Title

INCORPORATION OF l,3-DIMETHn.-l-PYRROLINIUM CHLORIDE IN NICOTIANA GLUTINOSA. BIOSYNTHESIS OF A SUBSTITUTED NICOTINE

Authors

Rueppel, Melvin L.
Rapoport, Henry

Publication Date

1970-08-01

Submitted to Journal of the
American Chemical Society

UCRL-20065
Preprint

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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

AEC Contract No. W-7405-eng-48

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-20065 c.2

DISCLAIMER

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

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 $^{14}CO_2$ 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 product[4] 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-$^{14}CH_3$ 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-$^{14}CH_3$ (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 x 10^7 dpm/mmole)

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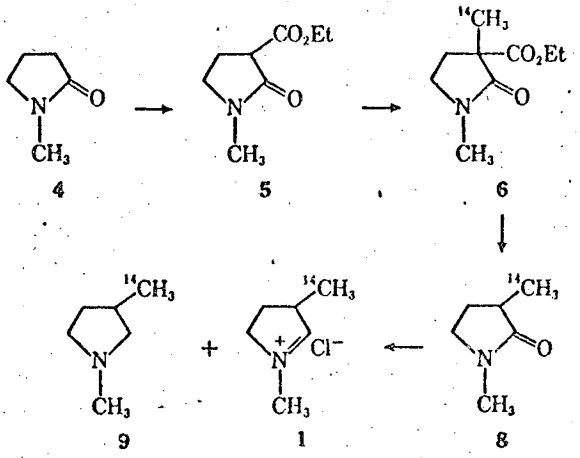

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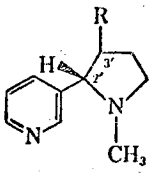

3, R = H
10, R = CH_3

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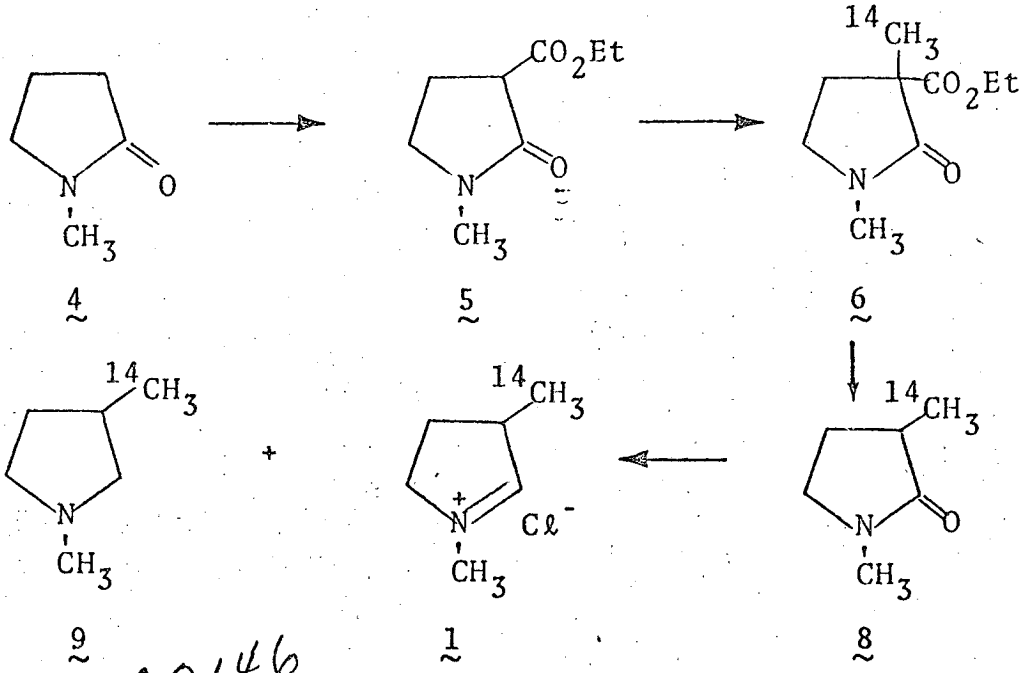

