grid[43].

Laboratory analogy for secodine formation from salt 64 was obtained by reduction of 64 with sodium borohydride which gave (±)-dihydrosecodine (68) as a crystalline racemic base (35% yield), identical in all spectroscopic properties with the amorphous natural alkaloid obtained from *Rhazya stricta* (A.I. Scott *et al.*, unpublished results). Further (catalytic) reduction of 68 afforded tetrahydrosecodine (40) which also occurs in *R. stricta*. This mode of formation of dihydrosecodine may be contrasted as a biosynthetic model with the extremely facile formation of (±)-tetrahydrosecodine (40) from stemmadenine acetate in 75% yield where it was suggested that rupture of the C-15,C-17 bond was facilitated by platinum-catalyzed isomerization of the 19,20-double bond to the endo position thereby allowing collapse to the secodine system, as discussed earlier.

Finally we note that the earlier difficulties in rationalizing the lack of biochemical conversion of (+)-catharanthine (34) to its 15,20-dihydro derivative coronaridine (57) can now be understood, since in *Catharanthus roseus* it has been found that these *Iboga* alkaloids bear antipodal absolute stereochemistry at C-14,C-17 and C-21[45]. The co-occurrence

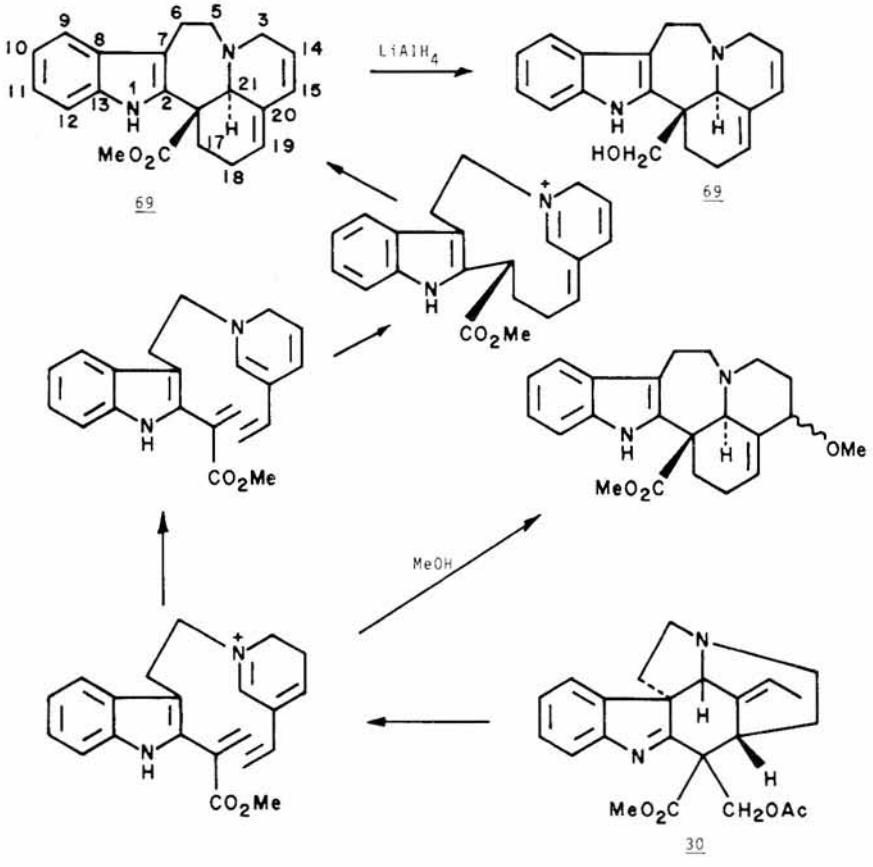
of such antipodal species in the same plant may once again signify the onset of different synthetases acting on the same intermediate. Thus (-)-coronaridine is detectable at very early stages of germination of *C. roseus* whereas (+)-catharanthine is found only after prolonged germination and seedling growth[13]. The achiral ester dehydrosecodine A (31) or its isomer B (32) could serve as an intermediate which can undergo cyclization (Scheme 19) to either coronaridine (57) or (+)-catharanthine (34) and, predictably, the *pseudo* series represented by 35, discovery of which from a natural plant source has just been reported.*

Andranginine, Pseudocatharanthine and Vindolinine

During the *in vitro* conversion of precondylocarpine (30) and related pentacyclic alkaloids, it was observed that thermolysis of 30) in ethyl acetate solution afforded (in 28% yield) a new racemic pentacyclic compound (69), M.W. 334, corresponding to dehydrogenation of the 336 series. The reaction was interpreted as shown in Scheme 20, where the stability of the vinyl dihydropyridine species is believed to have mediated in a relatively high yielding reaction to produce the new system (69) corresponding to the ionic addition depicted. In the course of structural investigation of a Madagascar species undertaken some months after this experiment was completed it was discovered that the compound andranginine (MW 334, $[\alpha]_{300-600}0^{o}$) was in fact identical in every respect with this novel, partially synthetic alkaloid[46]. The full power of biogenetic type synthesis was thus revealed in two independent studies which converged in a most satisfying manner. The implications of this and related discoveries of racemic alkaloids is discussed in the final section of this review.

