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Biosynthesis of Tropic Acid in *Datura innoxia* Root Tissue

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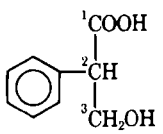
Abstract □ DL-Phenylalanine-1-^{14}C, phenylacetic acid-1-^{14}C, DL-tryptophan-(2-indolyl)-^{14}C, DL-tryptophan-(benzene ring)-U-^{14}C, L-serine-3-^{14}C, and formic acid-^{14}C were all utilized by *Datura innoxia* root tissue as precursors of tropic acid. All of these compounds were incorporated in a specific manner.

Keyphrases □ *Datura innoxia*—biosynthesis of tropic acid, studied using various radiolabeled precursors □ Tropic acid—biosynthesis in *Datura innoxia* root tissue, studied using various radiolabeled precursors □ Alkaloid biosynthesis—*Datura innoxia* root tissue, tropic acid pathways studied

Tropic acid (I) is a constituent of the alkaloids scopolamine (hyoscine) and hyoscyamine. Phenylalanine, phenylacetic acid, and tryptophan have been demonstrated to be the most efficient precursors for this acid. In contrast to phenylalanine (1–7), phenylacetic acid (1) and tryptophan (8) have received relatively little attention by subsequent investigators.

It is obvious that phenylacetic acid requires the addition of one carbon atom (Scheme I) for incorporation into tropic acid. Previous attempts (1) to clarify the origin of the one-carbon fragment using sodium bicarbonate-^{14}C or sodium formate-^{14}C resulted in random labeling of tropic acid. These experiments were, however, conducted on whole plants. The results probably were due to photosynthetic fixation of these compounds into precursors of the aromatic amino acids, resulting in randomization of the label. It is known that sodium formate rapidly degrades to carbon dioxide and water *in vivo*.

In the present investigation, sources of "active formate" were generated within the plant. These sources were serine-3-^{14}C and tryptophan-(2-indolyl)-^{14}C, which produce formate during their normal metabolic degradation (Scheme II). Since tryptophan could also be incorporated into tropic acid *via* another route (8) (Scheme III), it was expected to yield tropic acid labeled in positions C_1 and C_3. Formic acid-^{14}C was also investigated as a source of the C_1 of tropic acid. Vacuum infiltration of root tissue of *Datura innoxia* was used to eliminate the problem of photosynthesis and to lessen the required metabolism time. The biogenesis of the alkaloids occurs primarily in root tissue. Degradation to determine the position of the label was done by known methods, and liquid scintillation counting was employed to determine the activity of the degradation products.