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quantitatively gave acid 7 (sp act 2.68 x 10^7 dpm/mmole) which on decarboxylation at 150-160° gave 1,3-dimethyl-2-pyrrolidinone (8).[9] Reduction of 8 with lithium aluminum hydride gave in 92% yield a mixture of pyrrolinium salt 1 (70%) and pyrrolidine 9 (30%). Chromatographies on silica gel, eluting with ethanol-0.1N HCl (2:1), followed by ion exchange gave pure 1 in 40% overall yield from 5.

In order to examine the possibility of biosynthesizing 3'-methylnicotine (10),[10] 1 was administered in portions over a period of several days to an aerated hydroponic solution

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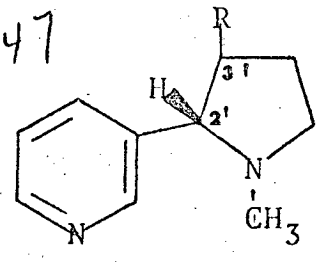

3, R=H

10, R=CH_3

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

Table I. Administration of 1,3-Dimethyl-1-Pyrrolinium-3-$^{14}CH_3$ Chloride (1) to Nicotiana Glutinosa[a] and Incorporation into 3-Methylnicotine (10)

Experiment	Pyrrolinium salt 1 fed		Incorpn into 3-methylnicotine (10)	
	mg	dpm	dpm	%
1	40.4[b]	8.10×10^6	4.22×10^5	5.2
2	200.4[c]	3.97×10^7	4.34×10^6	10.9[d]

[a] For the preparation of the plants, see footnote 11. [b] Administered over a 5-day period followed by one day of growth. Total weight of the four plants was 261 g; their age was 66 days. [c] Administered in increasing amounts over a period of 8 days to 59 day-old plants. Total weight of the four plants was 54 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-methylnicotine (10).

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

The new substance has been characterized as 3-methylnicotine (10). Its ultraviolet spectrum shows a $\lambda_{max}^{C_2H_5OH}$ of 261 nm and is identical in all respects with that of nicotine.[13] The specific activity of 10 was determined by a combination of uv absorption and liquid scintillation counting to be 2.76×10^7 dpm/mmole, in

excellent agreement with its precursor 1. The mass spectrum of 10 gave a molecular ion at m/e 176 (22% of base) along with peaks at m/e 175 (7), 133 (100), 119 (6), and 98 (53), all analogous with those of nicotine.[14] High-resolution mass spectroscopy established the molecular formula as $C_{11}H_{16}N_2$ for m/e 176 (Calcd:[17] 176.1313; Found:[17] 176.1323) and $C_6H_{12}N$ for m/e 98 (Calcd:[17] 98.0970; Found:[17] 98.0974) for the 1,3-dimethyl-1-pyrrolinium fragment formed by α-cleavage. The nmr (CCl_4) shows peaks at δ 8.40 (m, 2H), 7.60 (m, 1H), 7.17 (m, 1H), 3.20 (m, 1H), 1.4-2.6 (m, 5H), 2.10 (s, 3H, N-CH_3), and 0.97 (d, 3H, >CHCH_3) consistent with structure 10.

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

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

which catalyzes the biosynthesis of nicotine from 1-methyl-Δ1-pyrrolinium salt 2 and a nicotinic acid derivative is not completely specific, and its requirements may become definable through experiments such as these. In addition the formation of unnatural products from unnatural precursors *in vivo* should be useful in the preparation of analogs of biologically active natural products (with high specific activity if desired) and in the study of metabolism and interrelationships among alkaloids.

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Melvin L. Rueppel[18a] and Henry Rapoport[18b]
Department of Chemistry, University of California
Berkeley, California 94720

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

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

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

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LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720