[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

The Biogenesis of Tropic Acid and Related Studies on the Alkaloids of *Datura stramonium*[1]

BY EDWARD LEETE

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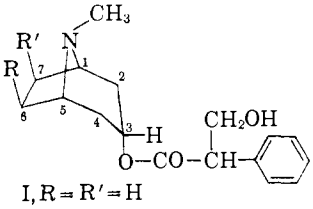
The administration of DL-phenylalanine-3-C^{14} to 2-month old *Datura stramonium* plants yielded radioactive hyoscyamine and hyoscine. Hydrolysis of these alkaloids yielded tropine and oscine which had negligible activity. All the activity resided in the tropic acid moiety of these ester alkaloids, and degradation indicated that tracer was all located on the carbon adjacent to the benzene ring. This result strongly suggested that phenylalanine is a direct precursor of tropic acid. The adminstration of sodium formate-C^{14} and formaldehyde-C^{14} to plants of the same age also produced radioactive hyoscyamine and hyoscine. In this case almost all the activity was located in the tropane bases. In particular it was shown that the hyoscyamine was labeled mainly on the N-methyl group when sodium formate-C^{14} was fed to the plants.

Tropic acid (IX) is the acid moiety of the ester alkaloids hyoscyamine (I) and hyoscine (II) which occur together in several solanaceous plants (*Atropa belladonna, Datura stramonium, Duboisia leichhardtii, Hyoscyamus niger* and many others).[2]



I, R = R′ = H
II, R–R′ = –O–

Trautner[3] suggested that tropic acid is related biogenetically to the terpenes because of the branched nature of the three-carbon side chain. More recently Wenkert[4] adumbrated the formation of tropic acid from prephenic acid, a well established intermediate in the biosynthesis of aromatic compounds. This second hypothesis seemed very plausible and the various routes whereby tropic acid could be formed from prephenic acid are illustrated in Fig. 1. An aldol condensation between prephenic acid and formaldehyde (or its biological equivalent) would yield compound IV which would afford the aromatic acid VII on decarboxylation and dehydration. Oxidative decarboxylation of VII would give tropic acid. Alternate routes, which differ only in the stage at which the hydroxymethyl group is introduced, would be *via* phenylpyruvic acid (VI) or phenylacetic acid (VIII). If phenylpyruvic acid is a precursor of tropic acid we would also expect incorporation of phenylalanine (V), since these two compounds are readily interconvertible by a transamination reaction.

In the present work, we administered DL-phenylalanine-3-C^{14}, sodium formate-C^{14} and formaldehyde-C^{14} to 2-month old *Datura stramonium* plants growing in an inorganic nutrient solution. The tracers were rapidly absorbed by the roots and the alkaloids were isolated after 12 days. Activity was found in both the hyoscyamine and hyoscine isolated from all the experiments. Hydrolysis of the hyoscyamine and hyoscine derived from the phenylalanine-3-C^{14} yielded tropic acid, which had essentially the same specific activity as the original alkaloids, and the bases tropine and oscine,[5] which had very low activity. The radioactive tropic acid was oxidized with alkaline permanganate to yield benzoic acid which was decarboxylated, the evolved carbon dioxide being absorbed in barium hydroxide solution. The resultant barium carbonate and the benzoic acid had the same specific activity as the tropic acid. This indicated that the tropic acid was labeled only on the α-carbon and strongly suggested that it was derived directly from phenylalanine-3-C^{14}.

Hydrolysis of the radioactive hyoscyamine and hyoscine derived from formate-C^{14} and formaldehyde-C^{14} yielded radioactive tropine and oscine. The tropic acid was essentially inactive, a result certainly *not* in accord with our preconceived biogenetic scheme. The origin of the hydroxymethyl group of tropic acid thus remains an unsolved problem. The tropine derived from the sodium formate feeding experiment was demethylated with hydrogen iodide, the evolved methyl iodide being absorbed in triethylamine. The resultant quaternary ammonium salt had almost the same specific activity as the tropine indicating that formate was serving as a precursor of N-methyl groups in the plant. It seems probable that the formaldehyde was also incorporated into the N-methyl groups.[6]

The percentage incorporation of formate into the tropane bases was extremely small. However the low efficiency of formate as a source of methyl groups in plants seems to be a general phenomenon (*cf.* Table I). In all these experiments formate was added to the nutrient solution in which the roots of the intact plant were growing. It is suggested that the bulk of the formate does not reach the site of alkaloid synthesis when it is administered to plants *via* the roots.