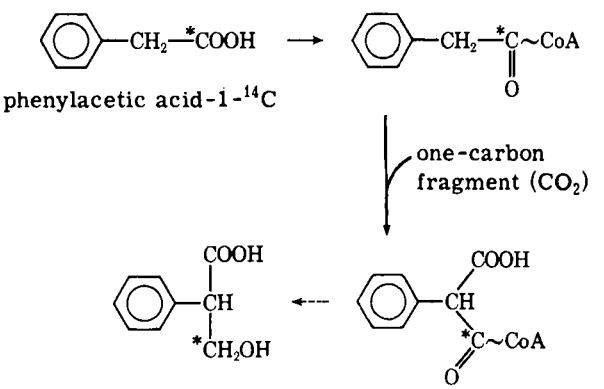


I

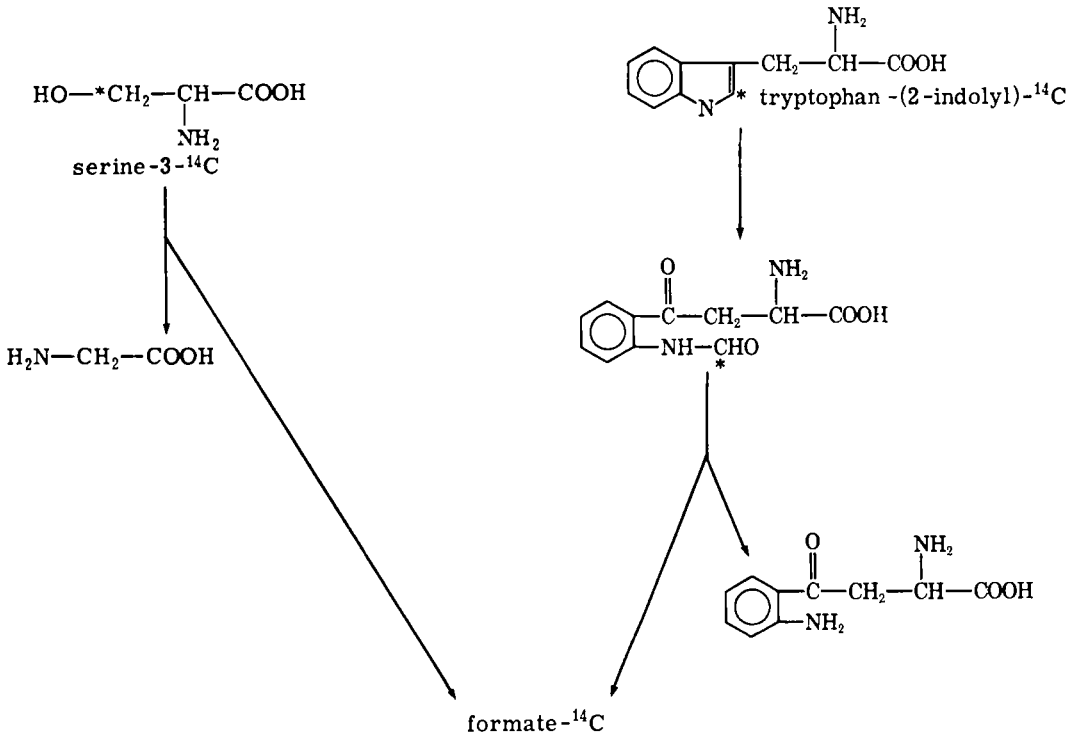
EXPERIMENTAL

Growth Conditions—*D. innoxia* plants were germinated and grown in a controlled-environment room which maintained them at 29.4° (85° F.) for 16 hr. under 1200 ftc. of light followed by 18.3° (65° F.) for 8 hr. in darkness. Relative humidity was 60 and 75%, respectively.

Isotope Administration—Plants (90 days old) were removed from their pots. The root tissue was removed and washed carefully with tap water followed by sterile distilled water, and the secondary roots were weighed into 2-g. samples. Two separate 2-g. samples

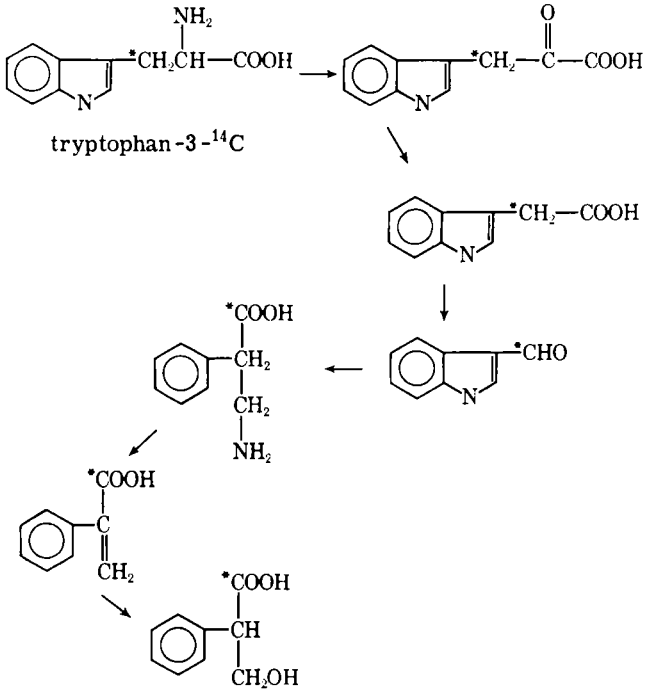


Scheme I—Incorporation of phenylacetic acid into tropic acid



Scheme II—Labeled formate production from tryptophan-(2-indolyl)-^{14}C and serine-3-^{14}C

were used for each radioactive compound administered. Each root sample was placed in a small beaker and weighted down by glass rods. The radioactive compounds were added with a known amount of sterile distilled water. The samples were placed in a vacuum desiccator, and the pressure inside was reduced using a vacuum pump. When sufficient air was removed from the tissue, the vacuum was released. Metabolism was then allowed to continue for 4 hr. The choice of a 4-hr. metabolism period was based on preliminary work in which the incorporation of phenylalanine-1-^{14}C into tropic acid was studied as a function of time. Each root sample was then removed, washed with sterile distilled water, and frozen with liquid nitrogen. The amount of uptake of the radioisotope was determined by the difference in activity between the original and the remaining solutions of radioactive compounds.



Scheme III—Incorporation of tryptophan-3-^{14}C into tropic acid (8)

Alkaloid Isolation—Each root sample was ground in a glass mortar with sand, using a mixture of 10% ammonium hydroxide–ethanol–ethyl ether (1:1:2) as a moistening agent. The resulting pulp was placed in a soxhlet apparatus and macerated for 18 hr. with more of this mixture. Ethyl ether was then added and extraction was carried out for 24 hr.

The ether extracts were transferred to a separator and shaken with successive 20-, 10-, and 10-ml. portions of 0.5 *N* H_2SO_4. These acid portions were combined, made distinctly alkaline with dilute ammonium hydroxide, and extracted with successive 20-, 10-, and 10-ml. portions of chloroform. These chloroform extracts were combined, evaporated to dryness, and redissolved in ethanol.

