STUDIES ON THE BIOSYNTHESIS OF
INDOLE ALKALOIDS

BY

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

DOCTOR OF PHILOSOPHY

in the Department

of

CHEMISTRY

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1973
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In Part A of this thesis a study of the later stages of the biosynthesis of indole alkaloids is described. This study is divided into three sections, the first centres on a versatile synthesis of secodine (67) through which certain derivatives can be prepared.

Ethyl indole-2-carboxylate was reduced and homologated to methyl indole-2-acetate (89) which was converted to β-[3-(2-carbomethoxymethyl)-indolyl]-ethanol (90). Treatment of the alcohol (90) with p-toluene-sulphonylchloride in 3-ethylpyridine followed by reduction gave N-β-[3-(2-carbomethoxymethyl)-indolyl]-ethyl-3'-ethyl-3'-piperideine (81) and β-[3-(2-carbomethoxymethyl)-indolyl]-ethylchloride (85). A pathway is proposed for the formation of the latter compound. The ester (81) was converted to secodine (67) by a known sequence.

Reduction of 3-acetylpyridine (106) with sodium borotritide produced an alcohol (107) which was acetylated and hydrogenated to yield [1-^3H]-1-(3'-pyridyl)-ethane. Condensation of this molecule with the alcohol (90) gave the ester (81) which was converted to [19-^3H]-secodine.

The biological evaluation of secodine (67) as a potential precursor for the indole alkaloids, catharanthine (16) and vincamine (7) is described in Sections B and C.

Using various labelled forms of secodine (67), double isotope studies with the plant species *Vinca minor* and *Vinca rosea* established the specific incorporation of the molecule into catharanthine (16) and vincamine (7). Degradation of these alkaloids confirmed that the entire secodine molecule (67) was incorporated intact.
Part B describes a preliminary investigation into the isolation of enzymes concerned with the biosynthesis of eleagnine (5). The isolation of this molecule from *Eleagnus angustifolia* and synthesis of possible labelled precursors is outlined in Section A. A study of the nuclear magnetic resonance spectrum of $N_b$-acetyeleagnine is also described. Section B reveals the initial procedures used to isolate protein extracts and their assay.
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It is my sincere pleasure to thank Dr. James P. Kutney for his excellent guidance and encouragement throughout the course of my research.

I am also grateful to Mr. Harald H. Hanssen and Mrs. Gloria A. Cook for their collaboration with me in parts of this research and also the other members of the group for helpful suggestions and discussions.

I also wish to express the gratitude owing to my wife for her support and understanding throughout this study.
PART A

STUDIES RELATED TO THE BIOSYNTHESIS
OF INDOLE ALKALOIDS
INTRODUCTION

From ancient times man has attributed various herbs with poisonous, magical and medicinal properties.\(^1\) With the development of science in the modern sense of the word, it has been found that alkaloids are often the cause of this biological activity.\(^2\) Alkaloids are nitrogenous bases, which have complex molecular structures and manifest significant pharmacological activity, that usually occur in plants. Today, somewhat over two thousand alkaloids are known. It is estimated that they are present in 10-15\% of all vascular plants\(^2-5\) and at least one quarter of these bases contain the indole or reduced dihydroindole nucleus.\(^5,6\) Although the distribution of indole alkaloids is widespread in flowering plants, the family Apocynaceae is particularly rich in them, especially the genera \textit{Rauwolfia}, \textit{Vinca} (\textit{Catharanthus}) and \textit{Aspidosperma}.\(^6-8\)

The biological activity associated with some of these bases comes from their tendency to upset the balance of endogenous amines associated with the central nervous system.\(^6,9\) The range of effects produced by the alkaloids is detailed in pharmacological collections\(^10\) and many have been medically useful.

A number of alkaloids have been isolated from animals, for example the psychotomimetic agents bufotenine (1) from the toad \textit{Bufo marinus}
and samandarine (2) which occurs with several related alkaloids as the skin poison of two salamanders (*Salamandra maculosa* Laurenti and *Salamandra atra* Laurenti).

Among the biologically active known indole alkaloids are the two hallucinogens, harmine (3) and psilocybine (4), the cardiac principle and stimulant strychnine (5), the veterinary aphrodisiac yohimbine (6), the antihypertensive vincamine (7) and the antihypertensive and central nervous system depressant reserpine (8). Reserpine was widely used in native medicine, usually as a sedative. The alkaloids of ergot have an oxytocic effect useful in childbirth and the well known psychotomimetic agent D-lysergic acid diethylamide - LSD (9) is a synthetic derivative of this fungus (*Claviceps purpurea*). 11 Vinblastine 12 - VLB (10) and vincristine - VCR (11), dimeric alkaloids produced by *Vinca rosea* Linn, are used clinically as tumor inhibitory drugs. Serotonin (12) is found in the human brain and is considered to function as a neurotransmitter. The biological action of many of the indole alkaloids is considered to arise from an interaction with this centrally active endogenous amine.

The study of the biosynthesis of alkaloids has interested scientists for many years. The early studies were based on natural compounds whose structures resembled proposed intermediates and the many chemical reactions which were believed to be of biogenetic significance. It was not until radioactive labelled materials became readily available that these biogenetic proposals could be tested. It is generally considered that the majority of alkaloids are secondary metabolites, "however some authorities conceive of alkaloids as reservoirs for protein synthesis; as protective materials discouraging animal or
Figure 1a. Examples of Alkaloids.
Figure 1b. Examples of Alkaloids.
insect attacks; as plant stimulants or regulators in such activities as growth metabolism and reproduction; or as detoxicating agents."

One can only conclude that while an individual alkaloid may be assigned a biological function, their ecological importance remains an unanswered question.³

This thesis is a study of the biosynthesis of the indole alkaloids within the cells of plant tissue.

The first forays into the field of biogenesis began with the recognition of common building blocks and in the alkaloidal case this common denominator was usually an amino acid. Specifically, Pictet¹³ in 1906 postulated that the amino acid, tryptophan (13) was the progenitor of indole alkaloids. With the development of tracer techniques in the last decade this hypothesis has received experimental justification. Labelled tryptophan was found to be successfully incorporated into a large variety of indole alkaloids, for example reserpine (8),¹⁴ ajmaline (14),¹⁵ vindoline (15)¹⁶,¹⁷ and catharanthine (16).¹⁷
The tryptophan hypothesis has never really been doubted, however, tryptamine (17) the other logical precursor of indole alkaloids and the decarboxylation product of tryptophan (13) was incorporated with mixed success. The exact nature of this result is difficult to interpret, it could suggest that the decarboxylation is delayed in some cases or that tryptamine (17) is not transported to the site of biosynthesis as efficiently as its amino acid.

The biogenetic origin of the remaining nine or ten carbon atoms, in contrast to the source of the 8-aminoethylindole moiety was the subject of considerable controversy. The earliest hypotheses on the origin of the nontryptophan unit were based on the structural similarities of indole alkaloids.

Forty years ago Barger and Hahn proposed that the indole alkaloids such as yohimbine (6) were formed by a Mannich type condensation of tryptamine (17) with 3,4-dihydroxyphenylacetaldehyde (18). The missing carbon atom needed to complete the D ring was derived from glycine via formaldehyde. Many years later Robinson suggested that the carboxyl group of yohimbine (6) resulted from a ring E expansion of (19) to a tropolone system followed by extrusion of the carboxyl on
collapse of the ring. A contribution of Woodward involved the suggestion that the catechol type ring E could undergo fission and after appropriate biomodifications this skeleton could give rise to the various structural types of indole alkaloids. With this scheme Woodward was able to rationalize the biogenesis of strychnine (5). The combined theory is outlined in Figure 2.

However a number of deficiencies arose with the above theory and in 1959 Wenkert and Bringi proposed an elegant alternative. They initially proposed that a hydrated prephenic acid was the intermediate, but later Wenkert modified this hypothesis so that prephenic acid itself was the direct progenitor of indole alkaloids. The latter rearranges according to the scheme shown in Figure 3 to afford a crucial intermediate, the seco-prephenate-formaldehyde (SPP) unit (21) which can be incorporated into yohimbine (6) and corynantheine (22). This ingenious alternative scheme uses the alicyclic precursors of phenylalanine directly to account for the oxidation state of the E ring. A key step in this hypothesis, the 1,2 migration of the pyruvate side chain of prephenic acid with retention of configuration, explains the absolute configuration at C in the indole alkaloids. The carbomethoxyl group is an integral part of the hydroaromatic progenitor instead of being attached whenever necessary.

Schlittler and Taylor in 1960 and Leete in 1961 postulated that the non-tryptophan portion of the indole alkaloids was derived via the acetate pathway. The suggestion utilized a six carbon chain derived from three acetate units which condensed with malonic acid and a one carbon unit (biologically equivalent to formaldehyde) yielding the desired C unit (see Figure 4).
Figure 2. The Barger-Hahn-Robinson-Woodward Hypothesis.
Figure 3. The Wenkert Prephenate Postulate.
Figure 4. The Acetate Postulate.

Figure 5. The Thomas-Wenkert Monoterpeno Postulate.
At this time many structure elucidations of cyclopentane monoterpenic glucosides had been achieved, examples being verbenalin (23) genipin (24), aucubin (25) and asperuloside (26). Wenkert commented on the fact that the terpenic compounds possess $C_{10}$ skeletons and the absolute stereochemistry of these monoterpenes as illustrated for genipin (24) at the starred carbon atom, is the same as that of the $C_{15}$ position of most indole alkaloids. Wenkert took the stand that either these monoterpenes were prephenic acid (20) derived or they were the progenitors of the $C_{9-10}$ unit.

In the same year, Thomas independently presented a monoterpenoid hypothesis. The non-tryptophan moiety of indole alkaloids may be derived from two units of mevalonate (27) (which is derived from acetate). The biosynthesis proceeds via a cyclopentanoid monoterpene (28) and the acyclic compound (29) to the cyclic unit (30).
which is the precursor for the non-tryptamine unit of the indole alkaloids (Figure 5). The cyclopentanoid monoterpenes genipin (24) and aucubin (25) have a related biosynthetic pathway.

The unit (30) is derived intact without the need for an additional carbon atom from formaldehyde or glycine as required by the earlier hypothesis. The C₉ unit in some indole alkaloids may be obtained through decarboxylation of the C₁₀ unit. Finally Thomas pointed out that aucubin (25) co-occurs with some indole alkaloids in the genus *Strychnos*.

The initial biosynthetic experiments using radioactive precursors disproved all the hypotheses concerning the genesis of the C₉-10 unit of the indole alkaloids. However further experiments by Battersby and co-workers with [2-¹⁴C]-mevalonate afforded low incorporations of activity into the ubiquitous non-tryptophan unit. These results were rapidly confirmed by two other research groups working with the plant species *Vinca rosea* Linn. and *Vinca major* Linn. The Thomas-Wenkert monoterpene postulate was considered valid within the year.

Through separate experiments by these same three research groups using sodium mevalonate labelled with carbon-14 at different sites and several plant systems, it was shown by means of rigorous degradations of the alkaloids involved that this precursor was specifically incorporated into these alkaloids with no prior catabolism. In addition, deuterium labelled mevalonolactone fed to *V. rosea* produced alkaloids whose mass spectral fragmentation patterns substantiated the radioactive label findings.

At this stage it is worth mentioning that three main groups of alkaloids had been recognized, (a) the Corynanthe-Strychnos type, e.g.
ajmalicine (31) and akuammicine (32), (b) the Aspidosperma type, e.g. vindoline (15), and (c) the Iboga type, e.g. catharanthine (16). The Iboga and Aspidosperma skeletons were considered to be rearrangement products of the Corynanthe type.

Now that a plausible theory had been substantiated the study of the biosynthesis of indole alkaloids made rapid progress and further precursors were immediately realized. In an elegant series of experiments by Battersby and co-workers the cyclopentanoid monoterpene previously proposed by Wenkert and Thomas, was shown to be loganin (33). Loganin (33) was specifically incorporated into the indole alkaloids of *V. rosea*, *Rauwolfia serpentina*, and *Cephaelis ipecacuanha*. In addition, the biosynthesis was shown to proceed via mevalonate and that loganin (33) co-occurred with the indole alkaloids in *V. rosea* and *Strychnos nux vomica*.

Geraniol (34), the progenitor of the monoterpenes was shown to be a precursor of loganin (33) and the indole alkaloids ajmalicine (31), catharanthine (16) and vindoline (15). Nerd (35), the cis isomer of geraniol (34), was incorporated with equal efficiency. Battersby proceeded to identify the precursors of loganin (33) arising from geraniol (34) in the biosynthetic pathway. The co-occurrence with loganin (33) of monoterpenes oxidized at C₁₀ in *Menyanthes trifoliata*
pointed to oxidation at this point as being the primary step. Consequently 10-hydroxygeraniol and 10-hydroxynerol were found to be incorporated specifically into loganin (33) and the indole alkaloids ajmalicine (31), vindoline (15), catharanthine (16), perivine (36) and serpentine (37) in V. rosea, with the cis compound generally being more effective than the trans isomer. However only 50% of the label was found to be in the methoxycarbonyls which come from positions 9 and 10 indicated in (34), showing the occurrence of randomization in the terminal unit. This would indicate that oxidation at both these positions is a necessary part of the biosynthetic pathway.

Deoxyloganin (38) was found to be a constituent of V. rosea and S. nux vomica and was also shown to be a precursor of loganin (33) and the indole alkaloids of V. rosea.  

The cleaved monoterpen derivative of loganin (33), secologanin (39), has been isolated from V. rosea and was found to be specifically incorporated into representative examples of all three families of indole alkaloids. Also loganin (33) was shown to be the precursor of this compound. 

An interesting side issue is that the central intermediates of Wenkert's prephenic acid hypothesis, the SPF unit, is almost
identical in structure, stereochemistry and oxidation level to the actual critical intermediate, although it was unsupported by experimental evidence.

Secologanin (39) is identical in stereochemistry to the Corynanthe and Strychnos alkaloids at the centre corresponding to C\textsubscript{15} of yohimbine. This work is outlined in Figure 6.

Now that the C\textsubscript{9-10} unit had been identified, researchers in the field directed their efforts towards studying the manner in which this species condensed with the aminoethylindole moiety and the ensuing rearrangements.

Simultaneously and independently, vincoside (40) and isovincoside (41) were isolated from \textit{V. rosea}\textsuperscript{64} and strictosidine (41) from \textit{Rhazya stricta}\textsuperscript{65}. Originally there was some doubt over the configuration at C\textsubscript{3} but this has been confirmed by X-ray analysis\textsuperscript{66,67}.

\begin{align*}
\text{Vincoside (40)} & \quad \text{Isovincoside (41)} \\
\begin{align*}
\text{Vincoside (40) was specifically incorporated by \textit{V. rosea} plants into all three types of indole alkaloids;\textsuperscript{64,68,70} isovincoside (41) was biologically inert and afforded no significant incorporations into any of the alkaloids.\textsuperscript{68} This result was unexpected from the point of view of the biosynthetic chemist because the configuration at C\textsubscript{3} of vincoside (40) is opposite to that of the Corynanthe alkaloids. Also the}
\end{align*}
\end{align*}
Figure 6. The Biosynthesis of the C₉-₁₀ Unit of Indole Alkaloids.
hydrogen at C₅ of loganin (33) (C₅ of loganin corresponds to C₃ of vincoside) is completely retained in the biosynthesis of the three main classes of indole alkaloids.⁶⁸,⁶⁹

It seems appropriate at this point to review the theories based on structural relations at the alkaloid level. It appears likely that the genesis of these higher order alkaloids is via the structurally simpler types rather than through any direct transformation of the tryptamine (17) entity. In his 1965 publication,⁷¹ Wenkert recognized this and based his arguments on the observation that there is almost complete stereochemical constancy at C₁₅ in the Corynanthe-Strychnos group and it was argued that the Strychnos, Aspidosperma and Iboga alkaloids could be derived from the Corynanthe systems. A possible route from the Corynanthe to the Strychnos systems was put forth (Figure 7, pathway A) and since then a second pathway has been proposed by Scott ⁷² (Figure 7, pathway B). No direct experimental evidence has yet been established to distinguish between these two mechanistic speculations.

Wenkert in 1962 ²₇ had proposed that the Strychnos system was transformed into both the Aspidosperma and Iboga types. As outlined in Figure 8 the proposal begins with a charged precursor (42) which is essentially equivalent to the imine (43) and of analogous origin. A retro-Michael reaction, reduction and dehydration of this intermediate lead to the piperideines (44) and (45), which via intramolecular Michael and Mannich condensations give the nine membered ring systems (46) and (47). Transannular cyclisation of these large rings leads to the Aspidosperma (48) and Iboga (49) skeletons. Peripheral oxidations,
Figure 7. The Postulated Origins of the Strychnos Family.
Figure 8. The Wenkert Postulate for the Biosynthesis of the Iboga and Aspidosperma Families.
reductions and alkylations could then account for all the alkaloids of these various families.

Returning to the pathway beyond vincoside (40); formation of the Corynanthe family requires no skeletal rearrangement and geissoschizine (51), corynantheine (22) and its aldehyde (50), and ajmalicine (31) are derived as in Figure 9.

Direct feeding experiments indicated that corynantheine aldehyde (50) was not significantly incorporated, however geissoschizine (51) was found to specifically label the _V. rosea_ alkaloids including the Strychnos alkaloid akuammicine (52). The occurrence of geissoschizine (51) in _V. rosea_ plants was established by direct isolation.

The Strychnos alkaloid stemmadenine (53), which resembles Wenkert's precursor (42), was found to be incorporated intact into the Iboga and Aspidosperma skeletons in _V. rosea_ and was also isolated from the young seedlings along with tabersonine (54) and preakuammicine (55). At present there is no direct evidence as to

\[
\text{CO}_2\text{CH}_3
\]

(54)

the relationship between stemmadenine (53), preakuammicine (55) and akuammicine (52) and as to whether stemmadenine (53) appears on the pathway as a precursor to the Strychnos systems or that it arises from them. However the stemmadenine (53) → preakuammicine (55). →
Figure 9. The Biogenesis of the Corynanthe Family.
akuammine (52) sequence has been demonstrated to be a facile \textit{in vitro} process.\textsuperscript{18}

To distinguish between static and dynamic constituents Scott studied the sequential uptake of labelled tryptophan into various alkaloids using \textit{V. rosea} seedlings.\textsuperscript{73} Within minutes of feeding \([2-\textsuperscript{14}C]-\)tryptophan, the Corynanthe type compounds appeared (vincoside (40) and geissoschizine (51)), these were followed by the Strychnos series (preakuammine (55), stemmadenine (53) and akuammine (52)) which in turn was followed by the Aspidosperma (tabersonine (54)), and finally by the Iboga (catharanthine (16)) alkaloids. This sequence suggests that the order Corynanthe \(\rightarrow\) Strychnos \(\rightarrow\) Aspidosperma \(\rightarrow\) Iboga, as originally postulated by Wenkert, is correct.
Our own experiments took a different approach from those previously mentioned. The transannular cyclisation required to form the pentacyclic systems of Aspidosperma and Iboga families as proposed by Wenkert in 1962 was examined and found to be a viable synthetic process.\(^ {76-80}\) Thus oxidation of quebrachamine (56) to the minimum salt, followed by cyclisation and reduction, yielded aspidospermidine (57), while catharanthine (16) was formed from carbomethoxycleavamine (58) in a similar manner.\(^ {78}\)

\[
\text{\includegraphics[scale=0.5]{image1.png}} \quad \rightarrow \quad \text{\includegraphics[scale=0.5]{image2.png}}
\]

However, neither carbomethoxycleavamine (58) or 6,7-dehydrovinca-dine (59) were converted to the Iboga or Aspidosperma compounds upon feeding radioactive forms of these alkaloids to \(V. \text{ rosea}\).\(^ {17}\) In an analogous manner the alkaloids quebrachamine (56) and vincaminoreine (60) resulted in negative incorporations into the Aspidosperma skeletons.\(^ {17}\) To lessen the hazard involved in interpreting negative
incorporations, a variety of methods was employed to administer the labelled compounds with no change in the initial result. Tabersonine (54) was found to be efficiently incorporated into the _V. rosea_ alkaloids, which establishes that the high molecular weight precursors were reaching the site of biosynthesis and therefore the feeding technique was effective. It was felt that the negative incorporations of the nine membered ring intermediates were most likely due to the lack of any real importance of the transannular cyclisation process in biosynthesis, rather than to experimental difficulty. To add strength to this argument, [3-^{14}C]-tryptophan was administered to _V. minor_ plants over varying time intervals. In each instance the tetracyclic alkaloids, vincadine (61) and vincaminoreine (60) as well as the pentacyclic alkaloids, vincadifformine (62) and minovine (63) were isolated and the total incorporations in these two series were determined. The results of these findings are illustrated in Table 1. As is evident the ratio of activity, B/A, of the pentacyclic versus the tetracyclic alkaloids remained fairly constant over the period, four hours to fourteen days. This is indicative of the lack of conversion of the latter to the former and vice versa. The possibility of equilibration in the plant which could also explain the stable ratio
Table 1.

<table>
<thead>
<tr>
<th>Time</th>
<th>Total % Incorporation</th>
<th>Ratio of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>4 hours</td>
<td>0.003</td>
<td>0.057</td>
</tr>
<tr>
<td>1 day</td>
<td>0.015</td>
<td>0.24</td>
</tr>
<tr>
<td>2 days</td>
<td>0.010</td>
<td>0.21</td>
</tr>
<tr>
<td>4 days</td>
<td>0.010</td>
<td>0.22</td>
</tr>
<tr>
<td>7 days</td>
<td>0.009</td>
<td>0.13</td>
</tr>
<tr>
<td>14 days</td>
<td>0.003</td>
<td>0.06</td>
</tr>
</tbody>
</table>

The Results of the Incorporation of [3-\textsuperscript{14}C]-D,L-Tryptophan into V. minor at Various Time Intervals.

(61) $R = H$
(60) $R = \text{CH}_3$
(vincadine + vincaminoreine)

(62) $R = H$
(63) $R = \text{CH}_3$
(minovine + vincadiformine)
was excluded from serious consideration when it was shown that labelled minovine (63) transferred no activity to the tetracyclic alkaloids after a one week feeding period. Thus it was concluded that the genesis of the quebrachamine-vincadine family is independent of the pathway leading to the rigid pentacyclic Aspidosperma series. Moreover, consideration of these results, in conjunction with other findings, suggested the existence of a common intermediate capable of generating Iboga and Aspidosperma tetracyclic and pentacyclic compounds without invoking the transannular cyclisation process. These results are summarized below utilizing the acrylic ester structure (64) as an attractive postulate. Thus bond formation between a-b leads to Aspidosperma tetracyclic compounds, and between a-b and c-d to the Aspidosperma pentacyclic compounds. Analogously bond formation between a-e leads to the tetracyclic members of the Iboga family, while a-e and f-d lead to the Iboga pentacyclic alkaloids.

It is also pertinent to note the resemblance of the acrylic ester (64) to Wenkert's acrylic ester (45) contained in his original proposal. Also the isolation from Tabernamontana cumminsii of the compound 2-ethyl-3-[2-(3-ethylpiperidine)-ethyl]indole (65) which can be regarded as the reduced nor-derivative of the acrylic ester (64), provided a strong in vivo suggestion of the existence of this pivotal intermediate.
With this evidence in mind, our laboratory set about the synthesis of the appropriate compounds. A direct synthesis of the acrylic ester (64) is by no means trivial since the established instability of dihydropyridine systems and the high reactivity of acrylic esters would suggest that the eventual isolation of such a compound would provide considerable difficulty. It was reasonable to assume that the more stable analogues (66) and (67) could be converted in vivo by the plant to intermediates such as (64) and they therefore became the targets for our synthetic efforts.

The acrylic ester (67) was given the name secodine by Smith, who also adopted a numbering system for this molecule based on the biogenetic assumption that the secodine type molecule is derived from the Corynanthe family of alkaloids, in which the "unrearranged" C₁₀ unit is involved.
It was at this time that Battersby and co-workers\textsuperscript{84} reported a synthesis of 16,17-dihydrosecodin-17-ol (66). On feeding [O-methyl\textsuperscript{3}H]-loganin to \textit{V. rosea} and \textit{Rhazya orientalis} and isolation of the alkaloids after adding synthetic (66), Battersby was able to show retention of radioactivity in the purified carrier alkaloid. Hence he proposed that 16,17-dihydrosecodin-17-ol (66) is a natural product, probably arising from a biosynthetic intermediate blocked by reduction of (68) or by hydration and reduction of (67).

Further to this publication\textsuperscript{84} several other papers appeared, in which the authors reported the isolation of 16,17,15,20-tetrahydrosecodine (69), 16,17-dihydrosecodine (70) and 16,17,15,20-tetrahydrosecodin-17-ol (71) from plant sources.\textsuperscript{83,85}
On this note [ar-^3H]-16,17-dihydrosecodin-17-ol was synthesized and fed to *V. rosea*, *V. minor*, and *Aspidosperma pyricollum*, from which the appropriate alkaloids were isolated. In each case negative incorporations were obtained and in fact this intermediate appeared to be toxic, since many of the plants deteriorated. It was assumed that the plant systems involved were unable to carry out the appropriate oxidations and dehydrations on this compound to give the actual intermediate. Consequently 16,17-dihydrosecodin-17-ol was dehydrated to secodine, as this compound was considered different from the proposed intermediate only in the oxidation level of the piperidine ring. When [ar-^3H]-secodine was fed to the two *V. rosea* species and *A. pyricollum*, low but definite incorporations were observed in three alkaloidal families.

Our initial attention was turned to the alkaloids, vindoline (15,87,89-91 *V. rosea*) and apparinine (72,83-91 *A. pyricollum*), as [ar-^3H]-secodine was found to be incorporated with reasonable efficiencies into these compounds. Thus different labelled forms of secodine were synthesized (73) and fed to these two plant systems. The results are shown in Tables 2 and 3.
The ([α-3H,22-14COOCH3]-secodine experimental results indicate that the indole portion of the secodine unit is not altered to a significant extent during its incorporation into the plant alkaloids. Both vindoline (15) and apparicine (72) were degraded and it was found that all of the carbon-14 label was located at the carbomethoxy groups in each case. The loss of tritium on feeding [22-14COOCH3,3,14,15,21-3H]-secodine for vindoline (15) 60% and apparicine (72) 48% is to be expected as the oxidation level of the piperidine ring in the secodine molecule must be raised in order to reach the proposed intermediate. Finally the little changed ratio, on feeding [22-14COOCH3,19-3H]-secodine to V. rosea and isolating vindoline (15) proves a complete incorporation of the secodine skeleton.