Although pseudocatharanthine (35) has never been described as a natural product, its synthesis from catharanthine, tabersonine, and preakuammicine are suggestive that the *Aspidosperma*-like structure of yet another mode of cyclization of the acrylic ester will appear as a natural series.* This postulate may well have extremely

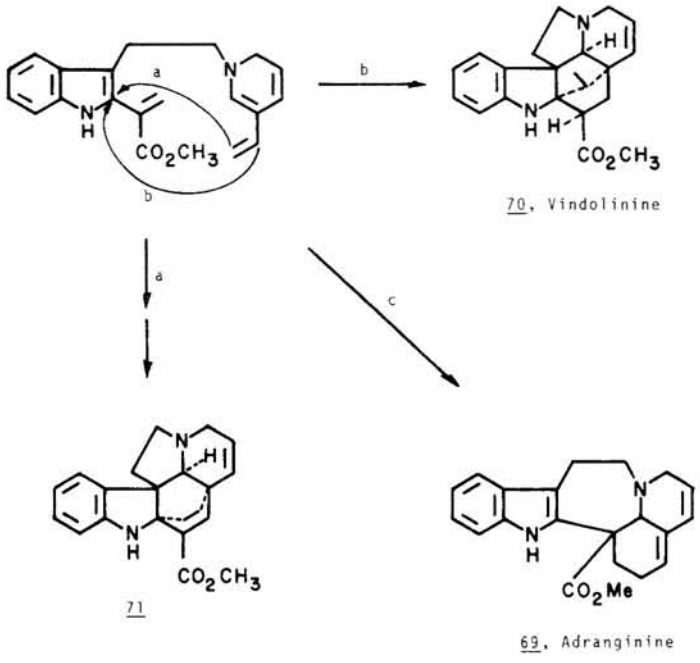
*Dr. P. Potier (Gif-sur-Yvette) has informed us that the system (35) has now been isolated from a natural source by Professor J. LeMen.



Scheme 20

important consequences for the biosynthesis of the "double" alkaloids discussed below.

In surveying the modes of cyclization of the secodine system, there exists a rather puzzling feature of the structure of a class of hexacyclic alkaloids exemplified by vindolinine (70) inasmuch as the stereochemistry required by the ethano-bridge (C-19, C-5) confers a *trans-syn* configuration on the fusion of rings D and E, a feature which is absent from all known *Aspidosperma* alkaloids. Elsewhere[47], we comment on this fact which has now been satisfactorily



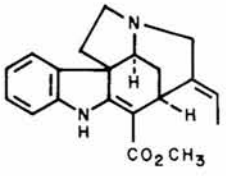
Scheme 21

explained by the correction of the structure of vindolinine to the bridged system (70)[48]. A possible derivation based on biogenetic condensations is shown in Scheme 21, which incidentally allows prediction of the relative stereochemistry of the asymmetric centers of vindolinine and also traces the connection with the hexacyclic series (71).

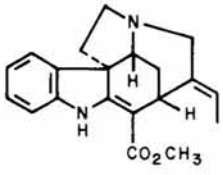
STEREOCHEMICAL AND STRUCTURAL RELATIONSHIPS WITHIN ALKALOID FAMILIES

Strychnos series

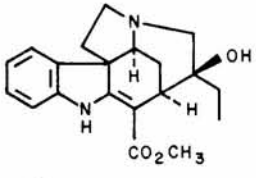
A perennial problem encountered in the theory of *Strychnos* biosynthesis has been the occurrence of enantiomeric and diastereoisomeric pairs of alkaloids exemplified by (+)-and (-)-akuammicine (67) and (-)-lochneridine and (+)-20 *epi*lochneridine. The crux of the stereochemical