Chromatography—Scopolamine and hyoscyamine were purified by separation of the crude alkaloid extract on silica gel G layers, using dimethylformamide–diethylamine–ethanol–ethyl acetate (1:1:6:12) as the solvent. The positions of radioactive spots were determined using a radiochromatogram scanner.

Table I—Incorporation of Precursors into the Combined Alkaloid Fraction and Tropic Acid

Precursor	Amount of ^{14}C Administered, d.p.m.	Activity of Combined Scopolamine and Hyoscyamine, d.p.m.	Activity of Tropic Acid, d.p.m.
L-Serine-3-^{14}C (25.0 mc./mmole)	2,053,200	53,875	43,180
DL-Tryptophan-(2-indolyl)-^{14}C (29.1 mc./mmole)	2,209,442	59,572	44,573
Formic acid-^{14}C (7.6 mc./mmole)	4,179,937	69,422	28,875
DL-Tryptophan(benzene ring)-U-^{14}C (100 mc./mmole)	5,246,000	37,264	30,948
Phenylacetic acid-1-^{14}C (52 mc./mmole)	1,619,555	40,087	37,104
DL-Phenylalanine-1-^{14}C (10.4 mc./mmole)	2,789,267	58,721	48,935

Table II—Position of Labeling in Tropic Acid

Precursor	Percent of Total Activity of Tropic Acid			
	C_1	C_2	C_3	Phenyl Ring
L-Serine-3-^{14}C	58	3	29	10
DL-Tryptophan-(2-indolyl)-^{14}C	20	4	61	15
Formic acid-^{14}C	41	8	21	30
DL-Tryptophan-(benzene ring)-U-^{14}C	2	1	3	94
Phenylacetic acid-1-^{14}C	12	6	64	18
DL-Phenylalanine-1-^{14}C	72	6	12	10

Degradation—The alkaloids were removed from the silica gel by soxhlet extraction, using ethanol as the solvent. They were identified by their R_f values and by preparation of their picrates. Hydrolysis of the combined scopolamine and hyoscyamine, as well as degradation of the resultant tropic acid to styrene or benzoic acid, was accomplished by known methods (1). Degradation of the benzoic acid to benzene and carbon dioxide was done by established methodology using quinoline and copper dust. Duplicate, individual degradations were carried out for each of the two plant samples/radioactive compound initially used.

Scintillation Counting—Samples were counted using 1 ml. of solubilizer[1] and 14 ml. of 0.4% 2,5-diphenyloxazole/0.005% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene in toluene. Individual samples were counted to a maximum of 100,000 counts or 300 min. Absolute count rates were determined using the method of channels ratio.

RESULTS AND DISCUSSION

All precursors were significantly incorporated into tropic acid (Table I). Serine and formic acid both showed high, specific incorporation into C_1 of tropic acid as anticipated (Table II). Tryptophan-(2-indolyl)-^{14}C also had good incorporation into the C_1 of tropic acid, but the bulk of labeling occurred at C_3 in agreement with the pathway outlined by Goodeve and Ramstad (8) (Table II).

Goodeve and Ramstad used tryptophan-3-^{14}C as a precursor for tropic acid. Labeling was determined to be in the C_1-position of the acid (Scheme III). A unique pathway for the biogenesis of tropic acid was based on this result. Criticism of this pathway arose from the fact that tryptophan-3-^{14}C could not show that tryptopan is capable of acting as a precursor for the entire carbon skeleton of tropic acid. To clarify this matter, tryptophan-(benzene ring)-U-^{14}C (U = uniformly labeled) was administered to the roots of the plant. The results indicated that this precursor was specifically incorporated into tropic acid to a significant extent, with the bulk of the label (94%) appearing in the phenyl ring of tropic acid (Tables I and II). This means that the phenyl ring from tryptophan probably is incorporated intact into tropic acid, illustrating the ability of tryptophan to act as a direct and specific precursor of tropic acid in *D. innoxia*.

Phenylalanine-1-^{14}C and phenylacetic acid-1-^{14}C were previously investigated as precursors for tropic acid. These two compounds were again used in this study for comparison with the other compounds. Both compounds were incorporated specifically into tropic acid in complete agreement with the literature (1, 4). The degree of incorporation as well as the specificity of incorporation was found to be comparable to the other compounds used in this investigation (Tables I and II).

Compounds capable of producing formate groups *in vivo* have been shown to be specifically incorporated into the C_1 of tropic acid. This result is likely accomplished by the reaction of these one-carbon fragments with a compound of the phenylacetate-type structure. Since the position of the label is predominately in the C_1 of tropic acid, rather than being equally divided between the C_1 and C_3 positions, no symmetrical intermediate in the pathway is likely. Tryptophan was capable of being incorporated in this manner, but the evidence also supports its direct role in tropic acid biosynthesis.

[1] Soluene-100, Packard Instrument Co., Downers Grove, Ill.

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