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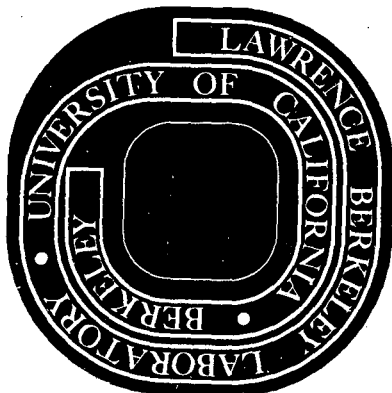
Melvin L. Rueppel and Henry Rapoport

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

3
4 Melvin L. Rueppel and Henry Rapoport

5
6 Contribution from the Department of Chemistry and Lawrence
7 Radiation Laboratory, University of California, Berkeley,
8 California, 94720.

9
10 Abstract: Several methyl derivatives of nicotine have
11 been biosynthesized using Nicotiana glutinosa plants and the
12 corresponding substituted pyrrolinium precursors. The
13 syntheses of the ¹⁴C-labeled pyrrolinium precursors, which
14 were utilized in the biosynthetic experiments, are also
15 described. For chromatographic and spectral comparisons,
16 authentic samples of some of the substituted nictines were also
17 synthesized. The stereochemistry and absolute configuration
18 of the biosynthesized nicotine analogs have been determined.
19 Incorporation results with 2- and 3-methyl substituted
20 pyrrolinium precursors allow some speculation on the specificity
21 and steric requirements of the enzyme(s) involved in the
22 latter stages of nicotine biosynthesis.

23 *****

24 The biosynthetic pathway of formation of nicotine (1)
25 in Nicotiana has been subject to a great deal of study. A
26 multitude of experiments have been carried out by means of
27 precursor feedings and short-term biosyntheses with ¹⁴CO₂;

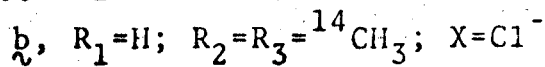
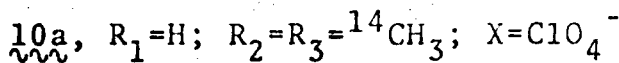
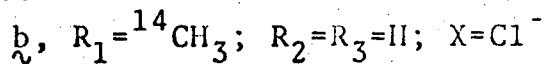
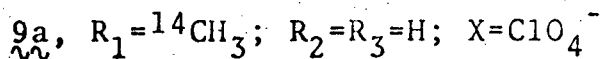
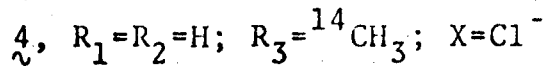
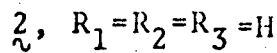
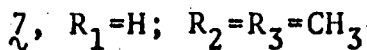
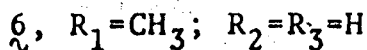
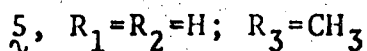
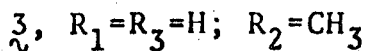
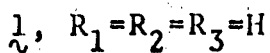
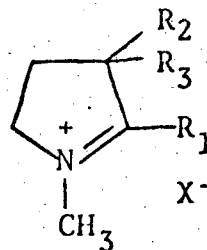
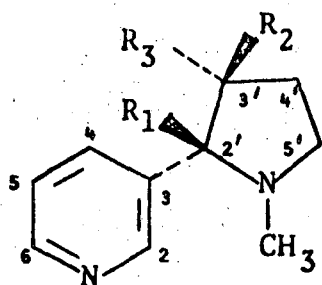
1 however, the precise biosynthetic pathway has yet to be
2 completely elucidated.² In conjunction with other biosynthetic
3 experiments with Nicotiana glutinosa, we became interested in
4 the possibility of biosynthesizing unnatural nicotine analogs
5 by using substituted precursors instead of the normal,
6 natural precursor.

7 The possibility of biosynthesizing unnatural nicotine
8 analogs using substituted natural precursors was interesting
9 for several reasons. First, the incorporation of an unnatural
10 precursor (i.e., a substituted natural precursor) into an
11 unnatural product (i.e., nicotine analog) had not been
12 previously reported in plants. Second, experiments with a
13 series of substituted precursors might define the specificity
14 of the enzyme system which catalyzes the biosynthesis of
15 nicotine from a 1-methyl-1-pyrrolinium salt $\overset{2}{\underset{\sim}{N}}$ and a nicotinic
16 acid derivative. Third, the formation of unnatural alkaloids
17 in vivo should be useful in the preparation of analogs of bio-
18 logically active natural products. Finally, since the unnatural
19 products possess a structural label in addition to the usual
20 radioactivity label, they should also be of great utility in the
21 study of metabolism and interrelationships among the various
22 alkaloids and other natural products in a given plant.

23 In a preliminary communication,³ we have reported the
24 incorporation of 1,3-dimethyl-1-pyrrolinium-3-¹⁴CH₃ chloride ($\overset{14}{\underset{\sim}{N}}$),
25 into the nicotine analog, 3'-methylnicotine ($\overset{3}{\underset{\sim}{N}}$). We now report
26 the full details for this preliminary communication and additional
27 related experiments concerning the formation of the nicotine

1 analogs, $\underset{\sim}{6}$ and $\underset{\sim}{7}$, from $\underset{\sim}{9b}$ and $\underset{\sim}{10b}$, respectively.

2 Precursor synthesis. In the present work, only derivatives
3 of the natural pyrrolidine ring precursor, 1-methyl-1-pyrrolinium
4 salt ($\underset{\sim}{2}$), have been examined as potential unnatural precursors
5 for analogs of nicotine. This choice was based on the fact



17
18 that $\underset{\sim}{2}$ has been reported to be a highly efficient precursor of
19 the pyrrolidine ring of nicotine.^{4,5} A priori, derivatives of
20 the pyridine ring precursor, nicotinic acid, could also have
21 been examined; however, nicotinic acid is a less efficient
22 precursor, probably due to its more wide spread metabolic
23 functions.⁶ Accordingly, it seemed reasonable to first con-
24 centrate on analogs of $\underset{\sim}{2}$.

25 The first candidate unnatural precursor examined was 1,3-
26 dimethyl-2-pyrrolinium-3-¹⁴CH₃ chloride ($\underset{\sim}{4}$). It was synthesized
27 by condensation of 1-methyl-2-pyrrolidinone ($\underset{\sim}{11}$) with diethyl

1 carbonate using sodium hydride as the base to give ester 12.

