Phytochemistry, Vol. 22, No. 4, pp. 933–935, 1983.
Printed in Great Britain.

0031–9422/83/040933–03$03.00/0

FAILURE OF 3-HYDROXY-3-PHENYLPROPANOIC ACID AND CINNAMIC ACID TO SERVE AS PRECURSORS OF TROPIC ACID IN *DATURA INNOXIA**

EDWARD LEETE

Natural Products Laboratory,† Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, U.S.A.

(*Received* 13 *July* 1982)

Key Word Index—*Datura innoxia*; Solanaceae; biosynthesis; tropic acid; phenylalanine precursor.

Abstract—*Datura innoxia* plants were fed the *R*- and *S*-isomers of [3-^{14}C]-3-hydroxy-3-phenylpropanoic acid, and [3-^{14}C]cinnamic acid along with DL-[4-^{3}H]phenylalanine. The hyoscyamine and scopolamine isolated from the plants 7 days later were labeled with tritium, but devoid of ^{14}C, indicating that 3-hydroxy-3-phenylpropanoic acid and cinnamic acid are not intermediates between phenylalanine and tropic acid. The [^{3}H]tropic acid obtained by hydrolysis of the hyoscyamine was degraded and shown to have essentially all its tritium located at the *para* position of its phenyl group, a result consistent with previous work.

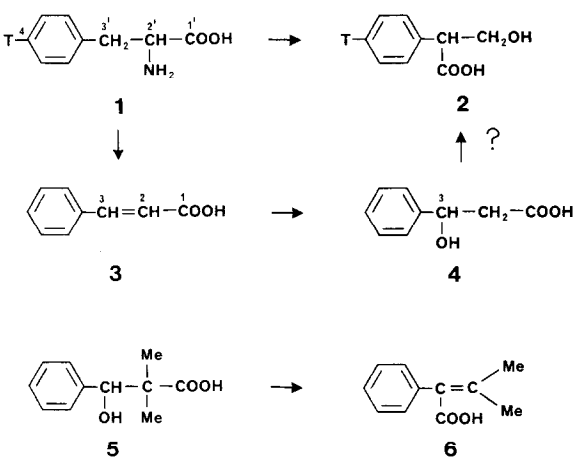
INTRODUCTION

It has been well established that tropic acid (**2**), the acid moiety of the alkaloids hyoscyamine and scopolamine, is formed from phenylalanine (**1**) by an intramolecular rearrangement of the side chain, the carboxyl group migrating from C-2′ to C-3′ [1]. No definitive work has appeared to clarify the mechanism of this remarkable rearrangement. Recently an analogous rearrangement has been demonstrated *in vitro* [2]. 3-Hydroxy-2,2-dimethyl-3-phenylpropanoic acid (**5**) was converted to 3-methyl-2-phenyl-2-butenoic acid (**6**) with superacid (HSO_3F), the 1,2-migration of the carboxyl group being intramolecular. We thus considered that 3-hydroxy-3-phenylpropanoic acid (**4**) would be a plausible intermediate between phenylalanine and tropic acid. It could be formed by the hydration of cinnamic acid (**3**) which is derived from phenylalanine by the action of phenylalanine-ammonia lyase. Labeled **4** was prepared from [3-^{14}C]cinnamic acid. Reaction of the cinnamic acid with hydrogen bromide yielded 3-bromo-3-phenylpropanoic acid, which was converted to **4** by boiling in water [3]. It was optimistically anticipated that only one of the enantiomers of **4** would be converted to tropic acid which occurs in the tropane alkaloids as the *S*-isomer [4, 5]. Accordingly, **4** was resolved via the morphine [6] and brucine [7] salts. The (−)-isomer has the *S* configuration [8]. These *R*- and *S*-isomers of [3-^{14}C]-3-hydroxy-3-phenylpropanoic acid were fed to *Datura innoxia* plants. DL-[4-^{3}H]Phenylalanine [9] was fed to the plants at the same time, so that the relative efficiency of 3-hydroxy-3-phenylpropanoic acid and phenylalanine as precursors of tropic acid could be measured.

