[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

The Biogenesis of the Nicotiana Alkaloids. VI. The Piperidine Ring of Anabasine[1]

BY EDWARD LEETE

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Cadaverine-1,5-C^{14} dihydrochloride was fed to intact *Nicotiana glauca* plants resulting in the formation of radioactive anabasine. Systematic degradation of the active anabasine indicated that the cadaverine was incorporated into the piperidine ring with maintenance of the integrity of the five carbon chain. There was negligible activity in the pyridine ring. The radioactive cadaverine was also incorporated into anabasine by excised shoots of *N. glauca*, the percentage incorporation being much higher than that of lysine-2-C^{14}. The significance of these results is discussed.

Introduction

We have shown previously[2] that lysine is a precursor of the piperidine ring of anabasine, an alkaloid of *N. glauca*. The metabolism of lysine in this plant was not analogous to that of ornithine in *N. tabacum* in which the main alkaloid is nicotine. In the latter species the administration of ornithine-2-C^{14} led to the production of nicotine labeled equally on the two α-positions of the pyrrolidine ring,[3] whereas lysine-2-C^{14} yielded anabasine labeled only on C-2 of the piperidine ring.[2] We have shown recently[4] that putrescine, like ornithine, is an efficient precursor of the pyrrolidine ring of nicotine. It was thus of interest to examine cadaverine (1,5-diaminopentane) as a possible precursor of the piperidine ring of anabasine.

Radioactive cadaverine previously has been prepared biosynthetically from lysine,[5,6] but in the present work it was obtained conveniently by the catalytic hydrogenation of 1,3-dicyanopropane which was derived from the reaction of 1,3-dibromopropane with potassium cyanide-C^{14}. The overall yield was 70%.

Aronoff[7] has reported that there was a negligibly small amount of radioactivity in the anabasine obtained from excised leaves of *N. glauca* which had been fed lysine-2-C^{14}. Since this result conflicts with our work using intact plants[2] we have also fed lysine-2-C^{14} to excised shoots of *N. glauca*. We also fed cadaverine-1,5-C^{14} to the excised shoots for varying lengths of time when we discovered that it was a good precursor of anabasine in the intact plant.

Experimental

Cadaverine-1,5-C^{14} Dihydrochloride.—1,3-Dibromopropane (0.55 ml.) was added to a solution of potassium cyanide (0.65 g., with an activity of 4.0×10^8 c.p.m.[8]) and potassium iodide (0.05 g.) in 75% ethanol (4 ml.) and the mixture refluxed for 45 minutes. The solution, which had deposited considerable solid, was diluted with 30 ml. of water and extracted with ether to remove unreacted dibromopropane. The aqueous solution was acidified with 20 ml. of 2 *N* sulfuric acid and extracted with chloroform in a continuous extractor for 24 hr. The chloroform extract was taken to dryness and the residue dissolved in a mixture of methanol (50 ml.) and concentrated hydrochloric acid (5 ml.) and hydrogenated at a pressure of 45 lb./sq. in. in the presence of Adams catalyst (0.5 g.) for 2 hr. The coagulated catalyst was filtered off and the filtrate taken to dryness *in vacuo* to leave a residue which was crystallized from a mixture of ethanol and ether yielding colorless hygroscopic needles of cadaverine-1,5-C^{14} dihydrochloride, m.p. 235–237° cor. having an activity of 4.3×10^5 c.p.m./mg. The yield was 0.610 g. (70%).

Anal.[9] Calcd. for $C_5H_{14}N_2 \cdot 2HCl$: C, 34.29; H, 9.21. Found: C, 34.26; H, 8.84.

