STUDIES IN THE FIELDS OF STEROIDS AND ALKALOIDS

BY

WALTER JAMES CRETNEY

B.Sc. Honours, The University of British Columbia, 1963

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
September, 1968
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemistry

The University of British Columbia
Vancouver 8, Canada

Date Sept 27, 1968
Abstract

In Part A of this thesis evidence is presented concerning the location and configuration of the bromine atom in each of the two isomeric monobromo derivatives (8a and 9a) of 5α,25R-spirostan (7, desoxytigogenin) and in each of the two isomeric monobromo derivatives (8b and 9b) of 3β-acetoxy-5α,25R-spirostan (tigogenin acetate) prepared by the action of bromine in acetic acid on the parent compounds. From a study of the mass spectra and nuclear magnetic resonance spectra obtained for the monobromotigogenins, it was established that the bromine atom was located at the C-23 site. In addition, the configuration of the bromine atom in each of the compounds studied was determined.

In Part B of this thesis the syntheses of several derivatives of 4β-dihydrocleavamine (116) having a substituent at the C-18 site are described. The method employed an apparent SN₂ displacement of chloride ion from the α-methyleneindoline form (118, R=H) of the chloroindolenine (113) of 4β-dihydrocleavamine. The chloroindolenine was prepared by the action of tert-butyl hypochlorite on 4β-dihydrocleavamine and was allowed to react with several nucleophiles under a variety of conditions. Using suitable conditions 18α-methoxy-4β-dihydrocleavamine (140), 18β-methoxy-4β-dihydrocleavamine (141), 18β-hydroxy-4β-dihydrocleavamine (142), and 18β-cyano-4β-dihydrocleavamine (143) were prepared. The last compound was transformed into 18β-carbomethoxy-4β-dihydrocleavamine (139) by unexceptional means. This transformation provided a crucial link in the total syntheses of the Vinca alkaloid, coronaridine (45) and its C-4 epimer dihydrocatharanthine (46).

In Part B of this thesis are also described the syntheses of dimeric compounds. The chloroindolenine of 4β-dihydrocleavamine was allowed to
react with deacetylindoline hydrazide (114) to give a dimer (115). The
coupling of the two units was shown to have taken place between the C-18
site of 4β-dihydrocleavamine and the C-15 site of deacetylindoline hydra-
zide. The dimeric Vinca alkaloids isoleurosine A (110) and vincaleuko-
blastine (as the methiodide salt, 109) are coupled in the same manner.
Isoleurosine A and vincaleukoblastine have in common a carbomethoxy group
at the C-18 site of the dihydrocleavamine portion. The syntheses of two
dimers (147 and 148) are described which also have this feature. The
syntheses were accomplished in the manner of the previous coupling using
the chloroindolenine (117, R=COOMe) of 18β-carbomethoxy-4β-dihydrocleavamine
in place of the chloroindolenine of 4β-dihydrocleavamine.

In Part C of this thesis an effective method for preparing tritium
and deuterium labelled indole alkaloids is described. Tritium labelled
trifluoroacetic acid or trifluoroacetic acid-d was used. A combination of
the methods of mass spectrometry and nuclear magnetic resonance spectro-
scopy was used to establish that the deuterium atoms were located primarily
in the benzene portion of deuterium labelled 18α-carbomethoxy-4α-dihydro-
cleavamine (67) and 18β-carbomethoxydihydrocleavamine (73).

Also in Part C of this thesis are described tracer experiments of a
preliminary nature in *Vinca rosea* L. plants using [22-\(^{14}\text{C}\)]-18β-carbomethoxy-
4β-dihydrocleavamine and [T-aromatic]-18β-carbomethoxydihydrocleavamine and tracer
experiments in *Vinca minor* L. plants using [T-aromatic]-vincadine (74) and
[T-aromatic]-vincaminoreine (75).
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xii</td>
</tr>
</tbody>
</table>

## PART A

I. Introduction ................................................. 2
II. Discussion of Results ................................. 10
III. General Conclusions ....................... 23
IV. Experimental ............................... 27
    References ............................................. 29

## PART B

I. Introduction ................................................. 32
II. Discussion .............................................. 76
III. Experimental ............................... 140
    References ............................................. 165

## PART C

I. Introduction ................................................. 170
II. Discussion .............................................. 198
III. Experimental ............................... 227
    References ............................................. 241
PART A

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The Iso reaction</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Mass spectrum of 23S-bromo-5α,25R-spirostan</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Mass spectrum of 23R-bromo-5α,25R-spirostan</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>High field region of nmr spectra at 60 Mcps</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Low field region of nmr spectra at 60 Mcps</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>Low field region of nmr spectra at 60 Mcps</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>Low field region of nmr spectra at 100 Mcps</td>
<td>22</td>
</tr>
</tbody>
</table>

PART B

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biosynthetic postulate for the production of Aspidosperma and Iboga skeletons</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Transannular cyclization giving the Aspidosperma skeleton</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Transannular cyclization giving the Aspidosperma and Iboga skeletons</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>Equilibria that would result in loss of optical purity</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>Interconversion of tubifolone and condifolone</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>A mechanism for tautomerization of iminium functions across nitrogen</td>
<td>41</td>
</tr>
<tr>
<td>7</td>
<td>Interconversion of veatchine and garryine</td>
<td>41</td>
</tr>
<tr>
<td>8</td>
<td>Kutney's syntheses of dl-quebrachamine, dl-4α-dihydrocleavamine and dl-4β-dihydrocleavamine</td>
<td>46</td>
</tr>
<tr>
<td>9</td>
<td>Stork's synthesis of dl-1,2-dehydroaspidospermidine</td>
<td>48</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>10.</td>
<td>Equilibrium with stereochemical implications and stereo-specific formation of dl-aspidospermine</td>
<td>49</td>
</tr>
<tr>
<td>11.</td>
<td>Stork's synthesis of tricyclic keto amine 62</td>
<td>51</td>
</tr>
<tr>
<td>12.</td>
<td>Ban's synthesis of tricyclic keto amine 63</td>
<td>51</td>
</tr>
<tr>
<td>13.</td>
<td>Kuehne's synthesis of tricyclic keto amines 64 and 65</td>
<td>52</td>
</tr>
<tr>
<td>14.</td>
<td>Harley-Mason's synthesis of dl-aspidospermidine</td>
<td>52</td>
</tr>
<tr>
<td>15.</td>
<td>Buchi's synthesis of dl-ibogamine and dl-epiibogamine</td>
<td>53,54</td>
</tr>
<tr>
<td>16.</td>
<td>Nagata's synthesis of dl-ibogamine</td>
<td>57</td>
</tr>
<tr>
<td>17.</td>
<td>Reaction of bridged aziridinium ions with nucleophiles</td>
<td>58</td>
</tr>
<tr>
<td>18.</td>
<td>Salley's synthesis of dl-ibogamine</td>
<td>59,60</td>
</tr>
<tr>
<td>19.</td>
<td>Taylor's hypothesis concerning an unusual reaction of indolenines</td>
<td>62</td>
</tr>
<tr>
<td>20.</td>
<td>Reactions of a hydroperoxyindoline</td>
<td>63</td>
</tr>
<tr>
<td>21.</td>
<td>Reaction giving substitution adjacent to the α-position of an indole</td>
<td>63</td>
</tr>
<tr>
<td>22.</td>
<td>Reaction giving substitution at the α-position of an indole</td>
<td>64</td>
</tr>
<tr>
<td>23.</td>
<td>Buchi's synthesis of voacangine</td>
<td>64</td>
</tr>
<tr>
<td>24.</td>
<td>Kutney's rationalization for the decarboxylation of catharanthine</td>
<td>67</td>
</tr>
<tr>
<td>25.</td>
<td>Dolby's rationalization for the reduction of 2-indolecarbinol derivatives</td>
<td>68</td>
</tr>
<tr>
<td>26.</td>
<td>Internal return within an ion pair</td>
<td>70</td>
</tr>
<tr>
<td>27.</td>
<td>An unusual rearrangement of the chloroindolenine of ibogaine</td>
<td>71</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Scheme envisaged for the preparation of 18-substituted dihydrocleavamines</td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Mass spectrum of the chloroindolenine of 4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>Nmr spectrum of the chloroindolenine of 4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Conceivable modes of reaction of the chloroindolenine of 4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Spiromidoether formation from a chloroindolenine</td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>An unusual SN₂' reaction involving nucleophilic attack on oxygen</td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>Flow sheet showing the partial separation of the products of the reaction of the chloroindolenine 113 under the Buchi conditions</td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>Mass spectrum of 18α-methoxy-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>36.</td>
<td>Nmr spectrum of 18α-methoxy-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>37.</td>
<td>Mass spectrum of 18β-methoxy-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>38.</td>
<td>Nmr spectrum of 18β-methoxy-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>40.</td>
<td>Nmr spectrum of 18β-hydroxy-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>41.</td>
<td>Mass spectrum of 18β-cyano-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>42.</td>
<td>Nmr spectrum of 18β-cyano-4β-dihydrocleavamine</td>
<td></td>
</tr>
<tr>
<td>43.</td>
<td>Ultraviolet spectrum of the dimer 115</td>
<td></td>
</tr>
<tr>
<td>44.</td>
<td>Mass spectrum of the dimer 115</td>
<td></td>
</tr>
</tbody>
</table>
### PART B continued

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.</td>
<td>Nmr spectrum of the dimer 115</td>
<td>119</td>
</tr>
<tr>
<td>46.</td>
<td>Nmr spectrum of 4β-dihydrocleavamine</td>
<td>121</td>
</tr>
<tr>
<td>47.</td>
<td>Nmr spectrum of deacetylvindoline hydrazide</td>
<td>122</td>
</tr>
<tr>
<td>48.</td>
<td>Nmr spectrum of the chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine</td>
<td>126</td>
</tr>
<tr>
<td>49.</td>
<td>Mass spectrum of the chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine</td>
<td>127</td>
</tr>
<tr>
<td>50.</td>
<td>Mass spectrum of the dimer 147</td>
<td>128</td>
</tr>
<tr>
<td>51.</td>
<td>Nmr spectrum of the dimer 147</td>
<td>130</td>
</tr>
<tr>
<td>52.</td>
<td>Nmr spectrum of 18β-carbomethoxy-4β-dihydrocleavamine</td>
<td>131</td>
</tr>
<tr>
<td>53.</td>
<td>Mass spectrum of the dimer 148</td>
<td>135</td>
</tr>
<tr>
<td>54.</td>
<td>Nmr spectrum of the dimer 148</td>
<td>136</td>
</tr>
</tbody>
</table>

### PART C

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Derivation of Aspidosperma and Iboga types of backbone from the Corynanthe type of backbone</td>
<td>172</td>
</tr>
<tr>
<td>2</td>
<td>Barger-Hahn-Robinson-Woodward hypothesis</td>
<td>174</td>
</tr>
<tr>
<td>3</td>
<td>Pathway from shikimic acid to dihydroxyphenylpyruvic acid incorrectly involving a hydrated prephenic acid intermediate</td>
<td>175</td>
</tr>
<tr>
<td>4</td>
<td>Essential features of hydrated prephenic acid hypothesis</td>
<td>176</td>
</tr>
<tr>
<td>5</td>
<td>Prephenic acid hypothesis</td>
<td>177</td>
</tr>
<tr>
<td>6</td>
<td>Acetate hypothesis for the production of a corynantheine-strychnine type of condensing unit</td>
<td>178</td>
</tr>
<tr>
<td>7</td>
<td>Examples of monoterpenic glucosides having corynantheine-strychnine type of backbone</td>
<td>179</td>
</tr>
</tbody>
</table>
PART C continued

Figure

8. Incorporation of geraniol into alkaloids representing the three structural types of indole alkaloids of the tryptamine + C\textsubscript{9-10} type .................................................. 182

9. Rearrangement in backbone of geraniol to give modification 1 of the C\textsubscript{9-10} condensing unit involving a cyclopentane monoterpenic unit .................................................. 183

10. Incorporation of loganin into various indole alkaloids .... 185

11. Proposal for the pathway from mevalonate to indole alkaloids of the tryptamine + C\textsubscript{9-10} type .................................................. 186

12. A cyclopentane cleavage reaction of possible biosynthetic importance .................................................. 187

13. Plausible pathway to ajmaline and related alkaloids ...... 189

14. Plausible pathway to the Akuamma alkaloids .................. 191

15. Alternative pathway to echitamine and related alkaloids ... 192

16. Final stages in Wenkert's proposal for the biosynthesis of alkaloids with the Aspidosperma and Iboga types of skeleton 193

17. Plausible variation on Wenkert's proposal for the biosynthesis of alkaloids with the Aspidosperma and Iboga types of skeleton .................................................. 194

18. A plausible pathway from stemmadenine to alkaloids with the Aspidosperma and Iboga types of skeleton ..................... 195

19. Plausible pathway to vincamine and related alkaloids and to the alkaloid vallesamidine .................................................. 196

20. Mass spectrum of unlabelled 18α-carbomethoxy-4α-dihydrocleavamine .................................................. 204
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Mass spectrum of deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine</td>
<td>205</td>
</tr>
<tr>
<td>22</td>
<td>Plausible pathway giving a fragment with an m/e value of 210</td>
<td>207</td>
</tr>
<tr>
<td>23</td>
<td>Nmr spectrum of deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine</td>
<td>209</td>
</tr>
<tr>
<td>24</td>
<td>Nmr spectrum of unlabelled 18α-carbomethoxy-4α-dihydrocleavamine</td>
<td>210</td>
</tr>
<tr>
<td>25</td>
<td>Nmr spectrum of deuterium labelled 18β-carbomethoxycleavamine</td>
<td>214</td>
</tr>
<tr>
<td>26</td>
<td>Bag-on-leaf method of feeding</td>
<td>222</td>
</tr>
<tr>
<td>27</td>
<td>Apparatus for small scale labelling experiments</td>
<td>230</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>215</td>
<td></td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Professor J. P. Kutney for being an unfailing source of advice, encouragement, and inspiration during the course of this research.

I am also grateful for having received a National Research Council of Canada Studentship during my studies.
PART A

STRUCTURE AND STEREOCHEMISTRY OF THE MONOBROMOTIGIGENINS
I. Introduction

The problem of conclusively establishing the structure and stereochemistry of bromosapogenins began almost three decades ago when Marker and Rohrmann in the course of their study of the steroidal sapogenin spiroketal system found that sarsasapogenin (1) yielded a monobromo derivative on treatment with bromine in acetic acid. Treatment of this monobromo derivative with sodium in alcohol regenerated sarsasapogenin. Since the bromination occurred readily, Marker rationalised that there must be a potential carbonyl group in sarsasapogenin and thus the bromination experiment provided a valuable clue in the eventual formulation of the spiroketal system. At the time of this work, Marker considered that there were two possible locations for the bromine, at C-20 or at C-23. In a later study, chromic acid oxidation of bromosarsasapogenin acetate provided 3β-acetoxy-16-oxo-5β-pregnane 20S-carboxylic acid (2). On the basis of having obtained this compound, Marker was able to rule out the C-20 site for the bromine leaving the C-23 site as the probable site for the bromine. Subsequently, other workers on the basis of Marker's assignment have assumed that other side-chain-brominated sapogenins have had the bromine at the C-23 site. Furthermore, when in the course of these bromination studies on steroidal sapogenins, isomeric side-chain-brominated sapogenins were isolated, they were rather arbitrarily assigned as 23a and 23b epimers. In fairness to the interested workers, however, they were careful to point out the rather
tenuous nature of their assignments. For instance, Wall and Jones in reporting a study of the side-chain bromination of diosgenin and tigogenin in which they chose the C-23 site for the location of the bromine were moved to record that "although this assignment is logical,..., it must be emphasized that the C-23 assignment is not based on a rigid structure proof." Earlier Mueller and Norton had reported that they were unable to oxidize 23a bromohecogenin acetate and obtain a 12,16-diketo-20-carboxylic acid in analogy with Marker's reported oxidation of bromosarsasapogenin acetate and hence could not obtain direct evidence to exclude the C-20 site as the location of the bromine. To add further confusion to the side-chain-bromination story, Wall and Jones were successful in preparing derivatives of diosgenin and tigogenin acetate that contained two rather than one bromine atom in the side chain in analogy with the work of others. In each case two products were obtained which had the desired extra bromine atom. The major product of these reactions was tentatively assigned the 23,23-dibromo structure, whereas the minor product was considered to have one bromine atom "located in some position other than C-23." By this
time the choice of C-23 as the site of side-chain bromination of steroidal sapogenins was indeed very arbitrary. Finally, work was culminating in an explanation of a seemingly unrelated reaction of the spiroketal side chain. Accompanying this explanation was the necessity to consider C-25 for valid chemical reasons as a possible site of bromination. Ironically, this reaction was first described by Marker and Rohrmann in the same paper in which they described the side-chain bromination of sarsasapogenin. Marker and Rohrmann found that sarsasapogenin on treatment with refluxing alcoholic hydrochloric acid was converted into isosarsasapogenin, which was identical with the naturally occurring smilagenin. These authors assumed that the two compounds differed only in configuration at C-22. Much later, Scheer, Kostic, and Mosettig repeated experiments which were purported to establish that these two compounds (and other similar pairs) were C-22 epimers and found that degradation products of sarsasapogenin and smilagenin in which the C-22 asymmetry had been destroyed were not the same as had been indicated by previous workers. In light of these results they carried out further experiments and showed that sarsasapogenin and smilagenin differed in configuration at C-25 although they were unable to establish whether or not the compounds differed in configuration at C-22. Working with another pair of compounds, neotigogenin and tigogenin, which are related in the same manner as sarsasapogenin and smilagenin, Callow and Massey-Beresford were able to destroy the asymmetry at C-25 without affecting the configuration at C-22 and thereby establish that neotigogenin and tigogenin have the same configuration at C-22. The Iso reaction was therefore shown to bring about an epimerization at C-25 without altering the rest of the molecule. This epimerization at first sight was most unusual and seemingly inexplicable since the position affected was β to an ether linkage and under ordinary
circumstances would be chemically inert to refluxing alcoholic hydrochloric acid. On the basis of the fact that the epimerization took place at all, consideration of the C-25 site as a possible one for bromination would have to be made although the bromination reactions were carried out under milder conditions. In the light, however, of the proposal put forth by Woodward to explain the Iso reaction, it became clear that C-25 as a site of bromination must be seriously considered. Woodward suggested as shown in Figure 1 that the isomerization proceeds through a reversible oxidation-reduction mechanism in which an intermediate C-26 aldehyde is formed which permits epimerization of the C-25 position via its enol form (6). In support of this mechanism Woodward reported that a mixture of 25-epimeric aldehydes, which had been prepared by dichromate oxidation of dihydrotigogenin acetate, on treatment under the usual conditions of the Iso reaction gave a mixture from which tigogenin and neotigogenin could be isolated. This showed that

Figure 1. The Iso reaction
the reaction as proposed would at least proceed in the direction 5 → 3. That the reaction could proceed in the direction 3 → 5 was demonstrated by Djerassi and coworkers who found that the aldehyde intermediate 5 could be trapped as its thioketal derivative when tigogenin acetate was treated with ethane dithiol or propane dithiol. The mechanism proposed by Woodward was, in addition, supported by deuterium exchange experiments carried out by Callow and Massey-Beresford. It was consequently quite apparent that through the intermediation of the enol form 6 of the aldehyde 5 bromination could take place at C-25 and that one or both of the monobromo-derivatives of a steroidal sapogenin could have the bromine atom located at C-25.

When the study of the bromotigogenins reported here was undertaken, it was apparent that although the C-23 site was still the most likely for the location of the bromine atoms, all the possible positions would have to be considered and the methods used would have to distinguish between all possible monobromo isomers if conclusive assignments were to be made. At the outset of the work it seemed that the problem would lend itself nicely to a mass spectrometric and nmr spectroscopic study, in particular the latter. Previously, Barton and coworkers had made an extensive infrared spectroscopic study of monobromosapogenins and were able to produce convincing evidence based on the position of the C-Br sketching frequencies that the so called 23a isomers had an equatorial bromine atom and the 23b isomers had an axial bromine atom. Unfortunately, the method was limited in that it could not establish that C-23 was the location of the bromine atoms. In comparison it was felt that an nmr spectroscopic study coupled with a mass spectrometric study would lead to an unequivocal assignment of the
location as well as the configuration of the bromine atoms in monobrominated steroidal sapogenins. The monobromo derivatives of 5α,25R-spirostan\(^{14}\) (7, desoxytigogenin)\(^6\) and 3β-acetoxy-5α,25R-spirostan (tigogenin acetate) were chosen for the purposes of the study. The study was a collaborative effort with Drs. G. N. Pettit and J. C. Knight at the University of Maine, Orono, Maine, U.S.A. Their work paralleled ours and they were able to supply us with samples of both bromo-isomers of tigogenin acetate for study purposes as well as samples of both bromo-isomers of desoxytigogenin for comparison with those prepared in our laboratory.
II. Discussion of Results

Bromination of desoxytigogenin gave two monobrominated products: one (8a) melting at 193-194°, \([\alpha]_D^{22} -643°\), and another (9a), previously unreported, melting at 215-217° to 225-226° (depending on rate of heating), \([\alpha]_D^{22} -87.4°\). Pure samples of each isomer could be conveniently obtained from the crude reaction mixture by column chromatography on basic (pH 8) silica gel. Nevertheless, the two isomers had very similar retention times and therefore the bulk of the material from any given chromatography still remained as a mixture. In Dr. Pettit's laboratory a much more tedious and time consuming fractionation of the mixture by crystallization had been carried out. This method had eventually brought about complete separation of the mixture. Elemental and mass spectral (Figures 2 and 3) analysis established that both isomers were products of monobromination. The fragmentation patterns of both isomers were very similar. Both exhibited a molecular ion at m/e 480 and of particular importance a fragment at m/e 331, which also appeared in the spectrum of desoxytigogenin. This particular fragment at m/e 331 has been assigned the structure 10. The appearance of the fragment 10 in both monobromodesoxytigogenins precluded C-20 as the location of the bromine atom in either isomer.

Tigogenin acetate was brominated and gave two isomeric bromotigogenin acetates: 8b, mp 206-208°, \([\alpha]_D^{22} -61.9°\) and 9b, mp 201-202°, \([\alpha]_D^{22} -57.0°\),
Figure 2. Mass spectrum of 23S-bromo-5α,25R-spirostan

m.p. 193-194°

\[ \alpha \] D 2 -64.3°

Relative Intensity

69 81 95 109 122 147 161 176 217

257 271 286 298 338 369 399 478 480 (M+1)
Figure 3. Mass spectrum of 23R-bromo-5α,25R-spirostan
which were shown to be monobrominated products by elemental analysis.

From a preliminary nmr study of steroidal sapogenins of both the 25D (C-25 equatorial methyl group) and 25L (C-25 axial methyl group) series conducted in our laboratories it appeared that two regions of the nmr spectra of the monobromotigogenins would be of particular value in solving the problem in hand: (a), the high field region encompassing the C-methyl signals and (b), the region between 200 cps and 300 cps (at 60 Mcps) in
which the signals of the protons at C-16, C-23 and C-26 (as well as C-3 in the monobromotigogenin acetates) occur. It was found to be useful to measure chemical shifts and chemical shift differences in cps. Chemical shifts quoted are relative to the TMS signal set at 0 cps.

The C-methyl region at 60 Mcps

The C-methyl region of compounds 8a, 8b, 9a and 9b is shown in Figure 4. By analogy with the assignments made for methyl protons in the study mentioned above, it was possible to make assignments with confidence in this region. In the sapogenins of the 25D series, of which the compounds presented in this study are members, the protons of the C-25 methyl group were observed to give rise to a doublet of which one signal, generally the only discernable member of the doublet, was found at highest field of all signals. In 8a and 8b the higher field member of this doublet was found at 44 cps and 45 cps, respectively. The signals from the angular methyl protons (C-18 and C-19) were observed to appear as two sharp spikes rising above the rest of the signals and were often quite close together. In 8a and 8b they were found at 47 and 51 cps, and 50 and 53 cps, respectively. In all the steroidal sapogenins of both the 25D and 25L series examined in the preliminary study, the C-19 signal occurred at higher field than the C-18 signal. In the particular instance of the monobromotigogenins it was difficult to assign with certainty the C-18 and C-19 methyl resonances to particular spikes because the effect of the bromine atom was difficult to ascertain. (This problem will be discussed more fully later.) Like the doublet due to the C-27 methyl protons, the doublet due to the C-21 methyl group usually was observed to show only one discernable signal at slightly lower field than the signals from the C-18 and C-19 methyl protons. In
the case of 8a, this signal occurred at .57 cps and in the case of 8b, at 58 cps. These assignments were assumed to describe only the twelve protons contained in the four methyl groups found in the molecules. That no signal from another proton in a molecule had been confused for one of these twelve protons described was verified by the observation that the integral over this region corresponded to exactly twelve protons.

In the case of compounds 9a and 9b there were some interesting features of the C-methyl region which were germane to the point of the study, which was to describe the location and configuration of the bromine atoms. In the spectra of both compounds, the C-21 methyl proton signals were shifted appearing as a distinct doublet (J = 7 cps) some 16 cps downfield from their position in 8a and 8b and were well removed from the signals due to the three other methyl groups. That this shift had taken place was supported by the integral of the spectra which showed that the doublet corresponded to 3 protons and the group of signals assigned to the other three angular methyl groups corresponded to nine protons. Confirmation for this assignment was obtained when the nmr spectrum of 9a was run at 100 Mcps. The separation in the signals assigned to the doublet from the C-21 methyl protons remained unchanged at 7 cps. It is interesting to note that because the protons of the C-18 and C-19 methyl groups in 9a occurred fortuitously as a single sharp spike at 47 cps, both signals attributable to the C-27 methyl doublet appeared, at 45 cps and 50 cps (J = 5 cps). In the case of 9b the situation was more typical and only the upper field member of the doublet attributable to the C-27 methyl protons was seen to occur at 46 cps. As an aside, the other member of this doublet, by assuming the same coupling as in the case of 9a, was expected at 51 cps and therefore
would have coincided with the methyl spike at 51 cps. Indeed, the spire at 51 cps in the spectrum of 9b did seem inordinately larger than the one at 48 cps by comparison with the same spikes in the spectra of 8a and 8b.

Figure 4. High field region of nmr spectra at 60 Mcps

Consideration of the facts above gained by examination of the methyl region of the various spectra made it possible to make a fairly reliable assignment of location and configuration of the bromine atom in the four compounds studied. For instance, C-20 and C-25 were ruled out as possible sites for the location of the bromine atom since if the bromine atom had resided at either of these sites the proton signals of the methyl group sharing the same carbon atom would have been shifted to lower field and
would have appeared as a singlet. The spectra obtained showed no such feature. Of the remaining sites in the spiroketal side chain, C-23 seemed the most logical since there appeared to be no chemical reason to assume that bromination could occur at C-24 and doubtful reason to assume that bromination could occur at C-26. Examination of molecular models of the monobromotigogenins showed that an axial C-23 bromine substituent would probably be in a 1,3 diaxial relation to the C-21 methyl group. Fairly complete data have been compiled\textsuperscript{17-19} concerning the effect of various substituents in steroid systems on the C-18 and C-19 angular methyl protons. For example, the effect of a bromine 1,3 to an angular methyl group has been determined for a 2β-bromo and a 6β-bromo substituent in the 5α,14α-androstane system and found to result in a downfield shift in the C-19 signal of 14.0 cps and 15.0 cps, respectively. The downfield shift in the proton resonance of the C-21 methyl group in 9a and 9b compared to their parent compounds was found to agree very well and was about 16 cps in each case. Since there was no noteworthy shift in the C-21 methyl resonance of compounds 8a and 8b, it was tentatively concluded that 9a and 9b had an axial bromine at C-23 and 8a and 8b had an equatorial bromine at C-23.

Although not directly concerned with the objective of the nmr study, it was an interesting excercise in the case of the 23-monobromo epimers to attempt to assign the C-18 and C-19 methyl proton resonances. In the spectrum of desoxytigogenin the C-18 methyl resonance occurred at 46 cps and the C-19 methyl resonance occurred at 48 cps. In the spectrum of tigogenin acetate, which can be considered as the 3β-acetate derivative of desoxytigogenin, the C-18 methyl resonance occurred at 46 cps and the C-19 methyl resonance occurred at 50 cps. Since the C-19 methyl group in the monobromotigogenins was seen to be far removed from the bromine substituent
in the sapogenin side chain, the C-19 methyl resonance was expected to occur in nearly the same position as it did in the parent compound; that is, at 48 cps in the case of 8a or 9a and at 50 cps in the case of 8b or 9b. On this basis the logical assignment of the C-19 methyl resonances required that the spike at 47 cps in the spectra of 8a and 9a and the spikes at 50 cps and 51 cps in the spectra of 8b and 9b, respectively, be due to the C-19 methyl protons. The remaining unassigned spike in each spectrum by default must have been due to the C-18 methyl resonance. In the case of compounds 9a and 9b, in which the bromine had been assigned the axial configuration, the C-18 methyl resonances were assigned as the peaks at 47 cps and 48 cps, respectively. Since as a general rule C-18 methyl resonances occur at higher field than C-19 methyl resonances, this assignment was quite unexceptional. Regarding compounds 8a and 8b, however, the situation was seen to depart from the ordinary. The C-18 methyl resonances had to be assigned to the signals at 51 cps and 53 cps, respectively, and consequently at lower field than the C-19 methyl resonances. The effect of an equatorial bromine substituent at C-23 on the C-18 methyl resonances must have caused a downfield shift of 5 cps in the case of 8a and of 7 cps in the case of 8b.

Since much had been deduced above on the basis of very small chemical shift differences, an alternate assignment was considered. For the sake of argument it was assumed that the C-18 methyl protons must always resonate at higher field than the C-19 methyl protons. The assignment for compounds 9a and 9b was unchanged, but in the case of compounds 8a and 8b a reassignment was required. The signal at 47 cps for 8a had to be due to the C-18 methyl protons and the one at 51 cps, to the C-19 methyl protons. This required that an equatorial bromine substituent at C-23 must have resulted in a downfield shift of 3 cps in the position of the C-19 methyl resonance and
only a downfield shift of 1 cps in the C-18 methyl resonance. Similarly, in the case of 8b, an equatorial bromine substituent must have resulted in a downfield shift of 3 cps in the position of the C-19 methyl resonance to 53 cps and a downfield shift in the C-18 methyl resonance of 4 cps to 50 cps. Examination of molecular models showed that the C-19 methyl group was about twice as far from the bromine substituent as the C-18 methyl group. Since long range magnetic anisotropic and dipole effects were known to be inversely proportional to the cube of the distance from a functional group, the effect of the bromine substituent on the C-19 methyl group was expected to be roughly one-eighth ($\frac{1}{2^3}$) the effect on the C-18 methyl group. Thus, it seemed that the alternative assignments above were untenable. It was thus clear that in compounds 8a and 8b the bromine substituent must have had a location and configuration that brought it near enough to the C-18 methyl group that the effect of bromine was to cause a downfield shift in the C-18 methyl resonance of one-third to one-half the downfield shift expected for a bromine atom in a 1,3 diaxial relationship to an angular methyl group. From an examination of molecular models it appeared that aside from a C-20 bromine substituent, which had been ruled out on other grounds, only a C-23 equatorial bromine substituent would be near enough to the C-18 methyl group to have an effect on its resonance of the amount and direction observed.

In summary, the direction and magnitude of the shift in the C-21 methyl resonance of 9a and 9b supported the assumption that these substances possess a C-23 axial bromine atom. Likewise the shift in the C-18 methyl resonance of 8a and 8b supported the assumption that they possess a C-23 equatorial bromine atom. Further evidence of an unequivocal nature, which supported the structural and stereochemical assignments made above on the basis of an analysis of the C-methyl region exhibited by the monobromotigogenins, was
found through an analysis of the 200-300 cps region of their nmr spectra.

