1,1-dimethylhydrazine was stored at room temperature for one week. There resulted 20 g. of crude solid which, after recrystallization from methanol, melted at 116–6.5°.

Anal. Calcd. for $C_5H_9N_3O$: C, 47.23; H, 7.13; N, 33.05. Found: C, 47.83; H, 7.27; N, 32.93.

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The Biogenesis of the Nicotiana Alkaloids. VIII. The Metabolism of Nicotine in *N. tabacum*[1]

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In order to investigate the metabolism of nicotine in the intact tobacco plant, methyl-C^{14} nicotine and nicotine-2,5-C^{14} were fed, in separate experiments, to *N. tabacum* plants. Nicotine was isolated from the plants at various times, from one to seven weeks after administration of the tracers. The recovered nicotine was radioactive in each case and systematic degradation indicated that there had been no randomisation of activity. The average recovery of the 2,5-labeled nicotine was about 6%, whereas the recovery of the methyl labeled nicotine was only about 1%, indicating extensive metabolic breakdown of the radioactive alkaloids. Radioactive choline was isolated from a plant which had been fed methyl labeled nicotine and degradation indicated that 90% of the activity was located on the methyl groups of the choline. Nicotine thus acts as a methyl donor in the tobacco plant.

The administration of ornithine-2-C^{14} to the roots of an intact *N. tabacum* plant produces nicotine labeled on the 2-and 5-carbons of the pyrrolidine ring.[2] By allowing the tobacco to grow for considerable lengths of time (up to 9 weeks) after administration of the ornithine, we found that there was very little decrease in the total radioactivity located in the nicotine.[3] These results suggested that there was no gross metabolic breakdown of nicotine in the healthy living plant. However, it was considered likely that the N-methyl group of nicotine would be in equilibrium with methyl acceptors such as ethanolamine or homocysteine. In order to test this hypothesis we have fed methyl-C^{14}-nicotine to *N. tabacum* plants, and then the nicotine was reisolated and assayed for activity 1, 3, 5 and 7 weeks after administration of the tracer. The methyl labeled nicotine was obtained by the reaction of *l*-nornicotine (isolated from *N. glutinosa*) with methyl iodide-C^{14}. The results are summarized in Table I, and it is seen that only about 1% of the administered nicotine was recovered from the plant. There was no significant difference in the specific activity of the nicotine isolated at different times after feeding the tracer. The small differences in the percentage recovery of activity, which were observed, were probably due to unavoidable variations in the individual plants. In view of the large loss of radioactive carbon from the nicotine, it was of interest to see whether there had been any randomization of activity. The method which has been previously used for the determination of activity on the methyl group of nicotine has been that of Brown and Byerrum.[4] This involves heating nicotine with hydriodic acid to yield methyl iodide which is absorbed in triethylamine to form the quaternary salt which is assayed. Since the accuracy of the counting is usually no better than 4%, this method of degradation fails to establish whether a small amount of activity is present in the rest of the nicotine molecule. Therefore in the present work we have demethylated the radioactive nicotine with silver hydroxide and isolated the resultant nornicotine by chromatography. The nornicotine was completely inactive, indicating that there had been no randomization of activity. In order to discover whether the methyl group of nicotine was transferred to methyl acceptors, choline was isolated from the plant harvested one week after feeding the radioactive nicotine. It was indeed radioactive and degradation indicated that 90% of the activity was located on the methyl groups.

Biosynthetic nicotine-2,5-C^{14} also was fed to a group of tobacco plants to serve as a control in our study of the lability of the N-methyl group. Rather surprisingly, we found that there was considerable metabolism of this ring labeled nicotine; however, it was not as extensive as with the methyl labeled nicotine. Again there was no significant change in the amount of activity recovered in the nicotine, isolated at various times after feeding the tracer (see Table I). Systematic degradation of the reisolated nicotine indicated that all the activity was still located on the 2- and 5-carbons of the pyrrolidine ring.

The high initial loss of activity from both labeled nicotines, with little subsequent decrease in the activity after one week, is compatible with the hypothesis that metabolism of the radioactive nicotine occurs in the roots, which are the main site of nicotine synthesis in *N. tabacum*, and then little further metabolism occurs after the nicotine has been translocated to the leaves, in agreement with our previous findings.[3] The significantly higher recovery of the ring labeled nicotine is presumably a measure of the greater stability of the pyrrolidine ring to metabolic breakdown.