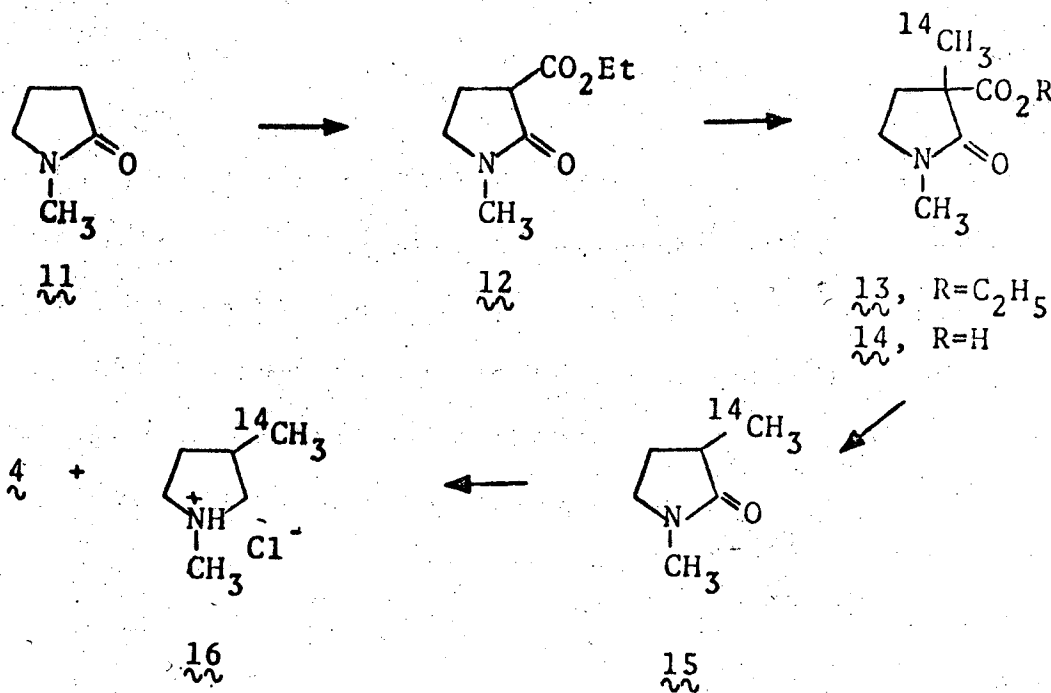
2 1,3-Dimethyl-3-carbethoxy-2-pyrrolidinone-3-¹⁴CH₃ (13)⁷

3 was obtained by alkylating the sodium enolate of 12 with methyl-
4 ¹⁴C iodide.⁸ The procedure utilized in the isolation of

5 3-methyl derivative 13 precluded the presence of unalkylated
6 compound 12; this was verified by glpc analysis. Hydrolysis

7 of the alkylated ester 13 (specific activity 2.71×10^7 dpm/mmol)
8 quantitatively gave the acid 14 (specific activity 2.68×10^7
9 dpm/mmol) which on decarboxylation gave 1,3-dimethyl-2-pyrroli-

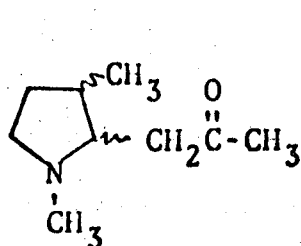
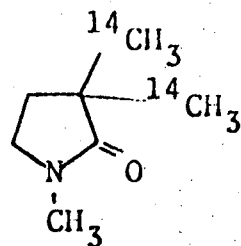
10 dinone (15).⁹ Stoichiometrically controlled reduction of 15



22 with lithium aluminum hydride gave in 92% yield a mixture of
23 pyrrolinium salt 4 (63%) and the pyrrolidine hydrochloride 16 (37%).

24 Chromatography on silica gel, followed by ion exchange gave
25 pure 4 in 40% overall yield from 12. The precursor 4 was

26 characterized spectrally, chromatographically, microanalytically
27 and by conversion to the hygrine derivative 17.

17
~18
~

7 The second candidate unnatural precursor (9b) also
 8 labeled with carbon-14, was synthesized as follows. Methyl-
 9 C¹⁴ iodide⁸ was converted to the corresponding Grignard reagent
 10 and subsequent addition of 1-methyl-2-pyrrolidinone (11)
 11 gave a mixture of 1,2-dimethyl-1-pyrrolinium-2-¹⁴CH₃ chloride
 10,11 (9b) and 1,2,2-trimethylpyrrolidine-2,2-¹⁴CH₃ hydrochloride.¹⁰⁻¹²
 12
 13 Conversion of the mixture to the corresponding free bases,
 14 followed by the addition of 70% perchloric acid gave the pure
 15 iminium perchlorate 9a (specific activity 9.57 x 10⁶ dpm/mmol)
 16 in an overall yield of 27%. Immediately prior to the actual
 17 feeding experiments, the perchlorate 9a was converted into the
 18 chloride 9b by ion exchange.

19 Synthesis of the third candidate unnatural precursor
 20 (10b) was also initiated with 1-methyl-2-pyrrolidinone (11).
 21 Alkylation of 11 with methyl-¹⁴C iodide⁸ in diethyl ether at
 22 -78° with lithium diisopropylamide as the base gave 1,3,3-
 13 trimethyl-2-pyrrolidinone-3,3-¹⁴CH₃ (18)^{9,13} in 78% yield.
 23 Controlled lithium aluminum hydride reduction of 18 (specific
 24 activity 8.63 x 10⁶ dpm/mmol) gave a mixture of 10b (66%) and
 25 1,3,3-trimethylpyrrolidine-3,3-¹⁴CH₃ hydrochloride (19; 34%).
 26 The pure perchlorate (specific activity 8.93 x 10⁶ dpm/mmol)

1 was obtained by the addition of perchloric acid to the correspond-
2 ing free base of 10a and 19. Prior to the feeding experiments
3 10a was reconverted into the chloride 10b.

4 Biosyntheses and Isolation. Each biosynthetic experiment
5 (Tables I and II) was carried out using four Nicotiana glutinosa
6 plants which were growing in an aerated hydroponic solution.¹⁴
7 The plants had been grown prior to the feeding experiment
8 as previously described.¹⁵ Appropriate precursor was added
9 daily in portions to the aerated hydroponic solution; the
10 rate of addition, age of plants, and the initial and final
11 weights of the plants are given in the footnotes to Table I.
12 The rate and amount of uptake of the precursors in experiments
13 1-4 were monitored continually by liquid scintillation
14 counting of aliquots of the hydroponic nutrient solution.
15 Particularly in experiments 3 and 4 in which an excess of the
16 appropriate precursor was constantly maintained in the hydro-
17 ponic solution, the rate and amount of precursor uptake was
18 directly proportional to the mass of the plants; that is,
19 as the mass of the plants increased by normal growth, a
20 corresponding increase in the rate and amount of precursor
21 was observed. The rate and amount of uptake was apparently
22 independent of the amount of precursor available in the hydro-
23 ponic solution. Finally, no harmful effects were noted in
24 plant growth although up to 40 mg of precursor was incorporated
25 daily.

26 Since the plants failed to completely absorb all the
27 radioactivity associated with each precursor from the hydroponic

1 solution, the residual nutrient solution was examined in order to
2 assess the stability of each precursor administered. In experi-
3 ments 3 and 4, greater than 95% of the radioactivity in the residual
4 nutrient was shown by nmr and tlc to be due to the presence of $9b$
5 and $10b$, respectively. In experiment 1, 90% of the total precursor
6 administered each day was absorbed into the plants in 24 hours.
7 The remaining 10% of the activity was due to chemical or biological
8 change of 4 and accumulated as the experiment proceeded. Since
9 these plants, and plants added to the nutrient solution after
10 removal of the previous plants, were incapable of absorbing the
11 transformation products of 4 , the observed incorporation most
12 probably is due to the uptake of 1,3-dimethyl-1-pyrrolinium-3- $^{14}CH_3$
13 chloride (4). Further support for the role of intact 4 as the
14 actual precursor is provided by the excellent agreement in specific
15 activities (see following) between 4 and the biosynthesized 3'-
16 methylnicotine (3).¹⁶

17 After each feeding experiment had proceeded for several days and
18 the desired amount of precursor had been incorporated, the plants
19 were fractionated as described previously¹⁵ to give the four fractions
20 indicated in Table I. The distribution of activity found in
21 these four fractions is of interest for several reasons. First,
22 essentially all ($100 \pm 3\%$) of the activity incorporated into the
23 plants in each experiment (with the exception of expt. 1 where
24 the activities of two fractions were not determined) has been accounted
25 for by these four fractions. Clearly no loss of activity has occurred
26 by metabolism of the administered precursors to respired $^{14}CO_2$.
27 Second, significant differences in metabolism of each of the
28 three precursors are indicated in the activity distributions.
29 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.