The 200-300 cps region

Examination of this region (Figures 5 and 6) showed that each compound exhibited a broad set of signals arising from the C-26 protons that approximated a doublet close to 200 cps. This feature was known to be characteristic of sapogenins of the 25D series and its presence excluded on its own merits C-26 bromo, C-25 bromo and C-23 bromo-25L compounds from consideration as possible alternatives to the structural assignments made above. A more interesting feature of the low field region of the spectra was that the nmr spectra of compounds 8a and 8b on one hand and compound 9a and 9b on the other differed markedly in the region 230-300 cps. Comparison of this region in the spectra of the monobromotigogenins with the same region in the parent compounds allowed assignment of the C-16 and C-3 proton resonances. Desoxytigogenin displayed a broad set of signals in the region 250-275 cps attributed to the C-16 proton and tigogenin acetate displayed, in addition, a broad set of signals which merged with the set from the C-16 proton on the low field side and was attributed to the C-3 proton. Comparison with the spectra of the parent compounds showed that the spectra of the monobromo derivatives exhibited an additional set of signals due to one proton on the upfield side of the signals from the C-16 proton. In the spectra of 8a and 8b there were in this set four distinct signals of nearly equal spacing and intensity which together resembled a doublet of doublets, whereas in the spectra of 9a and 9b the set of signals resembled a not-too-well-resolved triplet. In the case of each monobromotigogenin this set of signals must have arisen from a proton geminal to a bromine atom and the only sites in the side chain where a bromine could be geminal to a proton are C-23, C-24 and
C-26. Since C-24 and C-26 have been ruled out, the bromine atom must be at

- 19 -

C-23. On the basis of an analysis of the C-methyl region the bromine atom in compounds 8a and 8b had been tentatively assigned an equatorial configuration and the bromine atom in compounds 9a and 9b, an axial configuration. Interpretation of the set of signals arising from the C-23 proton permitted an unequivocal assignment of the orientation of the bromine atom.

Examination of molecular models of the monobromotigogenins indicated that ring F should exist as an undistorted chair in all cases since there were no exceptional interactions arising from the bromine substituents in either the axial or equatorial configuration at C-23. Consequently, it

Figure 5. Low-field region of nmr spectra at 60 Mcps

Figure 6. Low-field region of nmr spectra at 60 Mcps
appeared that the pattern of the C-23 proton in 8a and 8b should be interpretable by consideration of part structure 11 and the pattern of the C-23 proton in 9a and 9b by consideration of part structure 12.

It was apparent from the part structure 11 that the axial proton $H_X$ would enter into axial-axial spin-spin interaction with $H_A$ and axial-equatorial spin-spin interaction with $H_B$. In 12 the equatorial proton $H_X$ was expected to enter into axial-equatorial spin-spin interaction with $H_A$ and equatorial-equatorial spin-spin interaction with $H_B$. Since the coupling constants $J_{AX}$ and $J_{BX}$ in 12 were expected to be equal or nearly so, the expected pattern for the proton $H_X$ was a triplet. In addition what is probably more important, the half-height width ($J_{AX} + J_{BX}$) was expected to be less than 10 cps. The signals from the C-23 proton in 9a and 9b agreed quite well with this predicted pattern. The pattern of the signals was a triplet, although it was not too well defined at 60 Mcps, with half-height width of 7 cps.

A more complicated case was anticipated when the bromine substituent was equatorial as in 11. In this case the axial proton $H_X$ was expected to enter into axial-axial and axial-equatorial coupling with the vicinal protons $H_A$ and $H_B$. The pattern arising from the proton $H_X$, if the right conditions
prevailed,\textsuperscript{20,21} could also have been a triplet. Nevertheless, it was realized that the half-height width (or the difference between highest and lowest field signals) of the triplet must as in the previous case be equal to $J_{AX} + J_{BX}$. This sum would be expected to be greater than 9 cps.\textsuperscript{22-24} The half-height width (7 cps) for the triplet in 9a and 9b was too small to fit into this special case. The pattern observed for the C-23 proton resonances in 8a and 8b resembled a doublet of doublets, a more typical pattern for the "X" portion of an ABX system such as that shown in 11. The sum, $J_{AX} + J_{BX}$, which was about 17 cps, was also more typical of the case shown in 11.

\textbf{Nmr spectra at 100 Mcps}

The nature of the spectra obtained at 60 Mcps pointed out that it would be worthwhile to obtain spectra at 100 Mcps. Accordingly, desoxytigogenin and its monobromo derivatives were submitted for nmr analysis at 100 Mcps. At 100 Mcps in both 8a and 9a the C-23 proton signals were unmistakeably separated from the C-16 proton signals (see Figure 7) and the integral provided confirmation of the one proton nature of each group of signals. In the spectrum of 9a the splittings of the C-23 proton were measurable and $J_{AX}$ (apparent) was found to be about 11 cps and $J_{BX}$ (apparent), about 6 cps in close agreement with the values expected from the theory. In the spectrum of 9a the C-23 proton resonances, which at 60 Mcps appeared to form a poorly resolved triplet, formed a clear and unmistakeable triplet for which the following estimates, also in close agreement with the theory, could be made: $J_{AX} + J_{BX} \approx 3.5$ cps.

From the nmr data alone, enough information was obtained to permit complete structural and stereochemical assignments to be made in the case of
the four bromosapogenins studied. Similar nmr data would be expected to
provide enough information to allow valid structural and stereochemical
assignments to be made in the case of other bromosapogenins.

In summary, desoxytigogenin has been found to afford as bromination,
two monobromo derivatives which are epimeric at C-23. The compound melting
at 193-194° has been assigned the structure 8a and the one melting at 225-
226°, the structure 9a. Similarly, the monobromotigogenin acetate isomer
melting at 206-208° has been assigned structure 8b and the one melting at
201-202°, the structure 9b.
III. General Conclusions

Since the epimeric C-23 monobromotigogenins represented by far the bulk of the compounds produced in the bromination reaction, it seemed to be worth considering why the C-23 site was preferred for bromination. It was apparent that intermediate 4 (Figure 1), which was suggested as playing a role in the Iso reaction, must be involved in the bromination reaction as well. Such an intermediate would be expected, by loss of a proton from the C-20 carbon atom, to form the enol ether 13 or, by loss of a proton from the C-23 carbon atom, to form the enol ether 14. The enol ether could then have reacted with bromine to form a C-20 bromo derivative in the case of 13 or a C-23 bromo derivative in the case of 14. As a general rule, the acid catalysed bromination of an unsymmetrical ketone, \(^{25}\) which resembles the present problem, has led to preferential attack at the most substituted α carbon atom. Since
typical acid catalysis \((\text{Br}_2/\text{HOAc})\) was used in the present case, at first sight the expected products would have been C-20 epimeric bromotigogenins. Since only C-23 epimeric bromo derivatives were obtained, it was assumed that either the enol ether 13 was formed very slowly under the reaction conditions in comparison with the rates of formation and bromination of the enol ether 14 or, if it were formed at a competitive rate, it did not react with bromine at a competitive rate. It so happened that the proposed enol ether 13 was known to be a perfectly respectable compound, a member of the pseudosapogenin family, which could be formed simply by treatment of desoxygenation or tigogenin with acetic anhydride (though under fairly vigorous conditions) and base. Therefore there seemed to be no reason to deprecate 13 on the grounds that it would be a very high energy and hence unlikely intermediate. As far as the reactivity of an intermediate such as 13 was concerned, Mueller and Norton \(^3\) brominated pseudohecogenin under mild acid catalysis and obtained no product corresponding to either of the bromohecogenins obtained via the acid catalysed bromination of hecogenin acetate. Furthermore, it has been shown by several workers\(^{26}\) that pseudohecogenin could be oxidized by either chromic acid or peracid. Oxidation of pseudohecogenin with chromic acid led to a 3,12-dione-20-ol (15). Therefore, it seemed likely that if the enol ether 13 was formed in the bromination reaction it would react. The only plausible explanation of the results of the bromination reaction seemed to be that 13 was formed very slowly under the conditions employed.

Perhaps more direct evidence, which supported the proposition that 13 was not an intermediate, was that a pseudosapogenin under acetic acid catalysis equilibrates not with the parent sapogenin, but with a compound, having a
spirolketal side chain, called a cyclopseudosapogenin. The cyclopseudo-
sapogenins were known to be less stable than the parent sapogenins havin:
sapogenin. By the same token it was assumed that loss of the C-20 β proton in 4 would be expected to take place at much slower rate in acetic acid than the rates of loss of either of the C-23 protons and subsequent reaction with bromine of the enol ether (14) formed. In consequence of the forgoing reasoning the nature of the monobromosapogenins formed in the acetic acid-bromine bromination of steroidal sapogenins was considered to be controlled by kinetic factors.
IV. Experimental

Pet. ether refers to a petroleum ether fraction boiling at 40-50°. Mps were observed using a Kofler mp apparatus. Elemental microanalyses were performed in the laboratories of Dr. A. Bernhardt, Max Planck Institute, Mulheim, Germany. Mass spectral results were obtained using an Atlas-Mat Model CH4 mass spectrometer equipped with a direct inlet system. Ionizing energy of the mass spectrometer was maintained at 70 ev and the ionizing current at 50 μa.

The nmr spectra at 60 Mcps and 100 Mcps were recorded employing, respectively, Varian Associates Models A-60 and HR-100 nmr spectrometers. The spectra were taken in deuteriochloroform solutions with tetramethylsilane used as an internal standard. Values are given in cycles per second (cps) with respect to the TMS signal set at 0 cps. Ir spectral data were provided by Dr. R. A. Hill (University of Maine) and optical rotation (chloroform solution) measurements by Drs. Weiler and Strauss, Oxford, England.

Bromination of 5α,25R-spirostan (7) (by J.C.K.)

A solution of Br₂ (2.9 g) in glacial acetic acid (36 ml) was added (over 30 min) to a solution (maintained at 60°) composed of desoxytigogenin (6.0 g), 2 drops of 4N HBr in glacial acetic acid, and glacial acetic acid (800 ml). A deep blue colour formed at once and intensified upon further
addition of Br₂. The mixture of liquid and crystals (separated during bromination) was allowed to remain at room temp for ca. 24 hr. After filtration the crystalline product was separated into two principal components (3.7 g, mp 186-196° and 1.0 g, mp 211-222°) by fractional recrystallization from chloroform (Norit-A)-methanol. Repeated recrystallization of the lower melting mixture from chloroform-pet. ether eventually gave a pure specimen of 23S-bromo-5α,25R-spirostan melting at 193-194°; \([\alpha]_D^{22} -64.3°\) (c, 1.24), \(v_{\text{max}}^{\text{KBr}} 1008, 950, 918, 865\) and 730 cm⁻¹. (Found: C, 67.42; H, 8.75; Br, 16.91; mol wt, 480. C₂₇H₄₃BrO₂ requires: C, 67.60; H, 9.00; Br, 16.70%; mol wt 480.) Similar treatment of the higher melting fraction yielded an analytical sample of 9α melting at 215-217° to 225-226° (depending on rate of heating); \([\alpha]_D^{22} -87.4°\) (c, 1.36); \(v_{\text{max}}^{\text{KBr}} 1015, 972, 943, 905,\) and 880 cm⁻¹. (Found: C, 67.36; H, 8.84; Br, 17.01%; mol wt 480.)
References


22. Reference 18, p. 51.


27. Reference 26, pp. 825-828.

28. We wish to thank Professor C. Djerassi, Chemistry Department, Stanford University for running the mass spectra.

29. We wish to thank Professor W. A. Ayer, Chemistry Department, University of Alberta, for running the nmr spectra at 100 Mcps.

30. We wish to acknowledge the contributions of Dr. T. R. Kasturi during and early phase of this study.

PART B

SYNTHESIS OF C-18 SUBSTITUTED DIHYDROCLEAVAMINES
I. Introduction

In a general sense alkaloids are basic nitrogen containing compounds found in plants. The number of known alkaloids has been estimated to be about 4000. Many compounds which would appear to fit this description of an alkaloid are not numbered among these. Compounds such as putrescine (1) and tryamine (2), which are basic, nitrogen containing and found in plants, are not considered to be alkaloids and are often referred to as protoalkaloids since they often occur as structural units in alkaloids. Protoalkaloids are derived from amino acids. For instance, putrescine is derived from ornithine (3) and tyramine from tyrosine (4) by decarboxylation.

\[
\begin{align*}
\text{1} & : \text{NH}_2(CH_2)_4\text{NH}_2 \\
\text{2} & : \text{HO-CH}_2\text{CH}(\text{CH}_3)\text{NH}_2 \\
\text{3} & : \text{COOH} \quad \text{NH}_2\text{CH(\text{CH}_2)_2NH}_2 \\
\text{4} & : \text{COOH} \quad \text{HO-CH}_3\text{CHNH}_2
\end{align*}
\]
With some compounds the distinction between protoalkaloids and true alkaloids would appear to be somewhat hazy. If protoalkaloids are considered to include as well as biogenic amines, derivatives of biogenic amines, then compounds such as the toad poison, bufotenine (5) and the hallucinatory principle of "peyote" mescaline (6), might better be referred to as protoalkaloids rather than as alkaloids. With few exceptions the "alkaloids" such as bufotenine which are found in animals are of the protoalkaloid type. Two noteworthy true alkaloids which are found in animals are samandarine (7) and batrachotoxinin A (8). Samandarine occurs with several related alkaloids
as the skin poison of two salamanders (S. maculosa Laurenti and S. Atra Laurenti) of European habitat, whereas batrachotoxinin A is isolated from the skin of the Columbian arrow poison frog (Phyllobates aurotaenia). Batrachotoxinin A may be a secondary product formed from pseudobatrachotoxinin by the addition of water during isolation and purification.

There are many alkaloids which do not fit the description "basic". In these compounds the nitrogen forms part of an amide as in colchicine (9) or is quaternary as in rhazidine (10) and pleiocarpoline (11). Rhazidine is actually a conjugate acid form of the base 12 but the base form is observed only in strongly basic solutions or in hydrocarbon solvents.
A very large family of alkaloids, which number about 600, contains the indole system. The Aspidosperma, Vinca and Iboga alkaloids are members of this large group. In this part of this thesis a general method for the synthesis of the Aspidosperma, Vinca and Iboga alkaloids is discussed. This approach has been developed in our laboratories. A key step in this method was a transannular cyclization which is a laboratory analogy of a step proposed by Wenkert in a plausible scheme for the biosynthesis of these alkaloids. According to Wenkert's scheme nine-membered-ring intermediates, such as 13 and 15, are built up and undergo a transannular cyclization to give alkaloids possessing the Aspidosperma (e.g. 13 → 14) or the Iboga skeletons (e.g. 15 → 16) as shown in Figure 1.

Figure 1. Biosynthetic postulate for the production of Aspidosperma and Iboga skeletons
Kutney and coworkers were the first to demonstrate the feasibility of such cyclizations under laboratory conditions. They were able to convert dihydrocleavamine (17)\textsuperscript{8-10} which is conveniently obtained via a simple degradation of catharanthine\textsuperscript{11} in two steps to 7-ethyl-5-desethylaspidospermidine (18).\textsuperscript{12} Oxidation of dihydrocleavamine (Figure 2) provided the intermediate iminium ion 19 which cyclized to the indolenine 20. The indolenine without complete purification was then reduced with lithium aluminum hydride to provide the more stable 1,2-dihydro compound 18.

In the same manner these workers\textsuperscript{13} were able to convert carbomethoxydihydrocleavamine\textsuperscript{9,14} (21), which like dihydrocleavamine is easily obtained by a simple degradation of catharanthine, into 7-ethyl-5-desethylvincadifformine (24), and coronaridine,\textsuperscript{15} which is a known Iboga alkaloid, and the C-4 epimer, dihydrocatharanthine (26).\textsuperscript{11} Oxidation of carbomethoxydihydrocleavamine in the N\textsubscript{b}—C-19 direction led to the iminium ion 22 which cyclized in

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Transannular cyclization giving the Aspidosperma skeleton.}
\end{figure}
the reaction medium to provide 24. Alternately, oxidation of carbomethoxy-
dihydrocleavamine in the $N_b$—$C$-5 direction led to an iminium ion, which by
virtue of equilibration with the enamine 25 resulted in the mixture of
epimeric iminium ions 23. One epimer cyclized to coronaridine, the other,
to dihydrocatharanthine. The reaction sequence proposed is outlined in
Figure 3 below. It should be observed that a transannular cyclization of

Figure 3. Transannular cyclization giving the Aspidosperma and Iboga
skeletons
both types generated two new asymmetric centres and therefore each intermediate iminium ion above might have been expected to cyclize to a mixture of four diastereomers (see structures 27 to 30 for Aspidosperma skeleton).

\[ \begin{align*}
\text{27} & \quad \text{28} \\
\text{29} & \quad \text{30}
\end{align*} \]

The fact the above mentioned experiments showed that each of the intermediate iminium ions cyclized to give only one of the possible diasteriomers in sufficient quantity to isolate. This result was not unexpected since it was readily apparent (from examination of molecular models) that only one conformation of each iminium ion would permit the reacting centres to be close together and at the same time not be highly strained. It was not, however, immediately apparent that in each of the transannular cyclizations discussed that an optically active starting material would lead to an optically active product. Both dihydrocleavamine and carbomethoxydihydrocleavamine have a proton at C-2. If 19 and 22 could equilibrate with their enamine forms under the conditions of the reaction, racemic products would
be formed. A similar equilibration \((23 \rightleftharpoons 25)\) was shown to occur. Moreover, tautomerization of the iminium double bond across nitrogen might also occur. Thus a combination of equilibria (Figure 4) could serve to destroy the

![Equilibria Diagram](image)

Figure 4. Equilibria that would result in loss of optical purity

C-2 asymmetry independently of the direction of the initial oxidation and, therefore, destroy the optical activity of the compounds with the Iboga skeleton as well. There was precedence for tautomerization of an iminium ion across nitrogen\(^{17-20}\). However, it appeared that such a process was promoted by base and so would not likely be a factor in the mercuric acetate-acetic acid conversion of the dihydrocleavamines. For instance, Schumann and Schmid\(^ {17} \) found that condifoline (31) was converted into tubifoline (32) under a variety of neutral and basic conditions (e.g. neat at 117-120°, in tetralin at 160-180°, in triethylamine, in ammonia-isopropanol or in potassium t-butoxide-t-butanol, but not under acidic conditions (2-7N hydrochloric acid at 100-125°). They proposed the scheme shown in Figure 5 to account for their results. Edwards and Singh\(^ {18} \) found that both
Figure 5: Interconversion of tubifoline and condifoline

Atisine hydrochloride (33) and isoatisine hydrochloride (34) gave the same compound on treatment with acetic anhydride or acetic anhydride-pyridine mixtures. Pellitier and Jacobs\textsuperscript{19} showed that the compound was a diacetate hydrochloride (35) of atisine and proposed a mechanism "involving a concerted
abstraction and re-addition of a proton by acetate ion and acetic acid" as shown in Figure 6 below. In a closely related case Weisner and Edwards found that veatchine (36) was converted to garryine (37) by hot alkali and proposed that the quaternary forms of these compounds were in equilibrium with the ylid ion 38 under these conditions as shown in Figure 7.

Figure 6. A mechanism for tautomerization of iminium functions across nitrogen

Figure 7. Interconversion of veatchine and garryine
As far as the problem of the relative configuration of the synthetic Iboga compounds was concerned, the transannular cyclization of the iminium ions led to the natural skeleton as proven by the isolation of coronaridine and dihydrocatharanthine. In the case of the pseudoaspidosperma compounds no such direct comparison was possible. Kutney and coworkers and independently Schmid and coworkers, however, successfully carried out a conversion in the natural series. It was found that (-)-quebrachamine was transformed into (+)-aspidospermidine under suitable conditions. Thus, the transannular cyclization led to the natural diastereomer. Schmid suggested the absolute configuration of (+)-aspidospermine, based on optical studies of a large number of related alkaloids, shown in 40. It can be seen that the C-5 ethyl group and C-19 proton are cis in relationship to each other and that the C-19 proton bears a trans relationship with the C-10—C-11 bridge. This relative configuration was exactly the one that would be expected to arise on conformational grounds through cyclization of the intermediate iminium ion. The reduction of 1,2 dehydroaspidospermidine, which was the immediate product after cyclization, with hydride was also completely stereospecific since the natural C-2 epimer was obtained. Since
there was no possible means by which the C-5 configuration could be altered during the conversion of (-) quebrachamine to (+)-aspidospermidine, optically active product was obtained as expected. As pointed out above, the situation with regard to the absolute configuration of the pseudo-aspidosperma and Iboga compounds produced via transannular cyclization was not unambiguous because the optical activity of the C-2 carbon in the dehydrocleavamine molecule could conceivably have been destroyed in the process. Kutney and coworkers showed, however, that the asymmetry of the C-2 carbon atom was retained in the transannular cyclization reaction. 7-Ethyl-5-desethylaspidospermidine obtained from 4β-dihydrocleavamine (41) was established to have the absolute configuration as shown in 42 by X-ray diffraction analysis of its N<sub>a</sub>-acetyl-N<sub>b</sub>-methiodide derivative. The configuration of the C-2 carbon atom<sup>24,25</sup> in 4β-dihydrocleavamine, therefore, remained unaltered during the course of the conversion reaction. The configuration of the C-19, C-12 and C-2 carbon atoms relative to the configuration of the C-5 carbon atom of 7-ethyl-5-desethylaspidospermidine was exactly that expected from conformation consideration. In addition, the configuration of the C-4 carbon atom of 4β-dihydrocleavamine was unaltered during the course of the reaction. Consequently, of the equilibration
reactions shown in Figure 4 only "C" is important. Since the transannular cyclization of 4β-dihydrocleavamine to 7β-ethyl-5-desethylaspidospermidine took place in a stereospecific manner, it followed that the transannular cyclization of carbomethoxydihydrocleavamine to provide 7-ethyl-5-desethylvincadifformine, dihydrocatharanthine and coronaridine was also a stereospecific process. The absolute configuration of catharanthine, as shown in 43, ibogaine (44), coronaridine (45) dihydrocatharanthine (46), ibogamine (47) and voacangine (48) followed directly from the above cyclization when coupled with the fact that the relative configuration of ibogaine was available from the X-ray analysis\textsuperscript{26} and various interconversions were known.\textsuperscript{10,27,28}

Besides the intrinsic value of these results to the biosynthetic postulates of Wenkert, they had a special value from a synthetic point of view. The transannular cyclization step was incorporated into a general total synthesis of a number of alkaloids bearing the Aspidosperma or Iboga skeletons. Since this step was shown to be completely stereospecific with the configuration of each new asymmetric centre being completely determined by the configuration at C-2 in the nine-membered ring intermediates, the
total synthesis based on this step was not beset with stereochemical problems during the various stages of the synthetic sequence. Moreover, parallel total syntheses of the dihydrocleavamine and quebrachamine systems were feasible. Thus the total syntheses of alkaloids bearing the Aspidosperma skeleton on the one hand and the Iboga skeleton on the other complemented each other with obvious advantages over synthetic approaches that required a completely different method for each skeletal type. The important features of the syntheses of dl-quebrachamine$^{29}$ (57) and dl-dihydrocleavamine$^{30}$ (58) as carried out by Kutney and coworkers are shown in Figure 8. In each synthesis of appropriate succinate ester (49 or 50), which was condensed with tryptamine (51), was elaborated by separate and unexceptional means. It was seen that introduction of a carbomethoxy function at C-18 in dihydrocleavamine would provide a carbomethoxydehydrocleavamine and thereby complete the total syntheses of coronaridine and dihydrocatharanthine. Introduction of a carbomethoxy function at C-3 in quebrachamine would provide
Figure 8. Kutney's syntheses of dl-quebrachamine, dl-4α-dihydrocleavamine and dl-4β-dihydrocleavamine
a carbomethoxyquebrachamine. (+)-Vincadine (59) is a naturally occurring Vinca alkaloid as is (-)-vincadifforamine (60). A successful transannular cyclization of a carbomethoxyquebrachamine in the manner used to give the pseudo compounds previously would provide dl-vincadifformine. Part of this section is concerned with the introduction of a carbomethoxy group into dihydrocleavamine.

As mentioned above several syntheses have been elaborated for the preparation of alkaloids with the Iboga and Aspidosperma skeletons. Various syntheses carried out in other laboratories were peculiar to one of these skeletal types and are best discussed in two separate groups. Three approaches to the formation of the alkaloid aspidospermine, were particularly interesting. Each of these approaches involved the elaboration of a tricyclic keto-amine (61) which would be expected to yield the Aspidosperma skeleton when exposed to the Fisher indole reaction as shown in Figure 9. There are three asymmetric centres in 61 and therefore four diastereomerically related isomers are possible (62, 63, 64 and 65). In fact all four of these isomers have been synthesized. Stork and coworkers achieved the first total synthesis of dl-aspidospermine (66) and dl-quebrachamine. They synthesized a tricyclic keto amine shown later to have structure 62,
submitted it to Fisher indolization with o-methoxyphenylhydrazine and obtained \( \text{dl-1,2-dehydrodeacetylaspidospermine} \) (67). Reduction of 67 with lithium aluminum hydride in diethyl ether introduced the C-2 proton stereospecifically and the latter product upon acetylation provided \( \text{dl-aspidospermine} \) (66). Since the correct stereochemistry was obtained in the products even though the incorrect stereochemistry was possessed by the tricyclic keto amine 62, equilibration of the asymmetric centres at C-12 and C-19 must have occurred during the Fisher synthesis. Stork proposed the
Figure 10. Equilibrium with stereochemical implications and stereospecific formation of dl-aspidospermine

equilibrium $67 \rightleftharpoons 68$ (Figure 10) to account for the correct stereochemistry of the product. Stork and coworkers were able to extend their synthesis to provide a total synthesis of dl-quebrachamine by making use of this equilibrium. Application of the Fisher indole reaction to the keto amine 62 with phenylhydrazine provided d1-1,2-dehydroaspidospermidine (69) which was converted to dl-quebrachamine by bringing about the analogous equilibrium to $67 \rightleftharpoons 68$ in methanol and selectively reducing the very reactive iminium function in the tetracyclic cation with potassium borohydride.

Ban and coworkers$^{33}$ in the course of their studies related the total synthesis of aspidospermine produced a tricyclic keto amine which had the same planar structure (61) as the one described by Stork, but differed in chemical and physical properties. Comparison of both compounds allowed
these workers to propose the structure 63 for their compound and 62 for Stork's compound. The tricyclic keto amine 63 also provided dl-aspidospermine when subjected to the same sequence of reaction as mentioned above.

The remaining two possible tricyclic keto amines 64 and 65 were prepared by Kuehne and Sayha. Since the Fisher indole reaction required loss of the asymmetry at the site α to the ketone in the correct cyclization process, these two new isomers offered no additional stereochemical problems. Stork's synthesis of the tricyclic keto amine is outlined in Figure 11, while those of Ban and Kuehne are given in Figures 12 and 13, respectively.

Another interesting approach to the synthesis of the aspidosperma skeleton has been carried out by Harley-Mason and Kaplow. An entirely stereospecific synthesis of dl-aspidospermidine was achieved by these workers and is outlined in Figure 14.

Buchi and coworkers achieved the first total synthesis of an Iboga alkaloid. They were able to synthesize dl-ibogamine (70) and dl-epiibogamine (71) as shown in Figure 15. The cyclization rearrangement of 72 (73) to give 74 (75) is particularly noteworthy in view of some later discussion in this thesis. Buchi suggested that cyclization proceeds to give a compound (76) which has the desired iboga skeleton but which suffers a 1,2 shift of the amine (amide) nitrogen to give a rearranged product 74 (75). Buchi favored a non-classical carbonium ion (78) as an intermediate in the
Figure 11. Stork's synthesis of tricyclic keto amine 62

Figure 12. Ban's synthesis of tricyclic keto amine 63
Figure 13. Kuehne's synthesis of tricyclic keto amines 64 and 65

Figure 14. Harley-Mason's synthesis of dl-aspidospermidine
Figure 15. Buchi's synthesis of dl-ibogamine and dl-epiibogamine
Figure 15. Continued
1,2 shift of nitrogen. Although Buchi favored the non-classical ion, a classical bridged aziridinium ion could also have been postulated.

In connection with another approach to the synthesis of the iboga skeleton, Huffman and coworkers discovered an analogous rearrangement for which they postulated a bridged acylaziridinium intermediate. They found, for instance, that treatment of the isoquinuclidone tosylate 80 (R = Ts) with refluxing sodium acetate-acetic acid gave a mixture of the corresponding isoquinuclidone acetate 80 (R = Ac), and an isomeric acetate 81 (R = Ac). Similarly, the tosylate 81 (R = Ts) was converted to the same mixture under the same conditions. Heating either tosylate 80 (R = Ts) or tosylate 81 (R = Ts) to the melting point formed a mixture of the two tosylates in a ratio 80 (R = Ts):81(R = Ts) of about 5:1. Huffman proposed that these results were best explained in terms of an intermediate acylaziridinium ion (82).

Shortly after the publication of the work of the Buchi and Huffman groups, Nagata and coworkers described the first synthesis of bridged aziridine derivatives and demonstrated the ability of this approach to production of the isoquinuclidine position of the iboga skeleton by synthesizing desethyl-ibogamine. Recently, these workers have published a stereospecific total syntheses of dl-ibogamine and dl-epiibogamine. The racemic olefinic
amines 83 and 84 were synthesized by separate and rather lengthy procedures which will not be presented here. The reaction sequence which led to dl-ibogamine in the case of 83 and dl-epiibogamine in the case of 84 is shown in Figure 16 for the synthesis of the naturally occurring dl-ibogamine.

The mechanism of the cleavage of the aziridine system is worth considering especially as it relates to the proposals of Buchi and Huffman. Nagata and coworkers found that the cleavage reaction of the bridged aziridines with an acylating agent in an alkaline medium took place without incorporation of the solvent anion. They proposed that intermediate formation of an ion pair such as 87 took place with cleavage by ion-pair return from the opposite side of the nitrogen atom. Quaternization of the aziridines with poorer electron withdrawing groups than an acyl group lead to reasonably stable salts. For instance, the aziridine 88 reacted with methyl iodide at -50°C to give the methiodide 89 in good yield (Figure 17). The methiodide was readily cleaved by a variety of nucleophiles such as acetate, methoxide and cyanide. A mixture of the appropriately substituted isoquinuclidine 90 and its isomer 91 was obtained in each case in a ratio of about 2:1.

From the examples discussed above it became apparent that the isoquinuclidine system was ideally set up for nitrogen participation in reaction occurring
Figure 16. Nagata's synthesis of dl-ibogamine
Figure 17. Reaction of bridged aziridinium ions with nucleophiles at vicinal carbon atoms. This particular property of the isoquino-\ldots\)

Shortly before the Nagata group published their elegant synthesis of dl-ibogamine and dl-epiibogamine, Salley published a stereospecific total synthesis\textsuperscript{42} of dl-ibogamine. Salley's scheme is outlined in Figure 18.

In this particular synthesis the initial stereochemical relationship of the C-4, C-5, and C-18 protons in ibogamine was established at the outset of the synthesis. The Diels-Alder reaction between quinone and 1,3-hexadiene proceeded in the endo fashion.\textsuperscript{43} Woodward and Hoffman\textsuperscript{44} have shown that
Figure 18. Salley's synthesis of dl-ibogamine
Figure 18. Continued
endo addition allows maximum mixing of the highest occupied orbital of the one molecule with the lowest occupied orbital of the other. Thus the ethyl group in 92 bore a cis relationship to the ene-dione ring. This relationship was necessary in order that the eventually formed C-19—N-6 bridge would bear a cis relation to the same ethyl group. Also important to the synthesis was that the Beckman rearrangement of the oxime 93 proceeded to give the lactam 94 rather than the isomeric lactam which would be formed by migration of the secondary centre. Consequently, the oxime 93 must have had the trans configuration as shown since the Beckman rearrangement is known to proceed with preferential migration of the centre which bears an anti relationship to the hydroxyl of the oxime function. Sally's syntheses of dl-ibogamine provided a preparative proof for the cis relationship of the ethyl group to the C-19—N-6 bridge. Nagata's synthesis which was also stereospecific, provided the same proof.