Apparicine (72), it may be noticed, lacks the normal β-aminomethyl side chain which joins the β position of the indole nucleus to the tertiary nitrogen atom. However it does appear to possess the ubiquitous C9-10 unit.92,93 There have been a few postulates over the origin of this alkaloid,27,94,95 one of which assumes that this compound arises from stemmadenine (53).93 The data in Table 2 indicate that apparicine (72) in fact results from a secodine (67) type molecule. However, this does not rule out the possibility of an equilibrium between stemmadenine (53) and our proposed precursor.

In conclusion, the biosynthesis of the major classes of indole alkaloids may be summed up as in Figure 10. With the exception of the intermediacy of the stemmadenine → preakuanamicine sequence, the pathway has been verified through the specific incorporation of alkaloids into succeeding classes, i.e. Corynanthe → Strychnos → Aspidosperma → Iboga.
Table 2. The Results of the Incorporation of 16,17-Dihydrosecodin-17-ol (66) and Secodine(67) into *V. rosea* and Isolation of Vindoline (15).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound Fed</th>
<th>Ratio of Activity Fed $^3\text{H}/^{14}\text{C}$</th>
<th>Ratio of Activity Isolated $^3\text{H}/^{14}\text{C}$</th>
<th>% Incorporation $^3\text{H}$</th>
<th>$^{14}\text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[ar-$^3\text{H}$]-16,17-Dihydrosecodin-17-ol</td>
<td>-</td>
<td>-</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>[ar-$^3\text{H}$]-Secodine</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>[ar-$^3\text{H}$]-Secodine</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>[ar-$^3\text{H}$, $^{14}\text{COOCH}_3$]-Secodine</td>
<td>8.8</td>
<td>8.3</td>
<td>0.042</td>
<td>0.043</td>
</tr>
<tr>
<td>5</td>
<td>[3,14,15,21-$^3\text{H}$,$^{14}\text{COOCH}_3$]-Secodine</td>
<td>3.5</td>
<td>1.4</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>[$^{14}\text{COOCH}_3$,19-$^3\text{H}$]-Secodine</td>
<td>1.54</td>
<td>1.35</td>
<td>0.06</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 3. The Results of the Incorporation of 16,17-Dihydropseudocodein-17-ol (66) and Secodine (67) into *A. pyricollum* and Isolation of Apparicine (87).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound Fed</th>
<th>Ratio of Activity Fed $^3\text{H}/^{14}\text{C}$</th>
<th>Ratio of Activity Isolated $^3\text{H}/^{14}\text{C}$</th>
<th>% Incorporation $^3\text{H}$</th>
<th>% Incorporation $^{14}\text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$[^3\text{H}]$-16,17-Dihydropseudocodein-17-ol</td>
<td>$-$</td>
<td>$-$</td>
<td>$&lt;0.001$</td>
<td>$-$</td>
</tr>
<tr>
<td>2</td>
<td>$[^3\text{H}]$-Secodine</td>
<td>$-$</td>
<td>$-$</td>
<td>$0.01$</td>
<td>$-$</td>
</tr>
<tr>
<td>3</td>
<td>$[^{14}\text{C}]$-Secodine</td>
<td>$-$</td>
<td>$-$</td>
<td>$0.01$</td>
<td>$0.01$</td>
</tr>
<tr>
<td>4</td>
<td>$[^3\text{H},{^{14}\text{C}}]$-Secodine</td>
<td>8.7</td>
<td>8.4</td>
<td>0.014</td>
<td>0.015</td>
</tr>
<tr>
<td>5</td>
<td>$[3,14,15,21-^3\text{H},^{14}\text{C}]$-Secodine</td>
<td>4.2</td>
<td>2.2</td>
<td>0.005</td>
<td>0.009</td>
</tr>
</tbody>
</table>


Figure 11 relates to the latter stages of the biosynthesis. The intermediacy of the acrylic ester (64) has been shown to occur for the alkaloids vindoline (15) and apparicine (72) but has yet to be shown for catharanthine (16) and vincamine (7), a Hunteria alkaloidal skeleton.
Figure 10. The Biosynthesis of the Major Indole Alkaloid Families.
Figure 11. The Postulated Pathway for the Biogenesis of the Iboga and Hunteria Families.
DISCUSSION

Considerable effort has been put into the study of the biosynthesis of indole alkaloids in recent years and the pathway is now fairly well understood, particularly in the early stages. Our research group has been concerned with the elaboration of the Strychnos alkaloids into the various other families and it has been shown that a secodine (67) type intermediate is involved in the biosynthesis of vindoline (15) and apparicine (72).

For ease of presentation the work discussed here will be divided into three sections. An alternate synthesis of secodine (67) is discussed first while the later sections concentrate on the biosynthesis of the Iboga and Hunteria skeletons.

Section A

A synthesis of 16,17-dihydrosecodin-17-ol (66) has already been devised in our laboratories as mentioned in the introduction, and some initial biosynthetic results with this molecule have been obtained. The initial successful synthetic sequence is shown in Figure 12.

Condensation of the chloroethylindole derivative (74), obtained through a Fisher indole reaction, with 3-ethylpyridine (75) occurred in a sealed tube at 120° to give the pyridinium chloride (76). This salt
was subsequently converted to the piperideine (77) with sodium borohydride. The piperideine (77) was then reduced with lithium aluminum hydride to N-[2-(3-[2-hydroxymethyl]-indolyl)-ethyl]-3'-ethyl-3'-piperideine (78) and this compound was transformed into its benzoate derivative (79). Yields as high as 70% were obtained for the overall conversion from (74) to the benzoate (79). The position of the double bond in the piperideine ring was assigned on the basis of the nuclear magnetic resonance spectrum of (78) and by analogy with a publication by Wenkert. The purified benzoate was then displaced by cyanide to give the nitrile (80) which was hydrolyzed with methanolic hydrogen chloride to give the methyl ester (81). Formylation at C₁₆ of the ester (81) was then achieved with methyl formate and sodium hydride and the expected enol (82) was carefully reduced with sodium borohydride to afford 16,17-dihydrosecodin-17-ol (66). Normal sodium borohydride reduction of the enol (82) afforded the diol (83).

\[
\begin{array}{c}
\text{N} \\
\text{C} \\
\text{O} \\
\text{H}
\end{array}
\]

To obtain [ar⁻³H]-16,17-dihydrosecodin-17-ol the ester (81) was exchanged with tritiated trifluoroacetic acid since (66) was unstable to acidic conditions, and then formylated and reduced.

Very low, and generally considered insignificant, incorporations of of [ar⁻³H]-16,17-dihydrosecodin-17-ol were observed in feedings to
Figure 12. The Synthesis of 16,17-Dihydrosecodin-17-ol (66).
Thus while experiments aimed at producing the acrylic ester secodine (67) and obtaining more meaningful incorporation results were in progress, an alternative route to (66) was explored which would allow condensation of 3-ethylpyridine to the indole nucleus in the final stages of the sequence. Our reasons for this modification were twofold: (a) to produce isomers of 16,17-dihydrosecodin-17-ol (66) in which the position of the double bond in the piperideine ring is altered and, if possible, achieve the synthesis of (64) or the dienamine (84) in the hope that improved biosynthetic incorporations could be obtained; \(^{97}\)

\[\text{CH}_3\]

\[\text{CO}_2\text{CH}_3\]

(b) to introduce tritium into the ethyl side chain of the piperideine ring and consequently the handling of the radioactive compounds would be minimized and the cost of the sequence would be lowered. The desired alcohol (66) could be synthesized by the sequence shown in Figure 13.

The choice of this route was simplified by the fact that the chemistry induced in the elaboration of the side chain at the \(\alpha\) position of the indole nucleus had already been worked out \(^{86}\) (Figure 12) and in this manner the two routes would converge at the ester, \(N-[8-(3-[2-carboxymethoxymethyl]-indolyl)-ethyl]-3'-ethyl-3'-piperideine\) (81) stage.
Figure 13. The Proposed Synthesis of N-\(\beta\)-[3-(2-Carbomethoxymethyl)-indolyl]-ethyl]-3'-ethyl-3'-piperideine (81).
Wenkert in 1968 published a synthesis of dl-dihydrogambirtannine which involved the chloride, methyl 3-(β-chloroethyl)-2-indolylacetate (85) as an intermediate. However, it was proposed to remain with the route outlined in Figure 13.

The route proceeded via initial development of the side chain at the α position of the indole to give (89) and then attachment of a two carbon residue at the β-indole position. Finally, condensation of the latter with the appropriate pyridine derivative produces the ester (81). Reduction, formylation and lastly reduction again should give the desired secodinol derivative.

Ethyl indole-2-carboxylate was obtained commercially and reduction of this compound with lithium aluminum hydride in refluxing tetrahydrofuran gave 2-hydroxymethylindole (86). After chromatography, a white crystalline material was obtained, in 70% yield. The spectral data compared favourably with the assigned structure. In the infrared (ir) spectrum there was no absorption in the ester region and instead bands at 3380 cm⁻¹ and 1058 cm⁻¹ (-C-OH) were evident. The nuclear magnetic resonance (nmr) spectrum showed the newly formed hydroxymethyl group as a sharp singlet at δ 5.27. A single proton singlet at δ 7.62 which was found to be exchangeable with deuterium oxide was assigned to the hydroxyl proton.

Homologation of the side chain to the desired functionality was achieved via the route, (86) to (89) in Figure 13. The conversion of the alcohol (86) to the benzoate (87) was accomplished by dissolving 2-hydroxymethylindole (86) in tetrahydrofuran with added triethylamine and treating the mixture with benzoyl chloride. The crude benzoate was
purified by chromatography to yield a white crystalline product in quantitative yield. The spectral data for this compound (87) were in complete accord with the formulation. The ir spectrum showed the carbonyl of the benzoate ester at 1700 cm$^{-1}$, while in the nmr spectrum the aromatic protons of the benzoyl group although overlapping with the protons of the indole nucleus appeared between $\tau$ 1.95-3.00 (9H). The methylene protons now appeared as a singlet at $\tau$ 4.52. The mass spectrum showed a molecular ion peak at m/e 251 and a base peak at m/e 129 which was attributed to ready loss of benzoic acid. Finally, the molecular formula $C_{10}H_{13}O_2N$ was confirmed by high resolution mass spectrometry (Found: 251.0964; Calcd.: 251.0946) and elemental analysis.

If specific reaction conditions were followed carefully the nucleophilic displacement of the benzoate with cyanide anion could be achieved in good yield. The optimum reaction conditions required dissolving the benzoate (87) in N,N-dimethylformamide and adding a fivefold excess of solid potassium cyanide to the stirring solution under nitrogen. This heterogeneous mixture was stirred at room temperature for one hour and then slowly warmed to 75° over a further hour. The reaction mixture was stirred at this temperature until thin layer chromatography indicated no remaining starting material (about 1 1/2 hours). The reaction was stopped and after column chromatography, the nitrile (88) was obtained crystalline in 75% yield. Spectral data were indicative of 2-cyanomethylindole (88). The cyano functionality could be assigned to a peak at 2245 cm$^{-1}$ in the ir spectrum, and there was no absorption in the carbonyl region. In the nmr spectrum the methylene adjacent to the nitrile (-CH$_2$-CN) appeared as a sharp singlet at $\tau$ 6.10. The mass spectrum had a molecular ion peak at m/e 156 which underwent apparent
loss of hydrogen cyanide to give a peak at m/e 129 (85% relative intensity). The molecular formula, C_{10}H_{8}N_{2}, was confirmed by high resolution mass spectrometry (Found: 156.0688; Calcd.: 156.0688) and elemental analysis.

In the previous synthesis of 16,17-dihydrosecodin-17-ol (66) (see Figure 12), the best conversion of the nitrile (80) to the ester (81) was found to occur in methanolic hydrogen chloride. Thus this reaction was used on 2-cyanomethylindole (88). The nitrile (88) was dissolved in absolute methanol with 1% water added, and the resulting mixture was carefully saturated with hydrogen chloride gas under nitrogen. After standing at room temperature for 68 hours, followed by chromatography, a product resulted as white crystals, in 83% yield. The concentration of water in the reaction mixture was found to be critical. The spectral data on the reaction product agreed favourably with the structure for methyl 2-indolylacetate (89). The presence of an ester group was suggested by the ir spectrum (1719 cm^{-1}) and a sharp singlet in the nmr spectrum at τ 6.32 (-COOCH_{3}). The methylene protons adjacent to the indole ring and the ester carbonyl appeared as a sharp singlet at τ 6.29. The mass spectrum indicated a molecular ion peak at m/e 189 which appeared to undergo loss of the ester functionality to give an ion (92) at m/e 130.
Lastly the molecular formula $C_{11}H_{11}O_{2}N$ was supported by high resolution mass spectrometry (Found: 189.0781; Calcd.: 189.0790) and elemental analysis.

Now that the α side chain had been elaborated it became necessary to attach a two carbon unit to the β position of the indole nucleus. It was considered that the best way to do this was via a modified Friedel-Crafts reaction. A group of French workers$^{100}$ had taken indole (93) and treated it with ethylene oxide in the presence of a catalyst and obtained tryptophol (94).

![Chemical structures](image)

Consequently ethylene oxide was added to a solution of methyl 2-indolylacetate (89) in carbon tetrachloride and the mixture cooled. Fuming stannic chloride in carbon tetrachloride was added and after twenty minutes the reaction was quenched. Chromatography yielded starting material and the desired product as a brown oil in a 71% yield in terms of starting material consumed. Spectral data identified the product as β-[3-(2-carbomethoxymethyl)-indolyl]-ethanol (methyl 3-(β-hydroxyethyl)-2-indolylacetate) (90). In the ir spectrum a band at 3603 cm$^{-1}$ was assigned to the hydroxyl and the nmr spectrum had a hydroxyl proton occurring at $\tau$ 7.40. Also the methylene on the β-hydroxyethyl side chain and attached to the indole nucleus occurred as a two proton triplet at $\tau$ 7.12 (J = 7 Hz) while the hydroxymethylene
protons occurred at τ 6.25 (J = 7 Hz). The molecular ion was assigned to a peak at m/e 233 in the mass spectrum. Cleavage of this ion gave rise to a base peak at m/e 202 tentatively attributed to the ion (95).

![Chemical structure](image)

To confirm this structure, the 3,5-dinitrobenzoate derivative was formed. Spectral data on this compound are as follows. In the nmr spectrum the three dinitrobenzoate aromatic protons appeared between τ 0.85-1.00. The β-indolylmethylen protons had shifted to τ 6.71 (J = 7 Hz) while the protons due to the methylene adjacent to the dinitrobenzoate ester grouping occurred at τ 5.34 (J = 7 Hz). The mass spectrum showed a molecular ion, m/e 427, which appeared to undergo loss of \((-\text{CH}_2\text{OOC}(\text{NO}_2)_2\) to give a base peak at m/e 202, again assigned to the ion (95). Apparent loss of 3,5-dinitrobenzoic acid from the molecular ion also occurred to give an ion, m/e 215 (80% relative intensity). The molecular formula C_{20}H_{17}O_8N_2 was confirmed by high resolution mass spectrometry (Found: 427.1012; Calcd.: 427.1015) and elemental analysis.

Now that the appropriate side chains on the indole nucleus had been elaborated, the condensation of this indole derivative with 3-ethylpyridine was next considered. Wenkert and coworkers in 1968 had developed a synthesis of β-[3-(2-carbomethoxymethyl)-indolyl]-ethylchlorid
(85) in which this compound was condensed with various 3-acetylpyridine derivatives. Thus conversion of the alcohol (90) to the chloride (85) and reaction of this compound with 3-ethylpyridine or a derivative was thought to be a reasonable route to 16,17-dihydrosecodin-17-ol (66). On this basis treatment of the alcohol (90) with methanolic hydrochloric acid produced, after chromatography, a dark oil in low yield. Interpretation of the spectral data showed that this oil was 8-[3-(2-carbomethoxymethyl)-indolyl]-ethylchloride (85). The ir and nmr spectra compared favourably with that of Wenkert's, the nmr spectrum showing the β-indolylmethylole protons at δ 6.84 and the chloromethylene protons at δ 6.31. In the mass spectrum fragments at m/e 251, 253 (M⁺) underwent loss of [-CH₂-CI] to give a base peak at m/e 202 which could be due to the ion (95).

Under these reaction conditions the product contained impurities which were difficult to separate and this fact combined with the low yield indicated that the chloride should be formed by an alternative method. It was considered that the nucleophilic displacement of the hydroxyl group by chloride anion would proceed under less vigorous reaction conditions to give a cleaner product if the leaving capacity of the hydroxyl function was increased. Since primary tosylates are extremely labile, this derivative was attempted first and it was prepared in situ.

The alcohol (90) was dissolved in tetrahydrofuran containing triethylamine at 0° and p-toluenesulfonylchloride was then added. After stirring at 0° for 48 hours a sixfold excess of lithium chloride was
added and the reaction mixture was warmed to 50° until thin layer chromatography indicated that the tosylate had completely reacted. After chromatography the chloride was isolated crystalline and in low yield. This compound was found to be unstable and decomposed slowly to a black tar if left open to the air. The β-[3-(2-carbomethoxymethyl)-indolyl]-ethylchloride (85) isolated from this procedure had the same spectral characteristics as the previous chloride (85).

It was about this time that Battersby and co-workers\(^{84}\) published a communication on the synthesis of 16,17-dihydrosecodin-17-ol (66). The approach used is outlined in Figure 14 and as may be noticed this synthesis closely resembles our initial scheme.

Returning to our own work, condensation of β-[3-(2-carbomethoxymethyl)-indolyl]-ethylchloride (85) with 3-ethylpyridine under a variety of different conditions produced at best low yields of the anticipated salt (91), which after reduction with sodium borohydride gave small quantities of N-[β-{3-(2-carbomethoxymethyl)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (81). This compound was recognized by comparing it with the authentic material obtained from the previous synthesis\(^ {86}\) (refer to Figure 12).

Owing to the low yields in both the chloride (85) and salt (91) formation, another route from the alcohol (90) to the salt (91) was tried. Instead of displacing the alcohol tosylate (98) with chloride anion followed by a condensation it was realized that the direct displacement with 3-ethylpyridine might occur to give the salt (91).

Formation of β-[3-(2-carbomethoxymethyl)-indolyl]-ethyl-p-toluene-sulphonate (98) occurred when p-toluenesulphonylchloride was added to the
Figure 14. The Battersby Synthesis of 16,17-Dihydrosecodin-17-ol (66).
alcohol (90) dissolved in 3-ethylpyridine (75) at 0° and the resulting mixture was left at 0° for 10 hours. The tosylate was not isolated and the reaction mixture was then held at 75° for 60 hours, i.e. until the reaction had gone to completion by thin layer chromatography. The excess 3-ethylpyridine was removed under vacuum and the crude salt was then dissolved in methanol and reduced with sodium borohydride. Column chromatography produced two crystalline compounds, one of which proved to be the ester (81) in 35% overall yield from the alcohol (90).

Spectral data for this product were consistent with the structure N-[β-{3-(2-carbomethoxymethyl)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (81). The ir spectrum was superimposable with the ir spectrum of the ester (81) obtained from the previous synthesis. The nmr, ultraviolet (uv) and mass spectra of both esters were identical and the molecular formula $C_{20}H_{26}O_2N_2$ was confirmed by high resolution mass spectrometry (Found: 326.1971; Calcd.: 326.1994).

The spectral information from the other crystalline product (33% yield) isolated after chromatography of the above mixture was consistent with the chloride (85).

In an attempt to improve the yield in this reaction the tosylate (98) was now isolated instead of being formed in situ. The tosylate (98) was formed as before and worked up using cold sulphuric acid. After column chromatography (98) was found to be an oil. The spectral data compared favourably with the assigned structure. In the nmr spectrum the aromatic indole and tosylate protons occurred as a multiplet centred at $\tau$ 2.58 and the methyl protons were found at $\tau$ 7.57. On trying to repeat this reaction a crystalline material was isolated the
spectral characteristics of which were found to be consistent with 6-aza-7-carbomethoxymethylene-4,5-benzspirol[2.4]hepta-4-ene (99). Peaks at 1658 cm\(^{-1}\) and 1595 cm\(^{-1}\) in the IR spectrum were assigned to the \(\alpha\)-methyleneindolenine ester functionality. This chromophore also absorbed in the UV spectrum at 222 (log \(\epsilon\) 4.22), 302 (log \(\epsilon\) 4.01) and 323 (log \(\epsilon\) 4.12) \(\mu\)m. The NMR spectrum (Figure 15) exhibited a four proton multiplet centred at \(\tau\) 8.46. Assignment of these four protons to the cyclopropane ring in (99) is in agreement with the observed value of \(\tau\) 8.44 for the cyclopropane protons of (100).\(^{101}\)

![Chemical Structure](image)

The methine proton adjacent to the carbomethoxy group occurred as a one proton singlet at \(\tau\) 5.63. The presence of a methyl ester was suggested by the three proton singlet at \(\tau\) 6.34. The molecular formula \(\text{C}_{13}\text{H}_{13}\text{O}_{2}\text{N}\) was confirmed by high resolution mass spectrometry (Found: 215.0947; Calcd.: 215.0946).

Further chemical support for the structure of the spiromethylene-indolenine (99) is the fact that treatment of (99) with 2 M hydrochloric acid yields the chloride (85). This compound had the same spectral characteristics as \(\beta\)-(3-[2-carbomethoxymethyl]-indolyl)-ethylchloride (85) obtained by the previous two methods. Also the molecular formula \(\text{C}_{13}\text{H}_{14}\text{O}_{2}\text{NCl}\) was confirmed by high resolution mass spectrometry (Found:
Closson and co-workers\textsuperscript{101} have studied the solvolytic rearrangement of tryptophol tosylate (101) and have shown this reaction to proceed through the intermediate (100) to the appropriate tryptophyl derivatives.

Thus the reaction of 3-ethylpyridine with the tosylate (98) was interpreted as taking two possible pathways as shown in Figure 16. The first involves direct $S_N_2$ reaction with 3-ethylpyridine acting as a nucleophile to give the desired salt (91). The other route proceeds with abstraction of a carbomethoxymethyl proton by 3-ethylpyridine, followed by rearrangement to give (99). It is also possible to derive (99) from (91), however, this reaction was not attempted. The spiro-methyleneindolenine (99) could then be carried through the rest of the reaction and during workup of the borohydride reduction with 2 M hydrochloric acid be converted to the chloride (85).

The ester (81) is the convergence point of our two syntheses of 16,17-dihydrosecodin-17-ol (66) and the transformation of the ester into secodinol(66) has been reported elsewhere.\textsuperscript{86} Consequently only a brief outline of the succeeding reactions will be mentioned.

To obtain the secodine skeleton from the ester (81) it is necessary to add a one carbon unit to the methylene in the $\alpha$-indole position. This was achieved by treating an anhydrous benzene solution of the ester with sodium hydride and methyl formate. The resulting enol (82) was difficult to obtain pure and so was not isolated. The crude compound was very carefully reduced with sodium borohydride in methanol at $-30^\circ$ to yield the desired 16,17-dihydrosecodin-17-ol (66). At $0^\circ$ sodium borohydride attacked both the enol and the carbomethoxy functionalities.
Figure 16. The Synthesis of \( N\-{6\-\{3\-(2\-Carbomethoxymethyl)\-indolyl\}\-thyl}\)-3'-ethyl-3'-piperideine (81).
to yield a diol (83). \(^{84,86,89}\)

As already mentioned the biological evaluation of the \([\text{ar}^{-3}\text{H}]\)
form of the alcohol (66) was being carried out when this synthesis was
initiated. Negative incorporations were obtained from several plant
species and it was concluded that the biotransformation of the alcohol
(66) to the acrylic ester (64) was not taking place in the plant systems
studied. Consequently other derivatives of 16,17-dihydrosecondin-17-ol
(66), which plant enzymes might be capable of transforming to the
proposed intermediate (64), were considered.

The dehydrated analogue of 16,17-dihydrosecondin-17-ol (66),
secodine(67), was chosen as this molecule would require only an oxidation
in the piperidine ring to produce the intermediate (64) and this
appeared to be a likely reaction. \(^{36,89}\)

The experimental difficulties in handling secodine(67) were greatly
alleviated by the publication of a communication by Cordel, Smith and
Smith. \(^{102}\) In their investigations they describe the isolation and
properties of the dimeric alkaloids presecamine (102A or B), tetrahydro-
presecamine (103A or B) and dihydropresecamine (104A or B). \(^{102}\)
Presecamine and tetrahydropresecamine on sublimation yield secodine(67)
and 15,20-dihydrosecondine(105), respectively as the sole reaction products.
These products on standing in the absence of solvent reconvert to the
dimeric compounds.

Thus secodine(67) was prepared from the alcohol (66) in the
following manner; \(^{86,89}\) 16,17-dihydrosecondin-17-ol (66) was treated
with sodium hydride in dry benzene and the crude reaction mixture was
rapidly flushed through a column of neutral alumina to allow for the
(102) $R_1 = R_2 = X$
(103) $R_1 = R_2 = Y$
(104) $R_1 = X, R_2 = Y$ or $R_1 = Y, R_2 = X$

$x = -\text{CH}_2\text{CH}_2\text{N}\text{Cyclohexyl} \\
y = -\text{CH}_2\text{CH}_2\text{N}\text{Cyclohexyl}$
separation of the monomer from the rapidly forming dimeric materials. The benzene solution of secodine(67) thus obtained was then freeze dried to afford the pure acrylic ester (67) as an amorphous solid. The entire sequence avoids the use of acid and hydroxylic solvents which, as reported by Smith and co-workers,\textsuperscript{102} attack the monomer. The spectral characteristics of the isolated solid agreed with those reported by Smith and co-workers. Complete characterisation of this compound can be found in the doctoral thesis of R.S. Sood.\textsuperscript{86}

To assess the biological potential of secodine(67) it is necessary to incorporate a radioactive isotope into this molecule and the easiest and cheapest method of achieving this goal is to exchange the aromatic indole protons with tritium in acid. As both secodine(67) and 16,17-dihydrosecodin-17-ol (66) are unstable to acid conditions the ester (81) was chosen as the appropriate compound. Thus N-[β-{3-(2-carbomethoxy-methyl)-indolyl}-ethyl]-3'-ethyl-3'-piperideine (81) was equilibrated with tritiated trifluoroacetic acid, and after purification, was recovered in 80% yield, containing high specific radioactivity. This labelled compound was then converted to [ar-\textsuperscript{3}H]-secodine(67) as previously described.\textsuperscript{86}

The feeding experiments employing this material to assess whether secodine(67) is utilized in the biosynthetic pathway of vindoline (15) and apparicine (72) have already been mentioned in the introduction and were found to be successful. The other two radioactive forms of secodine (67) that were also used in the evaluation of these two bases were the \([22-\textsuperscript{14}COOCH\textsubscript{3}]\) and \([3,14,15,21-\textsuperscript{3}H]\) derivatives.

