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ABERRANT ALKALOID BIOSYNTHESIS. FORMATION OF NICOTINE ANALOGS FROM UNNATURAL PRECURSORS IN NICOTIANA GLUTINOSA.¹

Melvin L. Rueppel and Henry Rapoport

Contribution from the Department of Chemistry and Lawrence Radiation Laboratory, University of California, Berkeley, California, 94720.

Abstract: Several methyl derivatives of nicotine have been biosynthesized using Nicotiana glutinosa plants and the corresponding substituted pyrrolinium precursors. The syntheses of the ¹⁴C-labeled pyrrolinium precursors, which were utilized in the biosynthetic experiments, are also described. For chromatographic and spectral comparisons, authentic samples of some of the substituted nicotines were also synthesized. The stereochemistry and absolute configuration of the biosynthesized nicotine analogs have been determined. Incorporation results with 2- and 3-methyl substituted pyrrolinium precursors allow some speculation on the specificity and steric requirements of the enzyme(s) involved in the latter stages of nicotine biosynthesis.

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The biosynthetic pathway of formation of nicotine (1) in Nicotiana has been subject to a great deal of study. A multitude of experiments have been carried out by means of precursor feedings and short-term biosyntheses with ¹⁴CO₂;
however, the precise biosynthetic pathway has yet to be completely elucidated. In conjunction with other biosynthetic experiments with *Nicotiana glutinosa*, we became interested in the possibility of biosynthesizing unnatural nicotine analogs by using substituted precursors instead of the normal, natural precursor.

The possibility of biosynthesizing unnatural nicotine analogs using substituted natural precursors was interesting for several reasons. First, the incorporation of an unnatural precursor (i.e., a substituted natural precursor) into an unnatural product (i.e., nicotine analog) had not been previously reported in plants. Second, experiments with a series of substituted precursors might define the specificity of the enzyme system which catalyzes the biosynthesis of nicotine from a 1-methyl-1-pyrroline salt and a nicotinic acid derivative. Third, the formation of unnatural alkaloids in vivo should be useful in the preparation of analogs of biologically active natural products. Finally, since the unnatural products possess a structural label in addition to the usual radioactivity label, they should also be of great utility in the study of metabolism and interrelationships among the various alkaloids and other natural products in a given plant.

In a preliminary communication, we have reported the incorporation of 1,3-dimethyl-1-pyrroline-3-Cl3C14 chloride into the nicotine analog, 3'-methylnicotine. We now report the full details for this preliminary communication and additional related experiments concerning the formation of the nicotine
analogs, 6 and 7, from 9b and 10b, respectively.

Precursor synthesis. In the present work, only derivatives of the natural pyrrolidine ring precursor, 1-methyl-1-pyrrolinium salt (2), have been examined as potential unnatural precursors for analogs of nicotine. This choice was based on the fact that 2 has been reported to be a highly efficient precursor of the pyrrolidine ring of nicotine. A priori, derivatives of the pyridine ring precursor, nicotinic acid, could also have been examined; however, nicotinic acid is a less efficient precursor, probably due to its more widespread metabolic functions. Accordingly, it seemed reasonable to first concentrate on analogs of 2.

The first candidate unnatural precursor examined was 1,3-dimethyl-2-pyrrolinium-3-14CH3 chloride (4). It was synthesized by condensation of 1-methyl-2-pyrroolidinone (11) with diethyl
carbonate using sodium hydride as the base to give ester 12.

1,3-Dimethyl-3-carbethoxy-2-pyrrolidinone-3-\(^{14}\)CH\(_3\) (13)\(^7\)

was obtained by alkylating the sodium enolate of 12 with methyl-
\(^{14}\)C iodide.\(^8\) The procedure utilized in the isolation of
3-methyl derivative 13 precluded the presence of unalkylated
compound 12; this was verified by gcpc analysis. Hydrolysis
of the alkylated ester 13 (specific activity 2.71 \(\times\) 10\(^7\) dpm/mmol)
quantitatively gave the acid 14 (specific activity 2.68 \(\times\) 10\(^7\)
dpm/mmol) which on decarboxylation gave 1,3-dimethyl-2-pyrroli-
dinone (15).\(^9\) Stoichiometrically controlled reduction of 15

with lithium aluminum hydride gave in 92\% yield a mixture of
pyrrolinium salt 4 (63\%) and the pyrrolidine hydrochloride 16 (37\%).
Chromatography on silica gel, followed by ion exchange gave
pure 4 in 40\% overall yield from 12. The precursor 4 was
characterized spectrally, chromatographically, microanalytically
and by conversion to the hygrine derivative 17.
The second candidate unnatural precursor (9b) also labeled with carbon-14, was synthesized as follows. Methyl-$^{14}$C iodide$^8$ was converted to the corresponding Grignard reagent and subsequent addition of 1-methyl-2-pyrrolidinone (11) gave a mixture of 1,2-dimethyl-1-pyrrolinium-2-$^{14}$CH$_3$ chloride (9b) and 1,2,2-trimethylpyrrolidine-2,2-$^{14}$CH$_3$ hydrochloride.$^{10-12}$ Conversion of the mixture to the corresponding free bases, followed by the addition of 70% perchloric acid gave the pure iminium perchlorate 9a (specific activity 9.57 x $10^6$ dpm/mmol) in an overall yield of 27%. Immediately prior to the actual feeding experiments, the perchlorate 9a was converted into the chloride 9b by ion exchange.

Synthesis of the third candidate unnatural precursor (10b) was also initiated with 1-methyl-2-pyrrolidinone (11). Alkylation of 11 with methyl-$^{14}$C iodide$^8$ in diethyl ether at -78° with lithium diisopropylamide as the base gave 1,3,3-trimethyl-2-pyrrolidinone-3,3-$^{14}$CH$_3$ (18)$^9,13$ in 78% yield. Controlled lithium aluminum hydride reduction of 18 (specific activity 8.63 x $10^6$ dpm/mmol) gave a mixture of 10b (66%) and 1,3,3-trimethylpyrrolidine-3,3-$^{14}$CH$_3$ hydrochloride ($^{19}$; 34%). The pure perchlorate (specific activity 8.93 x $10^6$ dpm/mmol)
was obtained by the addition of perchloric acid to the corresponding free base of 10b and 19. Prior to the feeding experiments 10a was reconverted into the chloride 10b.

