1966

Biosynthetic studies on ambelline and tazettine

John Arthur Miller
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BIOSYNTHETIC STUDIES ON AMBElline AND TAZETTINE

by

John Arthur Miller

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Major Subject: Organic Chemistry

Approved:

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In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

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Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa
1966
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INTRODUCTION

Extensive studies on the biosynthesis of Amaryllidaceae alkaloids have been carried out during the past five years. It is apparent that the alkaloids are formed from the amino acids, phenylalanine and tyrosine, the former providing the aromatic $C_6-C_1$ portion of the alkaloid and the latter the $C_6-C_2$ unit. The role of phenyl-phenyl oxidative coupling and specifically the precursor norbelladine in the biosynthetic scheme is well understood. Because of the availability of the alkaloids and suitable degradative pathways, lycorine, galanthamine, belladine, haemanthamine and tazettine have been studied most completely. All of these alkaloids have two oxygenated functional groups on the aromatic ring. Since many Amaryllidaceae alkaloids have an aromatic ring substituted by three oxygenated groups, it was desirable to determine whether the same biosynthetic pathways held for these alkaloids as well.

It is well known that the alkaloidal one-carbon units such as methoxyl, N-methyl, and methylenedioxy groups are derived from formate or methionine. Anomalous results were found in the incorporation of formate into belladine and ambelline in *Nerine bowdenii*, where more than 40% of the radioactivity resided in the benzylic methylene group. To examine this result in more detail, radioactive formate and serine incorporation into the alkaloids of *Sprekelia formosissima* were studied.
HISTORICAL

Ring Systems

The Amaryllidaceae alkaloids (well over one hundred are known)\(^1\) may be divided into eight ring systems (Figure 1). Although a chemical

\[ R - N \]

\[ R - N \]

\[ R - N \]

\[ R - N \]

\[ R - N \]

\[ R - N \]

\[ R - N \]

Figure 1. Amaryllidaceae ring systems
name for each is known, for brevity each ring system is given the name of its most common alkaloid. The eight ring systems are lycorine (1), lycorenine (2), crinine (3) galanthamine (4), montanine (5), tazettine (6), belladine (7) and ismine (8). Each ring system, except those of belladine and ismine, may be divided into an aromatic C\textsubscript{6}-C\textsubscript{1} and a hydroaromatic C\textsubscript{6}-C\textsubscript{2} unit represented by the heavy lines. Belladine differs in that it has an aromatic rather than a hydroaromatic C\textsubscript{6}-C\textsubscript{2} unit. Ismine, having a biphenyl nucleus, is the only known Amaryllidaceae alkaloid which contains no C\textsubscript{6}-C\textsubscript{2} unit.

The large number of alkaloids found in the family can be attributed to the variations in oxygen substitution within these ring systems. The positions at which oxygen substituents may be attached in each nucleus are shown by arrows. The aromatic ring may be di- or tri-oxygenated, and the hydroaromatic ring may be mono- or di-oxygenated. The most common labelling pattern is a di-oxygenated aromatic ring and a mono-oxygenated hydroaromatic ring. The belladine type has only one oxygen
substituent on the aromatic ring of the C₆-C₂ unit. Haemultine (9 or 10), isolated from Haemanthus multiflorus by Boit³,⁴ contains no oxygen substituent on the hydroaromatic ring. However, there is some doubt that such an alkaloid exists⁵. Ismine has two oxygen substituents on the aromatic ring of the C₆-C₁ unit, as shown (Figure 1).

All of the alkaloids contain a tertiary nitrogen except for ismine and some members of the galanthamine and belladine types, which may have either a secondary or tertiary nitrogen atom.

Biogenesis of Amaryllidaceae Alkaloids

Robinson⁶ proposed that lycorine (11) and tazettine (12) were derived from a methylenedioxybenzyl amine (13) or from amino acid precursors. He assumed that the first stages of the biosynthesis of lycorine from amino acid precursors was oxidative coupling of one molecule of 3,4-dihydroxyphenylalanine, dopa (14), with a molecule of dihydroxydihydroindone (15a) or the carboxylic acid (15b) derived from dopa. Robinson did not describe the exact manner by which 15a couples
with 3,4-dihydroxyphenylalanine to form the Amaryllidaceae alkaloids or the type of intermediate formed.

In 1957 Steglich\(^7\) proposed a biogenetic scheme based on the condensation of a 3,4-dioxyphenylacetaldehyde (16) and \(\beta\)-keto-glutardialdehyde (17)
with glycine to form 18 and 19, which serve as precursors of the \( \text{C}_6-\text{C}_1 \)
and \( \text{C}_6-\text{C}_2 \) units (Figure 2). Caranine (20), lycorine (11), lycorenine
(21) and homolycorine (22) may be formed (Figure 3) from the condensa-
tion of 3,4-dioxyphenylacetaldehyde and 19, while tazzetteine (12), bupha-
manine (23), crinamidine (24), crinine (25) (Figure 4) and galanthamine
(26) may be formed from \( \beta \)-ketoglutaraldehyde and 18 (Figure 5).

In 1953 Wenkert proposed a biogenetic scheme (Figure 6) for Amaryllidaceae alkaloids based on the condensation of dioxyphenylethyl amine
(27) with dioxyphenylacetaldehyde (16) followed by phenyl-phenyl oxida-
tive coupling. The final step is reduction of one of the aromatic rings
to a hydroaromatic ring. Robinson criticized this theory on the grounds
that there is no reason for one aromatic ring to be reduced in preference
to the other following the phenyl-phenyl oxidative coupling.

In 1959 Wenkert replaced his original hypothesis by proposing a
shikimic-prephenic biogenetic scheme for the Amaryllidaceae alkaloids. He suggested that shikimic acid (28) serves as the precursor of the
\( \text{C}_6-\text{C}_1 \) unit and 29 serves as the precursor of the \( \text{C}_6-\text{C}_2 \) unit in the lyc-
corine (1) and lycorenine (2) systems. The double bond isomer (30)
serves as the precursor of the \( \text{C}_6-\text{C}_2 \) unit in crinine (3), galanthamine
(4) and tazzetteine (6) ring systems.

The ketamines (29 and 30) are derived from shikimic acid (28) by
reaction with pyruvic acid (31) followed by amination and condensation
(Figure 7).
Figure 2. Biogenesis of Amaryllidaceae alkaloid intermediates 
(Steglich)
Figure 2. (Continued)

Figure 3. Biogenesis of the lycorine-lycorenine group of Amaryllidaceae alkaloids (Steglich)
Figure 3. (Continued)
Figure 4. Biogenesis of the tazettine-crinine group of Amaryllidaceae alkaloids (Steglich)
Figure 4. (Continued)

Figure 5. Biogenesis of the galanthamine group of Amaryllidaceae alkaloids (Steglich)
Figure 5. (Continued)
Figure 6. Biogenesis of Amaryllidaceae alkaloids (Wenkert, 1953)
Figure 6. (Continued)
Figure 7. Biogenesis of the prephenates

The derivative (32) formed from shikimic acid and 29 undergoes an internal Michael condensation followed by hydration-dehydration and oxidation-reduction reactions (Figure 8) leading to lycorine (11) and lycorenine (21). The shikimyl derivative (33) of the ketamine (30) is a precursor (Figure 9) to crinine (25), narwedine (34) and tazettine (12).
Figure 8. Biogenesis of lycorine and lycorenine (Wenkert, 1959)
Figure 9. Biogenesis of crinine, narwedine and tazettine (Wenkert, 1959)
Figure 9. (Continued)
Barton and Cohen\textsuperscript{11} have proposed that many natural products may originate from coupling of phenol radicals. The carbon-carbon coupling of these radicals may be ortho-ortho, ortho-para or para-para. Examples of the first type (Figure 10) are shown by formation of dehydrodivanillin (35) from vanillin (36), dehydrodieugenol (37), dehydrodi-\textit{o}-cresol (38), dehydrodi-\textit{p}-cresol (39), dehydrodi-2,4-dimethyl phenol (40) and dehydrodi-\textit{\beta}-naphthol (41). The best example of the relatively unknown ortho-para coupling (Figure 11) is the formation of Pummerer's ketone (42) from para-cresol (43). Examples of the more common para-para coupling (Figure 12) are shown by the formation of 4,4'-dihydrodiphenyl (44) from lead tetraacetate oxidation of phenol (45), compounds (46 and 47) from 2,6-dimethylphenol (48), the ketone (49) from anthranol (50) and the dihydrophenanthrine (51) from oxidation of 52 via a diketone (53).

Extending the concept of phenol oxidative coupling to the Amaryllidaceae alkaloids, Barton and Cohen\textsuperscript{11} postulated that these alkaloids had a common phenolic precursor, norbelladine (54a). At the time that they postulated this phenolic precursor, the alkaloid belladine (54b) was unknown. The subsequent isolation of belladine\textsuperscript{12} from \textit{Nerine bowdenii} and \textit{Amaryllis belladonna} lent support to their hypothesis.

Barton and Cohen proposed a biogenetic scheme for lycorine (11) and caranine (55) based on their hypothesis (Figure 13). Applying their biogenetic hypothesis to the structure of galanthamine (Figure 14) they predicted the location of the hydroxyl group and double bond in the alkaloid to be as shown in structure 26. Chemical verification for this proposed structure of galanthamine was obtained by other methods\textsuperscript{13}.
Figure 10. Examples of ortho-ortho phenol-oxidative coupling
Figure 10. (Continued)

Figure 11. An example of ortho-para phenol-oxidative coupling
Figure 11. (Continued)

Figure 12. Examples of para-para oxidative coupling of phenol radicals
Figure 12. (Continued)
Figure 12. (Continued)

A biosynthetic type of synthesis of galanthamine has been accomplished. The galanthamine was formed in low yield from the norbelladine derivative (56) by manganese dioxide oxidation and lithium aluminum hydride reduction.

The biosynthesis of lycorenine (21) and tazettine (12) by this theory presented some difficulties. If the nucleus of an alkaloid of this type were to be formed by a simple phenyl-phenyl oxidative coupling reaction, two separate fragments would be required to assume a given orientation prior to being coupled to form the alkaloid nucleus. However, recent biosynthetic studies have shown (Figure 15) that tazettine is formed from haemanthidine (57), which in turn is derived from haemanthamine (58).
Figure 13. Biogenesis of lycorine and caranine (Barton and Cohen)
Figure 14. Biogenesis of galanthamine (Barton and Cohen)

Figure 15. Conversion of haemanthamine to tazettine *in vivo*
Figure 15. (Continued)

Biosynthesis of the Amaryllidaceae Alkaloids

The biosynthesis of various Amaryllidaceae alkaloids was investigated intensively during the period 1960 to 1965. The lycorine (1), crinine (3), galanthamine (4) and tazettine (6) group of alkaloids and the alkaloid belladine (54b) were the primary objects of study. The lycorenine (2) and montanine (5) groups of alkaloids have not been investigated due to their scarcity and the uncertainty of a suitable degradation scheme for the radioactive montanine.