As pointed out earlier the approach of Kutney and coworkers to the synthesis of Iboga, Aspidosperma and Vinca alkaloids required as an important step the introduction of a carbomethoxy group into the quebrachamine and dihydrocleavamine systems. In 1962 Taylor proposed a general reaction sequence (Figure 19) to explain some interesting and puzzling transformations present in the literature. It was apparent that such a sequence, if of a general nature, would provide the means of functionalizing the position adjacent to the α-position of an indole containing alkaloid. At the time Taylor made this postulate examples of such transformations were sparse. Nevertheless he was able to cite several examples from the previous literature which would fit into his general scheme. For instance, when 2,3-diethyl-3-hydroperoxyindolenine (95) which had been prepared by aerial oxidation
Figure 19. Taylor's hypothesis concerning an unusual reaction of indolenines
of the corresponding indole was subjected to refluxing water for 20 minutes, o-propionaminopropiophenone (96) was obtained as the only product (Figure 20). On the other hand, when 95 was heated at 100° for 30 minutes in the absence of solvent, only a small amount of 96 was obtained and the main product (52% yield) was 2-acetyl-3-ethylindole (97). Taylor proposed that the reaction could proceed via his mechanism (X=Y=OOH). In a subsequent step the unstable secondary hydroperoxide would decompose to the ketone 97 with loss of water. This particular example (and several others cited) involved an internal rearrangement. Another example of a reaction which Taylor believed could fit into his scheme was the bromine-acetic acid bromination of 2,3-dimethylindole and subsequent base treatment of the initial product to furnish 2-hydroxymethyl-3-methylindole (Figure 19: X = Br, Y = OH). 47
Figure 20. Reactions of a hydroperoxyindoline

A more recent example of a reaction (Figure 21) that likely proceeded according to Taylor's scheme was the conversion of tetrahydrocarbazole (98) into the pyridinium bromide 99 by the action of N-bromosuccinimide in the presence of pyridine. As a matter of interest to some later discussion workers in the same laboratory had shown that under similar conditions indoles unsubstituted at the α-position gave α-pyridinium salts as shown for 3-methyindole in Figure 22.

Figure 21. Reaction giving substitution adjacent to the α-position of an indole
Figure 22. Reaction giving substitution at the α-position of an indole

At the time that the introduction of a carbomethoxy group into the tetracyclic dihydrocleavamines was anticipated, there was available, however, what appeared to be an excellent and close analogy in the transformation of ibogaine (44) into voacangine (48) by Buchi and Manning⁵⁰ (Figure 23). According to the general scheme by Taylor, the chloroindolenine
100 would be in equilibrium with its enamine form 101 which could react with cyanide ion via a $\text{SN}_2^*$ mechanism. Regeneration of the indole system would provide a driving force for the reaction. Buchi and Manning, however, favored the imine 102 as the intermediate. They also suggested the non-classical carbonium ion 103 as a plausible alternative to the imine 102, but rejected a classical aziridinium intermediate on the grounds that such an intermediate would be severely strained in these cases and the nitrogen lone pair would not be available for displacement of the leaving group. Nevertheless at that time bridged aziridinium derivatives were completely unknown, but in view of the work of Nagata and coworkers cited previously, the classical ion 104 should also be considered as a plausible intermediate.
Of the four plausible intermediates above, it was apparent that either 101 or 102 would have to be a precursor to either of the other two plausible intermediates 103 and 104. There was some precedence for an intermediate like 101. For example, dihydrocatharanthine and related Iboga bases\textsuperscript{10,27,51-53} decarboxylate readily in acid and are believed to proceed through the indolenine 105 to give initially 106. Catharanthine itself does not decarboxylate under the same conditions. Molecular models revealed that the equivalent intermediate to 106 in the case of catharanthine would be extremely strained by comparison. Kutney and coworkers\textsuperscript{10} have postulated that the decarboxymethoxylation of catharanthine to descarbomethoxycatharanthine proceeded through several tetracyclic ions as indicated in Figure 24.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {105};
\node at (3,0) {106};
\end{tikzpicture}
\end{center}

2-Alkylidene-2H-indole intermediates such as 102 have been postulated in the lithium aluminum hydride reduction of 2-indolecarbinol derivatives.\textsuperscript{54} Dolby postulated that generation of the 2-alkylidine-2H-indole intermediates proceeded via the indole anion. Thus, the lithium aluminum hydride was required to act as a base in the initial step and as a reducing agent in the final step (Figure 25). The alternative mechanism would be direct replacement of the "X" group by hydride. Dolby and Booth demonstrated, however, that N-methylation effectively blocked the hydride reduction. They felt that this result supported their mechanism since it seemed to them that if
Figure 24. Kutney's rationalization for the decarboxylation of catharanthine
Figure 25. Dolby's rationalization for the reduction of 2-indolecarbinol derivatives

The reaction were to proceed by direct displacement by hydride the N-H compounds should be less reactive to reduction than the N-methyl compounds because formation of the indole anion would surely have preceded reduction and the anion would be expected to be less susceptible to nucleophilic attack.

It was apparent that intermediates 101 and 102 could not be ruled out as the species that initially react with the incoming nucleophile, although they both appeared to represent quite strained structures as indicated by molecular models and in the case of 102 there would be disruption of the
benzene resonance to some extent. It was attractive to postulate that 101 or 102 was initially formed, but suffered immediate attack by the N-6 lone pair of electrons (which incidently would also fit the Taylor scheme) to form either the non-classical ion 103 or the classical ion 104. The classical ion 104 appeared to be the better choice in the light of the work by Nagata and coworkers. They showed that alkylaziridinium ions such as 89 are reasonably stable entities. If 104 is redrawn to emphasize the similarity in their structures, it is seen that the indole portion in 104 forms a 6 membered ring along one of the C-N⁺ bonds of the aziridinium ring.

When a molecular model of the ion 104 was built the bridging triptyl function did not appear to bring any additional strain to bear on the bridged aziridinium system. In fact 104 appeared to be far less strained than either 101 or 102 and possess the full indole resonance as well. It could have been argued that although 104 appeared to be a nice looking intermediate, it seemed hardly necessary to postulate it since either 101 or 102 must be formed in a prior step and since the arguement has been to show that they are less stable (i.e. more reactive) why bother to postulate
The fact that internal attack by the basic nitrogen would seem to be possible hardly seemed to represent evidence that internal attack would be preferred to external attack by cyanide or another nucleophile. In this regard one piece of information was particularly interesting. The conversion of 72 (73) into 74 (75) in the Buchi synthesis proceeded without incorporation of an anion from the solvent. It appeared therefore that the steps in Figure 26 could offer the

\[
72(73) \rightarrow \text{Figure 26. Internal return within an ion pair} \rightarrow \text{76(77)}
\]

\[
107(108) \rightarrow \text{74(75)}
\]

only plausible explanation of this result. If intermediate 76 (77) gave rise to the ion pair 107 (108) then internal return within the pair would give 74 (75). Buchi and coworkers were able to incorporate solvent anions only under more vigorous conditions.

It thus appeared after close scrutiny that the successful substitution of a cyanide into the chloroindolenine of ibogaine was not a good analogy for a similar substitution into the chloroindolenines of dihydrocleavamine.
and quebrachamine if the formation of the aziridinium intermediate was an important feature of the reaction. On the other hand attack by the nitrogen's lone pair of electrons did appear to be in keeping with the Taylor hypothesis.

Buchi and Manning found that at elevated temperatures there was a serious side reaction also involving the lone pair of electrons on the isoquinuclidine nitrogen atom. These workers postulated a rearrangement as shown in Figure 27 to account for the product obtained. It was apparent that

Figure 27. An unusual rearrangement of the chloroindolenine of ibogaine in the tetracyclic series the electrons of the nitrogen atom could be involved in cleavage of the C ring in an analogous manner. Several other reactions of the chloroindolenine system were also considered to be also possible and will be discussed later. It was apparent, therefore, that the introduction of cyanide into the chloroindolenine of dihydrocleavamine in the desired manner would be difficult, if possible at all, to achieve. Nevertheless, the approach was attractive for several important reasons. It
required only three steps, in theory, to transform readily available dihydrocleavamine into a carbomethoxydihydrocleavamine. Radioactive \(^{14}\text{C}\) cyanide could be used and the radioactive carbomethoxydihydrocleavamines produced could be used in biosynthetic studies to test Wenkert's hypotheses for the biosynthesis of the Iboga alkaloids. Further, nucleophiles other than cyanide might be incorporated into the 18-position of dihydrocleavamine. In connection with the last possibility an entry into the dimeric Vinca alkaloids might be realized. Probably the most important member of this class is vincaleukoblastine (VLB), an effective anticancer agent. The structure of vincaleukoblastine has been determined by the X-ray method\(^5\)\(^5\)\(^5\) and its methiodide derivative is shown in 109. An important feature of the

![Chemical Structure](image)

X-ray diffraction analysis was that it showed that the upper portion of the dimer had a dihydrocleavamine skeleton rather than an Iboga skeleton as previously postulated.\(^5\)\(^6\)

Recently, a new dimeric alkaloid, called isoleurosine A\(^5\)\(^7\), has been reported and assigned the structure 110. The interesting feature about this compound is that the dihydrocleavamine portion is a carbomethoxydihydro-
cleavamine and it differs from vincaleukoblastine only in that it does not possess the 4-hydroxyl function. Several other dimeric Vinca alkaloids have also been isolated which also differ from vincaleukoblastine in oxidation level or placement of the oxygen atom in the piperidine ring of the dihydrocleavamine portion of the molecule. The important feature of all these compounds is that they all possess the dihydrocleavamine skeleton. From a biosynthetic point of view this is particularly interesting since neither dihydrocleavamine nor any monomeric derivatives of it have been isolated from plant sources to the present time. From a synthetic as well as a biosynthetic point of view the dimers could be thought of as arising through a coupling of a vindoline molecule with an appropriate carbomethoxy-dihydrocleavamine derivative. Indeed, similar dimerizations have been carried out in the laboratory. Buchi and coworkers\textsuperscript{28} were able to carry out an acid-catalyzed condensation of voacangine with vobasinol (111) to produce voacamine (112), an important member of the group of dimeric
Voacanga alkaloids. Vobosinol possesses a potential carbonium ion at the 3-position by virtue of the hydroxyl group and would be expected to lead to dimerization with voacangine at one of this compound's electron rich centres, i.e., at N-16, C-13, or C-11. Indeed, under mild conditions the condensation of voacangine with vobasinol yielded voacamine and a small amount of another known bisindole, voacamidine, which arose by coupling between the C-11 site of the voacangine molecule and the C-3 site of the vobasinol molecule.

Recently, Neuss, Gorman, and coworkers have reported a successful acid catalysed condensation between the chloroindolenine of 4β-dihydrocleavamine (113) and deacetylvindoline hydrazide (114) to give a dimer (115) coupled in the same fashion as vincaleukoblastine. In this portion of this thesis are described parallel experiments that were carried out in our
laboratories. In particular a description of the first successful experiments in which dimers possessing a C-18' carbomethoxy group is given. Because this functionality is present in the natural Vinca dimers these experiments will likely represent an important step in any subsequent synthesis of these alkaloids in our laboratories.
II. Discussion

The scheme that was envisaged for the introduction of a carbomethoxy group into dihydrocleavamine is shown in Figure 28 (Y = CN, R = H) (cf Figure 19). Conversion of the 18-cyano-4β-dehydrocleavamine (119; Y = CN, R = H)

![Chemical structure](image)

Figure 28. Scheme envisaged for the preparation of 18-substituted dihydrocleavamines

into carbomethoxy-4β-dehydrocleavamine was not expected to involve any difficulties. No difficulties were expected in the preparation of the chloroindolenine 113 either since the reagent t-butyl hypochlorite had been used successfully in several instances to convert indole alkaloids into
their corresponding chloroindolenines. On the other hand it was recognized that the sequence 117 → 118 → 119 might be difficult to achieve in reality.

Treatment of 4β-dihydrocleavamine (116; R = H) with t-butyl hypochlorite gave a mixture of products with one component in predominance. It was found, however, that the yield of the major product varied considerably according to the procedure used in the reaction and work up. In all experiments the reaction was carried out by adding a standard carbon tetrachloride solution of t-butyl hypochlorite to a chilled methylene chloride solution of 4β-dihydrocleavamine containing a catalytic amount of triethylamine. It was observed that as the t-butyl hypochlorite solution was added to the solution of the 4β-dihydrocleavamine, the latter solution became steadily more orange in colour, until an equivalent amount of the oxidant had been added. A greatly accelerated deepening of the orange colour then occurred as excess oxidant was added. The progress of the reaction could conveniently be followed by analytical thin-layer chromatography (alumina, benzene or silica gel, chloroform:ethyl acetate 1:1). It was observed that the sudden increase in coloration of the reaction medium was concomitant with the disappearance of 4β-dihydrocleavamine and what appeared to be an increase in materials that were very polar in nature. Washing the reaction solution with water was found to be ineffective in removing the coloured and polar impurities. It was discovered, however, that a colourless sample of the major product from the oxidation reaction, which showed only one spot by thin-layer chromatographic analysis with alumina and silica gel absorbents and several systems, could be obtained by first mixing the reaction solution with an equal volume of benzene and percolating the new solution at a rapid rate through a short, broad alumina (Woelm activity III) column. Evaporation of
the solvent from the eluant first in a rotatory evaporator at reduced pressure and room temperature and finally in vacuo at a pump provided a pure sample of the major reaction product as a colourless oil. It was found that the best yields were obtained when the percolation step above was aided by the application of positive pressure to the top of the column in order to maintain a steady stream of solution from the column. Use of the procedures described above resulted in consistent yields of the major oxidation product in the range of 80 to 90 percent. Although this material was found to run as one compound in a variety of thin-layer chromatography systems and so appeared to be a single compound, it could not be induced to crystallize. In fact all attempts to crystallize the material resulted in decomposition to a greater or lesser degree. It was realized that the material could be a mixture. In particular, it seemed very probable that it could be a mixture of the C-9 epimers of the desired chloroindolenine 113. The material however, always appeared to be a single compound when it was subjected to thin-layer chromatographic or spectral analysis. The spectral properties of the material were in accord with the formulation 113. The infrared spectrum showed bands at 1600 cm⁻¹ (m) and 1560 cm⁻¹ (s). This pattern is a characteristic feature of chloroindolenines₅₀,₅₈-₆₀. The ultraviolet spectrum and the nmr spectrum were also in keeping with the formulation 113. The low resolution mass spectrum was particularly informative and is shown in Figure 29. These were two molecular ion peaks at m/e 316 (³⁵Cl) and 318 (³⁷Cl) as would be expected. The base peak at m/e 281 corresponded to a loss of a chlorine atom from the molecular ion. Other peaks typical of the cleavamine system were also present. The molecular formula of the material was determined for the molecular ion at m/e 316 and was found to be C₁₉H₂₅N₂Cl. The material was, therefore,
Figure 29. Mass spectrum of the chloroindolenine of 4β-dihydrocoleamine.

RELATIVE ABUNDANCE

\[ 316(M^{+}(^{37}Cl)) \]

124

138
unquestionably a monochloro derivative of 4β-dihydrocleavamine. All the spectral data were in accord therefore with the formulation of a chloroindoleneine 113 assuming no rearrangement of the cleavamine skeleton had taken place. That no rearrangement had occurred was established when 4β-dihydrocleavamine was regenerated on treatment of the chlorinated material with lithium aluminum hydride in diethyl ether.

After the product of the chlorination reaction had been satisfactorily established as the desired chloroindolenine 113, the reaction of this substance with cyanide ion was contemplated. It was recognized that a successful conversion of the chloroindolenine 113 into an 18-cyano-4β-dihydrocleavamine (119; R = H, Y = CN) would depend, among other things, upon the equilibrium 117 $\rightleftharpoons$ 118 being established under the reaction conditions employed. It was clear from the nmr spectrum (Figure 30) of the chloroindolenine, which showed no resonances that might correspond to the α-methylene-indoline form (118; R = H), that the indolenine form (117; R = H) in deuteriochloroform at least, was by far the predominant form. Nevertheless, the ultraviolet spectrum of the chloroindolenine did show some pH dependency that could have been related to the desired imine-enamine tautomerization. In the absence of a more analogous sequence, the same conditions which had been used by Buchi and his coworkers to bring about the formation of 18-cyanoibogaine from the chloroindolenine of ibogaine were chosen for the study at hand. Thus, the chloroindolenine of 4β-dihydrocleavamine was allowed to react with a complex mixture of potassium cyanide, methanol, diethyl ether and water for 48 hours at room temperature under a nitrogen atmosphere. These conditions were found by the Buchi group to be optimum for the formation of the desired product in their sequence. In the
Figure 30. Nmr spectrum of the chloroindolenine of 4β-dihydrocleavamine
present case, however, a very complex mixture of products was obtained. Several modifications of the reaction conditions were used in an attempt to improve the reaction, but no discernable improvement could be made. Although the results were very discouraging, they were not really unexpected. It was apparent that the chloroindolenine had the potential to undergo many transformations other than the desired one. Several modes by which the chloroindolenine might conceivably have reacted are indicated in Figure 31. Reasonable arguments could be presented for the modes (a to i) of reaction shown. For example reaction mode "c" seemed to have a high probability of success. It has been demonstrated that the diastereomeric chloroindolenines derived from yohimbine are converted to imidoethers on treatment with acid or base. 7-Chloro-7H-yohimbine (120) on treatment with methanolic alkali was found to be converted into the corresponding spiroimidoether 121. The transformation was envisaged to proceed through attack of the methoxide in a cis manner to the chlorine atom allowing the migrating centre to displace chloride ion by trans attack (Figure 32). Buchi and coworkers found that
the chloroindolenine of ibogaine did not undergo such a rearrangement. On the basis of this result they tentatively proposed that the chlorine atom was \(\beta\)-oriented since of the two theoretically possible diasteriomers the one with the \(\alpha\) configuration of the chlorine atom would have led to an excessively crowded intermediate through "cis" attack by methoxide ion. In the case of either of the two possible chloroindolenines of 4\(\beta\)-dihydrocleavamine, which would have a more flexible nature, there seemed to be no steric inhibition to formation of an intermediate with the cis arrangement of the
chlorine atom and a methoxyl (or hydroxyl) group. Spiroimidoether or spirooxindole formation appeared to be a favorable reaction in the case of a chloroindolenine prepared from 4β-dihydro-α-avamine.

As far as reaction mode "a" was concerned it represented a simple $\text{SN}_2$ displacement of chloride ion albeit at a tertiary centre. A $\text{SN}_1$ displacement involving, perhaps, a 2-alkylidene-2H-indole intermediate was also conceivable. If the attacking nucleophile were water, the β-hydroxyindolenine 122 ($Y = \text{OH}$) would be formed which bears a striking resemblance to the conjugate base form 12 of the Aspidosperma alkaloid rhazidine (10). Rhazidine has been shown to exist in its salt form in acidic or neutral alcoholic solutions. The conjugate base form was observed in strongly basic alcoholic solutions or in hydrocarbon solvents such as heptane. It was tempting to consider that compounds such as 122 ($Y = \text{OH, Cl, CN, OMe}$) might also be represented by the structures 123 ($Y = \text{OH, Cl, CN, OMe}$) under the proper conditions.

Reaction mode "b" was considered although it represented a very unusual $\text{SN}_2'$ reaction. There was theoretical justification for such a reaction and a remote analogy which could be found in the literature is shown in Figure 33.
Figure 33. An unusual SN$_2$' reaction involving nucleophilic attack on oxygen.

Reaction mode "c" was expected to occur since the implied reaction was found to have been a serious competing reaction with the desired transformation in the conversion shown in Figure 27. In the case of the chloroindolenine of 4β-dihydrocleavamine it was felt that this mode would lead to an intermediate (124) which could undergo a variety of reactions.

Reaction mode "f" had to be considered as a possibility. It was felt that elimination of hydrogen chloride in the manner indicated would provide the α,β-unsaturated imine 125 which could undergo Michael addition of cyanide and regeneration of the indole would provide a strong driving force for the addition reaction. The product, 8-cyano-4β-dihydrocleavamine (126),
would be expected to be very difficult to distinguish from the desired 18-cyano isomer.

\[ \text{Diagram 125} \quad \text{Diagram 126} \]

Reaction mode "g" was an interesting possibility since such a trans-annular cyclization reaction would produce a quaternary salt (56) which was encountered as a key intermediate in Kutney and coworkers' synthesis of dihydrocleavamine (Figure 8).

From a cursory examination by thin-layer chromatography of the reaction mixture from the reaction of the chloroindolenine of 4β-dihydrocleavamine under the Buchi conditions, it seemed that every conceivable reaction and a few inconceivable ones as well might have taken place. There were a great many products formed and no one product seemed to be dominant. Chromatography of the reaction mixture on Woelm alumina (activity III) accomplished a resolution of the mixture into three groups of products. Elution with benzene provided group "A", elution with chloroform-benzene provided group "B" and elution with several solvent systems including methanol-water provided group "C". Each group represented about one-third of the total reaction mixture. After a cursory spectroscopic examination, it was decided that group A was the most likely group to contain the sought after 18-cyano-4β-dihydrocleavamine. Although the compounds in group A showed almost
identical rates of transport on alumina chromatoplates, there was a more distinct difference in the rates of transport on silica gel chromatoplates. Because of this difference, group A was chromatographed on Woelm silica gel (activity III). Elution with benzene-chloroform (3:1) provided a series of fractions called group A which were shown to be a mixture of at least two compounds by analytical thin-layer chromatography on freshly activated silica gel plates. Elution with 2% triethylamine in acetone provided a material \( A_3 \) which could be obtained in crystalline form from methanol. The chromatographic separation procedure is summarized in a flow sheet shown in Figure 34.

Figure 34. Flow sheet showing the partial separation of the products of the reaction of the chlorcindolene 113 under the Buchi conditions.
Group A resisted complete resolution into its component compounds. Nevertheless, a very small sample of the nitrile containing component of group A was obtained by carrying out several successive purifications on freshly activated silica gel preparative chromatoplates. The spectral data which could be obtained for this material was consistent with it being the sought for 18-cyano-4β-dihydrocleavamine. It exhibited a typical indole ultraviolet spectrum. Its infrared spectrum displayed absorptions at 3300 cm⁻¹ and 2220 cm⁻¹ which were consistent with the compound having an indole NH group and a nitrile group. The low resolution mass spectrum of the material displayed a molecular ion peak at m/e 307 and a typical cleavamine-type fragmentation pattern. High resolution mass analysis on the m/e 307 peak provided a molecular weight for the material of 307.205 which corresponded to the formula C₂₀H₂₅N₃ establishing that the material indeed was a cyano-4β-dihydrocleavamine. A sample of group A which had been purified to the extent of about 90% in the nitrile containing component gave a nmr spectrum which was consistent with that which was expected for the desired material.

Because it proved to be difficult to resolve group A into its components it was decided to proceed with the mixture in the next step of the syntheses; that is, to convert the nitrile to a methyl ester. It was hoped that purification of the ester would prove to be easier than purification of the nitrile. When a sample of group A was allowed to react with anhydrous methanolic hydrogen chloride and then with aqueous sodium carbonate, a complex mixture of compounds was obtained. Fortunately, a small amount of authentic 18β-carbomethoxy-4β-dihydrocleavamine, obtained as a minor product in the zinc-acetic acid reduction of catharanthine, was available. Comparison of the reaction mixture with the authentic sample by thin-layer
chromatography indicated that the compound was present in the mixture. A combination of the methods of column chromatography on alumina and preparative thin-layer chromatography on silica gel provided a pure sample of the compound believed to be 18β-carbomethoxy-4β-dihydrocleavamine. This material displayed the same rates of transport and colour reactions as the authentic material on analysis by thin-layer chromatography. This material also exhibited the same fragmentation pattern in its mass spectrum as the authentic sample when both compounds were run under the same conditions. High resolution mass analysis of the molecular ion peak at m/e 340 provided a molecular weight for the material of 340.212 (calcd mol wt of 18β-carbomethoxy-4β-dihydrocleavamine, 340.215).

Although there was no doubt that the methanolic hydrogen chloride treatment of a sample of group A had achieved the desired conversion, the yield of 18β-carbomethoxy-4β-dihydrocleavamine was terrible. Since one of the principle reasons for developing a method for introducing a carbomethoxy group into dihydrocleavamine via a cyano intermediate was that potassium cyanide-¹⁴C could be used to prepare ¹⁴C-labelled carbomethoxydihydrocleavamine for tracer experiments in plants, it was of particular importance to develop as high a yielding sequence as possible. It was clear that an attempt to improve the method for making the 18-cyano-4β-dihydrocleavamine would involve a considerable expenditure of time and effort with no guarantee of success. Therefore attention was directed to improving the yield of 18β-carbomethoxy-4β-dihydrocleavamine from the nitrile. It was found that when a sample of group A₁, which contained radioactive 18-cyano-4β-dihydrocleavamine from a preparation utilizing potassium cyanide-¹⁴C, was converted to 18β-carbomethoxy-4β-dihydrocleavamine in such a manner that all the radioactivity present in that compound was scavenged with inactive compound, the
activity present as 18β-carbomethoxy-4β-dihydrocleavamine represented only 5% of the activity present before the conversion. It was decided, therefore, to attempt a basic hydrolysis of the nitrile. Accordingly, a sample of group A containing radioactive nitrile was allowed to react with potassium hydroxide in diethylene glycol at 150° for 8 hours under a nitrogen atmosphere. The carboxylic acid formed was not isolated, but was immediately converted into its methyl ester by treatment of the mixture of reactants with methanolic hydrogen chloride and an ethereal solution of diazomethane. The 18β-carbomethoxy-4β-dihydrocleavamine formed was estimated as before to contain not less than 35% of the activity presented in the sample of radioactive group A. Thus base hydrolysis of the nitrile followed by esterification with diazomethane was shown to be a much better method of producing 18β-carbomethoxy-4β-dihydrocleavamine from the 18-cyano-4β-dihydrocleavamine component of group A. Using this method, enough synthetic 18β-carbomethoxy-4β-dihydrocleavamine in crystalline form was obtained for the purposes of melting point and infrared comparisons with an authentic sample. The synthetic and authentic samples had the same melting point, did not show depression of melting point on admixture and displayed identical infrared spectra. The synthetic carbomethoxydihydrocleavamine was thus irrefutably shown to be 18β-carbomethoxy-4β-dihydrocleavamine and not another possible isomer such as an 8-carbomethoxy-4β-dihydrocleavamine which would be formed from an 8-cyano-4β-dihydrocleavamine (116).

Compound A proved to be a very interesting compound. Although the yield (7%) of this compound was fairly small, it was nevertheless a major component of the complex mixture of products obtained when the chloroindolenine of 4β-dihydrocleavamine was allowed to react under the Buchi conditions. The compound was found to display a typical indole chromophore in it's
ultraviolet spectrum. The infrared spectrum showed a strong absorption band at 3280 cm\(^{-1}\) indicating the presence of an indole N-H grouping and displaying no bands in the region 2500-1500 cm\(^{-1}\). Of particular note was a strong band at 1070 cm\(^{-1}\) which indicated the presence of a -C-O-Me grouping. The thought that the compound might be an 18-methoxy-4\(B\)-dihydrocleavamine epimer (119, R = H, Y = OMe) was seriously entertained. The low resolution mass spectrum (Figure 35) of this compound supported this conjecture. It displayed a molecular ion peak at m/e 312 and a fragmentation pattern which was a typical cleavamine type. The molecular weight of the compound was found to be 312.220 (calcd mol wt, 312.220) by high resolution mass spectrometric determination. The nmr spectrum (Figure 36) displayed a prominent signal at \(\tau 6.8\) which corresponded to 3 protons in the integral. This feature verified the presence of a methoxyl function in the compound. The rest of the nmr spectrum was consistent with the compound being a methoxy-dihydrocleavamine. The compound was tentatively proposed to be 18-methoxy-4\(B\)-dihydrocleavamine although on the basis of the data C-8 could not be excluded as the site of the methoxyl function. Compelling nmr evidence to support the claim that this compound is 18\(a\)-methoxy-4\(B\)-dihydrocleavamine will be presented later.

Some attention was directed towards determining the nature of the compounds in group B. This mixture was found to behave almost as one compound during analysis by thin-layer chromatography. The spectral evidence, however, was not consistent with it being a single compound. The ultraviolet spectrum seemed to be made up of a superposition of chromophores. The infrared spectrum displayed bands which were indicative of an indole NH and a nitrile, probably conjugated judging by the intensity of the absorption.
Figure 35. Mass spectrum of 16a-methoxy-4β-dihydroclemamine

Relative Abundance

124
138
182 (M-130)
312 (M+)
Figure 36. Nmr spectrum of 18α-methoxy-4β-dihydrocleavamine
The nmr spectrum displayed four sharp singlets in the region \( \tau 5.0-7.0 \) and two broad "singlets" in the region \( \tau 1.0-2.0 \) in which protons on indole nitrogen often occur. A careful chromatography of the mixture provided a partial separation. The nmr spectrum of selected fractions showed there was a definite trend in the variation of the intensity of signals. It was determined, therefore, that there were at least three compounds in the mixture and because the rates of transport of the compounds were so nearly the same, no attempt was made to further resolve the mixture.

A cursory examination of group C by thin-layer chromatographic analysis showed that this was also a mixture which would not lend itself to easy resolution into its components.

It was clear from the results above that the 4β-dihydrocleavamine could be converted into derivatives possessing substitution at the C-18 site. Nevertheless, the conditions used were obviously not ideal. Enough 18-cyano-4β-dihydrocleavamine could not be obtained for complete characterization because of purification problems. In addition the yield of this material was very poor. It was felt that if the reaction 117 \( \rightarrow \) 124 was indeed a serious side reaction as it had been shown to be by Buchi and his coworkers in the pentacyclic series, then using conditions which were designed to suppress this undesirable side reaction might lead to a better yield and a cleaner reaction. In view of this, acidic conditions under which the basic nitrogen would be expected to be quaternary were chosen. An anhydrous methanolic hydrogen chloride solution was added to a mixture of potassium cyanide and the chloroindolenine of 4β-dihydrocleavamine in a flask fitted with an efficient condenser and cooled to ice-water temperature. Escaping hydrogen cyanide gas was passed into an aqueous potassium hydroxide solution.
The solution obtained was then heated to reflux temperature and refluxed for three hours. The reaction-product mixture obtained on work up was compared by thin-layer chromatography with the 18-cyano-4β-dihydrocleavamine and 18α-methoxy-4β-dihydrocleavamine. There was no sign of the former compound, but there was a major component of the mixture which corresponded to the latter compound. That none of the desired nitrile appeared to be formed was not unexpected because the concentration of cyanide ion would be expected to be very small under the acidic conditions employed. The crude reaction mixture was chromatographed on alumina (activity III). Elution with petroleum ether (30-60)-benzene (3:1) provided a mixture of the compound which corresponded to 18α-methoxy-4β-dihydrocleavamine and another compound which was slightly less polar. The nmr spectrum of the mixture indicated that it was a mixture of both epimeric 18-methoxy-4β-dihydrocleavamines. On this assumption the yield of the methoxydihydrocleavamines was calculated to be 39 percent. This result represented a more than five fold increase in the yield compared to the previous reaction. Chromatography of the mixture on silica gel (activity III) provided a pure sample of each isomer. On the basis of a comparison of the spectra of these compounds, the less polar compound (10% yield) was considered to be 18β-methoxy-4β-dihydrocleavamine and the more polar compound (24% yield) was shown to be 18α-methoxy-4β-dihydrocleavamine. The mass spectrum and the nmr spectrum of the less polar methoxydihydrocleavamine are shown in Figures 37 and 38, respectively. The nmr spectra of the substituted dihydrocleavamines are discussed and the reasons for the stereochemical assignments are presented later on in this section.

In view of the higher yield of the 18-methoxy-4β-dihydrocleavamines
Figure 37. Mass spectrum of 188-methoxy-4β-dihydrocleavamine
Figure 38. Nmr spectrum of 18β-methoxy-4β-dihydrocleavamine
obtained in the reaction described above, it was decided to attempt to prepare a derivative of 4β-dihydrocleavamine which possessed a good leaving group at C-18. The direct displacement of such a group with cyanide ion was anticipated. It was felt that a halogen would be a desirable leaving group. Consequently, an attempt to prepare an 18-iodo-4β-dihydrocleavamine was made. The hydrochloride salt of the chloroindolenine of 4β-dihydrocleavamine was prepared. To the salt in a small amount of anhydrous acetone was added a solution of sodium iodide in anhydrous acetone. A dark purple coloured solution formed instantaneously. The colour indicated the presence of iodine. The solution was stirred for 3 hours under a dry, oxygen-free atmosphere at room temperature and then worked up. The iodine colour was effectively removed by washing an ethereal solution of the reaction mixture with an aqueous sodium thiosulfate solution. Investigation of the crude reaction mixture by analytical thin-layer chromatography showed the presence of 4β-dihydrocleavamine and a mixture of compounds that were much too polar by comparison to contain the desired iodo compounds. Chromatography on alumina (activity III) provided 4β-dihydrocleavamine in 22 percent yield. It was recalled that Dolby and Gribble had reported a similar reduction of a chloroindolenine. They found that when the mixture of chloroindolenine epimers shown as 127 was treated with potassium t-butoxide the tetracyclic amine 128 was formed. They proposed that the reaction took place by nucleo-
philic attack on chlorine. The same sort of proposal could be made for the reduction of the chloroindolenine of 4β-dihydrocleavamine by iodide ion. The liberation of iodine on treatment of a haloindolenine with iodide has been previously reported. In 1933 Plant and Tomlinson reported that 2-phenyl-3-methylindole on bromination gave a compound (which was presumably the 3-bromoindolenine) that liberated iodine on treatment with aqueous potassium iodide.

