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BIOSYNTHESIS OF THE (+)-2-HYDROXY-3-PHENYLPROPIONIC ACID MOIETY OF LITTORINE IN *DATURA SANGUINEA* AND *ANTHOCERCIS LITTOREA**

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Abstract—DL-Phenylalanine-[1-^{14}C] and DL-phenylalanine-[3-^{14}C] were fed via the roots to *Datura sanguinea* plants and radioactive hyoscine, atropine and littorine isolated from the roots. Radioactivity was confined to the acid moieties of the alkaloid molecules and the specific activities decreased in the order littorine, atropine, hyoscine. Degradation of the (+)-2-hydroxy-3-phenylpropionic acid obtained from hydrolysis of the isolated littorine indicated a specific incorporation of phenylalanine-[1-^{14}C] and phenylalanine-[3-^{14}C] into the acid. *Anthocercis littorea* plants also incorporated phenylalanine into littorine, atropine and hyoscine.

INTRODUCTION

LITTORINE [(−)-3α-(2-hydroxy-3-phenylpropionyloxy)tropane] has recently been isolated from *Anthocercis littorea* Labill.[1] and from *Datura sanguinea* R. & P.;[2] the roots of the latter contain about 0·08 per cent of littorine.[3]

Early studies by Neish and his co-workers[4] on the importance of various phenylpropane compounds as lignin precursors showed that, in monocotyledons, both (+)- and (−)-phenyllactic acid (2-hydroxy-3-phenylpropionic acid) and L-phenylalanine were equally incorporated into lignin. In dicotyledons, however, only the (−)-acid was converted into lignin precursors. Further experiments[5] indicated that the α-hydroxy acids and α-oxo acids related to phenylalanine and tyrosine appear to form two pools, both arising irreversibly from shikimate by two separate routes; little importance was attached to the physiological significance of the α-hydroxy acids, although Gamborg[6] showed that cell-free preparations from wheat shoots oxidized α-hydroxy acids to α-oxo acids. Later Neish[7] reviewed the role of cinnamic acid and its hydroxy derivatives in the biosynthesis of phenolics and discussed the significance of phenyllactic acid in cinnamic acid formation. The following sequence of metabolites was proposed: phenylalanine→phenyllactic acid→cinnamic acid→lignin precursors→lignin. However, subsequent evidence from enzyme studies[8] suggested that

* Part VII in the series "Alkaloids of the genus *Datura*, section Brugmansia".

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[6] O. L. GAMBORG, Ph.D. Thesis, University of Saskatchewan, Saskatoon, Canada (1962).
[7] A. C. NEISH, *Ann. Rev. Plant Physiol.* **11**, 55 (1960).
[8] J. KOUKOL and E. E. CONN, *J. Biol. Chem.* **236**, 2692 (1961).

cinnamic acid is formed irreversibly by direct deamination of phenylalanine; an ammonia-lyase which catalyses this reaction has since been isolated from many higher plants. On this basis Neish[9] revised his earlier scheme to by-pass phenyllactic acid as a lignin intermediate (Fig. 1).

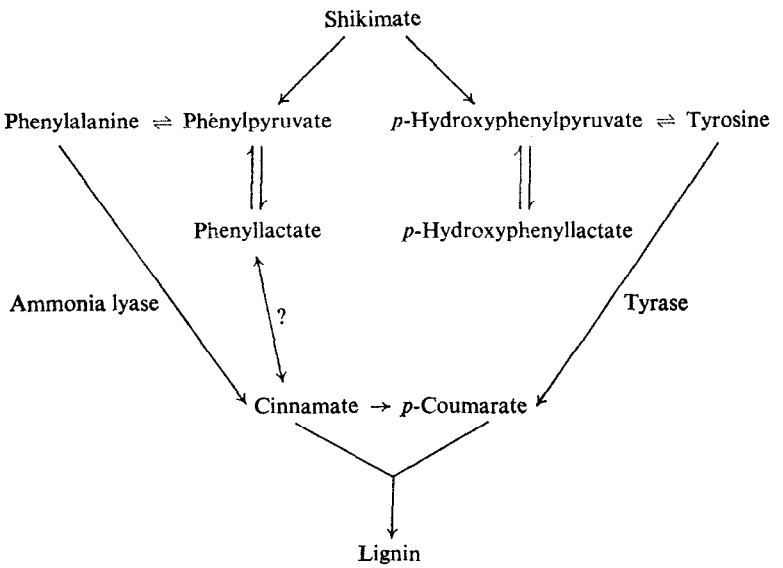

FIG. 1. THE ROLE OF PHENYLLACTIC ACID IN LIGNIN METABOLISM.