The synthesis of \([22-\textsuperscript{14}COOCH\textsubscript{3}]\)-secodine was achieved with the substitution of \([\textsuperscript{14}C]\)-potassium cyanide in the reaction to form the
- 59 -

nitrile (80).\(^{89}\) \([3,14,15,21-{\textsuperscript{3}}H]\)-Secodine was prepared by reducing the pyridinium chloride (76) with sodium borotritide.\(^{89}\) Both the labelled nitriles (80) and (76) were elaborated to the appropriate secodine form as in the sequence outlined in Figure 12.

One of the goals of the secodine (67) synthetic sequence was to introduce a tritium label into the ethyl side chain of the piperideine ring. This particular label is necessary to establish the intact incorporation of secodine (67) into the appropriate alkaloids, as successful feedings with \([\text{ar-}{\textsuperscript{3}}H, 22-{\textsuperscript{14}}COOCH_{3}]\)-secodine merely inform one that the indole portion is being incorporated intact while the piperideine ring could be biologically cleaved. A high loss of tritium is expected on feeding \([3,14,15,21-{\textsuperscript{3}}H]\)-secodine to a plant system as in this case the oxidation state of the piperideine ring must be altered and since one can make no predictions about the magnitude of this loss, again one cannot claim an intact incorporation of secodine (67). So it is essential to have a label from which little or no tritium is lost during plant feedings.

Tritiated 3-ethylpyridine (75) could be formed from a basic exchange of 3-acetylpyridine (106) with tritium oxide and then removal of the oxygen atom.

3-Acetylpyridine (106) is commercially available and a model system utilizing this material and deuterium oxide indicated that it was possible to achieve a 90% incorporation of deuterium. Successful elimination of the carbonyl was not so easy as the strongly basic conditions of the Wolff-Kishner reaction removed all the deuterium. However, a Raney-nickel desulfurization reaction on the thio-ketal of deuterated 3-acetylpyridine (107) proceeded in 45% yield with
retention of deuterium. The sequence is outlined below. It was 

\[
\begin{align*}
\text{106} & \xrightarrow{\text{D}_2\text{O} / \text{K}_2\text{CO}_3} \text{106} & \xrightarrow{\text{KOH}} & \text{75} \\
\downarrow & & \downarrow & \\
\text{107} & \xrightarrow{\text{Ra}/\text{Ni} / \text{H}_2} & \text{75}
\end{align*}
\]

decided to abandon this method in the light of a more suitable development. The reaction scheme outlined below involved reduction of 

\[
\begin{align*}
\text{106} & \xrightarrow{\text{NaBH}_4} \text{108} & \xrightarrow{\text{Ac}_2\text{O}} & \text{109} \\
\downarrow & & \downarrow & \\
\text{75}
\end{align*}
\]
the carbonyl and subsequent removal of the hydroxyl function by
derivative formation and reduction.

A solution of 3-acetylpyridine (106) in dry 1,2-dimethoxyethane
was warmed to 80° under nitrogen and sodium borohydride was then added.
The reaction mixture was stirred at this temperature for 20 hours and
the product isolated and purified by distillation. Spectral data
confirmed that this product was 1-(3'-pyridyl)-ethanol (108). The lack
of a carbonyl stretching frequency and the occurrence of a broad band
at 3200 cm\(^{-1}\) in the ir spectrum showed that reduction had occurred. A
broad one proton singlet in the nmr spectrum at \(\tau\) 3.44 which was
exchangeable with deuterium oxide was attributed to the hydroxyl
group while a single proton quartet at \(\tau\) 5.12 (\(J = 6.5\) Hz) was assigned
to the methine hydrogen. The methyl protons occurred as a three proton
doublet at \(\tau\) 8.60 (\(J = 6.5\) Hz). The molecular formula C\(_7\)H\(_9\)ON was
confirmed by high resolution mass spectrometry (Found: 123.0692; Calcd.:
123.0684) and elemental analysis.

Removal of the hydroxyl functionality was accomplished by
acetylation followed by hydrogenolysis. Formation of the acetate (109)
normally occurred readily at room temperature, however, when working
with the radioactive materials, neither the alcohol (108) nor the acetate
(109) were isolated and the crude reduction product required heating
in some cases. Thus acetic anhydride was added to the alcohol (108)
and the reaction monitored by thin layer chromatography. Distillation
of the resulting product yielded the acetate (109), the structure of
which was confirmed by spectral data. A carbonyl stretching frequency
at 1735 cm\(^{-1}\) and a peak at 1240 cm\(^{-1}\) due to the C-O-C bending mode in
the ir spectrum indicated that acetylation had taken place. The acetyl protons occurred as a three proton singlet at \( \tau 7.97 \) in the nmr spectrum. The single proton quartet due to the methine proton now occurred at \( \tau 4.05 \) (\( J = 7 \) Hz) and the methyl protons were found as a three proton doublet at \( \tau 8.46 \) (\( J = 7 \) Hz). Finally the molecular formula \( C_{9}H_{11}O_{2}N \) was confirmed by high resolution mass spectrometry (Found: 165.0807; Calcd.: 165.0790).

Hydrogenolysis of the acetate functionality of (109) occurred when 10% palladium on carbon was added to an aqueous solution of (109) and the resulting mixture hydrogenated for 24 hours. Distillation of the product gave 3-ethylpyridine (75) in 60% overall yield from 3-acetylpyridine (106). The 3-ethylpyridine required for the synthesis of 16,17-dihydrosecodin-17-ol (66) outlined in Figure 12, was prepared through a Wolff-Kishner reaction of 3-acetylpyridine. The spectral characteristics of 3-ethylpyridine prepared in this manner were identical with the hydrogenolysis product. The two ir spectra were superimposable. In the nmr spectrum of (75) the methylene protons occurred as a two proton quartet at \( \tau 7.36 \) (\( J = 8 \) Hz) and the methyl protons as a three proton triplet at \( \tau 8.76 \) (\( J = 8 \) Hz). The molecular formula \( C_{7}H_{9}N \) was confirmed by high resolution mass spectrometry (Found: 107.0732; Calcd.: 107.0735).

Introduction of the tritium atom was achieved using sodium borotritide during the reduction of 3-acetylpyridine (106). In practice the 3-acetylpyridine (106) was dissolved in dry 1,2-dimethoxyethane and the solution warmed to 80° under nitrogen. A small quantity of sodium borohydride was then added to remove any traces of easily reducible materials such as water and the reaction stirred for 1 hour. The sodium
borotritide was then added and the mixture stirred for 3 1/2 hours. The sodium borotritide was of high specific radioactivity and the mole ratio of 3-acetylpyridine to sodium borotritide was of the order of 20:1. At this time an excess of sodium borohydride was added and the reaction stirred at 80° for 20 hours. The crude alcohol (108) was then acetylated and hydrogenated as before. The distilled 3-ethylpyridine (75) was then condensed with β-[3-(2-carbomethoxymethyl)-indolyl]-ethanol (90) and the product was converted to [19-3H]-16,17-dihydrosecodin-17-ol (66) as already described.

Now that the required labelled forms of 16,17-dihydrosecodin-17-ol (66) were at hand, it was desirable to evaluate secodine (67) as a biointermediate. In our laboratories the most successful method of feeding a high molecular weight precursor was found to involve the water soluble acetate salt. Introduction of the aqueous salt into the plant normally required a maximum time of 2 hours. Thus it was obvious that dimerization of secodine (67) would occur in the aqueous, slightly acidic solution during the 2 hour feeding period. Consequently a blank experiment was performed in order to estimate the extent of the dimerization. It was found that after 2 hours the ratio of monomer to dimer was 2:1 and so the majority of the prepared secodine (67) could be successfully fed.

One other blank experiment was considered necessary. Under the feeding conditions used secodine (67) could be oxidized and then this molecule could rearrange to form the appropriate alkaloids before entering the plant system. The following conditions which describe this blank experiment, are those under which all subsequent feeding experiments were carried out.
A 32 milligram sample of [ar-\(^3\)H]-dihydrosecodin-17-ol was dehydrated by the established procedure. The secodine (67) after freeze drying, was immediately taken up in 0.5 ml of ethanol to which 0.5 ml of 0.1 M acetic acid were then added, followed by 1 ml of water. This slightly cloudy solution is the normal medium used to administer secodine (67) to plants. The solution was maintained at room temperature for 2 hours, quenched and diluted with vincamine (7) and catharanthine (16). These two alkaloids are the representatives of the Hunteria and Iboga families respectively, which are studied in Sections B and C. After counting the two bases to constant activity a percentage incorporation of less than 0.00008 was obtained for vincamine (7) and less than 0.00002 for catharanthine (16). The results are summarized below in Table 4.

Table 4. The Blank Feeding Experiment

<table>
<thead>
<tr>
<th>Compound Fed</th>
<th>Activity Fed</th>
<th>Specific Activity Fed</th>
<th>% Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>catharanthine (16)</td>
</tr>
<tr>
<td>[ar-(^3)H]- Secodine</td>
<td>5.19 (10^8) dpm</td>
<td>1.62 (10^{10}) dpm/mmol</td>
<td>&lt;0.00002</td>
</tr>
<tr>
<td>(67)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results suggest that secodine (67) is not transformed into vincamine (7) and catharanthine (16) and in fact during the feeding period most of the precursor (67) enters the plant in an intact form.
Section B

Amongst the spectrum of indole alkaloids found in the plant species *V. rosea* L. are three basic families, Corynanthe, Aspidosperma and Iboga. Secodine (67) would be expected to be on the biosynthetic pathway to both the Iboga and Aspidosperma families. Vindoline (15), a representative of the Aspidosperma alkaloids and a monomeric unit of the antineoplastic agent, vincristine (11), has already been studied in this plant species. However, no experimental data is available for the Iboga system and the following Section concentrates on the results obtained for catharanthine (16), a member of this family.

Vincamine (7), a member of the Hunteria family, is found in the tiny evergreen plant *V. minor*. This alkaloid is considered to arise from the Aspidosperma family and is discussed in Section C.

Wenkert²⁷ postulated that the Aspidosperma and Iboga type skeletons arise via rearrangements of the Strychnos skeleton as outlined in Figure 8. In order to evaluate this proposal Scott⁷³ fed labelled stemmadenine (53) and tabersonine (54) to seedlings of *V. rosea*.

The specific incorporation of stemmadenine (53) into both the Aspidosperma and Iboga alkaloids of this plant system has been discussed previously. Tabersonine (54) was found to be incorporated into vindoline
(15) and although this result was expected it confirmed the premise that minor functionalities were introduced into the molecule after the major skeletal modifications were complete. However, the incorporation of this Aspidosperma alkaloid into the Iboga compound catharanthine (16) was entirely unexpected and necessitated the proposal of a facile reversible reaction at the branching point of Wenkert's postulate. This work has been confirmed by our own group. 17

Labelled catharanthine (16), when administered to V. rosea seedlings, failed to be incorporated into any other alkaloid, indicating a possible lack of reversibility on the Iboga side of the branching point, or simply that catharanthine is too far from the main pathway to constitute an effective precursor (see Figures.17 and 18).

After considerable controversy it appears that the in vitro transformations of stemmadenine (53) to the Iboga and Aspidosperma skeletons and tabersonine (54) to the Iboga skeleton does proceed but in very low yields. 105-107 To explain these results Scott 107 has invoked the two isomers (84) and (110). Intermediate (84) leads to the Aspidosperma compounds and the Iboga skeleton is derived from intermediate (110). It is suggested that the iminium acrylic ester (111), which was
Figure 17. A Rationale for the Incorporation of Tabersonine (54) into Catharanthine (16).
formed from a preakuammicine derivative in the catharanthine (16) synthesis, has been trapped as two Diels Alder addends. The two isomers (110) and (84) are, of course, merely the enamine forms of (111) and the postulated acrylic ester (64). A parallel between the biological transformations of stemmadenine (53) and tabersonine (54) to the Aspidosperma and Iboga alkaloids and the laboratory biogenetic mode is drawn by Scott and he postulates that these conversions take place in V. rosea utilizing (84) as an intermediate.

A biosynthetic rationale for these transformations can be envisaged for catharanthine (16) and tabersonine (54) (see Figure 18).

Since the double bond migration [(53) to (112)] appears to be an essential step, saturation of this bond at some earlier stage would block the biosynthesis. In fact, dihydrovincoside (40, vinyl reduced) gave no significant incorporation into any of the alkaloidal types.

In order to analyze the mechanism in the later stages of the biosynthesis, Battersby studied the tritium loss or retention from a series of specifically labelled precursors.

It was established that (3R)-mevalonate (27) and not the (3S)-isomer is the specific precursor for these indole alkaloids. Also, from a series of experiments utilizing various double-labelled precursors
Figure 18. A Rationale for the Implication of the Acrylic Ester (64) in the Biosynthesis of the Aspidosperma and Iboga Alkaloids.
(some of which were optically active), the following conclusions were drawn: (a) the stereospecificity established for the formation of geraniol (34) double bonds in other biological systems holds in *V. rosea*; (b) removal of the C\textsubscript{21} proton during the transformation (53) → (112) is stereospecific, and furthermore, the proton derived from C\textsubscript{4} of loganin remains unaffected throughout the entire pathway; (c) tritium at C\textsubscript{2} of loganin (33) is lost in alkaloid formation; (d) tritium of C\textsubscript{7} of loganin (33), corresponding to C\textsubscript{15} of the Corynanthe-Strychnos alkaloids, is retained throughout; the configuration of C\textsubscript{7} of loganin (33) is thus stereochemically determining for this group; (e) tritium at C\textsubscript{5} of loganin (33) appears in the three alkaloidal systems and the label is found only at the expected position (C\textsubscript{3} in ajmalicine (31), vindoline (15) and catharanthine (16)).

These results are in full accord with the described biosynthetic pathway and the labels may be followed through this sequence using Figures 6, 9 and 18.

Recently an interesting result involving the oxidation state of the piperidine ring of the proposed secodine-like intermediate (64) has become available.\textsuperscript{108} On feeding mevalonic acid labelled with tritium
at C₅ and isolating catharanthine (16) and vindoline (15) in *V. rosea*, it has been found that both catharanthine (16) and vindoline (15) lose two of the four possible protons, as expected (see Figures 18 and 19). However, vindoline (15) was found to retain the two pro-5R protons of mevalonic acid while catharanthine (16) retained one pro-5R proton and one pro-5S proton.

The generation of the acrylic ester (64) from (-)-tabersonine (Hᵐ⁺Hᵐ⁻) (54) a proven precursor of catharanthine (Hᵣ⁺Hₛ⁻) (16), now appears difficult to interpret. However, one rationale provided by Scott,¹⁰⁸ is as follows, "the acrylic ester is generated from stemmadenine (53) (a proven precursor) with retention of the three C₅ mevalonic acid protons (2Hᵣ⁺Hₛ⁻). The dihydropyridinium system (64) then loses the Hₛ⁻ proton to give the dienamine (2Hᵣ⁺) (δ₄), and thence (-)-tabersonine (54) and vindoline (15) with the observed label (Hᵥ⁺Hᵥ⁻). If however the Hᵣ⁺ proton is lost from (64) in an enantiotopic process, the resultant acrylic ester (64) can form either catharanthine (Hᵣ⁺Hₛ⁻) (16), or by virtue of its enantiomeric conformation, generate a new alkaloid (+)-tabersonine (Hᵣ⁺Hₛ⁻) (54) which in turn suffers ring opening on the enzyme to that form of the acrylic ester (64) which can recyclize to catharanthine (Hᵣ⁺Hₛ⁻) (16) in an enzyme bound process". Refer to Figure 19.

Many labelled forms of secodine (67) were thus fed to *V. rosea* and the feeding technique employed to introduce the radioactive compounds was through a cotton wick method utilizing intact potted plants. The labelled precursors were administered as outlined in the discussion of the blank feeding experiments.

In order to estimate the most efficient feeding period for catharanthine (16) and to confirm that biosynthesis of this alkaloid was
Figure 19. Scott's Modified Postulate for the Biogenesis of the Aspidosperma and Iboga Alkaloids.
occurring on a measurable basis in the plant, an experiment was performed in which four very similar young *V. rosea* plants were each allowed to incorporate \([3-^{14}\text{C}]\)-D,L-tryptophan under identical growing conditions. The young plants were allowed to grow for different times and then the catharanthine (16) was isolated. The alkaloid was taken up in methanol and spotted on prepared Eastman-Kodak silica gel strips. After elution with chloroform containing 5% methanol, catharanthine (16) displayed an \(R_f\) value of 0.5. To obtain a quantitative determination of the radioactivity emitted by the catharanthine (16) sample, the strips were analyzed with a Nuclear-Chicago strip counter. The results are given in Table 5 below. It is evident from these results that catharanthine (16) incorporates tryptophan (13) relatively slowly with the largest incorporation being at the end of the experiment. Scott working with *V. rosea* seedlings obtained a similar result for catharanthine (16). However tabersonine (54), the Aspidosperma counterpart for catharanthine (16),

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Feeding Time (days)</th>
<th>% Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 5. The Results of the Incorporation of \([3-^{14}\text{C}]\)-Tryptophan (13) into Catharanthine (16).
was found to achieve a maximum incorporation of tryptophan (13) after only 9 hours.

Thus it was decided to feed secodine (67) for a time period of 20 days as the biosynthesis of this alkaloid is obviously taking place in the plant with reasonable efficiency. The results obtained for the various labelled forms of secodine (67) are summarized in Tables 6 and 7.

The initial results obtained with aromatically labelled secodine (67) (experiments one and two) indicated that the plant system was able to utilize the precursor in order to synthesize indole alkaloids.

Encouraged by these results the next experiment involved secodine (67) carrying a label in a different position. As aromatic protons are under certain circumstances quite labile, experiment three included a carbon-14 label in the carbomethoxy position. Again the results indicated that this molecule was incorporated into catharanthine (16).

Experiments four and five utilize doubly labelled secodine (67). The tritium/carbon-14 ratios of the secodine (67) fed for experiments four and five were 3.93 and 3.96 while the catharanthine (16) isolated showed isotopic ratios of 3.39 and 3.53 respectively. This indicated firstly that significant exchange or loss of tritium from the indole ring does not occur during the biosynthesis, only a small loss of 14% and 11% being observed. The second point established is that the \( \alpha \)-carbomethoxymethyleneindole portion of the secodine unit (67) is not significantly altered during its incorporation into the plant alkaloid catharanthine (16). Additional conformation of these conclusions comes from the degradation of this alkaloid which will be discussed shortly.

Although experiments four and five established the specific incorporation of the indole nucleus of secodine (67), so suggesting that
Table 6. The Results of the Incorporation of Secodine (67) into Catharanthine (16).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound Fed</th>
<th>% Incorporation $^{14}$C</th>
<th>$^{3}$H</th>
<th>Ratio of Activity Fed, $^{3}$H/$^{14}$C</th>
<th>Ratio of Activity Isolated, $^{3}$H/$^{14}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[ar-$^{3}$H]-Secodine</td>
<td>-</td>
<td>0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>[ar-$^{3}$H]-Secodine</td>
<td>-</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>$^{14}$COOCH$_{3}$-Secodine</td>
<td>0.006</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>[ar-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.0008</td>
<td>0.0007</td>
<td>3.93</td>
<td>3.39</td>
</tr>
<tr>
<td>5</td>
<td>[ar-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.001</td>
<td>0.0009</td>
<td>3.96</td>
<td>3.53</td>
</tr>
<tr>
<td>6</td>
<td>[19-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.001</td>
<td>0.001</td>
<td>1.91</td>
<td>1.95</td>
</tr>
<tr>
<td>7</td>
<td>[19-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.0006</td>
<td>0.0004</td>
<td>3.37</td>
<td>2.12</td>
</tr>
<tr>
<td>8</td>
<td>[19-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.0006</td>
<td>0.0004</td>
<td>2.39</td>
<td>1.64</td>
</tr>
<tr>
<td>9</td>
<td>[19-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.0006</td>
<td>0.0005</td>
<td>5.55</td>
<td>4.58</td>
</tr>
<tr>
<td>10</td>
<td>[3,14,15,21-$^{3}$H,$^{14}$COOCH$_{3}$]-Secodine</td>
<td>0.003</td>
<td>0.001</td>
<td>5.02</td>
<td>1.93</td>
</tr>
<tr>
<td>11</td>
<td>[ar-$^{3}$H]-Secodine</td>
<td>-</td>
<td>0.0004</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 7. Specific Activities Associated with Feeding Experiments in Table 6.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Activity Fed (dpm)</th>
<th>Specific Activity Fed (dpm/mmol)</th>
<th>Specific Activity Isolated (dpm/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{14}\text{C}$</td>
<td>$^{3}\text{H}$</td>
<td>$^{14}\text{C}$ $^{3}\text{H}$</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>$1.05\times10^8$</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>$1.05\times10^8$</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>$4.56\times10^7$</td>
<td>-</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>4</td>
<td>$2.02\times10^7$</td>
<td>$8.03\times10^7$</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>5</td>
<td>$2.00\times10^7$</td>
<td>$7.92\times10^7$</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>6</td>
<td>$1.95\times10^7$</td>
<td>$3.69\times10^7$</td>
<td>$4.94\times10^9$</td>
</tr>
<tr>
<td>7</td>
<td>$3.45\times10^7$</td>
<td>$1.19\times10^8$</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>8</td>
<td>$4.08\times10^7$</td>
<td>$9.75\times10^7$</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>9</td>
<td>$3.44\times10^7$</td>
<td>$1.92\times10^8$</td>
<td>$4.37\times10^9$</td>
</tr>
<tr>
<td>10</td>
<td>$1.75\times10^7$</td>
<td>$8.82\times10^7$</td>
<td>$4.94\times10^9$</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>$1.28\times10^8$</td>
<td>-</td>
</tr>
</tbody>
</table>
the entire molecule was being utilized by the plant system, unequivocal proof that the N-ethyl-3-ethyl-3-piperideine part of the alkaloid was also incorporated had yet to be obtained. It was felt extremely unlikely that the doubly labelled secodine molecule (67) could be catabolized, then biologically recycled and yet retain the same isotopic ratio.

To establish beyond doubt the specific incorporation of the entire secodine molecule (67), experiments six, seven, eight and nine were carried out. In these experiments, a dual isotopic secodine was used with the tritium in the ethyl-piperideine unit, in the 19 position and the carbon-14 again in the carbomethoxy group. Experiments six and nine show very little change in the ratio of tritium to carbon-14 fed and the ratio obtained from the isolated catharanthine (16). These results establish that the piperideine ring of the secodine molecule is incorporated intact into catharanthine (16). However experiments seven and eight show a significant loss of tritium (37% and 46% respectively) and these results are difficult to rationalize.

To establish unequivocally that the structural template for the bio-intermediate is of the secodine type, it was necessary to show that secodine (67) is incorporated specifically into catharanthine (16). In other words, it must be made clear that the carbomethoxy group of the secodine molecule (67) becomes the ester function in the alkaloid and that no randomization of the label occurs. A degradation was thus carried out by the established procedure outlined in Figure 20.

Catharanthine hydrochloride (16) was hydrogenated with Adam's catalyst in methanol for 24 hours. The crude dihydrocatharanthine hydrochloride (113) was crystallized from methanol, mp 216-217° (lit. mp 216-221°).
The nmr spectrum showed no olefinic proton signal and the molecular formula $[C_{21}H_{27}O_{2}N_{2}]^+$ was confirmed by high resolution mass spectrometry (Found: 339.2034; Calcd.: 339.2072).

Decarboxylation of dihydrocatharanthine (113) would then remove the pertinent carbon atom which should contain all the radioactivity from the catharanthine molecule (16). Refluxing dihydrocatharanthine (113) in a 1:1 mixture (v/v) of ethanol and hydrazine produced carbon dioxide and epi-ibogamine (114). The carbon dioxide was trapped in saturated barium hydroxide solution. The spectral characteristics of the crystalline product (mp 173-174°) compared favourably with those of epi-ibogamine (114) (lit. mp 162-164°). The ir spectrum showed no carbonyl absorption at 1735 cm$^{-1}$ while the three proton singlet at $\tau 6.35$ due to the carbomethoxy group in the nmr spectrum of dihydrocatharanthine had disappeared in the nmr spectrum of this compound. The molecular formula $C_{19}H_{24}N_2$ was confirmed by high resolution mass spectrometry (Found: 280.1928; Calcd.: 280.1938) and elemental analysis.

In practice, the pure samples of radioactive catharanthine hydrochloride (16) from experiments three, six and eight (after counting to constant radioactivity) were reduced to dihydrocatharanthine hydrochloride (113) and then this material was decarboxylated to give carbon dioxide. The radioactive carbon dioxide was trapped in 1 ml of a 1 M hydrazine hydroxide solution and was counted as such. The results are displayed in Table 8 and show that essentially all of the carbon-14 radioactivity in the catharanthine (16) from experiments three, six and eight was located in the carboxyl group.
Figure 20. The Degradation of Catharanthine (16).
Table 8. Specific Activities Related to the Degradation of Catharanthine (16).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Specific Activity Fed $^{14}C$ (dpm/mmol)</th>
<th>Specific Activity of Catharanthine $^{14}C$ (dpm/mmol)</th>
<th>Specific Activity of Carbon Dioxide $^{14}C$ (dpm/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.37 $10^9$</td>
<td>3.84 $10^3$</td>
<td>3.90 $10^3$</td>
</tr>
<tr>
<td>6</td>
<td>4.94 $10^9$</td>
<td>1.89 $10^3$</td>
<td>1.93 $10^3$</td>
</tr>
<tr>
<td>8</td>
<td>4.37 $10^9$</td>
<td>3.40 $10^3$</td>
<td>3.33 $10^3$</td>
</tr>
</tbody>
</table>

Thus the statements made earlier concerning the catabolism of the $\alpha$-carbomethoxymethylcyclohexadione portion of secodine (67) and of the N-ethyl-3-ethyl-3-piperideine unit of this molecule during the biosynthesis of catharanthine (16) are justified. The tritium to carbon-14 ratios obtained are shown to be a true indication of specific incorporation.