The investigations, summarized in Table 1, have shown that tyrosine (59a) is a precursor of the hydroaromatic C_6-C_2 unit but is never incorporated into the aromatic C_6-C_1 unit of the Amaryllidaceae alkaloids.
**Table 1. Incorporation of amino acids into Amaryllidaceae alkaloids**

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Plant</th>
<th>Alkaloid</th>
<th>% inc.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Cl4-Tyrosine</td>
<td><em>Narcissus</em> &quot;Twink&quot;</td>
<td>Haemanthamine (58)</td>
<td>0.078</td>
<td>16,17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norpluvine (60)</td>
<td>0.15</td>
<td>17,18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caranine (55)</td>
<td>0.079</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine (11)</td>
<td>0.23</td>
<td>17,18</td>
</tr>
<tr>
<td></td>
<td><em>Narcissus</em> &quot;King Alfred&quot;</td>
<td>Galanthamine (26)</td>
<td>----</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine (11)</td>
<td>----</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>Narcissus</em> &quot;Texas&quot;</td>
<td>Galanthamine (26)</td>
<td>----</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><em>Narcissus</em> &quot;Deanna Durbin&quot;</td>
<td>Lycorine (11)</td>
<td>----</td>
<td>19</td>
</tr>
<tr>
<td>3-Cl4-Tyrosine</td>
<td><em>Sprekelia</em> formossisima</td>
<td>Haemanthamine (58)</td>
<td>----</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tazettine (12)</td>
<td>0.16</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthidine (57)</td>
<td>0.20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><em>Haemanthus</em> natalensis</td>
<td>Haemanthamine (58)</td>
<td>0.97</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthidine (57)</td>
<td>0.19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-Hydroxycrinamine (61)</td>
<td>1.18</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><em>Nerine</em> bowdenii</td>
<td>Lycorine (11)</td>
<td>0.11</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belladine (54b)</td>
<td>0.82</td>
<td>22</td>
</tr>
<tr>
<td>2-Cl4-Phenylalanine</td>
<td><em>Narcissus</em> incomparibilis</td>
<td>Lycorine (11)</td>
<td>0.00</td>
<td>23</td>
</tr>
<tr>
<td>3-Cl4-Phenylalanine</td>
<td><em>Narcissus</em> incomparibilis</td>
<td>Lycorine (11)</td>
<td>0.18</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td><em>Nerine</em> bowdenii</td>
<td>Lycorine (11)</td>
<td>0.095</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belladine (54b)</td>
<td>0.42</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td><em>Narcissus</em> &quot;Deanna Durbin&quot;</td>
<td>Lycorine (11)</td>
<td>----</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pluvine (62)</td>
<td>----</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galanthine (63)</td>
<td>----</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine (58)</td>
<td>----</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Narcissidine (64)</td>
<td>----</td>
<td>19</td>
</tr>
</tbody>
</table>
Likewise, phenylalanine (59b) is shown to be incorporated only into the aromatic $C_6-C_1$ unit and is not incorporated into the hydroaromatic $C_6-C_2$ unit.
In the biogenetic scheme proposed by Steglich, the Amaryllidaceae alkaloids would contain radioactive labels in the hydroaromatic ring of the C₆-C₂ unit, if the 3,4-dioxyphenylacetaldehyde (16) or intermediate (18) was derived from 3-C¹⁴-phenylalanine (59b) or 3-C¹⁴-tyrosine (59a). Since no biosynthetic experiments using 3-C¹⁴-phenylalanine or 3-C¹⁴-tyrosine have shown incorporation of carbon-14 into the hydroaromatic ring of Amaryllidaceae alkaloids, Steglich's hypothesis must be discarded.

The hydroaromatic ring of the ketamines (29 and 30) which serve as the precursors of the C₆-C₂ unit in Amaryllidaceae alkaloids (Figure 8 and 9) is derived from shikimic acid (28), and the two-carbon unit of 29 and 30 is derived from pyruvic acid (Figure 7) by the biogenetic scheme proposed by Wenkert. The shikimic acid also serves as a precursor of the C₆-C₁ unit. At present this scheme must be considered as an alternate biosynthetic pathway of the Amaryllidaceae alkaloids.
There have been no investigations using labelled shikimic acid, pyruvic acid or the ketamines (29 and 30) to determine the validity of the Wenkert hypothesis. Evidence proving or disproving this biosynthetic pathway will be difficult to obtain, for shikimic acid and pyruvate are known precursors of phenylalanine (59b) and tyrosine (59a) in microorganisms.

A series of investigations, summarized in Table 2, have shown norbelladine (54a) to be incorporated intact into the Amaryllidaceae alkaloids. Further studies by Wildman and Battersby have shown negligible incorporation of 1'-C\(^{14}\)-bisdeoxynorbelladine (65) and of 1'-C\(^{14}\)-hydroxynorbelladine (66) into haemanthamine (58), or any alkaloid of the Amaryllidaceae family (Table 3). These data suggest that, prior to phenyl-phenyl oxidative coupling, the aromatic ring in the C\(_6\)-C\(_1\) unit must be di-oxygenated and the aromatic ring in the C\(_6\)-C\(_2\) unit of the phenolic precursor must be mono-oxygenated. The data also suggest that further oxygenation of the hydroaromatic ring of the C\(_6\)-C\(_2\) unit in the Amaryllidaceae alkaloids
Table 2. Incorporation of norbelladine into Amaryllidaceae alkaloids

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Plant</th>
<th>Alkaloid</th>
<th>% inc.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-C(^{14})-Norbelladine</td>
<td>Narcissus &quot;Twink&quot;</td>
<td>Lycorine (11)</td>
<td>0.24</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norplurine (60)</td>
<td>0.74</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine (58)</td>
<td>0.15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Galanthus elwese</td>
<td>Galanthamine (26)</td>
<td>0.053</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine (26)</td>
<td>----</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galanthamine (63)</td>
<td>----</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine (58)</td>
<td>----</td>
<td>31</td>
</tr>
<tr>
<td>1,1'-C(^{14})-Norbelladine</td>
<td>Nerine bowdenii</td>
<td>Lycorine (11)</td>
<td>0.07</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crinamine (67)</td>
<td>0.0009</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belladine (54b)</td>
<td>2.64</td>
<td>32</td>
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</table>
Table 3. Incorporation of norbelladine derivatives into Amaryllidaceae alkaloids

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Plant</th>
<th>Alkaloid</th>
<th>% inc.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'-C\textsuperscript{14}-Bisdeoxynorbelladine</td>
<td>Narcissus &quot;Twink&quot;</td>
<td>Tazettine</td>
<td>0.00</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>33</td>
</tr>
<tr>
<td>1'-C\textsuperscript{14}-Hydroxynorbelladine</td>
<td>Narcissus &quot;Twink&quot;</td>
<td>Tazettine</td>
<td>----</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>----</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>0.00068</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norpluvine</td>
<td>0.0017</td>
<td>33</td>
</tr>
<tr>
<td>N-C\textsuperscript{14}-Methyl norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.18</td>
<td>31,34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galanthine</td>
<td>0.00</td>
<td>31,34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>31,34</td>
</tr>
<tr>
<td>O-Methyl-N-C\textsuperscript{14}-Methyl norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.14</td>
<td>31,34</td>
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<td></td>
<td>Galanthine</td>
<td>0.00</td>
<td>31,34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>31,34</td>
</tr>
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<td>O-C\textsuperscript{14}-Methyl-N-C\textsuperscript{14}-Methyl norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.14</td>
<td>34,35</td>
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<td></td>
<td>Galanthine</td>
<td>0.00</td>
<td>34,35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>34,35</td>
</tr>
<tr>
<td>O-C\textsuperscript{14}-Methyl-N-C\textsuperscript{14}-Methyl 1-C\textsuperscript{14}-norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.018</td>
<td>35</td>
</tr>
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<td></td>
<td></td>
<td>Galanthine</td>
<td>0.00</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>35</td>
</tr>
<tr>
<td>O-C\textsuperscript{14}-Methyl norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.00</td>
<td>34,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galanthine</td>
<td>----</td>
<td>34,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>0.036</td>
<td>34,36</td>
</tr>
<tr>
<td>O-C\textsuperscript{14}-Methyl-1-C\textsuperscript{14}- norbelladine</td>
<td>Narcissus &quot;King Alfred&quot;</td>
<td>Galanthamine</td>
<td>0.00</td>
<td>34,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galanthine</td>
<td>----</td>
<td>34,36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemanthamine</td>
<td>1.00</td>
<td>34,36</td>
</tr>
</tbody>
</table>
must occur after oxidative coupling. The feeding of tritiated norpluvine (60) to "Deanna Durbin" and isolation of lycorine (11) containing 10.5% of the activity indicates that the oxygenation of the C₆-C₂ unit may occur after oxidative coupling of the phenolic precursor. A probable biosynthetic sequence (Figure 16) was considered to be: norpluvine (60) → caranine (55) → lycorine (11).

Figure 16. Biosynthetic sequence: norpluvine to lycorine
A series of feeding experiments using labelled methyl derivatives of norbelladine (54a) has been accomplished (Table 3). N-Methyl norbelladine (68) and O,N-dimethyl norbelladine (56) have been shown to be incorporated into galanthamine (26), but they were not incorporated into galanthine (63) and haemanthamine (58). However, O-methyl norbelladine (69) has been shown to be incorporated into haemanthamine but not into galanthamine. The data suggest a definite order of methylation for galanthamine (Figure 17): norbelladine (54a) → N-methyl norbelladine (68) → O,N-dimethyl norbelladine (56) → galanthamine (26). These

![Chemical structures](image.png)

*Figure 17. Order of methylation for galanthamine*
data also suggest a definite order of methylation for haemanthamine (Figure 18): norbelladine (54a) → O-methyl norbelladine (69) → haemanthamine (58).

Figure 18. Order of methylation for haemanthamine

There have been two pathways proposed (Figures 19 and 20) for the incorporation of phenylalanine (59b) into the C6-C1 unit of the Amaryllidaceae alkaloids: (1) phenylalanine (59b) → phenylserine (70) → benzaldehyde (71) → p-hydroxybenzaldehyde (72) →
3,4-di-hydroxybenzaldehyde (73) $\rightarrow$ norbelladine (54a) and (2) phenylalanine (59b) $\rightarrow$ trans-cinnamic acid (74) $\rightarrow$ p-hydroxycinnamic acid (75) $\rightarrow$ 3,4-dihydroxycinnamic acid (76) or p-hydroxybenzaldehyde (72) $\rightarrow$ 3,4-dihydroxybenzaldehyde (73) $\rightarrow$ norbelladine (54a).

Figure 19. Incorporation of phenylalanine into C$_6$-C$_1$ unit of the Amaryllidaceae alkaloids via phenylserine.
Figure 20. Incorporation of phenylalanine into \( \text{C}_6-\text{C}_1 \) unit of the Amaryllidaceae alkaloids via trans-cinnamic acid
<table>
<thead>
<tr>
<th>Precursor</th>
<th>Plant</th>
<th>Alkaloid</th>
<th>% inc.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-C\textsuperscript{14}-Phenylserine</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>------</td>
<td>37,38</td>
</tr>
<tr>
<td></td>
<td>Narcissus incomparabilis</td>
<td>Lycorine</td>
<td>0.00</td>
<td>38</td>
</tr>
<tr>
<td>7-C\textsuperscript{14}-Benzaldehyde</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Spekelia formossisima</td>
<td>Tazettine</td>
<td>0.005</td>
<td>33</td>
</tr>
<tr>
<td>p-Hydroxy-7-C\textsuperscript{14}-benzaldehyde</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>0.00</td>
<td>37,38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>------</td>
<td>37,38</td>
</tr>
<tr>
<td>trans-3-C\textsuperscript{14}-cinnamic</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>0.35</td>
<td>37-39</td>
</tr>
<tr>
<td></td>
<td>Nerine bowdenii</td>
<td>Lycorine</td>
<td>------</td>
<td>37,39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>0.02</td>
<td>33</td>
</tr>
<tr>
<td>p-Hydroxy-3-C\textsuperscript{14}-cinnamic acid</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>0.16</td>
<td>37-39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>------</td>
<td>37-39</td>
</tr>
<tr>
<td>3,4-Dihydroxy-3-C\textsuperscript{14}-cinnamic acid</td>
<td>Narcissus pseudonarcissus</td>
<td>Haemanthamine</td>
<td>------</td>
<td>23,38,40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>------</td>
<td>23,38,40</td>
</tr>
<tr>
<td>3,4-Dihydroxy-7-C\textsuperscript{14}-benzaldehyde</td>
<td>Narcissus incomparabilis</td>
<td>Lycorine</td>
<td>0.006</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lycorine</td>
<td>------</td>
<td>40,41</td>
</tr>
</tbody>
</table>
Negligible incorporation of phenylserine, benzaldehyde, and p-hydroxybenzaldehyde into haemanthamine (58) and tazettine (12) have been reported (Table 4). However, trans-cinnamic acid, p-hydroxycinnamic acid, 3,4-dihydroxycinnamic acid, and 3,4-dihydroxybenzaldehyde have been shown to be incorporated in good yield into various Amaryllidaceae alkaloids (Table 4). These data suggest that phenylalanine is incorporated into the C6-C1 unit of the Amaryllidaceae alkaloids by the phenylalanine-trans-cinnamic acid pathway (Figure 20) and not by the phenylalanine-phenylserine pathway (Figure 19).

