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BIOSYNTHESIS OF TROPIC ACID: FEEDING EXPERIMENTS WITH CINNAMOYLTROPINE AND LITTORINE

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Abstract—The administration of cinnamoyl-[2-^{14}C]-tropine-[*N-methyl*-^{14}C] to *Datura stramonium* plants resulted in the formation of labeled atropine and scopolamine However the atropine was found to have almost all its radioactivity located on the *N*-methyl group of the alkaloid, indicating that the administered ester had undergone hydrolysis in the plant affording tropine and cinnamic acid, the latter not being utilized for the biosynthesis of tropic acid Dual labeled *RS*-littorine (3α-(2-hydroxy-3-phenylpropionyloxy-[1-^{14}C]-tropane-[3β-^{3}H]) was also fed to *D stramonium* and radioactive atropine was obtained. However the drastic change in the ^{3}H.^{14}C ratio found in the atropine indicated that the littorine was not converted directly to the alkaloid, and it is suggested that the littorine is hydrolysed *in vivo* to tropine and phenyl-lactic acid, the latter undergoing rearrangement to tropic acid prior to esterification with tropine

TROPIC ACID is found in nature as the acid moiety of the ester alkaloids atropine (**7**) and scopolamine. It was discovered in 1960[1] that the administration of phenylalanine-[3-^{14}C] to intact *Datura stramonium* plants yielded radioactive atropine having essentially all its activity located at C-2 of its tropic acid moiety Later workers confirmed this result in *D. stramonium* (intact plants)[2] and *D. metel* (sterile root culture).[3] It was then established that the other carbons of the phenylalanine side chain are utilized for the production of the side chain of tropic acid.[4,5] The pattern of labeling found in tropic acid after feeding variously labeled phenylalanines indicated that a migration of the carboxyl group from C-2 to C-3 occurs during the formation of tropic acid. By using phenylalanine-[U-^{14}C] as a precursor it was established that the phenyl group is incorporated intact into tropic acid.[6] The mechanism of the rearrangement of the side chain of phenylalanine is currently unknown. Various metabolites of phenylalanine have been examined as precursors of tropic acid. Phenylpyruvic acid (**1**) was incorporated, and in competition with phenylalanine it was almost as an efficient a precursor of tropic acid.[7] However the expected facile interconversion of these two compounds (by transamination) does not enable us to make a decision

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[1] LEETE, E (1960) *J Am Chem Soc* **82,** 612
[2] UNDERHILL, E W and YOUNGKEN, H W (1962) *J Pharm Sci* **51,** 121
[3] GROSS, D and SCHUTTE, H R (1963) *Arch Pharm* **296,** 1
[4] LEETE, E and LOUDEN, M L (1961) *Chem Ind* 1405
[5] LOUDEN, M L and LEETE, E (1962) *J Am Chem Soc* **84,** 1510, 4507
[6] GIBSON, C A and YOUNGKEN, H W (1967) *J Pharm Sci* **56,** 854
[7] (a) LIEBISCH, H W, BHAVSAR, G C and SCHALLER, H J (1969) *Biochem Physiol Alkaloide, Int Symp* (MOTHES, K, ed), 4th Edn, p 233, Akad, Berlin, E Germany, (b) LIEBISCH, H W (1970) Abstracts of the *7th Int Sympos on the Chem Natural Products*, Riga, USSR, p 557

as to which is biosynthetically closer to tropic acid The same remarks apply to phenyllactic acid (**3**), the reduction product of phenylpyruvic acid This acid is esterified with tropine in the alkaloid littorine (**5**), and phenylalanine was shown to be its precursor in *D sanguinea*[8] and *D innoxia*.[9] Phenyl-lactic acid is also a good precursor of tropic acid[7,9] and it was reported to be superior to phenylalanine in competitive feeding experiments