Conflicting results have been reported on the conversion of cinnamic acid to tropic acid. Liebisch [10] and Evans [11, 12] reported that labeled cinnamic acid failed to serve as a precursor of tropic acid in *Datura* species. We fed [2-^{14}C]cinnamoyl-[*N*-methyl-^{14}C]tropine to *D. stramonium* plants [13]. Activity was found in both hyoscyamine and scopolamine, however, all of the activity was located in the *N*-methyl groups of the alkaloids. It was apparent that the administered ester had undergone hydrolysis to tropine and cinnamic acid, the latter not being utilized for the biosynthesis of tropic acid. Recently Prabhu *et al.* [14, 15] claimed that the administration of [2-^{14}C]cinnamic acid to *D. innoxia* plants yielded labeled atropine (*RS*-hyoscyamine) with activity present in the hydroxymethyl group of its tropic acid moiety. In view of this conflict we have repeated the feeding of cinnamic acid (labeled with ^{14}C at C-3), administered along with DL-[4-^{3}H]phenylalanine as an internal control.

RESULTS AND DISCUSSION

Details of the feeding experiments are recorded in Table 1. In each experiment the ratio of ^{3}H–^{14}C was 3.23. The plants were fed by the wick method and harvested after 7 days. To facilitate isolation and purification of the alkaloids, non-radioactive hyoscyamine and scopolamine were added at the time of extraction of the fresh plants



*Part 32 in the series "Chemistry of the Tropane Alkaloids". For Part 31 see Leete, E. (1983) *Phytochemistry* **22**, 699.

†Contribution No. 186 from this laboratory.

Table 1. Compounds administered to *Datura innoxia* and activity of the isolated alkaloids

Expt No.	Compound fed	Fr. wt plants(g)	Crude alkaloids ^3H–^{14}C	[^{3}H]Activity of the alkaloids (dpm/mmol × 10^{-5})*	
				Hyoscyamine	Scopolamine
1	*R*-(+)-[3-^{14}C]-3-Hydroxy-3-phenylpropanoic acid (33.2 mg, 0.2 mM, 7.28×10^7 dpm)	260	5.2	10.9 [0.23%]	9.29 [0.20%]
2	*S*-(−)-[3-^{14}C]-3-Hydroxy-3-phenylpropanoic acid (33.2 mg, 0.2 mM, 7.28×10^7 dpm)	240	5.1	9.32 [0.20%]	8.97 [0.19%]
3	[3-^{14}C]Cinnamic acid (29.6 mg, 0.2 mM, 7.28×10^7 dpm)	190	12.3	6.13 [0.13%]	9.02 [0.19%]

The above compounds were fed along with DL-[4-^{3}H]phenylalanine (3.0 mg, 0.018 mM, 2.35×10^8 dpm).
*Absolute incorporation values are given in brackets.

with chloroform. The crude alkaloids from all three experiments contained a significant amount of ^{14}C, however, subsequent separation of the alkaloids (by TLC on Si gel) and crystallization of their hydrochlorides yielded scopolamine and hyoscyamine which were devoid of ^{14}C. In all cases the absolute incorporation of ^{3}H into the alkaloids was good (0.13–0.23%). The hyoscyamine obtained from experiment 3 was degraded to determine the location of the ^{3}H. Hydrolysis yielded tropine (no ^{3}H) and tropic acid. The tropic acid was oxidized with permanganate to yield benzoic acid which was subjected to a Schmidt reaction affording aniline which was isolated as acetanilide. Bromination yielded *p*-bromoacetanilide containing less than 1% of the ^{3}H initially present in the tropic acid, indicating that essentially all the ^{3}H was located in the *para*-position of the tropic acid. This result is consistent with earlier work [16] in which it was established that the phenyl group of phenylalanine is incorporated into tropic acid. Our failure to observe the incorporation of [3-^{14}C]cinnamic acid into tropic acid is in agreement with all the previous work except that of Prabhu *et al.* [14, 15]. One possible explanation of the results obtained by these workers is that the isolated atropine was radiochemically impure, contaminated with some cinnamic acid derivative. The degradation carried out by Prabhu did not unequivocally determine the activity on the hydroxymethyl group of tropic acid. The tropic acid was oxidized to benzoic acid (inactive) and carbon dioxide (two equivalents), having half the specific activity of the tropic acid. A second sample of tropic acid was refluxed in quinoline in the presence of cupric oxide yielding carbon dioxide (inactive) and styrene (+ polystyrene), the latter having most of the activity of the original tropic acid. [2-^{14}C]Cinnamic acid subjected to this degradation would have given the same radiochemical results. The failure of 3-hydroxy-3-phenylpropanoic acid to serve as a precursor of tropic acid must be taken into account in elaborating a mechanism for the rearrangement of the phenylalanine side chain.