Expt.	Precursor	Activity (dpm x 10 ⁻⁶) in Fractions				
		Total plant ^a	Marc ^b	Acidic & neutral	Alkaloidal (basic)	Residual aqueous
1 ^c	4 ~	6.58	d	d	1.85	2.15
2 ^e	4 ~	31.40	2.52	4.00	14.30	9.95
3 ^f	9b ~	21.60	0.76	1.32	15.10	4.85
4 ^g	10b ~	12.30	0.16	0.21	10.90	0.59

^aBased on total activity fed minus activity remaining in nutrient solution. ^bThe activity present in the marc was determined by combusting an aliquot using a modification of the method of Kalberer and Rutschman¹⁷. ^cAdministered 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. ^dNot determined. ^eAdministered 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. ^fAdministered 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. ^gAdministered 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 $\overset{\sim}{4}$, $\overset{\sim}{9b}$, and $\overset{\sim}{10b}$ to Nicotiana glutinosa^a and Incorporation into Nicotine Analogs $\overset{\sim}{3}$, $\overset{\sim}{6}$, and $\overset{\sim}{7}$.

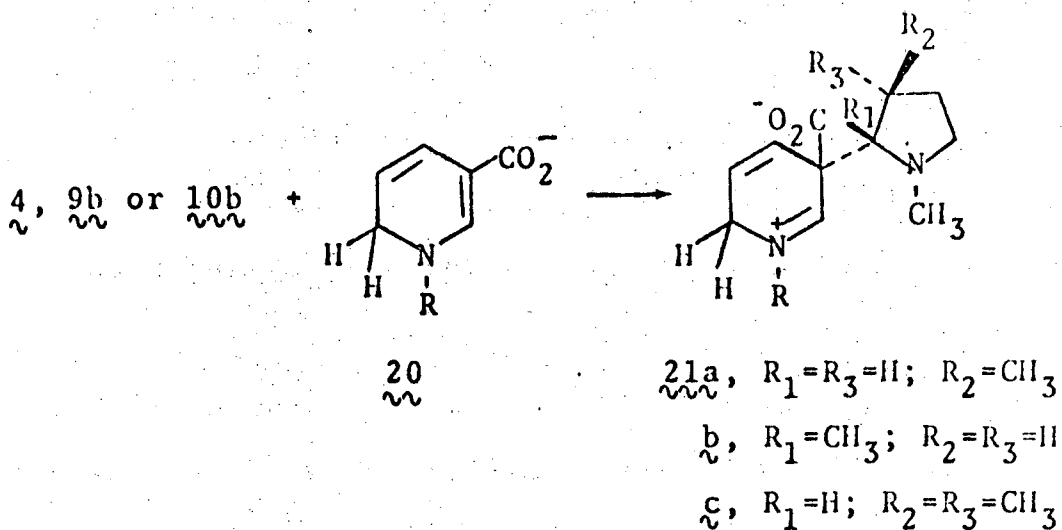
Expt.	Precursor fed	Incorporation wt. (mg)	act. (dpm)	Nicotine analog formed	Yield of analog dpm (%)
1	$\overset{\sim}{4}$	33	6.58×10^6	$\overset{\sim}{3}$	4.22×10^5 (6.4)
2	$\overset{\sim}{4}$	159	31.4×10^6	$\overset{\sim}{3}$	4.34×10^6 (13.8) ^b
3	$\overset{\sim}{9b}$	304	21.6×10^6	$\overset{\sim}{6}$	8.26×10^3 (0.04)
4	$\overset{\sim}{10b}$	202	12.3×10^6	$\overset{\sim}{7}$	9.50×10^4 (0.77) ^c

^aFor the preparation of the plants, see ref. 15. ^bUsing nicotine ($\overset{\sim}{1}$) as the standard, glpc analysis of the crude alkaloid fraction indicated the presence of 56.0 mg of $\overset{\sim}{1}$ and 21.6 mg (10.5%) of 3'-methylnicotine ($\overset{\sim}{3}$).

^cGlpc analysis of the crude alkaloid fraction indicated the presence of 17.0 mg of $\overset{\sim}{1}$ and 2.2 mg (0.8%) of 3',3'-dimethylnicotine ($\overset{\sim}{7}$).

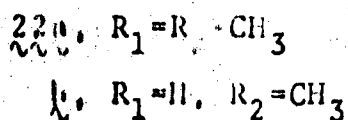
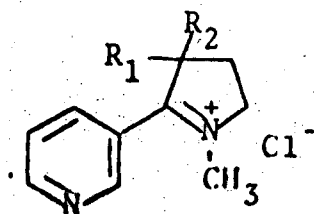
1 Table I should prove valuable in metabolism and precursor
2 feeding experiments. Although incorporations of the range
3 of .30 to 0.003% have been reported in biosynthetic experiments,
4 generally no attempt has been made to ascertain the fate of
5 the majority of the precursor administered. Undoubtedly,
6 significant metabolic and biosynthetic information could be
7 obtained in many precursor feeding experiments from such a
8 treatment.

9 The crude alkaloid fraction was analyzed by preparative
10 glpc on either a 15' x 1/4" column (expts. 1, 2, and 4) or
11 a 5' x 1/4" column (expt. 3) of 10% KOH, 10% polybutylene glycol
12 on 60/80 firebrick.¹⁸ In the latter case (expt. 3) no
13 fractionation of nicotine (1) and 2'-methylnicotine (6) was
14 attempted due to the presence of only approximately 150 μ g
15 of 6 in a total of 19.6 mg of 1. The yields of the three
16 analogs (3, 6, and 7) of nicotine are shown in Table II.
17 Relative incorporations of 1,3-dimethyl- (4), 1,2-dimethyl-
18 (9b), and 1,3,3-trimethyl-1-methyl pyrrolinium chloride (10b)
19 into the corresponding nicotine analogs (3, 6, and 7,
20 respectively) are in an approximate ratio of 360:1:20. The
21 differences in incorporation appear to be consistent with
22 the relative amount of steric hindrance expected in each case
23 in joining the substituted precursor with the hypothesized
24 1,6-dihydronicotinic acid derivative (20)¹⁹ to give the
25 intermediate 21a, b, or c, respectively. Oxidation decarboxylation
26 of 21 and subsequent loss of R affords the appropriate
27 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



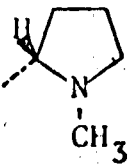
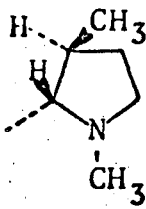
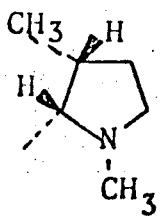
1 in Table III and the Experimental Section; however, the
 2 following points need emphasis for utilization in further
 3 discussion. In the nmr of $\underline{7}$ and $\underline{8}$ important resonances occur
 4 at δ 0.64 (s, 3H) and 1.08 (s, 3H). The assignment of the
 5 singlet at δ 0.64 to the cis-methyl in $\underline{7}$ and $\underline{8}$ follows from
 6 an examination of molecular models which indicate that the
 7 cis-methyl, in the most stable confirmation, is in the
 8 shielding cone of the pyridine ring; the trans-methyl in
 9 $\underline{7}$ and $\underline{8}$ is in the deshielding cone and occurs at significantly
 10 lower field, δ 1.08.

