any single process. The simple model, however, does provide a useful qualitative picture of the kinetics of helix formation as a nucleation and growth phenomenon.

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Further Studies on the Biosynthesis of Tropic Acid[1]

BY MARY L. LOUDEN AND EDWARD LEETE

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The administration of L-phenylalanine-1-C^{14} and DL-phenylalanine-2-C^{14} to *Datura stramonium* plants in separate experiments led to the formation of radioactive hyoscyamine and hyoscine which were labeled solely in their tropic acid moieties. Systematic degradation of the side chain of the radioactive tropic acids indicated that the carboxyl group was derived from the carboxyl group of phenylalanine, while the hydroxymethyl group arose from the α-carbon of the amino acid. Administration of sodium bicarbonate-C^{14} to *Datura* plants resulted in general labeling of the alkaloids. Plausible biogenetic schemes for tropic acid are discussed.

In our previous work on the biosynthesis[2] of tropic acid[3] (I), DL-phenylalanine-3-C^{14} was administered to two-month old *Datura stramonium* plants resulting in the formation of radioactive hyoscyamine and hyoscine which were labeled specifically at C-2 of their tropic acid moieties. This result has been recently confirmed by the work of Underhill and Youngken.[4] We had originally considered that the hydroxymethyl group (C-2) of tropic acid was derived from a one-carbon source. However, the administration of sodium formate-C^{14},[3] formaldehyde-C^{14},[3] methionine-methyl-C^{14}[5] or serine-3-C^{14}[4] to *D. stramonium* plants did not lead to tropic acid labeled at C-2.

We thus shelved the problem of the origin of the hydroxymethyl group and fed phenylalanine-2-C^{14} to three-month old *Datura* plants, fully expecting to obtain tropic acid which would be labeled on its carboxyl group. Radioactive tropic acid was indeed obtained and the degradative scheme which was used to determine the activities on the side chain carbons of tropic acid is illustrated in Fig. 1. Dehydration of tropic acid by refluxing with concentrated aqueous potassium hydroxide[6] yielded atropic acid (II) which was oxidized in alkaline solution with sodium metaperiodate and a catalytic amount of osmium tetroxide yielding formaldehyde collected as its dimedone derivative, and phenylglyoxylic acid isolated as its oxime (III). This oximino acid was refluxed in water yielding benzonitrile and carbon dioxide,[7] collected as barium carbonate. The benzonitrile was hydrolyzed with potassium hydroxide yielding benzoic acid which was subjected to the Schmidt reaction affording aniline, assayed as its benzoyl derivative, and carbon dioxide, collected as barium carbonate. We were agreeably surprised to discover that the tropic acid derived from phenylalanine-2-C^{14} was labeled solely on the hydroxymethyl group (*cf.* Table I).

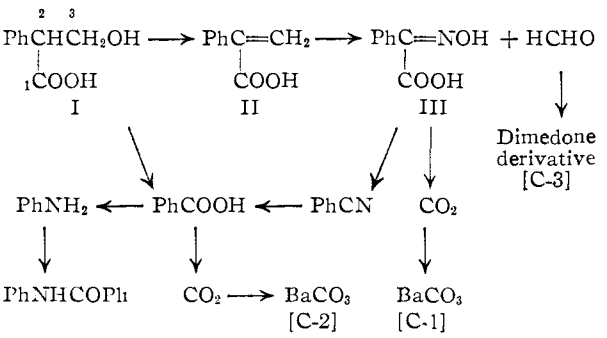

Fig. 1.—Degradative scheme for the radioactive tropic acid.