**Biosyntheses and Isolation.** Each biosynthetic experiment (Tables I and II) was carried out using four *Nicotiana glutinosa* plants which were growing in an aerated hydroponic solution. The plants had been grown prior to the feeding experiment as previously described. Appropriate precursor was added daily in portions to the aerated hydroponic solution; the rate of addition, age of plants, and the initial and final weights of the plants are given in the footnotes to Table I. The rate and amount of uptake of the precursors in experiments 1-4 were monitored continually by liquid scintillation counting of aliquots of the hydroponic nutrient solution. Particularly in experiments 3 and 4 in which an excess of the appropriate precursor was constantly maintained in the hydroponic solution, the rate and amount of precursor uptake was directly proportional to the mass of the plants; that is, as the mass of the plants increased by normal growth, a corresponding increase in the rate and amount of precursor was observed. The rate and amount of uptake was apparently independent of the amount of precursor available in the hydroponic solution. Finally, no harmful effects were noted in plant growth although up to 40 mg of precursor was incorporated daily.

Since the plants failed to completely absorb all the radioactivity associated with each precursor from the hydroponic
solution, the residual nutrient solution was examined in order to assess the stability of each precursor administered. In experiments 3 and 4, greater than 95% of the radioactivity in the residual nutrient was shown by nmr and tlc to be due to the presence of $^9b$ and $^{10b}$, respectively. In experiment 1, 90% of the total precursor administered each day was absorbed into the plants in 24 hours. The remaining 10% of the activity was due to chemical or biological change of 4 and accumulated as the experiment proceeded. Since these plants, and plants added to the nutrient solution after removal of the previous plants, were incapable of absorbing the transformation products of 4, the observed incorporation most probably is due to the uptake of 1,3-dimethyl-1-pyrrolinium-3-$^{14}$CH$_3$ chloride (4). Further support for the role of intact 4 as the actual precursor is provided by the excellent agreement in specific activities (see following) between 4 and the biosynthesized 3'-methylnicotine (3).$^{16}$

After each feeding experiment had proceeded for several days and the desired amount of precursor had been incorporated, the plants were fractionated as described previously$^{15}$ to give the four fractions indicated in Table I. The distribution of activity found in these four fractions is of interest for several reasons. First, essentially all (100 ± 3%) of the activity incorporated into the plants in each experiment (with the exception of expt. 1 where the activities of two fractions were not determined) has been accounted for by these four fractions. Clearly no loss of activity has occurred by metabolism of the administered precursors to respired $^{14}$CO$_2$. Second, significant differences in metabolism of each of the three precursors are indicated in the activity distributions. The compilation of activity distributions of the type given in
TABLE I. Distribution of Activity in Various Fractions of
Nicotiana glutinosa after Feeding Unnatural
Pyrrolinium Precursors 4, 9b and 10b.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Precursor</th>
<th>Activity (dpm x 10^-6) in Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total plant</td>
</tr>
<tr>
<td>1c</td>
<td>4</td>
<td>6.58</td>
</tr>
<tr>
<td>2e</td>
<td>4</td>
<td>31.40</td>
</tr>
<tr>
<td>3f</td>
<td>9b</td>
<td>21.60</td>
</tr>
<tr>
<td>4g</td>
<td>10b</td>
<td>12.30</td>
</tr>
</tbody>
</table>

*Based on total activity fed minus activity remaining in nutrient solution. *b*The activity present in the marc was determined by combusting an aliquot using a modification of the method of Kalberer and Rutschman. *c*Administered in equal portions over a period of 5 days with 1 day additional for growth. Total weight of the four plants was 261 g at the start and finish; their initial age was 66 days. *d*Not determined. *e*Administered in increasing amounts over a period of 8 days to 59-day-old plants. Total weight of the four plants was 54 and 139 g at the start and finish, respectively. *f*Administered in increasing amounts over a period of 13 days to 43-day-old plants. Total weight of the four plants was 13.7 and 53.3 g at the start and finish, respectively. *g*Administered in increasing amounts over a period of 8 days to 55-day-old plants. Total weight of the four plants was 27.2 and 54.5 g at the start and finish, respectively.
TABLE II. Administration of Pyrrolinium Precursors 4, 9b, and 10b to *Nicotiana glutinosa* and Incorporation into Nicotine Analogs 3, 6, and 7.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Precursor fed</th>
<th>Incorporation wt. (mg)</th>
<th>act. (dpm)</th>
<th>Nicotine analog formed</th>
<th>Yield of analog dpm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>33</td>
<td>6.58 x 10^6</td>
<td>3</td>
<td>4.22 x 10^5 (6.4)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>159</td>
<td>31.4 x 10^6</td>
<td>3</td>
<td>4.34 x 10^6 (13.8)</td>
</tr>
<tr>
<td>3</td>
<td>9b</td>
<td>304</td>
<td>21.6 x 10^6</td>
<td>6</td>
<td>8.26 x 10^3 (0.04)</td>
</tr>
<tr>
<td>4</td>
<td>10b</td>
<td>202</td>
<td>12.3 x 10^6</td>
<td>7</td>
<td>9.50 x 10^4 (0.77)</td>
</tr>
</tbody>
</table>

^a^ For the preparation of the plants, see ref. 15. ^b^ Using nicotine (1) as the standard, glpc analysis of the crude alkaloid fraction indicated the presence of 56.0 mg of 1 and 21.6 mg (10.5%) of 3'-methylnicotine (3). ^c^ Glpc analysis of the crude alkaloid fraction indicated the presence of 17.0 mg of 1 and 2.2 mg (0.8%) of 3',3'-dimethylnicotine (7).
Table I should prove valuable in metabolism and precursor feeding experiments. Although incorporations of the range of 30 to 0.003% have been reported in biosynthetic experiments, generally no attempt has been made to ascertain the fate of the majority of the precursor administered. Undoubtedly, significant metabolic and biosynthetic information could be obtained in many precursor feeding experiments from such a treatment.

The crude alkaloid fraction was analyzed by preparative glpc on either a 15' x 1/4" column (expts. 1, 2, and 4) or a 5' x 1/4" column (expt. 3) of 10% KOH, 10% polybutylene glycol on 60/80 firebrick. In the latter case (expt. 3) no fractionation of nicotine (l) and 2'-methylnicotine (6) was attempted due to the presence of only approximately 150 µg of 6 in a total of 19.6 mg of 1. The yields of the three analogs (3, 6, and 7) of nicotine are shown in Table II.