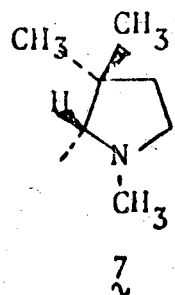
11 In an analogous manner, trans-3'-methylnicotine ($\underline{3}$) and
 12 cis-3'-methylnicotine ($\underline{5}$) were synthesized by sodium borohydride
 13 reduction of the iminium salt $\underline{22b}$ in 10 and 4% overall yield,
 14 respectively. By means of preparative glpc, $\underline{3}$ and $\underline{5}$ were
 15 separated and characterized as summarized in Table III and
 16 the Experimental Section. The most significant features arise
 17 from an examination of the nmr spectra of $\underline{3}$ and $\underline{5}$ along with
 18 that of nicotine ($\underline{1}$) and nicotine-5',5'-d₂ ($\underline{23}$).²¹

19 trans-3'-Methylnicotine ($\underline{3}$) was assigned trans stereo-
 20 chemistry with respect to the methyl group and the pyridine
 21 ring on the basis of the methyl doublet occurring at δ 0.97
 22 in analogy with the assignment for methyl groups in $\underline{7}$ and $\underline{8}$. In a
 23 similar manner, cis-3'-methylnicotine ($\underline{5}$) was assigned cis stereo-
 24 chemistry since its methyl resonance was centered at δ 0.55 as a doublet

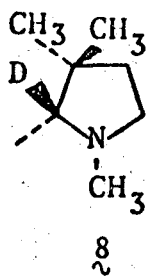
25 On the basis of the nmr spectra of $\underline{1}$, $\underline{7}$, $\underline{8}$ and $\underline{23}$, a
 26 very interesting difference in the shift of the C-2' hydrogen
 27 in $\underline{3}$ and $\underline{5}$ can also be noted. The nmr of nicotine ($\underline{1}$) has a

Table III. NMR, Mass Spectral, and Gas Chromatographic Data for Nicotine and its Pyrrolidine Ring Analogs.

Compound 3-Pyridyl-	NMR, δ^a	Assignment of NMR Resonances	Mass spectrum m/e ^b (rel. abund)	Gpc Retention time (min)
 1d	1.6-2.6 (m, 5H)	C-3' (2H) C-4' (2H) C-5' (1H)		20.6
	2.17 (s, 3H)	N-CH ₃	162 (36)	
	3.2 (m, 2H)	C-2' (1H), C-5' (1H)	134 (9)	
	7.21 (m, 1H)	C-5	133 (60)	
	7.68 (m, 1H)	C-4	119 (19)	
	8.43 (m, 1H)	C-2, C-6	84 (100)	
	 3	0.97 (d, J=6.3 Hz, 3H)	C-3'-CH ₃	
1.4-2.4 (m, 4H)		C-3' (1H), C-4' (2H), C-5' (1H)		22.4
2.10 (s, 3H)		N-CH ₃	176 (37)	
2.54 (d, J=7.5 Hz, 1H)		C-2'	134 (30)	
3.2 (m, 1H)		C-5' (1H)	133 (100)	
7.17 (m, 1H)		C-5	119 (9)	
7.60 (m, 1H)		C-4	98 (73)	
8.40 (m, 2H)		C-2, C-6		
 5		0.55 (d, J=6.3 Hz, 3H)	C-3'-CH ₃	176 (33)
	1.4-2.4 (m, 4H)	C-3' (1H), C-4' (2H), C-5' (1H)	134 (29)	
	2.14 (s, 3H)	N-CH ₃	133 (100)	
	3.2 (m, 2H)	C-2' (1H), C-5' (1H)	119 (12)	
	7.15 (m, 1H)	C-5	98 (64)	
	7.58 (m, 1H)	C-4		
	8.38 (m, 2H)	C-2, C-6		



0.64 (s, 3H)	C-3'-CH ₃ (cis)	190 (15)	
1.08 (s, 3H)	C-3'-CH ₃ (trans)	134 (48)	26.2
1.64 (m, 2H)	C-4'	133 (100)	
2.13 (s, 3H)	N-CH ₃	119 (6)	
2.43 (m, 1H)	C-5'	112 (7)	
2.88 (s, 1H)	C-2'		
3.21 (m, 1H)	C-5'		
7.18 (m, 1H)	C-5		
7.56 (m, 1H)	C-4		
8.41 (m, 2H)	C-2, C-6		



Same as 7 with resonance at 2.88 (s, 1H) absent		191 (18)	26.2
		135 (46)	
		134 (100)	
		120 (4)	
		113 (8)	

^aIn CCl₄ with TMS as the internal standard. ^bAt 70 eV.

^cGlpc 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).

^dFor 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.

1 multiplet integrating for two hydrogens centered at δ 3.2.
2 Since $\overset{\sim}{23}$ also shows a multiplet at 3.2 but integrating for
3 only one H, this resonance can be assigned in $\overset{\sim}{1}$ to the C-2'
4 hydrogen and to one of the C-5' hydrogens. The large
5 difference (> 0.8) in the shifts of the two hydrogens is no
6 doubt due to deshielding by the lone pair electrons of the
7 pyrrolidine ring nitrogen. In the nmr spectrum of $\overset{\sim}{7}$, the
8 multiplet at δ 3.21 integrates for one H, corresponding to
9 one of the C-5' hydrogens. The C-2' hydrogen has been
10 shifted upfield to δ 2.88 as confirmed by the absence of
11 this singlet in the spectrum of 3',3'-dimethylnicotine-2'-d
12 ($\overset{\sim}{8}$). The nmr spectrum of cis-3'-methylnicotine ($\overset{\sim}{5}$) shows a
13 multiplet at δ 3.2 integrating for two H's, assigned to the
14 C-2' hydrogen and one of the C-5' hydrogens in analogy to the
15 spectrum of $\overset{\sim}{1}$. In marked contrast, the nmr spectrum of
16 trans-3'-methylnicotine ($\overset{\sim}{3}$) has a multiplet at δ 3.2 which
17 integrates for only one H while a doublet ($J=7.5$ Hz, 1H)
18 is present at δ 2.54 and is assigned to the C-2' hydrogen
19 in analogy with the spectrum of $\overset{\sim}{7}$.