Cinnamic acid (**2**), a metabolite of phenylalanine in many plant species, was considered to be a prime candidate as an intermediate between phenylalanine and tropic acid.[10] In a model system it has been shown that phenyl *trans*-3-phenylthiolglycidate (**4**) rearranges on treatment with boron trifluoride etherate to 2-formylphenylthiol acetate (**6**)[11,12] This reaction involves a 1,2-migration of a thiol ester group, analogous to the migration of the carboxyl group which occurs during the biosynthesis of tropic acid *in vivo*. However cinnamic acid[7b,9] or its epoxide[13] failed to serve as precursors of tropic acid in *Datura* species

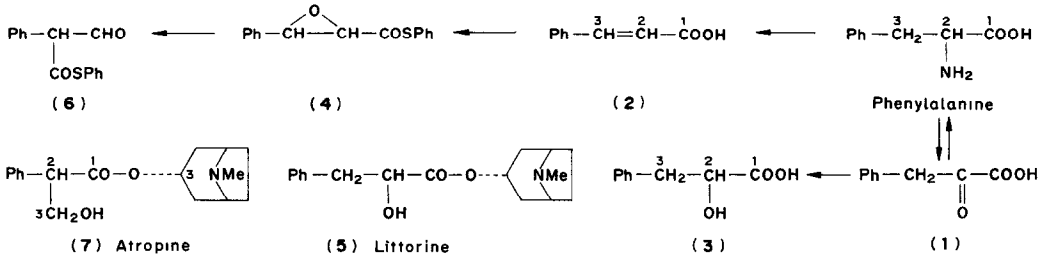
It was considered that perhaps rearrangement of the C_6 C_3 skeleton occurs after esterification of tropine with an acid derived from phenylalanine Cinnamoyl-tropine was thus prepared labeled with ^{14}C at C-2 of its cinnamoyl moiety, and on the *N*-methyl group of the tropine base This doubly labeled compound was fed to *D stramonium* plants by the wick method. After 5 days the plants were harvested and yielded radioactive atropine and scopolamine. However hydrolysis of the alkaloids yielded tropic acid having low activity (see Experimental), the bulk of the activity residing in the tropine bases Demethylation of the tropine obtained by hydrolysis of the atropine with phenyl chloroformate[14] yielded inactive *N*-carbophenoxynortropine. These results are consistent with the cinnamoyl-tropine being hydrolysed in the plant to tropine and cinnamic acid, the latter not being converted to tropic acid The incorporation of radioactivity into atropine and scopolamine based on the ^{14}C present in the tropine moiety of the administered cinnamoyl-tropine was 0·94 and 0·91% respectively

We then examined the ability of littorine to serve as a direct precursor of atropine. Phenyl-lactic-[1-^{14}C] acid was prepared from glycine-[1-^{14}C] by the sequence glycine → *N*-acetylglycine → azlactone of *N*-acetyl-α-aminocinnamic acid → phenylpyruvic acid → phenyl-lactic acid using modifications of established procedures[15] This phenyllactic acid was esterified with tropine yielding littorine[16] This material was mixed with littorine prepared from tropine-[3β-^{3}H][14] to afford dual labeled littorine ($^{3}H \cdot ^{14}C = 6\,75$) which was fed as before to *D stramonium* plants After 7 days the plants yielded radioactive atropine ($^{3}H \cdot ^{14}C = 33$) Hydrolysis of this atropine afforded tropine (labeled only with ^{3}H) which on oxidation to tropinone lost all its radioactivity indicating that all the tritium was located at C-3 The tropic acid was degraded as previously described[5] and was found to have essentially all its activity located on the carboxyl group The drastic change in the ^{3}H ^{14}C ratio of the isolated atropine indicates that the phenyl-lactic acid moiety of littorine does not undergo a rearrangement to tropic acid whilst it is esterified with tropine The specific labeling of the tropine and tropic acid with tritium and ^{14}C respectively