EXPERIMENTAL

General methods. Mps are corr. Radioactive materials were assayed by liquid scintillation counting using dioxane–EtOH as solvent with the usual scintillators [17]. *D. innoxia* seeds were obtained from the Zentral Institut für Genetick und Kulturpflanzenforschung, Gatersleben, East Germany.

(RS-[3-^{14}C]-3-*Hydroxy-3-phenylpropanoic acid* (**4**). [3-^{14}C]Cinnamic acid (296 mg, 2 mM, nominal activity 0.25 mCi) (Research Products International) was dissolved in a mixture of 48% HBr in H_2O (3 ml) and HOAc (10 ml) and cooled to 0°. The soln was satd with HBr gas. After 18 hr the mixture was evaporated to dryness and the residue refluxed in H_2O (20 ml) for 1 hr. The soln was cooled, when cinnamic acid (56 mg) separated. TLC of this material, on Si gel PF 254, developing with C_6H_6–HOAc (9:1) indicated that 99.8% of the activity was coincident with a spot having the same R_f as authentic cinnamic acid (R_f 0.6). This recovered cinnamic acid was used in the subsequent feeding expt. The aq. soln from which the cinnamic acid had been removed was extracted with Et_2O. The residue obtained on evaporation was crystallized from C_6H_6 to yield *RS*-[3-^{14}C]-3-hydroxy-3-phenylpropanoic acid (180 mg, 54%).

(R) *and* (S)-[3-^{14}C]-3-*Hydroxy-3-phenylpropanoic acid.* The *RS*-acid (180 mg) and morphine (309 mg) were dissolved in hot H_2O (20 ml). On cooling, the morphine salt of the *S*-(−)-3-hydroxy-3-phenylpropanoic acid separated (160 mg) mp 211–212° (lit. [6], mp 200°). This morphine salt was suspended in a little H_2O and made basic with NH_3. The morphine which separated was filtered off and the filtrate acidified with HCl. The soln was extracted with Et_2O which was evaporated (without drying) and the residue crystallized from C_6H_6 to yield *S*-(−)-[3-^{14}C]-3-hydroxy-3-phenylpropanoic acid (46.2 mg) mp 115–116°. Material obtained from a cold run had $[\alpha]_D^{25}$ −18.9° (EtOH; *c* 3.2). The aq. mother liquor obtained after the separation of the morphine salt of the (−)-acid was made basic with NH_3, the morphine filtered off and then acidified. The crude (+)-acid was extracted with Et_2O. Brucine (240 mg) was added to the soln of this acid in EtOAc (10 ml). On standing the brucine salt of the (+)-acid separated (190 mg). This was decomposed with NH_3 as described for the morphine salt of the (−)-acid, affording *R*-(+)-[3-^{14}C]-3-hydroxy-3-phenylpropanoic acid (51.2 mg) mp 117–118°. Material obtained from a cold run had $[\alpha]_D^{25}$ +18.0° (EtOH; *c* 2.4).