20 With reference to the spectra of $\overset{\sim}{5}$, $\overset{\sim}{7}$, and $\overset{\sim}{8}$, the large
21 shift of the C-2' hydrogen in $\overset{\sim}{3}$ is attributed primarily to
22 shielding by the trans methyl group rather than conformational
23 influences. Finally, it should be noted that coupling constant
24 ($J=7.5$ Hz) observed for the C-2' hydrogen in $\overset{\sim}{3}$ is consistent
25 with the assigned trans stereochemistry. ²⁴

26 Characterization of the Biosynthetic Nicotine Analogs.

27 The characterization of the biosynthetic product obtained

(6.4-13.8% yield) from the administration of 1,3-dimethyl-1-pyrrolinium-3-¹⁴CH₃ chloride as trans-3'-methylnicotine (3) has been established. High resolution mass spectroscopy established the molecular formula as C₁₁H₁₆N₂, m/e 176 (calcd: 176.1313; found: 176.1313) and C₆H₁₂N, m/e 98 (calcd: 98.0970; found: 98.0974) for the 1,3-dimethyl-1-pyrrolinium fragment formed by α 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 consideration. The specific activity of biosynthetic 3 was determined by a combination of uv absorption and liquid scintillation counting to be 2.76 x 10⁷ dpm/mmol, in excellent agreement with its precursor 4 (sp. act. 2.74 x 10⁷ dpm/mmol). The ultraviolet spectrum of biosynthetic 4 showed λ _{max} C₂H₅OH 261 nm as 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,²⁶ L-serine,²⁷ and optical rotary dispersion measurements.²⁸ The CD curve of biosynthetic 3 (in 95% C₂H₅OH) gave a molecular ellipticity [θ] at 260 nm of +22,800 (peak); 1 showed a [θ]₂₇₀ -7090 (trough) in addition to [θ]₂₆₁ +24,800 (peak). Although 1 showed a weaker negative Cotton effect at 273 nm in the ORD,²⁸ 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

1 assigned at the 2'-carbon. In addition, as a result of the
2 nmr spectral differences between cis- (5) and trans-3'-methyl-
3 nicotine (3) as discussed previously, the absolute configuration
4 at the 3'-carbon of biosynthetic 3 can also be assigned the
5 S chirality. Clearly, only one of the four possible diastereomers
6 was formed biosynthetically from precursor 4.

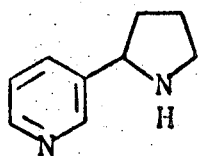
7 The product (6) arising from the administration of
8 1,2-dimethyl-1-pyrrolinium-2-¹⁴CH₃ chloride (9b) to N. glutinosa
9 was characterized solely on the basis of mass spectroscopy
10 due to the low incorporation (0.04%) of 9b. The mass spectrum
11 of 6 and 1 in a ratio of 1 to 130 showed m/e 176 and 98 in
12 addition to the normal mass spectrum of nicotine. High
13 resolution mass spectroscopy established a molecular formula
14 of C₁₁H₁₆N₂ for m/e 176 (calcd: 176.1313; found: 176.1313)
15 and C₆H₁₂N for m/e 98 (calcd: 98.0970; found: 98.0971) in
16 agreement with formulation of the biosynthetic product as 6.
17 No additional characterization was possible due to the small
18 amount of material available.

19 Administration of 1,3,3-trimethyl-1-pyrrolinium-3,3-¹⁴CH₃
20 chloride (10b) to the plants, subsequent isolation, and
21 preparative glpc gave 3',3'-dimethylnicotine (7) in 0.77% yield.
22 The characterization of biosynthetic 7 was by direct com-
23 parison with synthetic 7. Synthetic and biosynthetic 7 were
24 identical in glpc retention time (established by co-injection)
25 and mass spectrally. The CD curve of 7 (in 95% C₂H₅OH) gave
26 molecular ellipticities [θ] of +1950 (peak) and +2100 (peak)
27 at 263 and 270 nm, respectively. The large decrease in the

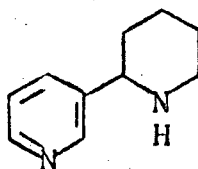
1 molecular ellipticity at 263 nm makes assignment of the S
2 configuration of the 2'-carbon of ζ tenuous although, bio-
3 genetically, the S configuration might be expected. The
4 possibility that the changes observed in the CD curve of ζ
5 are due to the presence of an unequal mixture of enantiomers
6 cannot be eliminated at this time.

7 Conclusions. The present work has shown that the enzyme
8 system which catalyzes the condensation of 1-methyl-1-
9 pyrrolinium salt with a 1,6-dihyronicotinic acid derivative
10 is not completely specific. Furthermore, its specificity
11 has been partially defined by the present experiments. The
12 great differences observed in the efficiency of incorporation
13 of the three substituted precursors examined can be rationalized
14 in a consistent manner on the basis of differences in steric
15 hindrance in the condensation reaction with a 1,6-dihydro
16 nicotinic acid derivative. The present experiments, therefore,
17 furnish additional support for this hypothesized step in
18 nicotine biosynthesis. Alternately, the differences in the
19 efficiency of incorporation of ζ , ζ b, and ζ b may reflect
20 differences in the metabolism of the precursors in vivo; how-
21 ever, we regard possible metabolic differences to be of secondary
22 importance. Finally, it seems possible that a single enzyme
23 system might produce the four common Nicotiana alkaloids,
24 nicotine (ζ), nornicotine (ζ), anabasine (ζ), and anatabine
25 (ζ), by similar condensations when provided with proper
26 substrates.

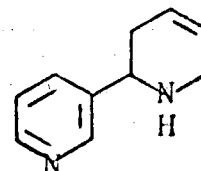
27 The present approach also has broad potential applications



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8 for the preparation of analogs of biologically active natural
9 products since, in general, it is easier to synthesize a
10 substituted precursor than to carry out a total synthesis of
11 an analog of a complex natural product. Additional experiments
12 are planned with Nicotiana and other species in order to
13 examine the generality of this latter concept.

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1 EXPERIMENTAL SECTION²⁹

2 trans-3'-Methylnicotine (3) and cis-3'-Methylnicotine
3 (5). The method of preparation of 5 and 3 is exactly as
4 described for the synthesis of 7 below with the following
5 exceptions: (a) 5 mmoles (565 mg) of 1,3-dimethyl-2-pyrrolidinone
6 were used in place of 18 and (b) all of the crude iminium
7 salt 22b was reduced with NaBH₄. Isolation as described for
8 7 and preparative glpc gave 86 mg (10%) of 3 and 36 mg (4%)
9 of 5.

10 Mol. Form.: Calcd. for C₁₁H₁₆N₂: 176.1313. Found: 176.1313.

11 3',3'-Dimethylnicotine (7). Under a nitrogen atmosphere
12 was placed 50 ml of anhydrous diethyl ether. After adding
13 5 mmoles (790 mg) of 3-bromopyridine, the reaction mixture
14 was cooled to -78°, and 3.1 ml (5 mmol) of n-butyllithium
15 in hexane was added, followed by stirring for 20 minutes at
16 -78° then addition of 5 mmoles (135 mg) of 1,3,3-trimethyl-2-
17 pyrrolidinone (18). After stirring at -78° for 5 hours and
18 room temperature for 13 hours, 20 ml of 6N NaOH was added,
19 the ether layer was separated, and the aqueous phase extracted
20 with ether (2 x 30 ml). The combined ethereal solutions were
21 then extracted with 30 ml of 10% HCl and evaporation of the
22 aqueous solution in vacuo gave the crude iminium salt 22a.
23 An aliquot (40%) of this iminium salt was dissolved in 10 ml
24 of H₂O, sodium borohydride was added until the solution
25 reached pH 8, and it was allowed to stand alkaline at room
26 temperature for 30 minutes. Excess borohydride was destroyed
27 by acidification with 10% HCl, and the resulting acidic

1 solution was made alkaline with 6N NaOH to pH 11. After
2 extracting with methylene chloride (3 x 25 ml), drying the
3 resulting methylene chloride solution over K_2CO_3 , and
4 filtering, concentration in vacuo to give the crude nicotine
5 analog. Preparative glpc gave 64 mg (17%) of pure 3',3'-
6 dimethylnicotine (7).