Studies of the incorporation of tyrosine (Table 1) and tyramine (77) into the Amaryllidaceae alkaloids (Table 1) suggest that tyrosine is incorporated into the alkaloids (Figure 21) by the following biosynthetic pathway: tyrosine (63a) \(\rightarrow\) tyramine (77) \(\rightarrow\) norbelladine (54a).

The combined biosynthetic pathway for incorporation of phenylalanine (59b) and tyrosine (59a) into norbelladine (54a) is given in Figure 22.

Other investigations have proved that the methylenedioxy group of haemantamine (58) originates from the O-methyl group of O-methyl norbelladine (69).
Figure 21. Incorporation of tyrosine into the C$_6$-C$_2$ unit of the Amaryllidaceae alkaloids
Figure 22. Incorporation of phenylalanine and tyrosine into norbelladine
RESULTS AND DISCUSSION

Ambelline

The structure of ambelline (78), a major alkaloid of *Amaryllis belladonna*\(^{43}\) and *Nerine bowdenii*\(^{44-47}\), was determined by Wildman in 1963 by degradative and spectroscopic methods\(^{48}\). A similar degradation was used in the present studies with radioactive material.

![Ambelline structure](image)

All feeding experiments (Table 5) used *Nerine bowdenii* as the plant host and the ambelline was isolated by standard procedures\(^{40}\).

Table 5. Feeding experiments with *Nerine bowdenii* as plant host

<table>
<thead>
<tr>
<th>Feeding experiment</th>
<th>Total act. used</th>
<th>Wgt. amb. isol.</th>
<th>Amb. act. (dpm/mM)</th>
<th>% inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-C(^{14})-Phenylalanine</td>
<td>0.3 mc.</td>
<td>70 mg.</td>
<td>4.40 \times 10^3</td>
<td>2.33 \times 10^{-3}</td>
</tr>
<tr>
<td>3-C(^{14})-Tyrosine</td>
<td>0.1 mc.</td>
<td>89 mg.</td>
<td>1.124 \times 10^4</td>
<td>1.50 \times 10^{-2}</td>
</tr>
<tr>
<td>2-C(^{14})-Tyrosine</td>
<td></td>
<td>77.5 mg.</td>
<td>4.465 \times 10^4</td>
<td></td>
</tr>
<tr>
<td>1,1'-C(^{14})-Norbelladine</td>
<td>0.355 mc.</td>
<td>118 mg.</td>
<td>7.73 \times 10^3</td>
<td>3.87 \times 10^{-3}</td>
</tr>
<tr>
<td>Sodium C(^{14})-formate</td>
<td>0.316 mc.</td>
<td>34 mg.</td>
<td>1.605 \times 10^4</td>
<td>2.64 \times 10^{-3}</td>
</tr>
</tbody>
</table>
Degradation of Ambelline

The radioactive precursors fed to Nerine bowdenii cited in Table 5 could yield ambelline (78) labelled in the methoxyl and/or the methylene-dioxy groups, as well as at the C₆, C₁₁ and C₁₂ positions. To isolate and identify each of these carbon atoms in ambelline as obtained from the various feeding experiments, degradation procedures were developed which permitted the determination of the amount of radioactivity at each position.

The total activity present in the two methoxyl groups of ambelline was determined by the Zeisel method (Figure 23). The radioactivity of the methylenedioxy group was determined by acid hydrolysis to formaldehyde₄⁹, (Figure 24), which was isolated as the dimedone adduct (79).

Figure 23. Methoxyl determination
Feedings of radioactive phenylalanine and tyrosine labelled in the aliphatic portions of these amino acids should, by biosynthetic prediction, lead to radioactive ambelline, which might be labelled at the \( C_6 \), \( C_{11} \) and/or \( C_{12} \) positions. It was imperative that a degradation scheme be devised which could isolate each of these carbon atoms specifically and in high yield. The carbon atoms \( C_6 \), \( C_{11} \) and \( C_{12} \) in ambelline are designated by a dagger, dot and asterisk in formula 78.

To determine the amount of radioactivity at the \( C_6 \), \( C_{11} \) and \( C_{12} \) positions, ambelline was converted (Figure 25) to \( N\)-(2-methoxy-6-
Figure 25. Degradation of ambelline

phenylpiperonyl) sarcosine hydrochloride (80a). This transformation involves the loss of the methoxyl group at the $C_3$ position of ambelline. There should be no loss of activity in 80a relative to ambelline which was obtained from a 3-$C^{14}$-phenylalanine, 2-$C^{14}$- or 3-$C^{14}$-tyrosine feeding experiment, due to the removal of the $C_3$ methoxyl group. Oxidation of
ambelline with chromium trioxide in pyridine gave o xoambelline (81), which was quaternized with methyl iodide to give o xoambelline methio- 
dide (82). Treatment of 84a with sodium hydroxide gave o xoambelline 
methine which was isolated as the hydrochloride (80a). From the hypothe-
tical labelling pattern assigned to 78, corresponding activity is shown 
in 80a, as would be expected.

No single degradative procedure allowed the simultaneous determina-
tion of the radioactivity in the two carbon atoms of the acetic acid 
group of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride. Al-
though the Kolbe electrolysis was successful for the determination of 
radioactivity in the methylene and carboxyl groups of 80b, the electrol-
ysis proved to be unsatisfactory for the determination for the non-
benzolic methylene carbon of 80a. If radioactivity was expected in the 
carboxylic acid group of 80a, it was most expedient to degrade by 
procedure A (Figure 26). This procedure could also be used to determine 
the activity present at the benzylic position of 80a. However, if the 
activity was present in the methylene group indicated by the asterisk, 
satisfactory results could be obtained only by use of a second procedure 
(Figure 26).

In procedure A, hydrogenolysis of N-(2-methoxy-6-phenylpiperonyl) 
sarcosine hydrochloride (80a) gave 3-methoxy-2-methyl-4,5-methylene-
dioxybiphenyl (83) and sarcosine (84), which was isolated as N-tosyl-
sarcosine (85) by reaction with p-toluene sulfonyl chloride. Kolbe 
electrolytic oxidation of 85 gave N-methyl-p-toluenesulfonamide (86), 
carbon dioxide and formaldehyde, the latter being isolated as the
Figure 26. Degradation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by procedures A and B.
dime done adduct (79). The quantities of radioactive ambelline degraded did not permit the isolation of 86. If the N-tosylsarcosine was labelled in the carboxylate carbon, the carbon dioxide liberated should contain all of the radioactivity. This result was supported by the model degradation of N-tosyl-1-C₁⁴-sarcosine (Table 6) synthesized from 1-C₁⁴-glycine (87). If 85 was labelled in the methylene group, the carbon

Table 6. Relative activities* of N-tosylsarcosine and degradation products

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oxidation of N-tosyl-1-C₁⁴-sarcosine in ethanol</th>
<th>Oxidation of N-tosyl-2-C₁⁴-sarcosine in ethanol</th>
<th>Oxidation of N-tosyl-2-C₁⁴-sarcosine in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Tosylsarcosine</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>0.88</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Formaldehyde dime done adduct</td>
<td>0.00</td>
<td>0.34</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*Relative activity is the specific radioactivity in dpm/mM of a compound compared to the specific radioactivity of the parent compound, which is given the value of 1.00 for convenience.

\[ H₂N-CH₂-CO₂H \]
dioxide contained no radioactivity and the formaldehyde isolated as the
dimedone adduct (79) would be labelled. However, in actual experiments
the formaldehyde dimedone adduct had less than 70% of the expected radio-
activity based on the level of the N-tosylsarcosine. This low relative
activity for the formaldehyde dimedone adduct has been attributed to the
formation of side products in the electrolytic oxidation of the N-tosyl-
sarcosine. This assumption was supported by electrolytic degration
of N-tosyl1-C\textsuperscript{14}-sarcosine (Table 6) prepared from 2-C\textsuperscript{14}-glycine, where
model experiments showed that only 60% of the radioactivity of the
methylene carbon was recovered as the dimedone adduct. These results
necessitated the development of a second procedure (B) which permitted
the determination of the activity at the C\textsubscript{12} position of ambelline. The
lead tetraacetate oxidation of amino acids appeared to be a promising
alternative.

Wieland and Bergel\textsuperscript{50} first reported the catalytic oxidative decar-
boxylation of amino acids with oxygen to give carbon dioxide, an alde-
hyde and ammonia. Spenser\textsuperscript{51} has shown that oxidation of \( \alpha-\beta-H_2^3 \)-valine
(88) and \( \alpha-\beta-H_2^3 \)-phenylalanine (59b) with sodium hypochlorite, hydrogen
peroxide, chloramine-T, ninhydrin and alloxan takes place without loss
of tritium to give 1,2-\( H_2^3 \)-isobutraldehyde (89) and 1,2-\( H_2^3 \)-phenylacetald-
dehyde (90), respectively. Spenser proposed that the reaction (Figure 27)
involved a carbinolamine intermediate (91) which rapidly decomposes to an
aldehyde and ammonia. Support for a carbinolamine intermediate was
found in the oxidation of \( \alpha-N \)-diphenylglycine (92) with potassium
Figure 27. Mechanism of oxidative decarboxylation of amino acids ferricyanide (Figure 28) to give the isolable Schiff base, benzalaniline (93). Presumably the carbinolamine (94) was formed and underwent rapid elimination.

In procedure B, lead tetraacetate oxidation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride (80a), labelled at the position indicated by the asterisk gave inactive carbon dioxide. The radioactivity of the formaldehyde which was isolated as the dimeredone adduct (79) was less than the predicted value. This result suggested that part of the formaldehyde collected as the dimeredone adduct was a decomposition
Figure 28. Mechanism of Schiff base formation in oxidative decarboxylation of amino acids

product of the lead tetraacetate-glacial acetic acid solution. This interpretation was found to be correct. Heating the lead tetraacetate-glacial acetic acid solution in the absence of N-(2-methoxy-6-phenyl-piperonyl)sarcosine hydrochloride afforded formaldehyde which was isolated as the dimedone adduct. In a second experiment lead tetraacetate solution was heated in the absence of 80a and the evolved gases were collected in water. The water solution was shown to contain formaldehyde by the method of Bricker and Vail.

A thorough investigation of the amount of formaldehyde produced by 10.0 ml. of 0.67% lead tetraacetate-glacial acetic acid solution at
various reaction times was undertaken (Table 7). The amount of formaldehyde obtained, within experimental error, was directly proportional to the length of time of heating and the age of the solution.