In previous work on the biogenesis of the tropane alkaloids ornithine-2-C^{14} and methionine–methyl-C^{14} were fed to mature (5-month old) *D.*

(1) This work was presented at the 136th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1959, and was supported by a Research Grant, M-2662, from the National Institute of Mental Health, Public Health Service.

(2) *Cf.* T. A. Henry in "The Plant Alkaloids," J. and A. Churchill London, 1949, p. 65.

(3) E. M. Trautner, *Austr. Chem. Inst. J. and Proc.*, **14**, 411 (1947).

(4) E. Wenkert, *Experientia*, **15**, 165 (1959).

(5) Oscine is a rearranged hydrolysis product of hyocine, resulting from a backside attack of the 3-hydroxyl of tropane on the 6,7-epoxide.

(6) Formaldehyde has been shown to be an excellent source of the N-methyl group of nicotine: R. U. Byerrum, R. L. Ringler, R. L. Hamill and C. D. Ball, *J. Biol. Chem.*, **216**, 371 (1955).

(7) E. Leete and L. Marion, *Can. J. Chem.*, **32**, 646 (1954).

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

TABLE I

INCORPORATION OF FORMATE-C^{14} INTO ALKALOIDS

Alkaloid		Incorporation, %	Reference
Hyoscyamine		0.00079	This article
Hyoscine		.00034	This article
Gramine		.0040	7
Hordenine		.029	7
Ephedrine	(NCH_3)	.00040	8
	(CCH_3)	.00036	
Protopine	(NCH_3 and methylenedioxy)	.0040	9

stramonium plants.[10,11] Only hyoscyamine became labeled (*cf.* Table II), and various hypotheses were advanced to explain the lack of activity in the hyoscine.[10-12] Since we obtained incorporation of tracers into both alkaloids in all of our experiments with 2-month old plants, it seems reasonable to assume that no synthesis of hyoscine was occurring during the feeding experiments with the 5-month old plants.

TABLE II

DISTRIBUTION OF ACTIVITY IN THE ALKALOIDS OF *Datura* AFTER FEEDING TRACERS AT DIFFERENT TIMES

Age of plant, mo.	Precursor	Distribution of activity, % Hyoscyamine	Hyoscine	Incorporation, %
2	DL-Phenylalanine-3-C^{14}	62	38	0.0029
2	Sodium formate-C^{14}	70	30	.0011
2	Formaldehyde-C^{14}	70	30	.00043
5	DL-Ornithine-2-C^{14}	100	0	.085
5	DL-Methionine-Me-C^{14}	100	0	.0063

Experimental

Administration of the Tracers to *D. stramonium* and Isolation of the Alkaloids.—The *D. stramonium* plants were grown from seed in soil and then transferred to a hydroponics set up with the roots immersed in an aerated nutrient solution.[13] The aerial parts of the plants were exposed to about 6 hr. of sunshine per day. Nine 2-month old plants were used in each feeding experiment. The amounts of tracer added to the nutrient solutions of the plants are given in Table III.

TABLE III

Tracer	Wt., mg.	Total activity, mc.	Fresh wt. of harvested plant, g.	Activity in aqueous sap (% of total fed)
DL-Phenylalanine-3-C^{14} [14]	7.13	0.12	25.0	2.5
Sodium formate-C^{14} [15]	34.0	.71	22.7	0.08
Formaldehyde-C^{14} [15]	14.0	.29	13.7	0.35