One final experiment was determined with catharanthine (16). Experiment ten again used a dual isotopic label, however, in this case the tritium was located throughout the piperideine ring. It was hoped that this experiment would provide useful information regarding the mechanism of the conversion of secodine (67) to catharanthine (16). The postulated conversion involves oxidation to the imine (64), rearrangement to the dienamine (84) and finally a Diels Alder type reaction to give catharanthine (16). The experiment revealed that the tritium to carbon-14 ratio dropped from 5.02 to 1.93. This represents a 61.5% loss of tritium and a loss of such magnitude was not expected. The same
experiment had been carried out during the study of the biosynthesis of
vindoline (15). \(^{87,89-91}\) An interesting point is that in this case a 60% loss of tritium was observed. Obviously the biosynthetic situation is more complicated than expected and one might predict that a similar enzymic process is involved for both catharanthine (16) and vindoline (15).

The actual values for the percentage incorporation of secodine (67) into catharanthine (16) are low compared to those values obtained for vindoline (15). \(^{87,89-91}\) These low incorporations can be ascribed to transportation phenomena occurring in \(V.\ rosea\). In other words, secodine (67) may be reaching the site of biosynthesis of vindoline (15) quite readily, considering that a presumably foreign body is being introduced into the plant system. However, the low incorporation of catharanthine (16) could arise from a lesser ability of the plant to transport secodine (67) to the area of the plant where catharanthine (16) is formed.

It has already been mentioned that Scott\(^{109}\) found that the Aspidosperma alkaloid tabersonine (54) was formed long before catharanthine (16) and he has suggested that catharanthine (16) is formed from (+)-tabersonine (54), as described in Figure 18. Thus these low incorporations could also be related to the fact that catharanthine (16) and vindoline (15) could be formed from similar but different intermediates. The biosynthetic system in \(V.\ rosea\) may be able to convert secodine (67) into (-)-tabersonine (54) but may have difficulty in forming the intermediate required for catharanthine (16).
During Scott's recent studies of the biosynthesis of strychnine (5) in the plant species *S. nux vomica*, it was found that to obtain significant incorporations, long term feeding periods of the order of three months were required. Consequently in our work a feeding period of 95 days was used in experiment eleven but as the results show in Tables 6 and 7 the incorporation of secodine (67) into catharanthine (16) is in fact lower than the other values obtained.

The above experiments have demonstrated that the plant *V. rosea* can utilize the acrylic ester secodine (67) in its biosynthesis of the Iboga alkaloids. The actual biological intermediate is probably very similar in structure to secodine (67) and the postulate that the critical intermediate in the biosynthesis of indole alkaloids is the dihydropyridinium (64) is in accord with our findings.
Section C

La Petite Pervenche or *Vinca minor* L. has been used for centuries as a popular therapeutic. The structures of many of the alkaloids of this plant have been elucidated, principally by J. Mokry and I. Kompis, and the majority of them can be classified as either Aspidosperma or Hunteria bases. The most abundant Aspidosperma alkaloid in *V. minor* is minovine (115) while the Hunteria alkaloids are represented by eburnamonine (116), vincine (117), vincaminine (118) and the major constituent vincamine (7).
Vincamine (7) has been used as a mild antihypotensive agent and more recently it has been recognized as a regulator of cerebral circulation. It reveals a comparable activity to the Ergot alkaloid derivatives. J. Le Men feels that vincamine (7) could be of great value in understanding the hemodynamic and biochemical mechanisms of the complex vascular regulation in the brain.
Only one postulate has been put forth with regard to the origin of vincamine (7) and the other co-occurring Hunteria alkaloids. Wenkert, in a paper on the synthesis of eburnamonine (116) suggested a simple mechanistic rationale for the biosynthesis of these compounds. He suggested that vincamine (7), the progenitor of the other Hunteria bases, is derived from an Aspidosperma alkaloid resembling a hydrated form of vincadifformine (62) (see page 83).

Thus these alkaloids can be linked with the Corynanthe, Strychnos group and consequently the acrylic ester intermediate (64) is thereby implicated in their biogenesis.

In 1972 a French group published a biomimetic synthesis of vincamine (7) which paralleled Wenkert's previous hypothesis in all respects. Oxidation of (-)-vincadifformine (62) gave (119) which on treatment with acetic acid and triphenylphosphine rearranged to give a good yield of (+)-vincamine (7), and (-)-16-epi-vincamine (120) as a minor product. The three Hunteria alkaloids (-)-vincadifformine (62), (+)-vincamine (7) and (-)-16-epi-vincamine (120) are all natural products isolated from V. minor.
In order to test the validity of Wenkert's hypothesis, our laboratory fed a series of labelled precursors to *V. minor* and vincamine (7) was isolated in each case. The results of the various experiments are presented in Table 9 below.
Table 9. Results of the Incorporation of Various Intermediates into *V. minor*.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>Feeding time(hr)</th>
<th>Activity fed(dpm)</th>
<th>% Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[ar-³H]-Tryptophan (13)</td>
<td>24</td>
<td>7.4 x 10⁷</td>
<td>0.089</td>
</tr>
<tr>
<td>2</td>
<td>[ar-³H]-Geissoschizine (51)</td>
<td>24</td>
<td>3.9 x 10⁷</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>[ar-³H]-Stemmadenine (53)</td>
<td>24</td>
<td>4.2 x 10⁷</td>
<td>0.076</td>
</tr>
<tr>
<td>4</td>
<td>[ar-³H]-Tabersonine (54)</td>
<td>24</td>
<td>1.6 x 10⁷</td>
<td>0.070</td>
</tr>
<tr>
<td>5</td>
<td>[ar-³H]-16,17-Dihydrosecodin-17-ol (66)</td>
<td>24</td>
<td>1.9 x 10⁷</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>[ar-³H]-16,17-Dihydrosecodin-17-ol (66)</td>
<td>96</td>
<td>2.4 x 10⁷</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>7</td>
<td>[ar-³H]-Secodine (67)</td>
<td>24</td>
<td>3.4 x 10⁸</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>[ar-³H]-Secodine (67)</td>
<td>96</td>
<td>2.6 x 10⁸</td>
<td>0.001</td>
</tr>
</tbody>
</table>

As anticipated tryptophan (13) was readily incorporated and the results from experiments 2, 3 and 4 suggest that the Hunteria alkaloids are derived from the Corynantheinoid family via the Strychnos and Aspidosperma bases as previously postulated. As in the case of vindoline (15) and apparicine (72) the feedings utilizing 16,17-dihydrosecodin-17-ol (66) resulted in negative incorporations and in fact this compound appeared to be toxic to the plant. However, secodine (67) was found to show a low but definite incorporation into vincamine (7).
To further examine the proposed biosynthesis a series of other labelled precursors has been fed to *V. minor*. A hydroponic feeding method was used with cuttings of this plant. The precursors were administered as outlined in the discussion of the blank feeding experiments. Experiments utilizing [ar-\(^3\)H]-secodine had already been carried out\(^{115}\) and indicated that the plant species *V. minor* metabolized secodine (67). Thus various labelled forms of secodine (67) were fed to *V. minor* and after a period of four days, vincamine (7) was isolated from the plant and analyzed for radioactivity. The results of these experiments are tabulated in Tables 10 and 11.
Table 10. The Results of the Incorporation of Secodine (67) into Vincamine (7).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>% Incorporation</th>
<th>Ratio of activity fed</th>
<th>Ratio of activity isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$^{14}$C</td>
<td>$^3$H</td>
<td>$^3$H/$^{14}$C</td>
</tr>
<tr>
<td>1</td>
<td>$[^{14}$COOCH$_3$]-Secodine</td>
<td>0.004</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>[ar-$^3$H, $^{14}$COOCH$_3$]-Secodine</td>
<td>0.0002</td>
<td>0.0002</td>
<td>4.52</td>
</tr>
<tr>
<td>3</td>
<td>[ar-$^3$H, $^{14}$COOCH$_3$]-Secodine</td>
<td>0.001</td>
<td>0.001</td>
<td>8.45</td>
</tr>
<tr>
<td>4</td>
<td>[19-$^3$H, $^{14}$COOCH$_3$]-Secodine</td>
<td>0.0009</td>
<td>0.0009</td>
<td>1.82</td>
</tr>
<tr>
<td>5</td>
<td>[19-$^3$H, $^{14}$COOCH$_3$]-Secodine</td>
<td>0.001</td>
<td>0.001</td>
<td>1.84</td>
</tr>
<tr>
<td>6</td>
<td>[19-$^3$H, $^{14}$COOCH$_3$]-Secodine</td>
<td>0.00008</td>
<td>0.00012</td>
<td>2.07</td>
</tr>
<tr>
<td>7</td>
<td>[ar-$^3$H]-Secodine</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>[ar-$^3$H]-Secodine</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 11. Specific Activities Associated with Feeding Experiments in Table 10.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Activity fed (dpm)</th>
<th>Specific activity fed (dpm/mmol)</th>
<th>Specific activity isolated (dpm/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{14}$C</td>
<td>$^{3}$H</td>
<td>$^{14}$C</td>
</tr>
<tr>
<td></td>
<td>$^{3}$H</td>
<td></td>
<td>$^{14}$C</td>
</tr>
<tr>
<td>1</td>
<td>6.35 $10^7$</td>
<td>-</td>
<td>4.37 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.39 $10^5$</td>
</tr>
<tr>
<td>2</td>
<td>3.25 $10^7$</td>
<td>1.44 $10^8$</td>
<td>4.37 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.62 $10^{10}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.12 $10^3$</td>
</tr>
<tr>
<td>3</td>
<td>1.79 $10^7$</td>
<td>1.51 $10^8$</td>
<td>1.28 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.10 $10^{10}$</td>
</tr>
<tr>
<td>4</td>
<td>1.95 $10^7$</td>
<td>3.79 $10^7$</td>
<td>4.94 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.22 $10^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>4.46 $10^7$</td>
<td>8.15 $10^7$</td>
<td>4.94 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.22 $10^{10}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.95 $10^4$</td>
</tr>
<tr>
<td>6</td>
<td>4.80 $10^7$</td>
<td>9.95 $10^7$</td>
<td>4.37 $10^9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.34 $10^{10}$</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>1.35 $10^8$</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1.35 $10^8$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.62 $10^{10}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.99 $10^4$</td>
</tr>
</tbody>
</table>
Experiment one shows that [22-¹⁴COCOCH₃]-secodine was incorporated into vincamine (7) to an extent of 0.004%. This result in conjunction with the already reported [ar-¹³C]-secodine feedings suggests that V. minor is capable of transforming secodine (67) into vincamine (7). One would anticipate, according to Wenkert's proposal, the carbomethoxy group of secodine (67) to become this same group in vincamine (7). Thus a degradation of vincamine (7) to isolate this particular carbon atom was performed. The reaction scheme is outlined in Figure 21 and first involves a reduction to give the diol (121) followed by acid cleavage to give formaldehyde.

Vincamine (7) was reduced with lithium aluminium hydride in dry tetrahydrofuran for 1 hour at room temperature to give a product which crystallized from methanol, mp 103-104°C. Analysis of the spectral data indicated that this compound was vincaminol (121). The ir, uv, and mass spectra were identical with those of the known compound. The carbomethoxyl protons which occurred at τ 6.23 in the nmr spectrum of vincamine (7) had disappeared in the spectrum of the reduction compound and in their place the hydroxymethylene protons of vincaminol (121) were found centered at τ 5.99. The nmr spectrum also showed that vincaminol (121) contained two protons which were exchangeable with deuterium oxide. The molecular formula C₂₀H₂₆O₂N₂ was confirmed by high resolution mass spectrometry (Found: 326.1963; Calcd.: 326.1994).

The diol (121) was then reacted with aqueous periodic acid in ethanol and the resultant mixture treated with dimedone. The reaction was left for 20 hours at which time a crystalline precipitate was filtered off and recrystallized from ethanol. Column chromatography of the crude compound obtained from the filtrate produced eburnamonine (116),
mp 174-174.5° (lit. mp 173°), mmp 172-173°. Spectral data on (116) was in accord with this formulation. The ir, mass, and uv spectra compared favourably with the spectra of the known compound. The molecular formula C_{19}H_{22}O_{2} was confirmed by high resolution mass spectrometry (Found: 294.1717; Calcd.: 294.1731) and elemental analysis.

Formaldehyde-bisdimedone (122) was prepared from formaldehyde and dimedone and a mixed melting point of this product with that obtained from the periodate cleavage of the diol (121) indicated that these two products were identical (mp 191-192°; mmp 190-192°). Spectral data confirmed this conclusion. The ir spectra of the two products were superimposable and the two nmr spectra were also identical. The molecular formula C_{17}H_{24}O_{4} was confirmed by high resolution mass spectrometry (Found: 292.1693; Calcd.: 292.1675) and elemental analysis.

This degradation scheme was then carried out with the purified vincamine (7) obtained from experiments one and five and the results are shown below in Table 12.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Specific activity of secodine (67)</th>
<th>Specific activity of vincamine (7)</th>
<th>Specific activity of formaldehyde-bisdimedone (122)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(dpm/mmol)</td>
<td>(dpm/mmol)</td>
<td>(dpm/mmol)</td>
</tr>
<tr>
<td>1</td>
<td>$4.37 \times 10^9$</td>
<td>$1.39 \times 10^5$</td>
<td>$1.26 \times 10^5$</td>
</tr>
<tr>
<td>5</td>
<td>$4.94 \times 10^9$</td>
<td>$1.95 \times 10^4$</td>
<td>$1.96 \times 10^4$</td>
</tr>
</tbody>
</table>
As may be noticed the formaldehyde derivative (122) contains essentially all of the carbon-14 radioactivity that was present originally in vincamine (7). Hence the incorporation of secodine (67) is specific (i.e. non-random) in that the ester function in secodine (67) becomes this same functionality in vincamine (7).

Experiments two and three utilized dual isotopic secodine (67) with tritium located in the aromatic indole nucleus and carbon-14 was present in the carboxyl group. As illustrated in Table 10, the ratio of tritium to carbon-14 in the secodine (67) fed to \( V. \text{minor} \) is effectively the same as the ratio of radioactivity in the isolated vincamine (7) in each experiment. Since \([22^{-14}\text{COOCH}_3]\)-secodine is specifically incorporated into vincamine (7), the constancy exhibited in this ratio confirms that \([\text{ar-}^3\text{H}]\)-secodine must also be incorporated specifically. Hence the \( \alpha \)-carbomethoxymethyleneindole unit of the secodine (67) molecule is incorporated intact into vincamine (7).

To unequivocally prove that the entire secodine (67) skeleton is biotransformed to the Hunteria alkaloid vincamine (7), it is necessary to show that the piperideine ring of secodine (67) is not catabolized by \( V. \text{minor} \) and is incorporated intact. Experiments four, five, and six were designed with this purpose in mind. Secodine (67) carrying tritium in the ethyl side chain of the piperideine ring (specifically at \( C_{19} \)) and carbon-14 in the carboxyl group was fed to \( V. \text{minor} \) and the vincamine (7) was isolated after four days. Experiments four and five showed very little change in the ratio of tritium to carbon-14 and these results coupled with the fact that \([22^{-14}\text{COOCH}_3]\)-secodine is incorporated specifically into vincamine (7), show that \([19^{-3}\text{H}]\)-secodine
Figure 21. The Degradation of Vincamine (7).
is also non-randomly incorporated. The results from experiment six indicate that a significant loss of tritium occurred during the metabolism of \([19-^3\text{H},22-^{14}\text{COOCH}_3]\)-secodine in this case. This situation is reminiscent of a similar experiment in Section B when this same secodine molecule (67) was fed to \(V. \text{rosea}\) and catharanthine (16) isolated. It is difficult to rationalize this tritium loss but it could also involve a different enzymic process pertaining to the elaboration of the piperideine ring in the secodine (67) molecule. The very low incorporation of secodine (67) in this experiment enhances this explanation.

The incorporation of secodine (67) into vincamine (7) was low but variable and it was noticed that higher incorporations could always be attained during the spring. These low values could also be attributed to the method of feeding the precursor to this creeping plant.

Owing to Scott's work\(^{119}\) with long term feeding periods, already mentioned in Section B, experiments seven and eight were performed. In this case a hydroponic feeding method was used with a whole plant of \(V. \text{minor}\) and after a feeding period of 96 days the shoots and roots of the plant were worked up separately. Experiment seven deals with the shoots and experiment eight with the roots of \(V. \text{minor}\). The results from Tables 10 and 11 show that after 3 months the incorporation of secodine (67) into vincamine (7) is similar to those values obtained after a 4 day feeding period with the stems and leaves of \(V. \text{minor}\). However, this is the first experiment (experiment eight) carried out on the roots of this plant and it may be noticed that after 3 months there was no activity found in the isolated vincamine (7). A possible explanation is that vincamine (7) is biosynthesized in the shoots of
V. minor and stored in the roots. Perhaps the transport of vincamine (7) to the roots is a slow process and in 3 months the vincamine (7) still remains in the above ground portion of the plant.

The experimental information obtained with the V. minor alkaloid vincamine (7) attests that the entire secodine (67) molecule can be utilized by this plant in a specific manner to afford the Hunteria skeleton.

These results provide preliminary support for the proposal of Wenkert 71 (see page 83) as to the origin of the Hunteria alkaloids.

In conclusion, the experimental results from Part A, Sections B and C, of this thesis prove that secodine (67) is incorporated intact into the V. rosea alkaloid, catharanthine (16) and into the V. minor alkaloid, vincamine (7). This information strongly supports the suggestion that there exists an intermediate resembling secodine (67) which plays a central role in the later stages of indole alkaloid biosyntheses. 87,90,91
EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet spectra (uv) were recorded in methanol (unless otherwise indicated) on a Cary 15 recording spectrometer. The infrared spectra (ir) were taken on a Perkin-Elmer Model 457 spectrometer utilizing a potassium bromide disc (unless otherwise indicated). Nuclear magnetic resonance spectra (nmr) were recorded in deuteriochloroform solution (unless otherwise indicated) at 100 MHz on a Varian HA-100 or a Varian XL-100 instrument and at 60 MHz on a Varian T-60 spectrometer. Chemical shifts are given in Tiers τ scale with reference to tetramethylsilane as the internal standard. The integrated peak areas, multiplicity and proton assignments are given in parentheses. Mass spectra were measured on a AEI-MS-902 high resolution mass spectrometer or on an Atlas CH-4B mass spectrometer. High resolution molecular weight determinations were measured on the AEI-MS-902 spectrometer. Analyses were carried out by Mr. P. Borda of the Microanalytical Laboratory, the University of British Columbia. Woelm neutral alumina and silica gel G (acc. to Stahl) containing 2% by weight General Electric Retma P-1, type 188-2-7 electronic phosphor were used for analytical and preparative thin layer chromatography (tlc), unless otherwise noted. Chromoplates were developed using the
spray reagent carbon tetrachloride-antimony pentachloride (2:1). Woelm neutral alumina (activity III – unless otherwise indicated) was used for column chromatography.

Radioactivity was measured with Nuclear-Chicago Mark I or Mark II Liquid scintillation counters in counts per minute (cpm). The radioactivity of a sample in disintergrations per minute (dpm) was subsequently calculated using the counting efficiency which was determined for each sample by the external standard technique utilizing the built-in barium-133 gamma source. The liquid scintillator solution used with the counters was made up of the following components: toluene (1 liter), 2,5-diphenyloxazole (4 g) and 1,4-bis[2-(5-phenyloxazoly)]benzene (0.05 g). In practice a sample was dissolved in benzene (1 ml) or in methanol (1 ml) or 1 M hyamine hydroxide in methanol (1 ml) if the compound was not sufficiently soluble in benzene, in a counting vial. The volume was then made up to 15 ml with the above scintillator solution. For each sample counted the background was determined for the counting vial to be used by filling the vial with the appropriate solvent and scintillator solution and counting to determine the background in cpm. The vial was then emptied, refilled with the sample to be counted and the scintillator solution and counted. The difference in the cpm between the background count and the sample count was used for subsequent calculations. Each vial was counted for a time period such that the total counts for the sample less the total counts for the background exceeded one thousand.

The radioactivity of labelled compounds absorbed on tlc plates was measured using a Nuclear-Chicago Actigraph Scanner, Model 1036, connected to a Nuclear-Chicago Model 8416 pen recorder and a Nuclear-
Chicago Model 8437 digital print-out recorder.

The *V. rosea* plants used in this study were grown in the Horticultural Department greenhouse, University of British Columbia. *V. minor* and *E. angustifolia* plants were grown in the gardens on the campus of the University of British Columbia or in the Horticultural Department greenhouse.
PART I

Section A

2-Hydroxymethylindole (86)

A solution of ethyl indole-2-carboxylate\(^\text{99}\) (10 g, 52.9 mmol) in THF (50 ml) was added slowly with stirring to an ice cooled suspension of lithium aluminum hydride (4 g, 105 mmol) in THF (250 ml). When the addition was complete, the mixture was refluxed for 2 hr, cooled, and the excess lithium aluminum hydride was decomposed by the addition of sodium hydroxide (5 ml, 15%) and water (15 ml). The solids were removed by filtration and washed with dichloromethane. The combined organic solution was washed with saturated sodium chloride solution, dried over sodium sulphate and evaporated to yield the crude alcohol (86) (10 g). This material was chromatographed on alumina (300 g). Elution with ethyl acetate followed by crystallization produced the alcohol (86) as white crystals (5.8 g, 70%), mp 73-74° (benzene) (lit.\(^\text{121}\) mp 75°); \(\nu\) max: 3380 (NH, OH), 1058 (CH\text{2-OH}) cm\(^{-1}\); \(\lambda\) max (log \(\epsilon\)):

218 (4.64), 271 (3.98), 281 (3.97), 290 (3.81) m\(\mu\); nmr: \(\tau\) 1.66 (1H, singlet, NH), 2.4-3.0 (4H, multiplet, indole protons), 4.62 (1H, doublet, indole-H\(_3\)), 5.27 (2H, singlet, -CH\text{2-OH}), 7.62 (1H, singlet, OH, disappears on addition of D\(_2\)O).

2-Benzoxymethylindole (87)

The alcohol (86) (5 g, 34.0 mmol) was dissolved in dry THF (100 ml) containing triethylamine (9.3 ml, 66.6 mmol) and cooled to 0°. To this solution benzoyl chloride (4.8 ml, 41.5 mmol) was slowly added.
After stirring this reaction mixture for 3 1/2 hr at 0°, dichloromethane and saturated potassium carbonate solution were added to quench the reaction. The water layer was separated and washed with dichloromethane and the combined organic solutions were dried over anhydrous sodium sulphate and evaporated. The crude benzoate was purified by chromatography on alumina (400 g) and elution with benzene yielded a white crystalline product (87) (8.5 g, 100%), mp 128-129° (benzene); ν<sub>max</sub>: 3355 (NH), 1700 (benzoate ester carbonyl) cm<sup>-1</sup>; λ<sub>max</sub> (log ε): 217 (4.79), 270 (4.19), 282 (4.14), 290 (3.90) μm; nmr: τ 1.0 (1H, singlet, NH), 1.95, 2.3-3.0 (9H, multiplet, aromatic benzoate and indole protons), 3.40 (1H, doublet, indole-H<sub>3</sub>), 4.52 (2H, singlet, -CH<sub>2</sub>O); ms: m/e 251 (M<sup>+</sup>), 130,129; high resolution mass spectrometry: Calcd. for C<sub>16</sub>H<sub>13</sub>O<sub>2</sub>N: 251.0946. Found: 251.0964. Anal. Calcd. for C<sub>16</sub>H<sub>13</sub>O<sub>2</sub>N: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.52; H, 5.27; N, 5.38.

2-Cyanomethylindole (88)

The benzoate (87) (5.7 g, 22.7 mmol) was dissolved in DMF (130 ml) and potassium cyanide (7.5 g, 136 mmol) was added. The reaction mixture was stirred at room temperature under nitrogen for 1 hr and then slowly warmed to 75° over another hour. After stirring at this temperature for 1 1/2 hr and then cooling to room temperature, the reaction was treated with water (200 ml), and dichloromethane (200 ml). The phases were separated, the water layer was washed with dichloromethane and the combined organic layers were evaporated under reduced pressure. The residual DMF was removed by freeze drying and the solid residues
were chromatographed on alumina (300 g). Elution with benzene-chloroform (1:1) produced the nitrile (88) as white crystals, (2.8 g, 75%), mp 103.5-104° (benzene); \( \nu_{\max} \): 3370 (NH), 2245 (CN) cm\(^{-1} \); \( \lambda_{\max} \) (log \( \varepsilon \)): 217 (4.83), 265 (4.15), 277 (4.09), 298 (3.94) mv; nmr: \( \tau \) 1.82 (1H, singlet, NH), 2.4-3.0 (4H, multiplet, indole protons), 3.56 (1H, doublet, indole-H\(_3\)), 6.10 (2H, singlet, -CH\(_2\)CN); ms: m/e 156 (M\(^+\)), 155, 130, 129; high resolution mass spectrometry: Calcd. for C\(_{10}\)H\(_8\)N\(_2\): 156.0688. Found: 156.0688.

Anal. Calcd. for C\(_{10}\)H\(_8\)N\(_2\): C, 76.90; H, 5.16; N, 17.94. Found: C, 76.86; H, 5.08; N, 17.91.

Methyl 2-Indolyl-acetate (89)

The nitrile (88) (2.1 g, 13.5 mmol) was dissolved in absolute methanol (200 ml) containing 1% water and saturated with hydrogen chloride gas. After standing at room temperature for 68 hr followed by evaporation of the methanol, the reaction was worked up with aqueous sodium bicarbonate and chloroform. The combined chloroform washings were dried over anhydrous sodium sulphate and the chloroform evaporated. Purification was achieved by chromatography on alumina (150 g). Elution with benzene yielded the acetate (89) as white crystals, mp 72-72.5° (benzene-petroleum ether, bp 65-110°) (2.1 g, 83%); \( \nu_{\max} \): 3350 (NH), 1719 (ester carbonyl) cm\(^{-1} \); \( \lambda_{\max} \) (log \( \varepsilon \)): 217 (4.69), 270 (4.04), 279 (4.01), 288 (3.92) mv; nmr: \( \tau \) 1.43 (1H, singlet, NH), 2.4-3.1 (4H, multiplet, indole protons), 3.68 (1H, doublet, indole-H\(_3\)), 6.29 (2H, singlet, -CH\(_2\)-COOCH\(_3\)), 6.32 (3H, singlet, -COOCH\(_3\)); ms: m/e 189 (M\(^+\)), 130; high resolution mass spectrometry: Calcd. for C\(_{11}\)H\(_{11}\)O\(_2\)N: 189.0790. Found: 189.0781.
Anal. Calcd. for C_{11}H_{11}O_{2}N: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.55; H, 5.81; N, 7.43.