Table 7. Formaldehyde produced by heating 0.67% lead tetraacetate-glacial acetic acid solution

<table>
<thead>
<tr>
<th>Trial no.</th>
<th>Age of solution</th>
<th>Length of time solution heated</th>
<th>Wgt. of dimedone adduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 hrs.</td>
<td>2 hrs. 30 min.</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>16 hrs.</td>
<td>3 hrs. 00 min.</td>
<td>0.26 mg.</td>
</tr>
<tr>
<td></td>
<td>16 hrs.</td>
<td>3 hrs. 45 min.</td>
<td>0.16 mg.</td>
</tr>
<tr>
<td>2</td>
<td>2 days 14 hrs. 10 min.</td>
<td>2 hrs. 30 min.</td>
<td>0.20 mg.</td>
</tr>
<tr>
<td></td>
<td>2 days 14 hrs. 10 min.</td>
<td>3 hrs. 15 min.</td>
<td>0.16 mg.</td>
</tr>
<tr>
<td></td>
<td>2 days 14 hrs. 10 min.</td>
<td>4 hrs. 05 min.</td>
<td>0.29 mg.</td>
</tr>
<tr>
<td>3</td>
<td>6 days 15 hrs. 40 min.</td>
<td>2 hrs. 30 min.</td>
<td>0.27 mg.</td>
</tr>
<tr>
<td></td>
<td>6 days 15 hrs. 40 min.</td>
<td>3 hrs. 20 min.</td>
<td>0.30 mg.</td>
</tr>
<tr>
<td></td>
<td>6 days 15 hrs. 40 min.</td>
<td>3 hrs. 50 min.</td>
<td>0.33 mg.</td>
</tr>
<tr>
<td>4</td>
<td>10 days 16 hrs. 30 min.</td>
<td>2 hrs. 40 min.</td>
<td>0.31 mg.</td>
</tr>
<tr>
<td></td>
<td>10 days 16 hrs. 30 min.</td>
<td>3 hrs. 00 min.</td>
<td>0.53 mg.</td>
</tr>
<tr>
<td></td>
<td>10 days 16 hrs. 30 min.</td>
<td>4 hrs. 05 min.</td>
<td>0.59 mg.</td>
</tr>
<tr>
<td>5</td>
<td>24 days 4 hrs. 20 min.</td>
<td>2 hrs. 35 min.</td>
<td>0.75 mg.</td>
</tr>
<tr>
<td></td>
<td>24 days 4 hrs. 20 min.</td>
<td>3 hrs. 15 min.</td>
<td>1.18 mg.</td>
</tr>
<tr>
<td></td>
<td>24 days 4 hrs. 20 min.</td>
<td>3 hrs. 50 min.</td>
<td>1.28 mg.</td>
</tr>
<tr>
<td>6</td>
<td>30 days 19 hrs. 30 min.</td>
<td>2 hrs. 30 min.</td>
<td>2.60 mg.</td>
</tr>
<tr>
<td></td>
<td>30 days 19 hrs. 30 min.</td>
<td>3 hrs. 10 min.</td>
<td>5.55 mg.</td>
</tr>
<tr>
<td></td>
<td>30 days 19 hrs. 30 min.</td>
<td>3 hrs. 55 min.</td>
<td>4.99 mg.</td>
</tr>
</tbody>
</table>
A corrected specific activity for the radioactive formaldehyde dimedone adduct \( (A_c) \) could be calculated by the following equation:

\[
\frac{A_1}{W_1 - W_2} = A_c
\]

where \( A_1 \) and \( W_1 \) are the specific radioactivity and weight, respectively, of the diluted dimedone adduct, and \( W_2 \) is the amount of formaldehyde dimedone adduct found to be produced in a blank experiment using 10.0 ml. of 0.67% lead tetraacetate-glacial acetic acid solution.

Ambelline Isolated From a 3-C\(^{14}\)-Phenylalanine Feeding Experiment

Previous biosynthetic studies have shown phenylalanine (59b) to be a precursor of the \( C_6-C_1 \) unit of the Amaryllidaceae alkaloids (Table 1) having two oxygen substituents on the aromatic ring. If 3-C\(^{14}\)-phenylalanine is a precursor of the \( C_6-C_1 \) unit of ambelline (78) which is tri-oxygenated in the aromatic ring, the radioactive label would be expected to occur at the benzylic (\( C_6 \)) position. Ambelline isolated from the 3-C\(^{14}\)-phenylalanine feeding experiment (Table 5) was degraded by procedure A (Figure 26) and shown to contain all its radioactivity at the \( C_6 \) position within experimental error (Table 8).

The low specific activity (4,400 dpm/mM) of the ambelline required complete radiochemical purity. The ambelline purity was checked by conversion to the hydrochloride which had the same specific activity as the original ambelline.
Table 8. Degradation of ambelline isolated from the 3-\textsuperscript{14}C-phenylalanine feeding experiment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td></td>
<td>650 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>Ambelline hydrochloride</td>
<td>---</td>
<td>---</td>
<td>1.04</td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>229 mg.</td>
<td>229 mg.</td>
<td>----</td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>220 mg.</td>
<td>200 mg.</td>
<td>1.05</td>
</tr>
<tr>
<td>N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride</td>
<td>164 mg.</td>
<td>164 mg.</td>
<td>----</td>
</tr>
<tr>
<td>N-Tosylsarcosine</td>
<td>38 mg.</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>3-Methoxy-2-methyl-4,5-methylenedioxybiphenyl</td>
<td>61 mg.</td>
<td></td>
<td>0.90</td>
</tr>
</tbody>
</table>

No further degradation was attempted to isolate the label in the methyl group of 3-methoxy-2-methyl-4,5-methylenedioxybiphenyl (83) because any migration of the C\textsubscript{3} atom in phenylalanine into the aromatic ring would be highly improbable.
These data prove that phenylalanine serves as a precursor of the tri-oxygenated aromatic C₆-C₁ unit of ambelline.

Ambelline Isolated From the 3-C¹⁴-Tyrosine Feeding Experiment

Previous biosynthetic studies have shown tyrosine (59a) to be a precursor of the hydroaromatic C₆-C₂ unit of the Amaryllidaceae alkaloids (Table 1). If 3-C¹⁴-tyrosine is a precursor of the C₆-C₂ unit of ambelline (78) the radioactive label would be expected to occur at the C₁₁ position. The ambelline isolated from the 3-C¹⁴-tyrosine feeding experiment (Table 5) was degraded by procedure A (Figure 26) and shown to contain all of its radioactivity at position C₁₁ (Table 9).

Table 9. Degradation of ambelline isolated from the 3-C¹⁴-tyrosine feeding experiment, procedure A

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td></td>
<td>650 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>450 mg.</td>
<td>450 mg.</td>
<td>---</td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>350 mg.</td>
<td>350 mg.</td>
<td>0.99</td>
</tr>
<tr>
<td>N-(2-Methoxy-6-phenylpiperonyl)sarcosine hydrochloride</td>
<td>231 mg.</td>
<td>200 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>3-Methoxy-2-methyl-4,5-methylenedioxybiphenyl</td>
<td>82 mg.</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>N-Tosylsarcosine</td>
<td>99 mg.</td>
<td>19.75 mg.</td>
<td>1.02</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct</td>
<td>5 mg.</td>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>
Ambelline Isolated From a $^{14}$C-Tyrosine Feeding Experiment

Since feeding $^{14}$C-tyrosine to Nerine bowdenii was shown to give radioactive ambelline labelled at C$_{11}$, it would be expected that $^{14}$C-tyrosine would give rise to ambelline labelled at C$_{12}$. This was found to be the case.

The ambelline obtained from the $^{14}$C-tyrosine feeding experiment was degraded twice by procedure A (Table 10). In each degradation the N-tosylsarcosine gave non-radioactive carbon dioxide. The formaldehyde dimedone adduct had a relative specific activity considerably less than acceptable (Table 10, trial 1). Two degradations of ambelline by procedure B (Table 11) gave formaldehyde dimedone adduct with relative specific activities of 0.62 and 0.72 without corrections for impurities. A third degradation, corrected for the extraneous formaldehyde produced according to the method prescribed in procedure B, provided the relative activity (0.97) for the formaldehyde dimedone adduct (Table 12).

Ambelline Isolated From the $^{14}$C-Norbelladine Feeding Experiment

Previous biosynthetic studies (Table 2) have shown norbelladine (54a) to be incorporated intact into the lycorine, galanthamine, crinine, belladine and tazettine types of Amaryllidaceae alkaloids. The norbelladine used in this feeding experiment had 78.2% of its total radioactivity at the C$_1$ position and 21.8% of its total radioactivity at the C$_{1'}$ position. If the norbelladine was incorporated intact into
Table 10. Degradation of ambelline isolated from the 2-C\(^{14}\)-tyrosine feeding experiment, procedure A

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trial 1</th>
<th></th>
<th></th>
<th>Trial 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td></td>
<td>824 mg.</td>
<td>1.00</td>
<td></td>
<td>1.3 g.</td>
<td>1.00</td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>692 mg.</td>
<td>692 mg.</td>
<td>----</td>
<td>990 mg.</td>
<td>990 mg.</td>
<td>----</td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>350 mg.</td>
<td>350 mg.</td>
<td>1.04</td>
<td>837 mg.</td>
<td>837 mg.</td>
<td>----</td>
</tr>
<tr>
<td>(N)-(2-Methoxy-6-phenylpiperonyl)sarcosine hydrochloride</td>
<td>185 mg.</td>
<td>180 mg.</td>
<td>0.94</td>
<td>435 mg.</td>
<td>200 mg.</td>
<td>0.98</td>
</tr>
<tr>
<td>3-Methoxy-2-methyl-4,5-methylenedioxybiphenyl</td>
<td>139 mg.</td>
<td></td>
<td>0.00</td>
<td>32 mg.</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>N-Tosylsarcosine</td>
<td>41 mg.</td>
<td>16 mg.</td>
<td>1.08</td>
<td>26 mg.</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>----</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct</td>
<td>3 mg.</td>
<td></td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11. Degradation of ambelline isolated from the 2-C\(^{14}\)-tyrosine feeding experiment, Procedure B

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td>750 mg.</td>
<td>1.00</td>
<td>750 mg.</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>472 mg.</td>
<td>472 mg.</td>
<td>472 mg.</td>
<td>472 mg.</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>466 mg.</td>
<td>466 mg.</td>
<td>466 mg.</td>
<td>466 mg.</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>N-(2-methoxy-6-phenylpiperonyl)sarcosine hydrochloride</td>
<td>250 mg.</td>
<td>14 mg.</td>
<td>1.06</td>
<td>250 mg.</td>
<td>14 mg.</td>
<td>1.06</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>0.00</td>
<td>-----</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct</td>
<td>6 mg.</td>
<td>0.72</td>
<td>6 mg.</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12. Corrected values of the relative activities of the formaldehyde dimedone adduct obtained by procedure B

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-C(^{14})-Tyrosine feeding experiment</td>
<td>5.55 mg.</td>
<td>1.81 mg.</td>
<td>0.66</td>
<td>0.97</td>
</tr>
<tr>
<td>1,1'-C(^{14})-Norbelladine feeding experiment</td>
<td>4.64 mg.</td>
<td>1.45 mg.</td>
<td>0.53</td>
<td>0.78</td>
</tr>
</tbody>
</table>
ambelline (78) as predicted by the Barton-Cohen hypothesis, the C\textsubscript{12} position should have 78.2% and position C\textsubscript{6} should have 21.8% of the total radioactivity of the ambelline (Figure 29).

\[ \text{Figure 29. Incorporation of norbelladine into ambelline} \]

The relative activities at the C\textsubscript{12} and C\textsubscript{6} positions were determined by degradation of ambelline by procedures A (Table 13) and B (Table 14). Degradation of the ambelline by procedure A gave 3-methoxy-2-methyl-4,5-methylenedioxybiphenyl having 20% of the total activity and N-tosyl-2-C\textsuperscript{14}\textsuperscript{-sarcosine containing 71% of the total activity, which is in good
### Table 13. Degradation of ambelline isolated from the 1,1'-C\textsuperscript{14}-norbelladine feeding experiment, procedure A

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td>836 mg.</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>573 mg.</td>
<td>573 mg.</td>
<td></td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>355 mg.</td>
<td>350 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>N-(2-Methoxy-6-phenylpiperonyl) sarcosine hydrochloride</td>
<td>158 mg.</td>
<td>150 mg.</td>
<td>0.93</td>
</tr>
<tr>
<td>3-Methoxy-2-methy-4,5-methylenedioxybiphenyl</td>
<td>16 mg.</td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>N-Tosylsarcosine</td>
<td>26 mg.</td>
<td></td>
<td>0.71</td>
</tr>
</tbody>
</table>

### Table 14. Degradation of ambelline isolated from the 1,1'-C\textsuperscript{14}-norbelladine feeding experiment, procedure B

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td>1.067 g.</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>660 mg.</td>
<td>660 mg.</td>
<td></td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>700 mg.</td>
<td>700 mg.</td>
<td></td>
</tr>
<tr>
<td>N-(2-Methoxy-6-phenylpiperonyl) sarcosine hydrochloride</td>
<td>14 mg.</td>
<td>14 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct</td>
<td>1.36 mg.</td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
agreement with the theory. The N-tosylsarcosine obtained from degrada-
tion of ambelline by procedure A was not subjected to electrolytic oxida-
tion. Lead tetraacetate oxidation of N-(2-methoxy-6-phenylpiperonyl)
sarcosine hydrochloride (Table 14) gave formaldehyde dimedone adduct
having a relative specific activity of 0.53, which could be corrected
to 0.78 (Table 12). These data prove that norbelladine is incorporated
intact into ambelline.

