Phytochemistry, 1972, Vol. 11, pp. 1737 to 1739. Pergamon Press. Printed in England.

SHORT COMMUNICATION

BIOSYNTHESIS OF THE PIPERIDINE NUCLEUS: METABOLISM OF D- AND L-LYSINE-2-^{14}C BY *NICOTIANA GLAUCA*

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(*Received* 12 *October* 1971)

Abstract—D- and L-lysine-2-^{14}C were administered to intact *Nicotiana glauca* plants. Anabasine and pipecolic acid were isolated. The results show that L-lysine is the preferred precursor of anabasine and D-lysine is the preferred precursor of pipecolic acid. This supports Spenser's hypothesis that pipecolic acid and the piperidine alkaloids derived from lysine do not have 6-amino-2-ketohexonoic acid as a common intermediate.

INTRODUCTION

RECENTLY Gupta and Spenser[1] have suggested that there are two routes for the metabolism of lysine. One route proceeds through 6-amino-2-ketohexanoic acid to pipecolic acid in the intact rat, in intact plants, and in excised shoots of *Sedum acre*. The second route proceeds through a non-symmetrical derivative of 1,5-diaminopentane or 5-aminopentanal to the piperidine alkaloids, anabasine, *N*-methylpelletierine, and sedamine.[2]

Since D-lysine has recently been shown to be a major precursor of pipecolic acid in the intact rat,[3] intact ryegrass, and intact corn seedlings,[4] we decided to find out if the route to anabasine preferred the D- or L-isomer of lysine.

RESULTS

The plants were able to distinguish the L-isomer from the D-isomer as shown by the fact that the L-isomer was 98% absorbed in two days while the D-isomer took six days to reach 98% absorption. The % incorporation of L-lysine-2-^{14}C into anabasine from this experiment was at least twice that previously reported from DL-lysine-2-^{14}C.[5] L-lysine-2-^{14}C was a 30 times better precursor of anabasine than D-lysine-2-^{14}C, and D-lysine-2-^{14}C was a 48 times better precursor of pipecolic acid than L-lysine-2-^{14}C as judged by the % specific incorporation of the products (Table 1).

[1] R. N. GUPTA and I. D. SPENSER, *Phytochem.* **9**, 2329 (1970).
[2] E. LEETE, *Acc. Chem. Res.* **4**, 100 (1970).
[3] J. A. GROVE, T. J. GILBERTSON, R. H. HAMMERSTEDT and L. M. HENDERSON, *Biochim. Biophys. Acta* **184**, 329 (1969).
[4] R. W. ALDAG and J. L. YOUNG, *Planta* **95**, 187 (1970).
[5] E. LEETE, *J. Am. Chem. Soc.* **80**, 4393 (1958).

TABLE 1. INCORPORATION OF L- AND D-LYSINE INTO ANABASINE AND PIPECOLIC ACID

Administration of L-lysine-2-^{14}C	Wt. (mg)	Activity (dpm)	Specific activity (dpm/mM)	% Specific incorporation
L-Lysine-2-^{14}C	55·2	$3{\cdot}00 \times 10^7$	$0{\cdot}988 \times 10^7$	
Anabasine diperchlorate	38·0	$2{\cdot}26 \times 10^4$	$2{\cdot}15 \times 10^5$	2·17
Pipecolic acid hydrochloride	34·2	$3{\cdot}01 \times 10^3$	$2{\cdot}26 \times 10^4$	0·22
Administration of D-lysine-2-^{14}C				
D-Lysine-2-^{14}C	45·3	$3{\cdot}00 \times 10^7$	$1{\cdot}20 \times 10^7$	
Anabasine diperchlorate	37·6	$9{\cdot}02 \times 10^2$	$8{\cdot}71 \times 10^3$	0·072
Pipecolic acid hydrochloride	35·7	$2{\cdot}62 \times 10^5$	$1{\cdot}26 \times 10^6$	10·5

DISCUSSION

The results confirm the previous work[3,4] that D-lysine is the preferred precurors of pipecolic acid. They show that L-lysine is the preferred precursor of anabasine. This lends support to Spenser's hypothesis[1] that pipecolic acid and the piperidine alkaloids derived from lysine do not have 6-amino-2-ketohexanoic acid as a common intermediate.

The fact that D-lysine is an extremely good precursor of pipecolic acid is not easy to rationalize. It may result from an aberrant metabolic pathway in the organisms efforts to convert D-lysine to L-lysine. The organism might convert the D-isomer to 6-amino-2-keto-hexanoic acid which may spontaneously cyclize to Δ^1-piperideine-2-carboxylic acid. The Δ^1-piperideine-2-carboxylic acid could be reduced to pipecolic acid. The pipecolic acid could then accumulate as it is known to be a relatively inert metabolite in the rat,[7] but it is readily metabolized by chickens and rabbits.[8] Fowden's work[9] suggests, however, that it is relatively inert in *Acacia homaphylla*. The other possibility is that plants make small amounts of D-lysine as a side product of L-lysine biosynthesis and that pipecolic acid represents a detoxification product.