Recent experiments on the biosynthesis of the fungal terphenylquinone, volucrisporin,[10] have demonstrated the incorporation of (±)-phenyllactic acid into phenylalanine and volucrisporin with phenylpyruvate as intermediate.

Thus the possibility exists that, in littorine, the phenyllactic acid moiety can be derived from phenylalanine, phenylpyruvate or cinnamate. We report here the results of an investigation into the role of phenylalanine as a precursor of littorine in *D. sanguinea* and *A. littorea*.

RESULTS

Feeding of Phenylalanine-[1-^{14}C] and -[3-^{14}C]

Two 15-month-old *Datura sanguinea* plants were fed, via the roots, with a solution of DL-phenylalanine-[3-^{14}C] (66·6 μc in 2 ml of water); two more plants were administered DL-phenylalanine-[1-^{14}C] (66·6 μc in 2 ml of water). Both groups were harvested after 72 hr. Two *Anthocercis littorea* plants (7 months old) were similarly supplied with DL-phenylalanine-[3-^{14}C] (33·3 μc in 1 ml of water) and harvested after 48 hr; a third plant received DL-phenylalanine-[1-^{14}C] (33·3 μc) and was harvested after 72 hr.

The alkaloids of the roots of *D. sanguinea* and the whole plants of *A. littorea* were fractionated by standard procedures;[2, 3, 11, 12] their specific activities are recorded in Table 1. In the case of *A. littorea* all alkaloids were isolated by dilution with inert carrier.

[9] A. C. NEISH, *Phytochem.* **1**, 1 (1961).
[10] P. CHANDRA, G. READ and L. C. VINING, *Can. J. Biochem.* **44**, 403 (1966).
[11] W. C. EVANS and M. W. PARTRIDGE, *J. Pharm. Pharmacol.* **4**, 769 (1952).
[12] W. C. EVANS, V. A. MAJOR and M. pe THAN, *Planta Medica.* **13**, 353 (1965).

TABLE 1. ISOLATION OF ALKALOIDS FROM *D. sanguinea* AND *A. littorea*

	D. sanguinea administered phenylalanine-		*A. littorea* administered phenylalanine-	
	1-^{14}C	3-^{14}C	1-^{14}C	3-^{14}C
Weight of dry roots (g)	25	19	—	—
Weight of whole plants (g)	—	—	5·7†	3·0‡
Hyoscine				
Base isolated (mg)	27	20	15	14·5
Picrate obtained (mg)*	28	12	19	17
Specific activity of picrate (dpm/mM × 10^{-4})	5·3	9·4	0·0	2·1
Hyoscyamine				
Base isolated (mg)	24	22	12	12
Picrate obtained (mg)*	11	15·3	16	15
Specific activity of picrate (dpm/mM × 10^{-4})	19·7	44·5	1·04	7·87
Littorine				
Base isolated (mg)	9·5	9·0	15	12
Picrate obtained (mg)*	6·7 ↓ 63·2	inactive carrier 9·6 ↓ 84·2	20·8	15
Specific activity of picrate (dpm/mM × 10^{-4})	8·3	8·7	2·1	7·5

* After recrystallization to constant specific activity.
† Inactive carrier (mg): hyoscine hydrobromide 19·8; littorine 16·4; atropine 10·4.
‡ Inactive carrier (mg): hyoscine hydrobromide 20·4; littorine 15·9; atropine 9·9.

Degradation of Atropine Isolated from D. sanguinea *Roots*

Radioactive atropine picrate prepared from plants fed with DL-phenylalanine-[1-^{14}C] and DL-phenylalanine-[3-^{14}C], after suitable dilution with inactive picrate, was subjected to alkaline hydrolysis and in both instances afforded inactive tropine and radioactive tropic acid (Table 2).