7 Mol. Form.: Calcd. for $C_{12}H_{18}N_2$: 190.1469. Found:
8 190.1449.

9 3',3'-dimethylnicotine-2'-d (8). The iminium salt 22a,
10 not utilized in the preparation of 7 was used to prepare 8.
11 The reduction and isolation were carried out as described for
12 7 except that $NaBD_4$ in D_2O solution was used instead of
13 $NaBH_4$ in H_2O . Preparative glpc of the crude nicotine analog
14 gave 90 mg (16%) of pure 8. The nmr indicated the presence
15 of 93% of the 2'-deutero species.

16 Mol. Form.: Calcd for $C_{12}H_{17}DN_2$: 191.1533. Found:
17 191.1531.

18 1,2-Dimethyl-1-pyrrolinium-2-¹⁴CH₃ Perchlorate (9a).
19 In a flask equipped with two dropping funnels, a condenser,
20 stir bar, and nitrogen sweep was placed 50 mmoles (1.22 g) of
21 magnesium and 100 ml of anhyd. diethyl ether. Then 0.25
22 millicuries of methyl-¹⁴C iodide⁸ (9.9 mg) and 50 mmoles
23 (7.1 g) of methyl iodide dissolved in 25 ml of ether was
24 added slowly with stirring. After nearly all the magnesium
25 had dissolved, 37.5 mmoles (3.72 g) of 1-methyl-2-pyrrolidinone
26 in 25 ml of diethyl ether was added over 30 minutes, the
27 solution was allowed to stand 20 hours at room temperature,

1 and 100 ml of 6N NaOH was added. The ether layer was
 2 removed, the aqueous phase was extracted with diethyl ether
 3 (6 x 50 ml), the combined ethereal solutions were extracted
 4 with 10% hydrochloric acid (3 x 50 ml), and the aqueous
 5 solution was evaporated in vacuo at 40° to give the crude
 6 pyrrolinium salt. This residue was dissolved in 50 ml of 3N
 7 NaOH, which was extracted with methylene chloride (4 x 25 ml).
 8 The methylene chloride extracts were added to 200 ml of
 9 absolute ethanol and 70% aqueous perchloric acid was added
 10 until the solution was slightly acidic (pH 3). Concentration
 11 in vacuo to 150 ml and cooling to 0° resulted in a precipitate
 12 which was dried at 10 μ for 18 hours giving 2.62 g (27%) of
 13 9a; mp (dec.) 225-30° (lit.^{10,11,12} mp 238°, 235-36°, 239-40°);
 14 nmr (D₂O) 4.17 (t, 2H), 3.46 (s, 3H), 3.23 (t, 2H), 2.44 (s,
 15 3H), 2.20 (m, 2H); tlc (EtOH:0.1N HCl = 2:1; I₂ detection)
 16 one spot at R_F = 0.27; tlc (n-BuOH:HOAc:H₂O = 4:1:5; I₂
 17 detection) one spot at R_F 0.10.

18 Anal. Calcd for C₆H₁₂ClNO₄: C, 36.5; H, 6.1; N, 7.1.
 19 Found: C, 36.2; H, 6.1; N, 7.2.

20 1,2-Dimethyl-1-pyrrolinium-2-¹⁴CH₃ Chloride (9b).
 21 Approximately 8 mmoles of 9a was dissolved in 50 ml of 3N
 22 NaOH and the alkaline solution was extracted with methylene
 23 chloride (3 x 25 ml). The combined extracts were shaken with
 24 25 ml of 10% HCl, and the aqueous solution was evaporated
 25 in vacuo. The residue was dissolved in 100 ml of distilled
 26 water prior to administering aliquots to N. glutinosa plants.

27 1,3,3-Trimethyl-1-pyrrolinium-3,3-¹⁴CH₃ Perchlorate (10a).

1 To 25 ml of anhydrous diethyl ether was added 20 mmoles
 2 (2.54 g) of 1,3,3-trimethyl-1-pyrrolidinone-3,3-¹⁴CH₃ and
 3 6.75 ml (20 mmoles) of lithium aluminum hydride in ether
 4 (0.74 mmole/ml). After refluxing for one hour, the reaction
 5 mixture was cooled, 50 ml of ether and 50 ml of 3N NaOH were
 6 added, the ether layer was removed, and the aqueous solution
 7 was extracted with ether (6 x 30 ml). The combined ether
 8 extracts were washed with 10% HCl (4 x 25 ml) and the aqueous
 9 solution was concentrated in vacuo at 40° to give a mixture
 10 of 10b (66%) and 1,3,3-trimethylpyrrolidine-3,3-¹⁴CH₃ hydro-
 11 chloride (19) (34%) as determined by nmr; tlc (n-BuOH:H₂O:HOAc
 12 [4:5:1]): 19 at R_F 0.14 and 10b at R_F 0.10; tlc (EtOH:0.1N
 13 HCl [2:1]): 19 at R_F 0.51 and 10b at R_F 0.37.

14 The mixture of 10b and 19 was dissolved in 50 ml of
 15 6N NaOH and extracted with methylene chloride (4 x 25 ml).
 16 The methylene chloride extracts were added to 150 ml of
 17 absolute ethanol, 70% aqueous perchloric acid was added until
 18 the ethanolic solution became acidic (pH 3), the methylene
 19 chloride was removed in vacuo, and the ethanolic solution
 20 was cooled to -10°. The resulting precipitate was removed and
 21 dried to give 1.14 g (27%) of 10a; mp 110-12°; nmr (D₂O) 8.41
 22 (s, 1H), 4.24 (t, 2H), 3.58 (s, 3H), 2.19 (t, 2H), 1.36 (s, 6H).

23 Anal. Calcd. for C₇H₁₄ClNO₄: C, 39.7; H, 6.7; N, 6.6.
 24 Found: C, 39.4; H, 6.7; N, 6.5.

25 1,3,3-Trimethyl-1-pyrrolinium-3,3-¹⁴CH₃ Chloride (10b)
 26 was obtained from the corresponding perchlorate 10a by the
 27 procedure given above for obtaining the chloride 9b from the

1 perchlorate 9a.