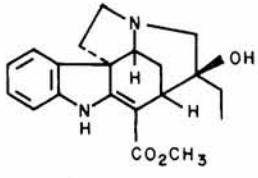
67, (-) - Akuammicine



67, (+) - Akuammicine



(-) - Lochneridine

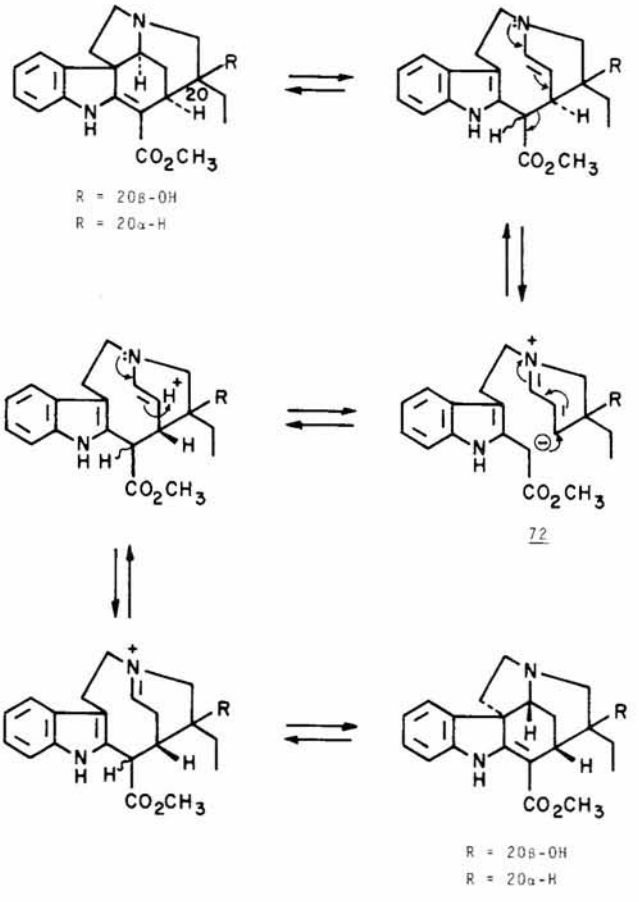


(+) - Lochneridine

enigma in this series concerns the apparent inversion at C-15 which, in the absence of any satisfactory rationale for the racemization or epimerization of this center, has led to the logical suggestion that C-15 retain its absolute stereochemical identity (αH or βH) from an earlier precursor of the *Corynanthe* series, or indeed that the inverted βH configuration comes all the way from a secoiridoid or "unnatural" configuration at this site. However, a recent series of experiments[49] portrayed in Scheme 22 has shown that epimerization of C-3, C-7 and C-15 is accomplished by a simple equilibration experiment in methanol solution at 95° (50 hr): this result now rationalizes the appearance of the so-called antipodal *Strychnos* family. The implications of these findings are discussed in the concluding section of this review. At this point we note that the intermediate *seco* system (71) may serve as a useful and easily accessible relay for total synthesis in this field. These experiments conducted with 19,20α-dihydroakuammicine (R=H) also constitute a model for the lochneridine series (R=OH).

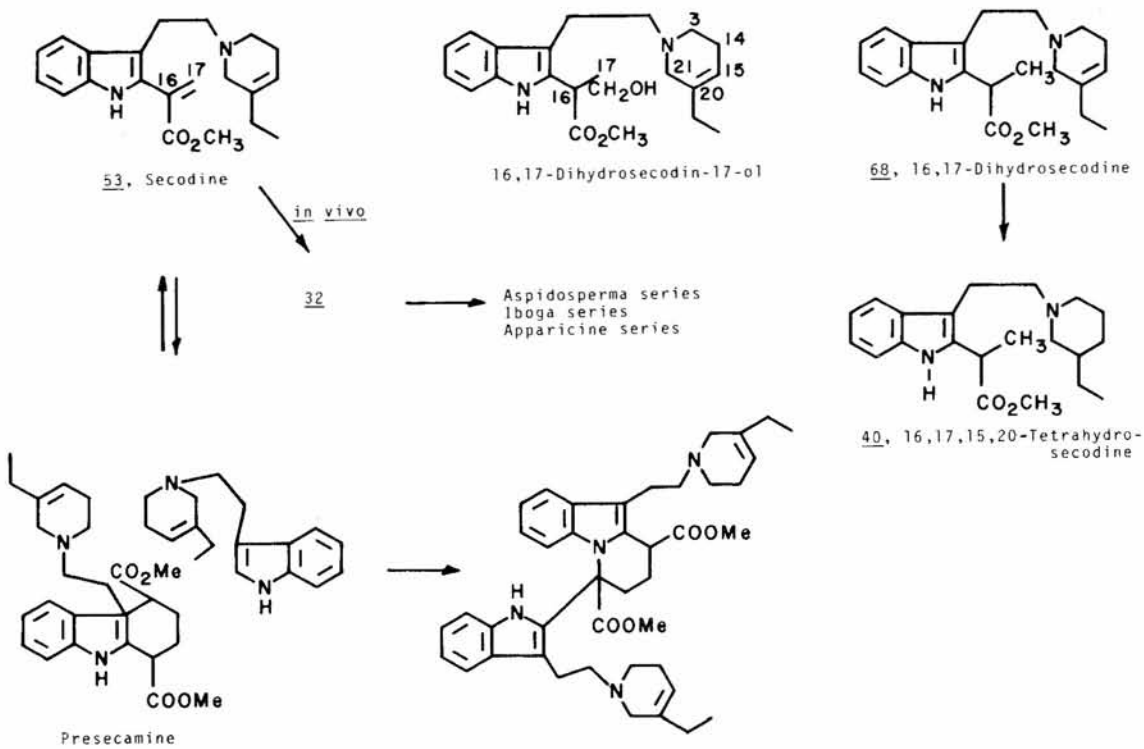
Secodines and related compounds

The discovery, beginning in 1968, of a whole new range



Scheme 22

of "*chano*" alkaloids based on the secodine array[50] was welcome testimony to the "336 concept" engendered by our preliminary experiments carried out the previous year. A selection of these structures and their chemical interrelationship is shown in Scheme 23. Secodine (53) is involved in several important biochemical transformations leading to the various classes (*Aspidosperma*, *Iboga*, and Apparicine series)[51]. The chemical manipulation of the fully activated dihydropyridine system in Scheme 23 has so far been studied indirectly[36-39] but a recent stabilization experiment[52] on some simpler models augurs well for more intensive and successful studies on the capture of 32 and its relatives.