Ambelline Isolated From the Sodium C\textsubscript{14}-Formate

Feeding Experiment to \textit{Nerine bowdenii}

There is ample evidence that formate serves as a precursor of one-
carbon units such as N-methyl, methoxyl and methylenedioxy groups in
natural products\textsuperscript{53-64}. Thus the ambelline (78) isolated from sodium
C\textsubscript{14}-formate feeding experiments would be expected to be labelled primar-
ily in the methoxyl and methylenedioxy groups.

As a routine experiment, sodium C\textsubscript{14}-formate was fed to \textit{Nerine}
bowdenii at the same time the feeding experiments with 3-C\textsubscript{14}-phenyl-
alanine, 3-C\textsubscript{14}-tyrosine and 1,1'-C\textsubscript{14}-norbelladine were carried out.
The degradation of belladine (54b) isolated from the formate feeding
experiment showed extremely peculiar isotopic incorporation. Radio-
activity was found to the extent of 27% in the methoxyl and N-methyl
groups. However, 73% remained unaccounted for and must be present in
the C\textsubscript{6}-C\textsubscript{1} and C\textsubscript{6}-C\textsubscript{2} units. Degradation of the belladine showed that
all remaining radioactivity unaccounted for was at the benzylic position
(H. Ishii, Chemistry Department, Iowa State University, Ames, Iowa,
Private Communication, 1965). Although belladine was the major alkaloid isolated from *Nerine bowdenii* in the sodium $^{14}$C-formate feeding experiment, it was possible to obtain ambelline at a very low level of activity ($1.605 \times 10^4$ dpm/mM). Because of the unusual results obtained in the degradation of belladine, it was deemed worthwhile to examine, within the limits of experimental validity, the pattern of incorporation present in ambelline. The pattern of isotopic incorporation in ambelline was in good agreement with that found for belladine.

One-carbon units in ambelline would consist of the methylenedioxy at C$_9$-C$_{10}$ and methoxys at the C$_3$ and C$_7$ positions. Specific degradations for these groups showed that 57% of the total radioactivity of the ambelline was present in these one-carbon groups (Table 15). The methylenedioxy group contained approximately 3% of the radioactivity, while a Zeisel determination showed that the C$_3$ and C$_7$ methoxyl groups contained approximately 54% of the total radioactivity. Since Hofmann degradation of ambelline to 80a occurred with loss of 47% of the total radioactivity, the C$_3$ methoxyl group contains 47% of the total one-carbon radioactivity and, by difference, C$_7$ contains about 7%.

<table>
<thead>
<tr>
<th>One-carbon units</th>
<th>% act. of amb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylenedioxy group</td>
<td>3.</td>
</tr>
<tr>
<td>C$_3$ Methoxyl group</td>
<td>47.</td>
</tr>
<tr>
<td>C$_7$ Methoxyl group</td>
<td>7.</td>
</tr>
<tr>
<td>Total of three groups</td>
<td>57.</td>
</tr>
</tbody>
</table>
Since degradation of the ambelline by procedure B (Table 16) showed no radioactivity at either the C_{11} or C_{12} positions, the remaining radioactivity (43%) in the ambelline must be in the C_{6}-C_{1} unit or in the hydroaromatic ring of the C_{6}-C_{2} unit. Because of the results obtained from belladine, it would be expected that a one-carbon unit would not be incorporated into the hydroaromatic ring of the C_{6}-C_{2} unit or the aromatic portion of the C_{6}-C_{1} unit of ambelline, and it is presumed that the remaining activity is at the benzylic carbon. Unfortunately, insufficient material was available to carry the degradation further.

Table 16. Degradation of ambelline isolated from Nerine bowdenii in the sodium C^{14}-formate feeding experiment, procedure B

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambelline</td>
<td></td>
<td>160 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>Oxoambelline</td>
<td>85 mg.</td>
<td>57 mg.</td>
<td>---</td>
</tr>
<tr>
<td>Oxoambelline methiodide</td>
<td>27 mg.</td>
<td>25 mg.</td>
<td>1.01</td>
</tr>
<tr>
<td>N-(2-Methoxy-6-phenylpiperonyl) sarcosine hydrochloride</td>
<td>--</td>
<td>8 mg.</td>
<td>0.53</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct</td>
<td>1.62 mg.</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>---</td>
<td></td>
<td>0.00</td>
</tr>
</tbody>
</table>

Radioactive formate could be incorporated into the C_{6} position of ambelline (78) and the C_{1} position of belladine (54b) by the following biosynthetic pathway (Figure 30).

The formation of serine in Zea mays, wheat, barley, and tobacco from sodium formate and glycine (87) is well established^{65-77}. 
Figure 30. Proposed biosynthesis of phenylalanine
Zelinková and Sorm\textsuperscript{73} proposed that the $\alpha$-carbon of glycine serves as a possible precursor of the C\textsubscript{3} position of serine (95). Sinhad and Cossins\textsuperscript{74} have shown that the C\textsubscript{3} position of serine does have varying amounts of radioactivity from 2-C\textsuperscript{14}-glycine feeding experiments. They proposed that serine could be formed by oxidation of the glycine to glyoxalate (96), which reacted with formate to give serine (Figure 31).

\[
\text{H}_2\text{N}-\text{CH}_2-\text{CO}_2\text{H} \quad \xrightarrow{87} \quad \text{OHC}-\text{CO}_2\text{H} + \]

\[
\text{HCO}_2\text{H} \quad \xrightarrow{96} \quad \text{HO-CH}_2-\text{CH}-\text{CO}_2\text{H} + \text{NH}_2
\]

Figure 31. Formation of serine from glycine and formate

The deamination of serine to pyruvic acid (97) has never been demonstrated in botanical specimens, but has been shown to occur in the microorganisms \textit{Escherichia coli}, \textit{Pseudomonos aeruginosa}, \textit{Proteus ox_{19}}, \textit{Clostridium welchii} and \textit{Neurospora spp}.\textsuperscript{78-82} The formation of phenylalanine (59b) from 5-phosphoshikimic acid (98) and pyruvic acid has been suggested by studies of the metabolic pathways of the microorganisms \textit{Neurospora crassa}, \textit{Escherichia coli} and \textit{Aerobacter aerogenes}\textsuperscript{24-29}. 
Further Studies on the Incorporation of One-Carbon Unit Precursors into the Amaryllidaceae Alkaloids

The unusual results obtained from the sodium C\textsuperscript{14}-formate feeding experiment with Nerine bowdenii as plant host were completely unexpected. Contrary to the previous biosynthetic experiments in which only members of the phenylalanine-cinnamic acid biosynthetic pathway (Figure 30) have been found to be incorporated into the C\textsubscript{6}-C\textsubscript{1} unit of the Amaryllidaceae alkaloids, it now appeared that formate was an excellent precursor of the benzyl position of the C\textsubscript{6}-C\textsubscript{1} unit of these alkaloids. To re-examine the pattern of incorporation of sodium C\textsuperscript{14}-formate in Amaryllidaceae alkaloids, further feeding experiments were conducted (Table 17). Sprekelia formosissima was chosen as plant host on the basis of its availability and alkaloid content\textsuperscript{83}. Sprekelia formosissima contains

Table 17. Feeding experiments with Sprekelia formosissima as plant host

<table>
<thead>
<tr>
<th>Feeding experiment</th>
<th>Wgt. taz. used (dpm/mM)</th>
<th>Total act. Taz. used (dpm/mM)</th>
<th>Taz. act. % inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium C\textsuperscript{14}-formate</td>
<td>263 mg. 1.0 mc.</td>
<td>6.62 x 10\textsuperscript{4}</td>
<td>1.15 x 10\textsuperscript{-2}</td>
</tr>
<tr>
<td>3-C\textsuperscript{14}-Serine</td>
<td>220 mg. 0.5 mc.</td>
<td>2.33 x 10\textsuperscript{4}</td>
<td>8.16 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>3-C\textsuperscript{14}-Phenylalanine*</td>
<td>150 mg. 0.5 mc.</td>
<td>2.66 x 10\textsuperscript{5}</td>
<td>1.00 x 10\textsuperscript{-2}</td>
</tr>
</tbody>
</table>

* The tazettine obtained from the 3-C\textsuperscript{14}-phenylalanine was not diluted with non-radioactive material prior to determining its activity.
minor amounts of haemanthamine and haemanthidine as well as the major alkaloid, tazettine. Each of these Amaryllidaceae alkaloids has a benzylic carbon in the C₆-C₁ unit. The alkaloids were isolated from Sprekelia formosissima by standard procedures. Tazettine was obtained in sufficient yield for degradative purposes, but only trace amounts of haemanthamine and haemanthidine were obtained. A trace amount of lycoramine also was obtained.

Since 3-C¹⁴-serine is a one-carbon unit precursor and may be a possible biosynthetic intermediate in the formation of Amaryllidaceae alkaloids, it was also fed to selected bulbs of Sprekelia formosissima at the same time as the sodium C¹⁴-formate. Because of previous biosynthetic experiments with 3-C¹⁴-phenylalanine, this substance was used as a reference; its incorporation into the C₆-C₁ unit of tazettine is well established (Table 1). In Sprekelia formosissima the levels of incorporation of the radioactive tracers, sodium C¹⁴-formate, 3-C¹⁴-serine and 3-C¹⁴-phenylalanine, were comparable.

Degradation of Tazettine

On the basis of the early experiments with the incorporation of formate into belladine and ambelline, it would be expected that tazettine (12) isolated from the sodium C¹⁴-formate and 3-C¹⁴-serine feeding experiments, would be labelled at the C₆ position as well as in the C₁ fragments.

The radioactivity present in the C³ methoxyl group of tazettine was determined by the Zeisel method. The radioactivity of the
methylenedioxy group was determined by acid hydrolysis (Figure 32) to formaldehyde, which was isolated as the dimedone adduct (70).

\[
\text{OCH}_3 \\
\text{N-CH}_3 \\
\text{OH}
\]

Figure 32. Methylenedioxy determination

In previous radioactive feeding experiments (Table 1) tazettine was degraded by the procedure given in Figure 33. If the tazettine was labelled at the C₈ position, the radioactivity would appear in the tazettine methine (99) at the position indicated by the dagger. Basic hydrolysis of 99 gave 6-phenylpiperonyl alcohol (100) and dimethylglycine, which was isolated as the hydrochloride (101).

Activity at the position indicated by the dagger in tazettine methine would appear in the benzylic carbon atom of 100. To determine the amount of radioactivity at the benzylic position of 6-phenylpiperonyl alcohol, it may be oxidized to 6-phenylpiperonylic acid (102) with potassium permanganate (Figure 34) and then decarboxylated with copper chromite to give carbon dioxide and 3,4-methylenedioxybiphenyl (103). The carbon
dioxide should contain all the activity at the C₈ position, and 103 would contain the radioactivity of the methylenedioxy group of tazettine.

