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BIOSYNTHESIS OF C_6-C_3 ACIDS IN *DATURA INNOXIA**

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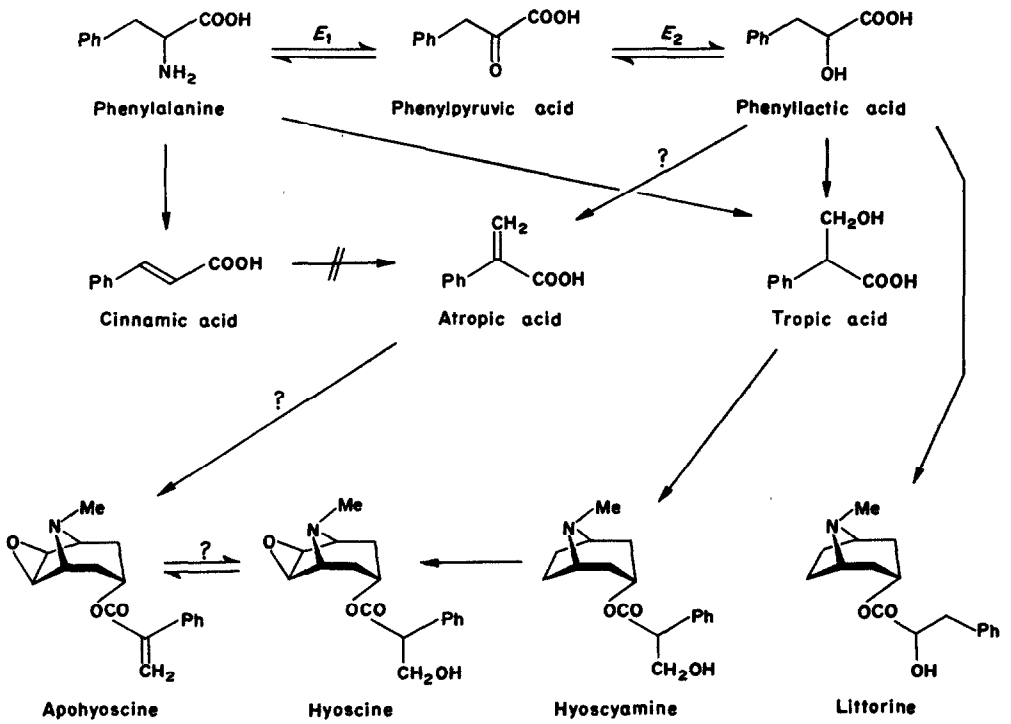
Key Word Index—*Datura innoxia*; Solanaceae; biosynthesis of tropic, atropic and phenyllactic acids; phenylalanine and cinnamic and phenyllactic acids as precursors.

Abstract—*Datura innoxia* plants were fed via the roots with cinnamic acid-[2^{14}C], (±)-phenyllactic (2-hydroxy-3-phenylpropanoic) acid-[2^{14}C] and phenylalanine-[2-^{14}C]. In each case apohyoscine, hyoscine, hyoscyamine and littorine were isolated from the aerial parts, and hyoscine, hyoscyamine and littorine from the roots. Cinnamic acid was not incorporated into the acid moieties of the alkaloids. Phenyllactic acid served as a better precursor than phenylalanine for tropic acid (hyoscine and hyoscyamine) and atropic acid (apohyoscine). Phenylalanine served as an effective precursor for the phenyllactic acid moiety of littorine.

INTRODUCTION

It is well known that all *Datura* spp. (Solanaceae) contain the tropic acid esters hyoscine and hyoscyamine. More recently, however, other C_6-C_3 esterifying acids e.g. atropic acid in apohyoscine [1] and (+)-2-hydroxy-3-phenylpropanoic acid in littorine [2,3] have been discovered (see Scheme 1 for structures). Phenylalanine is known to be a precursor of phenyllactic acid [4] but it also forms tropic acid in *Datura* [5,6] by an extraordinary

* A preliminary note concerning these findings has been published in *Abhandl. Deut. Akad. Wiss. Berlin* (1972) 227.