Because direct preparation of an 18-halo-4β-dihydrocleavamine from the chloroindolenine did not appear to be feasible on the basis of the above results, an attempt was made to prepare such a compound from the 18-methoxy-4β-dihydrocleavamines. The boron trihalides have been used successfully in many instances to cleave ethers to the corresponding alkyl halides with the direction of cleavage favoring the direction which would give rise to the most stable carbonium ion. In addition, boron tribromide was used successfully to cleave benzyl ethers 53 and 54 in the quebrachamine and dihydrocleavamine syntheses (Figure 8) developed in our laboratories. In the present case treatment of a mixture of 18-methoxy-4β-dihydrocleavamines with boron trichloride produced a complex mixture of products and the method was abandoned. An attempt to hydrolyse the 18-methoxy-4β-dihydrocleavamines with aqueous hydrochloric acid to give the corresponding 18-hydroxy-4β-dihydrocleavamines also gave a complex mixture of products. In view of these results it was apparent that the 18-methoxy-4β-dihydrocleavamines were acid sensitive in an undesirable fashion.

It was felt at this stage that direct formation of a 4β-dihydrocleavamine derivative with a suitable leaving group at the C-18 site from the chloroindolenine offered the best chance of success. It was decided that a
C-18 acetoxy group might be a suitable group. Although an acetoxy group under ordinary circumstances would not be a particularly good leaving group, it was felt that it might be in this case and, if not, there was good reason to believe that it could be converted by mild base hydrolysis into a C-18 hydroxy group. The latter substituent might in turn be converted into one of several good leaving groups. Dolby and Sakai\textsuperscript{71} found that the acetoxy lactam 129 gave the hydroxylactam 130 in 52 percent yield on standing for 2 hours at room temperature in a water-methanol (1:1) solution containing 5% sodium hydroxide. The hydroxylactam 130 was converted in quantitative yield to the acetoxy lactam 129 when it was treated with a sodium acetate-acetic acid solution for thirty minutes at room temperature.

The chloroindolenine of 4β-dihydrocleavamine was treated with a 10% solution of fused sodium acetate in glacial acetic acid. The reaction was carried out at 60°C in an atmosphere of dry, oxygen-free nitrogen and the progress of the reaction was followed by thin-layer chromatography (alumina, 3:1 benzene-ethylacetate; silica gel, 2.5% trimethylamine in ethyl acetate). In both thin-layer chromatography systems a reaction product whose rate of
transport was slightly larger than that of the chloroindolenine appeared. Aside from "baseline" material there was no indication of other products. After about 30 minutes the amount of this reaction product did not appear to increase in proportion to the amount of chloroindolenine whereas the amount of "baseline" material steadily increased in proportion to the chloroindolenine. After two hours only "baseline" material remained. Investigation by thin-layer chromatography (alumina, 3:1 ethyl acetate-ethanol) of the baseline material indicated that it was composed of two compounds. Development of a chromatoplate with antimony pentachloride revealed two overlapping spots that were slightly different shades of green. Before the reaction products obtained at 60° were investigated further, the reaction was repeated as before with the exception that it was carried out at room temperature. At room temperature the reaction proceeded at a slower rate as expected, but there was no noticeable change in the product distribution. Since it was apparent that the reaction could not be carried out without the formation of the very polar components even at room temperature, the nature of the polar compounds was investigated further. The behavior on thin-layer chromatography and solubility properties of the mixture of these polar compounds suggested that they were salts. In fact these properties were reminiscent of those possessed by the quaternary ammonium mesylate salts 55 and 56 which were prepared in the course of the synthesis of quebrachamine and dihydrocleavamine (see Figure 8) carried out in our laboratories. The ultraviolet spectrum of the mixture of these compounds was almost precisely the same as those obtained for the quaternary ammonium mesylate salts. A rationale could be made for the formation of a quaternary ammonium salt. Such a salt could conceivably be formed by reaction mode "g" or by "i" (Y = OAc) followed by "g" as indicated in Figure 31. To test the conjecture that the
polar mixture might consist of the two epimerically related quaternary salts 131 and 132, a small sample of it was subjected to treatment with lithium aluminum hydride in refluxing N-methylmorpholine. This procedure had been shown in our laboratory to result in the reduction of the quaternary salts in the quebrachamine series to give a fair yield of quebrachamine. In the present case the procedure resulted in the regeneration of some 4β-dihydrocleavamine. In view of this result the mixture was assumed to consist of 131 and 132, although polymeric systems such as the dimer 133 were conceivable by intermolecular rather than intramolecular attack of nitrogen. In this regard, however, a study of molecular models
of the epimerically related 18-acetoxy-4β-dihydrocleavamines, which were considered to be the most likely intermediates in the formation of the quaternary salts by analogy with the reaction of the chloroindolenine of 4β-dihydrocleavamine with methanol that was discussed earlier, showed that suitable conformations for intramolecular attack by nitrogen could be easily achieved. It was also observed that if the reaction were to proceed by a direct displacement mechanism, the conformation 134 in the case of the 18β-epimer would lead to the quaternary salt 131 and the conformation 135 in the case of the 18α-epimer would lead to the quaternary salt 132. There was good evidence from the nmr studies which will be mentioned later that in the

![Chemical structures](image)

134 135

case of the 18β-acetoxy epimer the conformation shown in 134 would be the preferred conformation of the molecule. Several attempts were made to show that the 18-acetoxy epimers were formed in the reaction of the chloroindolenine of 4β-dihydrocleavamine with sodium acetate in acetic acid. It was felt that the material which possessed a slightly larger rate of transport on thin-layer chromatography than the chloroindolenine might consist of one or a mixture of the 18-acetoxy-4β-dihydrocleavamine. Although elaborate
steps were taken to secure a pure sample of this material no success was achieved. A crude mixture of the desired material with unreacted chloroindolenine and the quaternary salts could be obtained by allowing the reaction to proceed for 30 minutes, then rapidly quenching the reaction in a rapidly stirred mixture of methylene chloride and aqueous ammonium hydroxide cooled to ice-acetone temperature, separating the organic phase, drying with anhydrous sodium sulfate, and removing the solvent at room temperature in a rotary evaporator. Chromatography on alumina resulted in complete loss of the desired material and the formation of a new material. Chromatography on silica gel was preferable. Elution with 2% triethylamine in ethyl acetate provided a material which was initially free of the quaternary salts but only partially free of the chloroindolenine. Some of the material encountered in the attempt to chromatograph the mixture on alumina was also formed. The longer the material was on the column the more the new material formed. Consequently, a careful chromatography could not be carried out. In addition quaterization of purified material occurred even as it stood at room temperature. Nevertheless, the ultraviolet, infrared, and nmr spectra of the partially purified material suggested the presence of an 18-acetoxy-4β-dihydrocleavamine. Since it was obvious that obtaining a pure sample of an 18-acetoxy-4β-dihydrocleavamine would be exceedingly difficult if not impossible, attention was directed to the material which was seen to be formed when the crude reaction product was chromatographed. This newly formed material must have arisen from the material whose rate of transport on thin-layer chromatography was seen to be slightly greater than that of the chloroindolenine since chromatography of a pure sample of the chloroindolenine on alumina did not give rise to any of it. It was felt therefore
that the new material might be a mixture of the two $18$-$\text{hydroxy}-4\beta$-$\text{dihydrocleavamines}$. In fact this material was single compound. All the evidence including that provided by the mass spectrum (Figure 39) was consistent with this compound being an $C$-$18$-$\text{hydroxy}$ epimer and on the basis of the nmr spectrum (Figure 40) it was proposed to be $18\beta$-$\text{hydroxy}-4\beta$-$\text{dihydrocleavamine}$. The nmr spectrum showed a broad signal at $\tau 1.4$ which was attributed to the OH proton and a poorly defined multiplet at $\tau 4.78$ which was attributed to the $C$-$18$ proton. Since in the spectra of related compounds the $C$-$18$ proton resonances occurred as a pair of doublets or in some cases, a doublet, the poorly defined nature of the multiplet in the nmr spectrum suggested that the $C$-$18$ proton was coupled to the OH proton. Therefore the deuteriochloroform solution was shaken with dueterium oxide and the nmr spectrum rerun. The signals attributable to the OH and NH protons disappeared and in addition the multiplet at $\tau 4.78$ became a distinct doublet in appearance. These results were entirely consistent with the compound being an $18$-$\text{hydroxy}-4\beta$-$\text{dihydrocleavamine}$. The reasons for assigning the $\beta$-configuration to the hydroxyl group in this compound will be discussed later.

In view of the problems encountered in the attempted preparation of an $18$-$\text{acetoxy}-4\beta$-$\text{dihydrocleavamine}$, it seemed that preparation of a compound with a suitable leaving group at $C$-$18$ for displacement by cyanide and not by the basic nitrogen would be difficult to achieve. Instead of pursuing this problem, it was decided to attempt to use the mixture of quaternary salts 131 and 132, which could be obtained in 85% yield, to prepare an $18$-$\text{cyano}-4\beta$-$\text{dihydrocleavamine}$. There was reason to believe that this approach would be successful. In our laboratory a fairly complete study of the reaction of the quaternary salts in the quebrachamine series with cyanide
Figure 39. Mass spectrum of 188-hydroxy-48-dihydrocleavamine
Figure 40. Nmr spectrum of 188-hydroxy-48-dihydrocleavamine
had been worked out which gave the desired nitriles in reasonable yield. In addition, Harley-Mason and coworkers has reported some success in a similar reaction in the dihydrocleavamine series.

Samples of the mixture of quaternary salts 131 and 132 were allowed to react with cyanide under a variety of conditions. The reactions were monitored by thin-layer chromatography using for comparison purposes a sample of the 18-cyano-4β-dihydrocleavamine prepared previously. The best yield of the compound which corresponded to the 18-cyano-4β-dihydrocleavamine was obtained when a solution of the mixture of quaternary salts and potassium cyanide in dry dimethylformamide was refluxed in a dry, oxygen-free nitrogen atmosphere for 100 minutes. In this manner a 24% yield of this compound was obtained after chromatography of the crude reaction product on silica gel. The compound was obtained in crystalline form from methanol. The spectral characteristics of this compound including those of the mass spectrum (Figure 41) were consistent with it being the same as the 18-cyano-4β-dihydrocleavamine which had been prepared before, but which could only be obtained in pure form after an extensive chromatographic separation procedure and then only in sub milligram amounts. On the basis of its nmr spectrum (Figure 42) which will be discussed later this compound was proposed to be 18β-cyano-4β-dihydrocleavamine. Using the better of the procedures developed previously for the conversion of the nitrile to the corresponding carbomethoxy derivative, a sample of pure 18β-cyano-4β-dihydrocleavamine was converted into 18β-carbomethoxy-4β-dihydrocleavamine in 53% yield.

The stereochemical assignments concerning the configuration of the C-18 function in the several derivatives of 4β-dihydrocleavamine discussed above were made on the basis of a comparison of the nmr spectra of these compounds.
Figure 44. Mass spectrum of 188-cyano-4b-dihydrocavamine.
Figure 42. Nmr spectrum of 18β-cyano-4β-dihydrocleavamine
and several others which were available in our laboratory. The shifts of the pertinent resonances are given in Table 1. Comparison of the resonances

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \text{CH}_2\text{-CH}_3(\tau) )</th>
<th>( \text{C}_{18}\text{H}(\tau) )</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>136, ( Y=\text{COOMe}; R=H, R'=\text{CH}_2\text{CH}_3; R''=H, R'''=H )</td>
<td>9.33</td>
<td>6.13</td>
<td>9,14,73</td>
</tr>
<tr>
<td>137, ( Y=\text{COOMe}; R=H, R'=H, R''=\text{CH}_2\text{CH}_3; R'''=H )</td>
<td>9.09</td>
<td>4.53</td>
<td>73</td>
</tr>
<tr>
<td>138, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.45</td>
<td>6.12</td>
<td>73</td>
</tr>
<tr>
<td>139, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.12</td>
<td>4.98</td>
<td>73</td>
</tr>
<tr>
<td>140, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.46</td>
<td>5.50</td>
<td></td>
</tr>
<tr>
<td>141, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.11</td>
<td>4.76</td>
<td></td>
</tr>
<tr>
<td>142, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.17</td>
<td>4.77</td>
<td></td>
</tr>
<tr>
<td>143, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.10</td>
<td>4.58</td>
<td></td>
</tr>
<tr>
<td>144, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.16</td>
<td>6.20</td>
<td>31,75</td>
</tr>
<tr>
<td>145, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.33</td>
<td>3.75</td>
<td>31,74</td>
</tr>
<tr>
<td>146, ( Y=\text{COOMe}; R=\text{CH}_2\text{CH}_3; R'=H, R''=H, R'''=H )</td>
<td>9.10</td>
<td>6.10</td>
<td>31,74</td>
</tr>
</tbody>
</table>
showed that whenever both C-18 epimers of a particular dihydrocleavamine or quebrachamine derivative were available the resonances of the C-18 proton in the epimer in which the substituent was assigned the \( \beta \)-configuration occurred downfield from the resonances of the C-18 proton in the \( 18\alpha \)-substituted epimer. The position of the resonances of the C-18 proton in the \( \alpha \)-epimers was that which would be considered as normal for the C-18 derivative in question. It was apparent that there must be some effect that was peculiar to the \( \beta \)-epimers which caused a chemical shift difference between the C-18 proton resonances of \( \tau \) 0.74 to \( \tau \) 2.35. The magnitude of this difference seemed to be extraordinarily large, but could be explained if the appropriate conformations for the members of the two series (\( 18\alpha \) and \( 18\beta \)) were assumed. In the compounds having the \( 18\beta \) configuration for the substituent it was seen that in only one of the possible conformations could the C-18 proton come in very close proximity to the basic nitrogen atom. In fact in molecular models the hydrogen and nitrogen atoms were seen to be in such close proximity that it was apparent that in these molecules there was likely to be a strong intramolecular van der Waals interaction. Because of the close proximity of the C-18 proton and the basic nitrogen of the piperidin moiety in the conformation shown, it followed that the C-18 proton would resonate at lower frequency. Mokry and Kompis who originally observed this effect\(^{76}\) in the epimeric pair, vincaminoreine (146) and vincacaninorine (145), also showed that these two compounds exhibited very slow rates of methiodide formation\(^{77}\) which could only be rationalized on the basis that these compounds possessed a conformation as shown in 145 and 146 in which the lone pair of electrons on the basic nitrogen atom is sterically shielded by the indolic bridge.

There was also a difference observed in the position of the methyl
proton resonances between the two groups of derivatives. In the case of the dihydrocleavamine derivatives the 18β epimers showed the resonances due to the methyl proton of the ethyl side chain at lower field than the resonances shown by the 18α-epimers. In the case of the quebrachamine derivatives, the opposite relationship was shown. Whatever the effect which resulted in the observed difference it did not seem to be dependent on the configuration of the 4-ethyl group.

Another striking difference between the 18α and 18β groups of epimers was that the nmr spectra of the former group showed in all the cases, which we have studied, a multiplet which corresponded to one proton and resembled a doublet in the region \( \tau 6.0-7.0 \). No such doublet was observed in this region in the 18β-epimers and presumably in this group the equivalent signals occur under the methylene envelope. The position of this "doublet" in the α-epimers is given in Table 2. The interesting feature of this difference

<table>
<thead>
<tr>
<th>Compound</th>
<th>Position of approx. doublet</th>
</tr>
</thead>
<tbody>
<tr>
<td>18α-carbomethoxy-4α-dihydrocleavamine</td>
<td>6.54</td>
</tr>
<tr>
<td>18α- &quot; -4β- &quot;</td>
<td>6.73</td>
</tr>
<tr>
<td>18α-methoxy-4β- &quot;</td>
<td>6.72</td>
</tr>
<tr>
<td>vincadine</td>
<td>6.77</td>
</tr>
<tr>
<td>vincaminoreine</td>
<td>6.89</td>
</tr>
</tbody>
</table>

between the 18α- and 18β-epimers was that the difference appeared to be independent of the configuration and site of the ethyl side chain and also appeared to be independent of the nature of the substituent at C-18.

The nmr spectra obtained for the methoxy, hydroxy, and cyano derivatives prepared via the chloroindolenine of 4β-dihydrocleavamine were consistent
with the position of substitution being at C-18 and not at C-8. Although
only one C-18 epimer has been synthesized in the case of the 18-cyano-4β-
dihydrocleavamine and 18-hydroxy-4β-dihydrocleavamine, it was clear from the
nmr data that the substituent is β in these compounds.

Now that reasonably satisfactory methods had been developed for intro­
ducing a substituent at C-18 of the dihydrocleavamine system, our attention
turned to the synthesis of dimeric systems. Aside from being a natural
extension to the total synthesis of Vinca alkaloids developed in our labora­
tories, the approach, if it were successful, would provide a convenient method
for synthesizing an almost unlimited number of potential anticancer agents
besides the well known naturally occurring dimers such as vincaleukoblastine
which have anticancer activity. Dimers of specific design would be useful
for structure-activity relationship studies.

The synthesis of the dimers 115, 147 and 148 are described and evidence
in support of the proposed structures are presented in this thesis. Because
it was anticipated that mass spectrometry would play an important role in the
characterization of any dimers formed, an attempt to prepare the dimer 115
which possesses no carbomethoxy groups, was made before the synthesis of any
other dimers was undertaken. It had been shown previously that dimers such
as vincaleukoblastine which possess a carbomethoxy group and have high
molecular weight and low volatility tend to undergo thermal reactions in the
inlet system of a mass spectrometer which results in "molecular ion" peaks
being observed which are multiples of 14 mass units larger than the true
molecular weight. These spurious peaks have been attributed to thermal
de decomposition products which arise through intermolecular methyl transfer
from a carbomethoxy group to nitrogen followed by Hofmann elimination.
Deacetylvindoline hydrazide (114) was prepared from vindoline (149) by refluxing it with anhydrous hydrazine for 3 hours. The coupling reaction of the deacetylvindoline hydrazide with the chloroindolenine of 4β-dihydrocleavamine was carried out in refluxing anhydrous methanolic hydrochloric acid (prepared from acetyl chloride (2.5 ml) and anhydrous methanol (100 ml)) under a nitrogen atmosphere for 3 hours. In this manner a 77% yield of the dimer 115 was obtained. This dimer proved to be virtually insoluble in methanol and was obtained in crystalline form from the crude reaction mixture simply by washing with hot methanol. Recrystallization from ethanol provided an analytical sample of the compound, mp 190-192 (rapid
rate of heating). All the evidence was in accord with this compound being a dimer. The ultraviolet spectrum (Figure 43) displayed in superimposition the characteristic absorptions of both an indole and a dihydroindole system. The low resolution mass spectrum (Figure 44) of this compound established beyond doubt is dimeric nature. High resolution mass analysis provided the empirical formula $C_{41}H_{54}O_6N$ (found: mol wt, 694.421; calcd mol wt, 694.421). These results clearly were in accord with the dimer being a product of coupling between deacetylvindoline and $4\beta$-dihydrocleavamine. Cleavage of the dimer in refluxing 2N aqueous hydrochloric acid in the presence of reducing agents (tin and stannous chloride) provided $4\beta$-dihydrocleavamine and deacetylvindoline hydrazide, the identities of which were established in the usual manner (mp, tlc, comparison ir). It was thus clear that the dimer contained deacetylvindoline hydrazide and $4\beta$-dihydrocleavamine as intact units. The site of attachment in each unit thus remained to be determined. The nmr spectrum (Figure 45) of the dimer provided the remaining
Figure 43. Ultraviolet spectrum of the dimer 115
information that was required to establish the sites of attachment. In fact the important resonances arising from both halves of the molecule were easily distinguishable from each other and the nmr spectrum in itself, virtually constituted a proof of structure. The nmr spectra of the monomers are shown in Figure 46 and 47. The important signals in the nmr spectra of deacetylvindoline hydrazide, 4β-dihydrocleavamine and the dimer are compared in Table 3. Deuterium exchange caused the signals attributed to the N-16', C-4 hydroxyl and C-3 hydroxyl protons in the nmr spectrum of the dimer to disappear and the integral showed that the three hydrazide protons must occur in the region above 6τ. Several important features of the dimer were seen from a comparison of the nmr resonances listed in Table 3. The signals attributed to the C-15 proton in deacetylvindoline hydrazide were absent in spectrum of the dimer. In addition, the signals attributed to the C-14 and C-17 protons, which in the deacetylvindoline hydrazide occurred as doublets, were found as sharp singlets in the dimer. The absence of signals attributable to the C-15 proton and the singlet nature of the C-14 and C-17 proton resonances could only be rationalized if the deacetylvindoline hydrazide portion of the dimer was coupled at the C-15 site. The appearance of a "doublet" at τ 5.60 in the dimer is consistent with the 4β-dihydrocleavamine portion of the dimer being coupled at the C-18 or C-8 site. Since in no experiment had it been shown that the C-8 site in the chloroindolenine of 4β-dihydrocleavamine was activated toward attack by nucleophiles and since it had been demonstrated in several experiments that the C-18 site was activated in this manner, it was virtually beyond doubt that C-18 was the site at which the 4β-dihydrocleavamine portion of the dimer was coupled to the deacetylvindoline hydrazide portion of the dimer.
Figure 46. Nmr spectrum of 4β-dihydrocleavamine
Figure 47. Nmr spectrum of deacylindoline hydrazide
<table>
<thead>
<tr>
<th>compound</th>
<th>116(R=H)</th>
<th>115</th>
<th>114</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>signals</strong></td>
<td>chemical shift( ), shape, no. of protons, coupling, constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>proton(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-21 H</td>
<td>9.28,t,3</td>
<td>9.34,t,3</td>
<td></td>
</tr>
<tr>
<td>C-21'H</td>
<td>9.16,t,3</td>
<td>9.18,t,3</td>
<td></td>
</tr>
<tr>
<td>C-19 H</td>
<td>7.34,s,1</td>
<td>7.36,s,1</td>
<td></td>
</tr>
<tr>
<td>N-1 CH₃</td>
<td>7.30,s,3</td>
<td>7.28,s,3</td>
<td></td>
</tr>
<tr>
<td>C-2 H</td>
<td>6.66,s,1</td>
<td>6.58,s,1</td>
<td></td>
</tr>
<tr>
<td>C-16 OCH₃</td>
<td>6.17,s,3</td>
<td>6.28,s,3</td>
<td></td>
</tr>
<tr>
<td>C-4 H</td>
<td>6.00,s,1</td>
<td>5.90,s,1</td>
<td></td>
</tr>
<tr>
<td>C-18'</td>
<td>under methylene envelope</td>
<td>5.60,&quot;d&quot;,1, J=10cps</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-6 H</td>
<td>4.35,d,1</td>
<td>4.24,m,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10cps</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-7 H</td>
<td>4.17, pair of m's,1, J₇,₆=10cps</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-17 H</td>
<td>3.97,s,1</td>
<td>3.98,d,1, J₁₇,₁₅=2.3cps</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-15 H'</td>
<td>3.79, pair of d's,1, J₁₅,₁₇=2.3cps, J₁₅,₁₄=8.5cps</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-14 H</td>
<td>3.35,s,1</td>
<td>3.19,d,1, J₁₄,₁₅=8.5cps</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-11' H to C-H¹ H</td>
<td>2.45-3.07,m,4</td>
<td>2.45-3.07,m,4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-16' H</td>
<td>2.24,s,1</td>
<td>1.75?,s,1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4 OH</td>
<td>2.05?,s,1</td>
<td>1.8,broad &quot;s&quot;,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3 OH</td>
<td>0.57?,s,1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In view of the success obtained in the reaction of the chloroindolenine of 4β-dihydrocleavamine with deacetylvindoline hydrazide, it became of immediate interest to see if the same reaction conditions would bring about reaction between the chloroindolenine of a carbomethoxydihydrocleavamine and deacetylvindoline hydrazide. If the coupling proved to be possible in this case, a dimer would be obtained which would possess a carbomethoxy group at C-18'. The naturally occurring dimers which have anticancer activity possess a carbomethoxy group at the C-18' position and it is possible that the activity of these compounds is dependent on such a functionality being present at that site.

Before such a coupling reaction could be tried it was necessary to prepare the chloroindolenine of carbomethoxydihydrocleavamine. It was possible through a series of reactions worked out in our laboratories to prepare in good yield 18β-carbomethoxy-4β-dihydrocleavamine. Treatment of this compound with t-butyl hypochlorite yielded a chloroindolenine in almost quantitative yield. This material was obtained as an amorphous solid which readily became a gum on standing in air. Like the chloroindolenine of 4β-dihydrocleavamine this compound resisted crystallization, but behaved as a single compound. Investigation by thin-layer chromatography and spectral analysis failed to show that the material was not a single compound. It was felt in the case of this compound that it might prefer to be in the α-methylene indoline form 118 (R=COOMe) rather than in the indolenine form 117 (R=COOMe) because alkaloids such as vincadifformine (60) possess the α-methylene indoline form in which the double bond is in conjugation with the ester group. Since it is the form 118 (R=COOCH₃) which can undergo substitution by an SN₂' mechanism, it would have been encouraging if the
compound possessed that form. The nmr spectrum (Figure 48), however, was strictly in accord with the structure 117 \( (R=\text{COOCH}_3) \) for this compound. The spectrum showed a "doublet" at \( \tau 5.53 \) which was attributed to a C-18 proton and did not show a signal that could be attributed to a proton on the indole nitrogen. In addition the infrared spectrum revealed an unconjugated ester absorption at \( 1727 \text{ cm}^{-1} \) and the characteristic absorptions at \( 1612 \text{ cm}^{-1} \) and \( 1575 \text{ cm}^{-1} \) observed in typical chloroindolenines. The mass spectrum (Figure 49) of the compound was in accord with it being a monochloro derivative of carbomethoxydihydrocleavamine with molecular ion peaks at m/e 374 \( (^{35}\text{Cl}) \) and m/e 376 \( (^{37}\text{Cl}) \). Regeneration of 18B-carbomethoxy-4B-dihydrocleavamine on reduction established that the chlorination had not brought about any backbone rearrangements.

The chloroindolenine prepared from 18B-carbomethoxy-4B-dihydrocleavamine and deacetylvinodoline hydrazide were allowed to react under the conditions above. A complex mixture of products was obtained. The dimeric product was identified by a combination of thin-layer chromatography, ultraviolet spectroscopy and mass spectrometry. Isolation of the dimeric product was carried out by preparative thin-layer chromatography (silica gel, 3:1 methanol-water). A 36.5% yield of dimer 147 as an amorphous powder was obtained in this manner. The ultraviolet spectrum displayed maxima characteristic of the indole and dihydroindole chromophores. The infrared spectrum was very similar to that obtained for the dimer 115, but showed an additional absorption attributable to an ester group at \( 1726 \text{ cm}^{-1} \). The low resolution mass spectrum (Figure 50) established the dimeric nature of the compound. It was noted that the mass spectrum of this compound, which has a carbomethoxy group, showed a series of spurious peaks spaced by multiples of 14
Figure 48. Nmr spectrum of the chloroindolenine of 18β-carbomethoxy-4β-dihydrocleavamine
Figure 49. Mass spectrum of the chloroindolene of 188-carbomethoxy-4β-di-hydrocleavamine
Figure 50. Mass spectrum of the dimer 147
mass units above the expected molecular ion peak at m/e 752. High resolution mass analysis provided the empirical formula C_{43}H_{56}N_{6}O_{6} (found mol wt, 752.427; calcd mol wt, 752.426) for the m/e 752 peak. Cleavage of the dimer in refluxing 2N aqueous hydrochloric acid in the presence of tin and stannous chloride, provided a carbomethoxydihydrocleavamine which was identified as 18β-carbomethoxy-4β-dihydrocleavamine by thin-layer chromatography and comparison of infrared spectra. Deacetylvindoline hydrazide could not effectively be separated from the hydrolysis products but its presence, was demonstrated by thin-layer chromatography. The nmr spectrum (Figure 51) of the dimer proved to be invaluable in establishing its structure. The important nmr signals of the dimer and those of 18β-carbomethoxy-4β-dihydrocleavamine (Figure 52) and deacetylvindoline hydrazide are given for comparison purposes in Table 4. Deuterium exchange made it possible to account for the two hydroxyl protons, the indole NH proton and the three hydrazide protons. Like the hydrazide protons of the dimer 115, the three hydrazide protons of this dimer were shown to be in the region above τ 6.0 by a comparison of the integrals obtained before and after the deuterium exchange. It was noted that no observable signals disappeared in the nmr spectrum above τ 6.0 and the integral above that region showed only a gradual change. Thus it appeared that the hydrazide protons occurred as a broad band or several broad bands above τ 6.0. The same phenomenon was observed in deacetylvindoline hydrazide itself except in this case the decrease in the integral over the range above τ 6.0 corresponded to 7 protons. This was consistent with the compound existing as a dihydrate with formula C_{22}H_{30}O_{4}N_{4}·2H_{2}O which was proposed for it by the Lilly group.80

It is apparent that these are only two "blanks" in the column for the dimer 147 in Table 4. These blanks correspond to the C-18 proton of the
Figure 52. Nmr spectrum of 18β-carbomethoxy-4β-dihydrocleavamine
<table>
<thead>
<tr>
<th>compound</th>
<th>signals</th>
<th>Chemical shift (τ), shape, no. of protons, coupling constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-21 H</td>
<td>9.20, t, 3</td>
<td>9.34, c, 3</td>
</tr>
<tr>
<td>C-21'</td>
<td>9.12, t, 3</td>
<td>9.08, t, 3</td>
</tr>
<tr>
<td>C-19 H</td>
<td>7.18, s, 1</td>
<td>7.36, s, 1</td>
</tr>
<tr>
<td>N-1 CH₃</td>
<td>7.33, s, 3</td>
<td>7.28, s, 3</td>
</tr>
<tr>
<td>C-2 H</td>
<td>6.64, s, 1</td>
<td>6.58, s, 1</td>
</tr>
<tr>
<td>C-16 OCH₃</td>
<td>6.18, s, 3</td>
<td>6.28, s, 3</td>
</tr>
<tr>
<td>C-18' COOCH₃</td>
<td>6.39, s, 3</td>
<td>6.30, s, 3</td>
</tr>
<tr>
<td>C-18' H</td>
<td>4.98, &quot;d&quot;, 1</td>
<td></td>
</tr>
<tr>
<td>C-4 H</td>
<td>6.01, s, 1</td>
<td>5.90, s, 1</td>
</tr>
<tr>
<td>C-6 H</td>
<td>4.28, d, 1</td>
<td>J₆,₇=10 cps</td>
</tr>
<tr>
<td>C-7 H</td>
<td>4.10 pair of m's, 1</td>
<td></td>
</tr>
<tr>
<td>C-17 H</td>
<td>4.07, s, 1</td>
<td>3.98, d, 1</td>
</tr>
<tr>
<td>C-15 H</td>
<td>3.79, pair of d's, 1</td>
<td>J₁₅,₁₇=2.3 cps, J₁₅,₁₄=8.5 cps</td>
</tr>
<tr>
<td>C-14 H</td>
<td>3.06, s, 1</td>
<td>3.18, d, 1</td>
</tr>
<tr>
<td>C-11' H to C-14' H</td>
<td>2.5-3.1, m, 4</td>
<td>2.5-3.1, m, 1</td>
</tr>
<tr>
<td>N-16' H</td>
<td>1.37, s, 1</td>
<td>1.84?, s, 1</td>
</tr>
<tr>
<td>C-4 OH</td>
<td>1.00?, s, 1</td>
<td></td>
</tr>
<tr>
<td>C-3 OH</td>
<td>0.73?, s, 1</td>
<td></td>
</tr>
</tbody>
</table>
carbomethoxydihydrocleavamine portion and to the C-15 proton of the deacetyl-
vindoline portion of the dimer. Assuming that the C-8' and C-18' proton 
"doublets" which would have to be present if the two halves of the dimer 
were coupled through the C-15 and C-8' sites are not shifted under the 
methylene envelope (a very unlikely occurrence), the two halves must be 
coupled through the C-18' and C-15 sites.