β-[3-(2-Carbomethoxymethyl)-indolyl]-ethanol (90)

A solution of the acetate (89) (1.0 g, 5.30 mmol) in carbon tetrachloride (100 ml) was cooled to 0° (internal temperature) and ethylene oxide (400 μl, 7.95 mmol) was added. At -15° to -18° stannic chloride (650 μl, 5.82 mmol) in carbon tetrachloride (20 ml) was added dropwise with rapid stirring, while maintaining the temperature at -18°. After the addition was complete, the stirring was continued for 20 min at a temperature below 0°, and then chloroform (35 ml) and saturated sodium carbonate (16 ml) were added rapidly with the temperature maintained below 10°. The organic phase was separated and the aqueous layer was washed with ether. The combined organic layers were dried over sodium sulphate and then the solvent was evaporated. The resulting oil was chromatographed on alumina (50 g). Elution with benzene produced recovered starting material (460 mg, 46%) and elution with dichloromethane yielded the alcohol (90) (465 mg, 38%), as a brown oil. The yield of the alcohol (90) in terms of starting material consumed was 71%; \( \nu_{\text{max}} \) : 3603 (OH), 3453 (NH), 1732 (ester carbonyl) cm\(^{-1} \); \( \lambda_{\text{max}} \) : 224, 276, 284, 292 μm; nmr: \( \tau \) 1.20 (1H, singlet, NH), 2.4-3.0 (4H, multiplet, indole protons), 6.25 (2H, triplet, \( J = 7 \) Hz, \(-\text{CH}_{2}\text{-OH}\)), 6.32 (2H, singlet, \(-\text{CH}_{2}\text{-COOCH}_{3}\)), 6.40 (3H, singlet, \(-\text{COOCH}_{3}\)), 7.12 (2H, triplet, \( J = 7 \) Hz, \( \beta \)-indole methylene protons), 7.40 (1H, singlet, OH); ms: m/e 233 (\( M^+ \)), 202, 142.
The alcohol (90) (100 mg, 0.430 mmol) was dissolved in pyridine (20 ml) and to this solution was added 3,5-dinitrobenzoyl chloride (115 mg, 0.5 mmol). The resulting solution was stirred over a steam bath for 10 min, quenched with water and the pyridine evaporated. The reaction was worked up with aqueous sodium bicarbonate and chloroform and the combined chloroform washings were dried over anhydrous sodium sulphate and the chloroform was evaporated. Purification was achieved by chromatography on alumina (5 g) and elution with benzene yielded the 3,5-dinitrobenzoate as orange crystals (184 mg, 100%), mp 188-189°; \( \nu_{\text{max}} \): 3390 (NH), 1720 (ester carbonyls) cm\(^{-1}\); \( \lambda_{\text{max}}^{\text{CH}_3\text{CN}} \) (log \( \varepsilon \)): 220 (4.77), 278 (3.97), 289 (3.88) \( \text{nm} \); nmr: \( \tau \): -0.03 (1H, singlet, NH), 0.85-1.00 (3H, multiplet, aromatic protons), 2.35-2.95 (4H, multiplet, indole protons), 5.34 (2H, triplet, \( J = 7 \text{ Hz} \), \(-\text{CH}_2\text{OCO-})\), 6.07 (2H, singlet, \(-\text{CH}_2\text{COOCH}_3\)), 6.35 (3H, singlet, \(-\text{COOCH}_3\)), 6.71 (2H, triplet, \( J = 7 \text{ Hz} \), \( \beta \)-indole methylene protons); ms: \( \text{m/e} \) 427 (N\(^+\)), 215, 202, 156, 142; high resolution mass spectrometry: Calcd. for \( \text{C}_{20}\text{H}_{17}\text{O}_8\text{N}_2 \): 427.1015. Found: 427.1012.

Anal. Calcd. for \( \text{C}_{20}\text{H}_{17}\text{O}_8\text{N}_2 \): C, 56.21; H, 4.01; N, 9.83. Found: C, 56.34; H, 4.02; N, 9.64.

The alcohol (90) (1.050 g, 4.51 mmol) was dissolved in absolute methanol (200 ml) containing 1\% water and saturated with hydrogen chloride gas. After standing at room temperature for 60 hr followed by evaporation of the methanol, the reaction was worked up with aqueous
sodium bicarbonate and dichloromethane. The combined dichloromethane washings were dried over anhydrous sodium sulphate and the dichloromethane evaporated. The crude chloride (978 mg) was purified by column chromatography on alumina (50 g). Elution with benzene produced the chloride (85) (331 mg, 29%) as a yellow oil; ν\textsubscript{\text{max}} \text{CHCl}_3: 3460 (NH), 1730 (ester carbonyl) cm\textsuperscript{-1}; λ\textsubscript{\text{max}} (log ε): 222 (4.41), 275 (3.75), 283 (3.78), 292 (3.75) μm; nmr: τ 0.95 (1H, singlet, NH), 2.20-3.00 (4H, multiplet, indole protons), 6.20 (2H, singlet, -CH\textsubscript{2}-COOCH\textsubscript{3}), 6.27 (3H, singlet, -COCCH\textsubscript{3}), 6.31 (2H, multiplet, -CH\textsubscript{2}-Cl), 6.84 (2H, multiplet, β-indole methylene protons); ms: m/e 253 (M\textsuperscript{+}), 251 (M\textsuperscript{+}), 215, 202, 156, 142. Elution with dichloromethane gave recovered starting material (275 mg, 26%). The overall yield of the chloride (85) in terms of starting material consumed was 40%.

**Alternative Synthesis of the Chloride (85)**

The alcohol (90) (94 mg, 0.374 mmol) was dissolved in THF containing triethylamine (56 μl, 0.4 mmol) at 0°. To this solution was added p-tosyl chloride (76 mg, 0.4 mmol) and the reaction was stirred until the p-tosyl chloride was dissolved. After storing at 0° for 48 hr, lithium chloride was added (102 mg, 2.40 mmol) and the reaction was warmed to 50° until tlc indicated that the reaction had reached completion. The mixture was then cooled, quenched with ice water (10 g) and extracted with dichloromethane. The organic phase was rapidly washed with 10% aqueous hydrochloric acid, followed by sodium carbonate, dried over anhydrous sodium sulphate and the dichloromethane evaporated. The resulting material was placed on a column of alumina
Elution with benzene produced the chloride (85) (33 mg, 35%). This chloride was identical on tlc and had the same spectral characteristics as the chloride (85) obtained earlier.

N-[β-(3-(2-Carbomethoxymethyl)-indolyl)-ethyl]-3'-ethyl-3'-piperideine (81)

p-Tosylchloride (390 mg, 2.05 mmol) was added to a solution of the alcohol (90) (333 mg, 1.43 mmol) and 3-ethylpyridine (5 ml). The reaction was stirred at 0° until the p-tosyl chloride was dissolved and left at this temperature for 10 hr. At this time tlc indicated that the reaction had gone to completion and had produced one product. The reaction was then warmed to 75° and maintained at this temperature for 60 hr. The 3-ethylpyridine was removed by molecular distillation and the crude salt (91) was dissolved in methanol (30 ml) containing triethylamine (0.3 ml, 2.15 mmol). Sodium borohydride (730 mg, 19.7 mmol) was added over 1/2 hr to the reaction mixture and the reaction was stirred at 0° for 2 3/4 hr. The reaction was quenched with water (100 ml) and acidified with aqueous hydrochloric acid. After stirring for 20 min and basifying the reaction with sodium carbonate the organic material was extracted with dichloromethane. The combined dichloromethane washings were dried over sodium sulphate and then the solvent was evaporated. Column chromatography on alumina (20 g) of the crude oil produced two compounds: Elution with benzene provided the chloride (85) (119 mg, 33%) as brown crystals. The spectral data on this compound were identical with the chloride (85). Elution with benzene-dichloromethane (1:1) yielded the ester (81) as a brown crystalline product, (163 mg, 35%). The spectral data on this compound
was identical with that data obtained from the ester synthesized in the manner outlined in Figure 12. In particular, the ir of both esters were superimposable in all respects; $\nu_{\text{max}}$: 3370 (NH), 1730 (ester carbonyl) cm$^{-1}$; $\lambda_{\text{max}}$ (log $\epsilon$): 222 (4.48), 275 (3.86), 283 (3.87), 292 (3.80) m$\mu$; nmr: $\tau$ 1.24 (1H, singlet, NH), 2.23-2.92 (4H, multiplet, indole protons), 4.52 (1H, multiplet, piperidine olefinic proton), 6.20 (2H, singlet, $-$CH$_2$-COOCH$_3$), 6.29 (3H, singlet, $-$COOCH$_3$), 8.98 (3H, triplet, $-$CH$_2$-CH$_3$); ms: m/e 326 (M$^+$), 124; high resolution mass spectrometry: Calcd. for C$_{20}$H$_{26}$N$_2$O$_2$: 326.1994. Found: 326.1971.

$\beta$-[3-(2-Carboxethoxymethyl)-indolyl]-ethyl-$p$-toluenesulphonate (98)

The alcohol (90) (200 mg, 0.86 mmol) was dissolved in dry pyridine (1.5 ml) and cooled to $-30^\circ$ under nitrogen. p-Tosyl chloride (180 mg, 0.95 mmol) in pyridine (1 ml) was added slowly while stirring. When addition was complete the temperature was raised to $-20^\circ$ and stirring continued for 3 hr. Ice cold 2 M sulphuric acid (10 ml) was added together with dichloromethane (10 ml). The layers were separated and the organic phase was washed with cold dilute sulphuric acid to remove excess pyridine. After washing with water the organic solution was dried over anhydrous sodium sulphate and the solvent evaporated. The resulting yellow oil was immediately chromatographed on alumina (15 g). The fraction eluted with benzene was rechromatographed on alumina (5 g) and the tosylate (98) was obtained as a yellow oil (183 mg, 55%); nmr $\tau$ 1.60 (1H, singlet, NH), 2.58 (12H, multiplet, indole and tosyl protons), 5.88 (2H, triplet, $J = 4.5$ Hz, $-$CH$_2$-O), 6.42 (7H, multiplet, $-$COOCH$_3$-, $-$CH$_2$COO and $\beta$-methyleneindole protons), 7.57 (3H, singlet, methyl protons); ms: m/e
252, 203, 171, 155, 91.

6-Aza-7-carboxymethylene-4,5-benzspiro[2.4]hepta-4-ene (99)

A solution of the alcohol (90) (238 mg, 1.02 mmol) in 3-ethylpyridine (5 ml) was cooled to -35° and recrystallized p-tosylchloride (253 mg, 1.33 mmol) was added rapidly to this solution. The reaction mixture was maintained at this temperature overnight. Cold dichloromethane (0°) was added to quench the reaction and the organic phase was washed (3 x) with cold 2 M sulphuric acid (0°). The dichloromethane layer was dried over sodium sulphate and the solvent evaporated to give a crude oil which was purified by chromatography on alumina (20 g). Elution with petroleum ether (bp 30-60°)-benzene (1:1) yielded the crystalline spiro product (99) (67 mg, 31%), mp 72-73.5°; ν_{max} \text{ cm}^{-1}: 3340 (\text{NH}), 1656, 1595 (α-methyleneindolenine ester carbonyl); λ_{max} (\log ε): 222 (4.22), 302 (4.01), 323 (4.13) μ; nmr: τ 0.03 (1H, singlet, NH), 2.83-3.26 (4H, multiplet, indole protons), 5.68 (1H, singlet, -CH_2-COOCH_3), 6.34 (3H, singlet, -COOCH_3), 8.26-8.65 (4H, multiplet, cyclopropyl protons); ms: m/e 215 (M^+), 183, 156; high resolution mass spectrometry: Calcd. for C_{13}H_{13}O_2N: 215.0946. Found: 215.0947. Elution with chloroform produced starting material (60 mg, 25%). The percentage yield of the spirocyclopropane (99) based on recovered starting material was 41%.

Alternative Synthesis of the Chloride (85)

The spirocyclopropane (99) (20 mg, 0.093 mmol) was dissolved in dichloromethane (20 ml) and washed with 2 M hydrochloric acid (20 ml). The organic phase was dried over sodium sulphate and the solvent removed
to yield the chloride (85) (23 mg, 98%). This compound had the same spectral characteristics as the chloride (85) obtained by the two previous methods; high resolution mass spectrometry: Calcd. for $\text{C}_{13}\text{H}_{14}\text{O}_2\text{NCl}$: 253.0684, 251.0713. Found: 253.0706, 251.0738.

**Formylation of Ester (81)**

A three-necked flask was equipped with a magnetic stirrer, a reflux condenser, a dropping funnel and a nitrogen inlet. All the glassware was flame dried and then thoroughly flushed with dry nitrogen. To the reaction flask a 65% suspension of sodium hydride in paraffin oil (50 mg, 1.3 mmol) was added. The suspension was washed (3 x) with 1-ml portions of dry benzene under nitrogen. The oil free sodium hydride was suspended in a fresh portion of dry benzene (2 ml) and to this freshly distilled methyl formate (dried first over calcium chloride and then over phosphorus pentoxide) (2 ml) was added. The ester (81) (50 mg, 0.15 mmol) was dissolved in dry benzene (3 ml) and added dropwise to the above suspension. The reaction mixture was stirred at room temperature for 15 min and at 35° for 2 hr. At this time tlc indicated completion of the reaction. The excess of sodium hydride was destroyed by cooling the mixture to 0°, adding methanol (0.1 ml), followed by the addition of some crushed ice. The mixture was acidified with 2 M aqueous hydrochloric acid. The excess acid was neutralized with aqueous sodium bicarbonate solution and the heterogeneous mixture was extracted with dichloromethane. The extract was washed with water, dried over anhydrous sodium sulphate and evaporated to afford the crude enol (82) as a white foam (70 mg). This material was used as such for the next reaction.
16,17-Dihydrosecodin-17-ol (66)

The crude enol (82) obtained above was dissolved in methanol (3 ml). The solution was cooled to -30° in a dry ice-acetone bath and sodium borohydride (50 mg, 1.35 mmol) was added in small portions. After stirring for 40 min at -30°, an additional amount of sodium borohydride (40 mg, 1.08 mmol) was again added in small portions to the reaction mixture. Ten minutes later tlc indicated no unreacted enol remained and traces of a more polar compound appeared. The excess of sodium borohydride in the reaction mixture was quenched by careful addition of a few drops of 2 M aqueous hydrochloric acid at -30°. The mixture was diluted with water (5 ml) at 0° and the methanol was evaporated under reduced pressure. The reaction product was acidified with 2 M aqueous hydrochloric acid, made basic with aqueous sodium bicarbonate and extracted with chloroform. The organic phase was dried and concentrated to a white foam (68 mg), which was chromatographed on alumina (2.5 g). Elution with benzene-chloroform mixtures and finally chloroform afforded the alcohol (66) (22 mg, 40% from (81)), mp 131.5-132° (dichloromethane); \( \nu_{\text{max}} \): 3400 (NH), 3050 (OH), 1718 (ester carbonyl) cm\(^{-1}\);
\( \lambda_{\text{max}} \) (log e): 222 (4.49), 274 (3.87), 284 (3.93), 292 (3.86) \( \mu \mu \); nmr:
\( \tau 1.16 \) (1H, singlet, NH), 2.48-3.00 (4H, multiplet, indole protons), 4.61 (1H, multiplet, piperidine olefinic proton), 6.00 (4H, multiplet, \( \text{CH}_2\text{-CH}_2\text{-OH} \)), 6.37 (3H, singlet, \( \text{COOCH}_3 \)), 9.04 (3H, triplet, \( \text{-CH}_2\text{-CH}_3 \));
ms: m/e 356 (M\(^+\)), 338, 326, 124; high resolution mass spectrometry:
Calcd. for C\(_{21}\)H\(_{28}\)O\(_3\)N\(_2\): 356.2091. Found: 356.2074.
Anal. Calcd. for C\(_{21}\)H\(_{28}\)O\(_3\)N\(_2\): C, 70.24; H, 7.93; N, 7.86. Found: C, 70.20; H, 7.83; N, 7.35.
Secodine (67)

A flask was equipped with a magnetic stirrer, a reflux condenser and a nitrogen inlet. All the glassware was first flame dried and then thoroughly flushed with dry nitrogen. To the reaction flask a 65% suspension of sodium hydride in mineral oil (25 mg, 0.65 mmol) was added. This suspension was washed with dry benzene (3 x 0.5 ml) and the oil free sodium hydride was suspended in dry benzene (0.5 ml). A solution of 16,17-dihydrosecodin-17-ol (66) (20 mg, 0.06 mmol) in dry benzene (2.5 ml) was dropped rapidly into the above suspension under nitrogen. The reaction mixture was stirred at 40° for 15 min and then rapidly flushed through a column of alumina (2 g, activity IV) with dry benzene. The first fraction (25 ml) was collected in a cold receiver (0°) and freeze dried under vacuum to afford secodine (67) as a light yellow gum (9.1 mg, 50%). Nmr: \( \tau \) 0.89 (1H, singlet, NH), 2.40-3.00 (4H, multiplet, indole protons), 3.55 (1H, doublet, \( J = 1 \) Hz, olefinic proton of acrylic ester), 3.91 (1H, doublet, \( J = 1 \) Hz, olefinic proton of acrylic ester), 4.58 (1H, multiplet, piperdine olefinic proton), 6.20 (3H, singlet, \(-\text{COOC}_3\)), 9.00 (3H, triplet, \(-\text{CH}_3\); ms: \( m/e \) 338 (M\(^+\)), 124.

\[2'_{-2H}\]-3-Acetylpyridine (106)

A solution of 3-acetylpyridine (106) (2.17 g, 17.9 mmol), potassium carbonate (0.3 g, 2.17 mmol) and deuterium oxide (4 ml, 221 mmol) in THF (4 ml) was stirred for 24 hr at room temperature. Ether (10 ml) was then added and the organic phase was separated. The deuterium oxide was washed with ether and the organic layers were combined, dried over
anhydrous sodium sulphate and the solvent evaporated to yield 3-acetylpyridine (106) (2.17 g, 100%) with a 90% incorporation of deuterium.
Nmr: \( \tau 0.76-2.73 \) (4H, multiplet, aromatic protons), \( 7.40 \) (0.3H, multiplet, \(-\text{CO-CH}_3\)).

\([2-^2\text{H}]-1-(3'-\text{Pyridyl})\text{-ethane (75)}\)

A mixture of 3-acetylpyridine (106) (2.02 g, 16.5 mmol; 90% deuterated), potassium hydroxide (1.6 g, 28.6 mmol) and 95% hydrazine hydrate (3 ml, 95 mmol) in TEG (dried over sodium hydride) (10 ml) was heated for 1 hr at 110-130°. The reaction mixture was cooled and gradually reheated with a takeoff condenser to an oil bath temperature of 180-190°. When the evolution of nitrogen had ceased, the volume collected (7 ml) was extracted with ether. The ether was dried over anhydrous sodium sulphate and concentrated under reduced pressure to yield 3-ethylpyridine (2.00 g, 88%) with a 25% incorporation of deuterium. Nmr: \( \tau 1.46-2.83 \) (4H, multiplet, aromatic protons), \( 7.36 \) (2H, multiplet, \(-\text{CH}_2\text{-CH}_3\)), \( 8.76 \) (2.25H, multiplet, \(-\text{CH}_2\text{-CH}_3\)).

\([2-^2\text{H}]-1-(3'-\text{Pyridyl})\text{-1-thioethyleneketal-ethane (107)}\)

A mixture of 3-acetylpyridine (106) (2.32 g, 19.2 mmol; 90% deuterated), ethane dithiol (10 ml) and boron trifluoride etherate (5 ml) was stirred for 24 hr at room temperature. The solvent was then removed under reduced pressure to yield the thioethyleneketal (107), with 88.5% deuterium in the 2'-position. The ir showed no carbonyl absorption; nmr: \( \tau 0.90-2.95 \) (4H, multiplet, aromatic protons), \( 6.61 \) (4H, multiplet, \(-\text{S-CH}_2\text{-CH}_2\text{-S}\)), \( 7.91 \) (0.345H, singlet, \(-\text{C-CH}_3\)).
[2-$^2$H]-1-(3'-Pyridyl)-ethane (75)

Number 28 Raney nickel (40 g) was washed (5 x) with dry methanol and to the Raney nickel was added the crude (107) dissolved in dry methanol (20 ml). The reaction mixture was refluxed for 20 hr and methanol (15 ml) saturated with sodium hydroxide was then added. The mixture was refluxed for 10 min, filtered and the 3-ethylpyridine and methanol distilled under reduced pressure. The distillate was acidified with hydrogen chloride gas and the methanol evaporated. After treating the residue with aqueous sodium carbonate and extracting with dichloromethane, the organic phase was dried over anhydrous sodium sulphate and the solvent removed to yield 3-ethylpyridine (0.925 g, 45% from 3-acetylpyridine) with 87% deuterium in the 2'-position. Nmr: $\tau$ 1.46-2.88 (4H, multiplet, aromatic protons), 7.36 (2H, broad singlet, $-\text{CH}_2\text{-CH}_3$), 8.76 (0.39 H, multiplet, $-\text{CH}_2\text{-CH}_3$).

1-(3'-Pyridyl)-ethanol (108)

A solution of 3-acetylpyridine (106) (21.7 g, 179 mmol) in dry 1,2-dimethoxyethane (40 ml) was warmed to 80° under nitrogen. Sodium borohydride (2 g, 23 mmol) was then added slowly and the reaction mixture was stirred at this temperature for 20 hr. After cooling the reaction to room temperature the excess sodium borohydride was quenched with methanol-hydrochloric acid and the solvent was evaporated. The crude product was basified with aqueous sodium carbonate and extracted with chloroform. The extract was dried over anhydrous sodium sulphate and the solvent evaporated carefully. The alcohol (108) was obtained by distillation (14.5 g, 66.5%), bp 97° at 0.5 mm; $v_{\text{max}}$: 3200 broad (OH).
cm⁻¹; \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 255 (3.37), 266 (3.29), 260 (3.42) \( \mu \); nmr: \( \tau \) 1.51-2.92 (4H, multiplet, aromatic protons), 3.44 (1H, singlet, \(-\text{OH}, \text{disappears on addition of D}_2\text{O}\)), 5.12 (1H, quartet, \( J = 6.5 \) Hz, \(-\text{CH}(\text{OH})-\text{CH}_3\)), 8.60 (3H, doublet, \( J = 6.5 \) Hz, \(-\text{CH}(\text{OH})-\text{CH}_3\)); ms: m/e 123 (\( M^+ \)), 108, 94; high resolution mass spectrometry: Calcd. for C\(_9\)H\(_9\)ON: 123.0684. Found: 123.0692.

Anal. Calcd. for C\(_9\)H\(_9\)ON: C, 68.27; H, 7.37. Found: C, 68.07; H, 7.50.

**1-Ethyl-(3'-pyridyl)-acetate (109)**

The alcohol (108) (5.0 g, 40.6 mmol) was dissolved in acetic anhydride (100 ml) and the reaction mixture was left at room temperature for 12 hr. The reaction was quenched with aqueous hydrochloric acid, basified with sodium hydroxide and extracted with dichloromethane. The dichloromethane extract was dried over sodium sulphate and the solvent was evaporated carefully. Distillation afforded the acetate (109) (6.3 g, 94%), bp 77-78° at 0.25 mm; \( v_{\text{film}}^{\text{max}} \): 1735 (acetate carbonyl), 1240 (C-O-C) cm⁻¹; \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 255 (3.41), 260 (3.46), 266 (3.33) \( \mu \); nmr: \( \tau \) 1.20-2.82 (4H, multiplet, aromatic protons), 4.05 (1H, quartet, \( J = 7.0 \) Hz, \(-\text{CH(OAc)}-\text{CH}_3\)), 7.97 (3H, singlet, \(-\text{OCOCH}_3\)), 8.46 (3H, doublet, \( J = 7.0 \) Hz, \(-\text{CH(OAc)}-\text{CH}_3\)); ms: m/e 165 (\( M^+ \)), 123, 105; high resolution mass spectrometry: Calcd. for C\(_9\)H\(_{11}\)O\(_2\)N: 165.0790. Found: 165.0807.
1-(3'-Pyridyl)-ethane (75)

The acetate (109) (1.8 g, 10.7 mmol) was titrated with 2 M aqueous hydrochloric acid to pH 7. To this solution was added water (10 ml) and 10% Pd/C (0.2 g) and the resulting suspension was hydrogenated at atmospheric pressure for 24 hr. The mixture was then filtered, basified with aqueous sodium carbonate and extracted with dichloromethane. The organic phase was then dried over anhydrous sodium sulphate and the solvent evaporated carefully. Distillation of the product yielded 3-ethylpyridine (75) (1.1 g, 96%), bp 27-28° at 0.7 mm. The spectral data on this compound were identical with that data obtained from 3-ethylpyridine synthesized by a Wolff-Kishner reaction on 3-acetylpyridine (106) as described in the thesis of R.S. Sood. In particular the ir of both compounds were superimposable; $\lambda_{\text{max}}$ (log $\varepsilon$): 256 (3.44), 262 (3.51), 268 (3.37) μm; nmr: δ 1.46-2.88 (4H, multiplet, aromatic protons), 7.36 (2H, quartet, $J = 8.0$ Hz, $-\text{CH}_2-\text{CH}_3$), 8.76 (3H, triplet, $J = 8.0$ Hz, $-\text{CH}_2-\text{CH}_3$); ms: m/e 107 (M$^+$), 92; high resolution mass spectrometry: Calcd. for C$_7$H$_9$N: 107.0735. Found: 107.0732.

[1-$^3$H]-1-(3'-Pyridyl)-ethanol (108)

A solution of distilled 3-acetylpyridine (106) (4.43 g, 36.6 mmol) in dry 1,2-dimethoxyethane (30 ml) was warmed to 80° under nitrogen. Sodium borohydride (10 mg, 0.26 mmol) was added and the reaction stirred for 1 hr at which time sodium borotritide (obtained from New England Nuclear) (63.5 mg, 1.68 mmol, 0.5 curie) was added and the reaction was stirred for 3 1/2 hr. To complete the reaction an excess of sodium borohydride (1.21 g, 32.0 mmol) was added slowly over 1/2 hr and
the mixture was stirred at 80° for 20 hr. Dry hydrogen chloride gas was bubbled through the reaction mixture to quench the excess sodium borohydride and neutralize the product. The solvent was then removed under reduced pressure to yield crude alcohol (108).

\[1-^3\text{H}\]-1-Ethyl-(3'-pyridyl)-acetate (109)

The crude alcohol (108) from the previous reaction was dissolved in acetic anhydride (50 ml) under nitrogen and the mixture was maintained at 100° for 3 hr. The acetic anhydride was then removed under reduced pressure and the product (109) was dried under vacuum for 12 hr.

\[1-^3\text{H}\]-1-(3'-Pyridyl)-ethane (75)

Distilled water (35 ml) and 10% Pd/C (0.5 g) was added to the dried radioactive acetate (109) and the resulting suspension was hydrogenated at atmospheric pressure for 24 hr. The mixture was filtered, washed with water, basified with aqueous sodium carbonate and extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the solvent evaporated carefully. Distillation of the product at 162-163° (lit. 86 bp 162-165°) afforded radioactive 3-ethylpyridine (75) (2.42 g, 62%). This reaction sequence was repeated using 3-acetylpyridine (106) (5.14 g, 42.5 mmol) and sodium borotritide (95 mg, 2.52 mmol, 0.5 curie) to yield 3-ethylpyridine (75) (2.78 g, 61%).