[8] EVANS, W C and WOOLLEY, V A (1969) *Phytochemistry* **8**, 2183
[9] EVANS, W C, WOOLLEY, J G and WOOLLEY, V A ref 7a p 227
[10] SPENSER, I D (1968) in *Comprehensive Biochemistry* (FLORKIN, M and STOTZ, E H eds) Vol 26, p 294 Elsevier, Amsterdam
[11] WEMPLE, J N (1970) *J Am Chem Soc* **92**, 6694
[12] DOMAGALA, J and WEMPLE, J (1973) *Tetrahedron Letters* 1179
[13] LETTE, E and BRAUNSTEIN, J D unpublished work
[14] LETTE, E (1972) *Phytochemistry* **11**, 1713
[15] HERBST, R M and SHEMIN, D (1943) *Organic Syntheses* (BLATT, A H, ed), Coll Vol 2, pp 1 11, 519
[16] TAKEUCHI, Y, KOGA, K SHIOIRI, T and YAMADA, S (1971) *Chem Pharm Bull (Japan)* **19**, 2603

strongly suggests that the littorine is hydrolysed in the plant to tropine and phenyl-lactic acid, the latter undergoing rearrangement to tropic acid prior to esterification with tropine to yield atropine.

Our results could also be rationalized by postulating that the administered radioactive littorine undergoes a facile reversible hydrolysis in the plant, the resultant tropine and phenyl-lactic acid being then diluted with non-radioactive pools of these materials already present in the plant. It has been demonstrated that esters of tropane alcohols are readily hydrolysed in *Datura* and related species.[17–19] The resynthesized littorine would then be expected to have a different $^{3}H:^{14}C$ ratio from the administered alkaloid. Rearrangement of the phenyl-lactic acid moiety of the littorine could then yield atropine having a $^{3}H:^{14}C$ ratio different from the administered littorine. This explanation seems unlikely since the littorine isolated from the plant at the end of the feeding experiment had essentially the same specific activity and $^{3}H:^{14}C$ ratio (6·9) as the administered alkaloid



Hypothetical metabolism of phenylalanine in *Datura*

EXPERIMENTAL

General methods Radioactivity measurements were carried out in a Nuclear Chicago Mark II liquid scintillation counter Radioactive compounds were dissolved in 1–2 ml EtOH or H_2O and diluted with 10 ml dioxane soln containing naphthalene (10%), 2,5-diphenyloxazole (0 7%), and 1,4-bis-2-(5-phenyloxazolyl)benzene (0 05%)

Cinnamoyl-[2-^{14}C]-tropine-[N-methyl-^{14}C] Cinnamic-[2-^{14}C] acid (ICN, Irvine, Calif, nominal activity 0 2 mCi, 85 mg 0 575 mM) dissolved in light petrol (10 ml) was refluxed with thionyl chloride (5 ml) for 3 hr The reaction mixture was evaporated, the residue dissolved in petrol and the evaporation repeated Tropine (100 mg, 0 71 mM) was dissolved in dry ether and the soln saturated with HCl gas Evaporation yielded tropine hydrochloride which was mixed with the cinnamoyl chloride and the mixture heated at 100° for 6 hr After cooling, the reaction mixture was dissolved in 2 N H_2SO_4, filtered and the filtrate made basic with Na_2CO_3 when cinnamoyl-[2-^{14}C]-tropine monohydrate separated (115 mg, 7 8 × 10^8 dpm/mM) m p 45° (lit [20] m p 45–46°) Cinnamoyl-tropine-[N-*methyl*-^{14}C] was similarly prepared using cinnamoyl chloride and tropine-[N-*methyl*-^{14}C] [14]

Administration of cinnamoyl-tropine to D stramonium *plants and isolation of the alkaloids* For administration to the plants a mixture of cinnamoyl-[2-^{14}C]-tropine (24 3 mg, 6 57 × 10^7 dpm) and cinnamoyl-tropine-[N-*methyl*-^{14}C] (21 1 mg, 8 75 × 10^7 dpm) was dissolved in H_2O (6 ml) containing 0 1 ml 2 N H_2SO_4 This soln was administered to 7 *D stramonium* plants (3-months-old) by means of cotton wicks inserted into the stems near to ground level After 5 days the whole plants (fr wt 250 g) were extracted as previously described,[1] inactive atropine (1 mM) and scopolamine (1 mM) being added as carriers to facilitate the isolation of the labeled alkaloids The crude alkaloids had an activity of 2 3 × 10^7 dpm (15% of the activity fed to the plants) TLC on a sample of this mixture (on Silica gel G, developing with $CHCl_3$–EtOH–conc NH_3, 85 14 1) showed the presence of the following alkaloids having the indicated distribution of radioactivity atropine (R_f 0 3) (15 5%), cinnamoyl-tropine (R_f 0 72) (71%), and scopolamine (R_f 0 85) (13 6%) The alkaloids were separated by partition chromatography on a celite column,[1] atropine (8 2 × 10^5 dpm/mM) and scopolamine (8 0 × 10^5 dpm/mM) being obtained