Scheme 23. Chano alkaloids and their relationships.

An extremely efficient method of arriving at the tetrahydro secodine alkaloids (as 40) was portrayed in Scheme 19 where the *in vitro* chemistry of tabersonine, and especially catharanthine, can be marshalled to afford first the pyridinium salt (64) and then, by further reduction, the naturally occurring 40 which is found in *Rhazya stricta*.

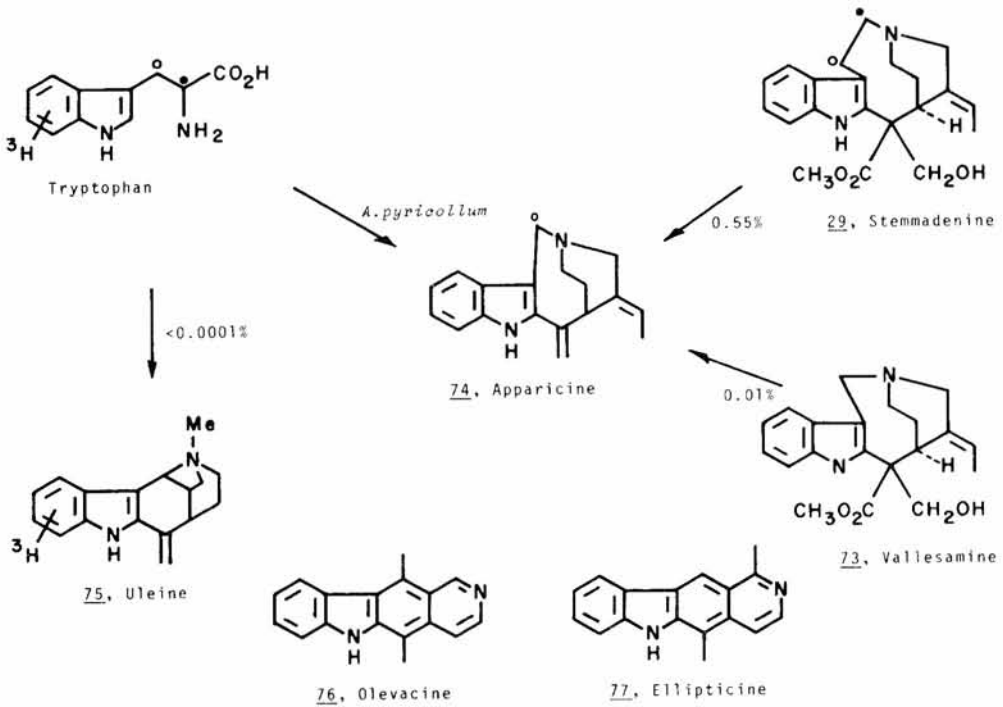
N-oxides as substrates for "Nor"-alkaloids

An intriguing reaction discovered recently by Potier and his colleagues provides a striking model for yet another puzzling feature of a set of alkaloids in which the ethanamino bridge of the original tryptophan moiety has been shortened by one methylene group[53]. Such alkaloids as vallesamine (73), apparicine (74), uleine (75), olivacine (76), and ellipticine (77) fall into this class. The work of Kutney[54] has shown that apparicine (74) is derived from tryptophan in *A. pyricollum* by loss of C-2 and retention of C-3 (Scheme 24). A most satisfactory rationale for this process has been provided by Potier[55] as summarized in Scheme 25 where a modified Polonovsky reaction serves as a model for the possible biogenetic relationships portrayed. An elegant experiment[56] has forged the link between the vobasine alkaloids (29) and the rearranged compound of the ervatamine series ($R=CHCH_3$) (Scheme 26).

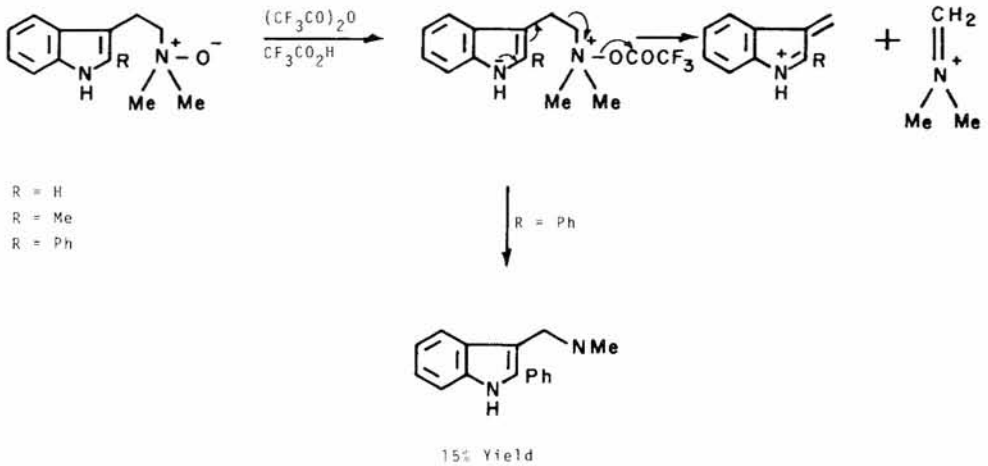
In another recent series of unpublished experiments, the transformation of stemmadenine (29) to vallesamine (73) in approximately 20% yield has been achieved (Scheme 27). Whether N-oxides turn out to be viable biochemical intermediates remains to be proved, however.