One of the N-methyl groups of the dimethylglycine hydrochloride is derived from the N-methyl group of tazettine, and the other arises from
Figure 34. Oxidation-decarboxylation of 6-phenylpiperonyl alcohol non-radioactive methyl iodide. All radioactivity not in the carboxyl or methylene group of 101 must be in the N-methyl groups. To determine the activity of the N-methyl groups, the dimethylglycine hydrochloride was oxidized with lead tetraacetate to obtain the carboxyl group as carbon dioxide and the methylene group as formaldehyde dimeredone adduct (Figure 35). The carbon dioxide and the formaldehyde dimeredone adduct were found to be non-radioactive in both the sodium C\textsuperscript{14}-formate and the 3-C\textsuperscript{14}-serine
Figure 35. Lead tetraacetate oxidation of dimethylglycine hydrochloride feeding experiments. Therefore the activity of the dimethylglycine hydrochloride obtained on degradation of the tazettine isolated from the sodium $^{14}C$-formate or the 3-$^{14}C$-serine feeding experiment could only be attributed to the N-methyl group derived from tazettine.

**Tazettine Isolated From the Sodium $^{14}C$-Formate Feeding Experiment**

The $^{13}C$ methoxyl group was shown to have 8% and the methylenedioxy group was shown to have 3% of the total radioactivity (Table 18) of the tazettine (12). The 6-phenylpiperonyl alcohol (101) obtained on degradation was shown to have only 4% of the total radioactivity of the tazettine. Since this amount of radioactivity, within experimental error, had already been shown to be in the methylenedioxy group, no incorporation of sodium-$^{14}C$-formate occurred at the $^{13}C_8$ position or in the aromatic or hydroaromatic rings of tazettine. The dimethylglycine hydrochloride obtained in the degradation was found to have 96% of the total
Table 18. Degradation of tazettine from Spekelia formosissima in the sodium C\textsuperscript{14}-formate feeding experiment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used next rctn.</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tazettine</td>
<td>1.25 g.</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Tazettine methiodide</td>
<td>1.58 g.</td>
<td>1.58 g.</td>
<td>0.93</td>
</tr>
<tr>
<td>6-Phenylpiperonyl alcohol</td>
<td>350 mg.</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Dimethylglycine hydrochloride</td>
<td>150 mg.</td>
<td>12.49 mg.</td>
<td>0.96</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct from dimethylglycine hydrochloride</td>
<td>4.69 mg.</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Carbon dioxide from dimethylglycine hydrochloride</td>
<td>---</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Methyltriethyl ammonium iodide</td>
<td>6.15 mg.</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct from methylenedioxy determination</td>
<td>19 mg.</td>
<td></td>
<td>0.03</td>
</tr>
</tbody>
</table>

activity present in the tazettine. Since the C\textsubscript{1} and C\textsubscript{2} positions contained no radioactivity, all of the activity must be in the N-methyl group of the dimethylglycine hydrochloride. These data (Table 19) prove that the major incorporation of sodium C\textsuperscript{14}-formate into tazettine occurred in the N-methyl group.

Table 19. Per cent of total activity of tazettine isolated from the sodium C\textsuperscript{14}-formate feeding experiment in the methoxyl, methylenedioxy and N-methyl groups and at position C\textsubscript{8}

<table>
<thead>
<tr>
<th>Pos. of carbon in comp.</th>
<th>% of total act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{3} Methoxyl group</td>
<td>8.</td>
</tr>
<tr>
<td>Methylenedioxy group</td>
<td>3.</td>
</tr>
<tr>
<td>N-Methyl group</td>
<td>96.</td>
</tr>
<tr>
<td>C\textsubscript{8} Position</td>
<td>0.</td>
</tr>
</tbody>
</table>
The different levels of incorporation of formate (Table 19) into the methoxyl, methylenedioxy and N-methyl groups may be explained by considering the metabolic pathway in Figure 36, which is based on previous biosynthetic studies.

A high level of incorporation would be expected in the N-methyl group of the tazettine for incorporation at this position occurs in the last step of the metabolic pathway. A smaller amount of incorporation would be expected from the oxidative coupling of O-methyl norbelladine (69) to form haemanthamine (58), which in turn undergoes oxidation and rearrangement to give tazettine. However, these data do not explain why ambelline (78) and belladine (54b) apparently incorporate a large amount of sodium C^{14}-formate at the benzylic position of the C_{6}-C_{1} unit while tazettine does not. If sodium C^{14}-formate were incorporated into tazettine at positions other than the one-carbon units, the process would presumably occur prior to formation of the norbelladine, since norbelladine is known to be incorporated intact into Amaryllidaceae alkaloids (Table 2). These data indicate such a metabolic sequence (Figure 30) either does not exist or is occurring at such a slow rate in Sprekelia formosissima during the period (7/8/1965 - 8/25/1965) of the sodium C^{14}-formate feeding experiment that detection of the process was unsuccessful.
Figure 36. Biosynthesis of tazettine
Tazettine Obtained From the $3-\text{C}^{14}$-Serine Feeding Experiment

As previously stated, the formation of serine (95) from sodium formate and glycine (87) in plant tissues is well established$^{67-77}$, but the initial studies of Tolbert and Cohan$^{75}$, designed to show the conversion of serine to glycine and formate, were unsuccessful. However, Wilkinson and Davies$^{70,77}$ have been able to show that serine can be cleaved to give formate and glycine in plant tissues. These studies are in agreement with experiments showing $3-\text{C}^{14}$-serine is an effective precursor of the one-carbon units of nicotine (104)$^{85-91}$ and gramine (105)$^{90,91}$.

![Chemical structures]

On the basis of the previous studies with nicotine and gramine, the expected location of the radioactive labels in tazettine (12) obtained from the $3-\text{C}^{14}$-serine feeding experiment would be the methoxyl, methylene-dioxy and N-methyl groups. Furthermore, if the biosynthetic pathway (Figure 30) observed for the incorporation of sodium-$\text{C}^{14}$-formate into ambelline (78) and belladine (54b) is valid, the tazettine would also
be expected to be labelled in the benzylic (C₈) position of the C₆-C₇ unit.

The radioactivities in the methoxyl, methylenedioxy and N-methyl groups and in position C₈ of tazettine were determined in a manner identical to that used in the sodium C¹⁴-formate feeding experiment (Table 20). Except for a slight increase in the per cent of radioactivity present in the methylenedioxy group and a slight decrease in the activity present in the N-methyl group (Table 21), the results were identical to those obtained for tazettine isolated from the sodium C¹⁴-formate feeding experiment (Table 19). These data prove that the 3-C¹⁴-serine served as a one-carbon unit precursor almost as effectively as formate. Since sodium C¹⁴-formate was not incorporated into the tazettine at the C₈ position, 3-C¹⁴-serine would not be expected to be incorporated into this position.

Table 20. Degradation of tazettine obtained from the 3-C¹⁴-serine feeding experiment

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Amount used</th>
<th>Rel. act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tazettine</td>
<td></td>
<td>901 mg.</td>
<td>1.00</td>
</tr>
<tr>
<td>Tazettine methiodide</td>
<td>906 mg.</td>
<td>906 mg.</td>
<td>1.03</td>
</tr>
<tr>
<td>6-Phenylpiperonyl alcohol</td>
<td>225 mg.</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Dimethylglycine hydrochloride</td>
<td>55 mg.</td>
<td>12 mg.</td>
<td>0.89</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct from dimethylglycine hydrochloride</td>
<td>3 mg.</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>Methyltriethyl ammonium iodide</td>
<td>5.47 mg.</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Formaldehyde dimedone adduct from methylenedioxy determination</td>
<td>6.82 mg.</td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 21. Per cent of the total activity of the tazettine isolated from the 3-$^{14}$C-serine feeding experiment in the methoxyl, methylenedioxy and N-methyl groups and the C$_8$ positions

<table>
<thead>
<tr>
<th>Pos. of carbon in comp.</th>
<th>% of total act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxy group</td>
<td>8.</td>
</tr>
<tr>
<td>Methylenedioxy group</td>
<td>7.</td>
</tr>
<tr>
<td>N-Methyl group</td>
<td>89.</td>
</tr>
<tr>
<td>C$_8$-position</td>
<td>0.</td>
</tr>
</tbody>
</table>

It is clear from the experiments performed with *Sprekelia formosissima* that both formate and serine serve solely as precursors of C$_1$ units such as methoxyl, methylenedioxy and N-methyl groups. This finding is in direct contrast to the sodium $^{14}$C-formate fed *Nerine bowdenii*, where a significant amount of the radioactivity appeared in the benzylic position on the C$_6$-C$_1$ unit. It would seem desirable to repeat the experiments with *Nerine bowdenii* to confirm the original observations of H. Ishii.
It has been shown that the Amaryllidaceae alkaloid ambelline, which has three oxygen substituents on the aromatic ring, is synthesized in the plant from the same precursors as those found for alkaloids containing only two aromatic oxygen atoms. Tyrosine was shown to be the precursor of the hydroaromatic \( C_6-C_2 \) unit of ambelline, and phenylalanine serves as the aromatic \( C_6-C_1 \) precursor. Evidence is presented that norbella-dine is incorporated intact into ambelline and does not undergo cleavage to smaller fragments prior to incorporation.

Sodium formate and serine serve as precursors of the methoxyl, N-methyl and methylenedioxy groups of tazettine. These precursors are shown not to be incorporated into either the \( C_6-C_1 \) or \( C_6-C_2 \) units of tazettine. This result is in contrast with that found previously in the incorporation of sodium formate into ambelline.
EXPERIMENTAL

Source of Plant Materials and Radioactive Precursors

The Nerine bowdenii bulbs were secured from the Walter Marx Gardens, Boring, Oregon. The Sprekelia formosissima bulbs were obtained from Robert D. Goedert, Jacksonville, Florida.

The $\text{C}^{14}_{\text{I}}\text{-norbelladine}$ used in the Nerine bowdenii feeding experiment was synthesized by R. J. Hight (National Heart Institute, Bethesda, Maryland)\textsuperscript{32}. All other radioactive precursors (Tables 5 and 17) were obtained from New England Nuclear Corp., Boston, Massachusetts. The radioactive precursors were assumed to have the purity established by New England Nuclear Corp.

The author received the radioactive ambelline after isolation and separation of the alkaloids from Nerine bowdenii by Dr. William C. Wildman, National Heart Institute, Bethesda, Maryland.

Measurement of the Radioactivities of the Compounds

To determine the specific radioactivity (dpm/mM) of a compound, other than carbon dioxide, the material (1-6 mg.) was dissolved in 1.0 ml. of methanol and 10.0 ml. of Bray's scintillation solution [60 g. of napthalene, 4 g. of 1,4-bis-2-(5-phenyloxazolyl)-benzene
(Packard) and 100 mg. of 2,5-diphenyloxazole (Packard) in 20 ml. of 1,2-ethanediol, 100 ml. of methanol and sufficient dry, peroxide-free dioxane to make 1.0 l. of solution. The carbon dioxide was trapped in a solution of 2.0 ml. of hydroxide of Hyamine 10-X (Packard) and 10.0 ml. of toluene POPOP scintillation solution [4 g. of 1,4-bis-2-(5-henyloxazoly)-benzene (Packard) and 100 mg. of 2,5-diphenyloxazole (Packard) in 1.0 l. of toluene]. To determine the relative specific activity of the carbon dioxide, the total disintegrations per minute for the trapping solution were compared with the theoretical disintegrations per minute attainable from a sample of the material being degraded. All measurements of the radioactivities of the compounds were obtained with a Packard Tri-carb Liquid Scintillation Spectrometer System (Model 314 X).