[17] Neumann, D and Tschope, K H (1966) *Flora (Jena)* **156,** 521
[18] Kaczkowski, J (1966) *Abh. Dtsch. Akad. Wiss. Berlin, Kl. Chem. Geol. Biol.* Nr. **3,** 521
[19] Achari, R, Evans, W C and Newcombe, F (1969) *Naturwissenschaften* **56,** 88
[20] Jowett, H A D and Pyman, F L (1909) *J Chem Soc* **95,** 1020

Degradation of the alkaloids Hydrolysis of the atropine yielded tropic acid (<0·02 × 10^5 dpm/mM) and tropine (8·1 × 10^5 dpm/mM). Reaction of the tropine with phenyl chloroformate[14] afforded *N*-carbophenoxynortropine (0·04 × 10^5 dpm/mM). The scopolamine on hydrolysis yielded tropic acid (<0·03 × 10^5 dpm/mM) and oscine (8·05 × 10^5 dpm/mM).

RS-*Phenyl-lactic*-[1-^{14}C] *acid*. Glycine-[1-^{14}C] (Amersham-Searle, nominal activity 0·5 mCi, 101 mg, 1·35 mM) was dissolved in H_2O (1 ml) and Ac_2O (1·1 ml) added. After stirring for 1 hr at room temp the mixture was diluted with H_2O and extracted with pentane in a continuous extractor for 18 hr. The aq. soln was then neutralized with NaOH and evaporated to dryness. The residual sodium aceturate was dissolved in a mixture of Ac_2O (2 ml) and benzaldehyde (0·5 ml) and refluxed for 1·5 hr in N_2. The reaction mixture was cooled and diluted with H_2O (2 ml) when the azlactone of *N*-acetyl-α-amino-cinnamic acid separated (72 mg). Recrystallization from CCl_4 afforded the azlactone as yellow needles (63·1 mg), m.p. 148·5–149·5° (lit.[15] m.p. 148–150°). This labeled azlactone (61·4 mg) was diluted with inactive material (52·2 mg) and refluxed in 2 N HCl (4 ml) in N_2 for 2 hr. The cooled, filtered soln was extracted with Et_2O (4 × 10 ml) which was then washed with satd. NaCl soln and evaporated to dryness. The residual crude phenylpyruvic-[1-^{14}C] acid was dissolved in a mixture of satd. Na_2CO_3 soln (5 ml) and H_2O (2 ml), cooled to 0°, and $NaBH_4$ (81 mg) added. After stirring for 2 hr the soln was acidified with HCl, and extracted with CH_2Cl_2 for 12 hr. The residue obtained on evaporation of the extract was dried by azeotroping off the H_2O with C_6H_6 and finally crystallized from CCl_4 to yield phenyl-lactic-[1-^{14}C] acid as colorless plates (75·9 mg), m.p. 94·5–96° (lit.[21] m.p. 94–95°), having a sp. act. of 5·15 × 10^8 dpm/mM (22% radiochemical yield from glycine-[1-^{14}C]).