Administration of Cadaverine-1,5-C^{14} to the Intact *N. glauca*.—The *N. glauca* plants were about 5 months old and were grown in an inorganic nutrient solution as previously described.[2] Cadaverine-1,5-C^{14} dihydrochloride (350 mg. (2.0 m*M*) with an activity of 1.50×10^8 c.p.m.) was divided equally between the nutrient solutions of three of the plants. The cadaverine was absorbed rapidly by the roots and after 5 days the nutrient solutions had only 2.5% of their original activity. Two weeks after feeding the cadaverine, the plants were harvested and the anabasine isolated and degraded by established methods.[2] The yield of anabasine from the three plants (wet wt. 800 g.) was 555 mg. The aqueous ammoniacal sap had an activity of 4.4×10^6 c.p.m. The activities of anabasine and its degradation products are recorded in Table I.

TABLE I

ANABASINE AND ITS DEGRADATION PRODUCTS

	Specific activity $\times 10^{-5}$, c.p.m./m*M*
Anabasine diperchlorate	1.49
Anabasine dipicrate	1.45
Anabasine dipicrolonate	1.39
Nicotinic acid	0.72
Nicotinic acid hydrochloride	0.69
Pyridine picrate[10]	0.01

Administration of Cadaverine-1,5-C^{14} and Lysine-2-C^{14} to Excised Shoots of *N. glauca*.—The shoots were obtained from the tops of 6 months old *N. glauca* plants. The shoots were cut at an angle of 45° with a razor blade and immediately placed in 25 ml. of distilled water containing only the radioactive compound. The average length of the shoots was 30 cm. and they had a fresh wt. of about 25 g. The shoots were exposed to continuous illumination. As the aqueous solution was absorbed by the shoots it was replenished with distilled water. The shoots were harvested at the times indicated in Table II. The fresh shoots were macerated in a Waring Blendor with a mixture of chloroform (100 ml.) and 15 *N* ammonia solution (20 ml.). Inactive anabasine (100 mg.) was added to the mixture as a carrier. The Blendor was washed out with a further 300 ml. of chloroform and the plant allowed to stand in contact with the chloroform and ammonia for 24 hr. The marc was then filtered off and the filtrate separated into aqueous and organic layers. The aqueous layer was assayed for activity. The anabasine was recovered from the chloroform in the usual way.[2] It was found that the anabasine

(1) The title of this series has been changed to the present one to include *Nicotiana* alkaloids other than nicotine. This work has been supported by a grant from the Research Corporation, New York, and was presented in part at the 133rd meeting of the American Chemical Society, San Francisco, April 13–18, 1958.

(2) E. Leete, THIS JOURNAL, **78**, 3520 (1956).

(3) E. Leete and K. J. Siegfried, *ibid.*, **79**, 4529 (1957).

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

(5) H. R. V. Arnstein, G. D. Hunter, H. M. Muir and A. Neuberger, *J. Chem. Soc.*, 1329 (1952).

(6) R. W. Schayer, R. L. Smiley and J. Kennedy, *J. Biol. Chem.*, **206**, 461 (1954).

(7) S. Aronoff, *Plant Physiol.*, **31**, 355 (1956).

(8) All counts were carried out in a windowless flow G.M. counter (Nuclear-Chicago Co. Model D-46 A) using "Q gas" as the quencher. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

(9) Carried out by Miss Heather King of these laboratories.

(10) Obtained from the decarboxylation of the nicotinic acid.[1]

from the cadaverine feedings was contaminated with a trace of cadaverine. Therefore the distilled anabasine was dissolved in benzene and chromatographed on alumina.[11] The anabasine was eluted with a solution of 1% methanol in benzene. Cadaverine remained on the column until pure methanol was used as the eluting solvent. The fractions containing anabasine (detected by paper chromatography) were combined and distilled. The recovery of anabasine from each experiment was about 80 mg. The incorporation of C^{14} (shown as a percentage in Table II) was calculated by dividing the total activity found in the anabasine by the total activity absorbed by the excised shoot. The small amount of anabasine (*ca.* 10 mg.) in the 25 g. of shoots was neglected in calculating the % incorporation which was based on a 100% recovery of the inactive anabasine added as a carrier. Details of these experiments are recorded in Table II. The radioactive anabasine diperchlorate obtained from the lysine-2-C^{14} fed had a specific activity of 2.5×10^3 c.p.m./m*M*. This was oxidized with nitric acid to yield nicotinic acid with an activity of 2.6×10^3 c.p.m./m*M*. If it is assumed that there is no activity in the pyridine ring of the nicotinic acid, this result is consistent with our previous findings.[2]