While our work was in progress, Goodeve and Ramstad[8] reported that the administration of tryptophan-3-C^{14} to *D. stramonium* yielded radioactive hyoscyamine which was labeled specifically on the carboxyl group of its tropic acid moiety. This was an astonishing result and difficult to reconcile with our observations. It seemed unlikely that tropic acid was being formed in the same plant by two quite different biosynthetic mechanisms, and we interpreted their work as follows. The radioactive tryptophan could undergo metabolic breakdown in the plant yielding some radioactive carbon dioxide. This could then participate in a carboxylation reaction with a metabolite derived from phenylalanine leading to carboxyl labeled tropic acid. We attempted to test this hypothesis by feeding sodium bicarbonate-C^{14} to *Datura* plants in the hope that we would obtain preferential incorporation of carbon-14 into the carboxyl group of tropic acid. Radioactive hyoscyamine and hyoscine were obtained. However, assay of the degradation products of the alkaloids (*cf.* Table I) indicated that the distribution of activity between the tropic acid and the tropane bases was almost proportional to the carbon content of the two halves of the alkaloids. The distribution of activity in the side chain of

(1) Part of this work has been published in preliminary communications: E. Leete and M. L. Louden, *Chemistry & Industry*, 1405 (1961); M. L. Louden and E. Leete, *J. Am. Chem. Soc.*, **84**, 1510 (1962). This investigation was supported by a research grant, MY-2662 from the National Institute of Mental Health, U. S. Public Health Service.

(2) We accept with enthusiasm the suggestion of Rapoport (F. R. Stermitz and H. Rapoport, *J. Am. Chem. Soc.*, **83**, 4045 (1961)) that the word biosynthesis should describe *in vivo* experimental studies on the mode of formation of natural products, whilst the word biogenesis should be applied to the hypothetical schemes which organic chemists are so fond of creating to rationalize the formation and structures of natural products.

(3) E. Leete, *ibid.*, **82**, 612 (1960).

(4) E. W. Underhill and H. W. Youngken, *J. Pharm. Sci.*, **51**, 121 (1962).

(5) L. Marion and A. F. Thomas, *Can. J. Chem.*, **32**, 1116 (1954).

(6) J. W. Baker and A. Eccles, *J. Chem. Soc.*, 2125 (1927).

(7) A. Ahmad and I. D. Spenser, *Can. J. Chem.*, **39**, 1340 (1961).

(8) A. M. Goodeve and E. Ramstad, *Experientia*, **17**, 124 (1961).

the tropic acid was not completely uniform. However, there was not a significantly larger amount of activity located in the carboxyl group. For some reason, the distribution of activity in the side chain of the tropic acids derived from hyoscyamine and hyoscine was not the same, a more uniform distribution being present in the tropic acid from the latter alkaloid.

We then considered that the branched side chain of tropic acid might arise by a rearrangement of the phenylalanine side chain. This possibility was tested by feeding L-phenylalanine-1-C^{14} to *Datura* plants. The incorporation[9] of tracer into the alkaloids was relatively high (0.23%) and degradation showed that the tropic acid was labeled solely on the carboxyl group. This significant result may indicate that an intramolecular rearrangement of the side chain of phenylalanine is occurring (reaction A, Fig. 2.). Alternatively, oxidative cleavage of the side chain could take place (reaction B) yielding carbon dioxide (perhaps attached to an enzyme or coenzyme such as biotin) and a C_6–C_2 unit such as phenylacetaldehyde. Carboxylation of the phenylacetaldehyde, or related metabolite, with the carbon dioxide complex (reaction C) followed by reduction of the aldehyde function would yield tropic acid. If reaction B does occur, one could claim that the radioactive carbon dioxide derived from phenylalanine-1-C^{14} never got very far away from the C_6–C_2 unit, and as a result never reached the photosynthetic cycle with consequent genera labeling.

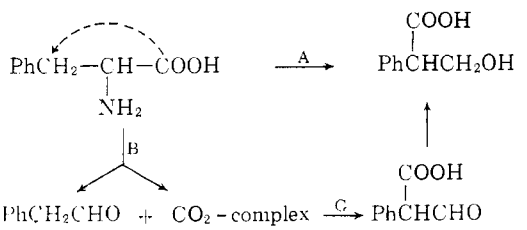

Fig. 2.—Plausible biogenetic pathways from phenylalanine to tropic acid.