EXPERIMENTAL

Labelled compounds. DL-Lysine-2-^{14}C was purchased from International Chemical and Nuclear Corporation. It was resolved by the method of Rothstein and Miller[6] as previously described.[3] The crude D-isomer was rendered free of the L-isomer by treatment with L-lysine decarboxylase. This should have given D- and L-isomers with the same specific activity. Since this was not the case both isomers were chromatographed on Dowex 50 ($H^{\oplus}$ form) and recrystallized. The specific activity did not change and no evidence for the presence of 1,5-diaminopentane-1,5-^{14}C in the D-isomer was obtained. L-Lysine-2-^{14}C monohydrochloride had a $[\alpha]_D^{25}$ $+19{\cdot}4 \pm 0{\cdot}5°$ (*c*, 2 in 0·6 N HCl) (lit.[10] $+18{\cdot}7$) and a specific activity of $0{\cdot}988 \times 10^7$ dpm/mM. D-Lysine-2-^{14}C monohydrochloride had a $[\alpha]_D^{25}$ $-19{\cdot}0 \pm 0{\cdot}5°$ (*c*, 2 in 0·6 N HCl) and a specific activity of $1{\cdot}20 \times 10^7$ dpm/mM.

Administration of the isomers to Nicotiana glauca. The *N. glauca* plants were grown from seed in soil until they were about 4 months old. They were transferred to an aerated hydroponic nutrient solution which was identical to that described by Leete[5] except that it was found necessary to increase the amount of $FeSO_4$ by 20 mg/l. to prevent chlorosis. After 2 weeks the plants had many new roots and had resumed growth. 2 plants of approximately the same size were chosen and their roots immersed in 1 l. of fresh nutrient solution. L-Lysine-2-^{14}C HCl was added to the nutrient solution of one plant and D-lysine-2-^{14}C HCl to the

[6] M. ROTHSTEIN and L. L. MILLER, *J. Biol. Chem.* **206,** 243 (1954).
[7] J. GROVE and L. M. HENDERSON, *Biochim. Biophys. Acta* **165,** 113 (1968).
[8] J. GROVE and H. G. ROGHAIR, *Arch. Biochem. Biophys.* **144,** 230 (1971); and personal communication with Dr. Grove.
[9] L. FOWDEN, *J. Exptl Bot.* **11,** 302 (1960).
[10] F. J. KEARLEY and A. W. INGERSOLL, *J. Am. Chem. Soc.* **73,** 5783 (1951).

other. The L-isomer was 98% absorbed in 2 days, while the D-isomer was not 98% absorbed until the 7th day. On the 7th day both plants were harvested.

Isolation of anabasine. Anabasine was isolated by preparative TLC on 1 mm Merck silica gel—PF with $CaSO_4$[11] in $CHCl_3$–MeOH–NH_4OH (60 : 20 : 1). The R_f for anabasine was 0·56 (lit.[11], 0·58), and it was extracted into EtOH and converted to the diperchlorate, m.p. 165–166°, which was recrystallized to constant activity with EtOH and Et_2O.

Isolation of pipecolic caid. The basic aqueous solution from which the alkaloids had been extracted with $CHCl_3$ was evaporated to dryness. L-Pipecolic acid HCl (150 mg) was added to the residue and it was allowed to stand with 200 ml MeOH overnight. The MeOH was heated to boiling, filtered, and the solvent evaporated. The residue was stirred with 10 ml H_2O and centrifuged to remove the undissolved solids. The aqueous solution was applied to a Dowex 50 × 8, 200–400 mesh (H^+ form) column of 1 × 40 cm. The column was washed with H_2O until the eluent gave a negative Molish test and contained little radioactivity. The amino acids were eluted with N NH_4OH. The amino acid containing fractions were combined and evaporated to dryness. The residue was dissolved in 3 ml of 50% v/v HCl and treated with $NaNO_2$ until the solution was ninhydrine negative. The solution was extracted 3 × Et_2O (10 ml) The Et_2O was dried (Na_2SO_4), evaporated, and the yellow oil which resulted was mixed with 4 ml conc. HCl and heated at 100° for 25 min. The HCl was evaporated and 5 ml H_2O was added and evaporated 3 × until a crystalline residue resulted. The residue was dissolved in 1 ml MeOH and the pipecolic acid HCl precipitated with Et_2O. The compound was recrystallized to constant specific activity from MeOH and Et_2O. m.p. 252–256° (lit.[12], 256–258°) and gave an IR spectra identical with that of an authentic specimen.

Acknowledgements—We thank Professor Edward Leete for providing the *N. glauca* seed and the National Institutes of Health for support through Research Grant 1 RO1 HE 13613-01 MCHA.

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[12] R. M. Zachavius, J. F. Thompson and F. C. Steward, *J. Am. Chem. Soc.* **74,** 2949 (1952).

Key Word Index—Nicotiana glauca; Solanaceae; biosynthesis; piperidines; L-lysine; D-lysine; anabasine; pipecolic acid.