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Authors
Rueppel, Melvin L.
Rapoport, Henry

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Melvin L. Rueppel and Henry Rapoport

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The study of the biosynthesis of natural products in plants has been carried out almost exclusively by means of precursor feeding experiments, although the importance of alternate methods such as short-term biosynthesis with $^{14}\text{CO}_2$ has been stressed. Ideally, only the natural precursor should be incorporated efficiently into the natural product; however, incorporation of an unnatural precursor into a natural product is well-documented. Although theoretically possible, neither the incorporation of a natural precursor into an unnatural product nor the incorporation of an unnatural precursor into an unnatural product, closely related to the natural one, has been previously reported. We now provide an example of biosynthesis involving the latter type of precursor incorporation.

Since 1-methyl-1-pyrrolinium chloride (2) has been found to be an efficient precursor of the pyrrolidine ring of nicotine (3), 1,3-dimethyl-1-pyrrolinium-3-$^{14}\text{CH}_3$ chloride (1) was selected as the candidate unnatural precursor and synthesized as shown. 1-Methyl-2-pyrrolidone (4), condensed with diethyl carbonate with sodium hydride as base, gave ester 5. 1,3-Dimethyl-3-carbethoxy-2-pyrrolidone-3-$^{14}\text{CH}_3$ (6) was obtained by alkylating the sodium enolate of 5 with methyl-$^{14}\text{C}$ iodide. Hydrolysis of the alkylated ester 6 (sp. act. $2.71 \times 10^7$ dpm/mmol)
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same size as shown here.

\[ \begin{align*}
4 & \rightarrow 5 \\
5 & \rightarrow 6 \\
9 & + \overset{\text{Cl}^-}{\text{N}} \quad \overset{\text{H}}{\text{N}} \rightarrow 8 \\
3, R = H \\
10, R = \text{CH}_3
\end{align*} \]
In order to examine the possibility of biosynthesizing 3\'-methylnicotine (10), it was administered in portions over a period of several days to an aerated hydroponic solution. Quantitatively, gave acid 7 (specific activity 2.68 x 10^7 dpm/mmol) which on decarboxylation at 150-160°C gave 1,3-dimethyl-2-pyrrolidinone 8. Reduction of 8 with lithium aluminum hydride gave in 92% yield a mixture of pyrrolinium salt 1 (70%) and pyrrolidine 9. Chromatography of the mixture on silica gel, eluting with ethanol-0.1N HCl (2:1), followed by ion exchange gave pure 1 in 40% overall yield from 5.
containing four N. glutinosa plants in each experiment

Table I. Administration of 1,3-Dimethyl-1-pyrrolinium-3\(^{14}\)CH\(_3\) Chloride (1) to Nicotiana glutinosa and Incorporation into 3\(^{\omega}\)-Methylnicotine (10)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pyrrolinium salt 1 fed</th>
<th>3-methylnicotine (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>dpm</td>
</tr>
<tr>
<td>1</td>
<td>40.4(^{b})</td>
<td>8.10(</td>
</tr>
<tr>
<td>2</td>
<td>200.4(^{c})</td>
<td>3.97(</td>
</tr>
</tbody>
</table>

For the preparation of the plants, see footnote 11. \(^{b}\)Administered over a 5-day period followed by one day of growth. Total weight of the four plants was 261 g; their age was 66 days. \(^{c}\)Administered in increasing amounts over a period of 8 days to 59 day-old plants. Total weight of the four plants was 54 g and 139 g at the start and finish, respectively. \(^{d}\)Using nicotine (3) as the standard, GLPC analysis indicated the presence of 56.0 mg of nicotine (3) and 21.6 mg (8.3%) of 3\(^{\omega}\)-methylnicotine (10).

At the end of the biosynthetic experiment, the alkaloidal fraction was isolated as described\(^{11}\) and fractionated by preparative gas liquid partition chromatography.\(^{12}\) In addition to the normal Nicotiana alkaloids,\(^{11}\) a peak at retention time 31.2 minutes was also present in a yield of \(5\)\(^{-11}\)% (Table I).