Tso and Jeffrey[5] have recently studied the fate of N^{15}-labeled tobacco alkaloids in *N. glauca* and *rustica*. They also found that there was extensive metabolism of nicotine when it was fed to the roots

(1) Part VII: E. Leete, *Chemistry and Industry*, 1477 (1958). This work was presented at the 135th meeting of the American Chemical Society, Boston, April 1959; and was supported in part by research grant M-2662 from the National Institute of Mental Health, Public Health Service.

(2) E. Leete and K. J. Siegfried, THIS JOURNAL, **79**, 4529 (1957).

(3) E. Leete, *ibid.*, **80**, 2162 (1958).

(4) S. A. Brown and R. U. Byerrum, *ibid.*, **74**, 1523 (1952).

(5) T. C. Tso and R. N. Jeffrey, *Arch. Biochem. Biophys.*, **80**, 46 (1959).

of these two species. In one experiment nicotine, labeled with N^{15} in both rings and with C^{14} in the methyl group, was fed to *N. glauca*. The change in the ratio of N^{15} to C^{14} in the nicotine, reisolated 10 days later, indicated that the methyl group was metabolized to a greater extent than the nitrogen containing rings, in agreement with our results. Bose, *et al.*,[6] have isolated a crude enzyme from *N. tabacum* which catalyzed the conversion of nicotine to nornicotine in the presence of the methyl acceptor ethanolamine.

The ability of the N-methyl groups of alkaloids to participate in reversible transmethylations may be a general phenomenon. Another example has been discovered by Frank and Marion,[7] who fed hordenine-α-C^{14} (N,N-dimethyltyramine), which is the main alkaloid of barley roots, to germinating barley seedlings. The hordenine reisolated 5 days after administration of the radioactive alkaloid contained only 14% of the initial activity. The N-methyltyramine isolated from the roots was radioactive, indicating that some demethylation of the hordenine had occurred in the plant.

Experimental

Methyl-C^{14}-nicotine.—*l*-Nornicotine was extracted from *N. glutinosa* plants by the method used to isolate anabasine from *N. glauca*.[8] Paper chromatography of the crude alkaloid indicated the presence of nicotyrine (1-methyl-2-(3-pyridyl)-pyrrole) and nicotine which were removed by chromatography on alumina. The *l*-nornicotine (130 mg., 0.88 m*M*) was dissolved in dry ether (30 ml.) and methyl iodide-C^{14} [9a] (69 mg., 0.49 m*M*, total activity, 4.06×10^8 c.p.m.)[9b] added and the mixture allowed to stand for 6 days at room temperature. Inactive *l*-nicotine (0.5 ml.) was added and the reaction mixture shaken with 2 *N* sodium hydroxide (50 ml.) and the whole extracted with dichloro-methane. The dried ether–dichloromethane extract was evaporated *in vacuo* and the residue chromatographed on alumina (activity II to III) using benzene as the eluting solvent. The fractions containing nicotine (detected by paper chromatography) were combined and distilled *in vacuo* to yield 427 mg. of methyl-C^{14} nicotine which was converted to the diperchlorate and had an activity of 3.36×10^7 c.p.m./m*M*. The low radiochemical yield (23%) is presumably due to a competing reaction leading to the formation of quaternary ammonium salts.

The nicotine-2,5-C^{14} was obtained from tobacco which had been fed ornithine-2-C^{14}.

Administration of the Tracers to the Tobacco.—The variety of tobacco used in these experiments was *Nicotiana tabacum* var. Maryland Mamoth. The plants were grown in an aqueous nutrient solution as previously described.[3,7] An aqueous solution of the methyl-C^{14}-nicotine (324 mg., 2 m*M*) was divided equally between the nutrient solutions of 4 plants of approximately equal size (4 months old). Similarly the nicotine-2,5-C^{14} (324 mg., 2 m*M*, total activity, 1.15×10^8 c.p.m.) was divided between four other plants of the same size and age. After 24 hr. there was negligible activity remaining in the nutrient solutions. At the end of one week a plant from each group was harvested and the nicotine isolated as previously described.[2] The nicotine was assayed as the dipicrate or the diperchlorate. Paper chromatography of the reisolated nicotine indicated the absence of any nornicotine. The remaining plants were harvested 3, 5 and 7 weeks after feeding the radioactive nicotine to the roots. The plants continued to increase in size and new roots and leaves were produced during the course of the experiments. The results are summarized in Table I.