The phenylalanine and sodium formate had no apparent deleterious effect on the growth of the plants; however, those which were in contact with the formaldehyde were definitely stunted. The plants were harvested after 12 days and were mascerated in a Waring blendor with chloroform (150 ml.), ether (150 ml.) and 15 *N* ammonia solution (10 ml.). Inactive hyoscyamine and hyoscine hydrochloride (200 mg. of each) were added to the mixture to serve as carriers for the radioactive alkaloids. After 48 hr. the mixture was filtered through cloth which was squeezed to remove as much liquid as possible. The plant residue was washed with more chloroform and ether. The brown aqueous layer ("aqueous sap" in Table III) was separated and assayed for activity. The organic layer was extracted with 0.5 *N* hydrochloric acid (4 × 50 ml.). This acid extract was made basic with 15 *N* ammonia solution and extracted with a 1:1 mixture of chloroform and ether. The mixture of crude alkaloids, which resulted from evaporation of this dried extract, was separated on an acid-treated Celite column. Since Marion and Thomas[11] reported that this method was unreliable, details are given since it is much simpler to use than the method of Evans and Partridge.[16] Celite (20 g.) was placed in a 250-ml. stoppered bottle and covered with chloroform (100 ml.). Hydrochloric acid (5.5 ml. *N*) was added and the stoppered bottle shaken vigorously for about 10 minutes. This mixture was then washed into a column (20 mm. diameter) with more chloroform. The Celite was impacted in the column by means of a glass rod and a plug of glass wool. The mixture of alkaloids dissolved in chloroform (10 ml.) was added to the top of the column and elution carried out with chloroform. The first 350 ml. of the chloroform eluted contained most of the hyoscyamine as the hydrochloride. The second 200 ml. of chloroform contained a trace of hyoscyamine. The column was then eluted with 200 ml. of chloroform which had been previously shaken with 50 ml. of 15 *N* ammonia solution. Evaporation of this fraction afforded hyoscine as the free base. Paper chromatography was used to determine the composition of the fractions. Using the organic phase obtained by shaking together *n*-butyl alcohol (250 ml.), concentrated hydrochloric acid (25 ml.) and water (100 ml.), the hyoscyamine and hyoscine hydrochlorides had R_f values of 0.79 and 0.50, respectively. The alkaloids were detected with Dragendoff reagent. The alkaloids obtained from the column were crystallized from ethanol–ether as their hydrochlorides.

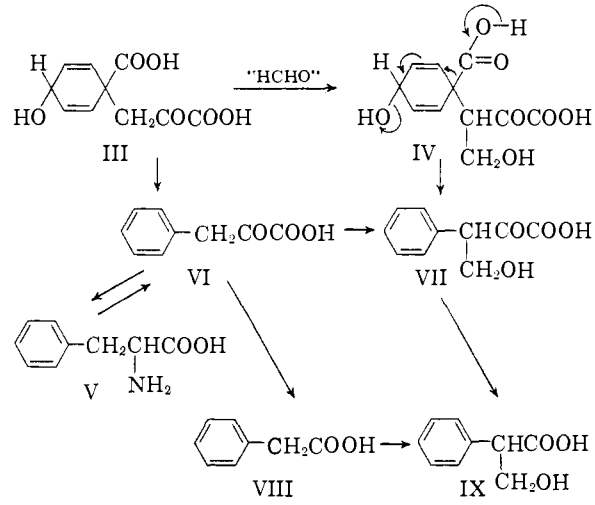


Fig. 1.—Possible biosynthetic routes to tropic acid.

Degradation of the Radioactive Alkaloids. (a) **Hydrolysis.**—In a typical hydrolysis, hyoscyamine hydrochloride (190 mg.) was refluxed with 2 ml. of 10% sodium hydroxide solution for 30 min. The cooled solution was then extracted with ether to yield tropine (64 mg.). The aqueous alkaline layer was acidified with hydrochloric acid and extracted with ether to yield tropic acid, which was purified by sublimation *in vacuo* (81 mg.).

(b) **Demethylation of the Tropine Derived from the Sodium Formate-C^{14}.**—The tropine was demethylated by heating with hydrogen iodide at 360° using the procedure of Brown and Byerrum.[17] The evolved methyl iodide was washed with cadmium sulfate and sodium thiosulfate, and

(9) M. Sribney and S. Kirkwood, *Nature*, **171**, 931 (1953).