The successful preparation of the dimer 115 and 147 demonstrated the 
utility of the reaction sequence used. Two other dimers 151 and 152, which 
are the vindoline containing analogues of these dimers, have been success-
fully prepared by other workers in our laboratories and work is in progress 
which will lead to dimeric systems which possess oxygen containing functions 
in the piperidine ring of the cleavamine portion of the dimers. The 
preparation of carbomethoxyvelbanamine (153) is being actively pursued at 
the present time. A successful dimerization of this compound through its

![Chemical Structure](image)

chloroindolenine with vindoline would provide vincaleukoblastine or its 
C-18 epimer or both.

Since the total synthesis of 6,7 dihydrovindoline (150) was being 
actively pursued in our laboratories and the syntheses of 18β-carbomethoxy 
4β-dihydrocleavamine had been accomplished, it was of interest to bring
about a coupling of these two compounds. In addition, the dimer obtained would be interesting for the purpose of biological testing. The chloroindolenine of 18\(\beta\)-carbomethoxy-4\(\beta\)-dihydrocleavaminenand 6,7-dihydrovindoline (150), which was obtained by catalytic reduction of vindoline, were allowed to react under the usual conditions. The pure dimer 148 was obtained by column chromatography in 42% yield. The expected ultraviolet and infrared spectra were obtained for this compound. High resolution mass analysis of the peak at m/e 796 provided the formula \(\text{C}_{46}\text{H}_{60}\text{O}_{8}\text{N}_{4}\) (found mol wt, 796.441; calcd mol wt, 796.441) for the compound which was in accord with the structure. Cleavage of the dimer under conditions which were designed to favor retention of the ester functions (1.5N methanolic hydrochloric acid, tin and stannous chloride) provided a mixture of cleavage products. These were identified by the usual means as 18\(\beta\)-carbomethoxy-4\(\beta\)-dihydrocleavamine, 18\(\alpha\)-carbomethoxy-4\(\beta\)-dihydrocleavamine and 6,7-dihydrovindoline. The low resolution mass spectrum and the nmr spectrum of the dimer 148 are shown in Figures 53 and 54, respectively. The pertinent signals of the dimer 148 18\(\beta\)-carbomethoxy-4\(\beta\)-dihydrocleavamine, and 6,7-dihydrovindoline are compared in Table 5. The nmr evidence was consistent with the two monomeric units being coupled between their respective C-18' and C-15 sites. The position of the proton resonances arising from the methoxyl functions were assigned after a comparison of the nmr spectra of the five dimers which had been prepared in our laboratories. The assignment of the C-15 methoxyl protons is undoubtedly correct, but the assignment for the protons of the two methyl esters might actually be the reverse.

There has been no discussion of the stereochemistry of the C-18' site in the synthetic dimers to this point. Two configurations about the C-18' carbon atom were considered to be possible in each of the dimers prepared.
Figure 53. Mass spectrum of the dimer 148
Figure 54. Nmr spectrum of the dimer 148
Table 5

<table>
<thead>
<tr>
<th>compound</th>
<th>139</th>
<th>148</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>signals</td>
<td>Chemical shift ((\tau)), shape, no. of protons, coupling constants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>proton(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-21 H</td>
<td>9.47, t, 3</td>
<td>9.52, t, 3</td>
<td></td>
</tr>
<tr>
<td>C-21' H</td>
<td>9.12, t, 3</td>
<td>9.04, t, 3</td>
<td></td>
</tr>
<tr>
<td>C-4 COOCH(_3)</td>
<td>7.92, s, 3</td>
<td>7.94, s, 3</td>
<td></td>
</tr>
<tr>
<td>C-19 H</td>
<td>7.84, s, 1</td>
<td>7.90, s, 1</td>
<td></td>
</tr>
<tr>
<td>N-1 CH(_3)</td>
<td>7.48, s, 3</td>
<td>7.42, s, 3</td>
<td></td>
</tr>
<tr>
<td>C-2 H</td>
<td>6.39, s, 1</td>
<td>7.32, s, 1</td>
<td></td>
</tr>
<tr>
<td>C-18' COOCH(_3)</td>
<td>6.39, s, 3</td>
<td>6.28, s, 3</td>
<td></td>
</tr>
<tr>
<td>C-3 COOCH(_3)</td>
<td>6.24, s, 3</td>
<td>6.27?, s, 3</td>
<td></td>
</tr>
<tr>
<td>C-16 OCH(_3)</td>
<td>6.18, s, 3</td>
<td>6.24?, s, 3</td>
<td></td>
</tr>
<tr>
<td>C-18' H</td>
<td>4.98, &quot;d&quot;, 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4 H</td>
<td>4.43, s, 1</td>
<td>4.38, s, 1</td>
<td></td>
</tr>
<tr>
<td>C-17 H</td>
<td>4.04, s, 1</td>
<td>3.98, d, 1</td>
<td></td>
</tr>
<tr>
<td>C-15 H</td>
<td>3.73, pair of d's, 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-14 H</td>
<td>3.08, s, 1</td>
<td>3.12, d, 1</td>
<td></td>
</tr>
<tr>
<td>C-11' to C-14; H</td>
<td>2.5-3.1, m, 4</td>
<td>2.54-3.1, m, 4</td>
<td></td>
</tr>
<tr>
<td>N-16&quot; H</td>
<td>0.07?, s, 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-3 OH</td>
<td>1.00?, s, 1</td>
<td>0.10, s, 1</td>
<td></td>
</tr>
</tbody>
</table>

When the dimerization reactions were considered it was felt that each reaction would provide two epimerically related dimers. In none of the five reactions which have been carried out to this time has a second dimer been detected. It would appear on the bases of this result that the coupling reactions were stereospecific. In this regard it was noted that both C-18
Epimers of 18-methoxy-4β-dihydrocleavamine were obtained when the chloro-indolenine of 4β-dihydrocleavamine was allowed to react in 1.5% methanolic hydrochloric acid for three hours under a nitrogen atmosphere in the absence of a vindoline derivative. A mixture of the two epimers was obtained in 35% overall yield. Before the mixture was separated it was determined by nmr that the ratio of the epimers was about 3:1 with the 18α-epimer predominating. Although it would require additional work to show whether this product ratio has any real significance, it would appear that attack on the β-face of the intermediate 118 (R=H) (or an equivalent intermediate) was preferred. In the dimerization reaction where the attacking group would be a bulky vindoline moiety any steric preference would be expected to be enhanced. In the case of dimerization reaction, however, it was realized there was a distinct possibility that the 18-methoxy-4β-dihydrocleavamines were intermediates. Harly-Mason and coworkers have recently reported a successful dimerization with the 18-hydroxydihydrocleavamine mixture 154.

\[ \text{154} \]

and vindoline in methanolic hydrochloric acid. The 18-methoxy derivatives would be expected to react in the same manner as the 18-hydroxy derivatives of dihydrocleavamine. Without there being a clear picture of the mechanism of the dimerization reaction, it would be risky to try and pick out the most likely configuration of the groups attached to the C-18' carbon atoms.
In regard to this stereochemical problem the synthetic dimer 143 and the naturally occurring isoleurosine B\(^{57}\) must either be the same compound or C-18' epimers. A fairly routine interrelation of the synthetic dimers and the isoleurosines with vincaleukoblastine whose absolute configuration is known from X-ray diffraction analysis, would be expected to be possible. Such an interrelation would provide the answer to the stereochemical problem of the synthetic dimers.

The chloroindolenines of 4\(\beta\)-dihydrocleavamine and 18\(\beta\)-carbomethoxy-4\(\beta\)-dihydrocleavamine have been shown to be extremely versatile substances for the preparation of a variety of new dihydrocleavamine derivatives displaying substitution at the C-18 site. Undoubtedly, the most important of these compounds from a synthetic and biological point of view are the dimeric compounds. As a whole the work presented in this thesis gives additional support to the postulate of Taylor shown in Figure 19. In the light of the work presented in this thesis it became apparent that given the right conditions substitution adjacent to the \(\alpha\)-position of any indole containing material could probably be achieved via the chloroindolenine.
III. Experimental

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet spectra were recorded with the use of methanol as solvent (unless otherwise specified) on a Cary 11 or Cary 14 recording spectrophotometer. Infrared (ir) spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer and potassium bromide pellets were used unless otherwise specified. Nuclear magnetic resonance (nmr) spectra were recorded using deuteriochloroform as solvent at 100 Mcps on a Varian HA-100 instrument by Mr. R. Burton and the chemical shifts are given in the Tiers $\tau$ scale. Mass spectra were recorded on an Atlas CH-4 or an AEI MS-9 mass spectrometer and high resolution molecular weight determinations were performed by either Mr. G. Eigendorf or Mr. G. Brown. Combustion analyses were carried out by Mr. P. Borda. Woelm neutral silica gel and alumina or Silica Gel G and Alumina G (according to Stahl) containing 5% by wt of General Electric Ratma p-1, Type 118-2-7 electronic phosphor were used for analytical and preparative thin-layer chromatography (tlc). Woelm neutral alumina and silica gel (activity III) were used for column chromatography. Chromatoplates were developed using 2:1 carbon tetrachloride-antimony pentachloride spray reagent. Solutions were dried exclusively with anhyd sodium sulfate. Some of the work was performed in collaboration with Dr. P. Le Quesne (P.L.Q.), Mr. J. Beck (J.B.) and Mr. F. Bylsma (F.B.).
18β-carbomethoxycleavamine\textsuperscript{73} (3,4 dehydro-139) (in part by JB)

In an efficient fume hood a three-necked, round bottom, 1 l flask was fitted with a reflux condenser and a reliable mechanical stirrer. A piece of rubber tubing leading to the fume hood air duct was connected to the top of the condenser. Acetic acid (300 ml) was introduced into the flask and heated to about 100°C. Catharanthine hydrochloride (11.02 g) and a further portion of acetic acid (100 ml) was introduced into the flask with rapid stirring. Immediately a portion of sodium borohydride was added and additional portions were added in rapid succession as soon as the vigorous reaction, which followed each addition, had subsided. It was intended by using this procedure that some borohydride would always be present in the solution. The rate of addition of sodium borohydride which was used was sufficiently slow that uncontrollable refluxing did not occur, but sufficiently rapid that the reaction temperature was maintained in the region 90°C-105°C. In this manner a total of 42 g of sodium borohydride was added over a period of an hour. After the addition was complete, the reaction mixture was immediately cooled in an ice-water bath. The reaction mixture which became extremely viscous on cooling, was treated with 9 N ammonium hydroxide (800 ml) and extracted with methylene chloride (three 400 ml portions). The methylene chloride extract was dried and the solvent was removed with the aid of a rotary evaporator and a vacuum pump to yield a white foam (10.85 g). The foam was taken up in hot methanol (20 ml) and almost immediately 4.4 g of 18β-carbomethoxycleavamine separated from the solution having mp 121-123°C (authentic sample 122-123°C). A further 1.3 g of material, which was shown to be almost pure 18β-carbomethoxycleavamine by tlc, was obtained by concentrating and chilling the methanol solution.
18β-carbomethoxy-4β-dihydrocleavamine \(^7\) \(^3\) (139)

18β-carbomethoxycleavamine (4.4 g) was hydrogenated at room temperature and atmospheric pressure over Adam's catalyst (368 mg) in ethyl acetate (51 ml). The uptake of hydrogen ceased after 130 minutes. The reaction mixture was filtered through a celite pad and solvent was removed to yield a foam which crystallized on trituration with methanol. After washing with methanol, 4.1 g of 18β-carbomethoxy-4β-dihydrocleavamine was obtained having mp 143-145°C (auth sample 146-148°C). A further 0.3 g with mp 142-145°C was obtained from the methanol washings.

4β-dihydrocleavamine \(^7\) \(^3\) (116, R=H)

A solution of 18β-carbomethoxy-4β-dihydrocleavamine (3.0 g) in 5 N hydrochloric acid (191 ml) was heated at about 90°C in a water bath for seven hours under a nitrogen atmosphere. The solution was cooled in an ice-water bath, made strongly basic by the addition of 15 N ammonium hydroxide and extracted with methylene chloride (three 125 ml portions). The methylene chloride extract was dried with anhyd sodium sulfate, the solvent was removed and the residue crystallized once from hot methanol to give 2.4 g of 4β-dihydrocleavamine with mp 134-138° (auth sample after several recrystallizations, mp 136-138).

Oxidation of 4β-dihydrocleavamine with tert-butyl hypochlorite

A solution of 0.050 M tert-butyl hypochlorite (7.1 ml, 0.36 mmol) in carbon tetrachloride was added over a period of 30 minutes to a solution of 4β-dihydrocleavamine (100 mg, 0.36 mmol) and triethylamine (0.07 ml) in methylene chloride (13.3 ml) cooled in an ice-acetone bath. After the addition was complete, the solution was stirred for a further 15 minutes at
the temperature of the ice-acetone bath. The orange coloured solution was then diluted with an equal volume of benzene and rapidly percolated through a column of alumina (1.5 g). The bulk of the solvent was removed at room temperature in a rotary evaporator and then the last traces were removed in vacuo to provide the chloroindolenine 113 as a pale yellow oil (101 mg): 

\[ \text{max}_{\lambda} \text{isoctane} = \begin{array}{c} 227, 260 \text{(broad)}, 303 \text{(broad)} \text{ m}\mu. \quad (\log \epsilon = 4.31, 3.55, 3.42, \text{ respectively}) \end{array} \]

\[ \nu_{\text{max}}^\text{CHCl}_3 = 2770 \text{ (Bohmann band)}, 1600 \text{ and } 1560 \text{ cm}^{-1} \text{ (indolenine C=N)}; \]

nmr \[ \tau = 2.5-3.1 \text{ (diffuse, 4H, aromatic protons), 8.79 (approx. quartet, 2H, CH}_2-\text{CH}_3, 9.14 \text{ (triplet, 3H, CH}_2-\text{CH}_3) \]; mass spectrum (Atlas) m/e (relative intensity) 318(72), 316(88), 281(100), 138(46.5), 124(38).

Anal. Calcd for C\textsubscript{19}H\textsubscript{25}N\textsubscript{2}Cl: mol wt, 316.171. Found: mol wt, 316.172 (mass spectrometry).

**Lithium aluminium hydride reduction of the chloroindolenine 113**

Lithium aluminum hydride powder (5 mg) was added slowly while stirring to a solution of the chloroindolenine (23 mg) in anhyd diethyl ether (20 ml). After 15 minutes ethyl acetate (saturated with water) was added until gas evolution ceased. The mixture was then filtered and the filtrate dried and evaporated to give a gummy residue (21 mg) which was chromatographed on alumina (2 g). Elution with 1:1 petroleum ether (bp 30-60°)-benzene provided 12.5 mg of a crystalline material, which on recrystallization from methanol gave 4β-dihydrocleavamine (116, R=H), mp 136-138°, identical with an authentic sample as shown by mp and mmp, comparison ir and tic (alumina benzene and silica gel, chloroform).

**Reaction of the chloroindolenine 113 with potassium cyanide**

A solution of the chloroindolenine 113 (559 mg), potassium cyanide (1.22 g), methanol (15.5 ml), water (1.67 ml) and diethyl ether (3.34 ml)
was stirred under a nitrogen atmosphere for 48 hours at room temperature. An aqueous potassium carbonate solution (10%, 25 ml) was then added and the solution extracted with methylene chloride (three 25 ml portions). The combined extracts were dried and evaporated to give a glassy residue (491 mg). This residue was chromatographed on alumina (50 g). Elution with benzene provided 118 mg of a mixture of compounds called group A. Tlc (alumina, benzene): chromatoplates showed one major spot which was blue in colour with a pink fringe at $R_f$ 0.5. Tlc (silica gel, 1:1 chloroform-ethyl acetate): chromatoplates showed two major spots: one pink in colour at $R_f$ 0.9 and the other grey-blue at $R_f$ 0.1. $\lambda_{\text{max}}$ 294, 285, 278 (sh), 225 (indicative of indole chromophore); $\nu_{\text{max}}^{\text{CHCl}_3}$ 3410 (NH), 2220 (nitrile group) cm$^{-1}$.

Further elution in the above chromatography with methylene chloride (vol %, from 12 to 30) in benzene provided 140 mg over 42 fractions of a mixture of compounds called group B: Tlc (alumina 3:1 benzene-ethyl acetate): chromatoplates of early fractions of mixture showed one green-brown spot at $R_f$ 0.5 and chromatoplates of late fractions of mixture showed one red-brown spot at $R_f$ 0.5. Tlc (silica gel, 1:1 chloroform-ethyl acetate): chromatoplates showed one brown spot at $R_f$ 0.5; $\lambda_{\text{max}}$ 290, 282, 278, 273, 222 (indicative of mixture of chromophores including an indole chromophore); $\nu_{\text{max}}^{\text{CHCl}_3}$ 3400 (NH), 2220 (very strong, indicating a conjugated nitrile group) cm$^{-1}$. Nmr spectra of select fractions indicated the spectra were composed of signals from three compounds: one with distinguishing signals at $\tau$ (approx) 6.63 (singlet), 5.41 (singlet), 1.63 (singlet, exchangeable proton); another with distinguishing signals at $\tau$ (approx) 6.35 (singlet), 1.45 (singlet, exchangeable proton); the third with distinguishing signal $\tau$ (approx) 6.6 (singlet).
Group A was chromatographed on silica gel (10 g). Elution with 3:1 benzene-chloroform provided a series of fractions which contained 36 mg of a mixture of compounds that was initially thought to be one compound: tlc (freshly activated silica gel, chloroform) chromatoplates showed two overlapping spots at $R_f$ 0.3. Preparative tlc (freshly activated silica gel, chloroform) provided a sample of both components of the mixture each of which contained about 10% of the other component as an impurity. The partially purified sample having the larger $R_f$ value had $\lambda_{\text{max}}$ 2.93, 240, 210 µm; $\nu_{\text{max}}$ 3300 and 2220 (both very weak and probably from contaminant) 1613 and 1595 cm$^{-1}$ (indolenine C=N?); nmr $\tau$ 9.12 (triplet, 3H, $\text{CH}_2\text{CH}_3$), 5.98 (singlet, exchangeable proton, 1H, OH or NH), 2.8-3.5 (diffuse, 4H, aromatic protons). Further purification was attempted without success.

The partially purified sample having the smaller $R_f$ value had $\nu_{\text{max}}$ 293.5, 284.5, 272(sh), 225 µm; $\nu_{\text{max}}$ 3300 (NH) 2670 (Bohlmann band), 2220 (CN) cm$^{-1}$. Nmr: $\tau$ 9.10 (triplet, 3H, $\text{CH}_2\text{CH}_3$), 4.58 (doublet, 1H, C-18 proton), 2.45-3.0 (diffuse, 4H, aromatic protons), 1.47 (singlet, 1H, NH). The sample was subjected to rigorous purification by preparative tlc. Freshly activated silica gel plates (5 x 20 cm, 0.25 mm thickness) were used and chloroform was used as the transporting solvent. The desired band was scraped off and eluted with ethyl acetate to provide a sample of 18β-cyano-4β-dihydrocleavamine (143) (ca. 1 mg). Mass spectrum (MS-9) m/e 307 (molecular ion), 138 and 124.


Further elution in the above chromatography with 2% triethylamine in acetone provided 40 mg of 18α-methoxy-4β-dihydrocleavamine (140) as a glass which became crystalline in form on standing. Recrystallization of this
compound from methanol provided a sample with mp 126-127°: $\lambda_{\text{max}}$ 292, 284, 277 (sh), 225 $\mu\text{m}$ (log $\varepsilon$ 3.86, 3.90, 3.86, 4.49, respectively); $\nu_{\text{max}}$ 3280 (N-H), 2780 (3ohlmann band), 1070 (C-O-Me) cm$^{-1}$; nmr $\tau$ 1.63 (singlet, 1H, N-H), 2.45-3.2 (diffuse, 4H, aromatic protons), 5.47 (pair of doublets, 1H, C-18 protons), 6.80 (singlet, 3H, COOCH$_3$), 9.46 (triplet, 3H, CH$_2$CH$_3$).

Mass spectrum: m/e (rel intensity) 312(58), 281(16), 280(15), 187(6), 182(49), 158(100), 124(29).

Anal. Calcd for C$_{20}$H$_{28}$N$_2$O: mol wt, 312.220. Found: mol wt, 312.220 (mass spectrometry).

18β-carbomethoxy-4β-dihydrocleavamine (139) via treatment of group A with anhyd methanolic hydrochloric acid (in part by P.L.Q.)

A solution of group A (113 mg) in anhyd saturated methanolic hydrochloric acid (35 ml) was heated under reflux for 30 minutes, let stand overnight at room temperature, and heated again under reflux for 4 1/2 hours. After the reaction solution had been evaporated almost to dryness, it was partitioned between diethyl ether and an aqueous solution of sodium carbonate. The organic layer was separated, washed with water and dried. Removal of the solvent provided a gummy residue (92 mg) which was chromatographed on alumina (4 g). Elution with benzene provided a mixture (30 mg) which contained 18β-carbomethoxy-4β-dihydrocleavamine as a major component. This mixture was rechromatographed on alumina (2.5 g). Elution with benzene provided several fractions containing 18β-carbomethoxy-4β-dihydrocleavamine. One of these fractions (3.3 mg) showed only one spot when subjected to an investigation on alumina chromatoplates (3:1 benzene-chloroform, 3:1 benzene-ethyl acetate). Further investigation by tlc (silica gel, 1:1 chloroform-ethyl acetate) showed that it was a mixture of several
compounds. Purification by preparative tlc using a silica gel plate (5 x 20 cm, 0.25 mm thickness) and the above solvent system provided 18β-carbomethoxy-4β-dihydrocleavamine (0.7 mg) as shown by comparison tlc and mass spectra (virtually identical fragmentation pattern as an authentic sample when both spectra were seen under the same conditions on the Atlas instrument).

Anal. Calcd for C_{21}H_{28}N_{2}O_{2}: mol wt, 340.215. Found: mol wt, 340.212 (mass spectrometry).

[22-^{14}C]-18β-carbomethoxy-4β-dihydrocleavamine (139) via methanolysis of group A

Group A (5.1 mg, 1.4 x 10^-5 mCi), which had been obtained by the method described above, was dissolved in an anhyd saturated methanolic hydrochloric acid solution (1 ml) and heated under reflux for one hour under a nitrogen atmosphere. Then, the solvent was rotary evaporated to yield a glassy residue which was partitioned between methylene chloride (20 ml) and a saturated aqueous sodium bicarbonate solution (5 ml). The organic layer was dried and rotary evaporated to give a gummy residue (1.2 mg). One-half (0.6 mg) of this material was mixed with inactive 18β-carbomethoxy-4β-dihydrocleavamine (5.4 mg) and the mixture was crystallized to constant activity (148 dpm/mg). The radioactivity of the 18β-carbomethoxy-4β-dihydrocleavamine was estimated to be 7.1 x 10^-7 mCi. Only 5% of the radioactivity in group A was present after reaction as 18β-carbomethoxy-4β-dihydrocleavamine.
18g-carbomethoxy-4g-dihydrocleavamine (139) via treatment of group A_1 with potassium hydroxide and then diazomethane

A sample of group A_1 (10.0 mg) was dissolved in a 20% solution (0.1 ml) of potassium hydroxide in diethylene glycol and heated at 150°C for 8 1/2 hours under a nitrogen atmosphere. The solution was then allowed to cool to room temperature and diluted with methanol (0.2 ml). This methanolic solution was cooled in an ice-water bath and treated with a saturated solution of hydrogen chloride in methanol until it had become slightly acidic as shown by indicator paper. An ethereal solution (1 ml) of diazomethane (ca. 20 mg) was added immediately and the resulting mixture was allowed to stand in an ice-water bath for 15 minutes. In the same manner as above the mixture was re-acidified and treated with excess diazomethane two more times before the ether, methanol and excess diazomethane were removed with the aid of a nitrogen stream and a warm water bath. The residue obtained was shaken with an aqueous 10% solution of potassium carbonate (1 ml) and extracted with diethyl ether (three 5 ml portions). After the ethereal extract had been dried, the ether was removed to provide a viscous material (14.5 mg) containing diethylene glycol which was chromatographed on alumina (2 g). Elution with 4:1 petroleum ether (bp 30-60°)-benzene provided 18g-carbomethoxy-4g-dihydrocleavamine (3.1 mg) which upon recrystallization from methanol had a mp of 145-148° and was found to be identical with an authentic sample as shown by mp, mmp, comparison ir and tic (alumina, 3:1 benzene-chloroform and silica gel, 1:1 chloroform ethyl acetate).

[22-14C]-18g-carbomethoxy-4g-dihydrocleavamine (139) via potassium hydroxide and diazomethane treatment of group A_1

Group A_1 (10.15 mg, 6.76 x 10^-4 mCi) was used and the above procedure
repeated. The crude reaction product (47 mg) was diluted with inactive 18β-carbomethoxy-4β-dihydrocleavamine (10.3 mg) and chromatographed on alumina (2 g). Elution with 4:1 petroleum ether (bp 30-60°)-benzene provided [22-14C]-18β-carbomethoxy-4β-dihydrocleavamine (13.5 mg, 2.3 x 10^{-4} mCi). The radioactivity represented 35% of that present in group A.

The reaction of the chloroindolenine 113 in a methanolic solution of hydrogen chloride and potassium cyanide

An anhydrous methanolic solution (19.5 ml) containing 1.5% hydrogen chloride was added slowly with stirring to a mixture of the chloroindolenine 113 (229 mg) and potassium cyanide (378 mg) in a flask which had been fitted with an efficient condenser and was cooled in an ice-water bath. Escaping hydrogen cyanide gas was passed into an aqueous potassium hydroxide solution and the entire experiment was carried out in an efficient fume hood. After the addition of the methanolic hydrogen chloride had been completed, the resulting solution was refluxed for three hours under a nitrogen atmosphere. Then the reaction solution was cooled in an ice-water bath and solid sodium carbonate was added until the solution was neutral to indicator paper. The solution was diluted with water (29.5 ml), made quite basic by the addition of sodium carbonate and extracted with methylene chloride (five 20 ml portions). The methylene chloride solution was dried and rotary evaporated to yield a glassy residue (219 mg). The major portion (196 mg) of this residue was chromatographed on alumina (20 g). Elution with 3:1 petroleum ether (bp 30-60°)-benzene provided a mixture (79 mg) of two compounds. A portion (62 mg) of this mixture was chromatographed on silica gel (6 g). Elution with 1:1 chloroform-ethyl acetate provided a compound (15 mg) which immediately crystallized on trituration with methanol.
Recrystallization of this compound from methanol-diethyl ether provided a sample, mp 175-178\(^\circ\) of 18\(^\alpha\)-methoxy-4\(\beta\)-dihydrocleavamine (141): \(\lambda_{\text{max}}\) 294, 286, 279(sh), 227 (log \(e\) 3.85, 3.91, 3.87, 4.48, respectively); \(\nu_{\text{max}}\) 3250 (N-H) 2780 (Bohlmann band) 1075 cm\(^{-1}\) (C-O-Me); nmr \(\tau\) 1.70 (singlet, 1H, NH), 2.44-3.06 (diffuse, 4H, aromatic protons), 4.76 (pair of doublets, 1H, C-18 proton), 6.86 (singlet, 3H, COOCH\(_3\)), 9.11 (triplet, 3H, CH\(_2\)CH\(_3\)). Mass spectrum (Atlas) m/e (rel intensity) 312(85), 281(22), 280(11), 187(100), 182(35), 138(97), 126(35), 124(40).


Elution in the above chromatography with 2% triethylamine in acetone provided a compound (37 mg) which slowly crystallized on standing. Recrystallization of this compound from methanol provided a sample with mp 126-127\(^\circ\), which was identical (mp, nmr, ir, tlc) with authentic 18\(\alpha\)-methoxy-4\(\beta\)-dihydrocleavamine (140).

Anal. Calc for C\(_{20}\)H\(_{28}\)N\(_2\)O: C, 76.88; H, 9.03; N, 8.97. Found: C, 77.15; H, 9.28; N, 8.75 (combustion).

The reaction of the chloroindolenine 113 in a solution of sodium iodide in acetone - an attempt to prepare an 18-iodo-4\(\beta\)-dihydrocleavamine

The chloroindolenine 113 (79 mg) was converted into hydrochloride salt by dissolving it in ether-methylene chloride and passing hydrogen chloride gas over the surface of the solution. Removal of the solvent gave the salt as a white powder. Acetone (1 ml), which had been dried by percolation through and storage over Linde 4A molecular seives, was added to the salt and then a sodium iodide-acetone solution (2 ml), which had been prepared by dissolving sodium iodide (6.6 g) in dry acetone (30 ml).
Immediately upon the latter addition the solution became dark purple-brown in colour. The coloured solution was stirred at room temperature for three hours under a dry oxygen free (Fieser's solution) nitrogen atmosphere. Then, the purple-brown coloured solution was diluted with diethyl ether (25 ml) and the resulting solution was washed with an aqueous solution of sodium thiosulfate until it had become colourless. The aqueous washings were made strongly basic by the addition of potassium carbonate and were extracted with methylene chloride (three 10 ml portions). The methylene chloride solution and the ether solution were combined and dried. Removal of the solvent provided a gummy residue (77 mg) which was chromatographed on alumina (8 g). Elution with 3:1 petroleum ether (bp 30-60°)-benzene afforded 15.4 mg of a product which gave crystals, mp 136-138°, from methanol and proved to be identical with 4β-dihydrocleavamine (116, R=H)-as shown by mp and mmp, comparison ir and tlc.

The reaction of 18-methoxy-4β-dihydrocleavamine with boron trichloride - an attempt to prepare an 18-chloro-4β-dihydrocleavamine

Boron trichloride gas was condensed in a flask which had been cooled in a dry ice-acetone bath. A solution of a mixture (5 mg) of 18α-methoxy-4β-dihydrocleavamine and 18β-methoxy-4β-dihydrocleavamine (ratio of the former compound to the latter, 3:1) in anhydrous methylene chloride (1 ml) was added slowly to the liquid boron trichloride. After the reaction solution had stood at the dry-ice-acetone bath temperature for ten minutes under a dry nitrogen atmosphere, the methylene chloride and excess boron trichloride was removed on a rotary evaporator. The residue obtained was mixed thoroughly with a mixture of an aqueous sodium bicarbonate solution (1 ml) (saturated) and diethyl ether (10 ml). The ether phase was separated
and replaced with methylene chloride (10 ml). Methylene chloride was mixed thoroughly with the mixture of aqueous sodium bicarbonate and undissolved residue. Both the ether and methylene chloride extracts were dried and evaporated separately to yield 1.9 mg and 2.3 mg, respectively, of a gummy residue. Investigation by tlc of these residues in each case failed to show the presence of unreacted material, but did show that each mixture was composed of a minimum of five compounds.

The reaction of 18-methoxy-4β-dihydrocleavamine with aqueous 1.5% hydrochloric acid - an attempt to prepare a 18-hydroxy-4β-dihydrocleavamine

A solution of a 3:1 mixture (5 mg) of 18α-methoxy-4β-dihydrocleavamine to 18β-methoxy-4β-dihydrocleavamine in 1.5% hydrochloric acid (0.5 ml) was stirred under a nitrogen atmosphere at room temperature while aliquots were taken periodically and analysed by tlc (alumina, ethyl acetate). It appeared that the 18-methoxy-4β-dihydrocleavamines were being slowly hydrolysed to a series of more polar compounds of which the amount of "baseline" material increased progressively. Only a small amount of unreacted material remained as shown by tlc after 3 1/2 hours.

The reaction of the chloroindolenine 113 in a solution of sodium acetate in acetic acid - the preparation of a mixture of the quaternary nitrogen acetate salts 131 and 132.