The new substance has been characterized as 3\(^{\omega}\)-methylnicotine (10). Its ultraviolet spectrum shows a \(\lambda_{max}\) of 261 nm and is identical in all respects to that of nicotine.\(^{13}\) The specific activity of 10 was determined by a combination of uv absorption and liquid scintillation counting to be \(2.76 \times 10^{7}\) dpm/m mole, in
excellent agreement with its precursor 1. The mass spectrum
of 10 gave a molecular ion at \( m/e \) 176 (22% of base) along
with peaks at \( m/e \) 175 (7), 133 (100), 119 (6), and 98 (53), all
analogous with those of nicotine.\(^{14}\) High-resolution mass
spectroscopy established the molecular formula as \( C_{11}H_{16}N_2 \) for
\( m/e \) 176 (Calcd: \( 97.1313; \) Found: \( 97.1323 \)) and \( C_6H_{12}N \) for \( m/e \) 98
(Calcd: \( 98.0970; \) Found: \( 98.0974 \)) for the 1,3-dimethyl-1-pyrrolinium
fragment formed by \( \Delta \) cleavage. The nmr (CCl\(_4\)) shows peaks at
\( \delta \begin{align*}
8.40 \text{ (m, 2H),} & \hspace{1cm} 7.60 \text{ (m, 1H),} \\
7.17 \text{ (m, 1H),} & \hspace{1cm} 3.20 \text{ (m, 1H),} \\
1.4 \text{ (m, 3H, N-CH}_3& \text{), and,} 0.97 \text{ (d, 3H, >CHCH}_3 \text{) consistent}
\end{align*} \)
with structure 10.

Biogenetically, \( 3\delta \)-methylnicotine (10) would be expected
to have the same absolute configuration at the \( 2\delta \)-carbon as
nicotine (3) which has been assigned the \( S \) configuration with
reference to L-proline,\(^{15}\) L-serine,\(^{16}\) and optical rotary
dispersion measurements.\(^{17}\) The CD curve of 10 (in 95% EtOH)
gave a molecular ellipticity \( \theta \) at 260 nm of +22,800 (peak);
3 showed a \( \theta \) -7090 (trough) in addition to \( \theta \) +24,800
(peak). Although 3 showed a weaker negative cotton effect, at
273 nm in the ORD,\(^{17}\) this absorption was absent in both the CD
and ORD of 10 due possibly to the presence of an adjacent
assymmetric center. As a consequence, 10 is tentatively assigned
the \( S \) configuration at the \( 2\delta \)-carbon; the presence of the alkyl
methyl in 10 as a single doublet in the nmr indicates that
only one of the possible diastereomers was formed biosynthetically.

The biosynthesis of 3\( \delta \)-methylnicotine (10) from 1,3-
1,3-dimethyl-1-pyrrolinium salt 1 demonstrates that the enzyme system
which catalyzes the biosynthesis of nicotine from 1-methyl-
1-pyrrolium salt and a nicotinic acid derivative is not
completely specific, and its requirements may become definable
through experiments such as these. In addition the formation
of unnatural products from unnatural precursors in vivo should
be useful in the preparation of analogs of biologically active
natural products (with high specific activity if desired) and
in the study of metabolism and interrelationships among
alkaloids.
(1) This investigation was supported in part by Grant MH 12797 from the National Institute of Mental Health, U.S. Public Health Service, and the U.S. Atomic Energy Commission.


(3) G. Blaschke, H. I. Parker, and H. Rapoport, ibid., 89, 1540 (1967); T. J. Gilbertson and E. Leete, ibid., 89, 7085 (1967).

(4) Preliminary results indicate that examples of this type have been observed in studies on nicotine and morphine metabolism.


(6) All compounds in this report have been fully characterized spectrally (uv, ir, nmr) and analytically (elemental analysis, mass spec).


(8) Purchased from New England Nuclear.


(10) D. R. Hoagland and D. I. Arnon, California Agricultural Experimental Station Circular 347, revised 1950, College of Agriculture, University of California, Berkeley.
1 (11) W. L. Alworth, R. C. DeSelms, and H. Rapoport, J. Amer.
Chem. Soc., 86, 1608 (1964); W. L. Alworth, A. A. Liebman,
and H. Rapoport, ibid., 86, 3375 (1964).

2 (12) Chromatography was on a 15 x 1/4 m column of 10% KOH, 10%
polybutyleneglycol on 60-80 firebrick, column temperature
176°, flow rate of 90 ml/min. The retention times of
nicotine and nornicotine were 28.2 and 47.5 minutes, respectively.

3 (13) M. I. Swain, A. Eisner, C. F. Woodward, and B. A. Brice,

4 (14) A. M. Duffield, H. Budzikiewicz, and C. Djerassi, J. Amer.


8 (18) National Science Foundation Graduate Fellow, 1967-70.
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