Reactions of Aspidosperma Alkaloids

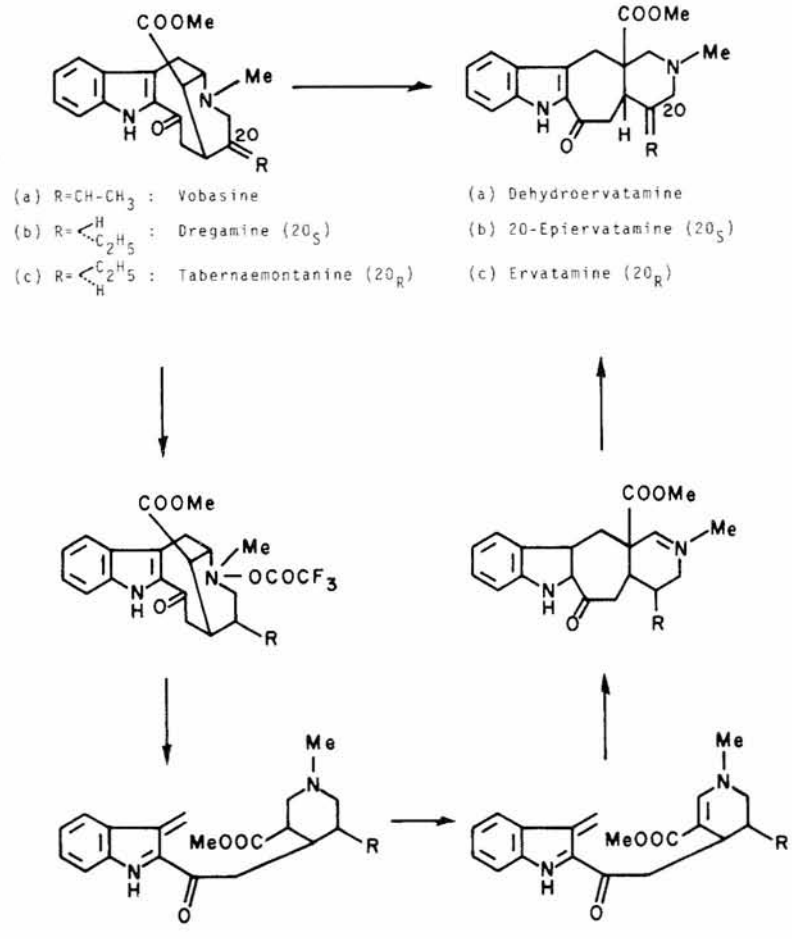
A series of rearrangement products derived from the structure of the *Aspidosperma* alkaloid tabersonine (33) can only be described as spectacular[57]. Several of these are reproduced in Scheme 28 together with the suggested mechanism. Of particular interest is the biogenetic-like connection made with the vincamine (78) series.


Tryptophan
A.pyricollum
<0.0001%
0.55%
0.01%
74, Apparicine
29, Stemmadenine
73, Vallesamine
75, Uleine
76, Olevacine
77, Ellipticine