(10) E. Leete, L. Marion and I. D. Spenser, *Can. J. Chem.*, **32**, 1116 (1954).

(11) L. Marion and A. F. Thomas, *ibid.*, **33**, 1853 (1955).

(12) P. Reinouts van Haga, *Biochim. et Biophys. Acta*, **19**, 562 (1956).

(13) The composition of the nutrient solution was the same as that used in our experiments with tobacco: E. Leete, THIS JOURNAL, **78**, 3520 (1956).

(14) Purchased from Research Specialties Co., Berkeley, Calif.

(15) Purchased from Isotopes Specialites Co., Glendale, Calif.

(16) W. C. Evans and M. W. Partridge, *J. Pharm. Pharmacol.*, **4**, 769 (1952).

(17) S. A. Brown and R. U. Byerrum, THIS JOURNAL, **74**, 1523 (1952).

TABLE IV

ACTIVITIES OF THE HYOSCYAMINE (I) AND HYOSCINE (II) AND THEIR DEGRADATION PRODUCTS (C.P.M./MM.[18])

Precursor fed	Phenylalanine-3-C^{14} I	Phenylalanine-3-C^{14} II	Sodium formate-C^{14} I	Sodium formate-C^{14} II	Formaldehyde-C^{14} I	Formaldehyde-C^{14} II
Alkaloid hydrochloride	7.6×10^3	4.5×10^3	2.1×10^4	9.0×10^3	3.1×10^3	1.4×10^3
Tropine or oscine picrate	$<0.3 \times 10^3$	$<0.2 \times 10^3$	1.8×10^4	9.2×10^3	2.8×10^3	1.5×10^3
Triethylmethylammonium iodide	.....	.....	1.5×10^4	.....	.....	.....
Tropic acid	7.1×10^3	4.3×10^3	$<0.05 \times 10^4$	0	$<0.05 \times 10^3$	$<0.06 \times 10^3$
Benzoic acid	7.1×10^3	4.2×10^3	.....	.....	.....	.....
Barium carbonate	6.9×10^3	4.0×10^3	.....	.....	.....	.....

then absorbed in a cooled ethanolic solution of triethylamine to yield triethylmethylammonium iodide.

(c) **Oxidation of the Tropic Acid.**—Tropic acid (50 mg.), obtained from the phenylalanine feeding experiment was refluxed with sodium hydroxide (0.2 ml. of 10%), and potassium permanganate (0.15 g.) in 5 ml. of water for 2 hr. The boiling solution was filtered, and the filtrate made acid with hydrochloric acid and extracted with ether. Evaporation of the dried ether extract yielded benzoic acid (29 mg.) which was purified by sublimation. The benzoic acid (20 mg.) was decarboxylated in boiling quinoline (5 ml.) in the presence of copper chromite catalyst (10 mg.). The evolved carbon dioxide was absorbed in barium hydroxide to yield barium carbonate (14 mg.). The activities of the degradation products are summarized in Table IV.

(18) Counts were carried out in a Nuclear-Chicago model D-47 Q gas flow counter using a "Micromil" window. Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

MINNEAPOLIS 14, MINN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Nature of the Intermediary Ketols in the Robinson Annelation Reaction[1]

BY WILLIAM S. JOHNSON, JAMES J. KORST,[2] R. A. CLEMENT[3] AND J. DUTTA[4]

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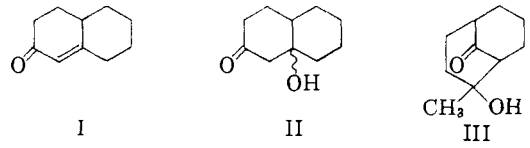
The epimeric ketols-a and -b produced from the condensation of the tricyclic ketone IV (R = H) with methyl vinyl ketone have been shown through n.m.r. spectroscopic and degradation studies to have the bridged-ring structure VIII rather than the formerly proposed constitution VI and VII, respectively. On the basis of this study doubt is cast on the latter type of structural assignments that have been made in other cases (see ref. 7). Further studies with the ketols-a and -b have shown that their configurations and conformations correspond to those shown by expressions XIIa and XIIb, respectively. N.m.r. spectroscopic studies have proved that a ketol obtained from cyclohexanone and methyl vinyl ketone has the normal structure II, but a pair of ketols derived from the octalindione XX has the bridged-ring constitution XXII.

