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THE METABOLISM OF NICOTINE-2'-^{14}C IN *NICOTIANA GLAUCA*

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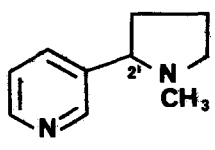
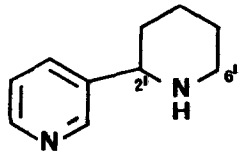
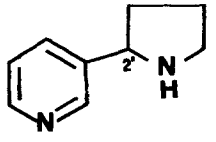
There has been considerable speculation (1) and some ambiguous experimental work on the question of the conversion of nicotine (I) to anabasine (II) in *Nicotiana* species . Tso and Jeffrey (2) administered nicotine-^{15}N-methyl-^{14}C to *N. glauca* plants growing in hydroponics and obtained radioactive anabasine which contained both excess ^{15}N and ^{14}C , however the incorporation of the ^{14}C was about half that of the ^{15}N. Schröter (3) administered nicotine-U-^{14}C (obtained biosynthetically by growing *N. rustica* plants in an atmosphere containing $^{14}CO_2$) to excised *N. glauca* shoots. The anabasine isolated four days later was not radioactive . However when putrescine-1,4-^{14}C, an established



I



II



III

precursor of the pyrrolidine ring of nicotine, was administered to *N. glauca* plants, radioactive anabasine, nicotine, and nornicotine (III), were apparently produced (4) . A mechanism involving incorporation of the N-methyl group of nicotine into C-6' of the piperidine ring of anabasine was considered plausible (5) . Recently Alworth and Rapoport (6) carried out biosyntheses with $^{14}CO_2$ in *N. glutinosa* plants, and determined the specific activities of nicotine, nornicotine, and anabasine, after relatively short times. They came

to the conclusion that the conversion of nicotine to anabasine is potentially possible.

In view of these conflicting results it seemed desirable to investigate the metabolism of specifically labeled nicotine in N. glauca . We allowed ten excised N. glauca shoots (20 - 30 cm. tall) to absorb a solution of nicotine-2'-^{14}C (2.7 mg., having a total activity of 6.86 x 10^7 dpm.) (7) in dilute hydrochloric acid. After five days essentially all the activity had been absorbed by the shoots. Crude alkaloids were isolated from the fresh shoots (188 g.) by previously described methods (8) . Thin layer chromatography on preparative plates of Silica Gel F_{254} (Merck), eluting with a 4:1 mixture of chloroform and methanol yielded nornicotine (R_f 0.15), anabasine (0.50) and nicotine (0.82) . The alkaloids were isolated as their diperchlorates and crystallized to constant activity after dilutions with inactive alkaloids . The amounts of alkaloids isolated and their activities are recorded in the Table.

Table Alkaloids isolated from N. glauca which had been fed Nicotine-2'-^{14}C

Alkaloids	Weight isolated	Activity (dpm/mM.)	% Incorporation
Anabasine	85 mg.	0.00	0.00
Nicotine	2.1 mg.	2.16 x 10^7	0.41
Nornicotine	8.5 mg.	6.80 x 10^7	5.7

The anabasine was completely inactive . We thus feel confident in stating that nicotine is not a direct precursor of anabasine in excised N. glauca shoots. It is of course possible that the nicotine-2'-^{14}C could be degraded to nicotinic acid-7-^{14}C (9) . However if this were utilized for the biosynthesis of anabasine, no activity would appear in the alkaloid, since it has been established (10) that the carboxyl group of nicotinic acid is lost when it is incorporated into anabasine. The radioactive nicotine underwent extensive metabolism in the N. glauca , only 0.41 % being recovered after five days. We are currently searching for metabolites of the nicotine , which are apparently

non-alkaloidal. The demethylation of nicotine to nornicotine has ample precedent in N. glauca and other species (2, 3, 6, 11) . The nornicotine (6.8×10^7 dpm/mM.) was oxidized with concentrated nitric acid affording nicotinic acid (6.7×10^7 dpm/mM.), which was decarboxylated by refluxing in boiling quinoline with copper chromite, yielding carbon dioxide collected as barium carbonate (6.5×10^7 dpm/mM.) . Thus essentially all the activity of the nornicotine was located at C-2' , indicating that nicotine is a direct precursor of this alkaloid in N. glauca.

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