**Nerine bowdenii Feeding Experiments**

**Purification of radioactive ambelline**

The radioactive ambelline (Table 5), isolated from a feeding experiment, was diluted with non-radioactive material and crystallized from absolute ethanol to give ambelline having a constant activity in the range 4,400-45,000 dpm/mM (Table 5).
Degradation of ambelline

Ambelline was degraded to N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by a general procedure in which the ratio of reagents to reactants was held constant in each experiment. All weights quoted were those obtained in the degradation of the ambelline from the 3-C¹⁴-tyrosine feeding experiment.

Oxoambelline. A solution of 650 mg. of ambelline in 26 ml. of dry pyridine was added to a slurry of 1.3 g. of dry chromium trioxide in 13 ml. of dry pyridine. The reaction mixture was stirred for 20 hours at room temperature and poured over ice. The excess chromium trioxide was reduced with sodium sulfite. The reaction mixture was made basic (pH=9) with sodium carbonate and extracted with chloroform until a negative silicotungstic acid test was obtained. The chloroform extract was washed with water, dried with anhydrous magnesium sulfate and evaporated to give 985 mg. of a brown oil. The oil was chromatographed on 29.4 g. of Florisil packed in 25% ethyl acetate - 75% benzene. Elution with 25% ethyl acetate - 75% benzene gave 540 mg. of oxoambelline, which was not crystalline. The material was identified by its infrared spectrum which was identical with authentic material of oxoambelline.

Oxoambelline methiodide. To a solution of 450 mg. of oxoambelline in 5 ml. of acetone was added a large excess of methyl iodide. The
solution was allowed to stand for 12 hours and the precipitated oxoambelline was removed by filtration. The oxoambelline methiodide was crystallized from 95% ethanol to give white crystals (350 mg.), m.p. 246-250°C. (dec.); reported, m.p. 250-254°C. (dec.).

N-(2-Methoxy-6-phenylpiperonyl) sarcosine hydrochloride A solution of 350 mg. of oxoambelline in 5 ml. of hot water was treated with 1.5 ml. of 50% sodium hydroxide. The solution was heated on the steam bath for 1 hour and allowed to cool. The excess alkali was decanted from the brown gum that had formed and the brown gum was dissolved in 3 ml. of 6N hydrochloric acid. The resulting solution was saturated with sodium chloride and extracted five times with chloroform. Evaporation of the chloroform gave 231 mg. of a product which was crystallized from methanol-acetone, m.p. 185-187°C, mixed m.p. 185-187°C. with an authentic sample of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride.

Degradation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by procedure A

The ratio of reagents to reactants was held constant for each degradation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by procedure A. All weights quoted were those obtained in the degradation of the ambelline from the 3-C^{14}-tyrosine feeding experiment.

3-Methoxy-2-methyl-4,5-methylenedioxybiphenyl and N-tosyl-sarcosine A solution of 200 mg. of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride in 30 ml. of absolute ethanol and 0.5 ml. of glacial acetic acid was hydrogenated with 833 mg. of pre-equilibrated palladium-
on-charcoal (10%). The reduction was stopped after a period of 48 hours at room temperature. The uptake of hydrogen was 1.5 equivalents. The reaction mixture was filtered. The filtrate was diluted with 20 ml. of water and extracted five times with ether. The ether extract was washed three times with water and dried with anhydrous magnesium sulfate.

Evaporation of the ether gave 82 mg. of a brown oil, which was distilled at 110°C. (0.05 mm.). The oil had an infrared spectrum identical with an authentic sample of 3-methoxy-2-methyl-4,5-methylenedioxybiphenyl.

The aqueous raffinate was condensed to one-third of its volume, and the solution was made basic (pH=11) with 2.5N sodium hydroxide. A solution of 1.838 g. of p-toluene sulfonyl chloride in 54 ml. of benzene was added and the emulsion was stirred for 24 hours at room temperature. The alkaline water layer was separated from the benzene layer. The aqueous solution was extracted five times with ether, made acidic (pH=1) with 1.5N hydrochloric acid and extracted five times with chloroform.

Evaporation of the chloroform gave 99 mg. of a solid which was crystallized from acetone-cyclohexane, m.p. 144-146°C., mixed m.p. 144-145°C. with an authentic sample of N-tosyl sarcosine.

**Electrolytic degradation of N-tosylsarcosine** To 8 ml. of commercial ethanol (95%) in a three necked, round-bottomed flask (Figure 37) was added 19.75 mg. of N-tosylsarcosine and 3 mg. of sodium hydride.

The 250 ml., three-necked, round-bottomed flask (Figure 37) contained two platinum electrodes (0.5 cm. x 1 cm.), 1 cm. apart and mounted 0.5 cm. from the bottom of the flask. The flask (Figure 37) was fitted with a nitrogen inlet and an outlet attached to a trap containing 2 ml. of
Hyamine hydrodide 10-X (Packard) and 10 ml. of standard toluene POPOP scintillation solution. The reaction mixture was electrolyzed for 60 hours at 90 m. amps. The carbon dioxide generated was trapped in the scintillation solution and its total radioactivity (765 dpm) determined.

In a second electrolytic degradation a solution of 50 mg. of N-tosylsarcosine and 6 mg. of sodium hydride in 20 ml. of water was electrolyzed for 16.5 hours at 20 m. amps. To the solution was added 5 ml. of concentrated hydrochloric acid and 100 mg. of dimedone, and the solution was refluxed for 3.5 hours. The solution was allowed to cool and
the cold solution was extracted with chloroform. Evaporation of the chloroform gave a residue which was heated in water to remove excess dimedone. The brown gum remaining after filtration was sublimed to give a solid. The sublimate was heated in water, filtered and resublimed to give white crystals (5 mg.), m.p. 189-191°C., mixed m.p. 187-188°C with an authentic sample of formaldehyde dimedone adduct.

**Degradation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by procedure B**

The ratio of reagents to reactants was held constant for each degradation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride by procedure B. All weights quoted were those obtained in the degradation of the ambelline from the 2-C\(^{14}\)-tyrosine feeding experiment.

**Lead tetraacetate oxidation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride**

To 15 mg. of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride in a three-necked, round-bottomed flask (Figure 38) was added 10.0 ml. of freshly prepared 0.67% lead tetraacetate-glacial acetic acid solution. The three-necked flask (Figure 38) was fitted with a nitrogen inlet and a condenser. The condenser was fitted with a nitrogen outlet leading to a trap containing a 90% saturated dimedone solution and a second trap containing 2 ml. of Hyamine hydroxide 10-X (Packard) and 10 ml. of standard toluene POPOP scintillation solution. The reaction mixture was heated on a steam bath for 2 hours 38 minutes. The formaldehyde dimedone adduct was separated from the dimedone solution by filtration, heated with water and sublimed at 140°C. (0.01 mm.), to give 5.55 mg. of radioactive formaldehyde dimedone.
Figure 38. Apparatus for lead tetraacetate oxidation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride adduct, m.p. 190-191°C., mixed m.p. 190-191°C. with authentic material.

The carbon dioxide produced in the reaction was trapped in toluene POPOP scintillation solution.

Determination of per cent impurities in radioactive formaldehyde dimeredone adduct obtained from the lead tetraacetate oxidation of N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride To a three-necked round-bottomed flask connected to apparatus previously described (Figure 38) was added 10.0 ml. of the freshly prepared 0.67% lead
tetraacetate-glacial acetic acid solution used in the oxidation of the N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride. The solution was heated on the steam bath for 2 hours 38 minutes. The non-radioactive formaldehyde generated was trapped in 90% saturated dimedone solution. The non-radioactive formaldehyde dimedone adduct was separated from the dimedone solution by filtration, heated with water and sublimed at 140°C (0.01 mm.) to give 1.81 mg. of material, m.p. 191-192°C. The non-radioactive formaldehyde dimedone adduct (1.81 mg.) was equivalent to an impurity of 32.6% in the radioactive formaldehyde dimedone adduct obtained in the lead tetraacetate oxidation of the N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride.

In the 1,1'-C\textsuperscript{14}-norbelladine feeding experiment, 4.64 mg. of radioactive formaldehyde dimedone adduct was obtained from the lead tetraacetate oxidation of the N-(2-methoxy-6-phenylpiperonyl) sarcosine hydrochloride and was shown to contain 1.45 mg. of non-radioactive formaldehyde dimedone adduct, equivalent to an impurity of 31.3% in the radioactive formaldehyde dimedone adduct.

Ambelline hydrochloride

Hydrogen chloride was bubbled into 0.75 ml. of an ethanolic solution of ambelline (60 mg.) obtained from the 3-C\textsuperscript{14}-phenylalanine feeding experiment. On evaporation of the ethanol with a stream of air gave crystals of ambelline hydrochloride (66 mg.) which were crystallized from acetone to constant activity (4,580 dpm/mM), m.p. 227-231°C. (dec.); reported, m.p. 227-230°C. (dec.).
A solution of the radioactive ambelline hydrochloride in water was made basic (pH=11) with 10% sodium hydroxide and extracted with chloroform. Evaporation of the chloroform gave 56 mg. of ambelline, (m.p. 259-261°C.); reported, m.p. 260-261°C. (dec.).

**Methylenedioxy determination**

To 110 mg. of ambelline and 336 mg. of dimedone in a centrifuge tube was added 5.5 ml. of sulfuric acid (60 ml. of concentrated sulfuric acid per 100 ml. of water). The reaction mixture was heated on a steam bath for 15 hours, diluted with 20 ml. of water and extracted five times with ether. The ether extract was dried with calcium chloride and evaporated to give a brown residue. The residue was heated with 10 ml. of water to remove water-soluble material and filtered. The brown residue was sublimed to give 34 mg. of a solid, m.p. 189-191°C. The sublimate was heated with 5 ml. of water for 15 minutes, filtered and resublimed to give white crystals, m.p. 190-191°C., identical with an authentic sample of formaldehyde dimedone adduct.

**Zeisel determination**

A solution of 0.5 ml. of 5% cadmium sulfate and 0.5 ml. of 5% sodium thiosulfate was placed in the iodine trap B (Figure 3). A solution of 5 ml. of 5% triethyl amine in absolute ethanol was placed in trap C (Figure 39) to trap the methyl iodide generated in the reaction as methyltriethyl ammonium iodide. Trap C (Figure 39) was emersed in a dry ice-chloroform bath at -30°C.

To 18 mg. of ambelline and 500 mg. of phenol in flask A (Figure 39)
Figure 39. Apparatus for Zeisel determination
was added 2.0 ml. of 57% hydroiodic acid. Flask A was then connected to the nitrogen source and heated 45 minutes at 125-132°C. in a Wood's metal bath. After the reaction was completed, trap C (Figure 39) was detached from the apparatus at point D (Figure 39) -- a rubber joint -- and stored under nitrogen for 12 hours. The ethanol-triethyl amine solution was evaporated by vacuum (aspirator) distillation to give a solid. The material was crystallized from methanol-ether to give 12.5 mg. of methyltriethyl ammonium iodide, m.p. 277-280°C. (dec.), identical with an authentic sample of the material.

**Synthesis of 2-C\textsuperscript{14}-N-tosylsarcosine**

To a solution of 1 g. of non-radioactive glycine and 0.8 mg. of 2-C\textsuperscript{14}-glycine in 300 ml. of water, adjusted to pH=11 with 10% sodium hydroxide, was added a solution of 20 g. of \( p \)-toluene sulfonyl chloride in 600 ml. of benzene. The emulsion was stirred for 20 hours at room temperature. The water layer was separated from the benzene layer. The aqueous solution was made basic (pH=11) with 10% sodium hydroxide and extracted with ether. The water layer was made acidic (pH=1) with 6N hydrochloric acid and extracted with chloroform. Evaporation of the chloroform gave a solid, which was dissolved in 95% ethanol. To the ethanol solution was added 1 ml. of 50% potassium hydroxide and 2 ml. of methyl iodide. White crystals precipitated and were removed by filtration. A solution of the precipitate in water was acidified (pH=1) with 6N hydrochloric acid and the solution was extracted with chloroform. Evaporation of the chloroform gave 7 mg. of material which was sublimed to give white crystals, m.p. 145-148°C., mixed m.p. with N-tosylglycine,
115-140°C., mixed m.p. with N-tosysarcosine 145-148°C. The crystals were diluted with 300 mg. of non-radioactive N-tosylsarcosine and crystallized to constant activity (209,500 dpm/mM) from acetone-cyclohexane.