The Robinson annelation reaction is typified, in its simplest form, by an example described in the present work, namely the condensation of methyl vinyl ketone with cyclohexanone to produce $\Delta^{1,9}$-octalone-2 (I).[5] Of all of the known methods for the synthesis of fused hydroaromatic ring systems this reaction holds a unique position of importance. Thus every one of the successful total syntheses of natural non-aromatic steroids to date[6] have depended upon the use of this reaction at some stage.



I II III

In several instances, when the Robinson annelation method was being used, intermediary ketols have been isolated.[7] These substances have invariably been formulated as that ketol (*e.g.*, II) which is the direct precursor of the unsaturated ketone formed by a β-elimination process (*e.g.*, II → I). In the present work it is demonstrated that such ketols may be, on the contrary, correctly represented by a bridged-ring expression like that represented by formula III.[8] Indeed,

(1) This represents paper XI of the series entitled "Steroid Total Synthesis—Hydrochrysene Approach." For paper X see W. S. Johnson, B. Bannister, R. Pappo and J. E. Pike, THIS JOURNAL, **78**, 6354 (1956).

(2) Wisconsin Alumni Research Foundation Research Assistant 1957–1958; Allied Chemical and Dye Co. Fellow, 1958–1959.

(3) National Science Foundation Postdoctoral Project Associate, 1954–1955.

(4) Upjohn Co. Postdoctoral Project Associate, 1955–1956.

(5) A variation that is frequently employed involves the use of a progenitor of methyl vinyl ketone such as the Mannich base methiodide derived from acetone. A number of such cases have been summarized by J. H. Brewster and E. L. Eliel in Adams', "Organic Reactions," Vol. VII, 1953, p. 99. In this form the annelation process has sometimes been referred to as the "Robinson-Mannich base reaction."

(6) For a summary see paper I, W. S. Johnson, THIS JOURNAL, **78**, 6278 (1956). For subsequent work see A. Lardon, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **40**, 666 (1957); W. J. Van Der Burg, D. A. Van Dorp, O. Schindler, C. M. Siegmann and S. A. Szpilfogel, *Rec. trav. chim.*, T **77**[2], 171 (1958); K. Heusler, P. Wieland, H. Ueberwasser and A. Wettstein, *Chimia*, **12**, 121 (1958); and W. S. Johnson, J. C. Collins, R. Pappo and M. B. Rubin, THIS JOURNAL, **80**, 2585 (1958).

(7) See for example (a) C. Mannich, W. Koch and F. Borkowsky, *Ber.*, **70**, 355 (1937); (b) P. Wieland, H. Ueberwasser, G. Anner and K. Miescher, *Helv. Chim. Acta*, **36**, 1231 (1953); (c) V. Georgian, *Chemistry & Industry*, 930 (1954); (d) J. Colonge, J. Dreux and J. P. Kehlstadt, *Bull. soc. chim. France*, 1404 (1954); (e) G. Stork, *ibid.*, 256 (1955); (f) F. J. McQuillin, *J. Chem. Soc.*, 528 (1955); (g) R. Howe and F. J. McQuillin, *ibid.*, 2423 (1955); (h) W. S. Johnson, J. Ackerman, J. F. Eastham and H. A. DeWalt, Jr., THIS JOURNAL, **78**, 6302 (1956); (i) P. Wieland and K. Miescher, *Helv. Chim. Acta*, **33**, 2215 (1950).

(8) K. D. Zwahlen, W. J. Horton and G. I. Fujimoto, *ibid.*, **79**, 3131 (1957), have described the formation of this type of ketol by the reaction of phenylmagnesium bromide with an enol lactone, i → ii, which they considered to proceed through rearrangement of the primary Grignard adduct. The same ketol ii was produced by the condensation of 1-methyl-2-tetralone with β-dimethylaminopropiophenone—a reaction which is quite analogous to that described in the