Relative incorporations of 1,3-dimethyl- (4), 1,2-dimethyl- (9b), and 1,3,3-trimethyl-1-methyl pyrrolinium chloride (10b) into the corresponding nicotine analogs (3, 6, and 7, respectively) are in an approximate ratio of 360:1:20. The differences in incorporation appear to be consistent with the relative amount of steric hindrance expected in each case in joining the substituted precursor with the hypothesized 1,6-dihydronicotinic acid derivative (20) to give the intermediate 21a,b, or c, respectively. Oxidation decarboxylation of 21 and subsequent loss of R affords the appropriate nicotine analog.
In Vitro Synthesis and Characterization of Nicotine Analogs. To aid in the characterization of the biosynthesized nicotine analogs 3 and 7, authentic samples of 3 and 7 were synthesized along with cis-3'-methylnicotine (5) and 3,3'-dimethylnicotine-2'-d (8). Adapting a method previously utilized for synthesizing nicotine (1) 20, 1,3,3-trimethyl-2-pyrrolidinone was added to an ethereal solution of 3-pyridyl-lithium at -78°; isolation gave the iminium salt 22a which was not purified or characterized. The sample was divided into two portions, and reduction with NaBH₄ in one case and NaBD₄ in the other gave 7 and 8 in 17 and 16% overall yield, respectively. Complete characterization of 7 and 8 is given
in Table III and the Experimental Section; however, the following points need emphasis for utilization in further discussion. In the nmr of 7 and 8 important resonances occur at δ 0.64 (s, 3H) and 1.08 (s, 3H). The assignment of the singlet at δ 0.64 to the cis-methyl in 7 and 8 follows from an examination of molecular models which indicate that the cis-methyl, in the most stable confirmation, is in the shielding cone of the pyridine ring; the trans-methyl in 7 and 8 is in the deshielding cone and occurs at significantly lower field, δ 1.08.

In an analogous manner, trans-3'-methylnicotine (3) and cis-3'-methylnicotine (5) were synthesized by sodium borohydride reduction of the iminium salt 22b in 10 and 4% overall yield, respectively. By means of preparative glpc, 3 and 5 were separated and characterized as summarized in Table III and the Experimental Section. The most significant features arise from an examination of the nmr spectra of 3 and 5 along with that of nicotine (1) and nicotine-5',5'-d2 (23).

trans-3'-Methylnicotine (3) was assigned trans stereochemistry with respect to the methyl group and the pyridine ring on the basis of the methyl doublet occurring at δ 0.97 in analogy with the assignment for methyl groups in 7 and 8. In a similar manner, cis-3'-methylnicotine (5) was assigned cis stereochemistry since its methyl resonance was centered at δ 0.55 as a doublet.

On the basis of the nmr spectra of 1, 7, 8 and 23, a very interesting difference in the shift of the C-2' hydrogen in 3 and 5 can also be noted. The nmr of nicotine (1) has a
Table III. NMR, Mass Spectral, and Gas Chromatographic Data for Nicotine and its Pyrrolidine Ring Analogs.

<table>
<thead>
<tr>
<th>Compound 3-Pyridyl-</th>
<th>NMR, δ&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Assignment of NMR Resonances</th>
<th>Mass spectrum m/e&lt;sup&gt;b&lt;/sup&gt; (rel. abund)</th>
<th>Glpc Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>1.6-2.6 (m, 5H)</td>
<td>C-3&lt;sup&gt;'&lt;/sup&gt;' (2H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-4&lt;sup&gt;'&lt;/sup&gt;' (2H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-5&lt;sup&gt;'&lt;/sup&gt;' (1H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.17 (s, 3H)</td>
<td>N-CH₃</td>
<td>162 (36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2 (m, 2H)</td>
<td>C-2&lt;sup&gt;'&lt;/sup&gt;' (1H),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-5&lt;sup&gt;'&lt;/sup&gt;' (1H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.21 (m, 1H)</td>
<td>C-5</td>
<td>133 (60)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.68 (m, 1H)</td>
<td>C-4</td>
<td>119 (19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.43 (m, 1H)</td>
<td>C-2, C-6</td>
<td>84 (100)</td>
<td></td>
</tr>
<tr>
<td>3&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.97 (d, J=6.3 Hz, 3H)</td>
<td>C-3&lt;sup&gt;'&lt;/sup&gt;'-CH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4-2.4 (m, 4H)</td>
<td>C-3&lt;sup&gt;'&lt;/sup&gt;' (1H),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-4&lt;sup&gt;'&lt;/sup&gt;' (2H),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-5&lt;sup&gt;'&lt;/sup&gt;' (1H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.10 (s, 3H)</td>
<td>N-CH₃</td>
<td>176 (37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.54 (d, J=7.5 Hz, 1H)</td>
<td>C-2&lt;sup&gt;'&lt;/sup&gt;'</td>
<td>134 (30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2 (m, 1H)</td>
<td>C-5</td>
<td>133 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.17 (m, 1H)</td>
<td>C-5</td>
<td>119 (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.60 (m, 1H)</td>
<td>C-4</td>
<td>98 (73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.40 (m, 2H)</td>
<td>C-2, C-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.55 (d, J=6.3 Hz, 3H)</td>
<td>C-3&lt;sup&gt;'&lt;/sup&gt;'-CH₃</td>
<td>176 (33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4-2.4 (m, 4H)</td>
<td>C-3&lt;sup&gt;'&lt;/sup&gt;' (1H),</td>
<td>134 (29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-4&lt;sup&gt;'&lt;/sup&gt;' (2H),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-5&lt;sup&gt;'&lt;/sup&gt;' (1H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.14 (s, 3H)</td>
<td>N-CH₃</td>
<td>133 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2 (m, 2H)</td>
<td>C-2&lt;sup&gt;'&lt;/sup&gt;' (1H),</td>
<td>119 (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-5&lt;sup&gt;'&lt;/sup&gt;' (1H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.15 (m, 1H)</td>
<td>C-5</td>
<td>98 (64)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.58 (m, 1H)</td>
<td>C-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.38 (m, 2H)</td>
<td>C-2, C-6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
$\text{CH}_3 \quad \text{CH}_3$

$\text{CH}_3$

$\text{N}$

0.64 (s, 3H) \quad \text{C-3'}-\text{CH}_3 (\text{cis}) \quad 190 (15)

1.08 (s, 3H) \quad \text{C-3'}-\text{CH}_3 (\text{trans}) \quad 134 (48) \quad 26.2

1.64 (m, 2H) \quad \text{C-4'} \quad 133 (100)

2.13 (s, 3H) \quad \text{N-CH}_3 \quad 119 (6)

2.43 (m, 1H) \quad \text{C-5'} \quad 112 (7)

2.88 (s, 1H) \quad \text{C-2'}

3.21 (m, 1H) \quad \text{C-5'}

7.18 (m, 1H) \quad \text{C-5}

7.56 (m, 1H) \quad \text{C-4}

8.41 (m, 2H) \quad \text{C-2, C-6}

$\text{CH}_3 \quad \text{CH}_3$

$\text{N}$

Same as 7 with resonance at 2.88 (s, 1H) absent

191 (18) \quad 26.2

135 (46) \quad 134 (100)

120 (4) \quad 113 (8)

$\text{CH}_3 \quad \text{CH}_3$

$\text{N}$

$\text{CH}_3$

$\text{D}$

$\text{a}$ In CCl$_4$ with TMS as the internal standard. $\text{b}$ At 70 eV.

