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BIOSYNTHESIS OF THE PYRROLIDINE RING OF NICOTINE : FEEDING EXPERIMENTS WITH N^{15}-LABELLED ORNITHINE-2-C^{14}

Edward Leete[a], Eduardo G. Gros[b] and Terry J. Gilbertson
Department of Chemistry, University of Minnesota, Minneapolis.

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When ornithine-2-C^{14} was fed to intact tobacco plants radioactive nicotine (VII) was obtained in which essentially all the activity was located at C_2 and C_5 of the pyrrolidine ring and was equally divided between these positions (1,2). A similar result was obtained when glutamic acid-2-C^{14} (3,4) or proline-2-C^{14} (5) were fed to Nicotiana plants, although these amino acids were less efficient precursors of nicotine than ornithine. Putrescine-1,4-C^{14} (III) was also found to be a reasonably efficient precursor of the pyrrolidine ring of nicotine (3).

It was of considerable interest to determine whether the nitrogen atoms of ornithine participate in the formation of the pyrrolidine ring, especially as it has been suggested (6) that they do not. In the present work we have used sterile root cultures of Nicotiana tabacum plants since Dawson and coworkers, using such a system, obtained much higher incorpor-

[a]Alfred P. Sloan Fellow, 1962-64.
[b]Fellow of the Consejo de Investigaciones Científicas y Técnicas, Argentina.

ation of tracers into nicotine than we obtained with intact plants. The nutrient solution and the experimental procedures were the same as those used by Dawson (7). The α- and δ-N^{15} labelled ornithines were prepared by previously described methods (8). Each culture flask containing 30 ml. of nutrient solution was fed approximately 1 mg. of a mixture of DL-ornithine-2-C^{14} and the N^{15} labelled ornithine. The roots were allowed to grow for one month and then harvested , the nicotine being isolated as its dipicrate . For N^{15} assay in a mass spectrometer* the nicotine was converted to its diperchlorate. Oxidation of the nicotine with potassium permanganate yielded nicotinic acid which was isolated as its methyl ester . The distribution of C^{14} in the isolated nicotines was determined as previously described (1) , and was found to be located solely at C_2 and C_5 of the pyrrolidine ring and was equally divided between these positions . The degree of incorporation of C^{14} and N^{15} into nicotine is recorded in Table I.

In both experiments we found a high specific incorporation of C^{14} into the pyrrolidine ring of nicotine, substantiating the previously described results using intact plants. The difference in specific incorporation of C^{14} in the two experiments may have been due to the fact that the second experiment was carried out at a somewhat higher average room temperature.

In experiment 1 where a mixture of ornithine-2-C^{14} and ornithine-α-N^{15} was fed , no excess N^{15} was detected in the nicotine. In the second experiment involving ornithine-δ-N^{15}

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* We thank Dr. Adrian Swanson for the assay of N^{15}. Atmospheric nitrogen had a 29/28 ratio of 0.00733 ± 0.00001.

TABLE I

Experiment 1

Precursors fed:	wt(mg.)	% excess N^{15}	Specific activity[a] d.p.m./mM.
DL-Ornithine-α-N^{15}.HCl	50.17	88 (in N_α)	1.83×10^8
DL-Ornithine-2-C^{14}.HCl	4.53		
Nicotine isolated	2.05	0	0.68×10^8

Specific incorporation $[C^{14}] = 37\%$

Absolute incorporation[b] $[C^{14}] = 1.5\%$

Experiment 2

Precursors fed:	wt(mg.)	% excess N^{15}	Specific activity
DL-Ornithine-δ-N^{15}.HCl	71.03	88 (in N_δ)	1.97×10^8
DL-Ornithine-2-C^{14}.HCl	7.05		
Nicotine isolated	3.10	13.5[c]	1.20×10^8
Methyl nicotinate		0	0.56×10^8

Specific incorporation $[C^{14}] = 61\%$

Absolute incorporation $[C^{14}] = 2.5\%$

Specific incorporation $[N^{15}] = \frac{2 \times 13.5}{88} = 31\%$

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[a] Radioactivities were determined in a Nuclear Chicago Model 720 liquid scintillation system.

[b] Absolute incorporation is defined as the total activity found in the isolated nicotine divided by the total activity fed to the roots.

[c] This value is the average of the pyridine and pyrrolidine nitrogen.