A solution of the chlorindolenine 113 (101 mg) in a glacial acetic acid solution (6.5 ml), which contained 10% fused sodium acetate by weight, was heated at 60° for two hours under a nitrogen atmosphere. The reaction solution was then poured into a mixture of 15 N ammonium hydroxide (8 ml) and methylene chloride (26 ml) with rapid stirring. The organic phase was separated and saved and the aqueous phase was made strongly basic by the
addition of 15 N ammonium hydroxide, saturated with ammonium acetate and extracted with methylene chloride (three 10 ml portions). The methylene chloride extracts were then combined and dried. Removal of the solvent provided 93 mg of a white powder: tlc (alumina, 3:1 ethyl acetate - ethanol): chromatoplates showed two overlapping green coloured spots at 

\[ R_f \, 0.12; \lambda_{max} \, 220, 270, 280, 289 \] (cf \[ \lambda_{max} \, 220, 270(sh), 280, 289 \] for mixture 55 and \[ \lambda_{max} \, 226, 273(sh), 282, 289 \] for mixture 56).

The reaction of the chloroindolenine 113 in a solution of sodium acetate in acetic acid - an attempt to obtain an 18-acetoxy-4β-dihydrocleavamine (134 and/or 135)

A solution of the chloroindolenine 113 (367.5 mg) in a glacial acetic acid solution (23.3 ml), which contained 10% fused sodium acetate by wt, was heated at 60°C for 30 minutes under a nitrogen atmosphere. Then, the reaction solution was immediately poured into a rapidly stirred mixture of 15 N ammonium hydroxide (30 ml) and methylene chloride (93 ml) which was maintained at low temperature in an ice-acetone bath. The organic phase was separated and saved. A further portion of methylene chloride (46.5 ml) was added to the aqueous phase and while the mixture was being rapidly stirred at the temperature of the ice-acetone bath, the weakly basic aqueous phase was made strongly basic by the addition of 15 N ammonium hydroxide. The organic phase was separated and combined with the previously separated organic phase. The combined solution was dried and rotary evaporated to give a gummy residue (416 mg) which was chromatographed almost immediately on silica gel (20 g). Elution with ethyl acetate provided 67 mg of material over several fractions. Each fraction was shown to contain the chloroindolenine 113 and another compound(s) believed to be
an 18-acetoxy-4β-dihydrocleavamine. The spectral properties of the purest fractions were determined: $\lambda_{\text{max}}$ 292 (sh), 284, 278 (sh), 226 (slightly distorted indole spectrum); $\nu_{\text{max}}^{\text{CHCl}_3}$ (PE 137), 3300 (indole NH), 1720 cm$^{-1}$ (ester C=O); nmr $\tau$ 1.72 (singlet, NH), 2.5-3.2 (diffuse, aromatic protons), 3.97 (pair of doublets, C-18 proton), 7.96 (singlet, CH$_3$COO), 9.15 (triplet, CH$_2$CH$_3$) (signals which could be attributed to an acetoxy-4β-dihydrocleavamine).

Further elution in the above chromatography with 3% triethylamine in ethyl acetate gave a further 116 mg of material consisting mainly of the chloroindolenine 113 and the compound(s) believed to be an 18-acetoxy-4β-dihydrocleavamine. Finally, the column was washed with 5% acetic acid in methanol and 1:1 methanol-water. The washings were combined, rotary evaporated to give a residue which was treated with a saturated aqueous ammonium acetate solution (10 ml) containing ammonium hydroxide and extracted with methylene chloride (three 25 ml portions). The combined extracts were dried and evaporated to provide 164 mg of a mixture of compounds which consisted mainly of the quaternary ammonium salts 131 and 132 as shown by tlc.

A mixture (65.5 mg) containing the alleged 18-acetoxy-4β-dihydrocleavamine from the chromatography above was chromatographed on alumina (6 g). Elution with 1:1 petroleum ether (bp 30-60°)-benzene gave 27 mg of a mixture which was shown by tlc to contain the chloroindolenine 113 as the major component. Elution with 1:1 benzene-ethyl acetate gave 14.3 mg of 18β-hydroxy-4β-dihydrocleavamine (142). Recrystallization from methanol provided a sample, $\text{mp}$ 202-205°: $\lambda_{\text{max}}$ 292, 284, 278 (sh), 226 mp (log ε 3.86, 3.91, 3.87, 4.50, respectively); $\nu_{\text{max}}$ 3200 (broad, NH and OH), 2700 cm$^{-1}$ (Bohlmann band); nmr $\tau$ 1.42 (broad singlet, 1H, OH), 2.19 (singlet, 1H, NH), 2.5-3.15 (diffuse, 4H, aromatic protons) 4.77 (unresolved multiplet becoming a doublet after treatment of nmr sample with D$_2$O, 1H, C-18 proton),
9.17 (triplet, 3H, CH₂CH₂); mass spectr. m/e (Atlas) m/e (rel intensity) 298(46), 281(13.5), 280(13.5), 173(11.5), 168(43), 138(100), 124(51).


**Reaction of the mixture of the quaternary ammonium salts 131 and 132 with lithium aluminium hydride in N-methylmorpholine**

A mixture (25 mg) of salts 131 and 132 and lithium aluminium hydride (101 mg) were allowed to react in refluxing anhyd N-methylmorpholine (10 ml) under a dry, oxygen free nitrogen atmosphere. Aliquots (ca. 0.5 ml) were taken periodically. Each aliquot was treated with ethyl acetate (saturated with water) until gas evolution ceased. The mixture was filtered and the residue washed with methylene chloride (ca. 2 ml). The filtrate and washings were combined, dried, and evaporated with the aid of a nitrogen stream and a hot water bath. The residue was examined by tlc (alumina, 3:1 benzene-chloroform and 3:1 ethyl acetate-ethanol, and silica gel, chloroform). After one hour the presence of 48-dihydrocleavamine was detected. After 4 1/2 hours, the chromatoplates on development showed a major spot which corresponded in colour and Rf value to a spot from an authentic sample of 48-dihydrocleavamine (116, R=H).

**18β-cyano-4β-dihydrocleavamine (143)**

A mixture of the quaternary ammonium salts 131 and 132 (73.3 mg) was allowed to react with potassium cyanide (56 mg) in refluxing dimethylformamide (9 ml) for 1 2/3 hours under a nitrogen atmosphere. The solvent was then removed by distillation at reduced pressure (50°C and 6 mm of Hg). The residue obtained was treated with 6 N ammonium hydroxide (1 ml) and the aqueous mixture was extracted with methylene chloride (three 5 ml portions).
The extract was then dried and the solvent removed to provide a gummy residue (64.4 mg). The residue was then chromatographed on silica gel (7 g). Elution with 1:1 benzene-chloroform provided 15.7 mg of 18β-cyano-4β-dihydrocleavamine which gave crystals from methanol with mp 150-152°C: 
\[ \lambda_{\text{max}} 294, 285, 277, 226 \text{ nm} \quad (\log \varepsilon 3.86, 3.93, 3.89, 4.44, \text{ respectively}); \]
\[ \nu_{\text{max}} 3300 \text{ (NH)}, 2760 \text{ (Bohlmann band)}, 2220 \text{ cm}^{-1} \text{ (CN); } \]
\[ \text{nmr } \delta 1.72 \text{ (singlet, } 1\text{H, NH)}, 2.48-3.04 \text{ (diffuse, } 4\text{H, aromatic protons)}, 4.58 \text{ (pair of doublets, } 1\text{H, C-18 proton}), 9.10 \text{ (triplet, } 3\text{H, CH}_2\text{CH}_3); \]
\[ \text{mass spectrum (MS-9) } m/e \text{ (rel intensity) 307(32), 281(<3), 280(<3), 182(14), 177(77), 138(100), 124(75)}. \]

Anal. Calcd for C\textsubscript{20}H\textsubscript{25}N\textsubscript{3}: mol wt, 307.205. Found: mol wt, 307.205 (mass spectrometry).

18β-carbomethoxy-4β-dihydrocleavamine (139) from 18β-cyano-4β-dihydrocleavamine (143)

A solution of 18β-cyano-4β-dihydrocleavamine (5.21 mg) in a solution (0.05 ml) of diethylene glycol containing 20% KOH by wt was heated at 150°C for nine hours under a nitrogen atmosphere. Then, the solution was allowed to cool to room temperature and diluted with methanol (0.1 ml). While this new solution was kept cool in an ice-water bath, a saturated solution of methanolic hydrochloric acid was added to it until it had become slightly acidic to test with indicator paper. A solution (0.5 ml, approx conc 20 mg/ml) of diazomethane in ether was immediately added and the resulting mixture was allowed to stand in an ice-water bath for 15 minutes. In the same manner as above the reaction mixture was re-acidified and treated with excess diazomethane two more times before the ether, methanol and excess diazomethane were removed with the aid of a nitrogen stream and a warm
water bath. The residue obtained was shaken with an aqueous 10% potassium carbonate solution and extracted with diethyl ether. After the ethereal extract had been dried, the ether was removed to provide a viscous material (23 mg) which was mainly diethylene glycol. This material was chromatographed on alumina (1 g). Elution with 4:1 petroleum ether (bp 30-60°)-benzene provided 18β-carbomethoxy-4β-dihydrocleavamine (2.97 mg). Recrystallization provided a sample, mp 146-148, which was identical with an authentic sample of 18β-carbomethoxy-4β-dihydrocleavamine as shown by mp, mmp, comparison ir and tlc (alumina, 3:1 benzene-chloroform and silica gel, 1:1 chloroform-ethyl acetate).

Oxidation of 18β-carbomethoxy-4β-dihydrocleavamine (139) with tert-butyl hypochlorite (in part by FB & JB)

A solution of 18β-carbomethoxy-4β-dihydrocleavamine (400 mg) and triethylamine (0.2 ml) in methylene chloride (40 ml) was cooled in an ice-water bath. While the solution was being stirred under a nitrogen atmosphere a solution (250 ml, 0.05 M) of tert-butyl hypochlorite in carbon tetrachloride was added over a period of 45 minutes. Then the solution was washed with ice-water (two 30 ml portions), dried and rotary evaporated at room temperature to give the chloroindolenine 117 (R=COOMe) as an amorphous solid (440 mg): $\lambda_{max}$ dioxane 292, 275, 227 μ (log ε 3.44, 3.44, 4.30, respectively); $\nu_{max}$ CHCl$_3$ 2775 (Bohlmann band), 1727 (ester C=O), 1612 and 1575 cm$^{-1}$ (indolenine C=N); nmr $\tau$ 2.40-2.98 (diffuse, 4H, aromatic), 5.53 (doublet, 1H, C-18 proton), 6.41 (singlet, 3H, COOMe), 9.14 (triplet, 3H, CH$_2$CH$_3$); mass spectrum (MS-9) m/e (rel intensity) 376(8), 374(22), 138(100), 124(85).

Anal. Calcd for C$_{21}$H$_{27}$N$_2$O$_2$Cl: mol wt, 374.176. Found: mol wt, 374.174 (mass spectrometry).
Catalytic reduction of the chloroindolenine 117 (R=COOMe)

The chloroindolenine 117 (R=COOMe) (23 mg) was hydrogenated over Adam's catalyst (37 mg) in ethyl acetate (10 ml) at room temperature and atmospheric pressure for one hour. Then, the reaction mixture was filtered through a celite pad and the pad was washed with methanol. The methanol and ethyl acetate solutions were combined and evaporated. The material obtained was dissolved in methylene chloride and washed with a saturated aqueous potassium carbonate solution. The methylene chloride solution was then dried with anhyd sodium sulfate and evaporated to give 19 mg of a material, which on recrystallization from methanol gave 18β-carbomethoxy-4β-dihydrocleavamine, mp 146-148° identical with an authentic sample as shown by mp and mmp, comparison ir and tlc (alumina, 3:1 benzene-chloroform and silica gel, 1:1 chloroform-ethyl acetate).

Deacetylvindoline hydrazide (114)

About 8 ml of anhydrous hydrazine was distilled from sodium hydroxide into a round bottom flask containing vindoline (1.03 g). The mixture was refluxed under a dry nitrogen atmosphere for three hours. (The foregoing steps were carried out in a fume hood behind a shield.) Then the reaction solution was allowed to cool to room temperature and methylene chloride (30 ml) was added with stirring. Next water was added with stirring in a dropwise fashion to the solution until it separated into two phases. The upper hydrazine hydrate phase was separated from the lower methylene chloride phase and extracted with an additional amount of methylene chloride. The methylene chloride solutions were combined and shaken with water, which was added a drop at a time, until a fluffy white crystalline material (228 mg) was seen to suddenly separate. After this material had been
separated by filtration, the methylene chloride solution was washed with an additional small amount of water and dried. Removal of the solvent gave deacetylvindoline hydrazide as a white powder (687 mg) which gave crystals from hot 95% ethanol with mp 130-180° (lit. mp 130-180°): \[\alpha\]_d^{22} +17.3 (CHCl₃) (lit. \[\alpha\]_d^{180} +18.6); \lambda_{\text{max}} 308, 248 (sh), 213 \mu \text{ (log } \varepsilon 3.71, 4.11, 4.66, \text{ respectively). } \nu_{\text{max}} 3380, 3260, 3170 (\text{NH and OH}), 2810 (\text{Bohlmann band}), 1650 and 1615 cm⁻¹ (CONHNH₂); \text{nmr } \tau 1.80 (\text{broad, 2H, C-4 OH and C-5 OH}), 3.19 (\text{doublet, 1H, C-14 proton}), 3.79 (\text{pair of doublets, 1H, C-15 proton}), 3.98 (\text{doublet, 1H, C-17 proton}), 5.90 (\text{singlet, 1H, C-4 proton}), 6.28 (\text{singlet, 3H, OCH₃}), 7.28 (\text{singlet, 3H, N-CH₃}), 9.34 (\text{triplet, 3H, CH₂CH₃}).


The dimer 115

A solution of the chloroindolenine 113 (601 mg) and deacetylvindoline hydrazide (521.5 mg) in an anhydrous methanolic 1.5% hydrochloric acid solution (52 ml) was refluxed under a dry nitrogen atmosphere for three hours. After this period of time had passed and the solution had been allowed to cool to room temperature, it was diluted with water (78.5 ml) and made slightly basic by the addition of sodium carbonate. The basic solution was extracted with methylene chloride (three 130 ml portions). The methylene chloride extract was dried and the solvent was removed to yield 985.5 mg of a powder. Some of this powder (894.5 mg) was washed several times with hot methanol to provide 543.6 mg of small white crystals. Concentration of the methanol washings provided a further 18.2 mg of the same crystalline material. This material displayed rather unusual melting properties. When the
temperature was raised slowly (ca. 2°/min) no melting point could be observed but rather a progressive darkening beginning at about 190°C. When the temperature was raised rapidly (ca. 10°/min) the sample appeared to melt and immediately solidify in the range 189-194°C. Recrystallization from 95% ethanol provided a sample which melted and solidified in the same manner in the range 190-192°C: $\lambda_{\text{max}}$ 309(sh), 294, 285, 263, 224(sh), 215 $\mu$m ($\log \varepsilon$ 3.85, 4.04, 4.05, 4.17, 4.60, 4.64 respectively); $\nu_{\text{max}}$ 3400 and 3280 (NH and OH), 1668 and 1616 cm$^{-1}$ (CONHNH$_2$); nmr $\tau$ 2.45-3.07 (diffuse, 4H, aromatic protons of cleavamine portion), 3.35 (singlet, 1H, C-14 proton of vindoline portion), 3.97 (singlet, 1H, C-17 proton of vindoline portion), 5.60 (doublet, 1H, C-18 proton of cleavamine portion, $J=10$cps), 9.18 (triplet, 3H, CH$_2$CH$_3$ of cleavamine portion), 9.40 (triplet, 3H, CH$_2$CH$_3$ of the vindoline portion).

Analytical. Calcd for C$_{41}$H$_{54}$O$_4$N$_6$: mol wt, 694.421. Found: mol wt, 694.420.

Calcd for C$_{41}$H$_{52}$O$_4$N$_6$ ($M^+ - 2$): mol wt, 692.405. Found: 692.403 (mass spectrometry).

Cleavage of the dimer 115

A mixture of the dimer 115 (51 mg), tin (205 mg), stannous chloride dihydrate (205 mg) and 2 N hydrochloric acid (10 ml) was refluxed for two hours under a nitrogen atmosphere. After this period of time had passed and the mixture had been cooled to room temperature, a saturated aqueous solution of potassium carbonate was added until the mixture was basic. The mixture was extracted with methylene chloride (five 5 ml portions). The methylene chloride extract contained a fine white suspension which was effectively removed by centrifugation. The methylene chloride centrifugate was dried and rotary evaporated to give 41 mg of a brown coloured residue which was
chromatographed on alumina (4 g). Elution with 1:1 petroleum ether (bp 30-60°)-benzene provided a material (8 mg) which on crystallization from methanol gave 4β-dihydrocleavamine (116, R=H), mp 135-138, identical with an authentic sample as shown by mp, mmp, comparison ir and tlc (alumina, 3:1 benzene-chloroform and silica gel, chloroform). Elution with methanol provided a material (22 mg) which on crystallization from ethanol-water and recrystallization from 95% ethanol provided a sample that was shown to be identical with deacetylvindoline hydrazide by comparison ir and tlc (silica gel, 95% ethanol and alumina, 95% ethanol).

The dimer 147 (in part by JB)

A solution of deacetylvindoline hydrazide (294 mg) and the chloroindolenine 117 (R=COOME)(386 mg) in an anhydrous methanolic 1.5% hydrochloric acid solution (2.9 ml) was refluxed for three hours under a dry nitrogen atmosphere. After this period of time had passed and the solution had been allowed to cool to room temperature, the solution was diluted with water (44 ml) and made slightly basic by the addition of sodium carbonate. The basic solution was extracted with methylene chloride (three 75 ml portions). The methylene chloride extract was dried and the solvent removed to give a powdery material (621 mg). Some of this material (409.5 mg) was subjected to separation by preparative tlc. Silica gel (Woelm) plates (20 x 20 cm, 0.5 mm thickness) were used, with about 60 mg of material being applied to each plate. After transport with 3:1 methanol-water, the desired band was scraped off each plate and extracted, first with methanol at room temperature and then with boiling methanol. The solvent was then removed and the residue was extracted with methylene chloride. Filtration of the methylene chloride extracts and removal of the solvent provided 118 mg of the dimer
147 as an amorphous solid: $\lambda_{\text{max}}$ 313(sh), 296, 290, 269, 218 mp (log ε 3.88, 4.05, 4.06, 4.11, 4.69, respectively); $\upsilon_{\text{max}}$ 3410 and 3290 (NH and OH), 1726 (ester C=O), 1663 and 1615 cm$^{-1}$ (CONHNH$_2$); $\delta_{\text{nmr}}$ 2.48-3.10 (diffuse, 4H, aromatic protons on cleavamine portion), 3.06 (singlet, 1H, C-14 proton of vindoline portion), 4.07 (singlet, 1H, C-17 proton on vindoline portion), 6.30 (singlet, 3H, COOCH$_3$ at C-18' of cleavamine portion), 9.08 (triplet, 3H, CH$_2$CH$_3$ of cleavamine portion), 9.20 (triplet, 3H, CH$_2$CH$_3$ of vindoline portion).

Anal. Calcd for C$_{43}$H$_{56}$O$_6$N$_6$: mol wt, 752.426. Found: mol wt, 752.427 (mass spectrometry).

**Cleavage of the dimer 147 (by JB)**

A mixture of the dimer 147 (13 mg), tin (750 mg), stannous chloride dihydrate (750 mg) and 2 N hydrochloric acid (25 ml) was refluxed for one hour. The mixture was then diluted with water, cooled and neutralized with sodium bicarbonate. The solid material was separated by filtration. Both the solid and filtrate were extracted with chloroform. The chloroform extract was dried and the solvent removed to give a residue (9 mg). This residue was subjected to separation by preparative tlc. A silica gel (Woelm) plate (5 x 20 cm, 0.5 mm) was used. After transport with 1:1 ethyl acetate-acetone, the desired bands were scraped off and extracted with methanol. In this manner 2 mg of a material was obtained which was shown to be 18β-carbomethoxy-4β-dihydrocleavamine (139) by comparison (tlc and ir) with an authentic sample. Another 3 mg of a material was obtained which was impure but displayed the tlc properties of deacetylvindoline hydrazide.

**The dimer 148 (in part by FB & JB)**

A solution of 6,7-dihydropindoline (150) and the chloroindolenine 117
(R=COOMe) (440 mg) in anhydrous methanolic 1.5% hydrochloric acid (57 ml) was refluxed for three hours under a dry nitrogen atmosphere. The solvent was then removed in a rotary evaporator and the residue was dissolved in methylene chloride (100 ml). Water (100 ml) was added and then potassium carbonate with mixing until the mixture was basic. The organic phase was then separated from the aqueous phase and the aqueous phase was extracted with an additional quantity of methylene chloride (two 30 ml portions). After the methylene chloride extracts had been combined and dried, the solvent was removed to give a glassy residue (694.4 mg). The residue was chromatographed on alumina (70 g). Elution with 4:1 benzene-diethyl ether provided 248.4 mg of the dimer 148 as an amorphous solid: $\lambda_{\text{max}}$ 307(sh), 295, 287, 263, 217 nm ($\log \epsilon$ 4.01, 4.14, 4.15, 4.19, 4.64, respectively); $\nu_{\text{max}}$ 3430 (NH and OH), 1735 cm$^{-1}$ (ester C=O); nmr $\tau$ 2.54-3.10 (diffuse, 4H, aromatic protons of cleavamine portion), 3.08 (singlet, 1H, C-14 proton of vindoline portion), 4.04 (singlet, 1H, C-17 proton of vindoline portion), 6.28 (singlet, 3H, COOCH$_3$ at C-18' on cleavamine portion), 9.04 (triplet, 3H, CHCH$_3$ of cleavamine portion), 9.47 (triplet, 3H, CH$_2$CH$_3$ of vindoline portion).

Anal. Calcd for $C_{46}H_{60}O_{10}N_4$: mol wt, 796.441. Found: mol wt, 796.441 (mass spectrometry).

**Cleavage of the dimer 148**

A mixture of the dimer 148 (50.1 mg), tin (100 mg), stannous chloride dihydrate (100 mg) and anhyd 1.5 N (6.5%) methanolic hydrochloric acid (10 ml) was refluxed for one hour under a nitrogen atmosphere. Then, after the mixture had been cooled to room temperature, an aqueous 10% potassium carbonate solution (15 ml) was added and the resulting mixture was extracted...
with methylene chloride (five 10 ml portions). The extract was centrifuged to precipitate the suspended white solid and the clear centrifugate was dried. Removal of the solvent provided a gummy residue (55 mg). The residue was subjected to chromatography on alumina (5 g). Elution with 4:1 petroleum ether (bp 30-60°)-benzene provided 4.2 mg of a crystalline compound. Recrystallization of this compound provided a sample with mp 146-148°C which was identical with an authentic sample of 18β-carbomethoxy-4β-dihydrocleavamine (139) as shown by mp and mmp, comparison ir and tlc (alumina, 3:1 benzene-chloroform and silica gel, 1:1 chloroform-ethyl acetate).

Further elution in the above chromatography with 4:1 petroleum ether (bp 30-60°)-benzene provided 8.0 mg of a material which could not be induced to crystallize but was shown to be identical with 18α-carbomethoxy-4β-dihydrocleavamine (138) by comparison ir and tlc (above systems).

Further elution in the above chromatography with 4:1 benzene-chloroform provided 25.6 mg of a foam which was shown to be identical with an authentic sample of 6,7-dihydrovindoline (150) by comparison ir and tlc (silica gel, ethyl acetate and the above systems).
References


62. J. Shavel, Jr., and H. Zinnes, J. Amer. Chem. Soc. 84, 1320 (1962); see also ref. 60.

63. N. Finch and W. I. Taylor, J. Amer. Chem. Soc. 84, 3871 (1962); 84, 1318 (1962); see also W. I. Taylor, et. al. ibid. 87, 2229 (1965).


74. Isolation of the compounds and nmr spectra at 100 Mcps by Vern Nelson.

75. Prepared by C. Gletsos.


PART C

STUDIES RELATED TO THE BIOSYNTHESIS OF INDOLE ALKALOIDS
I. Introduction

Since the beginning of this decade, a considerable amount of effort by several groups of workers has been expended in the direction of solving the mystery of the biosynthesis of the indole alkaloids. A fairly complete biosynthetic scheme has emerged, although much of the scheme is still speculative in nature. Tracer studies have established many key steps in the biosynthesis. Some key steps, which have yet to be confirmed by tracer studies, have been performed under laboratory conditions and therein derive support. Other key steps, which have been proposed, have derived support from the isolation of new alkaloids or other natural compounds whose structures closely resemble proposed intermediates. In some cases a unique structure, which is possessed by a natural compound, has led to new and more viable postulates. In this section of this thesis are described tracer experiments which were undertaken in an attempt to verify a key step proposed by Wenkert in his scheme for the biosynthesis of alkaloids possessing the Iboga and Aspidosperma types of skeleton.\(^1\) This key step, which involved a transannular cyclization, was supported by several successful laboratory conversions performed by Kutney and coworkers\(^2\text{--}^5\), which were described in some detail in the introduction to part B of this thesis.

Alkaloids possessing the Iboga and Aspidosperma types of skeleton are of the tryptamine + C\(_{9\text{--}10}\) unit structural type; that is, they can be envisaged to arise from the union of tryptamine and a unit that contains
9 or 10 carbon atoms. There are several hundred indole alkaloids which fit this description. Nevertheless, a close look at the nature of the C$_{9-10}$ unit in these alkaloids reveals that there are only three basic modifications of the carbon backbone. One modification (1) (in which the dotted line represents the bond cleaved when the C$_{10}$ unit becomes the C$_9$ unit) of the C$_{9-10}$ unit is found in alkaloids which are exemplified as a group by corynantheine (2) and strychnine (3). The second modification (4) of the C$_{9-10}$ unit is found in alkaloids exemplified as a group by tabersonine (5). The third modification (6) of the C$_{9-10}$ unit is found in alkaloids exemplified as a group by coronaridine (7). It can also be seen that the two modifications 4 and 6 are related quite simply to the modification 1. Modifications 4 and 6 could be envisaged as being derived from modification 1 at some stage in the biosynthesis by a transformation such as "a" in the case of 4 and "b" in the case of 6 as shown in Figure 1.
Figure 1. Derivation of Aspidosperma and Iboga types of backbone from the Corynanthe type of backbone.
The indole portion of alkaloids of the tryptamine + C₉-₁₀ type and, indeed, all other indole alkaloids would logically be derived in part from tryptophan (8) or its decarboxylation product tryptamine (9). Tracer studies have borne out this conjecture and in all cases reported tryptophan has been shown to be a direct precursor of indole alkaloids. As might be expected several hypotheses concerning the biosynthesis of the C₉-₁₀ portion in indole alkaloids were proposed. The earliest scheme, which can be considered as the Barger ¹⁰-Hahn ¹¹,¹²-Robinson ¹³-Woodward ¹⁴,¹⁵ hypothesis after the workers who proposed or elaborated on the scheme, involved dihydroxy-phenylpyruvic acid (10) or an equivalent compound and two C₁ units in the production of the non-tryptamine portion of alkaloids of the corynanthine-strychnine type. The essential details of this scheme as it emerged are shown in Figure 2. Several features of the scheme are worthy of note. It was implied that the direct precursors of the corynanthine-strychnine group of alkaloids were aromatic in their non-tryptamine portion. Two C₁ units were required at different stages in the synthesis. Since alkaloids of the corynanthine-strychnine type are characterized by constant stereochemistry at C-15, a stereospecific hydrogenation of the aromatic ring was required.

In 1959¹⁶,¹⁷ Wenkert proposed that the condensing unit with tryptamine might be derived from a direct precursor of the phenylpyruvic acid of the
Figure 2. Barger-Hahn-Robinson-Woodward hypothesis
earlier theory. At that time the sequence shikimic acid (11) → prephenic acid (12) → 4-hydroxyphenylpyruvic acid (13) → 3,4-dihydroxyphenylpyruvic acid (10) (Figure 3) was thought to include a hydrated prephenic acid (14) as an intermediate. Wenkert proposed that this intermediate gave rise to the condensing unit 15 after some stereospecific rearrangements as shown in Figure 4. The important features of this hypothesis were that the ring never became aromatic, the stereochemistry at C-15 was predetermined by the stereochemistry of the hydrated prephenic acid molecule and the carbomethoxy function was retained so that only one C₁ unit was required in the synthesis of the alkaloids. When it was shown ¹⁸,¹⁹ that a hydrated

Figure 3. Pathway from shikimic acid to dihydroxyphenylpyruvic acid incorrectly involving a hydrated prephenic acid intermediate
Figure 4. Essential features of hydrated prephenic acid hypothesis

Prephenic acid was not involved in the shikimic acid-prephenic acid transformation, Wenkert\textsuperscript{1} modified his scheme to utilize prephenic acid itself to give a condensing unit as shown in Figure 5. Corynantheine could arise, according to this scheme, by condensation of tryptamine with 17 and a related alkaloid, yohimbine (18), by condensation of tryptamine with either 16 or 17. This modified prephenic acid theory of Wenkert embodied the same features as the earlier one; that is, in particular, the establishment of the configuration at C-15 and the required inclusion of one C\textsubscript{1} unit. Wenkert referred to 17 as the seco-prephenate-formaldehyde (SPF) unit. Its relationship to 1 is obvious.

Another scheme\textsuperscript{8} that had been proposed for the biosynthesis of the non-tryptamine portion of the indole alkaloids is illustrated in Figure 6. In this scheme the condensing unit 19 was to have been built up by condensation of a poly-\(\beta\)-keto ester of six carbon atoms in length with malonate and a C\textsubscript{1} unit. This scheme received some experimental support\textsuperscript{20-22} which was subsequently withdrawn.\textsuperscript{23}
Figure 5. Prephenic acid hypothesis
Figure 6. Acetate hypothesis for the production of a corynantheine-strychnine type of condensing unit

Another hypothesis for the biosynthesis of the non-tryptamine portion of the indole alkaloids was proposed independently by Wenkert and Thomas. The "Monoterpenoid hypothesis", as it is called, was suggested to these people by the striking structural similarities that several monoterpenic glucosides bore to the non-tryptamine portion of the corynantheine-strychnine type of alkaloids and to the hypothetical SPF unit proposed by Wenkert. Some monoterpenic glucosides are shown in Figure 7. The backbone of each compound is emphasized by a heavy line to point out the similarity of the backbone to the C_{9-10} unit 1. It was observed that the terpenic portion of these compounds possessed the requisite number of carbon atoms and in the compounds, for which the absolute configuration at the starred centres was known, the stereochemistry was the same as that found in the majority of indole alkaloids. In addition, the
necessary backbone was intact in these compounds and they were seen to be simply related to the hypothetical condensing units 17 and 19. Cleavage (dashed lines) and rotation (curved arrows) would give in the case of each of the examples in Figure 7 structures whose backbones are superimposable with the corynantheine-strychnine $C_{9-10}$ unit (1). An important feature of the monoterpenoid hypothesis not possessed by any other hypothesis was that a $C_1$ unit was not required to be involved in the biosynthesis. The Barger-Hahn-Robinson-Woodward hypothesis, the Wenkert hydrated prephenic acid and prephenic acid hypotheses, and the Taylor acetate hypothesis, all required
that C-21 in yohimbine, corynantheine and related alkaloids should come from the one-carbon pool in plants.

In order to discover which, if indeed any, of the hypotheses most closely resembled the biosynthetic pathway, tracer experiments were carried out by several groups of workers. Unfortunately, the initial results of the tracer experiments led to confusion. Indeed for a time it could be construed that all the hypotheses had been ruled out. One group of workers\textsuperscript{22} reported that when sodium formate\textsuperscript{14}C was fed to \textit{Rauwolfia serpentina} plants, C-21 of ajmaline (21) became labelled (12\% of activity). This was in keeping with a hypothesis that required the inclusion of a C\textsubscript{1} unit from the one-carbon pool of the plant. Further experiments\textsuperscript{20,21} were reported which could be purported to disprove all the hypotheses except the Taylor acetate hypothesis. In particular mevalonic acid-2-\textsuperscript{14}C (22) was found not to be incorporated into ajmaline as required by the monoterpenoid hypothesis. Other workers\textsuperscript{25} who carried out feeding experiments in \textit{Rauwolfia serpentina} obtained results which were consistent with a C\textsubscript{1} unit not being involved in the biosynthesis except in trivial ways. As a result of experiments that were carried out by several groups\textsuperscript{25-29} of workers, it was apparent that neither the Barger-Hahn-Robinson-Woodward hypothesis, nor the Wenkert...
hydrated prephenic acid and prephenic acid hypotheses, nor the Taylor acetate hypothesis described the biosynthesis of the indole alkaloids. In addition, the reported failure to incorporate mevalonic acid into ajmaline as required by the monoterpenoid hypothesis seemed to indicate that this hypothesis was not tenable either.