$\text{c}$ G1pc was carried on a column of 10% KOH, 10% polybutylene glycol on 60/80 firebrick (column length: 15' x 1/4''; column temperature: 182°, flow rate: 100 ml He/min). $\text{d}$ For the reported mass spectrum and partially assigned nmr of 1, see references 22 and 23, respectively. The nmr spectrum of 1 reported above was obtained by us.
multiplet integrating for two hydrogens centered at δ 3.2.
Since 23 also shows a multiplet at 3.2 but integrating for
only one H, this resonance can be assigned in 1 to the C-2'
hydrogen and to one of the C-5' hydrogens. The large
difference (> 0.8) in the shifts of the two hydrogens is no
doubt due to deshielding by the lone pair electrons of the
pyrrolidine ring nitrogen. In the nmr spectrum of 7, the
multiplet at δ 3.21 integrates for one H, corresponding to
one of the C-5' hydrogens. The C-2' hydrogen has been
shifted upfield to δ 2.88 as confirmed by the absence of
this singlet in the spectrum of 3',3'-dimethylnicotine-2'-d
(8). The nmr spectrum of cis-3'-methylnicotine (5) shows a
multiplet at δ 3.2 integrating for two H's, assigned to the
C-2' hydrogen and one of the C-5' hydrogens in analogy to the
spectrum of 1. In marked contrast, the nmr spectrum of
trans-3'-methylnicotine (3) has a multiplet at δ 3.2 which
integrates for only one H while a doublet (J=7.5 Hz, 1H)
is present at δ 2.54 and is assigned to the C-2' hydrogen
in analogy with the spectrum of 7.

With reference to the spectra of 5, 7, and 8, the large
shift of the C-2' hydrogen in 3 is attributed primarily to
shielding by the trans methyl group rather than confirmational
influences. Finally, it should be noted that coupling constant
(J=7.5 Hz) observed for the C-2' hydrogen in 3 is consistent
with the assigned trans stereochemistry.

Characterization of the Biosynthetic Nicotine Analogs.
The characterization of the biosynthetic product obtained
(6.4-13.8% yield) from the administration of 1,3-dimethyl-1-
pyrrolinium-3-\(^{14}\)C\(\text{H}_3\) chloride as trans-3'-methylnicotine (3)
has been established. High resolution mass spectroscopy
established the molecular formula as \(\text{C}_{11}\text{H}_{16}\text{N}_2\), \(m/e\ 176\)
(calcd: 176.1313; found: 176.1313) and \(\text{C}_6\text{H}_{12}\text{N}\), \(m/e\ 98\) (calcd:
98.0970; found: 98.0974) for the 1,3-dimethyl-1-pyrrolinium
fragment formed by a cleavage. The nmr, mass spectrum, and
glpc retention time of biosynthetic 3 are identical to those
of synthetic 3 (see Table III); these comparisons eliminate
alternative structures such as 4'-methylnicotine from con-
sideration. The specific activity of biosynthetic 3 was
determined by a combination of uv absorption and liquid
scintillation counting to be \(2.76 \times 10^7\) dpm/mmol, in excellent
agreement with its precursor 4 (sp. act. \(2.74 \times 10^7\) dpm/mmol). The
ultraviolet spectrum of biosynthetic 4 showed \(<\text{C}_2\text{H}_5\text{OH}\> 261\ nm\ as
\(\text{max}\) expected for a derivative of nicotine. 25

Biogenetically, trans-3'-methylnicotine (3) would be
expected to have the same absolute configuration at C-2'
as nicotine (1) which has been assigned the S configuration
with reference to L-proline, \(\text{L-serine},\ 26\) \text{and optical rotary}
dispersion measurements. 28 The CD curve of biosynthetic 3
(in \(95\%\ \text{C}_2\text{H}_5\text{OH}\)) gave a molecular ellipticity \([\theta]\) at 260 nm
of +22,800 (peak); \(\text{I}\) showed a \([\theta]_{270} -7090\) (trough) in addition
to \([\theta]_{261} +24,800\) (peak). Although \(\text{I}\) showed a weaker negative
Cotton effect at 273 nm in the ORD, \(\text{I}\) this absorption was
absent in both the CD and ORD of biosynthetic 3. On the basis
of the CD curve of biosynthetic 3, the S configuration is
assigned at the 2'-carbon. In addition, as a result of the
nmr spectral differences between cis- (5) and trans-3'-methyl-
nicotine (3) as discussed previously, the absolute configuration
at the 3'-carbon of biosynthetic 3 can also be assigned the
S chirality. Clearly, only one of the four possible diastereomers
was formed biosynthetically from precursor 4.

The product (6) arising from the administration of
1,2-dimethyl-1-pyrrolinium-2-14CH₃ chloride (9b) to N. glutinosa
was characterized solely on the basis of mass spectroscopy
due to the low incorporation (0.04%) of 9b. The mass spectrum
of 6 and 1 in a ratio of 1 to 130 showed m/e 176 and 98 in
addition to the normal mass spectrum of nicotine. High
resolution mass spectroscopy established a molecular formula
of C₁₁H₁₆N₂ for m/e 176 (calcd: 176.1313; found: 176.1313)
and C₆H₁₂N for m/e 98 (calcd: 98.0970; found: 98.0971) in
agreement with formulation of the biosynthetic product as 6.
No additional characterization was possible due to the small
amount of material available.