**Determination of formaldehyde with chromotropic acid**

To a three-necked, round-bottomed flask connected to the apparatus previously described (Figure 38) was added 10.0 ml. of 0.67% freshly prepared lead tetraacetate-glacial acetic acid solution. The solution was heated on the steam bath for 3.00 hours. The formaldehyde generated was trapped in 10.0 ml. of water.

To 104 mg. of chromotropic acid in a 30 ml. beaker was added 1.00 ml. of the formaldehyde-water solution of unknown concentration. The solution was heated on a hot plate at 175°C. until all of the moisture had disappeared from the beaker. The material in the beaker was heated for an additional 5 minutes at 175°C. The beaker was allowed to cool and 5.0 ml. of concentrated sulfuric acid were added. The material was heated for 30 minutes in boiling water to give a purple solution. The purple solution was allowed to cool and was diluted to 50 ml. in a volumetric flask. The absorption of the purple solution at 570 mμ measured.

The above procedure was repeated with three formaldehyde-water solutions having concentrations of 78 μg./ml., 39 μg./ml. and 19.5 μg./ml. The three purple solutions obtained showed a linear relationship between absorption at 570 mμ and concentration of the formaldehyde-water solution from which they were made. The formaldehyde-water solution of unknown concentration was shown to contain 63.1 μg. of formaldehyde per milliliter.
Sprekelia formosissima Feeding Experiments

The same experimental procedures were used in isolating and degrading tazettine from the sodium C^{14}-formate and the 3-C^{14}-serine feeding experiments. All weights quoted were those obtained in the isolation and degradation of tazettine from the sodium-C^{14}-formate feeding experiment.

Feeding experiments

The sodium C^{14}-formate (1.0 mc., 17.0 mg.) was dissolved in 1.0 ml. of water. Into each of ten Sprekelia formosissima bulbs was injected, with a microsyringe, 0.1 ml. of the sodium C^{14}-formate solution. The radioactive vial was washed with 0.2 ml. of water and the water injected into an eleventh bulb. After allowing the plants to grow for a period of 48 days (7/8/1965 - 8/25/1965) the bulbs (667 g.) were harvested and the alkaloids isolated in the usual manner.

The 3-C^{14}-serine (0.5 mc. 13.7 mg.) was dissolved in 1.0 ml. of water. The 3-C^{14}-serine feeding experiment was identical to the sodium C^{14}-formate feeding experiment in all other respects. The bulbs (643 g.) were harvested and the alkaloids isolated in the usual manner.

General procedure for isolation of the crude alkaloids from Sprekelia formosissima bulbs

The eleven bulbs (667 g.) were homogenized with 2 l. of 95% ethanol in a Waring Blender. The mixture was allowed to settle for 30 minutes and the solid material removed by filtration. The filter cake was stirred in 2 l. of 95% ethanol and allowed to stand overnight. The solid
material was removed by filtration. The process was repeated a third time. The three filtrates were combined and concentrated to 1.0 l. by aspirator vacuum distillation under a nitrogen atmosphere. The remaining ethanol was removed on a rotatory evaporator to yield 21 g. of residue from the sodium C\(^{14}\)-formate feeding experiment and 11.7 g. of residue from the 3-C\(^{14}\)-serine feeding experiment.

The crude residue (21 g.) was stirred at 50\(^{\circ}\) C. with 250 ml. of 2N hydrochloric acid and filtered to remove the acid-insoluble material. The insoluble material was heated with 100 ml. of 2N hydrochloric acid and refiltered. The filtrates were combined and extracted 5 times with chloroform. The chloroform extract gave a negative alkaloid test with silicotungstic acid and was discarded.

The 2N hydrochloric acid solution was made basic (pH=12) with 50% sodium hydroxide and extracted with chloroform until the aqueous solution gave a negative alkaloid test. The chloroform extract of the basic solution was evaporated on a rotovaporator to give 646 mg. (0.097% of the total weight of the bulbs) of a crude alkaloid mixture from the sodium C\(^{14}\)-formate feeding experiment. The 3-C\(^{14}\)-serine feeding experiment afforded 440 mg. (0.069% of the total weight of the bulbs) of a crude alkaloid mixture.

Thin-layer and gas-phase analysis proved the crude alkaloid mixtures to contain primarily tazettine and traces of haemanthamine, haemanthidine and lycoramine.
Separation of tazettine from the crude alkaloid mixture

The crude alkaloid mixture (646 mg.) was dissolved in acetone and allowed to stand for a period of 3 days. The tazettine that had precipitated was removed by filtration. The filtrate was evaporated to give a residue, which was dissolved in 25% benzene-75% chloroform and chromatographed on an alumina (Merck-neutral) column (17.5 cm x 1.5 cm) packed in 25% benzene-75% chloroform. Elution with 25% benzene-75% chloroform gave lycoramine first, haemanthamine second and then tazettine. Elution with chloroform and 1%, 2%, 5% and 10% methanol-chloroform solutions gave no characterizable products. Elution with 20% methanol-80% chloroform gave a mixture containing haemanthidine.

A total yield of 263 mg. of tazettine, m.p. 207-209°C., (317,000 dpm/mM) obtained from the sodium C\textsuperscript{14}-formate feeding experiment was diluted with 1.017 g. of non-radioactive tazettine and crystallized to constant activity, 66,200 dpm/mM (1.25g.), m.p. 209-210°C.; reported m.p. 208-210°C.

A total yield of 220 mg. of impure tazettine (m.p. 204-209°C., 224,100 dpm/mM) was isolated from the crude alkaloid mixture (440 mg.) from the 3-C\textsuperscript{14}-serine feeding experiment. This material was recrystallized from acetone. To the filtrate was added 100 mg. of non-radioactive tazettine, and the material was recrystallized to give a second crop of radioactive tazettine. The process was repeated with the second filtrate to give a third crop of radioactive tazettine. The three crops of radioactive tazettine were combined, diluted with 880 mg. of non-radioactive tazettine and crystallized to constant activity, 23,300
dpm/mM (1.223 g.), m.p. 209-210°C.; reported, m.p. 208-210°C.

Zeisel determination

The Zeisel determination was conducted in the apparatus shown in
Figure 39 with the traps B and C containing the prescribed reagents. To
22 mg. of tazettine and 500 mg. of phenol in flask A (Figure 39) was
added 2.0 ml. of 57% hydroiodic acid. Flask A (Figure 39) was then
connected to the nitrogen source and heated 45 minutes at 125-132°C.
in a Wood's metal bath. After the reaction was completed, trap C
(Figure 39) was detached from the apparatus at point D (Figure 39)—
a rubber joint — and stored under nitrogen for 12 hours.

The ethanol-triethyl amine solution was evaporated under reduced
pressure. The methyltriethyl ammonium iodide (6.15 mg.) was crystallized
from methanol-ether and dried under vacuum at room temperature.

Methylenedioxy determination

To 103 mg. of tazettine and 308 mg. of dimedone in a centrifuge
tube was added 5 ml. of sulfuric acid (60 ml. of sulfuric acid in 100 ml.
of water). The reaction mixture was heated on a steam bath for 16 hours,
added to 25 ml. of water and heated to dissolve excess dimedone. The
hot solution was filtered and the brown residue reheated in 5 ml. of
water to remove further dimedone. The hot solution was filtered and the
brown residue sublimed at 140°C. (0.01 mm.). The sublimate was heated
in water, filtered and resublimed to give white crystals (19 mg.), m.p.
191-192°C., identical with an authentic sample of formaldehyde dimedone
adduct.
Degradation of tazettine

Tazettine (850 mg., 66,200 dpm/mM) from the sodium C$^{14}$-formate feeding experiment was diluted with non-radioactive tazettine (702 mg.) and crystallized from acetone to give tazettine having an activity of 36,100 dpm/mM. The tazettine (23,300 dpm/mM) obtained from the 3-C$^{14}$-serine feeding experiment was not diluted further prior to degradation.

Tazettine methiodide To a solution of 1.25 g. of tazettine in 50 ml. of acetone was added a large excess of methyl iodide, and the solution was allowed to reflux for 2 hours. The acetone solution was concentrated until tazettine methiodide precipitated. The solution was cooled and filtered. The tazettine methiodide (1.58 g.) was recrystallized from methanol-acetone-ether to give white crystals, m.p. 238-239°C. (dec.); reported, m.p. 238°C. (dec.).

Dimethylglycine hydrochloride and 6-phenylpiperonyl alcohol To a solution of 1.58 g. of tazettine methiodide in water was added a large excess of freshly prepared neutral silver hydroxide and the mixture stirred with a magnetic stirrer for 15 minutes. The reaction mixture was filtered. The filtrate gave a negative iodide ion test with acidic silver nitrate. The filtrate was evaporated on a rotovaporator and pyrolyzed 1 hour on the steam bath under aspirator vacuum to give 1.1 g. of tazettine methine, which was not crystalline. The material was identified by its infrared spectrum which was identical with an authentic sample of tazettine methine.

To a methanolic solution (15 ml.) of the methine was added 3 ml. of
2.5N sodium hydroxide. The reaction mixture was heated for 1 hour on
the steam bath and added to 20 ml. of cold water. The water emulsion
was extracted six times with ether. Evaporation of the ether gave a
solid which was crystallized from ether to give 350 mg. of 6-phenyl-
piperonyl alcohol, m.p. 98-99°C., identical with an authentic sample.

The water raffinate, remaining after the ether extraction, was
acidified with 6N hydrochloric acid and evaporated on a rotovaporator
to give a mixture of sodium chloride and dimethylglycine hydrochloride.
The dimethylglycine hydrochloride was removed from the sodium chloride
by sublimation at 145-150°C. (0.01 mm.). Resublimation of the dimethyl-
glycine hydrochloride yielded white crystals (150 mg.), m.p. 184-185°C.,
identical with an authentic sample of dimethylglycine hydrochloride.

**Lead tetraacetate oxidation of dimethylglycine hydrochloride**

To 12.49 mg. of dimethylglycine hydrochloride in a three-necked, round-
bottomed flask connected to the apparatus previously described (Figure
38) was added 10.0 ml. of 0.67% lead tetraacetate-glacial acetic acid
solution. The first trap contained a 90% saturated dimedone solution,
and the second trap contained a 90% barium hydroxide solution. The
flask was heated on the steam bath for 30 minutes.

The formaldehyde dimedone adduct was separated from the dimedone
solution by filtration, heated with water and sublimed at 140°C.
(0.01 mm.). The sublimed material was heated in water, filtered and
resublimed to give 4.69 mg. of formaldehyde dimedone adduct, m.p. 191-
192°C., identical with an authentic sample of the material.
The carbon dioxide trapped as barium carbonate was separated from the barium hydroxide solution by filtration, washed with distilled water and then washed with methanol. The barium carbonate was dried under vacuum. To 12.31 mg. of the barium carbonate in a three necked, round-bottomed flask (Figure 38) was added a large excess of 6N hydrochloric acid. The carbon dioxide, generated in an apparatus identical to that used in the lead tetraacetate oxidation reaction (Figure 38), was trapped in 1.0 ml. of Hyamine hydroxide 10-X (Packard) and 10 ml. of toluene POPOP scintillation solution. The nitrogen stream was allowed to bubble into the scintillation solution for a period of 30 minutes following disappearance of the barium carbonate in the reaction flask (Figure 38).


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