Scheme 24



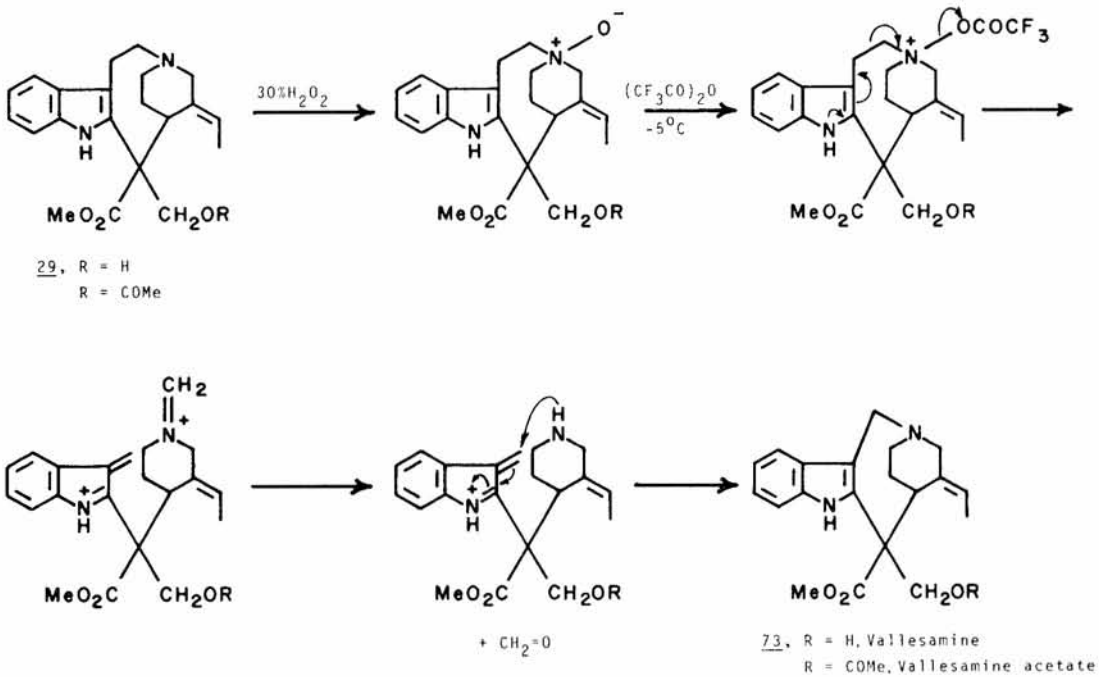
Scheme 25



Scheme 26

"Double" or Dimeric Indolic Alkaloids

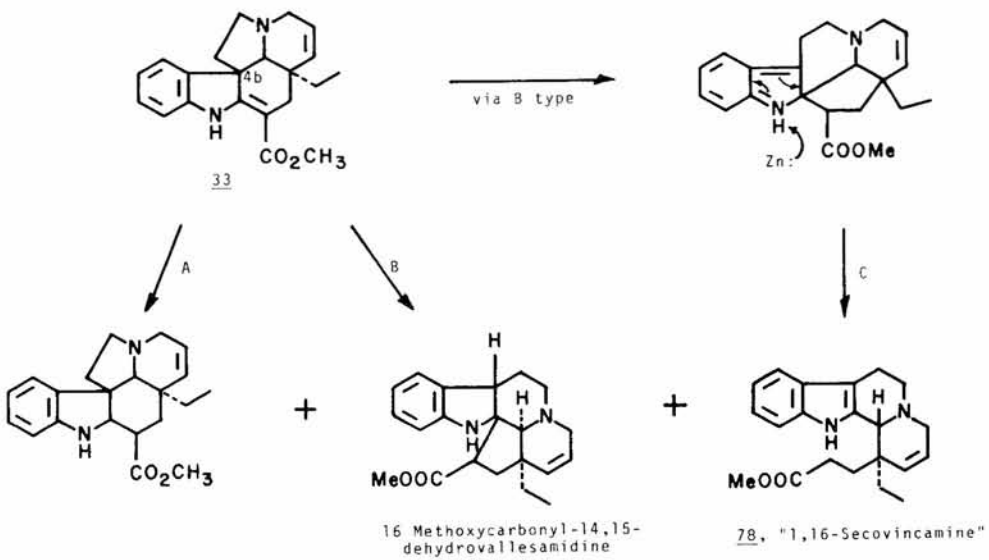
The chemistry of these complex substances which have gained prominence due to the anti-tumor properties of several members of the *Aspidosperma-Iboga* double alkaloids, e.g. vinblastine, VLB (79) and vincristine, VLC (80) has been treated in an excellent recent review[58]. From the standpoint of biogenetic-type synthesis it appears certain that many (but by no means all) of these structures could be readily formed as artefacts. Examples of successful



29, R = H
R = COMe

73, R = H, Vallesamine
R = COMe, Vallesamine acetate

Scheme 27



Scheme 28. Reaction of tabersonine with $Zn/CuSO_4/AcOH/110^{o}$.