The two products were combined and the activity of the resulting 3-ethylpyridine (75) was found to be $2.19 \times 10^8$ dpm/mg or $2.34 \times 10^{10}$ dpm/mmol.

\[19-^3\text{H}\]-16,17-dihydrosecodin-17-ol (66) was subsequently obtained following the normal procedure as outlined previously.
The Preparation of Labelled Secodine (67)

The lability of secodine (67) necessitated a sequence which would bring about the formation of this compound and introduce it into the plant system as rapidly as possible. The following is the procedure employed to form and administer the secodine (17) in all of its labelled forms. The labelled compound used for the blank feeding experiment will be used to illustrate the procedure.

All glassware was dried overnight at 110°. A flask equipped with a magnetic stirrer, a reflux condenser, and a dry nitrogen inlet was flame dried and then thoroughly flushed with nitrogen. A 65% suspension of sodium hydride in mineral oil (20 mg) was added to the reaction vessel. The suspension was washed with dry benzene (3 x 0.5 ml) and the oil free compound was then suspended in a fresh portion of dry benzene (0.5 ml). In a small dry test tube [ar-\textsuperscript{3}H]-16,17-dihydrosecodin-17-ol (66) (32.0 mg, 0.09 mmol, 1.62 \times 10^{10} dpm/mmol) was dissolved in dry benzene (0.6 ml). This solution was added rapidly to the sodium hydride-benzene suspension. An additional portion of dry benzene (0.6 ml) was used to rinse the test tube and was added to the reaction. The reaction mixture was stirred at 40° for 15 min and then immediately chromatographed on alumina (2.0 g, activity IV). The column was eluted with dry benzene which was collected in a cold volumetric flask (25 ml). Of this measured volume, 1 ml was diluted to 100 ml with benzene and this diluted solution was used for radioactive counting purposes. The remaining benzene fraction (24 ml) was frozen at -10° and dried under vacuum to yield [ar-\textsuperscript{3}H]-secodine (11.2 mg, 5.24 \times 10^{8} dpm) as a pale yellow gum.
The Blank Feeding Experiment

[ar-\(^3\)H]-16,17-Dihydrosecodin-17-ol (32.0 mg, 0.09 mmol, \(1.62 \times 10^{10}\) dpm/mmol) was dehydrated to [ar-\(^3\)H]-secodine as just described. The resulting gum was taken up in ethanol (0.5 ml) followed by 0.1 M acetic acid (0.5 ml) and water (1 ml). This solution was maintained at room temperature for 2 hr after which time it was basified with aqueous sodium carbonate and catharanthine (16) (31.5 mg) and vincamine (7) (38.3 mg) were added. The mixture was extracted with chloroform and the organic phase dried over anhydrous sodium sulphate. After evaporation of the solvent, the two alkaloids, catharanthine (16) and vincamine (7) were purified and separated from the residue by silica gel preparative tlc. Catharanthine (16) was then crystallized to a constant activity of less than \(1.32 \times 10^{3}\) dpm/mmol representing less than a 0.000022% incorporation of [ar-\(^3\)H]-secodine (67) and vincamine (7) was found to have a value of less than \(3.64 \times 10^{3}\) dpm/mmol which represents less than a 0.000076% incorporation (see Table 13).

Table 13. The Blank Feeding Experiment.

<table>
<thead>
<tr>
<th>Compound fed</th>
<th>Activity fed</th>
<th>Specific activity fed</th>
<th>Specific activity isolated</th>
<th>% Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vincamine (7)</td>
<td>Catharanthine (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dpm</td>
<td>dpm/mmol</td>
<td>dpm/mmol   %</td>
</tr>
<tr>
<td>[ar-(^3)H]-Secodine (67)</td>
<td>(5.19 \times 10^8)</td>
<td>(1.62 \times 10^{10})</td>
<td>(3.64 \times 10^{3})</td>
<td>(1.32 \times 10^{3})</td>
</tr>
</tbody>
</table>
Section B

The Administration of Labelled Secodine (67) to \textit{V. rosea}

The particular labelled secodine (67) to be fed was prepared from 16,17-dihydrosecodin-17-ol (66) as described in Section A. The pale yellow gum was then dissolved in ethanol (0.5 ml) and to this solution was added 0.1 M acetic acid (0.5 ml) and distilled water (1.0 ml). This solution was administered to mature, 1-2 year old \textit{V. rosea} plants by the cotton wick method. This method required the threading of a cotton string through the stem of the growing plant at a point above the ground, but below the branching point. The intertwined ends of the wick were placed in a small vial (capacity 1 ml) located at the base of the plant. This vial was filled with the precursor solution. When this solution was absorbed into the plant, the original container of the labelled compound was washed with distilled water (2 ml), and the washings used to incorporate any remaining compound into the plant. The plants were placed under fluorescent lamp illumination. After 2 hr the plants had absorbed the precursor and the washings. At this time, the cotton wick was removed from the stem and along with the vial and the original container of the labelled material, was rinsed with methanol (100 ml). The activity of the methanol solution was determined and this residual activity was subtracted from the activity estimated for the secodine (67). The residual activity normally amounted to about 1% of that fed. The feeding period for \textit{V. rosea} was 20 days.
The Administration of Labelled Tryptophan to \textit{V. rosea} \\

The commercial tryptophan (13) was suspended in ethanol (1 ml) by means of an ultrasonic cleaner and 0.1 M acetic acid (usually 0.5 ml) was added until the tryptophan (13) was in solution. This solution was then administered to mature 1-2 year old \textit{V. rosea} plants by the cotton wick method as just described. Various feeding periods were used.

Extraction of Alkaloids from \textit{V. rosea} \textsuperscript{122} \\

The following procedure was employed to extract and purify catharanthine (16) from \textit{V. rosea}.

The \textit{V. rosea} plants (3 or 4 plants, approximately 20-200 g fresh plant) were mascerated with methanol in a Waring blender, filtered and remascerated until the filtrate was colourless. The solvent was removed under reduced pressure and the aqueous residue taken up in 2 M aqueous hydrochloric acid (150 ml). This mixture was extracted with benzene (3 x 75 ml). The combined benzene extracts were washed with 2 M aqueous hydrochloric acid (50 ml) and the combined aqueous extracts were cooled in ice, basified with 15 M ammonium hydroxide and extracted with chloroform (3 x 100 ml). The combined chloroform extracts were washed with water (100 ml), dried over anhydrous sodium sulphate and evaporated under reduced pressure to give a brown foam.

The foam was dissolved in a small volume of methylene chloride and chromatographed on alumina (25 g). The column was eluted with petroleum ether (bp 30-60°C)-benzene mixtures and fractions of 25 ml were taken. The petroleum ether-benzene (1:1) fractions were combined and evaporated to dryness. The residue was taken up in methanol containing a trace of
hydrochloric acid. Catharanthine hydrochloride (16) crystallized from this solution on reducing the initial volume of solvent. The alkaloid salt was recrystallized from methanol-ether.

**Tryptophan Feeding Experiments 1, 2, 3, and 4**

In these experiments four very similar *V. rosea* plants of age 1-2 yr and approximately equal weight were fed [3-\(^{14}\)C]-D,L-tryptophan (obtained from New England Nuclear). The precursor was administered and catharanthine (16) was isolated as already described. However, the catharanthine (16) isolated from each experiment was not crystallized as the hydrochloride salt but was dissolved in methanol (100 µl) and 10 µl of this solution was placed on Eastman-Kodak silica gel strips (5 x 20 cm) containing phosphor. After development with chloroform containing 5% methanol, catharanthine (16) displayed an \( R_f \) value of 0.5. The silica plates were then scanned for radioactivity using a tlc plate scanner connected to a pen recorder and a digital print-out recorder. The activity of the catharanthine (16) was measured and the percent incorporation calculated. The experimental details and results are presented in Table 14.

**Secodine (67) Feeding Experiments 1 to 11**

The precursor for experiments 1 to 11 was administered and catharanthine (16) isolated as previously described. The specific details of these experiments are listed in Tables 15 and 16. Experiment 11 had a feeding period of 95 days.
Table 14. The Incorporation of $[^{14}\text{C}]-\text{D,L-Tryptophan}$ (13) into *V. rosea*.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Feeding time (day)</th>
<th>Activity fed (dpm)</th>
<th>Weight fed (mg)</th>
<th>Wet weight of plant (g)</th>
<th>% Incorporation of Catharanthine (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.88</td>
<td>$5.49 \times 10^7$</td>
<td>5.04</td>
<td>17.8</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>2.96</td>
<td>$5.59 \times 10^7$</td>
<td>5.12</td>
<td>18.0</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>6.90</td>
<td>$5.63 \times 10^7$</td>
<td>5.16</td>
<td>20.6</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>17.0</td>
<td>$5.60 \times 10^7$</td>
<td>5.14</td>
<td>10.6</td>
<td>0.80</td>
</tr>
</tbody>
</table>
Table 15. The Feeding of Labelled Secodine (67) to V. rosea.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>Weight fed (mg)</th>
<th>Activity fed</th>
<th>Specific activity fed (dpm/mmol)</th>
<th>Ratio fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[ar-³H]-Secodine</td>
<td>2.4</td>
<td>-</td>
<td>1.05 \times 10^8</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>[ar-³H]-Secodine</td>
<td>2.4</td>
<td>-</td>
<td>1.05 \times 10^8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>([¹⁴COOCH₃])-Secodine</td>
<td>3.7</td>
<td>4.56 \times 10^7</td>
<td>-</td>
<td>4.37 \times 10^9</td>
</tr>
<tr>
<td>4</td>
<td>[ar-³H,¹⁴COOCH₃]-Secodine</td>
<td>4.1</td>
<td>2.02 \times 10^7</td>
<td>8.03 \times 10^7</td>
<td>4.37 \times 10^9</td>
</tr>
<tr>
<td>5</td>
<td>[ar-³H,¹⁴COOCH₃]-Secodine</td>
<td>3.6</td>
<td>2.00 \times 10^7</td>
<td>7.92 \times 10^7</td>
<td>4.37 \times 10^9</td>
</tr>
<tr>
<td>6</td>
<td>[19-³H,¹⁴COOCH₃]-Secodine</td>
<td>2.5</td>
<td>1.95 \times 10^7</td>
<td>3.69 \times 10^7</td>
<td>4.94 \times 10^9</td>
</tr>
<tr>
<td>7</td>
<td>[19-³H,¹⁴COOCH₃]-Secodine</td>
<td>4.5</td>
<td>3.45 \times 10^7</td>
<td>1.19 \times 10^8</td>
<td>4.37 \times 10^9</td>
</tr>
<tr>
<td>8</td>
<td>[19-³H,¹⁴COOCH₃]-Secodine</td>
<td>4.8</td>
<td>4.08 \times 10^7</td>
<td>9.75 \times 10^7</td>
<td>4.37 \times 10^9</td>
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<tr>
<td>9</td>
<td>[19-³H,¹⁴COOCH₃]-Secodine</td>
<td>5.8</td>
<td>3.44 \times 10^7</td>
<td>1.92 \times 10^8</td>
<td>4.37 \times 10^9</td>
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<tr>
<td>10</td>
<td>[3,14,15,21-³H,¹⁴COOCH₃]-Secodine</td>
<td>2.9</td>
<td>1.75 \times 10^7</td>
<td>8.82 \times 10^7</td>
<td>6.94 \times 10^9</td>
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<tr>
<td>11</td>
<td>[ar-³H]-Secodine</td>
<td>3.0</td>
<td>-</td>
<td>1.28 \times 10^8</td>
<td>1.62 \times 10^{10}</td>
</tr>
</tbody>
</table>
Table 16. The Feeding of Labelled Secodine (67) to *V. rosea*.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Wet weight of plants (g)</th>
<th>Weight of catharanthine isolated (mg)</th>
<th>Specific activity isolated (dpm/mmol)</th>
<th>% Incorporation</th>
<th>Ratio isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( ^{14}C )</td>
<td>( ^{3}H )</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>106.2</td>
<td>27.5</td>
<td>-</td>
<td>7.78 ( 10^4 )</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>129.7</td>
<td>25.3</td>
<td>-</td>
<td>2.83 ( 10^4 )</td>
<td>0.002</td>
</tr>
<tr>
<td>3</td>
<td>147.0</td>
<td>25.3</td>
<td>3.84 ( 10^3 )</td>
<td>-</td>
<td>0.0006</td>
</tr>
<tr>
<td>4</td>
<td>168.8</td>
<td>32.4</td>
<td>1.82 ( 10^3 )</td>
<td>6.18 ( 10^3 )</td>
<td>0.0008</td>
</tr>
<tr>
<td>5</td>
<td>95.4</td>
<td>26.9</td>
<td>2.64 ( 10^3 )</td>
<td>9.26 ( 10^3 )</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>143.5</td>
<td>41.4</td>
<td>1.90 ( 10^3 )</td>
<td>3.70 ( 10^3 )</td>
<td>0.001</td>
</tr>
<tr>
<td>7</td>
<td>143.0</td>
<td>29.1</td>
<td>2.52 ( 10^3 )</td>
<td>5.31 ( 10^3 )</td>
<td>0.0006</td>
</tr>
<tr>
<td>8</td>
<td>216.0</td>
<td>28.0</td>
<td>3.40 ( 10^3 )</td>
<td>5.56 ( 10^3 )</td>
<td>0.0006</td>
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<tr>
<td>9</td>
<td>95.0</td>
<td>19.7</td>
<td>3.98 ( 10^3 )</td>
<td>1.82 ( 10^4 )</td>
<td>0.0006</td>
</tr>
<tr>
<td>10</td>
<td>77.6</td>
<td>12.7</td>
<td>1.50 ( 10^4 )</td>
<td>2.90 ( 10^4 )</td>
<td>0.003</td>
</tr>
<tr>
<td>11</td>
<td>64.0</td>
<td>12.9</td>
<td>-</td>
<td>1.33 ( 10^4 )</td>
<td>0.0004</td>
</tr>
</tbody>
</table>
Dihydrocatharanthine Hydrochloride (113)

Catharanthine hydrochloride (16) (99.8 mg, 0.268 mmol) was dissolved in methanol (7 ml) and to this solution Adam's catalyst (9.4 mg) was added. This suspension was hydrogenated at atmospheric pressure for 24 hr. The reaction mixture was filtered and the methanol evaporated from the filtrate to yield crystalline dihydrocatharanthine hydrochloride (113) (100 mg, 99%). For analytical purposes this material was recrystallized from methanol, mp 216-217° (lit. mp 216-221°); \( \nu_{\text{max}} \) 2440 (broad, quaternary salt), 1735 (ester carbonyl), 1240 (C-O-C) cm\(^{-1}\); \( \lambda_{\text{max}} \) (log ε): 222 (4.56), 277 (3.95), 283 (3.97), 293 (3.90) μm; nmr (taken on the free base): \( \tau 2.18 \) (1H, singlet, NH), 2.46-3.00 (4H, multiplet, indole protons), 6.19 (1H, doublet, N-CH-CHO), 6.36 (3H, singlet, -COOCH\(_3\)), 9.06 (3H, triplet, -CH\(_2\)-CH\(_2\)-); ms: m/e 339 (M\(^+\)), 338, 323, 309, 214, 154, 136, 130, 124; high resolution mass spectrometry: Calcd. for \( [\text{C}_{21}\text{H}_{27}\text{O}_{2}\text{N}_{2}]^+ \): 339.2072. Found: 339.2034.

Epi-ibogamine (114)

Dihydrocatharanthine hydrochloride (113) (233 mg, 0.624 mmol) was dissolved in a mixture of ethanol (10 ml) and hydrazine hydrate (10 ml, 95%) and the solution was refluxed for 24 hr. The solvents were removed under reduced pressure and the resulting colourless gum was purified by chromatography on alumina (10 g). Elution with chloroform afforded epi-ibogamine (114) (160 mg, 92%), which crystallized from ethanol, mp 173-174° (lit. mp 162-164°); \( \nu_{\text{max}} \): 3410 (NH), 2850-2950
(C-H stretch), 1455 (H-C-H bend) cm\(^{-1}\); \(\lambda_{\text{max}} (\log e): 226 (4.47), 280 (3.80), 283 (3.81), 290 (3.76) \text{ m}_{\mu}; \text{nmr: } \tau 1.95 (1H, \text{ singlet, } \text{NH}), 2.44-3.00 (4H, \text{ multiplet, indole protons}), 9.11 (3H, \text{ triplet, } -\text{CH}_2-\text{CH}_3); \text{ms: } m/e 280 (M^+) , 265, 251, 195, 156, 136, 129, 124; \text{high resolution mass spectrometry: Calcd. for } C_{19}H_{24}N_2: 280.1938. \text{ Found: 280.1928.} \text{Anal. Calcd. for } C_{19}H_{24}N_2: C, 81.38; H, 8.63; N, 9.99. \text{Found: C, 81.73; H, 8.66; N, 9.70.}

Degradation of Dihydrocatharanthine Hydrochloride (113) on a Small Scale

Dihydrocatharanthine hydrochloride (113) (3.55 mg, 9.50 \text{ \mu mol}) was dissolved in a mixture of ethanol (1 ml) and hydrazine hydrate (1 ml, 95%) in a three-necked round bottom flask and the solution was refluxed for 12 hr. The solvents were removed under reduced pressure. The flask was then flushed with dry nitrogen and a gentle flow of dry nitrogen was passed through the flask, and then through two saturated solutions of aqueous barium hydroxide. The hydrazine carbonate salt was carefully neutralized using 2 M aqueous sulphuric acid. The solution was refluxed for 15 min and the barium hydroxide from the first trap was filtered to afford barium carbonate (1.40 mg, 75%). The residue from the flask was basified with aqueous sodium carbonate and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate and the solvent evaporated. The crude product was flushed through a column of alumina (150 mg, activity IV) with chloroform (50 ml) as the eluent and the solvent was evaporated. The residue was dissolved in chloroform (1.0 ml) and 15 \text{ \mu l} of this solution was injected into a high pressure liquid chromatography instrument.
(Waters Associates, ALC 202, Differential U.V. Detector) using a column of alumina (Waters Associates, cat. no. 26981) with chloroform as the eluant. After injection of the appropriate standards, using known concentrations of epi-ibogamine (114) in chloroform, comparison of peak areas and retention times indicated the yield of epi-ibogamine (114) to be 68.5% (1.82 mg). This experiment was repeated with dihydrocatharanthine hydrochloride (113) (3.83 mg, 10.2 μmol) to afford epi-ibogamine (114) (2.27 mg, 72.8%).

Reduction of Radioactive Catharanthine Hydrochloride (16)

Catharanthine hydrochloride (16), of constant specific activity, from experiments 3, 6 and 8 was hydrogenated to give dihydrocatharanthine hydrochloride (113) as already described. This material was recrystallized from ethanol.

Degradation of Radioactive Dihydrocatharanthine Hydrochloride (113)

A known amount of radioactive dihydrocatharanthine hydrochloride (113) from experiments 3, 6 and 8 was dissolved in ethanol (1 ml) and hydrazine hydrate (1 ml, 95%) in a three-necked flask. The mixture was refluxed for 12 hr and the ethanol and hydrazine were removed under reduced pressure. The flask was flushed with dry nitrogen and a gentle stream of dry nitrogen was passed through the flask and then bubbled into 1M hyamine hydroxide in methanol (1 ml) contained in a counting vial. The hydrazine carbonate salt was carefully neutralized using 2 M aqueous sulphuric acid and the solution was refluxed for 15 min after which time the activity of the hyamine hydroxide solution was determined. The results and experimental details are shown in Table 17.
Table 17. The Degradation of Radioative Catharanthine (16).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Specific activity of $[^{14}C]COOCH_3$-secodine (dpm/mmol)</th>
<th>Specific activity of catharanthine isolated (dpm/mmol)</th>
<th>Weight of dihydro-catharanthine HCl degraded (mg)</th>
<th>Specific activity of hyamine carbonate $^{14}C$ (dpm/mmol)</th>
<th>Corrected specific activity of carbon dioxide $^{14}C$ (dpm/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$4.37 \times 10^9$</td>
<td>$3.84 \times 10^3$</td>
<td>0.213</td>
<td>$2.76 \times 10^3$</td>
<td>$3.90 \times 10^3$</td>
</tr>
<tr>
<td>6</td>
<td>$4.94 \times 10^9$</td>
<td>$1.89 \times 10^3$</td>
<td>2.10</td>
<td>$1.36 \times 10^3$</td>
<td>$1.93 \times 10^3$</td>
</tr>
<tr>
<td>8</td>
<td>$4.37 \times 10^9$</td>
<td>$3.40 \times 10^3$</td>
<td>2.91</td>
<td>$2.35 \times 10^3$</td>
<td>$3.33 \times 10^3$</td>
</tr>
</tbody>
</table>

* Corrected for the yield of the reaction.
Section C

The Administration of Labelled Secodine (67) to V. minor

The procedure employed to administer radioactive compounds to V. minor consisted of a hydroponic feeding to cut stems of the plant, unless otherwise indicated.

The particular labelled secodine (67) to be fed was prepared from 16,17-dihydrosecodin-17-ol (66) as described in Section A. The pale yellow gum was then dissolved in ethanol (0.5 ml) and to this solution was added 0.1 M acetic acid (0.5 ml) and distilled water (1 ml). This solution was then distributed among 10 test tubes (10 x 70 mm). Into each of these test tubes were placed three freshly cut shoots (25-50 cm in length) of V. minor. The test tubes were placed in a rack and the shoots were exposed to fluorescent illumination. The original container of the labelled compound was rinsed with distilled water (2 ml) and this solution was used to refill the feeder tubes after the initial precursor solution had been absorbed by the stems. The solution of labelled secodine (67) and the washings from the secodine (67) container were absorbed by V. minor within a 2 hr period. Following these washings, the ends of the shoots were covered with distilled water for the duration of the experiment. V. minor feedings were carried out for 96 hr. At the completion of the experiment, the 10 test tubes used to incorporate the secodine (67) and the original secodine (67) container were washed with methanol (100 ml) and the radioactivity of this solution was determined. This residual activity was then subtracted from the activity estimated for the secodine (67). The residual activity normally amounted to about 1% of that fed.
Extraction of Alkaloids from *V. minor* 122

The following procedure was used in all cases to extract and purify the alkaloids of *V. minor*.

The stems and leaves of *V. minor* (approximately 25-80 g, wet weight) were macerated with methanol in a Waring blender, filtered and remacerated until the filtrate was colourless. The solvent was removed in vacuo and the aqueous residue taken up in 2 M aqueous hydrochloric acid (150 ml). This solution was extracted with benzene (3 x 75 ml) and the combined benzene extracts were washed with 2 M aqueous hydrochloric acid (50 ml). The combined aqueous phases were cooled in ice, basified with 15 M ammonium hydroxide and extracted with chloroform (3 x 100 ml). The combined chloroform extracts were washed with water (100 ml), dried over anhydrous sodium sulphate and evaporated under reduced pressure to give a brown gum.

The gum was dissolved in a small volume of dichloromethane and chromatographed on alumina (10 g). The column was eluted with petroleum ether (bp 30-60°)-benzene mixtures. Fractions of 25 ml in volume were taken. Petroleum ether-benzene (1:1) eluted vincamine (7) from the column. This alkaloid crystallized readily from methanol.

Secodine (67) Feeding Experiments 1 to 8

The precursor for experiments 1 to 8 was administered and the vincamine (7) isolated as described above. The specific details of these experiments are listed in Tables 18 and 19. Experiments 7 and 8 utilized the hydroponic feeding method with a whole plant to administer the secodine (67). The plant was fed for a period of 96 days
Table 18. The Feeding of Labelled Secodine (67) to V. minor.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>Weight fed (mg)</th>
<th>Activity fed (dpm)</th>
<th>Specific activity fed (dpm/mmol)</th>
<th>Ratio fed $^{3}\text{H} / ^{14}\text{C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$[^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>5.0</td>
<td>$6.35 \times 10^7$</td>
<td>$4.37 \times 10^9$</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>$[\text{ar-}^{3}\text{H},^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>5.8</td>
<td>$3.25 \times 10^7$</td>
<td>$1.44 \times 10^8$</td>
<td>$4.37 \times 10^9 \quad 1.62 \times 10^{10}$</td>
</tr>
<tr>
<td>3</td>
<td>$[\text{ar-}^{3}\text{H},^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>5.0</td>
<td>$1.79 \times 10^7$</td>
<td>$1.51 \times 10^8$</td>
<td>$1.28 \times 10^9 \quad 1.10 \times 10^{10}$</td>
</tr>
<tr>
<td>4</td>
<td>$[19-^{3}\text{H},^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>2.8</td>
<td>$1.95 \times 10^7$</td>
<td>$3.79 \times 10^7$</td>
<td>$4.94 \times 10^9 \quad 1.22 \times 10^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>$[19^{3}\text{H},^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>5.5</td>
<td>$4.46 \times 10^7$</td>
<td>$8.15 \times 10^7$</td>
<td>$4.94 \times 10^9 \quad 1.22 \times 10^{10}$</td>
</tr>
<tr>
<td>6</td>
<td>$[19-^{3}\text{H},^{14}\text{COOCH}_3]\text{-Secodine}$</td>
<td>5.2</td>
<td>$4.80 \times 10^7$</td>
<td>$9.95 \times 10^7$</td>
<td>$4.37 \times 10^9 \quad 2.34 \times 10^{10}$</td>
</tr>
<tr>
<td>7</td>
<td>$[\text{ar-}^{3}\text{H}]\text{-Secodine}$</td>
<td>3.0</td>
<td>-</td>
<td>$1.35 \times 10^8$</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>$[\text{ar-}^{3}\text{H}]\text{-Secodine}$</td>
<td>3.0</td>
<td>-</td>
<td>$1.35 \times 10^8$</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 19. The Feeding of Labelled Secodine (67) to V. minor.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Wet weight of plants (g)</th>
<th>Weight of vincamine (mg)</th>
<th>Specific activity isolated (dpm/mmol)</th>
<th>% Incorporation</th>
<th>Ratio isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.9</td>
<td>6.5</td>
<td>$1.39 \times 10^5$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>43.8</td>
<td>11.5</td>
<td>$2.12 \times 10^3$</td>
<td>0.0002</td>
<td>3.83</td>
</tr>
<tr>
<td>3</td>
<td>43.4</td>
<td>10.6</td>
<td>$6.22 \times 10^4$</td>
<td>0.001</td>
<td>8.62</td>
</tr>
<tr>
<td>4</td>
<td>72.6</td>
<td>10.1</td>
<td>$4.00 \times 10^3$</td>
<td>0.0009</td>
<td>1.88</td>
</tr>
<tr>
<td>5</td>
<td>48.1</td>
<td>8.1</td>
<td>$1.95 \times 10^4$</td>
<td>0.001</td>
<td>2.02</td>
</tr>
<tr>
<td>6</td>
<td>52.2</td>
<td>14.8</td>
<td>$1.32 \times 10^3$</td>
<td>0.00008</td>
<td>1.47</td>
</tr>
<tr>
<td>7</td>
<td>106.4</td>
<td>13.4</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>157.8</td>
<td>12.5</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
and the stems (experiment 7) were worked up independently of the roots (experiment 8).