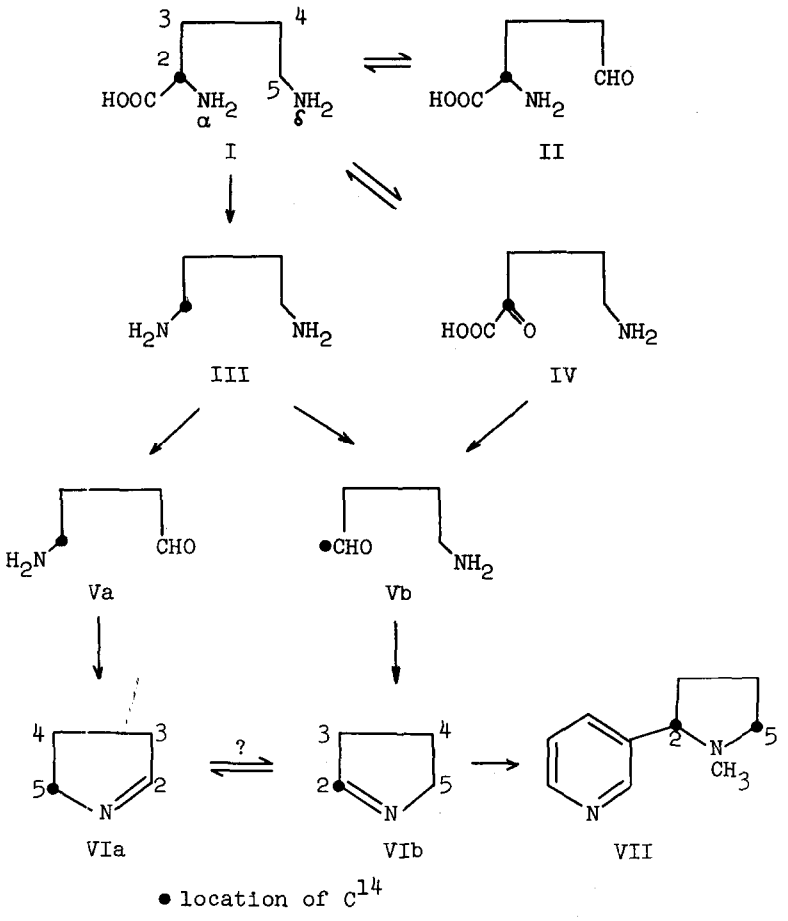

Figure 1. Hypothetical conversion of ornithine (I) to nicotine

nicotine was obtained having excess N^{15} in the pyrrolidine ring, no excess N^{15} being detected in the pyridine ring. These results were somewhat unexpected because in most biological systems which have been studied transamination reactions with ornithine involve the δ-amino group (9).

We have previously suggested (10) that Δ^1-pyrroline (VI) is an intermediate between ornithine and nicotine. We still favor

this hypothesis, however its postulated formation via glutamic semialdehyde (II) must now be abandoned since such a mechanism would involve loss of all the δ-nitrogen.

Our results can be explained by postulating that tobacco roots contain an ornithine-α-transaminase which catalyses the formation of α-keto-δ-aminovaleric acid (IV) from ornithine. Such a reaction would result in loss of N^{15} from ornithine-2-C^{14}-α-N^{15}. Transamination of the α-keto-δ-aminovaleric acid with amino acids containing normal nitrogen (there was an abundant supply of nitrogenous compounds in the nutrient solution) would lead ultimately to ornithine-2-C^{14} containing essentially no excess N^{15}.

Decarboxylation of ornithine affords putrescine (III) , this reaction having been observed in barley (11). Δ^1-Pyrroline (VIa, VIb) labelled equally at C_2 and C_5 could then be formed via 4-aminobutanal (V) by the oxidative deamination of putrescine (12). In this oxidation half of the nitrogen of the putrescine is lost and this could account for the result obtained in experiment 2, where the specific incorporation of the N^{15} was half that of the C^{14}.

An alternate explanation of our results would be to suggest that the ornithine-2-C^{14}-δ-N^{15} undergoes transamination to α-keto-δ-aminovaleric acid which is then decarboxylated to the 4-aminobutanal Vb. Cyclization would then afford the Δ^1-pyrroline (VIb) labelled only at C_2 with C^{14}. However, this pyrroline may be in tautomeric equilibrium with the Δ^1-pyrroline (VIa) labelled at C_5*. If these were the sole reactions occuring the specific incorporation of the N^{15} should have been the same as that of the C^{14} . The lower incorporation of the N^{15} may be rationalized by suggesting that some δ-transamination

occurs leading to glutamic semialdehyde, followed by resynthesis of ornithine utilizing unenriched nitrogen.

We are currently attempting to isolate from tobacco roots enzymes which catalyse the metabolism of ornithine.

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* We are investigating whether such a tautomeric equilibrium exists.

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