Scheme 1. The biosynthesis of C_6-C_3 acids in *Datura*.

rearrangement of the side-chain. The intermediates, if any, remain obscure despite many attempts to solve the problem (see references 7, 8 and 9 for reviews), and it has recently been shown [10] that the rearrangement does not occur at the ester level. Since cinnamic and trimethoxycinnamic acids are the only other C_6-C_3 acids found in association with the tropane ring system, e.g. in *Erythroxylum* spp [11,12], it was decided to compare the roles of cinnamic and phenyllactic acids in the biosynthesis of tropic and atropic acids.

RESULTS AND DISCUSSION

The alkaloids apohyoscine, hyoscine, hyoscyamine and littorine were isolated from the roots and aerial parts of plants from each tracer experiment. Cinnamic acid-[2-^{14}C] was not incorporated into any of the bases isolated and it would appear that it is not involved in the biosynthesis of tropic or atropic acids. Phenylalanine-[2-^{14}C] and phenyllactic acid-[2-^{14}C] both served as precursors for tropic and atropic acids which were degraded according to the excellent scheme devised by Leete [5]. Phenyllactic acid (from littorine) from the phenylalanine-[2-^{14}C] feeding experiment was degraded to phenylacetaldehyde [4] which was oxidized to the acid with alkaline permanganate and then decarboxylated to yield C(2) using the Schmidt reaction [13].

In all cases phenyllactic acid-[2-^{14}C] was incorporated to a higher degree into the tropic and atropic moieties than was phenylalanine-[2-^{14}C]. One may interpret these results in several ways. First, phenyllactic acid may be the rearranging acid giving tropic acid directly (Scheme 1). Secondly, if phenylpyruvic acid rearranges then the reaction rate for E_2 is faster than that for E_1. Thirdly, the higher incorporation of phenyllactic acid may merely be due to greater competition for phenylalanine. Thus, although the results establish that phenyllactic acid is a precursor of tropic and atropic acids, it is still impossible to say unequivocally which of the three compounds undergoes side-chain rearrangement.

Romeike has established that hyoscine is formed from hyoscyamine [14] and if the ratios of the specific activities of these alkaloids in the roots and aerial parts from the phenylalanine-[2-^{14}C] and phenyllactic acid-[2-^{14}C] are compared, then it is clear that more radioactivity has been carried over from hyoscyamine to hyoscine in the latter case than in the former (Table 2). This again strongly suggests that phenyllactic acid is a more efficient precursor than phenylalanine. Surprisingly, with both precursors the specific activity of 'root' hyoscine is higher than 'top' hyoscine. Whereas Romeike has concluded that hyoscine is synthesized in the aerial parts of *Datura ferox* here in *D. innoxia* the root must be considered as an alternative biosynthetic site.

When the specific activity ratios for apohyoscine/hyoscine ('root' and 'top') are examined, then one must conclude that apohyoscine is not formed from 'top' hyoscine although the former base is only present in the aerial parts. Since it is unlikely that these bases are formed via entirely different routes the most likely explanation is that 'root' hyoscine is converted into apohyoscine during translocation to the aerial parts.

Phenyllactic acid-[2-^{14}C] was prepared by the oxidation of phenylalanine-[2-^{14}C] with hot nitrous acid according to the method of Neish [15]. At ambient temperatures it has now been shown that (−)-tropic acid is produced in 2% yield from phenylalanine in nucleophilic solvents by aryl rather than carboxyl migration which occurs *in vivo* [16,17]. However, TLC/autoradiography failed to detect any tropic acid in the phenyllactic acid used and satisfactory recoveries of label were obtained by degradation.

