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INCORPORATION OF 1,3-DIMETHYL-1-PYRROLINIUM CHLORIDE IN NICOTIANA GLUTINOSA.
BIOSYNTHESIS OF A SUBSTITUTED NICOTINE

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INCORPORATION OF
1,3-DIMETHYL-1-PYRROLINIUM CHLORIDE IN
NICOTIANA GLUTINOSA.
BIOSYNTHESIS OF A SUBSTITUTED NICOTINE

Melvin L. Rueppel and Henry Rapoport

August 13, 1970

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Incorporation of 1,3-Dimethyl-1-Pyrrolinium Chloride in Nicotiana Glutinosa. Biosynthesis of a Substituted Nicotine

JUN 15 1970

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

REC 362G
Sir:

The study of the biosynthesis of natural products in plants has been carried out almost exclusively by means of precursor feeding experiments, although the importance of alternate methods such as short-term biosynthesis with $^{14}\text{CO}_2$ has been stressed.² Ideally, only the natural precursor should be incorporated efficiently into the natural product; however, incorporation of an unnatural precursor into a natural product is well documented.³ Although theoretically possible, neither the incorporation of a natural precursor into an unnatural product⁴ nor the incorporation of an unnatural precursor into an unnatural product, closely related to the natural one, has been previously reported. We now provide an example of biosynthesis involving the latter type of precursor incorporation.

Since 1-methyl-1-pyrrolinium chloride (2) has been found to be an efficient precursor of the pyrrolidine ring of nicotine (3),⁵ 1,3-dimethyl-1-pyrrolinium-3- $^{14}\text{CH}_3$ chloride (1) was selected as the candidate unnatural precursor and synthesized as shown.⁶ 1-Methyl-2-pyrrolidone (4), condensed with diethyl carbonate with sodium hydride as base, gave ester 5. 1,3-Dimethyl-3-carbethoxy-2-pyrrolidone-3- $^{14}\text{CH}_3$ (6)⁷ was obtained by alkylating the sodium enolate of 5 with methyl- ^{14}C iodide.⁸ Hydrolysis of the alkylated ester 6 (sp. act. 2.71×10^7 dpm/mmol)