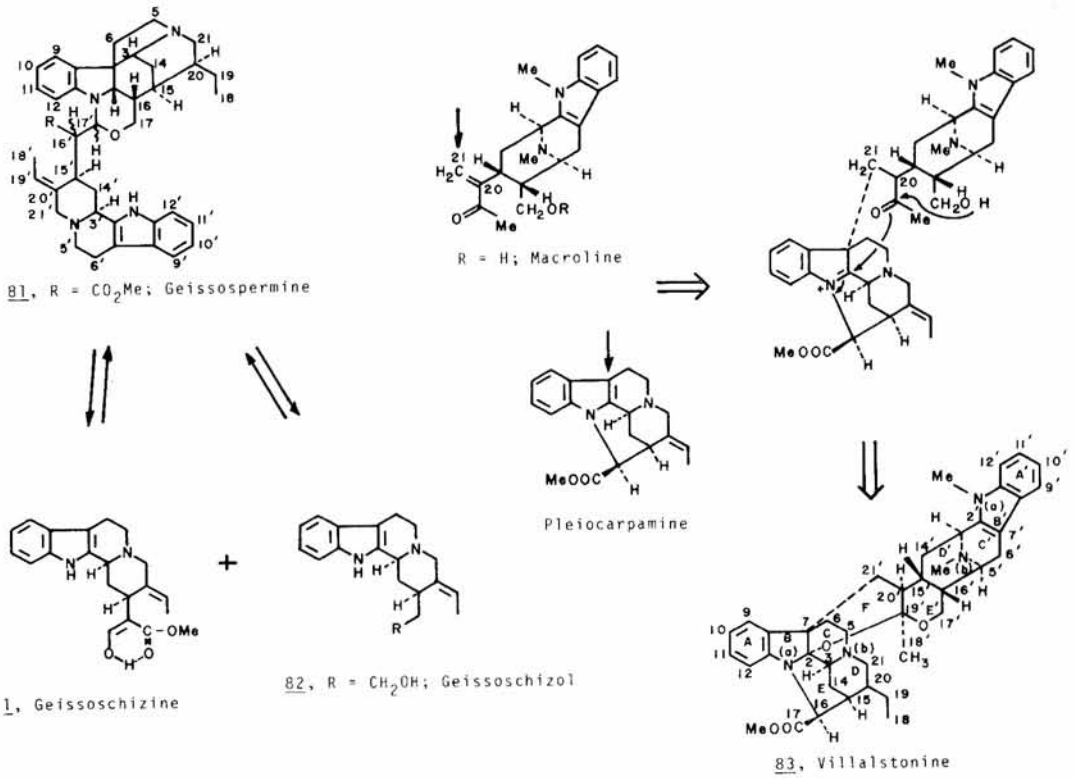
coupling reactions include the preparation of geissospermine (81) which is formed from geissoschizine (1) and geissoschizol (82) under extremely mild conditions, the classical work on the Calebash-curare dimers[58] and a more recent biogenetic-type synthesis[59] of villalstonine (83) as illustrated in Scheme 29.

It seems unlikely that the anti-tumor series exemplified by VLB (79), VLC (80) and leurosidine (84) occurs without enzymic intervention in *C. roseus*, since a coupling reaction which leads to the desired stereochemistry at C-18 in the dimer remains to be described. Considerable progress has been made however in refining the coupling reactions of substituted, modified structures such as 85 and 86 with vindoline (88) to give dimeric products whose stereochemistry is under investigation (Scheme 30, *in vitro*)[60-61-62-63]. Preliminary feeding experiments (Scheme 30, *in vivo*) have not settled the problem in *C. roseus*; the specific incorporation of vindolinine (88) and catharanthine (34) into VLB is approximately 0.05% and 0.005% respectively. An intriguing possibility, which is currently being tested in the author's laboratory, is that the *pseudo* series (35) may be involved.

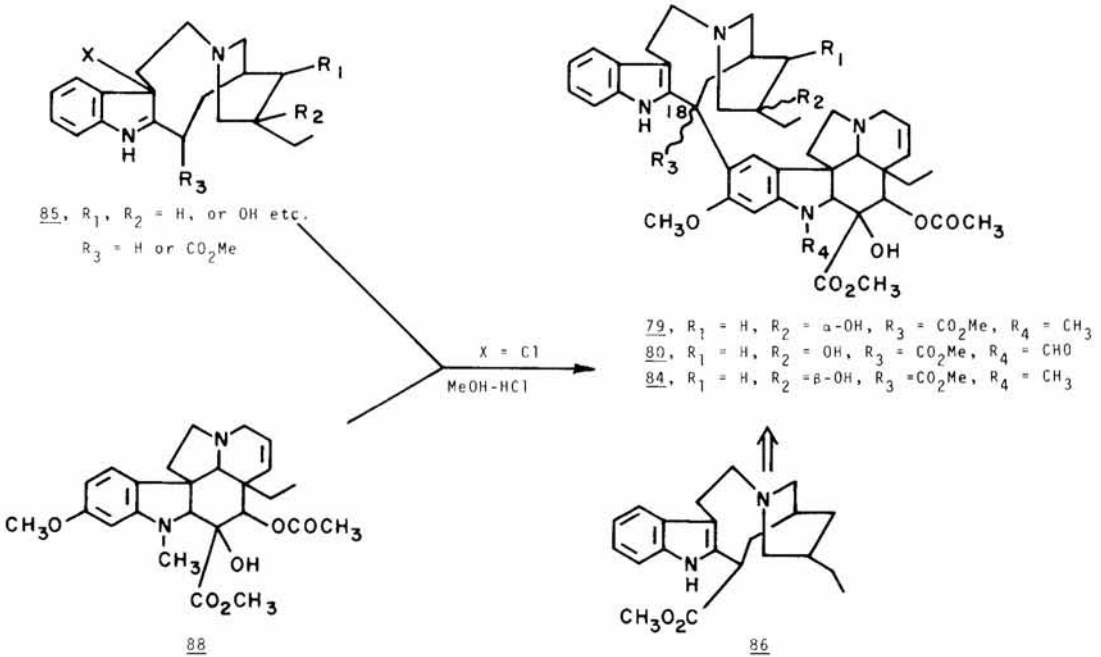
SUMMARY, CONCLUSIONS AND PROGNOSIS

Within the last decade, and particularly since 1968, the apparent diversity and complexity of the approximately 1000 members of the mevalonate-derived indole alkaloids has been rationalized in terms of a main pathway beginning with vincoside and passing through geissoschizine, stemmadenine≠ preakuammicine, tabersonine and their more highly oxygenated, rearranged and degraded derivatives. The important fact that all of the observed skeletal rearrangements take place on a preformed alkaloidal template such as geissoschizine and stemmadenine can now be used not only to clear up several remaining mysteries, e.g. the *Corynanthe → Strychnos* mechanism, but also to predict within each category new classes of alkaloid based for example on the "336" concept, which represent highly reactive species upon which the rearrangement mechanisms may operate.