Table 1. The specific activities of alkaloids and their degradation products from *Datura* plants fed with DL-phenylalanine-[2-^{14}C] and phenyllactic acid-[2-^{14}C]

	Phenylalanine -[2-^{14}C] Aerial parts		Phenyllactic acid -[2-^{14}C] Aerial parts		Roots
	I*	II	I	III	IV
Picrate derivative	0·32†	1·61	0·84	2·09	1·57
Tropic acid	—	—	—	1·93	1·48
Atropic acid	0·30	—	0·83	—	—
Phenyllactic acid	—	1·50	—	—	—
Oscine picrate	0	—	0	0	—
Tropine picrate	—	0	—	—	0
Formaldehyde dimedone deriv.	0·28 (88)	—	0·74 (88)	1·76 (84)	1·34 (85)
CO_2		1·54 (96)			

*I—Apohyoscine; II—littorine; III—hyoscine; IV—hyoscyamine. † Dpm/mM × 10^{-4} ‡ Percentage recoveries in parentheses.

Table 2. The ratios of specific activities of biosynthetically related alkaloids obtained from *Datura* plants fed with DL-phenylalanine-[2-^{14}C] and phenyllactic acid-[2-^{14}C]

Compounds	Phenylalanine-[2-^{14}C] Roots	Phenylalanine-[2-^{14}C] Aerial parts	Phenyllactic acid-[2-^{14}C] Roots	Phenyllactic acid-[2-^{14}C] Aerial parts
Hyoscine/hyoscyamine	$0·58 \left(\frac{0·17}{0·3}\right)$*	$0·11 \left(\frac{0·32}{2·79}\right)$	$1·22 \left(\frac{20·18}{16·47}\right)$	$0·55 \left(\frac{4·03}{7·27}\right)$
Apohyoscine/'top' hyoscine		$6 \left(\frac{1·92}{0·32}\right)$		$1·08 \left(\frac{4·35}{4·03}\right)$
Apohyoscine/'root' hyoscine		$0·30 \left(\frac{1·92}{6·32}\right)$		$0·22 \left(\frac{4·35}{20·18}\right)$

* The actual sp. act. (dpm/mM × 10^{-4}) of the alkaloids are given by the values in parentheses.

EXPERIMENTAL

Counting procedures. Duplicate samples were counted in commercially available dioxane or toluene based POP/POPOP scintillators.

Tracer compounds. DL-Phenylalanine-[2-^{14}C] was purchased from the Radiochemical Centre, Amersham, and cinnamic acid-[2-^{14}C] was obtained from Tracerlab, Waltham, Mass., U.S.A.

(±)-2-*Hydroxy*-3-*phenylpropanoic(phenyllactic acid)*-[2-^{14}C]. DL-Phenylalanine-[2-^{14}C] (30 μCi, 300 mg) in H_2O (30 ml) and 50% H_2SO_4 (20 ml) was heated to 90° with stirring, then $NaNO_2$ (500 mg) in H_2O (20 ml) was added over 1 hr. The cooled soln was basified with KOH pellets, washed several times with Et_2O, reacidified to pH 1 with 50% H_2SO_4 and extracted again with Et_2O (6 × 20 ml). After evaporation of the solvent the remaining residue was dried over P_2O_5 and recrystallised 3× from dry C_6H_6 (*ca* 3 ml) to give phenyllactic acid-[2-^{14}C] 222 mg (74% yield) mp and mmp 96°, IR (KBr) identical with authentic material, and sp. act. 3·63 × 10^7 dpm/mM. The acid was examined by TLC (Kieselgel G, cyclohexane–$CHCl_3$–HOAc (glac.) 3:1:1, locating reagent $Ce(SO_4)_2$ spray) and autoradiograms prepared by exposing the TLC plates to Ilford X-Ray G film for 2 weeks revealed only one radioactive spot corresponding to phenyllactic acid.

Feeding experiments. Forty 3-month-old *Datura innoxia* plants which had been grown in pots under glass were carefully uprooted, washed free from soil, divided into 4 groups and suspended in the appropriate tracer soln for 9 days. Group 1 received 20 μCi (200 mg) DL-phenylalanine-[2-^{14}C]; group 2, 22 μCi (220 mg) (±)-phenyllactic acid-[2-^{14}C] (as the Na salt): group 3, 25 μCi (250 mg) cinnamic acid-[2-^{14}C] (as the Na salt); and group 4, 25 μCi cinnamic acid-[2-^{14}C] (2·2 mg).