specific activity

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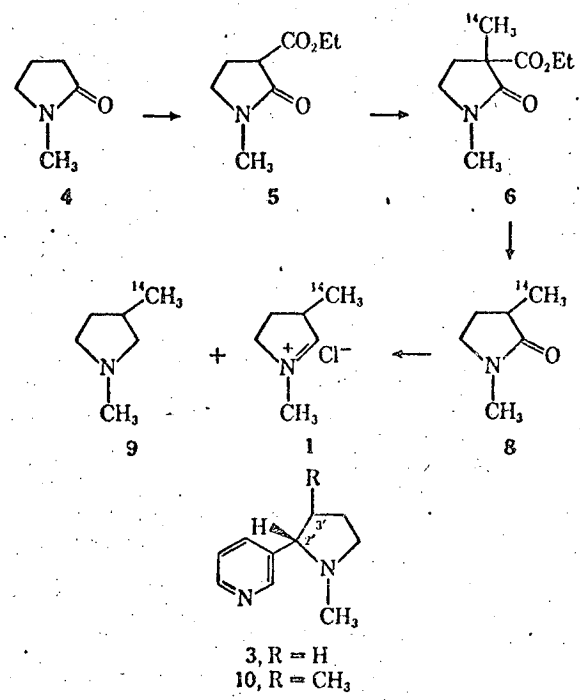
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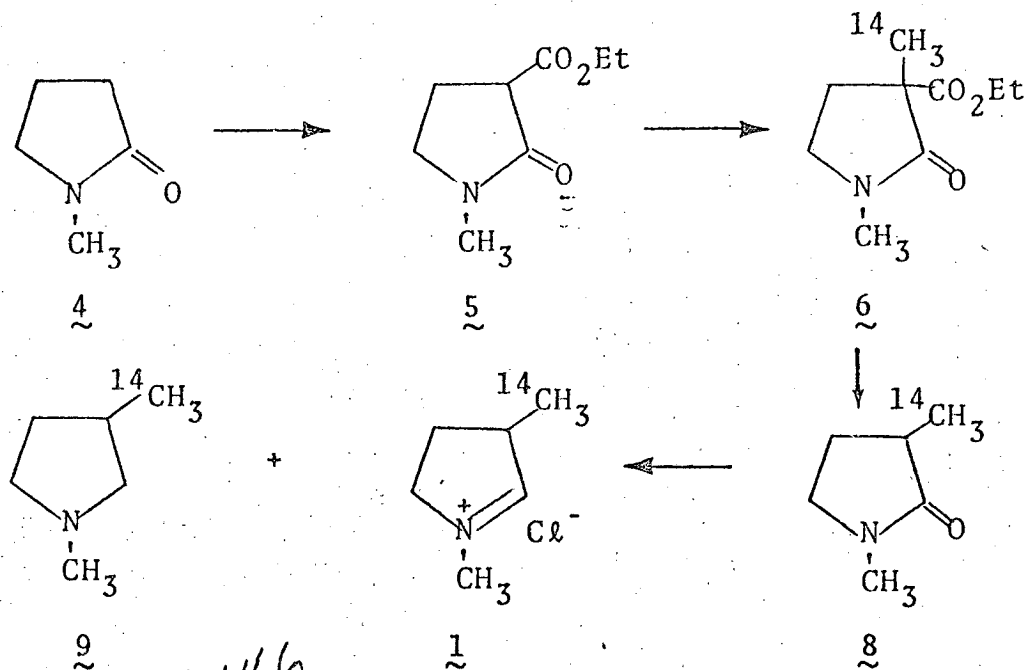
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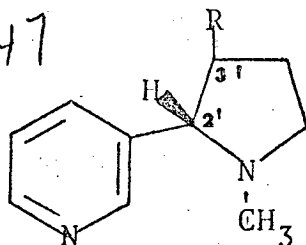


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12 quantitatively gave acid 7 (sp/act/2.68 x 10⁷ dpm/mmol) which
13 on decarboxylation at 150-160° gave 1,3-dimethyl-2-pyrrolidinone
14 (8).⁹ Reduction of 8 with lithium aluminum hydride gave in 92%
15 yield a mixture of pyrrolinium salt 1 (70%) and pyrrolidine 9
16 (30%). Chromatographies on silica gel, eluting with ethanol-0.1N
17 HCl (2:1), followed by ion exchange gave pure 1 in 40% over-all
18 yield from 5.

19 In order to examine the possibility of biosynthesizing
20 3^v-methylnicotine (10), 1 was administered in portions over a
21 period of several days to an aerated hydroponic solution 10



1 containing four N. glutinosa plants in each experiment
 2 (Table I).

3 *filed 8/9 575 & 362E file*
 4 Table I. Administration of 1,3-Dimethyl-1-Pyrrolinium-3-¹⁴CH₃
 5 Chloride (1) to Nicotiana Glutinosa^a and
 6 Incorporation into 3^V-Methylnicotine (10)^b *set 3 rules*

Experiment	Pyrrolinium salt 1 fed		Incorp ⁿ into 3-methylnicotine (10)	
	mg	dpm	dpm	%
1	40.4 ^b	8.10/x/10 ⁶	4.22/x/10 ⁵	5.2
2	200.4 ^c	3.97/x/10 ⁷	4.34/x/10 ⁶	10.9 ^d

8/9 ^a For the preparation of the plants, (see ^{ref} footnote 11. ^b Administered over a 5-day period followed by ~~one~~ day of growth. Total weight of the four plants was 261 g; their age was 66 days. ^c Administered in increasing amounts over a period of 8 days to 59 day-old plants. Total weight of the four plants was 54 ~~g~~ and 139 g at the start and finish, respectively. ^d Using nicotine (3) as the standard, glpc analysis indicated the presence of 56.0 mg of nicotine (3) and 21.6 mg (8.3%) of 3^Vmethylnicotine (10).