The recent results of Underhill and Youngken[4] favor the participation of a C_6–C_2 compound in the biosynthesis of tropic acid. They found that phenylacetic acid-1-C^{14} was a precursor of tropic acid. Furthermore they found that the tropic acid was not labeled on the carboxyl group. Unfortunately, they did not carry out a more extensive degradation of the radioactive tropic acid.

It should be mentioned that the plants used in the present investigation were between two and three months old, and in all cases both the hyoscyamine and the hyoscine became labeled, indicating that both alkaloids were being synthesized in plants of this age. This result is in agreement with earlier studies.[10]

Experimental

Administration of Tracers to *D. stramonium* plants and Isolation of the Alkaloids.—The tracers were administered to the plants which were growing in soil in a greenhouse[11] by means of a cotton wick inserted through the stems with an English sewing needle. The alkaloids were extracted from the plants without dilution by methods previously described,[3,10] two weeks after administration of the tracers. Details of the amount of tracers fed, and activities of the alkaloids are recorded in Table I. The radioactive alkaloids were diluted prior to degradation.

TABLE I

Tracer fed	DL-Phenylalanine-2-C^{14} hydrochloride[12]	L-Phenylalanine-1-C^{14} [13]	Sodium bicarbonate-C^{14} [14]
Wt., m.g	36.4	1.21	24.8
Activity,[15] mc.	0.1	0.05	1.0
Month of expt.	May	Dec.	June
No. of plants	20	10	20
Fresh wt. of plants, g.	409	109	418
Act. of aq. sap (% of total fed)	16	7	2.1
Hyoscyamine and its degradation products (Act. ind.p.m./m*M*.)			
Incorporation, %	0.046	0.11	0.005
Hyoscyamine, HCl	1.5×10^6	3.6×10^6	5.1×10^5
Tropine picrate	0.00	0.00	2.1×10^5
Tropic acid	1.5×10^6	3.6×10^6	3.1×10^5
Formaldehyde-dimedone	1.6×10^6	0.00	0.5×10^5
Phenylglyoxylic acid oxime	0.03×10^6	3.4×10^6	2.5×10^5
Barium carbonate (from oxime)	[a]	3.5×10^6	0.5×10^5
Benzoic acid	[a]	0.00	2.0×10^5
$BaCO_3$ (from benzoic acid)	[a]	[a]	0.1×10^5
N-Phenylbenzamide	[a]	[a]	2.1×10^5
Hyoscine and its degradation products			
Incorporation, %	0.022	0.13	0.003
Hyoscine.HCl	4.7×10^5	3.0×10^5	4.2×10^5
Oscine picrate	0.00	0.00	1.7×10^5
Tropic acid	4.8×10^5	3.0×10^6	2.6×10^5
Formaldehyde-dimedone	4.3×10^5	0.00	0.2×10^5
Phenylglyoxylic acid oxime	[a]	3.0×10^6	2.2×10^5
$BaCO_3$ (from oxime)	[a]	3.1×10^6	0.3×10^5
Benzoic acid	[a]	0.00	1.6×10^5
$BaCO_3$ (from benzoic acid)	[a]	[a]	0.2×10^5

[a] Not isolated, or degradation not carried out to this stage because of negligible activity or lack of material.

Degradation of the Radioactive Alkaloids. (a) Hydrolysis.—The following typical procedure gave more reliable results than the method previously described.[3] Hyoscine hydrochloride (570 mg.) was refluxed with 3% barium hydroxide solution (21 ml.) in a nitrogen atmosphere for 45 min. The cooled solution was acidified with hydrochloric acid and extracted with ether. The residue obtained on evaporation of the dried ether extract was crystallized from a mixture of benzene and petroleum ether (b.p. 60–70°) yielding tropic acid (219 mg., 81%), m.p. 116.5–118°. The solution from which the tropic acid had been extracted was made alkaline with potassium hydroxide and extracted with ether yielding oscine which was purified by sublimation *in vacuo*; 102 mg., 39%, m.p. 108–111°. The picrate crystallized from ethanol without difficulty (*cf.* ref. 4), m.p. 237–240°. Hyoscyamine was hydrolyzed with dilute aqueous methanolic potassium hydroxide yielding tropine and tropic acid.