Administration of labeled compounds to D. innoxia *plants and isolation of alkaloids.* The amounts of labeled compounds and their activities are recorded in Table 1. All three expts were carried out at the same time. Each separate expt involved five *D. innoxia* plants which were 3 months old, growing in soil in a greenhouse. Cotton wicks were inserted in the stems near to ground level. Feeding was started on 1st June and after 7 days the plants were harvested and chopped up in a Waring blendor with a mixture of $CHCl_3$ (750 ml), Et_2O (750 ml) and conc. NH_3 (50 ml). At this time hyoscyamine (0.5 mM) and scopolamine (0.5 mM) were

added to the plant extract. In all expts, the residual activity (both ^{14}C and ^{3}H) left in the beakers supplying aq. solns of the labeled compounds to the plants, was $< 0.01\%$ of the amount initially fed. The mixture of alkaloids was isolated from the organic layer as previously described [18] and then separated by TLC on Si gel PF-254 (Merck) developing with $CHCl_3$–EtOH–conc.NH_3 (80:20:1). In this system hyoscyamine and scopolamine have an R_f of 0.2 and 0.7, respectively, (visible as dark zones in UV). The alkaloids were extracted from the Si gel with MeOH which was then acidified with HCl. The resultant alkaloid hydrochlorides were crystallized several times from a mixture of EtOH and Et_2O.

Degradation of hyoscyamine hydrochloride obtained from expt 3. The hyoscyamine hydrochloride (6.13×10^5 dpm/mmol [^{3}H]) was hydrolysed with aq. 10% NaOH yielding tropine (inactive) and tropic acid (5.90×10^5 dpm/mmol) [19]. The tropic acid was oxidized with permanganate [19] yielding benzoic acid (5.81×10^5 dpm/mmol). The benzoic acid was degraded as previously described [9] affording acetanilide (5.40×10^5 dpm/mmol) and *p*-bromoacetanilide (0.05×10^5 dpm/mmol).

Acknowledgement—This work was supported by a research grant GM-13246 from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

1. Leete, E., Kowanko, N. and Newmark, R. A. (1975) *J. Am. Chem. Soc.* **97**, 6826.
2. Berner, D., Cox, D. P. and Dahn, H. (1982) *J. Am. Chem. Soc.* **104**, 2631.
3. Fittig, R. and Binder, F. (1879) *Justus Liebigs Ann. Chem.* **195**, 131.
4. Fodor, G. and Csepreghy, G. C. (1961) *J. Chem. Soc.* 3222.
5. Watson, M. B. and Youngson, G. W. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1597.
6. McKenzie, A. and Humphries, H. B. P. (1910) *J. Chem. Soc.* **97**, 121.
7. Kenyon, J., Philips, H. and Shutt, G. R. (1935) *J. Chem. Soc.* 1663.
8. Schöpf, C. and Wüst, W. (1959) *Justus Liebigs Ann. Chem.* **626**, 1950.
9. Leete, E., Bowman, R. M. and Manuel, M. F. (1971) *Phytochemistry* **10**, 3029.
10. Liebisch, H. W. (1970) *Abstr. 7th IUPAC Meeting on Natural Products.* Riga, USSR, p. 557.
11. Evans, W. C., Woolley, J. G. and Woolley, V. A. (1972) *Abhandl. Deut. Akad. Wiss. Berlin* 227.
12. Evans, W. C. and Woolley, J. G. (1976) *Phytochemistry* **15**, 287.
13. Leete, E. and Kirven, E. P. (1974) *Phytochemistry* **13**, 1501.
14. Prabhu, B. V., Gibson, C. A. and Schramm, L. C. (1976) *Lloydia* **39**, 79.
15. Prabhu, B. V. (1975) *Diss. Abstr.* **35**, 4974-B.
16. Gibson, C. A. and Youngken, H. W. (1967) *J. Pharm. Soc.* **56**, 854.
17. Friedman, A. R. and Leete, E. (1963) *J. Am. Chem. Soc.* **85**, 2141.
18. Leete, E. (1972) *Phytochemistry* **11**, 1713.
19. Leete, E. (1960) *J. Am. Chem. Soc.* **82**, 612.