**Vincaminol (121)**

A suspension of lithium aluminum hydride (32 mg, 0.84 mmol) in dry THF (1 ml) was warmed to 45° under nitrogen. Vincamine (7) (37.2 mg, 0.10 mmol) was dissolved in dry THF (1 ml) and added dropwise to the suspension and dry THF (1 ml) was added. The reaction mixture was maintained at 45° for 1 hr. The reaction was quenched with wet ether (2.5 ml) and water (2.5 ml). The aqueous and ether phases were separated and the aqueous phase was extracted with chloroform. The ether and chloroform layers were combined, dried over anhydrous sodium sulphate and the solvent evaporated to afford vincaminol (121) (34.2 mg, 100%). The diol was crystallized from methanol, mp 103-104°; ν\text{CHCl}_3: 3600 (OH), 3000-2850 (C-H) cm\(^{-1}\); λ\text{max} (log ε): 229 (4.48), 276 (3.82), 281 (3.83), 290 (3.75) μm; nmr: τ 2.28-3.00 (4H, multiplet, indole protons), 5.99 (2H, AB quartet, hydroxymethyl protons), 9.13 (3H, triplet, -CH\(_2\)-CH\(_3\)), integration after addition of D\(_2\)O showed that two protons had exchanged with deuterium; ms: m/e 326 (M\(^+\)), 308, 295, 279, 267, 238; high resolution mass spectrometry: Calcd. for C\(_{20}\)H\(_{26}\)O\(_2\)N\(_2\): 326.1994. Found: 326.1963.

**Formaldehyde-bisdimedone (122)**

A 36% formalin solution (1 ml) was added dropwise to an ethanol solution (1 ml) of dimedone (100 mg, 0.715 mmol). The reaction vessel was sealed and maintained at room temperature for 18 hr. The white precipitate was filtered off, washed with water and crystallized from
ethanol to afford formaldehyde-bisdimedone (122) (110 mg, 53%) as white needles, mp 192-193° (lit. 192°); $\nu_{\text{max}}$: 2960 (C-H), 1610, 1593 ($\delta$-diketone, enol form) cm$^{-1}$; nmr: $\tau$ 6.85 (2H, singlet, exocyclic methylene), 7.71 (8H, singlet, cyclic methylenes), 8.96 (12H, singlet, gem dimethyls).

(-)-Eburnanone (116)

Crude vincaminol (121) (111 mg, 0.342 mmol) was dissolved in ethanol (2 ml) and 0.3 M aqueous periodic acid (2 ml) was added to this solution. The reaction vessel was sealed and maintained at room temperature for 4 hr. The reaction was then cooled in ice, and a saturated aqueous solution of dinedone (20 ml, 0.0297 M) was added. The reaction vessel was again sealed and maintained at room temperature for 18 hr. The reaction was filtered and the filtrate washed with chloroform. The aqueous phase was basified with aqueous sodium carbonate and extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulphate and the solvent evaporated. The product was crystallized from dichloromethane-methanol to afford (-)-eburnanone (116) (83.5 mg, 83%), mp 174-174.5° (lit. 173°), mmp 172-173°; $\nu_{\text{max}}$: 2950, 2920, 2880, 2840 (CH), 1690, 1610 (amide carbonyl) cm$^{-1}$; $\lambda_{\text{max}}$ (log $\varepsilon$): 242 (4.29), 266 (4.02), 293 (3.69) nm; nmr: $\tau$ 1.62, 2.67 (4H, multiplet, indole protons), 6.09 (1H, singlet, $\gamma$CH-N), 9.12 (3H, triplet, $-\text{CH}_2$-$\text{CH}_3$); ms: m/e 294 ($M^+$), 293, 265, 237, 224; high resolution mass spectrometry: Calcd. for $C_{19}H_{22}ON_2$: 294.1731. Found: 294.1717.

Anal. Calcd. for $C_{19}H_{22}ON_2$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.25; H, 7.60; N, 9.34.
The filtered precipitate from the reaction was washed with water and air dried. The white solid was dissolved in methanol, filtered to remove insoluble material and the methanol removed in vacuo. The residue was crystallized from ethanol to afford after three such crystallizations, formaldehyde-bisdimedone (122) (19.5 mg, 19.5%), mp 191-192°, mmp 190-192°; the ir of this material was superimposable on the ir of authentic formaldehyde-bisdimedone (122), \( \nu_{\text{max}} \): 2960 (CH), 1610, 1593 (β-diketone, enol form) cm\(^{-1}\); \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 257 (4.41) \( \mu\mu \); nmr: \( \tau \) 6.85 (2H, singlet, exocyclic methylene), 7.72 (8H, singlet, cyclic methylenes), 8.96 (12H, singlet, gem dimethyls); ms: m/e 292 (M\(^+\)), 165, 124; high resolution mass spectrometry: Calcd. for \( C_{17}H_{24}O_4 \): 292.1675. Found: 292.1693.

Anal. Calcd. for \( C_{17}H_{24}O_4 \): C, 69.84; H, 8.27. Found: C, 69.66; H, 8.19.

Reduction of Radioactive Vincamine (7)

Vincamine (7) of constant specific activity, from experiments 1 and 5 was reduced to give the diol, vincaminol (121), as already described.

Degradation of Radioactive Vincaminol (121)

The crude product from the reduction of radioactive vincamine (7) was dissolved in ethanol (0.25 ml) and to this solution 0.3 M aqueous periodic acid (0.2 ml) was added. The reaction vessel was sealed and maintained at room temperature for 4 hr. The reaction was cooled in ice and saturated aqueous dimedone solution (2 ml) was added. The reaction vessel was again sealed and maintained at room temperature for
18 hr. The resultant precipitate was filtered off, washed with water and air dried. The white solid was dissolved in dichloromethane-methanol (1:1) (1 ml), filtered to remove insoluble material and the solvent removed in vacuo. The formaldehyde-bisdimedone (122) was then crystallized from ethanol to constant specific activity. The results and experimental details are shown in Table 20.

Table 20. The Degradation of Radioactive Vincamine (7).

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Specific activity of [14COOCH₃]-secodine fed (dpm/mmol)</th>
<th>Specific activity of vincamine (dpm/mmol)</th>
<th>Weight of vincamine (mg)</th>
<th>Specific activity of formaldehyde-degraded bisdimedone (122) (dpm/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.37 \times 10^9</td>
<td>1.39 \times 10^5</td>
<td>6.5</td>
<td>1.26 \times 10^5</td>
</tr>
<tr>
<td>5</td>
<td>4.94 \times 10^9</td>
<td>1.95 \times 10^4</td>
<td>6.9</td>
<td>1.96 \times 10^4</td>
</tr>
</tbody>
</table>


91. J.P. Kutney, J. Heterocyclic Chem. 9, Supplementary Issue, S-1 (1972).


99. Ethyl indole-2-carboxylate was obtained from Aldrich Chemical Company Inc.


108. A.I. Scott, private communication.
111. E. Schlittler, Lloydia, 27, 278 (1964).
118. H. Budzikiewicz, C. Djerassi, D.H. Williams, Structure Elucidation of Natural Products by Mass-Spectrometry, 1, 89 (1964), Holden-Day San Francisco.


PART B

A PRELIMINARY INVESTIGATION INTO THE
ISOLATION OF ENZYMES FROM ELEAGNUS ANGUSTIFOLIA
INTRODUCTION

Among the simplest known indole alkaloids are those based upon the β-carboline ring system and these alkaloids have been reported as occurring in fourteen genera. Many of these compounds possess significant biological activity, as for example psychotomimetic activity. Harmine (1), harmaline (2) and 1,2,3,4-tetrahydroharmine (3) have been identified as major constituents of "Yage", an extract of *Banisteria caapi* used by South American Indians for the production of ritual hallucinations. Other neurological effects which could be attributed to these alkaloids are those which arise from smoking and drinking. Harman (4) has been isolated from cigarette smoke and 1,2,3,4-tetrahydroharmine (3) has been identified in the urine of rats treated with ethanol.

![Chemical structures]

(1) $R = \text{CH}_3$
(2) $R = \text{OCH}_3$
(4) $R = \text{H}$
(6) $R = \text{H}$
The biosynthesis of these compounds poses an interesting dilemma and over the years a number of different biogenetic postulates have been proposed utilizing different precursors. The first of these proposals was put forward by Perkin and Robinson who suggested that the skeleton of these molecules could be formed from tryptamine (7) (derived from tryptophan (8)) and acetaldehyde. In support of this scheme it was shown that eleagnine (5) can be synthesized readily from tryptamine hydrochloride (7) and acetaldehyde in dilute aqueous solution at 25° using an acetate (pH 5.2) or phosphate (pH 6.2) buffer, as shown below.

A biogenetic scheme for harman (4) in *Passiflora edulis* was proposed by Slaytor and McFarlane in 1967. They showed that tryptophan (8) is decarboxylated to tryptamine (7) which after N-acetylation is cyclodehydrated to harmalan (6). Harmalan (6) can
then be oxidized to harman (4) or reversibly reduced to eleagnine (5). This scheme is presented in Figure 1. However they concluded that these results did not exclude the existence of an alternative pathway. That same year, O'Donovan and Kenneally\textsuperscript{8} working with \textit{Eleagnus angustifolia} L. plants showed that eleagnine (5) is specifically derived from tryptophan (8) and acetate.

In 1968 Stolle and Gröger\textsuperscript{9} published a paper on the biosynthesis of harmine (1) in \textit{Peganum harmala}. Both tryptophan (8) and tryptamine (7) were specifically incorporated into this alkaloid, as was pyruvate. Acetate, however, was found to produce a non-specific labelling pattern in harmine (1). The authors proposed that the origin of the C\textsubscript{1}, C\textsubscript{10} two carbon unit in harmine (1) could be pyruvate. Condensation of 7-methoxytryptamine (10) with pyruvate may yield the amino acid (11) which would then decarboxylate to produce harmaline (2). Oxidation of (2) would then yield harmine (1) as outlined below.
Figure 1. Slayton and McFarlane's Postulate for the Biosynthesis of Harman (4).
However one cannot rule out the possibilities that pyruvate is initially decarboxylated to acetate or is a source of acetaldehyde. Stolle and Gröger also found that 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (12) was not incorporated into harmine (1).

The biosynthesis of the isoquinoline alkaloids such as anhalonidine (13) provides a close parallel to the biogenesis of the β-carboline ring system. These isoquinoline alkaloids are derived from an appropriately hydroxylated or methoxylated phenylethylamine and a two carbon unit. Again there has been much controversy over the origin of this two carbon unit. However, Kapadia and co-workers in a 1970 publication showed that the biosynthesis of anhalonidine (13) in the
cactus *Lophophora williamsii* proceeds via the amino acid, peyorrubic acid (14). Pyruvate and 3-demethylmescaline (15) condense to give (14).

As further evidence McFarlane and Slaytor in a communication published in 1972 demonstrated that the biosynthesis of salsoline (16) in *Echinocereus merkeri* proceeds from 3-hydroxy-4-methoxyphenyl-ethylanine (17) through 1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (18) to (16).
In a succeeding paper McFarlane and Slaytor\textsuperscript{12} found that under conditions when \textit{E. angustifolia} was synthesizing eleagnine (5), \(N_{b}\)-acetyltryptamine (9) was not incorporated.

In summary, many compounds have been proposed as possible intermediates for the biosynthesis of the 1-methyl-\(\beta\)-carboline skeleton. Some have been successfully incorporated while others have not and it may well be that there exists more than one major pathway in the different plant species. Some attempts at clarification of the above situation were undertaken in our laboratory with the specific objective to isolate plant enzymes. We hope that establishment of the actual enzymic reactions will serve to elaborate more accurately the biosynthetic pathways in these relatively simple indole alkaloids. The results obtained thus far are discussed in the following sections.
DISCUSSION

As the biosynthetic pathway of the indole alkaloids containing the C\(_{9-10}\) unit was fairly well mapped out and many of the intermediates identified, the next step pertained to the isolation of the particular enzymes. Naturally the first reaction in the pathway that held our interest was the condensation of tryptamine with secologanin to form vincoside (Refer to Figure 10 in Part A of this thesis). However, it was felt that initial attempts at the isolation of this type of enzyme should be carried out on a simpler system and the system of choice was the 1-methyl-\(\beta\)-carboline skeleton.

As already noted in the previous section the biogenesis of these simple alkaloids is still far from being fully understood, yet it was felt that each of the possible precursors could be easily obtained and tested.

The preliminary work to be discussed here will be divided into two sections, the first discusses the synthesis of possible intermediates and the second concentrates on the attempts at enzyme isolation.
Section A

Russian olive or *E. angustifolia* was readily available and provided a source of 1-methyl-β-carboline alkaloids. The most abundant alkaloid in this plant species was found to be eleagnine (5), and as this compound is one of the simplest of the 1-methyl-β-carboline alkaloids it was decided to initially study the biosynthesis of (5). Eleagnine (5) isolated from *E. angustifolia* was crystallized from ethyl acetate, mp 178-179° (lit. mp 178-180°) and gave spectral data identical to that of synthetic material. The ir spectra were superimposable and the uv spectrum ($\varepsilon_222$ 34,500, $\varepsilon_274$ 7150, $\varepsilon_280$ 7390, $\varepsilon_289$ 6120) demonstrated that the compound contained the indole nucleus. The nmr spectrum showed the indolic NH proton occurring at $\tau$ 0.16 and the aromatic indole protons were found between $\tau$ 2.54-3.18. The four methylene protons exhibited a multiplet centred at $\tau$ 7.05 while the methyl group occurred as a doublet at $\tau$ 8.60 ($J = 7$ Hz) and was coupled to the methine proton which appeared as a broadened quartet at $\tau$ 5.88 ($J = 7$ Hz). The mass spectrum exhibited a molecular ion peak at m/e 186.

The synthetic eleagnine (5) was formed via a Bischler-Napieralski reaction as shown in the scheme outlined below. This scheme is identical to McFarlane and Slaytor's hypothesis concerning the biosynthesis of the 1-methyl-β-carboline alkaloids.
Treatment of tryptamine (7) with excess acetic anhydride produced (9) as a brown crystalline material. The crude product was normally used as such for the next reaction, however, for analytical purposes it was purified by chromatography on alumina. The spectral data compared favourably with the assigned structure (9). The IR spectrum showed the amide carbonyl at 1660 cm\(^{-1}\) while in the NMR spectrum the methyl protons of the acetyl group were found at \(\tau\) 8.14. The mass spectrum indicated a molecular ion peak at m/e 202, which is in agreement with the molecular formula \(C_{12}H_{14}ON_2\) as found by elemental analysis.

Harmalan (6) was prepared by cyclizing (9) under Bischler-Napieralski conditions.\(^{14}\) \(N_b\)-Acetyltryptamine (9) was refluxed in dry toluene and phosphorus oxychloride for 1 1/2 hours. The crude product was purified by column chromatography on alumina and the resulting harmalan (6)
was recrystallized as the hydrochloride salt, mp 236-239° (lit. 7 mp 243-5°). The spectral data was consistent with the proposed formulation. The ir spectrum showed no amide carbonyl while the uv spectrum exhibited a pattern which was not characteristic of the indole chromophore. The nmr spectrum showed the methyl protons occurring as a singlet at \( \tau 7.61 \). The mass spectrum suggested a molecular ion peak at \( m/e 184 \), which agreed with the molecular formula \( C_{12}H_{13}N_{2}Cl \) obtained by elemental analysis.

Reduction of the 1,2 double bond of harmalan (6) gave eleagnine (5), mp 178-180° (lit. 1 mp 178-180°) in 33% overall yield. Comparison of the spectral data with that of authentic material confirmed the structure while the nmr spectrum showed the methyl protons as a doublet \((J = 7 \text{ Hz})\) at \( \tau 5.60 \) and the methine proton occurred as a broad quartet at \( \tau 5.89 \) \((J = 7 \text{ Hz})\). The mass spectrum indicated a molecular ion at \( m/e 136 \) and the molecular formula \( C_{12}H_{14}N_{2} \) was confirmed by elemental analysis.

The \( N_{b} \)-acetyl derivative of eleagnine (5) was prepared and recrystallized from ethanol to provide white needles, mp 206-206.5°. The spectral data were in accord with the proposed formulation. The ir spectrum showed the amide carbonyl at 1610 cm\(^{-1}\). However the nmr spectrum was quite complex. The acetyl protons occurred at \( \tau 7.86 \) and the methyl protons appeared as a pair of doublets, one pair \((\tau 8.43, J = 7 \text{ Hz})\) being one-third of the intensity of the other \((\tau 8.56, J = 7 \text{ Hz})\). A pair of quartets \((\tau 4.35, J = 7 \text{ Hz}; \tau 4.80, J = 7 \text{ Hz})\) were assigned to the methine proton. Decoupling experiments showed that the doublet at \( \tau 8.56 \) was coupled to the quartet at \( \tau 4.35 \) and the doublet at \( \tau 8.43 \) to the quartet at \( \tau 4.80 \). The methylene protons
adjacent to the N\textsubscript{b}-acetyl group exhibited a complex multiplet (\(\tau\) 5.06-6.78) while the \(\beta\)-methyleneindole protons occurred as a multiplet (\(\tau\) 7.30) (Figure 2). At 160\(^\circ\) both of the methyl and methine resonances had merged to give a single doublet (\(\tau\) 8.53, \(J = 7\) Hz), and a single quartet (\(\tau\) 4.52, \(J = 7\) Hz) respectively. The complex multiplet assigned to the methylene protons adjacent to the N\textsubscript{b}-acetyl group was simplified (\(\tau\) 5.60-6.90) (Figure 3). This evidence suggests that N\textsubscript{b}-acetylleaginine exists as two rotational isomers due to restricted rotation about the CO-N bond. In the mass spectrum a molecular ion peak occurred at m/e 228 and the molecular formula \(C_{14}H_{17}ON_2\) was confirmed by elemental analysis.

Now that a suitable 1-methyl-\(\beta\)-carbolinem alkaloid had been identified as occurring in \textit{E. angustifolia} the next step in the study of the biosynthesis of these compounds was to synthesize possible precursors. The intermediates in the original proposal of Slaytor and McFarlane for the biosynthesis of eleagnine (5) and harman (4)\(^7\) have already been prepared. However, Stolle and Gröger have proposed that 1,2,3,4-tetrahydroharmine-1-carboxylic acid (11) is a precursor of harmine (1)\(^9\) and the analogous amino acid in the biosynthesis of eleagnine (5) would be eleagnine-1-carboxylic acid (19) as outlined below.
Thus eleagnine-1-carboxylic acid (19) was synthesized by dissolving tryptamine hydrochloride (7) and pyruvic acid in distilled water and adjusting the pH to 4.0. After allowing the reaction mixture to stand for 48 hours, (19) the product crystallized as brown flakes, mp 219-221° dec. The spectral data were consistent with the proposed structure. The presence of a carboxylic acid functionality was demonstrated in the IR spectrum by a broad band between 2300 cm\(^{-1}\) and 3040 cm\(^{-1}\) (OH stretching) and the carbonyl stretching mode at 1620 cm\(^{-1}\). The UV spectrum exhibited the typical indole chromophore while in the NMR spectrum the methyl protons occurred as a singlet at \(\tau\) 8.4. The mass spectrum showed a molecular ion peak at m/e 230 which underwent apparent loss of carbon dioxide to give a base peak at m/e 186. The molecular formula \(\text{C}_{13}\text{H}_{14}\text{O}_{2}\text{N}_{2}\) was confirmed by high resolution mass spectrometry (Found: 230.1054;
Calcd.: 230.1045) and elemental analysis.

Radioactive labels were required for each of the possible precursors of eleagnine (5) as it was decided to use tracer methods as an initial assay procedure for the enzymes.

Tryptamine (7) was tritiated in the aromatic indole ring according to the procedure described in Part A for the tritiation of the ester (see page 58). The resultant [ar-3H]-tryptamine (7) was then utilized in the syntheses of appropriately tritiated compounds.

Introduction of a carbon-14 label into N_b-acetyltryptamine (9) was achieved by reacting tryptamine (7) with one mole of [1-14C]-acetic anhydride in a closed system. After 15 minutes one mole of cold acetic anhydride was carefully distilled into the reaction mixture. The resulting [14COCH_3]-N_b-acetyltryptamine (7) was purified as before and this material was cyclized to form [1-14C]-harmalan (6) in the manner previously described.

Section B

Before any enzyme assay experiments were performed it was necessary to confirm that the biosynthesis of eleagnine (5) was occurring on a regular and measurable basis in E. angustifolia.

This experiment was carried out by feeding young shoots of E. angustifolia with [3-14C]-tryptophan (13). The radioactive compound was introduced into the shoots by the hydroponic feeding method and the shoots were allowed to grow for three days at which time eleagnine (5) was isolated. The results for experiment 1 are displayed in Table 1, and it may be seen that eleagnine (5) incorporated tryptophan (8)
to an extent of 0.035%. Thus our plant system was biosynthesizing eleagnine (5) and the next experiments involved feeding \([\text{ar}-^3\text{H}]-\text{N}_b^-\text{acetyltryptamine} \) (4) (experiment 2) and \([\text{ar}-^3\text{H}, ^1\text{H}^{14}\text{COCH}_3]-\text{N}_b^-\text{acetyltryptamine} \) (9) (experiment 3). The information from Table 1 shows that this material is incorporated into \textit{E. angustifolia} almost as efficiently as tryptophan (8). Thus it was presumed that \text{N}_b^-\text{acetyltryptamine} (9) was the precursor for eleagnine (5) as McFarlane and Slaytor\(^7\) had proposed and our first enzyme assays utilized this compound.

Table 1. The Results of the Incorporation of Tryptophan (13) and \text{N}_b^-\text{Acetyltryptamine} (9) into \textit{E. angustifolia}.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>Activity fed (dpm)</th>
<th>% Incorporation</th>
<th>Ratio fed</th>
<th>Ratio isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>([3-^1\text{C}])-Tryptophan (13)</td>
<td>(3.23\times10^7)</td>
<td>-</td>
<td>0.035</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>([\text{ar}-^3\text{H}]-\text{N}_b^-\text{Acetyltryptamine} ) (9)</td>
<td>-</td>
<td>(5.51\times10^8)</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>([\text{ar}-^3\text{H}, ^1\text{H}^{14}\text{COCH}_3]-\text{N}_b^-\text{Acetyltryptamine} ) (9)</td>
<td>(1.85\times10^7) (2.00\times10^8)</td>
<td>0.021 0.017</td>
<td>11.05 8.98</td>
<td>-</td>
</tr>
</tbody>
</table>
As our plant material was available only in the spring it was decided to prepare acetone powders so it would be possible to assay the year around. Young shoots of *E. angustifolia* were macerated with acetone in a Waring blender at -15° and after filtration the residues were washed thoroughly with cold acetone. The resulting acetone powder was stored at 0° in a sealed container. Assays were carried out on a soluble protein preparation utilizing the following procedure. The acetone powder suspended in a phosphate buffer was stirred at 0° in the presence of polyvinylpyrrolidone, glutathione (reduced) and sodium isoascorbate for 1 hour. The mixture was pressed through cheese cloth and the filtrate centrifuged at 10,000 g for 10 minutes. Protamine sulphate was added to the supernatant which was stirred for 10 minutes at 0° and then centrifuged at 20,000 g for 10 minutes (for one assay with \( \text{N}_b \)-acetyltryptamine (9) this step was left out). The pH of the mixture remained at 7.0. The supernatant was then incubated with the appropriate labelled precursor at 37° for 1 hour in the presence of the following cofactors, ATP, magnesium chloride, KADH, NADPH and FAD. Sodium hydroxide was used to quench the reaction and after dilution with eleagnine (5), this alkaloid was isolated and counted to constant activity.

In this manner no activity was detected when \(^{14}\text{COCH}_3\text{-N}_b\)-acetyltryptamine (9), [\( \text{ar-}^3\text{H}\)]-harmalan (6) and [\( \text{ar-}^3\text{H}\)]-eleagnine-1-carboxylic acid (19) were used as precursors.

It was decided that our acetone powders might not contain the active enzyme so a source of plant material was obtained with which it was possible to obtain young shoots throughout the year. The first experiment involved incubation of \( \text{N}_b \)-acetyltryptamine (9) and the
preceding cofactors with a homogeniate of the young shoots (prepared in a Virtus blender at 0°) at 37° for 1 hour. Again no activity was detected in the isolated eleagnine (5).