TABLE 2. SPECIFIC ACTIVITIES (dpm/mM × 10^{-4}) OF THE DEGRADATION PRODUCTS OF HYOSCYAMINE AND LITTORINE ISOLATED FROM *D. sanguinea* ROOTS AFTER ADMINISTRATION OF DL-PHENYLALANINE-[1-^{14}C] AND -[3-^{14}C]

Derivative or degradation product of alkaloid	Hyoscyamine		Littorine	
	Phenylalanine-		Phenylalanine-	
	1-^{14}C	3-^{14}C	1-^{14}C	3-^{14}C
Diluted picrate	9·84	11·10	8·30	8·73
Tropine picrate	Inactive		Inactive	
Tropic acid	10·50	9·00	—	—
Phenyllactic acid	—	—	9·80	7·50
Diluted phenyllactic acid	—	—	2·45	3·75
Barium carbonate	—	—	2·08	—
Phenylacetaldehyde	—	—	Inactive	—
Benzoic acid	—	—	—	3·23

Degradation of Littorine Isolated from Roots of D. sanguinea *Plants Fed with Phenylalanine-*[1-^{14}C]

Littorine, isolated as the picrate, furnished on hydrolysis inactive tropine and radioactive phenyllactic acid (Table 2). The latter, after dilution with inactive carrier, was decarboxylated with lead tetra-acetate[13] and the evolved CO_2 collected as $BaCO_3$ (86 per cent activity recovered). Phenylacetaldehyde produced in the above reaction was converted to the 2,4-dinitrophenylhydrazone which was inactive (Table 2).

Degradation of Littorine Isolated from Roots of D. sanguinea *Plants Fed with Phenylalanine-*[3-^{14}C]

Hydrolysis of the littorine afforded inactive tropine and radioactive phenyllactic acid. The acid, after dilution, was oxidized with alkaline permanganate; it yielded radioactive benzoic acid (86 per cent of total activity recovered) (Table 2).

DISCUSSION

Phenylalanine was incorporated into hyoscine and atropine in *Anthocercis littorea* and *Datura sanguinea*; thus the plants were producing alkaloid at the time of the feeding experiments. The incorporation of tracer was low in these studies ranging from 0·002–0·013 per cent, but was generally comparable with results obtained by other workers in similar feeding experiments with phenylalanine.

Phenylalanine-[1-^{14}C] and [3-^{14}C] were incorporated into the acid moiety of littorine in both experiments and in each case the specific activity relationship was littorine > hyoscyamine > hyoscine (see Table 1). The high specific activity of littorine could indicate that phenylalanine is a more immediate precursor of phenyllactic acid than of tropic acid; the relationship, if any, between tropic and phenyllactic acids is at present under investigation.

Degradation studies on littorine from *D. sanguinea* indicated that the phenylalanine was incorporated without alteration of the 3-carbon side-chain. This is the expected result if phenyllactic acid is synthesized from phenylalanine via either phenylpyruvic acid or cinnamic acid (Fig. 1). We have yet to establish whether one or both of these routes are involved.

The occurrence of (+)-phenyllactic acid as a tropane-ester alkaloid is not without interest in the light of Neish's observations that dicotyledons could not utilize this isomer for lignin formation. If phenyllactic acid is involved in lignin metabolism at all, it is possible that its (±)-form is produced during normal metabolism and that in *Datura* and *Anthocercis* the (+)-form is removed in part, as an alkaloidal product, whereas the (−)-form is built into lignin. Further studies are required in order to clarify the physiological significance of phenyllactic acid.

EXPERIMENTAL

Radioactivity Measurements

All activities were determined using a Labgear 4π counting chamber (Type D1426) and a Philips electronic counter, operating in the Geiger–Muller region (1350 V). The counter was used with 2π geometry and had a geometrical efficiency of 46 per cent.

Samples of 1 mg [(±) 0·05 mg] were spread as thin films over a dimpled planchet (area of dimple 1·31 cm^2). Alkaloids were counted as picrates and organic acids as sodium salts. Under these conditions the self-absorption losses were found to be for picrates 14 per cent, for sodium salts 40 per cent, and for $BaCO_3$ 32 per cent.

[13] H. OEDA, *Bull Soc. Chim., Japan* **9**, 8 (1934).