Isolation of alkaloids. The alkaloids were separated by the previously published method, [18] isolated as the picrates and crystallised to constant sp. act.

Degradation of alkaloids. All picrates were diluted to *ca* 60 mg which was found to be the minimum workable quantity. In the following typical degradation hyoscine picrate from the aerial parts of the plants fed with phenyllactic acid-[2-^{14}C] (35·44 mg) sp. act. 4·03 × 10^4 dpm/mM was diluted with 32·41 mg authentic carrier (calc. sp. act. 2·09 × 10^4 dpm/mM), made alkaline with dil NH_4OH and extracted with $CHCl_3$. After removal of the solvent the residue was redissolved in *ca* 1 ml EtOH, diluted with 5% $Ba(OH)_2$ (20 ml) and heated in a sealed tube at 100° for 3 hr. The cooled hydrolysate was acidified with 50% H_2SO_4 and extracted with Et_2O (6 × 2 ml). Evaporation of the dried solvent gave a residue which was crystallised from C_6H_6/petrol to give tropic acid (18·98 mg, 89% yield) m.p. 122°, sp. act. 1·98 × 10^4 dpm/mM. The alkamine, oscine, was recovered from the remaining hydrolysate as the picrate by established procedures [19]. Tropic acid (18 mg) was refluxed with 10 N KOH (5 ml) for 45 min under N_2. Extraction of the cooled, acidified mixture with Et_2O gave atropic acid which was not isolated, but immediately redissolved in 1 ml H_2O containing Na_2CO_3 (10 mg). The soln was cooled in ice, mixed with OsO_4 (*ca* 5 mg) and then a soln of 50 mg $NaIO_4$ in 2 ml H_2O was added drop-wise with stirring over 25 min. After 20 hr at 4° the mixture was extracted with Et_2O to remove the OsO_4, acidified with dil HCl and extracted with a further quantity of Et_2O. All attempts to prepare the oxime of phenylglyoxylic acid from this Et_2O extract failed, probably because of the extremely small quantity present. The remaining aq. phase was distilled and the distillate (*ca* 3 ml) was mixed with a soln of dimedone (40 mg) in H_2O (10 ml) and allowed to stand for 24 hr when the formaldehyde dimedone derivative formed (3·8 mg) mp 190°, sp. act. 1·76 × 10^4 dpm/mM (84% recovery of radioactivity). The remaining tropic and atropic acid esters were degraded by the same procedures. Littorine picrate (13·25 mg, sp. act. 3·3 × 10^4 dpm/mM) from the aerial parts of plants fed with DL-phenylalanine-[2-^{14}C] was diluted with authentic carrier (13·89 mg) to give sp. act. 1·61 × 10^4 dpm/mM. The base recovered from the picrate in the usual way was hydrolysed by boiling with 5% $Ba(OH)_2$ and gave phenyllactic acid, recrystallised from C_6H_6, mp 120°, sp. act. 1·50 × 10^4 dpm/mM, yield 7·2 mg (82%), and inactive tropine isolated as the picrate mp 270° (12·8 mg). Phenyllactic acid (7 mg) was decarboxylated by heating at 70° for 45 min with lead tetraacetate (20 ml) [20]. The cooled soln was filtered, evaporated to dryness under an air-stream and the residue (crude phenylacetaldehyde) was allowed to stand over-night with a soln of $KMnO_4$ (20 mg) and Na_2CO_3 (10 mg) in 2 ml H_2O. The phenylacetic acid thus produced was extracted from the filtered, acidified mixture with Et_2O, and decarboxylated by heating to 80° with conc. H_2SO_4 (5 ml) containing NaN_3 (5 mg). The liberated CO_2 was collected in freshly prepared 5% $Ba(OH)_2$ and gave 2·481 mg $BaCO_3$ which was then acidified with 50% aq. citric acid in an enclosed, evacuated vessel, the liberated CO_2 (sp. act. 1·54 × 10^4 dpm/mM) being absorbed in Hyamine10-X soln for counting.

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