2 3-Ethoxycarbonyl-1-methyl-2-pyrrolidinone (12). A
3 mixture of 500 g of diethyl carbonate, 99.1 g (1 mole) of
4 1-methyl-2-pyrrolidinone and 2500 ml of anhydrous benzene
5 was refluxed overnight under a water separator. The mixture
6 was cooled to room temperature, 85.3 g of 56.3% NaH dispersion
7 was slowly added, and reaction was allowed to proceed at room
8 temperature for 15 minutes and then refluxed for 12 hours
9 at which time hydrogen evolution had ceased. Cooling in an
10 ice bath was followed by addition of 130 g of glacial
11 acetic acid and 200 ml of benzene to decompose the excess
12 sodium hydride and sodium enolate. The resulting slurry was
13 filtered, the precipitate was washed with methylene chloride,
14 and the filtrate and washings were concentrated in vacuo and
15 then fractionally distilled at 119° and 1.8 mm to give the
16 desired product contaminated with a small amount of mineral
17 oil. Column chromatography on silica gel using benzene and
18 benzene:ethanol (1:1) as eluants followed by redistillation
19 gave 70.4 g (41.2%) of 12; ir (thin film) 1680 (amide C=O) and
20 1740 cm^{-1} (ester C=O); nmr (neat) 4.17 (q, 2H), 3.42 (m, 3H),
21 2.83 (s, 3H), 2.35 (m, 2H), 1.27 (t, 3H).

22 Anal. Calcd for $\text{C}_8\text{H}_{13}\text{NO}_3$: C, 56.1; H, 7.7; N, 8.2.

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

24 3-Ethoxycarbonyl-1,3-dimethyl-2-pyrrolidinone-3-¹⁴CH₃ (15).
25 Petroleum ether was added to 1.71 g (40 mmoles) of 56% sodium
26 hydride dispersion and then drained, leaving sodium hydride
27 free of mineral oil. A solution of 5.13 g (30 mmoles) of 3-

1 ethoxycarbonyl-1-methyl-2-pyrrolidinone (12) and 165 ml of
 2 tetrahydrofuran was added and stirred with the sodium hydride
 3 for two hours. Then 2.18 ml (35 mmoles) of methyl-¹⁴C iodide⁸
 4 was added, the mixture was stirred overnight, and the
 5 solvent was removed in vacuo. The product was extracted
 6 from the sodium salts with benzene (4 x 25 ml), and distillation
 7 at 105-09° and 1.4 mm gave 3.73 g (66%) of 13; ir (thin film)
 8 1680 (amide C=O) and 1740 cm⁻¹ (ester C=O); nmr (CDCl₃) 4.17
 9 (q, 2H), 3.40 (m, 2H), 2.84 (s, 3H), 1.7-2.5 (m, 2H), 1.27 (m,
 10 6H); glpc on 30% QF-1 on chromosorb P (5' x 1/4"; 100 ml/min;
 11 146°) gave one peak, retention time 6.4 min (starting material
 12 12, 8.2 min retention time).

13 Anal. Calcd. for C₉H₁₅NO₃: C, 58.4; H, 8.2; N, 7.6.
 14 Found: C, 58.3; H, 8.0; N, 7.5.

15 3-Carboxy-1,3-dimethyl-2-pyrrolidinone-3-¹⁴CH₃ (14).
 16 3-Carboethoxy-1,3-dimethyl-2-pyrrolidinone-3-¹⁴CH₃, 1.85 g
 17 (10 mmoles), and 25 ml of 10% NaOH were stirred at room
 18 temperature for 16 hours. The reaction mixture was adjusted
 19 to pH 1 with concentrated hydrochloric acid and continuously
 20 extracted with methylene chloride for 90 hours. Removal of
 21 the solvent in vacuo gave 1.57 g (100%) of acid 14, melting
 22 at 142-44° (dec) after recrystallization from ethyl acetate;
 23 nmr (CDCl₃) 10.91 (s, 1H), 3.42 (m, 2H), 2.90 (s, 3H), 1.8-2.6
 24 (m, 2H), 1.42 (s, 3H).

25 Anal. Calcd. for C₇H₁₁NO₃: C, 53.5; H, 7.1; N, 8.9.
 26 Found: C, 53.5; H, 7.2; N, 8.8.

27 1,3-Dimethyl-2-pyrrolidinone-3-¹⁴CH₃ (15). The acid 14,

1 1.16 g (7.4 mmoles) was heated at 150-60° until decarboxylation
2 was complete to give 836 mg (100%) of 15; nmr (CDCl₃) 3.25 (m,
3 2H), 2.80 (s, 3H), 1.5-2.6 (m, 3H), 1.18 (d, 3H).

4 Anal. Calcd. for C₆H₁₁NO: C, 63.7; H, 9.8; N, 12.4.

5 Found: C, 63.6; H, 10.0; N, 12.3.

6 Lithium Aluminum Hydride Reduction of 1,3-Dimethyl-2-
7 pyrrolidinone-3-¹⁴CH₃ (15). The acid 14, 3.144 g (20 mmoles)
8 was heated at 150-60° until the evolution of carbon dioxide
9 had ceased. After cooling, 25 ml of anhydrous ether was added
10 followed by 5 ml of a 1.2M ethereal lithium aluminum hydride
11 solution and the solution was refluxed for one hour.
12 Water (5 ml) was added after cooling, then 100 ml of 6N NaOH.
13 The ether layer was removed, the aqueous solution was extracted
14 with ether (7 x 25 ml), the combined ethereal extracts were
15 extracted with 10% HCl (6 x 25 ml), and aqueous acid solution
16 was evaporated to dryness in vacuo. The residue was dissolved
17 in 2 ml of D₂O and its nmr showed a doublet at 1.33 (3H), a
18 singlet at 3.61 (3H), a triplet at 4.17 (2H), and a singlet at
19 8.6 (1H) assignable to 4; signals assignable to 16 occurred
20 at 1.10 (overlapping doublets, 3H) and 2.92 (s, 3H). Thus,
21 nmr analysis indicated that the product was a mixture of 4
22 (63%) and 16 (37%). Scintillation counting of an aliquot
23 of the aqueous solution of the reduction products gave a
24 yield of 92%; tlc [I₂ detection, EtOH:0.1N HCl (2:1)] gave 16
25 and 4 at R_F's of 0.54 and 0.41, respectively; tlc [I₂ detection,
26 n-BuOH:HOAc:H₂O (4:1:5)] gave, after two elutions, 16 and 4
27 at R_F's of 0.45 and 0.33, respectively.

1 1,3-Dimethylpyrrolidine-3-¹⁴CH₃ Hydrochloride (16).

2 In a Parr hydrogenation bottle was placed 3 mmoles of a
 3 mixture of 4 (63%) and 16 (37%) in 50 ml of H₂O and 50 mg
 4 of 10% Pd/C was added. After hydrogenation at 40 psi of
 5 hydrogen for 18 hours, the solution was filtered through
 6 celite, and the filter pad was washed with 100 ml of hot water.
 7 The solvent was removed in vacuo at 40° to give a 97% yield
 8 of 16 by radioactive assay; nmr (D₂O) 3.0-3.9 (m, 4H), 2.92
 9 (s, 3H), 1.6-2.8 (m, 3H), 1.10 (overlapping doublets, 3H);
 10 tlc [I₂ detection, EtOH:0.1N HCl (2:1)] one spot at R_F 0.53;
 11 tlc [I₂ detection, n-BuOH:HOAc:H₂O (4:1:5)] one spot at
 12 R_F 0.47 after two elutions.