The lack of activity could be due to a variety of factors, two of which might be (a) utilization of the incorrect precursors or (b) low molecular weight substances causing enzyme inhibition. To overcome each of these problems it was decided to isolate a soluble protein fraction from the homogenate of plant material and to assay this fraction, using four possible precursors, for eleagnine (5). N-b-acetyltryptamine (9) and harmalan (6) have already been mentioned, as has eleagnine-l-carboxylic acid (19) as intermediates in biosynthetic proposals by McFarlane and Slaytor, and Stolle and Groger respectively. Stolle and Groger reported the specific incorporation of pyruvate in P. harmala into harmine (1). Three pathways with the intermediacy of pyruvate could be (a) decarboxylation to acetate and the incorporation of a C2 unit such as acetyl coenzyme A, (b) the direct reaction of pyruvate with tryptamine (7) to give eleagnine-l-carboxylic acid (19), and (c) the interaction of the cofactor thiamine pyrophosphate with pyruvate to produce "active acetaldehyde" which may then undergo a Pictet Spengler type of reaction to give eleagnine (5) as proposed by Perkin and Robinson.5

Young shoots of E. angustifolia were frozen in liquid nitrogen, ground to a powder and suspended in a borate buffer (pH = 7) containing mercaptoethanol, glutathione, and sodium isoascorbate for 1/2 hour at 0°. The mixture was pressed through cheese cloth and centrifuged at 30,000 g. The supernatant was brought to 70% saturation
with ammonium sulfate to precipitate the protein and centrifuged at 10,000 g. The pellet was redissolved in buffer and flushed through a Sephadex G25 column. The concentrated protein solution was divided into four equal volumes and each was assayed for 1 hour at 30°. Each assay contained the cofactors ATP, magnesium chloride, NADH, NADPH, FAD. Assay 1 contained [ar-3H]-N-acetyltryptamine (9) as the substrate and assay 2, [ar-3H]-harmalan (6). Assay 3 involved pyridoxal phosphate as an additional cofactor and eleagnine-1-carboxylic acid (19) as the precursor. Assay 4 utilized sodium pyruvate and thiamine pyrophosphate and after incubation for 30 minutes [ar-3H]-tryptamine (7) was added as the substrate. The eleagnine (5) isolated from each assay was found to be essentially inactive.

As little success was achieved in this approach to isolate an enzyme, i.e. the extraction of a soluble protein fraction and assaying for activity, it was considered that a more thorough examination would be required.

Tryptophan (13) would be fed to a crude homogenate of E. angustifolia. If activity was detected this homogenate would be centrifuged and both the pellet and the supernatant assayed. It has been assumed that the enzymes that hold our interest have been soluble in the buffer, however, it is possible that these proteins are membrane bound and are to be found in the pellet.

This approach is now being carried out in our laboratories.
EXPERIMENTAL

For general information refer to page 97.

Section A

Isolation of Eleagnine (5) from *E. angusifolia*

The plant material (young shoots and leaves, 500 g fresh plant) was macerated with methanol in a Waring blender, filtered and remacerated until the filtrate was colourless. The solvent was removed under reduced pressure and the residue was taken up in 2 M hydrochloric acid (300 ml). The aqueous phase was washed with chloroform (3 x 150 ml) and the combined chloroform washings were extracted with 2 M hydrochloric acid (100 ml). The combined aqueous extracts were cooled in ice, basified with 15 M aqueous ammonia and extracted with chloroform (3 x 150 ml). The combined chloroform extracts were washed with water (100 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure.

Eleagnine (5) (10 mg) was isolated from the crude extract utilizing preparative tlc employing alumina as the absorbant and chloroform-methanol (95:5) as the eluting solvent (R$_f$ 0.3). The free base was recrystallized from ethyl acetate, mp 178-179° (lit. mp 178-180°). The spectral data were identical with that of synthetic
eleagnine (5). The two ir were superimposable; $\nu_{\text{max}}$: 3280 (NH), 2920 (CH), 1445 (CH$_2$) cm$^{-1}$; $\lambda_{\text{max}}$ (log $\varepsilon$): 222 (4.54), 274 (3.86), 280 (3.87), 289 (3.79) mp; nmr (CD$_3$COCD$_3$): $\tau$ 0.16 (1H, singlet, NH), 2.54-3.18 (4H, multiplet, indole protons), 5.88 (1H, broad quartet, $J = 7$ Hz, methine proton), 7.05 (4H, multiplet, methylene protons), 8.60 (3H, doublet, $J = 7$ Hz, methyl protons); ms: m/e 186 (M$^+$), 185, 171, 157, 156.

$N_b$-Acetyltryptamine (9)

Tryptamine (7) (300 mg, 1.88 mmol) was dissolved in acetic anhydride (2 ml) containing a catalytic amount of sodium acetate (1 mg) and maintained at 80° for 3 min. Removal of the acetic anhydride under reduced pressure produced a brown crystalline material, which was dissolved in chloroform and washed with 2 M hydrochloric acid, followed by water. The chloroform extract was dried over anhydrous sodium sulphate and the solvent removed under reduced pressure. Purification was achieved by chromatography on alumina (20 g) and elution with chloroform yielded $N_b$-acetyltryptamine (9) (379 mg, 95%), mp 73-74.5°; $\nu_{\text{CHCl}_3}$: 3480, 3450 (NH stretching frequency), 1665 (amide carbonyl stretching mode) cm$^{-1}$; $\lambda_{\text{max}}$ (log $\varepsilon$): 222 (4.54), 275 (3.74), 281 (3.77), 290 (3.71) mp; nmr (CD$_3$COCD$_3$): $\tau$ 2.32-3.12 (4H, multiplet, indole protons), 6.50 (2H, broad triplet, $J = 8$ Hz, $-\text{CH}_2$-NH-,), 7.08 (2H, broad triplet, $J = 8$ Hz, $\beta$-indole methylene protons), 8.14 (3H, singlet, acetyl protons); ms: m/e 202 (M$^+$), 143, 130.

Anal. Calcd. for C$_{12}$H$_{14}$ON$_2$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.19; H, 6.96; N, 13.59.
N\textsubscript{b}-Acetyltryptamine (9) (306 mg, 1.51 mmol) was refluxed in dry toluene (20 ml) and phosphorus oxychloride (3 ml) for 1 1/2 hr. The toluene was decanted and the residue taken up in a minimum volume of methanol and ice water (200 ml) was added. After acidifying with concentrated hydrochloric acid (10 ml), the aqueous phase was washed with ether, made basic with 15 M aqueous ammonia and extracted with ether. The combined ether extracts were dried over anhydrous sodium sulphate and the solvent was evaporated. The crude product was purified by column chromatography on alumina (20 g) and elution with chloroform produced harmalan (6) which was recrystallized as the hydrochloride salt from methanol-ether, mp 236-239° (lit.\textsuperscript{7} mp 243-245°) (147 mg, 53%); \(\nu_{\text{max}}\) (free base): 2930 (C=O) cm\textsuperscript{-1}; \(\lambda_{\text{max}}\) (log \(\varepsilon\)) (free base): 228 (4.15), 235 (4.16), 240 (4.15), 317 (4.13), 346 sh. mu; nmr (free base): \(\tau\) -0.07 (1H, broad singlet, NH), 2.30-3.10 (4H, multiplet, indole protons), 6.10 (2H, broad triplet, \(J = 8\) Hz, \(-\text{CH}_2-N=C^\parallel\)), 7.15 (2H, broad triplet, \(J = 8\) Hz, \(\beta\)-indole methylene protons), 7.61 (3H, singlet, methyl protons); ms: m/e 184 (M\textsuperscript{+}), 183.

Anal. Calcd. for C\textsubscript{12}H\textsubscript{12}N\textsubscript{2}HCl: C, 65.31; H, 5.94; N, 12.70.

Found: C, 65.21; H, 5.70; N, 12.69.

Eleagnine (5)\textsuperscript{7}

Harmalan (6) (108 mg, 0.587 mmol) was dissolved in glacial acetic acid (30 ml) and platinum oxide (60 mg) was added to the solution. The suspension was hydrogenated at 95 psi on a Parr hydrogenator for 3 hr. The mixture was filtered and the acetic acid evaporated under reduced
pressure. The residue was dissolved in a minimum volume of methanol, diluted with water, basified with 15 M aqueous ammonia and extracted with benzene. The combined benzene extracts were dried over anhydrous sodium sulphate and the solvent evaporated. The crude crystalline product was further purified by chromatography on alumina (10 g). Elution with chloroform and recrystallization from ethyl acetate produced eleagnine (5), mp 178-180° (lit. 178-180°) (71 mg, 65%); ν max: 3280 (NH), 2920 (CH), 1445 (CH₂) cm⁻¹; λ max (log ε): 222 (4.54), 274 (3.86), 280 (3.87), 289 (3.79) μ; nmr (CD₃COCD₃): τ 0.16 (1H, singlet, NH), 2.54-3.16 (4H, multiplet, indole protons), 5.89 (1H, broad quartet, J = 7 Hz, methine proton), 7.05 (4H, multiplet, methylene protons), 8.60 (3H, doublet, J = 7 Hz, methyl protons); ms: m/e 186 (M⁺), 185, 171, 157, 156.


N₂-acetyeleagnine

Eleagnine (5) (26 mg, 0.14 mmol) was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) was added. The mixture was stood at room temperature for 16 hr at which time the solvent was evaporated under reduced pressure. Recrystallization of the crude product from ethanol produced N₂-acetyeleagnine as white needles, mp 206-206.5° (31 mg, 98%); ν max: 1610 (amide carbonyl) cm⁻¹; λ max (log ε): 224 (4.62), 276 (3.89), 282 (3.89), 289 (3.79) μ; nmr (DMSO, 41°): τ 2.46-3.08 (4H, multiplet, indole protons), 4.35 (0.75H, quartet, J = 7 Hz, methine proton, coupled to the doublet at τ 8.56), 4.80 (0.25H, quartet,
J = 7 Hz, methine proton, coupled to the doublet at τ 8.43), 5.13, 5.26, 5.89, 6.03, 6.56 (2H, complex multiplet, \(-\text{CH}_2\text{-N}^\cdot\)), 7.30 (2H, multiplet, \(\beta\)-methyleneindole protons), 7.86 (3H, singlet, acetyl protons), 8.43 (0.75H, doublet, \(J = 7\) Hz, methyl protons, coupled to the quartet at τ 4.80), 8.56 (2.25H, doublet, \(J = 7\) Hz, methyl protons, coupled to the quartet at τ 4.35); nmr (DMSO, 160°): τ 2.52-3.12 (4H, multiplet, indole protons), 4.52 (1H, quartet, \(J = 7\) Hz, methine proton), 5.67, 5.80, 6.74 (2H, multiplet, \(-\text{CH}_2\text{-N}^\cdot\)), 7.28 (2H, multiplet, \(\beta\)-methyleneindole protons), 7.90 (3H, singlet, acetyl protons), 8.53 (3H, doublet, \(J = 7\) Hz, methyl protons); ms: \(m/e\) 228 (M\(^+\)), 213, 185, 171, 157, 156.

Anal. Calcd. for C\(_{14}\)H\(_{17}\)O\(_2\): C, 73.65; H, 7.06; N, 12.27. Found: C, 73.89; H, 7.13; N, 11.96.

Eleagnine-1-carboxylic Acid (19)

Tryptamine hydrochloride (7) (500 mg, 2.55 mmol) was dissolved in distilled water (30 ml) and pyruvic acid (245 mg, 2.79 mmol) was added. The pH was adjusted to 4.0 with aqueous ammonia and the mixture left at room temperature for 48 hr. Eleagnine-1-carboxylic acid (19) was collected by filtration as brown flakes mp 219-221° dec. (523 mg, 89%); \(v\) \(_\text{max}\): 3390 (NH), 3040-2300 (hydrogen bonded carboxylate), 1620, 1565 (carboxylate anion) \text{cm}^{-1}; \(\lambda\) \(_{\text{max}}\) \(2\) \text{M NH}_4\text{OH} (log ε): 225 (4.46), 275 (3.85), 280 (3.87), 289 (3.82) \text{nu}; nmr (NaOD/D\(_2\)O): τ 2.38-3.02 (4H, multiplet, indole protons), 7.11 (4H, multiplet, methylene protons), 8.43 (3H, singlet, methyl protons); ms: \(m/e\) 230 (M\(^+\)), 215, 186, 185, 171, 157, 156, 144, 130; high resolution mass spectrometry: Calcd. for C\(_{13}\)H\(_{14}\)O\(_2\)N\(_2\): 230.1054. Found: 230.1045.
Anal. Calcd. for \( \text{C}_{13}\text{H}_{14}\text{O}_2\text{N}_2\cdot\text{H}_2\text{O} \): C, 62.89; H, 6.49; N, 11.29.
Found: C, 62.58; H, 6.53; N, 11.18.

**Tritiated Trifluoroacetic Acid**

Tritiated water (0.125 g, 6.94 mmol, 1 curie/g) was combined with trifluoroacetic anhydride (1.45 g, 6.94 mmol) utilizing a vacuum transfer system. The resultant tritiated acid (1.57 g, 9 mcurie/mmol) was kept in a sealed vial under dry nitrogen at -10° until required.

**[ar-\(^3\)H]-Tryptamine (7)**

Tryptamine hydrochloride (7) (287 mg, 1.46 mmol) was combined with tritiated trifluoroacetic acid (1.57 g, 13.88 mmol, 9 mcurie/mmol) by means of a vacuum transfer system. The acid solution was maintained under a dry nitrogen atmosphere at room temperature for 48 hr. The tritiated trifluoroacetic acid was then removed by molecular distillation and the product was taken up in methanol (5 ml) and the solvent removed under reduced pressure. More methanol (5 ml) was added and the process was repeated (4 x) to remove any readily exchangeable tritium atoms in the molecule. The tryptamine hydrochloride (7) was then taken up in water, basified with 15 M aqueous ammonia and extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulphate and the solvent evaporated. The [ar-\(^3\)H]-tryptamine (7) (279 mg, 97%, \(3.92 \times 10^{10}\) dpm/mmol) was used as such for the synthesis of [ar-\(^3\)H]-\(\text{N}_b\)-acetyltryptamine (9) and [ar-\(^3\)H]-eleagnine-1-carboxylic acid (19).
[ar-\(^3\)H]-N\(_b\)-Acetyltryptamine (9), [ar-\(^3\)H]-harmalan (6), and [ar-\(^3\)H]-eleagnine-1-carboxylic acid (19) were subsequently obtained following the normal procedure.

\[ ^{14}\text{COCH}_3\text{-N}_b\text{-Acetyltryptamine (9)} \]

Tryptamine (7) (33.5 mg, 0.21 mmol) was combined with \([1-{\text{\textsuperscript{14}}C}]\)-acetic anhydride (25.8 \(\mu\)l, 0.27 mmol, 0.25 mcurie) by means of a vacuum transfer system. The mixture was warmed to 50° and held at that temperature for 1/2 hr, at which time another mole of acetic anhydride (20 \(\mu\)l, 0.21 mmol) was distilled into the reaction mixture. After 1/2 hr at 50° the excess acetic anhydride was removed by molecular distillation and the resulting oil was taken up in aqueous sodium carbonate and chloroform. The aqueous phase was extracted with chloroform and the chloroform extracts combined, dried over anhydrous sodium sulphate and the solvent evaporated. After column chromatography on alumina (2 g), \([^{14}\text{COCH}_3\text{-N}_b\text{-Acetyltryptamine (9)}\) (37.3 mg, 88.5\%, 7.75 \(10^8\) dpm:mmol) was obtained pure.

The synthesis of \([1-{\text{\textsuperscript{14}}C}]\)-harmalan (6) was achieved in the normal manner.

Section B

The Administration of Labelled Compounds to \(E.\) angustifolia

The compound to be fed was dissolved in ethanol (1 ml) by means of an ultrasonic cleaner and 0.1 M acetic acid (usually 0.5 ml) was added. This solution was administered to young cut shoots of \(E.\) angustifolia by the hydroponic feeding method. The solution was distributed among ten test tubes (10 x 70 mm) and into each of these test tubes was
placed a freshly cut shoot of *E. angustifolia*. The test tubes were placed in a rack and the shoots were exposed to fluorescent illumination. The original container of the labelled compound was rinsed with a minimum volume of ethanol and this solution was used to refill the feeder tubes after the initial precursor solution had been absorbed by the stems. The solution of labelled compound and the washings were absorbed by *E. angustifolia* within a 2 hr period. The ends of the shoots were then covered with distilled water for the duration of the experiment. *E. angustifolia* feedings were carried out for 72 hr. At the completion of the experiment the ten test tubes used to incorporate the labelled compound and the original container for this compound were washed with methanol (100 ml) and the radioactivity of this solution was determined. The residual activity was then subtracted from the activity estimated for the labelled compound. The residual activity normally amounted to about 1% of that fed.

**Extraction of Alkaloids from *E. angustifolia***

The procedure utilized for the isolation of eleagnine (5) was adopted for all precursor feedings and enzyme assays. This procedure was scaled down according to the wet weight of plant used for the feedings or the dry weight of assay residue. For further purification of the crystalline eleagnine (5), the free base was converted to its N-acetyl derivative and crystallized from ethanol.
Feeding Experiments 1, 2, and 3

The precursors for experiments 1, 2, and 3 were administered and the eleagnine (5) isolated as described above. The specific details of these experiments are listed in Tables 2 and 3. Eleagnine (5) was added to the plant material before extraction as a carrier for *E. angustifolia* contained only small amounts of this alkaloid.

Preparation of Acetone Powders from *E. angustifolia*

The young shoots of a mature Russian olive (*E. angustifolia*) (20 g) were macerated in a Virtis blender with acetone (150 ml) at -40° in the presence of 1% polyethylene glycol 4000 (1.5 g) and 0.1% mercaptoethanol in acetone (10 ml). The resulting suspension was filtered and washed thoroughly with cold acetone (-20°). The procedure was repeated on the residue and the light green powder was dried in vacuo. The acetone powder was stored at 0° in a sealed container.

Preparation of a Soluble Protein Extract from the Acetone Powder

Acetone powder (6 g) was stirred at 0° with 0.04 M phosphate buffer, pH 7.0 (150 ml), in the presence of polyvinylpyrrolidone (6 g), glutathione (reduced) (305 mg) and sodium isoascorbate (200 mg) for 1 hr. The mixture was pressed through cheese cloth and the filtrate was centrifuged at 10,000 g for 10 min. To the supernatant was added 2% protamine sulphate in 0.04 M phosphate buffer pH 7 (1 ml) and the mixture was stirred for 10 min at 0° and centrifuged at 20,000 g for 10 min. The pH remained at 7.0.
Table 2. The Feeding of Labelled Compounds into *E. angustifolia*.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Compound fed</th>
<th>Weight fed (mg)</th>
<th>Activity fed (dpm)</th>
<th>Specific activity fed (dpm/mmol)</th>
<th>Ratio fed</th>
<th>Wet weight plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>[3-(^{14}\text{C})]-Tryptophan (8)</td>
<td>1.2</td>
<td>3.23 (10^7)</td>
<td>-</td>
<td>-</td>
<td>63.5</td>
</tr>
<tr>
<td></td>
<td>[ar-(^{3}\text{H})]-N(_p)-Acetyltryptamine (7)</td>
<td>-</td>
<td>2.9</td>
<td>5.51 (10^8)</td>
<td>-</td>
<td>108.5</td>
</tr>
<tr>
<td>3</td>
<td>[ar-(^{3}\text{H},^{14}\text{COCH}_{3})]-N(_p)-Acetyltryptamine (7)</td>
<td>7.0</td>
<td>1.7</td>
<td>1.85 (10^7)</td>
<td>7.65 (10^8)</td>
<td>3.92 (10^{10})</td>
</tr>
</tbody>
</table>
Table 3. The Feeding of Labelled Compounds into E. angustifolia.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Weight oleagine (5) added (mg)</th>
<th>% Incorporation 14C</th>
<th>3H</th>
<th>3H/14C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.4</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>34.0</td>
<td>-</td>
<td>0.003</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>35.0</td>
<td>0.021</td>
<td>0.017</td>
<td>8.98</td>
</tr>
</tbody>
</table>
Assay of a Homogenate of *E. angustifolia*

Young shoots of *E. angustifolia* (19 g) were macerated in a Virtis blender at 0° with 0.04 M phosphate buffer pH 7 (150 ml) and polyvinylpyrrolidone (8 g), glutathione (reduced) (305 mg) and sodium isoascorbate (200 mg). The suspension was pressed through cheese cloth and the filtrate was incubated with [14COCH₃]-N₆-acetyltryptamine (9) (27.4 mg, 1.06 \(10^7\) dpm), ATP (2.0 mg, 4 \(\mu\)mol), magnesium chloride (0.1 mg, 0.5 \(\mu\)mol), FAD (3.0 mg, 4 \(\mu\)mol), NADPH (2.5 mg, 4 \(\mu\)mol) and NADH (2.0 mg, 4 \(\mu\)mol) at 37° for 1 hr. The assay was quenched with 10 M sodium hydroxide (10 ml) and eleagnine (5) (12.5 mg) was added. After freeze drying, the eleagnine (5) was isolated and counted to constant activity. \(N_6\)-Acetyltryptamine (9) was incorporated to an extent of less than 0.002%.
Table 4. The Assay of a Soluble Protein Extract of an Acetone Powder of *E. angustifolia*.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Weight of eleagnine added (mg)</th>
<th>Weight of substrate fed (mg)</th>
<th>Activity fed (dpm)</th>
<th>% Incorporation</th>
<th>Ratio fed</th>
<th>Ratio isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>14_C</em></td>
<td><em>3_H</em></td>
<td><em>14_C</em></td>
<td><em>3_H</em></td>
<td><em>14_C</em></td>
<td><em>3_H</em></td>
</tr>
<tr>
<td>[ar-^3_H,^14_COCH_3]-N_b-Acetyltryptamine (9)</td>
<td>41.6</td>
<td>4.8</td>
<td>1.0</td>
<td>2.12 (10^7)</td>
<td>2.58 (10^8)</td>
<td>0.002</td>
</tr>
<tr>
<td>[^14_COCH_3]-N_b-Acetyltryptamine (9)</td>
<td>33.4</td>
<td>0.47</td>
<td>-</td>
<td>2.08 (10^7)</td>
<td>-</td>
<td>0.007</td>
</tr>
<tr>
<td>[ar-^3_H]-harmalan (6)</td>
<td>37.0</td>
<td>-</td>
<td>2.1</td>
<td>-</td>
<td>7.32 (10^7)</td>
<td>-</td>
</tr>
<tr>
<td>[ar-^3_H]-Eleagnine-l-carboxylic acid (19)</td>
<td>49.7</td>
<td>-</td>
<td>23.9</td>
<td>-</td>
<td>1.60 (10^8)</td>
<td>-</td>
</tr>
<tr>
<td>Substrate</td>
<td>Weight of eleagnine added (mg)</td>
<td>Weight of substrate fed (mg)</td>
<td>Activity fed (dpm)</td>
<td>% Incorporation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>-----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$COCH$_3$-N$_b$-Acetyltryptamine (9)</td>
<td>30.5</td>
<td>0.44</td>
<td>$1.95 \times 10^7$</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{3}$H]-Harmalan (6)</td>
<td>32.5</td>
<td>2.2</td>
<td>$7.6 \times 10^7$</td>
<td>&lt; 0.035</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Blank Experiments Run Concurrently with the Experiments in Table 4.
Preparation of a Purified Soluble Protein Extract

The young shoots of a mature Russian olive (30 g) were frozen in liquid nitrogen and ground to a fine light green powder. The powder was stirred at 0° with 0.2 M borate buffer, pH 7.0 (240 ml) in the presence of glutathione (reduced) (100 mg), sodium isoascorbate (100 mg) and mercaptoethanol (0.05 ml) for 30 min. The mixture was pressed through cheese cloth and the filtrate was centrifuged for 20 min at 30,000 g. The protein was precipitated from the supernatant by adding ammonium sulphate (109.0 g) to 70% saturation. The suspension was then centrifuged for 20 min at 10,000 g. The pellet was redissolved in buffer (3 ml) and was chromatographed on a Sephadex G25 column (void volume 25 ml). The protein extract (28 ml) was divided into four equal volumes and each volume was assayed utilizing a different precursor.

Assay #1, N₅-Acetyltryptamine (9)

The supernatant (7 ml) was incubated with \(^{14}\text{COCH}_3\)-N₅-acetyltryptamine (9) (2.4 mg, 2.4 \(10^7\) dpm) and ATP (2.0 mg, 4 \(\mu\)mol), magnesium chloride (0.1 mg, 0.5 \(\mu\)mol), FAD (3.0 mg, 4 \(\mu\)mol), NADPH (2.5 mg, 4 \(\mu\)mol), and NADH (2.0 mg, 4 \(\mu\)mol) for 1 hr at 30°. Eleagnine (5) (28.8 mg) was added and the reaction mixture was freeze dried. After isolation and counting to constant activity, the eleagnine (5) showed a 0.0006% incorporation.

Assay #2, Harmalan (6)

The supernatant (7 ml) was incubated with \(^3\text{H}\)-harmalan hydrochloride (6) (4.8 mg, 2.1 \(10^7\) dpm) and ATP (2.0 mg, 4 \(\mu\)mol), magnesium
chloride (0.1 mg, 0.5 µmol), FAD (3.0 mg, 4 µmol), NADPH (2.5 mg, 4 µmol), and NADH (2.0 mg, 4 µmol) for 1 1/2 at 30°. Eleagnine (5) (30.9 mg) was added and the reaction mixture was freeze dried. After isolation and counting to constant activity the eleagnine (5) showed a 0.092% incorporation. A blank utilizing buffer (7 ml) instead of supernatant was run concurrently and showed a 0.112% incorporation.

Assay #3, Eleagnine-1-carboxylic Acid (19)

The supernatant (7 ml) was incubated with [ar-^3H]-eleagnine-1-carboxylic acid (19) (3.33 mg, 2.95 10^8 dpm) and pyridoxal phosphate (1.0 mg, 4 µmol), ATP (2.0 mg, 4 µmol), magnesium chloride (0.1 mg, 0.5 µmol), FAD (3.0 mg, 4 µmol), NADPH (2.5 mg, 4 µmol) and NADH (2.0 mg, 4 µmol) for 1 hr at 30°. Eleagnine (5) (16.1 mg) was added and the reaction mixture was freeze dried. After isolation and counting to constant activity the eleagnine (5) showed a 0.0051% incorporation. The appropriate blank experiment showed a 0.009% incorporation.

Assay #4, Tryptamine (7)

The supernatant (7 ml) was incubated with sodium pyruvate (2.3 mg, 22 µmol), thiamine pyrophosphate (1.6 mg, 4 µmol), ATP (2.0 mg, 4 µmol), magnesium chloride (0.1 mg, 0.5 µmol), FAD (3.0 mg, 4 µmol), NADPH (2.5 mg, 4 µmol) and NADH (2.0 mg, 4 µmol) at 30°. After 30 min, [ar-^3H]-tryptamine (7) (2.70 mg, 3.45 10^8 dpm) was added and the assay was incubated for a further 30 min. Eleagnine (5) (23.4 mg) was added and the reaction mixture was freeze dried. After isolation and counting to constant activity the eleagnine (5) showed a 0.000075% incorporation. The appropriate blank experiment was run concurrently.
BIBLIOGRAPHY