Administration of 1,3,3-trimethyl-1-pyrrolinium-3,3-14CH₃
chloride (10b) to the plants, subsequent isolation, and
preparative glpc gave 3',3'-dimethylnicotine (7) in 0.77% yield.
The characterization of biosynthetic 7 was by direct com-
parison with synthetic 7. Synthetic and biosynthetic 7 were
identical in glpc retention time (established by co-injection)
and mass spectrally. The CD curve of 7 (in 95% C₂H₅OH) gave
molecular ellipticities [θ] of +1950 (peak) and +2100 (peak)
at 263 and 270 nm, respectively. The large decrease in the
molecular ellipticity at 263 nm makes assignment of the S configuration of the 2'-carbon of \( \lambda \) tenuous although, biogenetically, the S configuration might be expected. The possibility that the changes observed in the CD curve of \( \lambda \) are due to the presence of an unequal mixture of enantiomers cannot be eliminated at this time.

**Conclusions.** The present work has shown that the enzyme system which catalyzes the condensation of 1-methyl-1- pyrrolinium salt with a 1,6-dihydronicotinic acid derivative is not completely specific. Furthermore, its specificity has been partially defined by the present experiments. The great differences observed in the efficiency of incorporation of the three substituted precursors examined can be rationalized in a consistent manner on the basis of differences in steric hindrance in the condensation reaction with a 1,6-dihydr nicotine acid derivative. The present experiments, therefore, furnish addition support for this hypothesized step in nicotine biosynthesis. Alternately, the differences in the efficiency of incorporation of 4, 9b, and 10b may reflect differences in the metabolism of the precursors in vivo; however, we regard possible metabolic differences to be of secondary importance. Finally, it seems possible that a single enzyme system might produce the four common Nicotiana alkaloids, nicotine (1), nornicotine (24), anabasine (25), and anatabine (26), by similar condensations when provided with proper substrates.

The present approach also has broad potential applications
for the preparation of analogs of biologically active natural products since, in general, it is easier to synthesize a substituted precursor than to carry out a total synthesis of an analog of a complex natural product. Additional experiments are planned with *Nicotiana* and other species in order to examine the generality of this latter concept.
EXPERIMENTAL SECTION

trans-3'-Methylnicotine (3) and cis-3'-Methylnicotine (5). The method of preparation of 5 and 3 is exactly as described for the synthesis of 7 below with the following exceptions: (a) 5 mmoles (565 mg) of 1,3-dimethyl-2-pyrrolidinone were used in place of 18 and (b) all of the crude iminium salt 22b was reduced with NaBH₄. Isolation as described for 7 and preparative glpc gave 86 mg (10%) of 3 and 36 mg (4%) of 5.


3',3'-Dimethylnicotine (7). Under a nitrogen atmosphere was placed 50 ml of anhydrous diethyl ether. After adding 5 mmoles (790 mg) of 3-bromopyridine, the reaction mixture was cooled to -78°, and 3.1 ml (5 mmol) of n-butyllithium in hexane was added, followed by stirring for 20 minutes at -78° then addition of 5 mmoles (135 mg) of 1,3,3-trimethyl-2-pyrrolidinone (18). After stirring at -78° for 5 hours and room temperature for 13 hours, 20 ml of 6N NaOH was added, the ether layer was separated, and the aqueous phase extracted with ether (2 x 30 ml). The combined ethereal solutions were then extracted with 30 ml of 10% HCl and evaporation of the aqueous solution in vacuo gave the crude iminium salt 22a.

An aliquot (40%) of this iminium salt was dissolved in 10 ml of H₂O, sodium borohydride was added until the solution reached pH 8, and it was allowed to stand alkaline at room temperature for 30 minutes. Excess borohydride was destroyed by acidification with 10% HCl, and the resulting acidic
solution was made alkaline with 6N NaOH to pH 11. After extracting with methylene chloride (3 x 25 ml), drying the resulting methylene chloride solution over K$_2$CO$_3$, and filtering, concentration in vacuo to give the crude nicotine analog. Preparative glpc gave 64 mg (17%) of pure 3',3'-dimethylnicotine (7).

Mol. Form.: Calcd. for C$_{12}$H$_{18}$N$_2$: 190.1469. Found: 190.1449.

3',3'-dimethylnicotine-2'-d (8). The iminium salt 22a, not utilized in the preparation of 7 was used to prepare 8. The reduction and isolation were carried out as described for 7, except that NaBD$_4$ in D$_2$O solution was used instead of NaBH$_4$ in H$_2$O. Preparative glpc of the crude nicotine analog gave 90 mg (16%) of pure 8. The nmr indicated the presence of 93% of the 2'-deutero species.

Mol. Form.: Calcd for C$_{12}$H$_{17}$DN$_2$: 191.1533. Found: 191.1531.

1,2-Dimethyl-1-pyrrolinium-2-$^{14}$CH$_3$. perchlorate (9a). In a flask equipped with two dropping funnels, a condenser, stir bar, and nitrogen sweep was placed 50 mmoles (1.22 g) of magnesium and 100 ml of anhyd. diethyl ether. Then 0.25 millicuries of methyl-$^{14}$C iodide (9.9 mg) and 50 mmoles (7.1 g) of methyl iodide dissolved in 25 ml of ether was added slowly with stirring. After nearly all the magnesium had dissolved, 37.5 mmoles (3.72 g) of 1-methyl-2-pyrrolidinone in 25 ml of diethyl ether was added over 30 minutes, the solution was allowed to stand 20 hours at room temperature,
and 100 ml of 6N NaOH was added. The ether layer was removed, the aqueous phase was extracted with diethyl ether (6 x 50 ml), the combined ethereal solutions were extracted with 10% hydrochloric acid (3 x 50 ml), and the aqueous solution was evaporated in vacuo at 40° to give the crude pyrrolinium salt. This residue was dissolved in 50 ml of 3N NaOH, which was extracted with methylene chloride (4 x 25 ml). The methylene chloride extracts were added to 200 ml of absolute ethanol and 70% aqueous perchloric acid was added until the solution was slightly acidic (pH 3). Concentration in vacuo to 150 ml and cooling to 0° resulted in a precipitate which was dried at 10° for 18 hours giving 2.62 g (27%) of 9a; mp (dec.) 225-30° (lit. 10,11,12 mp 238°, 235-36°, 239-40°); 

nmr (D₂O) 4.17 (t, 2H), 3.46 (s, 3H), 3.23 (t, 2H), 2.44 (s, 3H), 2.20 (m, 2H); tlc (EtOH:0.1N HCl = 2:1; I₂ detection) one spot at Rₚ = 0.27; tlc (n-BuOH:HOAc:H₂O = 4:1:5; I₂ detection) one spot at Rₚ 0.10.