One unifying feature of the experimental work described above was that the results were negative in nature; that is, they appeared to disprove rather than prove. Nevertheless the evidence against the inclusion of a fragment from the one-carbon pool was strong since incorporation of sodium formate-14C was achieved, but the crucial carbon atom (C-21) was essentially inactive. For example, when Battersby and coworkers fed sodium formate-14C to Rauwolfia serpentina plants in repetition of the earlier work, they found that in the ajmaline isolated the N-methyl group carried not less than 25% of the activity and that the carbon atom, C-21, had little or no activity. The evidence against the monoterpenoid hypothesis, on the other hand, was weak. In the attempt to show incorporation of mevalonate-14C into ajmaline by Rauwolfia serpentina plants, the ajmaline that was isolated was completely inactive and, therefore, there was no guarantee that the labelled mevalonic acid ever reached the site of synthesis or, if it did, that it reached the site of synthesis at a period when synthesis of ajmaline was taking place. Other work has demonstrated that negative results should be interpreted with extreme care.

Scott and coworkers were the first to report a successful incorporation of mevalonate into an alkaloid of the tryptamine + Cg type. Subsequent publication by several groups of workers established that specifically labelled mevalonic acid was incorporated into indole alkaloids
in a manner which was completely consistent with the monoterpenoid hypothesis. Additional irrefutable evidence for the terpenoid origin of the C₉₋₁₀ condensing unit was found independently by four research groups. Labelled geraniol (23) was found to be incorporated as an intact unit into vindoline (24), catharanthine (25), and ajmalicine (26) in *Vinca rosea* L. plants (Figure 8). Each of these three alkaloids are representative of one structural type of indole alkaloids of the tryptamine + C₉₋₁₀ type.

---

**Figure 8.** Incorporation of geraniol into alkaloids representing the three structural types of indole alkaloids of the tryptamine + C₉₋₁₀ type.
of the three types of tryptamine + C₉₋₁₀ alkaloids. The location of the radioactive label in the alkaloids was in all cases consistent with the formation of an intermediate cyclopentane monoterpenic unit as shown in Figure 9 and the transformations shown in Figure 1. Strong evidence for

\[ \text{Figure 9. Rearrangement in backbone of geraniol to give modification 1 of the C₉₋₁₀ condensing unit involving a cyclopentane monoterpenic unit} \]

the intermediacy of cyclopentane intermediates in the pathway was first obtained by Battersby and coworkers⁴¹ who succeeded in showing that loganin (27) which had been labelled in the ester methyl, was incorporated into ajmaline, vindoline and catharanthine by *Vinca rosea* L. plants. These workers fed a variety of cyclopentane monoterpenic compounds all of which were labelled in the ester methyl group. They found that there was no incorporation of activity when radioactive verbenalin (20), dihydroverbenalin (28), or monotropeine methyl ester (29) were fed and therefore they felt that the good incorporation of loganin obtained (ca. 1% into vindoline) did not come about by simple transmethylation. Recently, any possibility of ambiguity was removed when loganin, labelled biosynthetically with ¹⁴C in the 2-position⁴²,⁴³ and in the 4-position⁴⁴,⁴⁵ was incorporated by *Vinca rosea* L. plants into several alkaloids representative of the three
main structural types of indole alkaloids. In addition, a successful incorporation of [1-\textsuperscript{3}H]-loganin by \textit{Rauwolfia serpentina} plants into ajmaline was also reported,\textsuperscript{43} thereby establishing loganin as a precursor of a Corynanthe type alkaloid in another plant species. These results are summarized in Figure 10. Several compounds are shown as multiply labelled entities, but it should be understood that each label corresponds to a separate experiment.

Battersby\textsuperscript{46} has proposed a reasonable pathway from mevalonate to the indole alkaloids of the tryptamine + C\textsubscript{9-10} type (Figure 11). He was careful to point out, however, that "several closely similar schemes could be written in which the sequence of operations is altered." For example, there has been published no evidence concerning precisely where in the biosynthesis the union of tryptamine and the C\textsubscript{9-10} unit comes about, although Battersby indicated\textsuperscript{46} that there was some recent experimental evidence that indicated that "the conversion of the corynantheine-strychnine C\textsubscript{9-10} unit into the Aspidosperma and Iboga C\textsubscript{9-10} units occurs after the introduction of the nitrogen..."

Of several possible intermediates which could undergo cyclopentane
Figure 10. Incorporation of loganin into various indole alkaloids
Figure 11. Proposal for the pathway from mevalonate to indole alkaloids of the tryptamine + C<sub>9-10</sub> type
cleavage, hydroxyloganin (30) is particularly attractive. A cleavage mechanism is indicated in Figure 12 (where "X" could be a phosphate residue to provide a good leaving group.)

It has been reported very recently that the monoterpenic glucoside, sweroside (33), was incorporated very efficiently (11%) into vindoline by *Vinca rosea* L. plants. Sweroside bears a striking resemblance to the hypothetical condensing unit 31, having the same stereochemistry at C-2 and C-7 and a potential aldehyde function at C-5. The incorporation of sweroside into vindoline was more efficient than the incorporation of loganin into vindoline and this would tend to suggest that sweroside is further along the biosynthetic pathway than loganin. In this regard there was good evidence that loganic acid (34) is a precursor of gentiopicroside (35) in *Swertia carboliniensis* plants and sweroside is an extremely efficient precursor (incorporation ratio 40%) of gentiopicroside in *Gentiana scabra* plants.

There are a few alkaloids in which the hypothetical condensing unit 31 occurs as an intact or nearly intact unit. One of these is ipecoside (36). It has been shown that the monoterpenic portion of ipecoside is derived...
from loganin. Another is the indole alkaloid cordifoline\(^{50}\) (37). In this compound the sugar portion was found to be monoacetylated. A third alkaloid, macrosalhine\(^{51}\) (38), whose structure has been determined by X-ray diffraction analysis,\(^{52}\) has a unique structure in which the hypothetical condensing unit 31 is disguised. If macrosalhine is written in a distorted form, as in 39, the relationship between rings D and E of macrosalhine and loganin is
clearly revealed. The configurations of the centres 15, 20 and 21 in macrosalbine are the same as the configurations of the corresponding centres in loganin.

A plausible pathway by which an intermediate such as 32 of geissoschizine (40) might be transformed into ajmaline-type alkaloids is shown in Figure 13.

Figure 13. Plausible pathway to ajmaline and related alkaloids
Wenkert has proposed a rather interesting pathway to Akuamma (e.g., akuammicine (45)) and pleiocarpamine-like bases. The unusual structure of pleiocarpamine (44) suggested the pathway. Wenkert envisaged an oxidation of a corynantheine-type intermediate such as geissoschizine to give rise to a diradical cation (42) as the key step. The pathway suggested by Wenkert is shown in Figure 14. Two addition alkaloids which might be derived from intermediate 43 are pseudoakuammigine (46) and echitamine (47). It should be emphasized that the pathway in Figure 14 is purely speculative in nature and is meant only to provide a plausible biosynthetic sequence. Alternative schemes are also conceivable. For example, another plausible route\textsuperscript{55} from geissoschizine to echitamine is illustrated in Figure 15. The key reaction of this pathway is an intramolecular Michael condensation. In this regard it is interesting to note that the 2-acyl indole system does not seem to be susceptible to nucleophilic attack at the carbon atom\textsuperscript{56}.

Wenkert\textsuperscript{1} has postulated that the alkaloids of the Iboga- and Aspidosperma-type are related biosynthetically to the Akuamma alkaloids and has suggested the scheme shown in Figure 16 to account for their biogenesis. It should be observed that whereas Wenkert has chosen to postulate the formation
Figure 14. Plausible pathway to the Akuamma alkaloids
Figure 15. Alternative pathway to echitamine and related alkaloids
Figure 16. Final stages in Wenkert's proposal for the biosynthesis of alkaloids with the Aspidosperma and Iboga types of skeleton.
of bond "b" prior to the formation of bond "a", it is equally plausible that bond "a" could be formed prior to bond "b" as indicated in Figure 17.

Very recently there has been some indication that the Aspidosperma and Iboga bases are derived from an Akuamma-type intermediate.\textsuperscript{57} Radioactive stemmadenine (54) was incorporated into catharanthine (25) and tabersonine (5). If these results should be confirmed, then the determination of the
precise biosynthetic pathway from the Akuamma skeleton to the Aspidosperma and Iboga skeletons will become even more interesting. A plausible variation on the Wenkert scheme utilizing stemmadenine is outlined in Figure 18.

The conversion of 55 into tabersonine and catharanthine is shown to be

![Chemical Structure Diagram]

Figure 18. A plausible pathway from stemmadenine to alkaloids with the Aspidosperma and Iboga types of skeleton
completely concerted in Figure 18 and is a plausible alternative to a stepwise formation of bonds. The enamine form (56) of 55 would also be a plausible intermediate and could react to give catharanthine or tabersonine in either a two step reaction or a concerted reaction (Diels-Alder?) as shown.

Wenkert has extended his previously proposed biosynthetic pathway to the Aspidosperma-type alkaloids to include alkaloids such as vincamine (59) (Figure 19). The key step in this pathway is the formation of the intermediate

Figure 19. Plausible pathway to vincamine and related alkaloids and to the alkaloid vallesamidine
by transannular cyclization in 57 through coupling of the iminium function to the α-position of the indole. In this regard it is interesting to note that the structure of an alkaloid called vallesamidine (60) has recently been established by X-ray diffraction analysis.
II. Discussion

THE PREPARATION OF LABELLED COMPOUNDS

The demonstration in our laboratories that the transformations 50 → 52 and 51 → 53, which were proposed by Wenkert as key steps in the biosynthesis of the Aspidosperma and Iboga alkaloids, were chemically feasible and, moreover, proceeded in the laboratory with complete stereospecificity stimulated a study to determine whether or not the transformations were of any significance in living Vinca plants. Several possible precursors were available in our laboratories, but it was necessary to prepare them in radioactive form before any tracer experiments could be carried out. A method for preparing $^{14}\text{C}$-labelled 18β-carbomethoxy-4β-dihydrocleavamine (61), a plausible precursor for Iboga alkaloids, had been worked out as part of the total synthesis of that compound. At the time that tracer experiments in Vinca plants were being contemplated, however, 18β-carbomethoxy-4β-dihydrocleavamine was available only via a poor yielding sequence and it was recognized that the level of activity obtainable might be too low for definitive results. In addition, attempts to prepare a carbomethoxyquebrachamine (62) as a possible precursor of vincadifformine (63) and a carbomethoxy-4α-dihydrocleavamine (64), which possesses the same configuration of the 4-ethyl group as coronaridine (7), by the same method were unsuccessful. It thus appeared that the method would be applicable to the preparation of
18β-carbomyloxy-4β-dihydrocleavamine only. At any rate the method would obviously not be applicable to the preparation of labelled compounds which do not possess a carbomethoxy group, but which could be incorporated, in theory at least, into several Aspidosperma alkaloids. For these and other reasons several other methods for preparing labelled compounds were investigated in our laboratories in order that their applicability to the preparation of the desired labelled compounds might be assessed. Since all the potential precursors which were to be used in this study had the indole moiety in common, the task set to this worker was to investigate methods of exchanging the hydrogen atoms of the benzene portion of the indole moiety with tritium atoms. Although no information could be found in the literature concerning tritium labelling of indole alkaloids by exchange of the aromatic protons, there were, however, some examples of deuterium exchange. Buchi and coworkers\textsuperscript{59} were able to exchange the aromatic hydrogen atoms in voacangine
(65) with deuterium atoms to a reasonable extent by treating the compound with a solution of deuterium oxide, methanol-0-d, and hydrogen chloride. Voacangine, however, possesses a methoxyl group in the aromatic ring and as a result exchange would be expected to take place much more easily than the exchange in compounds like the carbomethoxydihydrocleavamines which possess no such activating group. These same workers, however, also showed that dregamine (66) could be labelled in this manner. Dregamine was treated with a 1:1 mixture of methanol-0-d and deuterium oxide which had been saturated with hydrogen chloride gas. After the solution was heated under reflux for 23 hours under a nitrogen atmosphere, the dregamine was recovered in 95% yield and the aromatic hydrogen atoms, as shown by the nmr spectrum, had been replaced by deuterium atoms to the extent of about 50%. It was felt that this method could be modified to give tritium labelled compounds. The practical problems that were anticipated in making such a change were formidable. Tritium labelled water could not be directly substituted in the recipe above because an unknown quantity of tritium labelled hydrogen chloride gas would escape in the initial saturation and subsequent reflux steps. This problem would necessitate the use of efficient traps for
escaping gar. Also the extent of dilution of the label might be serious. Although it was felt that these problems might be overcome, it was also felt that there might be more suitable conditions for bringing about the acid catalyzed exchange reaction in which a non gaseous acid was substituted for hydrogen chloride. One possible acid that came to mind immediately was acetic acid. The carbomethoxydihydrocleavamines were known to retain their ester functions in refluxing glacial acetic acid because these compounds are formed by the treatment of catharanthine with a zinc-acetic acid mixture. Tritium labelled glacial acetic acid could be easily prepared from acetic anhydride by hydrolysis of the anhydride with tritium labelled water. There were two possible drawbacks that came to mind concerning the use of tritium labelled acetic acid. One was that it was a much weaker acid than hydrochloric acid and consequently the rate of exchange might be too slow for the labelling method to be practical. The other was that the label might become diluted through exchange of the α-hydrogen atoms of the acetic acid with the acidic hydrogen atom. Consideration of these possible drawbacks led to the belief that the ideal acid for the exchange reaction might be trifluoroacetic acid. Trifluoroacetic acid appeared to possess several desirable properties. It was known to be a fairly strong acid (pKa 0.3) and to possess only one hydrogen atom, the acidic one. The boiling point (75°C) was considered to be sufficiently low that the labelled acid could be easily removed from samples by distillation at reduced pressure for subsequent use, but high enough that there would be little danger from radioactive vapours. Finally, tritium labelled trifluoroacetic acid could be prepared easily from trifluoroacetic anhydride because the hydrolysis of the anhydride was known to take place virtually instantaneously.
When it was decided to test the ability of trifluoroacetic acid to promote exchange, a plentiful supply of 18α-carbomethoxy-4α-dihydrocleavamine was on hand in our laboratories and consequently this compound was chosen as the test compound. Since it seemed to be advisable to use fairly vigorous conditions, a solution of 18α-carbomethoxy-4α-dihydrocleavamine in trifluoroacetic acid-tritium labelled water (specific activity, ca. 0.1 mCi/mmol) was heated at 50°C for four hours under a nitrogen atmosphere. Investigation of the crude product by thin-layer chromatography showed only the presence of 18α-carbomethoxy-4α-dihydrocleavamine (67) and its epimer 18β-carbomethoxy-4α-dihydrocleavamine (68). Preparative thin-layer chromatography and recrystallization from methanol provided a pure sample of

![Chemical Structures](67_68)

18α-carbomethoxy-4α-dihydrocleavamine in 72% yield. The specific activity of the 18α-carbomethoxy-4α-dihydrocleavamine was found to be 0.103 mCi/mmol. These were particularly encouraging results since they meant that the method would likely be suitable for labelling other potential precursors with a high level of activity without fear of extensive decomposition. It remained of course to be shown that the position of the label was in the aromatic system. There was little doubt that the label was not at the site of the indole nitrogen atom since the conditions used in the work up would cause
an immediate exchange of any tritium atoms at that site for hydrogen atoms. There was the possibility, however, that the label could reside at the C-18 site.

In order to resolve the problem of the location of the label, deuterated 18a-carbomethoxy-4a-dihydrocleavamine was prepared. Trifluoroacetic acid-d, which had been prepared by hydrolyzing trifluoroacetic anhydride with an equivalent amount of deuterium oxide, was allowed to react with 18a-carbomethoxy-4a-dihydrocleavamine for 4 hours at 50°C under a nitrogen atmosphere. Again thin-layer chromatography showed that the reaction mixture contained both C-18 epimers. Preparative thin-layer chromatography and crystallization provided a pure sample of deuterated 18a-carbomethoxy-4a-dihydrocleavamine in 67% yield. An estimate of the average number of dueterium atoms and their location in the molecule was made with the aid of the nmr and mass spectra of the compound in its labelled and unlabelled forms. It was quite simple to distinguish the fragments which corresponded to portions of the molecule which had become labelled from those which had not by comparing the mass spectrum of the deuterium labelled compound with that of the unlabelled compound. Each spectrum was obtained under as nearly identical conditions as possible. Since the deuterium labelled compound was in actuality a mixture of hydrogen-isotope compounds \( \text{C}_{21}\text{H}_{28-n}\text{D}_{n}\text{N}_2\text{O}_2 \)\), it displayed a composite mass spectrum. In the case of the spectrum of the unlabelled compound (Figure 20) there were four peaks at m/e 338, 339, 341, and 342 that were associated with the molecular ion peak at m/e 340. In the spectrum of the labelled compound (Figure 21) there were a cluster of peaks stretching to an m/e value of 346 (and perhaps to 347). Assuming that the peak at 346 represented the M+2 peak of the most highly substituted molecular ion, there could have been a mixture of hydrogen-isotope compounds
Figure 20. Mass spectrum of unlabelled 18α-carbomethoxy-4α-dihydrocleavamine
Figure 21. Mass spectrum of deuterium labeled 18a-carboxymethyl-4a-dihydrotestosterone.
corresponding to the formulae: \( \text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_2 \), \( \text{C}_{21}\text{H}_{27}\text{D}_2\text{N}_2\text{O}_2 \), \( \text{C}_{21}\text{H}_{26}\text{D}_2\text{N}_2\text{O}_2 \), \( \text{C}_{21}\text{H}_{25}\text{D}_3\text{N}_2\text{O}_2 \) and \( \text{C}_{21}\text{H}_{24}\text{D}_2\text{N}_2\text{O} \) in the sample of labelled carbomethoxydihydro-

-cleavamine. Although a series of simultaneous equations might have been

set up from which the relative abundance of each of the species \( \text{C}_{21}\text{H}_{28-n}\text{D}_n\text{N}_2\text{O}_2 \)

could theoretically have been calculated, to do so seemed to be impractical. Only the average number of deuterium atoms at each site in the molecule was of interest for our purposes and this number was easily obtained with a certain degree of precision from the nmr spectra. Some useful information was available from the mass spectra, however. A comparison of the spectrum of the unlabelled compound with that of the labelled compound revealed that there is a relative abundance of the peaks in the vicinity of the peaks at m/e 138 and m/e 124 were virtually the same in both spectra. This was consistent with there being no deuterium containing fragments corresponding to ions 69 and 70 in the deuterium labelled compound. Hence the protons in these fragments were not exchangeable under the conditions employed. It was

![Diagram of molecular structures](image)

observed that both spectra displayed a strong peak at m/e 210. The strength of this peak in the mass spectrum of the labelled compound meant that the fragment arose from a portion of the molecule which was not easily labelled. By comparison the peak at m/e 340 was almost non existent in the spectrum
of the labelled compound. Since most of the label was in the indole system (as shown by nmr below), it was apparent that this fragment must not contain the indole moiety. The ion 71 would display an m/e value of 210 and might arise from 18α-carbomethoxy-4α-dihydrocleavamine as shown in Figure 22.

Figure 22. Plausible pathway giving a fragment with an m/e value of 210

It was also observed that the peak at m/e 211 in the spectrum of the deuterated compound was slightly larger by comparison with the same peak in the spectrum of the unlabelled compound. This would be consistent with there
being a small contribution to the m/e 211 peak from the species 72.

The nmr spectrum of the deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine provided substantiation of the information obtained from the mass spectrum. In addition, the sites in which deuterium was located and the amount of deuterium in each site could be determined by careful integration. The spectrum of the deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine is shown in Figure 23. The spectrum of the unlabelled compound is shown in Figure 24. It was immediately apparent even without the aid of the integral that the aromatic portion contained most of the deuterium atoms. The spectrum showed strong signal due to the proton on the indole nitrogen atom and the proton at C-18. In addition, the portion of the spectrum above \( \tau \) 6 was virtually superimposable with the same portion of the spectrum of the unlabelled compound. The region of the aromatic protons, however, was radically different. The multiplet at about \( \tau \) 2.55 in the spectrum of the unlabelled compound had collapsed to a singlet at \( \tau \) 2.53 in the spectrum of the labelled compound. The other groups of multiplets appeared to be simplified. These factors were in accord with deuterium being present in the aromatic portion of the molecule in a substantial amount. The apparent
Figure 23. Nmr spectrum of deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine
Figure 24. Nmr spectrum of unlabelled 18α-carbomethoxy-4α-dihydrocleavamine
simplification of the region would not be expected if only a small number of molecules containing deuterium atoms were present. Comparison of the integrals in the spectra of the labelled and unlabelled compounds in the region above \( \tau 7 \) did not reveal any sites that were suspected to have been labelled to some extent were determined by assuming the integral of the signal from the protons of the methyl ester to be exactly equivalent to 3 protons. This assumption was substantiated by carefully comparing the integral of the signal from the methyl ester to the integral of the signals from the methyl group of the ethyl side chain. As near as could be determined both integrals were the same. The integral over the aromatic region, after correction for the presence of chloroform had been made, corresponded to 1.55 protons. This meant that 61.5% of the aromatic hydrogen atoms had been exchanged for deuterium atoms. The integral over the region of the C-18 proton resonances corresponded to \( 0.89 \pm 0.03 \) protons. This meant that about 10% of the C-18 hydrogen atoms had been exchanged for deuterium atoms and this result agreed with the observation made from the mass spectrum concerning the C-18 site. The integral over the indole NH corresponded to about 0.9 protons as well, but this integral in the spectrum of the undeuterated compound also corresponded to about 0.9 protons. The nmr evidence was consistent with the aromatic hydrogen atoms and the C-18 hydrogen atoms being the only atoms for which there was net exchange under the conditions of the deuterium labelling experiment and also therefore during the tritium labelling experiment. Only about 4% of the label was at the C-18 site, with the remainder of the label being in the aromatic sites.

Since the deuterium labelling experiment proceeded very well at 50°C, the reaction was repeated at room temperature. In this instance a solution
of 18α-carbomethoxy-4α-dihydrocleavamine in trifluoroacetic-d was presented to the nmr spectroscopist who ran the spectrum every so often when it was convenient to do so. The nmr spectra obtained were of very good quality. The number of deuterium atoms that had replaced aromatic hydrogen atoms over several different time intervals are given in Table 1. The number of deuterium atoms was estimated by assuming that the integral over the proton resonances of the methyl ester corresponded to 3 protons. Even after two weeks the nmr spectrum revealed barely any signs of decomposition although the solution had become red-purple in colour. Investigation of the material obtained after two weeks by thin-layer chromatography showed the presence of 18α-carbomethoxy-4α-dihydrocleavamine, some 18β-carbomethoxy-4α-dihydrocleavamine and only a small amount of unidentified impurities.

Since 18β-carbomethoxycleavamine (73) seemed to be the most likely

![Chemical structure of 18β-carbomethoxycleavamine (73)]
precursor of the naturally occurring alkaloid catharanthine (25), it was of interest to see how this compound was affected by trifluoroacetic acid-d. It was anticipated that the presence of the olefinic double bond might make the compound sensitive to acid. In fact this conjecture was borne out by the experiment. When the labelling reaction was carried out at 50°C, a complex mixture of compounds as shown by thin-layer chromatography was obtained. Chromatography on alumina provided quite pure 18β-carbomethoxy-cleavamine in 40% yield. Further purification by crystallization provided an analytically pure sample. The nmr spectrum (Figure 25) revealed that as in the case of the 18α-carbomethoxy-4α-dihydrocleavamine the aromatic hydrogen atoms were exchanged to the greatest extent. Because the signals arising from the C-18 and C-3 protons overlapped to some extent, it was not possible to distinguish between these two sites. The integral over the aromatic region, after correcting for the presence of chloroform, corresponded to 1.85 protons and the integral over the region in which the C-18 and C-3 protons resonances occurred corresponded to 1.55 protons. These results meant that the deuterium atoms were distributed with 82.5% of the deuterium atoms being on the aromatic ring and 17.5% of them being in one or both of the other two sites at C-18 and C-3.

Although the yield of deuterium labelled 18β-carbomethoxy-cleavamine was poor when the labelling reaction was done at 50°C, several other workers in our laboratories have been able to obtain nearly quantitative yield of the labelled compound by carrying out the reaction at room temperature for periods longer than four hours.

Because the aromatic hydrogen atoms of 18α-carbomethoxy-4α-dihydrocleavamine and 18β-carbomethoxy-cleavamine were exchanged for deuterium atoms
Figure 25. Nmr spectrum of deuterium labelled 18β-carbomethoxyclavamine
(and hence, tritium atoms) in preference to the other hydrogen atoms in these molecules, the location of label in related compounds, which were to be prepared for use in tracer experiments by treatment with tritium labelled trifluoroacetic acid, could be predicted. Since several of the compounds which were to be used in the tracer experiments were available in amounts of only a few milligrams, it was not practical to label them with deuterium for the purposes of mass spectrometry and nmr spectroscopy and it was imperative in these cases that the position of the label could be predicted with certainty.

On this basis 18α-carbomethoxy-4α-dihydrocleavamine, vincadine (74) and vincaminoreine (75) were labelled with tritium labelled trifluoroacetic acid (specific activity, 0.88 mCi/mmol), the latter two compounds for the purposes of tracer experiment with Vinca minor L. plants. The method used was a modification of the method used to prepare deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine. Whereas a 65:1 molar ratio was used in the deuterium labelling experiments, a smaller ratio of trifluoroacetic acid to the compound being labelled was used in the tritium labelling experiments, entirely in the interest of conserving tritium labelled trifluoroacetic acid. The specific activities obtained for the three compounds are given in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>molar ratio of reactants</th>
<th>specific activity (mCi/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18α-carbomethoxy-4α-dihydrocleavamine</td>
<td>8.6</td>
<td>0.205</td>
</tr>
<tr>
<td>vincaminoreine</td>
<td>9.3</td>
<td>0.282</td>
</tr>
<tr>
<td>vincadine</td>
<td>18.0</td>
<td>0.548</td>
</tr>
</tbody>
</table>
Other workers in our laboratories have used tritium labelled trifluoroacetic acid to prepare tritium labelled samples of a great many indole containing compounds for tracer experiments in plants. As a rule the compounds were recovered virtually unchanged from the acidic medium.

When the labelling experiments described in this thesis were being done, it was realized that it would be economically more feasible to recover the labelled trifluoroacetic acid for reuse. A simple vacuum system was constructed in which the tritium labelled trifluoroacetic acid could be distilled at reduced pressure to and from the compound which was to be labelled. In a typical labelling experiment one vessel containing the compound and another containing the acid was attached to the system. The trifluoroacetic acid was then frozen with a liquid nitrogen bath and the system evacuated. The liquid nitrogen bath was then transferred to the vessel containing the compound to be labelled and the trifluoroacetic acid was distilled over. When all the trifluoroacetic acid had been transferred nitrogen was allowed into the system and the liquid nitrogen bath was removed from the vessel containing the compound to be labelled and the frozen acid. The reactants were allowed to come to room temperature. After the reaction had proceeded
at room temperature for several hours (usually overnight), the excess trifluoroacetic acid was transferred from the labelled compound using the vacuum system. Since a large excess of acid was used, the dilution of the label in the reaction was very small and the recovered tritium labelled trifluoroacetic acid was suitable for reuse.

TRACER EXPERIMENTS USING VINCA PLANTS

Experiment using $[^{14}\text{C}]-18\beta$-carbomethoxy-4$\beta$-dihydrocleavamine as tracer

Each of the four epimerically related carbomethoxydihydrocleavamines was considered as a possible precursor of the alkaloids bearing the Iboga skeleton. One of these epimers, $18\beta$-carbomethoxy-4$\beta$-dihydrocleavamine, was available from 4$\beta$-dihydrocleavamine by a synthetic route in which the carbonyl carbon atom of the ester group came from the cyanide ion. Therefore $[^{14}\text{C}]-18\beta$-carbomethoxy-4$\beta$-dihydrocleavamine was available for the initial tracer experiment in our laboratories to test the postulate put forward by Wenkert for the biosynthesis of alkaloids possessing the Iboga skeleton. $\text{Vinca rosea}$ L. plants were used for this experiment. The foliage on the plants was about two months old, whereas the root system was older. The plants were not flowering when used. $[^{14}\text{C}]-18\beta$-carbomethoxy-4$\beta$-dihydrocleavamine (6.823 mg, $1.67 \times 10^{-4}$ μCi) was converted to its hydrochloride salt and an aqueous solution of the salt was fed hydroponically to $\text{Vinca rosea}$ L. plants. Each plant was cut on the diagonal across its stem and the severed end was immediately placed in the solution of the labelled compound. Each plant was allowed to take up the solution of the labelled compound and an additional volume of distilled water. When a particular plant refused to take up any liquid or did so at a rate that was no comparable with the average rate, its
stem was recut. The bits of stem that were cut off were saved and included in the work-up. The plants were maintained for a period of eight days by addition of distilled water when it was necessary except towards the end of the period when the plants were allowed to take in all the water which was available to them. The plants were then immediately macerated in the presence of a mixture of benzene and 15 N ammonium hydroxide. The alkaloids that were present in the benzene phase after this treatment were extracted into 2N hydrochloric acid. The acidic solution was then made basic and extracted with benzene. The benzene extract provided an alkaloid containing mixture which was found to possess 30% of the activity that had been fed.

Preparative thin-layer chromatography (alumina, 3:1, benzene-chloroform) of the crude extract on a 20x60 cm plate, which was run in the direction of its longer measurement, provided a partial separation of the mixture into nine groups of alkaloids. Thin-layer chromatography confirmed the presence of 18β-carbomethoxy-4β-dihydrocleavamine in group 4 (av R_f 0.55), coronaridine in group 5 (av R_f 0.45), and catharanthine in group 8 (av R_f 0.1), but dihydrocatharanthine, which is not a known alkaloid, could not be detected by thin-layer chromatography in group 7 (av R_f 0.2) in which it would have been present. The activity in each of the groups of alkaloids was counted separately and the total activity obtained from the plate corresponded to 59% of that which had been put on the plate. The activity in group 7, in which dihydrocatharanthine might have been present, accounted for only 0.2% of the total activity fed and a small amount of cold dihydrocatharanthine which was added to the mixture represented by group 7 and reisolated by preparative thin-layer chromatography was found to show background activity only. Catharanthine and coronaridine were purified by preparative thin-layer
chromatography until analytical thin-layer chromatography indicated that they were pure. At each stage in the purification the activity associated with these compounds diminished. When further purification by thin-layer chromatography became impractical, the total activity associated with coronaridine was 0.09% of the fed activity and the total activity associated with catharanthine was 0.02% of the fed activity. These results indicated that the level of incorporation of [22-14C]-18β-carbomethoxy-4β-dihydrocleavamine, if it was a precursor of the Iboga alkaloids, was going to be very low under the conditions in which this tracer experiment was run and helped stimulate a study of different methods of labelling and feeding compounds.

The above described feeding experiment was conducted in collaboration with Dr. Stan Hall and he determined the level of activity associated with catharanthine. He was also a collaborator in two of the other tracer experiments that will be described in this thesis and his contributions will be signified by the letters "S.H."