TABLE I

Tracer fed, methyl-C^{14} nicotine; amount fed, 81 mg., 1.68×10^7 c.p.m./plant.

Time of harvesting, wk.	Wet wt. of plant (g.)	Nicotine reisolated Wt., mg.	Specific activity (c.p.m./m*M* $\times 10^{-5}$)	% Recovery of activity
1	540	154	1.91	1.1
3	570	196	1.27	0.9
5	650	202	0.63	0.5
7	617	177	2.28	1.3

Tracer fed, nicotine-2,5-C^{14}; amount fed, 81 mg., 2.88×10^5 c.pm./plant.

Time of harvesting, wk.	Wet wt. of plant, g.	Nicotine reisolated Wt., mg.	Specific activity (c.p.m./m*M* $\times 10^{-4}$)	% Recovery of activity
1	550	208	1.23	5.5
3	540	132	1.88	5.3
5	670	167	2.35	8.4
7	690	136	1.49	4.4

Degradation of the Radioactive Nicotine, (a) the Nicotine Isolated from the Methyl-C^{14}-nicotine Fed Plants.—A modification of the method of Späth, *et al.*,[10] was used. The active nicotine diperchlorate (250 mg., total activity, 1.29×10^5 c.p.m.), which was obtained from the tobacco plant which had been fed radioactive nicotine one week previously, was diluted with an equal weight of inactive nicotine diperchlorate. This diluted nicotine was dissolved in water (5 ml.) and refluxed with freshly prepared silver hydroxide, obtained from 1.5 g. of silver nitrate, for 17 hr. The mixture was then filtered and the filtrate extracted with chloroform after making it strongly basic with sodium hydroxide. The mixture of bases obtained on evaporation of the dried chloroform extract was dissolved in benzene and chromatographed on alumina (activity II to III). Initial elution with benzene yielded nicotyrine, then nicotine. On changing the eluting solvent to a mixture of benzene and methanol (50:1), three unidentified substances were eluted together and were visible as an orange zone on the column. Finally nornicotine was eluted. The fractions containing nornicotine were combined and distilled *in vacuo* (140° (0.1 mm.)) to yield nornicotine (20 mg.) as a pale yellow oil. The picrate was obtained as yellow plates from aqueous ethanol, m.p. 188–189° (lit.[10] 190–191°).

Anal. Calcd. for $C_9H_{12}N_2.2C_6H_3N_3O_7$: C, 41.59; H, 2.99. Found: C, 41.65; H, 3.00.

Two samples of more than 10 mg. each showed no trace of activity in the internal flow counter.

(b) **The Nicotine Isolated from the Plant Fed Nicotine-2,5-C^{14}.**—This nicotine was obtained from the plant which was allowed to grow for 5 weeks after administration of the tracer. The method of degradation was the same as that previously used.[2] Nicotine diperchlorate (2.35×10^4 c.p.m./m*M*) on oxidation with nitric acid yielded 4-nitro-5-(3-pyridyl)-pyrazole (1.20×10^4 c.p.m./m*M*) and nicotinic acid (1.10×10^4 c.p.m./m*M*). Decarboxylation of the nicotinic acid yielded pyridine isolated as the picrate which was inactive.

Isolation and Degradation of the Choline.—Choline was isolated from the plant which was harvested one week after feeding the methyl-C^{14}-nicotine. The choline was separated as the reineckate from the aqueous ammonical sap from which the nicotine had been extracted with chloroform, using the method described by Kirkwood and Marion.[11] The reineckate was decomposed with silver sulfate and the choline reisolated as the chloroplatinate, m.p. 228–230°, having an activity of 3.1×10^4 c.p.m./m*M*. The choline chloroplatinate was oxidized with potassium permanganate using the procedure of du Vigneaud, *et al.*,[12] yielding trimethylamine picrate which had an activity of 2.8×10^4 c.p.m./m*M*.

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