Anal. Calcd for C₆H₁₂ClN0₄: C, 36.5; H, 6.1; N, 7.1.

Found: C, 36.2; H, 6.1; N, 7.2.

1,2-Dimethyl-1-pyrroline-2-CH₃ Chloride (9b).

Approximately 8 mmoles of 9a was dissolved in 50 ml of 3N NaOH and the alkaline solution was extracted with methylene chloride (3 x 25 ml). The combined extracts were shaken with 25 ml of 10% HCl, and the aqueous solution was evaporated in vacuo. The residue was dissolved in 100 ml of distilled water prior to administering aliquots to N. glutinosa plants.

1,3,3-Trimethyl-1-pyrroline-3,3-CH₃ Perchlorate (10a).
To 25 ml of anhydrous diethyl ether was added 20 mmol of 1,3,3-trimethyl-1-pyrroolidinone-3,3-\textsuperscript{14}CH\textsubscript{3} and 6.75 ml (20 mmol) of lithium aluminum hydride in ether (0.74 mmol/ml). After refluxing for one hour, the reaction mixture was cooled, 50 ml of ether and 50 ml of 3N NaOH were added, the ether layer was removed, and the aqueous solution was extracted with ether (6 x 30 ml). The combined ether extracts were washed with 10% HCl (4 x 25 ml) and the aqueous solution was concentrated in vacuo at 40° to give a mixture of 10b (66%) and 1,3,3-trimethylpyrrolidin-3,3-\textsuperscript{14}CH\textsubscript{3} hydrochloride (19) (34%) as determined by nmr; tlc (n-BuOH:H\textsubscript{2}O:HOAc [4:5:1]): 19 at RF 0.14 and 10b at RF 0.10; tlc (EtOH:0.1N HCl [2:1]): 19 at RF 0.51 and 10b at RF 0.37.

The mixture of 10b and 19 was dissolved in 50 ml of 6N NaOH and extracted with methylene chloride (4 x 25 ml). The methylene chloride extracts were added to 150 ml of absolute ethanol, 70% aqueous perchloric acid was added until the ethanolic solution became acidic (pH 3), the methylene chloride was removed in vacuo, and the ethanolic solution was cooled to -10°. The resulting precipitate was removed and dried to give 1.14 g (27%) of 10a; mp 110-12°; nmr (D\textsubscript{2}O) 8:4.24 (s, 1H), 4.24 (t, 2H), 3.58 (s, 3H), 2.19 (t, 2H), 1.36 (s, 6H).

**Anal.** Calcd. for C\textsubscript{7}H\textsubscript{14}ClNO\textsubscript{4}: C, 39.7; H, 6.7; N, 6.6.

Found: C, 39.4; H, 6.7; N, 6.5.

1,3,3-Trimethyl-1-pyrroolidinium-3,3-\textsuperscript{14}CH\textsubscript{3} Chloride (10b) was obtained from the corresponding perchlorate 10a by the procedure given above for obtaining the chloride 9b from the.
perchlorate 9a.

3-Ethoxycarbonyl-1-methyl-2-pyrrolidinone (12). A mixture of 500 g of diethyl carbonate, 99.1 g (1 mole) of 1-methyl-2-pyrrolidinone and 2500 ml of anhydrous benzene was refluxed overnight under a water separator. The mixture was cooled to room temperature, 85.3 g of 56.3% NaH dispersion was slowly added, and reaction was allowed to proceed at room temperature for 15 minutes and then refluxed for 12 hours at which time hydrogen evolution had ceased. Cooling in an ice bath was followed by addition of 130 g of glacial acetic acid and 200 ml of benzene to decompose the excess sodium hydride and sodium enolate. The resulting slurry was filtered, the precipitate was washed with methylene chloride, and the filtrate and washings were concentrated in vacuo and then fractionally distilled at 119° and 1.8 mm to give the desired product contaminated with a small amount of mineral oil. Column chromatography on silica gel using benzene and benzene:ethanol (1:1) as eluants followed by redistillation gave 70.4 g (41.2%) of 12; ir (thin film) 1680 (amide C=O) and 1740 cm⁻¹ (ester C=O); nmr (neat) 4.17 (q, 2H), 3.42 (m, 3H), 2.83 (s, 3H), 2.35 (m, 2H), 1.27 (t, 3H).

Anal. Calcd for C₁₆H₁₃NO₃: C, 56.1; H, 7.7; N, 8.2.

Found: C, 55.9; H, 7.7; N, 8.3.

5-Ethoxycarbonyl-1,3-dimethyl-2-pyrrolidinone-3⁻¹⁴CH₃ (15).

Petroleum ether was added to 1.71 g (40 mmoles) of 56% sodium hydride dispersion and then drained, leaving sodium hydride free of mineral oil. A solution of 5.13 g (30 mmoles) of 3-
ethoxycarbonyl-1-methyl-2-pyrrolidinone (12) and 165 ml of
tetrahydrofuran was added and stirred with the sodium hydride
for two hours. Then 2.18 ml (35 mmole) of methyl-\(^{14}\)C iodide was added, the mixture was stirred overnight, and the
solvent was removed in vacuo. The product was extracted
from the sodium salts with benzene (4 x 25 ml), and distillation
at 105-09° and 1.4 mm gave 3.73 g (66%) of 13; ir (thin film)
1680 (amide C=O) and 1740 cm\(^{-1}\) (ester C=O); nmr (CDCl\(_3\)) 4.17
(q, 2H), 3.40 (m, 2H), 2.84 (s, 3H), 1.7-2.5 (m, 2H), 1.27 (m,
6H); glpc on 30% QF-1 on chromosorb P (5' x 1/4''; 100 ml/min;
146°) gave one peak, retention time 6.4 min (starting material
12, 8.2 min retention time).

Anal. Calcd. for C\(_9\)H\(_{15}\)NO\(_3\): C, 58.4; H, 8.2; N, 7.6.
Found: C, 58.3; H, 8.0; N, 7.5.