Experiment using [T-aromatic]-18β-carbomethoxydihydrocleavamine as tracer

We felt that 18β-carbomethoxydihydrocleavamine would be the most likely precursor of catharanthine. The conversion required that the plant be able to bring about oxidative cyclization between the C-18 and C-5 atoms in the carbomethoxydihydrocleavamine molecule. An analogous cyclization had been shown to take place after mercuric acetate oxidation of 18α-carbomethoxy-4α-dihydrocleavamine. Therefore, 18β-carbomethoxydihydrocleavamine (3.105 mg, 8.38 x 10^-4 mCi), which had been labelled in the aromatic system, was fed to Vinca rosea L. plants in the same manner as above. In this case, however, the foliage on the plants was about 5 months old and the plants were worked up after 46 hours. The extraction procedure was the same as described above.
and the crude alkaloidal extract contained 35% of the activity that had been fed. The crude extract was chromatographed on alumina (activity III). Elution with 3:1 petroleum ether (30-60)-benzene provided [T-aromatic]-18β-carbomethoxycleavamine. This material was diluted with inactive 18β-carbomethoxycleavamine and the mixture crystallized to constant activity. About 11% of the fed activity was recovered in the form of unchanged 18β-carbomethoxycleavamine. Elution with 1:1 petroleum ether (30-60)-benzene provided catharanthine. Analytical thin-layer chromatography of the catharanthine obtained showed that it contained several impurities. Consequently, the catharanthine was diluted with a measured amount of inactive catharanthine and chromatographed on alumina. Elution as before provided catharanthine which was seen to be pure by thin-layer chromatography except for some impurities which were observable only by their fluorescence under ultraviolet light. These impurities were transported at or nearly at the same rate as catharanthine in alumina thin-layer chromatoplates but were seen to be transported in the main at a much slower rate on silica gel chromatoplates. Accordingly the catharanthine was chromatographed on silica gel (activity III). A trial chromatography showed that a considerable amount of catharanthine would be lost to the column if the chromatography was carried out slowly. Therefore the catharanthine was chromatographed quickly. The catharanthine obtained (80% recovery) was seen to be free from most of the fluorescent impurities. It was then combined with an additional portion of inactive catharanthine and the mixture recrystallized a total of five times from methanol. After each crystallization a sample was counted. The activity in the catharanthine was seen to decrease with each recrystallization to a counting rate of only 2.2 dpm/mg. This corresponded to a
maximum total activity in the isolated catharanthine of 208 dpm which corresponded to 0.011% of the activity fed.

Experiment using [T-aromatic]-vincaminoreine as tracer

Three alkaloids which could conceivably be biosynthesized from vincaminoreine (75) by *Vinca minor* L. plants are minovine (76), vincamine (59) and 1,2-dehydroaspidospermidine (77). Accordingly, an experiment was conducted to see whether these transformations were carried out by *Vinca minor* L. plants. A different method of feeding than that described above was used. Since it was felt that the site of biosynthesis of the alkaloids might be in the leaves of the *Vinca* plants, there was some concern that the negative results obtained in the previous experiments could have been caused by failure of the tracer to be transported to the leaves. For this reason a method of feeding the leaves was sought. One method generally used for feeding leaves directly is to make a slit along the central vein of the leaf from the apex towards the heel and bend the triangular peninsula that is formed into a vessel containing a solution of the tracer. In our experiments small triangular bags, made from pliable plastic sheeting, were used.
as the vessels. The placement of the bags is illustrated below in Figure 26.

![Diagram of bag-on-leaf method of feeding](image)

**Figure 26. Bag-on-leaf method of feeding**

Mature greenhouse grown *Vinca minor* L. plants were fed with an aqueous solution of [T-aromatic]-vincaminoreine as the acetate salt by the bag-on-leaf method. The tritium labelled vincaminoreine (1.507 mg, $1.2 \times 10^{-3}$ mCi) as the acetate salt was dissolved in distilled water. The aqueous solution was divided into two portions. One portion (15%, $1.45 \times 10^{-4}$ mCi) was saved as a blank and the other portion (85%, $1.05 \times 10^{-3}$ mCi) was used to fill the bags. After the feeding was complete the activity remaining in the bags was determined and found to be 21% of that put in them. The amount of activity as [T-aromatic]-vincaminoreine that was taken into the plant was determined by difference to be $8.25 \times 10^{-4}$ mCi. Caution was taken to assure that as little time as possible was wasted from the time a leaf was cut until a bag was put in place and filled with some of the solution of the tracer. The bags were never allowed to be sucked to dryness. Distilled water was injected into the bags as required. The plants were worked up after four days. The alkaloids were extracted using the procedure above except that methanol was used in place of the benzene - 15N ammonium hydroxide mixture and methylene chloride was used in place of benzene in the back extraction
step. The total activity recovered in the crude alkaloidal extract was 46.5% of the activity fed. The crude extract was chromatographed on alumina (activity III). Elution with 1:1 petroleum ether (30-60) provided vincaminoreine in the initial fractions and minovine in the later fractions with some overlap in the middle fractions. Elution with 3:1 benzene-petroleum ether (30-60) provided a mixture of compounds in which 1,2-dehydroaspidospermidine was a major component. Elution with an additional quantity of this solvent mixture provided vincamine. The vincamine was recrystallized from methanol and its counting rate determined. The percent of the activity which had been fed that was associated with the vincamine when further purification was impractical was 0.0021.

The mixture of compounds that contained 1,2-dehydroaspidospermidine was reduced with lithium aluminum hydride in ether. The crude reduction product was chromatographed on alumina. Elution with 3:1 benzene-petroleum ether (30-60) provided partially pure aspidospermidine. Further purification of the aspidospermidine, first as the free base and then as the hydrochloride salt brought the level of activity that was associated with the aspidospermidine down to 0.008% of the activity which had been fed.

The minovine was purified and its rate of incorporation was found to be 0.7%(SH). When the blank was worked up, it was found that the vincaminoreine had been converted to minovine in 0.3% yield (SH). This behavior was also observed in the case of quebrachamine and it was established that quebrachamine was converted into 1,2-dehydroaspidospermidine simply by standing in air. In one experiment (SH), quebrachamine was allowed to stand in solution for 10 days, and then treated with lithium aluminum hydride. The yield of aspidospermidine was shown to be 3.4%.
Experiment using [T-aromatic]-vincadine as tracer

All the tracer experiments described above and others conducted in our laboratories at the same time by other workers gave negative results or questionable positive results. It was felt at this stage that it would be difficult in the case of the conversion of a precursor to an aspidosperma skeleton by transannular cyclization to establish whether the cyclization was brought about by enzymic oxidation in the plant or by aerial oxidation. Nevertheless, it was hoped that an actual incorporation of a tracer by the plant could be demonstrated so that the question of whether the tracers were actually being transported into a site of syntheses in the plants could be answered. If labelled vincadine (74) were to be incorporated into minovine (76) by the Vinca minor L. plants, this question could be answered in the affirmative since only the plant could bring about an N-methylation. Vincadine was also of interest as a probable precursor of vincamine. The conversion could be envisaged as being brought about by oxidation of vincadine at the C-3 and C-19 sites followed by a rearrangement such as that shown in Figure 19.

[T-aromatic]-vincadine was fed to mature Vinca minor L. plants as before by the bag-on-leaf method. A solution of [T-aromatic]-vincadine (1.133 mg, 1.79 x 10^{-3} mCi) was used. After the feeding was over, the activity remaining in the bags was determined to be 31% of that contained in the solution used. The total activity which had gone into the leaves was determined by difference to be 1.23 x 10^{-3} mCi and this amount was considered to be the activity fed. The crude alkaloidal extract was found to contain 43% of the activity which had been fed to the plants. Purification of the alkaloids of interest was carried out in the same fashion as in the experiment
above. The maximum total activity which could have been present as the isolated 1,2-lehydroaspidospermidine was found to be 0.003% of the activity which had been fed to the plant. Purification of minovine until it was impossible to purify it further brought the level of the maximum total activity which could have been present as isolated minovine down to 0.001%. In this experiment the vincamine displayed a rate of incorporation, but the blank experiment also showed a rate of incorporation that was comparable. Because no attempt had been made to obtain a radiochemically pure sample of [T-aromatic]-vincadine for the feeding experiment, it was suspected that the vincadine which was labelled contained a small amount of vincamine which could not be detected as an impurity by thin-layer chromatography. When inactive vincamine was added to the tritium labelled vincadine and reisolated it was found to have become radioactive (SH). Repetition (SH) of the feeding experiment using purified tritium labelled vincadine showed that there was no incorporation of vincadine into vincamine by Vinca rosea L. plants which could be detected in our experiments.

In summary, none of the experiments recounted in this thesis were of a positive nature. The activities given for the most of the compounds signify the degree to which the different samples could be purified before further purification was no longer possible or practical. In cases where a definite specific activity could be determined for a compound an explanation which did not involve the plant was possible. It appeared on the basis of the results obtained that the transannular cyclization as envisaged was not a step in the biosynthesis of the Iboga and Aspidosperma alkaloids. It must be emphasized that the above experiments were really of a preliminary nature to simply investigate whether high levels of incorporation could be easily
achieved by the use of the above-mentioned large precursors. Clearly these results were not conclusive and more definitive experiments were essential. Due to lack of time I was unable to conduct such experiments but other workers in this laboratory have now done this work. A recent communication on all of these results has now appeared. It is concluded that the transannular cyclization reaction is not of biosynthetic significance at least in *Vinca* plants.
III. Experimental

Melting points were determined on a Kofler block and are uncorrected. Nuclear magnetic resonance (nmr) spectra were recorded using deuteriochloroform as solvent on a Varian HA-100 instrument by Mr. R. Burton and the chemical shifts are given in the Tiers \( \tau \) scale. Mass spectral were recorded on an Atlas CH-4 mass spectrometer and high resolution molecular weight determinations were performed by either Mr. G. Eigendorf or Mr. G. Brown on an AEI MS-9 mass spectrometer. Woelm neutral silica gel and alumina without binder or Silica Gel G and Alumina G (according to Stahl) containing 5\% by wt of General Electric Ratma p-1, Type 118-2-7 electronic phosphor were used for analytical and preparative thin-layer chromatography (tlc). Chromatoplates were developed with 2:1 carbon tetrachloride-antimony pentachloride spray reagent. Woelm neutral silica gel and alumina (activity III) were used for column chromatography. Solutions were dried using anhyd sodium sulfate. Radioactivity was measured with a Nuclear-Chicago Mark 1 Model 6860 Liquid Scintillation counter in counts per minute (cpm). The radioactivity of a sample in disintegrations per minute (dpm) was calculated using the counting efficiency which was determined for each sample by the external standard technique\(^{61}\) utilizing the built-in barium-133 gamma source. The standard deviations in the radioactivity of samples which are quoted were calculated\(^{62}\) so that the probability of a number being within the prescribed limits was 68\%. The radioactivity of alkaloids as the free
base was determined using a scintillator solution made up according to the recipe:

\[
\begin{align*}
\text{toluene} & \quad 1 \text{ ml} \\
2,5\text{-diphenyloxazole (PPO)} & \quad 4 \text{ g} \\
1,4\text{-bis[2-(5-phenyloxazolyl)] benzene (POPOP)} & \quad 0.05 \text{ g}
\end{align*}
\]

The radioactivity of the salts of alkaloids and the radioactivity of crude extracts were determined using a scintillator solution made up according to the recipe:

\[
\begin{align*}
toluene & \quad 0.385 \text{ ml} \\
dioxane & \quad 0.385 \text{ ml} \\
methanol & \quad 0.230 \text{ ml} \\
naphthalene & \quad 80 \text{ g} \\
PPO & \quad 5 \text{ g} \\
POPOP & \quad 0.0625 \text{ g}
\end{align*}
\]

In practice a sample of an alkaloid as the free base was dissolved in 1 ml of benzene in a counting vial using heat if necessary and then the volume was made up to 15 ml with scintillator solution. In the case of the salt of an alkaloid the sample was dissolved initially in 1 ml of methanol.

**Tritium labelled 18α-carbomethoxy-4α-dihydrocleavamine (67)**

18α-carbomethoxy-4α-dihydrocleavamine (20.5 mg) was dissolved in trifluoroacetic acid (0.65 ml). Approx. 1 mCi of tritium labelled water (0.01 ml) was added and the solution was heated at 50°C for four hours under a nitrogen atmosphere. The solution was then added slowly with stirring to a saturated sodium bicarbonate solution (5 ml) which was cooled in an ice-water bath. When the addition was complete, the solution was made fairly strongly basic by the addition of sodium carbonate and extracted with methylene chloride. The methylene chloride extract was dried and the solvent was removed to yield a residue which was comprised of 18α-carbomethoxy-4β-dihydrocleavamine and a small amount of 18β-carbomethoxy-4β-dihydrocleavamine as shown by tlc. Preparative tlc (silica gel, 20 x 20 cm plate,
0.5 thickness, chloroform as transporting solvent, extraction with ethyl acetate) and crystallization from methanol provided 14.8 mg of tritium labelled 18α-carbomethoxy-4α-dihydrocleavamine mp 171-172.5°C, with a specific activity of 0.103 mCi/mmol.

Deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine (67)

18α-carbomethoxy-4β-dihydrocleavamine (50.0 mg) was dissolved in trifluoroacetic acid-d (0.7 ml) which had been prepared by hydrolysing trifluoroacetic anhydride with deuterium oxide. The solution was heated at 50°C for four hours under a nitrogen atmosphere. The solution was then added slowly with stirring to a saturated sodium bicarbonate solution (10 ml) which was maintained at the temperature of an ice-water bath. A little sodium carbonate was added to the solution to make it definitely basic and the solution was extracted with methylene chloride (three 10 ml portions). After the extract had been dried, the solvent was removed to yield a gummy residue (61 mg). Crystallization of the residue yielded 17.4 mg of deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine. The mother liquors from the above crystallization were subjected to separation by preparative tlc (silica gel, 20 x 20 cm plate, 0.5 mm thickness, chloroform as transporting solvent, extraction with ethyl acetate) and in this manner an additional quantity of deuterium labelled 18α-carbomethoxy-4α-dihydrocleavamine (16.0 mg) and some deuterium labelled 18β-carbomethoxy-4α-dihydrocleavamine (68) (10.5 mg) as shown by tlc comparison with an authentic sample were obtained.

Deuterium labelled 18β-carbomethoxycleavamine (73)

Some 18β-carbomethoxycleavamine (152.3 mg) was dissolved in trifluoroacetic acid-d and the solution was heated for four hours at 50°C under a
nitrogen atmosphere. The same work up as given in the previous experiment with the appropriate adjustment of quantities was used and a gummy residue obtained (180.4 mg). This residue was chromatographed on alumina (18 g). Elution with benzene provided 61.2 mg of deuterium labelled 18β-carbomethoxy-cleavamine, which showed some minor impurities on analysis by tlc (silica gel, chloroform). A combination of preparative tlc (silica gel, chloroform, extraction with ethyl acetate) and crystallization from methanol and then methanol-water provided 16.1 mg of 18β-carbomethoxycleavamine with mp 119-123 °C.

Tritium labelled 18α-carbomethoxy-4α-dihydrocleavamine (67) - a small scale experiment

A sample of 18α-carbomethoxy-4α-dihydrocleavamine (10.7 mg) was weighed in a piece of glass tubing which was sealed at one end. Tritium labelled trifluoroacetic acid (20 μl, 0.88 mCi/mmol) was added and the glass tubing was attached to a glass "T" joint as shown in Figure 27. After the solution

![Diagram of apparatus for small scale labelling experiments]

Figure 27. Apparatus for small scale labelling experiments
had been heated at 50°C for four hours under a nitrogen atmosphere, it was added to a cooled aqueous 10% sodium bicarbonate solution (0.5 ml) with stirring and the basic solution obtained was extracted with methylene chloride (three 1 ml portions). After the methylene chloride extract had been dried, the solvent was removed with the aid of a nitrogen stream and a warm water bath and a vacuum pump to yield a foam (9.5 mg). Crystallization from methanol to constant activity gave a sample of 18α-carbomethoxy-4α-dihydrocleavamine with specific activity of 0.205 mCi/mmol.

Tritium labelled vincaminoreine (75)

The experiment was carried out as described above using vincaminoreine (10.29 mg) and labelled trifluoroacetic acid (20 μl, 0.88 mCi/mmol). After the work up had been completed, labelled vincaminoreine (9.10 mg, 0.282 mCi/mmol), which showed only one spot on analysis by tlc (alumina, benzene and silica gel, 1:1 ethyl acetate-chloroform), was obtained.

Tritium labelled vincadine (74)

The experiment was carried out as described above using vincadine (5.14 mg) and labelled trifluoroacetic acid (20 ml, 0.88 mCi/mmol). After the work up, which included recovering the vincadine from a large volume of methanol to ensure that only an insignificant percentage of the label would remain on the indole nitrogen atom, had been completed, labelled vincadine (5.00 mg, 0.548 mCi/mmol), which appeared to be chemically pure as shown by tlc, was obtained.

Tracer experiment in Vinca rosea L. plants using [22-14C]-18β-carbomethoxy-4β-dihydrocleavamine (61)

A sample of [22-14C]-18β-carbomethoxy-4β-dihydrocleavamine (6.823 mg,
1.67 \times 10^{-4} \text{ mCi/mmol}) was dissolved in diethyl ether and precipitated as its hydrochloride salt by blowing hydrogen chloride vapours over the solution. After the diethyl ether and excess acid had been removed by evaporation, the salt was dissolved in water (10 ml) and the aqueous solution was divided among ten 5 ml test tubes. Ten *Vinca rosea* L. plants with about two month old foliage and an old root system were cut on an angle through the stem. Each plant as it was severed from its root in this fashion was immediately placed in one of the test tubes containing a solution of the tracer. After each plant had been permitted to take up the solution of tracer until there was just enough solution to keep the cut end moist, the volume was made up to 1 ml with distilled water. After this procedure was repeated several times to ensure that nearly all the tracer had been taken up by the plant, the test tubes were filled to the top with distilled water and more water was added when it was required over a period of eight days. During this period of time between 10 ml and 30 ml of liquid was taken up by each plant and light was supplied by a bank of neon tubes.

After eight days the plants (67.3 g) were chopped up with a pair of scissors and macerated in the presence of 10:1 benzene - 15 N ammonium hydroxide (500 ml) in a Waring blender. The green mash was filtered through a celite pad in a Buchner funnel. The marc was separated from the celite pad and macerated again with 10:1 benzene - 15 N ammonium hydroxide (250 ml) and filtered as before. The filtrates were combined and the organic phase was separated from the aqueous phase. After the bulk of the solvent had been removed in a rotary evaporator, benzene (150 ml) was added and the solution extracted with 2 N hydrochloric acid (five 50 ml portions). After the acid extract had been washed with benzene (five 50 ml portions), an additional quantity (100 ml) of benzene was added to it and it was made
strongly basic by the addition of 15 N ammonium hydroxide. The organic phase was drawn off and the aqueous phase was extracted with a further quantity (three 50 ml portions) of benzene. The aqueous phase was then saturated with potassium carbonate and again extracted with benzene (two 50 ml portions). The benzene extracts were combined, washed with water (three 50 ml portions) and salt brine (one 50 ml portion), and dried. Removal of the solvent provided 139.0 mg of a gummy residue. The radioactivity ($3.44 \times 10^{-5}$ mCi) of this residue represented 30% of the total activity fed.

Most of the crude alkaloidal extract (133.2 mg) was subjected to a separation by preparative tlc. An alumina (Woelm) plate (20 x 60 cm, 0.5 mm thickness) was used and the crude extract was applied in a narrow band at one end of the plate. After being transported in the direction of the longer measurement with 3:1 benzene-chloroform, the alkaloids were located as dark bands by observation of the plate under ultraviolet light. The plate was divided into nine sections, from baseline to solvent front with the bands corresponding to 18β-carbomethoxy-4β-dihydrocleavamine, coronaridine, dihydrocatharanthine and catharanthine coinciding with a different section. Each section was scraped off the plate and eluted with methanol. Removal of the solvent gave nine groups of alkaloids. The activity in each group was determined and the total radioactivity was $1.95 \times 10^{-5}$ mCi (59% of that placed on the plate). Attention was directed towards investigating groups 4 (48% of recovered activity, av. $R_f$ 0.55), 5 (25% of recovered activity, av. $R_f$ 0.45) and 8 (1.5% of recovered activity, av. $R_f$ 0.1), which consisted mainly of 18β-carbomethoxy-4β-dihydrocleavamine, coronaridine and catharanthine, respectively, as shown by comparison tlc using several systems, and group 7 (1.2% of recovered activity, av $R_f$ 0.2) in which
dihydrocatharanthine would occur, if it were present.

Group 5 was again subjected to preparative tlc on an alumina (Woelm) plate (5 x 20 cm, 0.25 mm thickness). After being transported with 3:1 benzene-chloroform, the bands corresponding to 18β-carboamethoxy-4β-dihydrocleavamine, coronaridine and the interval between these were scraped off the plate and eluted with methanol. Removal of the solvent provided three residues which corresponded to 37.5%, 9.3% and 36.0%, respectively, of the activity in group 5. The residue which appeared to contain only coronaridine as shown by comparison tlc using alumina plates was shown to be composed of two compounds on silica gel plates. Tlc (silica gel, 3:1 chloroform-ethyl acetate): chromatoplate showed a green spot of Rf 0.7, which corresponded in Rf and colour reaction to coronaridine, and a pink spot at Rf 0.55. The mixture was then resolved by preparative tlc using the above system. At this stage it was no longer practical to attempt a further purification and it was determined that the radioactivity of the coronaridine was 242 dpm (0.09% of the radioactivity that was fed) whereas the radioactivity of the unknown material was 2000 dpm (0.8% of the radioactivity that was fed). The spectral data of this unknown material: $\lambda_{\text{Max}}^{\text{MeOH}}$ 327, 297, 228; mass spectrum m/e (rel intensity) 338(35), 124(100) indicated that it might be 7β-ethyl-5-desethylvincadifformine (7β-ethyl-5-desethyl-63).

Anal. Calcd for C$_{21}$H$_{26}$N$_{6}$O$_{2}$: mol wt, 338.199. Found: mol wt, 338.197 (mass spectrometry).

Group 7 was diluted with a small amount of authentic dihydrocatharanthine and subjected to preparative tlc. The reisolated dihydrocatharanthine after preparative tlc (alumina, 3:1 benzene-chloroform and silica gel, 1:1 chloroform ethyl acetate) was found to have no radioactivity.

The catharanthine component in group 8 was subjected to purification by
tlc (by SH) and when purification was found to be no longer practical, the radioactivity of the catharanthine was 75 dpm (0.02% of the activity which had been fed).

Tracer Experiment in *Vinca rosea* L. plants using [T-aromatic]-18β-carbomethoxycalevamine (73)

A sample of [T-aromatic]-18β-carbomethoxycalevamine (3.105 mg; 8.38 x 10^-4 mCi) was fed as its hydrochloride salt in the same manner as was described in the preceding experiment. Ten *Vinca rosea* L. plants which had five month old foliage and were not flowering at the time of the experiment were used. The plants were worked up after 46 hours during which time each had taken up an average of 13 ml of solution.

The plants (124.2 g) were worked up in the same fashion as was described in the previous experiment and in this case 213.1 mg of a crude alkaloidal extract was obtained, the radioactivity of which was 2.9 x 10^-4 mCi (35% of the radioactivity which had been fed). About 1% of the radioactivity fed was recovered in the test tubes after the feeding. Most of the crude alkaloidal extract (212 mg) was chromatographed on alumina (20 g). Elution with 3:1 to 2:1 petroleum ether (bp 30-60)-benzene provided crude [T-aromatic]-18β-carbomethoxycalevamine. Dilution with inactive 18β-carbomethoxycalevamine and recrystallization to constant activity from methanol showed that 11% of the activity which had been fed or 31% of the activity which was recovered in the crude alkaloidal extract was due to unchanged 18β-carbomethoxycalevamine. Elution with 1:1 petroleum ether (bp 30-60)-benzene provided 25.6 mg of crude catharanthine which was seen to contain several minor impurities by tlc (alumina, 3:1 benzene-chloroform) The crude catharanthine was combined with inactive catharanthine to give
53.2 mg of which 46.6 mg was chromatographed on alumina (5 g). Elution with 1:1 petroleum ether (bp 30-60)-benzene provided 39.3 mg of catharanthine which showed only fluorescent (ultraviolet light) impurities when subjected to thin-layer chromatographic analysis using the above mentioned system. The catharanthine from this chromatography was chromatographed on silica gel (4 g) at a very rapid rate (almost a percolation). Most of the fluorescent impurities were eluted with 10% diethyl ether benzene. Elution with 15% to 50% diethyl ether benzene provided 31.4 mg of catharanthine shown to be free of much of the fluorescent impurity. This material was then diluted with inactive catharanthine to give 56.6 mg of catharanthine which was recrystallized five times from methanol to provide a sample with a counting rate of 2.2 ± 0.3 dpm/mg. The radioactivity of the catharanthine was 208 ± 24 dpm (0.011 ± 0.001% of the radioactivity that was fed).

Tracer experiment in Vinca minor L. plants using \([T\text{-aromatic}]\)-vincaminoreine (75)

Vinca minor L. plants, which were mature and greenhouse grown, were fed by the bag-on-leaf method with \([T\text{-aromatic}]\)-vincaminoreine as the acetate salt. The acetate salt was prepared from a sample of \([T\text{-atomic}]\)-vincaminoreine (1.507 mg, 1.20 x 10^{-3} mCi) and dissolved in distilled water. The aqueous solution was divided into two portions, the larger portion (85%, 1.05 x 10^{-3} mCi) of which was fed to the plants and the smaller portion (15%, 1.45 x 10^{-4} mCi) of which was put aside as a blank. The period of time used in feeding the plants was four days and during this period of time the plants took in 79% of the radioactivity or 8.25 x 10^{-4} mCi.

The method used in the isolation of the alkaloids from the plants was similar to the method used in the experiments using Vinca rosea L. plants
described in the preceding experiments. The only differences were that methanol was used in place of the benzene ammonium hydroxide mixture in the maceration step and that methylene chloride was used in the final extraction step. Using the modified procedure 111.1 mg of crude alkaloidal extract was obtained from about 35 g of plant material. It should be emphasized that the entire plant was extracted - roots, stems, and leaves. The radioactivity recovered in the crude alkaloidal was $3.38 \times 10^{-4}$ mCi (46.5% of the activity which was determined to have been taken in by the plants).

Most of the crude alkaloidal extract (110 mg) was chromatographed on alumina (10 g). Elution with 1:1 petroleum ether (bp 30-60)-benzene provided unchanged vincaminoreine in the early fractions and minovine in the late fractions with some overlap of the two in the middle fractions. Elution with 3:1 benzene-petroleum ether (bp 30-60) provided 1,2-dehydroaspidospermidine in a mixture with other unknown alkaloids as shown by tlc. Further elution with 3:1 benzene-petroleum ether (bp 30-60) provided a mixture (4.65 mg) which was almost entirely composed of vincamine. Crystallization of this mixture from methanol provided 3.30 mg of vincamine with a counting rate of $8.5 \pm 0.9$ dpm/mg. Assuming 4.64 mg to be the wt of vincamine that had been isolated, the percent of the radioactivity fed that was still associated with the vincamine was $0.0021 \pm 0.0002$.

The mother liquors from the vincamine crystallization, which were shown by tlc to contain some 1,2-dehydroaspidospermidine, and the appropriate fractions from the chromatography were combined to give 7.8 mg of a mixture which was taken up in diethyl ether (3 ml) and mixed with a solution of lithium aluminum hydride (30 mg) in diethyl ether (3 ml). The reaction solution was stirred overnight at room temperature under a nitrogen atmosphere.
After the excess lithium aluminum hydride had been destroyed by the addition of wet ethyl acetate, the inorganic material was removed by filtration and the filtrate was dried with anhyd sodium sulfate. Removal of the solvent provided a gummy material (7.3 mg) which was chromatographed on alumina (1 g). Elution with 3:1 benzene-petroleum ether (bp 30-60) provided 0.58 mg of nearly pure aspidospermidine. The aspidospermidine was diluted to 7.55 mg with inactive aspidospermidine and crystallized from methanol to give 5.28 mg with a counting rate of 57.6 dpm/mg corresponding to an associated level of activity of 0.023%. The aspidospermidine (4.24 mg) was then diluted again to 16.95 mg with an inactive sample, recrystallized once from methanol and converted to the hydrochloride salt. The salt was washed several times with hot acetone and dried vacuo overnight to provide a sample with a counting rate of 3.95 ± 0.45 dpm/mg. The radioactivity of the aspidospermidine was, therefore, 154 ± 17 dpm (0.0083 ± 0.0009% of the radioactivity which was fed).

The minovine (76) containing fractions (3.47 mg) were combined and diluted to 21.97 mg with inactive minovine. Through three crystallizations the level of activity remained constant. The third crystallization gave a sample with a counting rate of 615 dpm/mg. The radioactivity of the isolated minovine was 6.0 x 10^-6 mCi (apparent incorporation 0.7%) (by SH).

The work up of the blank, which had stood for ten days in an open test tube, was accomplished by reversing the pH with ammonium hydroxide and extracting with methylene chloride. The extracted material (40% of the activity) was mixed with 2 mg of inactive minovine and chromatographed on alumina. Elution with 1:1 petroleum ether-benzene provided 0.7 mg of minovine which was diluted to 22.0 mg with inactive minovine and recrystallized three times. The counting rate was seen to rise slightly but not
significantly to give a final counting rate of 66 dpm/mg which corresponded to an apparent intraconversion of vincaminoreine to minovine of 0.3% (by SH).

Tracer experiment in Vinca minor L. plants using [T-aromatic]-vincadine (74)

Vinca minor L. plants, which were mature and greenhouse grown, were fed by the bag-on-leaf method with [T-aromatic]-vincadine (1.133 mg, 1.79 x 10^{-3} mCi) as its acetate salt. After seven days had passed, the radioactivity taken in by the plant was 1.23 x 10^{-3} mCi (69%). The alkaloids were isolated in the same manner that was used in the previous experiment and 26 g of plant material afforded 89.0 mg of crude alkaloidal extract, the radioactivity of which was 5.45 x 10^{-4} mCi (43% of the radioactivity taken in by the plants). The crude alkaloidal extract (87.8 mg) was chromatographed in the same fashion as in the previous experiment. In this experiment the fractions which contained vincamine weighed 4.26 mg and crystallization from methanol provided 1.36 mg of vincamine (59) with a counting rate of 1173 dpm/mg. Some of this sample of vincamine (0.83 mg) was then diluted to 10.17 mg with inactive vincamine (dilution factor, 1225) and crystallized twice from methanol to give a sample with a counting rate of 89.8 ± 3.7 dpm/mg (89.8 ± 2.7 dpm/mg x 12.25 = 1100 ± 33 dpm/mg). This result meant that the apparent rate of incorporation was between the limits 0.054% and 0.17% depending on whether 1.36 mg or 4.26 mg is taken to be the amount of vincamine obtained from the plant.

The blank was worked up in the following fashion. After inactive vincamine (2.0 mg) had been added to the blank, it was made basic with ammonium hydroxide and extracted with methylene chloride. The crude extract which contained 80% of the activity was chromatographed on alumina. Elution with 2:1 petroleum ether (bp 30-60)-benzene was carried out until all the
activity as vincadine had been eluted as indicated by counting selected fractions. Elution with 3:1 benzene-petroleum ether then provided 1.85 mg of vincamine which was diluted to 11.65 mg with inactive vincamine (59) and crystallized twice from methanol. The first crystallization gave 7.88 mg with counting rate of $94 \pm 4$ dpm/mg and the second, 6.02 mg with a counting rate of $88.2 \pm 1.7$ dpm/mg. The radioactivity of the vincamine was therefore $1100 \pm 21$ dpm ($0.068 \pm 0.001\%$ of the radioactivity of the blank).

The 1,2-dehydroaspidospermidine (77) was converted into aspidospermidine in the same fashion that had been employed in the case of the previous experiment. The partially purified aspidospermidine (0.34 mg) obtained from the column chromatography was diluted to 10.27 mg with inactive aspidospermidine and recrystallized from methanol to afford 4.4 mg of crystalline aspidospermidine with a counting rate of $9.25 \pm 1.6$ dpm/mg. The radioactivity of the aspidospermidine was therefore $95 \pm 13$ dpm ($0.0034 \pm 0.0006\%$ of the radioactivity which had been taken up by the plants).

The minovine containing fractions (ca. 1 mg) were combined and diluted to 18.3 mg with inactive minovine. The minovine was recrystallized from methanol three times. After each crystallization the level of activity fell until after the third crystallization the counting rate was barely distinguishable from the background and the estimated level of activity was $0.001\%$ of that which had been fed (by SH).
References


57. Private communication from Professor A. I. Scott to Professor J. P. Kutney.


62. Ref. 61, p. 49, section IV.