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

16 Anal. Calcd for C₁₂H₁₆N₄O₇: C, 43.9; H, 4.9; N, 17.1.
 17 Found: C, 44.2; H, 4.9; N, 17.1.

18 Separation of 1,3-Dimethyl-1-pyrrolinium-3-¹⁴CH₃ Chloride
 19 (4) and 1,3-Dimethylpyrrolidine-3-¹⁴CH₃ Hydrochloride (16).

20 A column (5 x 64 cm) was prepared using 550 g of silica gel
 21 slurried in EtOH:0.1N HCl (2:1), the eluting solvent.
 22 Four mmoles of a mixture of 4 (63%) and 16 (37%) was applied
 23 to the column in 25 ml of the eluting solvent, using two 25-ml
 24 portions of the eluting solvent for rinsing the compounds
 25 onto the column. Fractions of approximately 50 ml were
 26 collected at a flow rate of approximately 50 ml/hr and the
 27 chromatography was followed by scintillation counting; 200 μ l \pm

1 10 μ l of each fraction was dissolved in 15 ml of dioxane
2 scintillation counting solution. The pyrrolidine 16 was
3 eluted in fractions 18-26 and the pyrrolinium salt 4 in
4 fractions 26-50. Fractions 27-50 were combined, evaporated
5 to dryness in vacuo at 40° and reappplied to the silica gel
6 column as a slurry in EtOH:0.1N HCl (2:1), carrying out the
7 chromatography in the same manner. The desired product 4
8 was eluted in fractions 39-71 which were combined, concentrated
9 to approximately 200 ml in vacuo at 40°, and applied to a
10 cation exchange column (AG-50W-X8; H⁺ form; 200-400 mesh;
11 approximately 150 ml resin). The column was washed until
12 neutral with distilled water; elution of 4 from the column
13 followed with 1.5N HCl, the volume of each fraction being 75 ml
14 and the flow rate 75 ml/hr., monitored by scintillation
15 counting as described above. Pyrrolinium salt 4 was eluted
16 in fraction 16-24; in addition, each of these fractions
17 gave positive Dragendorff's test.³⁰ Fractions 16-24 were
18 combined and evaporated at 40° in vacuo to give 85% (2.08
19 mmoles) of the 4 applied to the initial column; $\lambda_{\text{max}}^{\text{C}_2\text{H}_5\text{OH}}$ 262 nm
20 (ϵ 21); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 249 nm (ϵ 14); nmr (D₂O) 8.55 (s, 1H), 4.18 (t,
21 2H), 3.63 (singlet methyl superimposed on multiplet, 4H),
22 1.7-2.8 (m, 2H), 1.33 (d, 3H); tlc [I₂ detection, EtOH:0.1N
23 HCl (2:1)] one spot, R_F 0.43; tlc [I₂ detection, n-BuOH:HOAc:H₂O
24 (4:1:5)] one spot, R_F 0.31 after two elutions; mass spectrum
25 (70 eV) m/e (rel intensity) 97 (54, M⁺-HCl), 96 (100), 82 (34).
26 Mol. Form. Calcd. for C₆H₁₁N (M⁺-HCl): 97.0891.
27 Found: 97.0887.

1,3,3-Trimethyl-2-pyrrolidinone-3,3-¹⁴CH₃ (18).

To 500 ml of anhydrous diethyl ether and 220 mmoles (31 ml) of diisopropylamine (freshly distilled from BaO), cooled to -70°, 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°, and 13.8 ml (220 mmoles) of ¹⁴CH₃I⁸ was added. Stirring was continued at room temperature for 16 hours, 150 ml of H₂O was added, the ether layer was removed and evaporated in vacuo, and the residue was dissolved in 100 ml of H₂O. 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° (27 mm) gave 4.79 g (78%) of 18; nmr (CDCl₃) 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°; 100 ml/min; 10' x 1/4") one peak, 9.0 min.

Anal. Calcd. for C₇H₁₃NO: C, 66.1; H, 10.3; N, 11.0.
Found: C, 65.9; H, 10.2; N, 10.9.

2-Acetyl-1,3-dimethylpyrrolidine (17). To 6 mmoles of 1,3-dimethyl-1-pyrrolinium chloride and 6 ml of H₂O were added 35 ml of 1N NaOH, 10 ml of H₂O, 20 ml of ethanol, and 15 ml of ethyl acetoacetate. After stirring in the dark under nitrogen for 17 days, 50 ml of concentrated HCl 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

1 methylene chloride for 4 days. Removal of the solvent
2 gave 440 mg (47%) of 17; ir (thin film) 1730 cm^{-1} (C=O); nmr
3 (CDCl_3) 2.7-3.7 (m, 5H), 2.69 (s, 3H), 1.4-2.5 (m, 3H),
4 2.30 (s, 3H), 1.12 (d, 3H); mass spectrum (70 eV) m/e (rel.
5 intensity) 155 (4, M^+), 140 (2), 124 (28), 109 (21), 98 (100).

6 Warming 17 with a saturated ethanolic solution of picric acid
7 gave the picrate which was recrystallized from absolute
8 ethanol; mp 147-51 (dec); nmr (pyridine- d_5) 8.97 (s, 2H),
9 3.1-4.0 (m, 5H), 3.08 (s, 3H), 1.6-2.6 (m, 3H), 2.25 (s, 3H),
10 1.01 (overlapping doublets, 3H).

11 Anal. Calcd. for $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_8$: C, 46.9; H, 5.3;
12 N, 14.6; O, 33.3. Found: C, 47.1; H, 5.3; N, 14.7.

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Footnotes

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- 2 (1) This investigation was supported in part by Grant No.
3 NIH 12797 from the National Institute of Mental Health,
4 U. S. Public Health Service, and the U.S. Atomic
5 Energy Commission.
- 6 (2) For complete reviews of the present status of nicotine
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8 transformation product cannot be completely excluded.
9 However, such an hypothesis would require rapid and total
10 absorption of the transformation product and would be
11 extremely difficult to subject to experimental test.
12 It would also require reconversion in the plant to a
13 form capable of incorporation into the observed substituted
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19 temperature of 162° and flow rate of 100 ml/min gave a
20 retention time of 9.3 minutes for nicotine (γ).
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26 Pharmaceutical Chemistry, University of California,
27 San Francisco, for helpful discussions on nicotine nmr

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1 Chicago Corporation Mark I Liquid Scintillation Computer
2 (Model 6880) and are in disintegrations per minute (dpm)
3 relative to an external standard, corrected for back-
4 ground. Counting was carried out with 15 ml aliquots
5 of either a solution of 18.0 g of 2,5-diphenyloxazole
6 (PPO), 0.4 g of p-bis[2-(5-phenyloxazolyl)]benzene (POPOP),
7 and 4 μ l of toluene or a solution of 18.0 g of PPO, 0.4 g
8 of POPOP, 200 g of naphthalene, 1 μ l of ethanol, 1.4 μ l of
9 toluene, and 1.6 μ l of dioxane. Whenever necessary, 1 ml
10 of NCS Solubilizer (Nuclear Chicago Corp.) was added to
11 the liquid scintillation sample vial in order to insure
12 complete solubility. All elemental analyses were
13 performed by the Analytical Laboratory, Department of
14 Chemistry, University of California, Berkeley. CD and
15 ORD spectra were run on a Cary 60 instrument. Glpc's
16 were carried out on a Varian Aerograph A-90-P instrument.
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