3-Carboxy-1,3-dimethyl-2-pyrrolidinone-3-\(^{14}\)CH\(_3\) (14).
3-Carboxy-1,3-dimethyl-2-pyrrolidinone-3-\(^{14}\)CH\(_3\), 1.85 g
(10 mmole), and 25 ml of 10% NaOH were stirred at room
temperature for 16 hours. The reaction mixture was adjusted
to pH 1 with concentrated hydrochloric acid and continuously
extracted with methylene chloride for 90 hours. Removal of
the solvent in vacuo gave 1.57 g (100%) of acid 14, melting
at 142-44° (dec) after recrystallization from ethyl acetate;
nmr (CDCl\(_3\)) 10.91 (s, 1H), 3.42 (m, 2H), 2.90 (s, 3H), 1.8-2.6
(m, 2H), 1.42 (s, 3H).

Anal. Calcd. for C\(_7\)H\(_{11}\)NO\(_3\): C, 53.5; H, 7.1; N, 8.9.
Found: C, 53.5; H, 7.2; N, 8.8.

1,3-Dimethyl-2-pyrrolidinone-3-\(^{14}\)CH\(_3\) (15). The acid 14,
1.16 g (7.4 mmols) was heated at 150-60° until decarboxylation was complete to give 836 mg (100%) of 15; nmr (CDCl₃) 3.25 (m, 2H), 2.80 (s, 3H), 1.5-2.6 (m, 3H), 1.18 (d, 3H).

Anal. Calcd. for C₆H₁₁NO: C, 63.7; H, 9.8; N, 12.4.
Found: C, 63.6; H, 10.0; N, 12.3.

Lithium Aluminum Hydride Reduction of 1,3-Dimethyl-2-
pyrrolidinone-3-¹⁴CH₃ (15). The acid 14, 3.144 g (20 mmols) was heated at 150-60° until the evolution of carbon dioxide had ceased. After cooling, 25 ml of anhydrous ether was added followed by 5 ml of a 1.2M ethereal lithium aluminum hydride solution and the solution was refluxed for one hour.

Water (5 ml) was added after cooling, then 100 ml of 6N NaOH.

The ether layer was removed, the aqueous solution was extracted with ether (7 x 25 ml), the combined ethereal extracts were extracted with 10% HCl (6 x 25 ml), and aqueous acid solution was evaporated to dryness in vacuo. The residue was dissolved in 2 ml of D₂O and its nmr showed a doublet at 1.33 (3H), a singlet at 3.61 (3H), a triplet at 4.17 (2H), and a singlet at 8.6 (1H) assignable to 4; signals assignable to 16 occurred at 1.10 (overlapping doublets, 3H) and 2.92 (s, 3H). Thus, nmr analysis indicated that the product was a mixture of 4 (63%) and 16 (37%). Scintillation counting of an aliquot of the aqueous solution of the reduction products gave a yield of 92%; tlc [I₂ detection, EtOH:0.1N HCl (2:1)] gave 16 and 4 at Rₚ's of 0.54 and 0.41, respectively; tlc [I₂ detection, n-BuOH:HOAc:H₂O (4:1:5)] gave, after two elutions, 16 and 4 at Rₚ's of 0.45 and 0.33, respectively.
1. 3-Dimethylpyrrolidine-3-\( ^{14}\)CH\( _3 \) Hydrochloride (16).

In a Parr hydrogenation bottle was placed 3 mmoles of a mixture of 4 (63%) and 16 (37%) in 50 ml of H\( _2 \)O and 50 mg of 10% Pd/C was added. After hydrogenation at 40 psi of hydrogen for 18 hours, the solution was filtered through celite, and the filter pad was washed with 100 ml of hot water. The solvent was removed in vacuo at 40° to give a 97% yield of 16 by radioactive assay; nmr (D\( _2 \)O) 3.0-3.9 (m, 4H), 2.92 (s, 3H), 1.6-2.8 (m, 3H), 1.10 (overlapping doublets, 3H); tlc [I\( _2 \) detection, EtOH:0.1N HCl (2:1)] one spot at R\( _F \) 0.53; tlc [I\( _2 \) detection, n-BuOH:HOAc:H\( _2 \)O (4:1:5)] one spot at R\( _F \) 0.47 after two elutions.

A portion of 16 was converted to the free base to which was added picric acid. The picrate was crystallized from isopropanol, mp 180-83° (lit.\( ^{12} \) mp 183-84°).

Anal. Calcd for C\( _{12} \)H\( _{16} \)N\( _4 \)O\( _7 \): C, 43.9; H, 4.9; N, 17.1.

Found: C, 44.2; H, 4.9; N, 17.1.

Separation of 1,3-Dimethyl-1-pyrrolinium-3-\( ^{14}\)CH\( _3 \) Chloride (4) and 1,3-Dimethylpyrrolidine-3-\( ^{14}\)CH\( _3 \) Hydrochloride (16).

A column (5 x 64 cm) was prepared using 550 g of silica gel slurred in EtOH:0.1N HCl (2:1), the eluting solvent. Four mmoles of a mixture of 4 (63%) and 16 (37%) was applied to the column in 25 ml of the eluting solvent, using two 25-ml portions of the eluting solvent for rinsing the compounds onto the column. Fractions of approximately 50 ml were collected at a flow rate of approximately 50 ml/hr and the chromatography was followed by scintillation counting; 200 \( \mu \)l ±
10 µl of each fraction was dissolved in 15 ml of dioxane scintillation counting solution. The pyrrolidine 16 was eluted in fractions 18-26 and the pyrrolinium salt 4 in fractions 26-50. Fractions 27-50 were combined, evaporated to dryness in vacuo at 40° and re-applied to the silica gel column as a slurry in EtOH:0.1N HCl (2:1), carrying out the chromatography in the same manner. The desired product 4 was eluted in fractions 39-71 which were combined, concentrated to approximately 200 ml in vacuo at 40°, and applied to a cation exchange column (AG-50W-X8; H+ form; 200-400 mesh; approximately 150 ml resin). The column was washed until neutral with distilled water; elution of 4 from the column followed with 1.5N HCl, the volume of each fraction being 75 ml and the flow rate 75 ml/hr., monitored by scintillation counting as described above. Pyrrolinium salt 4 was eluted in fraction 16-24; in addition, each of these fractions gave positive Dragendorf's test. Fractions 16-24 were combined and evaporated at 40° in vacuo to give 85% (2.08 mmol) of the 4 applied to the initial column; \( \lambda_{\text{max}}^{C_{2}H_{5}OH} = 262 \text{ nm} \) \( \varepsilon \quad 21 \); \( \lambda_{\text{max}}^{H_{2}O} = 249 \text{ nm} \) \( \varepsilon \quad 14 \); nmr (D_{2}O) 8.55 (s, 1H), 4.18 (t, 2H), 3.63 (singlet methyl superimposed on multiplet, 4H), 1.7-2.8 (m, 2H), 1.33 (d, 3H); tlc [I_{2} detection, EtOH:0.1N HCl (2:1)] one spot, \( R_{F} \) 0.43; tlc [I_{2} detection, n-BuOH:HOAc:H_{2}O (4:1:5)] one spot, \( R_{F} \) 0.31 after two elutions; mass spectrum (70 eV) \( m/e \) (rel intensity) 97 (54, M+HCl), 96 (100), 82 (34). Mol. Form. Calcd. for \( C_{6}H_{11}N(M^{+HCl}) \): 97.0891. Found: 97.0887.
1,3,3-Trimethyl-2-pyrrolidinone-3,3-\(^{14}\)CH\(_3\) \((18)\).  