11 At the end of the biosynthetic experiment, the alkaloidal fraction
 12 was isolated as described¹¹ and fractionated by preparative gas
 13 liquid partition chromatography.¹² In addition to the normal
 14 Nicotiana alkaloids,¹¹ a peak at retention time 31.2 minutes
 15 was also present in a yield of 5¹-11% (Table I).

16 The new substance has been characterized as 3^V-methylnicotine
 17 (10). Its ultraviolet spectrum shows a ⁽³⁵⁾ C₂H₅OH of 261 nm and is
 18 identical in all respects ^{with} to that of nicotine.¹³ The specific
 19 activity of 10 was determined by a combination of uv absorption
 20 and liquid scintillation counting to be 2.76/x/10⁷ dpm/m³mmole³, in
 21 *(times)*

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1 excellent agreement with its precursor 1. The mass spectrum
 2 of 10 gave a molecular ion at m/e 176 (22% of base) along
 3 with peaks at m/e 175 (7), 133 (100), 119 (6), and 98 (53), all
 4 analogous with those of nicotine.¹⁴ High-resolution mass
 5 spectroscopy established the molecular formula as $C_{11}H_{16}N_2$ for
 6 m/e 176 (Calcd: ^H176.1313; Found: ^H176.1323) and $C_6H_{12}N$ for m/e 98
 7 (Calcd: ^H98.0970; Found: ^H98.0974) for the 1,3-dimethyl-1-pyrrolinium
 8 fragment formed by α -cleavage. The nmr (CCl_4) shows peaks at
 9 δ 8.40 (m, 2H), 7.60 (m, 1H), 7.17 (m, 1H), 3.20 (m, 1H), 1.4-2.6
 10 (m, 5H), 2.10 (s, 3H, N-CH₃), and 0.97 (d, 3H, >CHCH₃) consistent
 11 with structure 10.

12 Biogenetically, 3 ν -methylnicotine (10) would be expected
 13 to have the same absolute configuration at the 2 ν -carbon as
 14 nicotine (3) which has been assigned the S configuration with
 15 reference to L-proline,¹⁵ L-serine,¹⁶ and optical rotary
 16 dispersion measurements.¹⁷ The CD curve of 10 (in 95% EtOH)
 17 gave a molecular ellipticity $[\theta]$ at 260 nm of +22,800 (peak);
 18 3 showed a $[\theta]_{271}$ -7090 (trough) in addition to $[\theta]_{261}$ +24,800
 19 (peak). Although 3 showed a weaker negative cotton effect at
 20 273 nm in the ORD,¹⁷ this absorption was absent in both the CD
 21 and ORD of 10 due possibly to the presence of an adjacent
 22 asymmetric center. As a consequence, 10 is tentatively assigned
 23 the S configuration at the 2 ν -carbon; the presence of the alkyl
 24 methyl in 10 as a single doublet in the nmr indicates that
 25 only one of the possible diastereomers was formed biosynthetically.

26 The biosynthesis of 3 ν -methylnicotine (10) from 1,3-
 27 dimethyl-1-pyrrolinium salt 1 demonstrates that the enzyme system

1 which catalyzes the biosynthesis of nicotine from 1-methyl-
 2 1-pyrrolinium salt 2 and a nicotinic acid derivative is not
 3 completely specific, and its requirements may become definable
 4 through experiments such as these. In addition the formation
 5 of unnatural products from unnatural precursors in vivo should
 6 be useful in the preparation of analogs of biologically active
 7 natural products (with high specific activity if desired) and
 8 in the study of metabolism and interrelationships among
 9 alkaloids.

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

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