Plant Material and Administration of Tracers

Six 15-month-old plants of *Datura sanguinea*, grown from seed, were dug up from open land in Nottingham and the roots carefully washed free of soil. The plants were supported in light-proof beakers and the roots covered with water. Two plants were kept as controls; another pair was supplied with 2 ml of an aqueous solution containing 66·6 μc of DL-phenylalanine-[3-^{14}C], the solution being divided equally between the two plants; the third pair received 2 ml of a solution containing 66·6 μc of DL-phenylalanine-[1-^{14}C]. Uptake of tracer was complete in 6–7 hr in all cases. The plants were supplied with distilled water as necessary for 3 days and then harvested. Aerial parts and roots were dried separately at 60° in a Mitchell Air Stream Oven.

A similar technique was adopted for plants of *Anthocercis littorea* (7 months old) grown from seed in Nottingham. Thus two plants were supplied with 1 ml of solution containing 33·3 μc of DL-phenylalanine-[3-^{14}C] and one plant was given 1 ml of solution containing 33·3 μc of DL-phenylalanine-[1-^{14}C]. The first group showed signs of wilting after 2 days and was therefore harvested at this stage, the remaining plant was harvested after 3 days.

Extraction and Fractionation of the Alkaloids

The plant material was extracted and fractionated by standard techniques. Thus, by partition chromatography at pH 6·8, hyoscine was eluted in ether and characterized by its R_f on TLC plates and by conversion to its picrate which was crystallized to constant specific activity. Littorine was eluted as a sharp peak in $CHCl_3$; the following peak contained hyoscyamine and/or atropine. On some occasions the littorine and atropine peaks tended to overlap and some fractions which were mixtures were rejected. The two bases were characterized by their R_fs on silica gel plates run in $CHCl_3/NHEt_2$ (9:1), and by picrate derivatives crystallized to constant activity. The results for all the feeding experiments are set out in Table 1. Dilutions were necessary at certain stages and these are indicated in the table.

Hydrolysis of Littorine and Hyoscyamine from D. sanguinea *Feeds*

For hydrolysis, the bases were liberated from samples of the picrates and refluxed with 5% $Ba(OH)_2$ (5 ml) for 3 hr at 100°. The mixture was then acidified with 10 N H_2SO_4 and the organic acid extracted into ether. Removal of the solvent afforded either tropic or phenyllactic acid from hyoscyamine and littorine respectively; the acids were crystallized to constant radioactivity. Tropine was obtained as its picrate from the original acid-aqueous solution, and its activity determined. The activities of these hydrolysis products are summarized in Table 2.

Permanganate Oxidation of (+)-*Phenyllactic Acid* (3-^{14}*C-phenylalanine Feeds*)

Phenyllactic acid (5 mg), s.a. $7{\cdot}5 \times 10^4$ dpm/mM was diluted with inactive phenyllactic acid to give s.a. $3{\cdot}75 \times 10^4$ dpm/mM. The acid was then refluxed under N_2 with 0·5 ml of 10% Na_2CO_3 solution and 5 ml of 1% $KMnO_4$ solution for 2 hr. The permanganate solution was cooled, filtered, and acidified with dil. HCl, and the benzoic acid extracted into ether. The crude acid was crystallized from hot water and counted as its sodium salt, s.a. $3{\cdot}23 \times 10^4$ dpm/mM (86 per cent recovery of counts from phenyllactic acid).

Decarboxylation of Phenyllactic Acid with Lead Tetra-acetate[13]

Phenyllactic acid (2·5 mg), s.a. $9{\cdot}8 \times 10^4$ dpm/mM, was diluted to s.a. $2{\cdot}45 \times 10^4$ dpm/mM with inactive acid (7·5 mg). The acid was dissolved in benzene (2 ml), flushed with N_2 for 10 min and then connected to two $Ba(OH)_2$ traps. A fine suspension of lead tetra-acetate (20 mg) in benzene (4 ml) was then added dropwise over a period of 45 min. The acid solution was maintained at 70–75° during this time. The mixture was then flushed with N_2 and the $BaCO_3$ filtered off and dried (yield 12 mg), it had s.a. $2{\cdot}08 \times 10^4$ dpm/mM (counts recovered = 86 per cent). Phenylacetaldehyde was recovered from the benzene solution as its 2,4-dinitrophenylhydrazone (3 mg) and was found to be non-radioactive.