To 500 ml of anhydrous diethyl ether and 220 mmoles (31 ml) of diisopropylamine (freshly distilled from BaO), cooled to \(-70^\circ\), was added 131 ml (210 mmoles) of n-butyllithium (1.6 M in hexane). Then 4.95 g (50 mmoles; 4.85 ml) of 1-methyl-2-pyrrolidinone was added, the solution was stirred for 15 minutes at \(-70^\circ\), and 13.8 ml (220 mmoles) of \(^{14}\)CH\(_3\)I was added. Stirring was continued at room temperature for 16 hours, 150 ml of H\(_2\)O was added, the ether layer was removed and evaporated in vacuo, and the residue was dissolved in 100 ml of H\(_2\)O. The combined aqueous solutions were continuously extracted with methylene chloride for 24 hours. Removal of the solvent and distillation of the residue at 95-97\(^\circ\) (27 mm) gave 4.79 g (78%) of 18; nmr (CDCl\(_3\)) 3.29 (t, 2H), 2.79 (s, 3H), 1.81 (t, 2H), 1.04 (s, 6H); glpc on 30% QF-1 on Chromosorb P (168\(^\circ\); 100 ml/min; 10' x 1/4") one peak, 9.0 min.

Anal. Calcd. for C\(_7\)H\(_{13}\)NO: C, 66.1; H, 10.3; N, 11.0. Found: C, 65.9; H, 10.2; N, 10.9.

2-Acetonyl-1,3-dimethylpyrroldine (17). To 6 mmoles of 1,3-dimethyl-1-pyrrolinium chloride and 6 ml of H\(_2\)O were added 35 ml of 1N NaOH, 10 ml of H\(_2\)O, 20 ml of ethanol, and 15 ml of ethyl acetoacetate. After stirring in the dark under nitrogen for 17 days, 50 ml of concentrated H\(_2\)SO\(_4\) was added. The reaction mixture was warmed on a steam bath for 5 hours, and concentrated to 5 ml in vacuo. The residue was dissolved in 50 ml of water and made strongly alkaline with 6N NaOH; the resulting aqueous solution was continuously extracted with
methylene chloride for 4 days. Removal of the solvent
gave 440 mg (47%) of 17; ir (thin film) 1730 cm⁻¹ (C=O); nmr
(CDCl₃) 2.7-3.7 (m, 5H), 2.69 (s, 3H), 1.4-2.5 (m, 3H),
2.30 (s, 3H), 1.12 (d, 3H); mass spectrum (70 eV) m/e (rel.
intensity) 155 (4, M⁺), 140 (2), 124 (28), 109 (21), 98 (100).
Warming 17 with a saturated ethanolic solution of picric acid
gave the picrate which was recrystallized from absolute
ethanol; mp 147-51 (dec); nmr (pyridine-d₅) 8.97 (s, 2H),
3.1-4.0 (m, 5H), 3.08 (s, 3H), 1.6-2.6 (m, 3H), 2.25 (s, 3H),
1.01 (overlapping doublets, 3H).
Anal. Calcd. for C₁₅H₂₀·₄O₈: C, 46.9; H, 5.3;
N, 14.6; O, 33.3. Found: C, 47.1; H, 5.3; N, 14.7.
Footnotes

(1) This investigation was supported in part by Grant No. MH 12797 from the National Institute of Mental Health, U. S. Public Health Service, and the U. S. Atomic Energy Commission.


(8) Purchased from New England Nuclear.


The possible incorporation of $\Delta$ as a chemical or biological transformation product cannot be completely excluded. However, such an hypothesis would require rapid and total absorption of the transformation product and would be extremely difficult to subject to experimental test. It would also require reconversion in the plant to a form capable of incorporation into the observed substituted nicotine, a most improbably event.

See footnote c in Table III for the conditions utilized with the 15' x 1/4" column. The 5' x 1/4" column at a temperature of 162° and flow rate of 100 ml/min gave a retention time of 9.3 minutes for nicotine (1). We are indebted to Dr. Neal Castagnoli, Department of Pharmaceutical Chemistry, University of California, San Francisco, for helpful discussions on nicotine nmr.
spectra and for the spectrum of 23, prepared by the
method of A. M. Duffield, H. Budzikiewicz and C. Djerassi,
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photometer, ultraviolet (uv) spectra were recorded on
a Cary Model 14 instrument, and nuclear magnetic
resonance (nmr) spectra were obtained with either a
Varian A-60 or T-60 spectrometer and are reported as δ
values downfield from internal tetramethylsilane or
sodium trimethylsilylpropanesulfonate (δ 0). Mass
spectra were obtained on a CEC 103C or 21-110B instrument.
All radioactive counting was performed on a Nuclear
Chicago Corporation Mark I Liquid Scintillation Computer (Model 6880) and are in disintegrations per minute (dpm) relative to an external standard, corrected for background. Counting was carried out with 15 ml aliquots of either a solution of 18.0 g of 2,5-diphenyloxazole (PPO), 0.4 g of p-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 4 ml of toluene or a solution of 18.0 g of PPO, 0.4 g of POPOP, 200 g of naphthalene, 1 ml of ethanol, 1.4 ml of toluene, and 1.6 ml of dioxane. Whenever necessary, 1 ml of NCS Solubilizer (Nuclear Chicago Corp.) was added to the liquid scintillation sample vial in order to insure complete solubility. All elemental analyses were performed by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley. CD and ORD spectra were run on a Cary 60 instrument. G1pc's were carried out on a Varian Aerograph A-90-P instrument. (30) K. Randerath, "Thin Layer Chromatography", Academic Press, New York, N.Y. 1963, p. 74.
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