TABLE II

EXPERIMENTS WITH EXCISED SHOOTS OF *N. glauca*

Precursor	Wt., mg.	Activity $\times 10^{-7}$, c.p.m.	Duration of feeding, days	% of amount fed in: Activity Nutrient	Sap	Anabasine
Cadaverine-1,5-C^{14}-di-HCl	25	1.07	2	23	19	0.078
	25	1.07	4	15	22	.26
	25	1.07	6	0	20	.49
Lysine-2-C^{14} HCl	26	0.49	4	12	28	.036

Discussion

The incorporation of cadaverine-1,5-C^{14} into anabasine 14 days after feeding to the intact *N. glauca* plant was 0.33% which compares favorably with that of lysine-2-C^{14} which was only 0.0026 and 0.046% 5 and 16 days after feeding.[2] The excised shoots were also capable of synthesizing anabasine from cadaverine or lysine, confirming the conclusions of Dawson[12] that alkaloid synthesis occurs in the aerial parts of *N. glauca* as well as in the roots. The percentage incorporation of cadaverine in the shoots increased slowly with time, being highest after 6 days. Attempts to extend the time of feeding beyond this were unsuccessful since the shoots became flaccid. Previous results[3,4] have indicated that nicotine is synthesized at a slow rate in *N. tabacum*. It may be a general phenomenon that alkaloid synthesis occurs at a much slower rate than such processes as photosynthesis. The specific activity of the anabasine obtained after feeding lysine-2-C^{14} to the excised shoots for 4 days was extremely low and just within the limits of accurate counting with a G.M. flow counter. It is thus not surprising that Aronoff reported[7] negligible activity in the anabasine after feeding lysine-2-C^{14} to an excised leaf.

The activities of the degradation products (Table I) of the radioactive anabasine are consistent with the hypothesis that the cadaverine-1,5-C^{14} is incorporated into the piperidine ring without any breakdown of the 5-carbon chain. Half the activity of the anabasine was located at C-2 of the piperidine ring and we assume that the rest of the activity is at C-6. Work is proceeding to confirm this.

We consider that the immediate precursor of the piperidine ring of anabasine is Δ^1-piperideine which is derived from cadaverine by oxidation to 5-aminopentanal followed by cyclization. This oxidation has been accomplished by Mann and Smithies[13] using, as catalyst, an amine oxidase isolated from pea seedlings. More recently Hasse and Berg[14] have obtained anabasine by the oxidation of cadaverine in the presence of a pea extract. Schöpf, *et al.*,[15] have shown that Δ^1-piperideine will undergo dimerization *in vitro* to yield tetrahydroanabasine. However, since the anabasine obtained in our experiments had negligible activity in the pyridine ring, analogous reactions must not occur in *N. glauca*. Nicotinic acid has been shown to be a precursor of the pyridine ring of nicotine[16,17] and it seems reasonable to assume that it is also a precursor of the pyridine ring of anabasine.

The incorporation of lysine-2-C^{14} into the piperidine ring cannot proceed *via* free cadaverine since this would result in randomization of activity between C-2 and C-6 of anabasine, whereas all the activity was found at C-2. However it is conceivable that the lysine-2-C^{14} could be converted to Δ^1-piperideine-2-C^{14} *via* cadaverine if it is assumed that the cadaverine is bound to the enzyme surface so that randomization cannot occur, the α-carbon of lysine becoming the aldehyde group in 5-aminopentanal. Cadaverine may be a better precursor of the piperidine ring than lysine because of its metabolic proximity to 5-aminopentanal.

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(11) The alumina was digested with ethyl acetate, filtered, washed with methanol and then heated at 120° for 48 hr.

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