(9) Incorporation is defined as the total amount of radioactivity found in the isolated natural product divided by the total amount of radioactivity administered to the plant.

(10) E. Leete, *J. Am. Chem. Soc.*, **84**, 55 (1962).

(11) We thank Robert C. McLeester of the Botany Department of the University of Minnesota for the cultivation of the *Datura* plants.

(12) Purchased from Tracerlab, Inc., Waltham, Mass.

(13) Purchased from Nichem, Inc., Bethesda, Md. The L-isomer was fed since the DL-phenylalanine-1-C^{14} was not commercially available.

(14) Purchased from Isotopes Specialties Co., Inc., Burbank, Calif.

(15) Radioactivity measurements were carried out in a Nuclear-Chicago model C-115 low background Q gas flow counter. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self-absorption.

(b) **Atropic Acid.**[6]—Tropic acid (280 mg.) was refluxed with a solution of potassium hydroxide (0.8 g.) in water (2 ml.) in a nitrogen atmosphere for 40 min. The cooled solution was acidified with dilute hydrochloric acid; crude atropic acid (181 mg., 72%), m.p. 102.5–107.5°, separated. Recrystallization from aqueous methanol afforded colorless prismatic needles, m.p. 105–107.5°.

(c) **Cleavage of the Atropic Acid.**—Atropic acid (143 mg.) was dissolved in a solution of sodium carbonate (51 mg.) in water (5 ml.) and cooled to 0°. Osmium tetroxide (10 mg.) was added, the solution becoming pale brown and then purple. A solution of sodium metaperiodate (449 mg.) in water (10 ml.) was then added slowly to the stirred solution during 45 min. Stirring was continued for 24 hr. maintaining the temperature at 0°. The reaction mixture which was then pale yellow was extracted with ether to remove osmium tetroxide and then acidified with 2 *N* hydrochloric acid. Ether extraction of this solution yielded crude phenylglyoxylic acid which was converted to its oxime (84 mg., 53%), m.p. 141.5–144°, by the method of Ahmad and Spenser.[7] The oxime was sublimed prior to radioactive assay. The aqueous solution from which the phenylglyoxylic acid had been extracted was distilled and the aqueous distillate added to a solution of dimedone (280 mg.) in water (100 ml.). After standing overnight, colorless needles of the formaldehyde-dimedone derivative (153 mg., 55%), m.p. 192–193°, separated. It was crystallized from aqueous ethanol prior to assay.

(d) **Decarboxylation of Phenylglyoxylic Acid Oxime.**—The oxime (47 mg.) was suspended in water (18 ml.) which had been previously boiled to remove carbon dioxide. The mixture was then refluxed in a current of pure nitrogen which was subsequently passed through a solution of barium hydroxide to collect the evolved carbon dioxide. The yield of barium carbonate was 27 mg. (48%). Extraction of the residual aqueous solution with ether yielded benzonitrile which was hydrolyzed by heating with glycerol (0.6 ml.) and potassium hydroxide (0.4 g.) at 200° for 1 hr. Acidification of the reaction mixture with hydrochloric acid and extraction with ether yielded benzoic acid which was sublimed and crystallized from water; 3 mg., 9%, m.p. 121–122°, not depressed on admixture with an authentic specimen.

Benzoic acid was also obtained by the direct oxidation of the tropic acid with potassium permanganate.[3] The benzoic acid was decarboxylated by heating with sodium azide and concentrated sulfuric acid using established procedures.[16] The resultant aniline was isolated as its benzoyl derivative.

The activities of the degradation products of the alkaloids are recorded in Table I, calculated for undiluted material.

(16) S. Shibata, I. Imaseki and M. Yamazaki, *Pharm. Bull. (Japan)* **5**, 594 (1957).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY, EVANSTON, ILL.]