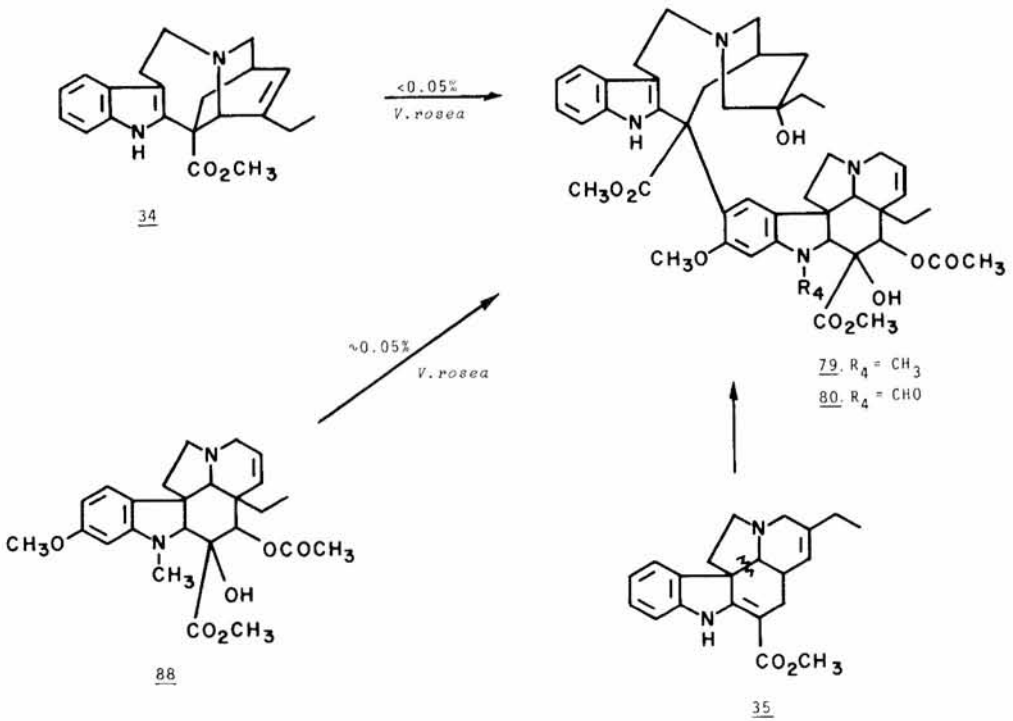
We feel that many short and eventually high-yielding syntheses of little-studied and rare alkaloidal structures await the development of methodology based on the pathway


81, R = CO2Me; Geissospermine
R = H; Macroline
Pleiocarpamine
1, Geissoschizine
82, R = CH2OH; Geissoschizol
83, Villalstonine

Scheme 29



Scheme 30 (*in vitro*)



Scheme 30 (*in vivo*)

followed by Nature in arriving at these rather complex and challenging structures. Now that the innate beauty of the biochemical pathway has been revealed (at least in broad detail) there seems no doubt that many improvements can and will be made on existing and still quite primitive methods of inter-familial connection. However, the facility of several of the biogenetic-type syntheses reviewed even in this rather selective survey leads to a highly speculative - even bizarre - suggestion that the occurrence of many racemic, enantiomeric and diastereomeric sets within the Apocynaceae may reflect either a lack of substrate specificity on the part of a rearranging enzyme or, indeed, the complete absence of any such enzyme. In other words the latter alternative implies that, especially in the tropical regions, where so many of the most rearranged structures occur in species subjected to long periods of warmth, a number of transformations including rearrangement, dimerization, and racemization may have taken place without the agency of enzyme control on a highly reactive substrate.

The final suggestion and many others implicit in our survey remain to be tested by rigorous experiment. Such experiments would, we believe, have profound implications for chemotaxonomy and perhaps reduce the apparent multitude of enzymes in plant alkaloid biochemistry to a reasonable number. At the same time the "artificial" nature of many complex alkaloids may have to be recognized, the examples of vallesiachotamine (10), geissospermine (31) and even andranginine (69) falling into this category.

In spite of the many frustrations and experimental difficulties encountered by the investigators of biogenetic-type synthesis in the field of indole alkaloids, there is emerging the most satisfying viewpoint that many of Nature's processes can be simulated in a significant way. Most importantly, work from various laboratories has made available substrates which can be tested in biochemical experiments and which may be used to test model reactions for enzymic and non-enzymic transformations.

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