RS-*Littorine*-[^{14}C] {3α(2-*hydroxy*)-3-*phenylpropionyloxy*-[1-^{14}C])-*tropane*}. Phenyl-lactic-[1-^{14}C] acid (58 mg, 0·35 mM, 5·15 × 10^8 dpm/mM) was mixed with tropine (56·7 mg, 0·41 mM) and the mixture heated at 120° in a current of dry HCl gas. After 4·5 hr the mixture was cooled and the pale yellow gum stirred with 10% aq. NH_3 (10 ml). This aqueous solution was extracted with CH_2Cl_2 (4 × 10 ml) which was then extracted with 2 N HCl (2 × 10 ml) and H_2O (10 ml). The combined aqueous extracts were brought to pH 8 by the addition of Na_2CO_3 and extracted with Et_2O. Evaporation yielded littorine as a colorless oil (67 mg) which was converted to its picrate and crystallized from EtOH (80·3 mg), m.p. 158·5–160° (lit.[16] m.p. 159·5–160°) (sp. act. 4·6 × 10^8 dpm/mM). Littorine-[^{3}H] was prepared similarly from phenyllactic acid and tropine-[3β-^{3}H][14] and had a specific activity of 1·02 × 10^9 dpm/mM.

Administration of RS-3α-(2-*Hydroxy*-3-*phenylpropionyloxy*-[1-^{14}C])-*tropane*-[3β-^{3}H] *to D. stramonium and isolation of the alkaloids*. *RS*-Littorine-[^{14}C] picrate (80 mg, 0·154 mM) and *RS*-littorine-[^{3}H] picrate (246 mg, 0·467 mM) were dissolved in 2 N HCl (60 ml) and the soln extracted with Et_2O until free of picric acid. The aq. soln was evaporated to dryness *in vacuo* at 30° and the residue exposed to high vacuum to remove traces of HCl and H_2O. The residual littorine hydrochloride was dissolved in H_2O (10 ml) and assayed. The soln contained ^{14}C 7·05 × 10^7 dpm, ^{3}H 4·76 × 10^8 dpm (^{3}H/^{14}C = 6·75). The sp. act. (^{14}C) 1·13 × 10^8 dpm/mM. This aqueous solution was administered to six *D. stramonium* plants (3-months-old) as before. The plants were harvested 7 days later (fr. wt. 695 g) and the alkaloids isolated without the addition of inactive atropine. Partition chromatography of the crude alkaloids (^{14}C 2·1 × 10^7 dpm, ^{3}H/^{14}C = 7·1) on a celite column[1] yielded a mixture of atropine and littorine which was separated by TLC on Silica gel PF-254, developing with $CHCl_3$–EtOH (19:1) saturated with anhydrous NH_3. The littorine (43 mg) was converted to its picrate and crystallized to constant activity: ^{14}C 1·14 × 10^8 dpm/mM (^{3}H/^{14}C = 6·9). The atropine (51·6 mg) was sublimed (95°, 5 × 10^{-4} mm) and crystallized to constant activity as its hydrobromide: ^{14}C 6·80 × 10^5 dpm/mM, ^{3}H 2·26 × 10^7 dpm/mM (^{3}H/^{14}C = 33).

Degradation of the atropine. Hydrolysis of the atropine yielded tropine assayed as its HBr salt (^{3}H 2·02 × 10^7 dpm/mM, negligible ^{14}C) and tropic acid (^{14}C 6·82 × 10^5 dpm/mM, negligible ^{3}H). The tropine dissolved in CH_2Cl_2 was oxidized with a soln of CrO_3 in pyridine[22] affording tropinone assayed as its picrate (negligible activity). The tropic acid was degraded as previously described.[5] Dehydration afforded atropic acid (6·54 × 10^5 dpm/mM) which was oxidized with $NaIO_4$ and OsO_4 yielding formaldehyde (inactive) and phenylglyoxylic acid isolated as its oxime (6·9 × 10^5 dpm/mM). This oxime was decomposed in boiling H_2O yielding CO_2 collected as $BaCO_3$ (6·64 × 10^5 dpm/mM) and benzonitrile which was hydrolysed to benzoic acid (inactive).

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[21] LIEBISCH, H. W., BHAVSAR, G. C. and SCHUTTE, H. R. (1971) *Z. Chem.* **12**, 220.
[22] RATCLIFFE, R. and RODEHORST, R. (1970) *J. Org. Chem.* **35**, 4000.