Pyrolytic Formation of Arenes. I. Survey of General Principles and Findings

BY CHARLES D. HURD, A. R. MACON, JUSTINE I. SIMON AND R. V. LEVETAN

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A survey is made of the pyrolytic formation of arenes from hydrocarbons and of various hypotheses designed to explain the findings. It is pointed out that C_3-fragments are important, hitherto unrecognized intermediates in the production of benzene and other arenes. In particular, there is the propadienyl–propargyl resonance hybrid (Ia) that is considered to arise from propylene, toluene, methylthiophene or picoline and the isomeric trimethine fragment Ib, ·CH=CH—CH:, both a radical and a carbene, that is formed from Ia by a 1,2-hydrogen shift or from hetero-arenes (as thiophene or pyridine) by direct rupture of the nucleus. It is shown how Ib may account for benzene by dimerization and for naphthalene, benzothiophene or quinoline by addition to benzyl, 2-thenyl or picolyl radicals, respectively. A resonance-stabilized C_4H_4 diradical II, ·CH=CH—CH=CH·, is considered to be formed along with Ia by thermal scission of benzyl, and it arises also from thiophene or pyridine by rupture of the nucleus. The role of II in giving rise to naphthalene from toluene, benzothiophene from thiophene, or quinoline from pyridine is outlined. The 2-thenyl radical not only ruptures into Ia but it also rearranges into 3-thenyl. A transition state involving a 3-membered ring is postulated to account for this 1,2-shift. Similarly, all of the picolyl radicals shift reversibly into their isomers, and undoubtedly the α-carbon of the benzyl radical wanders at high temperature to different nuclear carbons in parallel fashion. Experiments on which these conclusions are based are discussed. They deal with the pyrolysis of derivatives of thiophene and pyridine, and with labeled (^{14}C) toluene and 2-methylthiophene.

Work of long standing has demonstrated that the formation of arenes by pyrolysis may start with other arenes or with aliphatic substances. It will be shown in this and the following papers that arenes may be formed also from heterocyclic substances. To arrive at a common basis of understanding for these seemingly unrelated pyrolytic syntheses it is helpful to examine the pertinent past work.

Arenes from Aliphatics.—Of the several hypotheses that have been suggested starting with alkenes (or alkanes), these figure prominently.

a. Acetylene Hypothesis of Berthelot.—Berthelot[1] proposed that a hydrocarbon may pyrolyze into a denser hydrocarbon, ultimately into acetylene which may then polymerize in part into benzene or styrene, that the acetylene may continue to react yielding naphthalene or anthracene, and that for any particular high temperature there exists a complex equilibrium. Although this hypothesis has been shown to be unrealistic (see below), it has influenced thinking up to fairly recent times. Thus, in pyrolyzing pyridine at 900° Ruhemann[2] reported that no more than a trace of acetylene, if any appeared in the gaseous products. He added: "während Azetylenkondensationsprodukte aromatischer Natur überhaupt nicht auftreten." Thus, Ruhemann noted the absence of acetylene and arenes but he obviously accepted Berthelot's acetylene hypothesis as the basis of arene formation.

b. CH_2 and CH Radicals.—The suggestion that CH_2 and CH fragments serve as precursors of arenes was put forward by Bone and Coward.[3]

c. Butadiene or Conjugated Dienes as Precursors of Thermal Aromatization.—This concept was based on Staudinger's observation[4] that butadiene yielded a tar at 800° of which 30.6% was benzene and 25% was naphthalene. D. T. Jones[5]

(1) M. Berthelot, *Ann. chim. phys.*, [4] **9**, 453, 471 (1866); **12**, 143 (1867); **16**, 144 (1869); *Bull. soc. chim.*, [2] **22**, 137 (1874).

(2) S. Ruhemann, *Braunkohle*, **28**, 749 (1929).

(3) W. Bone and H. Coward, *J. Chem. Soc.*, **93**, 1201 (1908).

(4) H. Staudinger, R. Endle and J. Herold, *Ber.*, **46**, 2466 (1913).