Incorporation of Ornithine-2-C^{14} into Scopolamine

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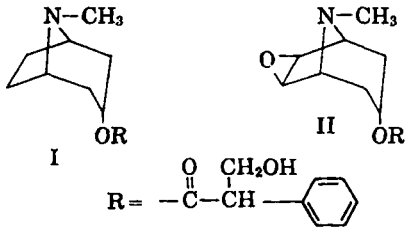
Radioactive hyoscyamine and scopolamine were isolated from 7-week-old *Datura stramonium* plants after the administration of ornithine-2-C^{14}. Degradation of the scopolamine showed that the radioactivity was located at the numbers 1 and 5 carbon atoms of the tropane ring.

THE ROLE OF ornithine as a precursor in the biosynthesis of hyoscyamine was demonstrated by Marion and co-workers (1), who isolated radioactive hyoscyamine (I) from 5-month-old *Datura stramonium* plants which had been fed ornithine-2-C^{14}. Degradation of the radioactive hyoscyamine showed that the radioactivity was located at carbons 1 and 5 of the tropane ring, and indication that at least most of the ornithine molecule was incorporated into hyoscyamine. Scopolamine (II) isolated from these plants proved to be inactive. These investigators suggested that either ornithine was not a direct precursor of scopolamine or that scopolamine was not being synthesized in 5-month-old plants.

Since further experiments by Marion (2) indicated that scopolamine synthesis had halted in mature plants, it seemed desirable to attempt to feed labeled ornithine to younger plants to establish its role in the biosynthesis of scopolamine. During the course of this investigation, a study by Leete (3) reported the isolation of radioactive scopolamine from 3-month-old *D. stramonium* plants which had been fed radioactive ornithine. Since no degradative studies of the alkaloid were reported, the study offers no evidence of whether ornithine is incorporated as a unit or is broken down into fragments before incorporation.

To determine the most favorable age for the administration of the radioactive ornithine, *D. stramonium* plants from the age of 2 to 12 weeks were analyzed for their alkaloidal content. The results of the analyses are listed in Table I.

The first alkaloid to be formed was scopolamine. Hyoscyamine was not formed in significant quantities until the fourth week, after which its concentration rapidly increased. After the seventh week, hyoscyamine was present in larger amounts than scopolamine.



From this study it appeared that a precursor must be fed to young plants if it is to be incorporated into scopolamine. Seven-week-old plants were chosen for the study since they were still synthesizing scopolamine and were of sufficient size to be handled conveniently.

When 7-week-old *D. stramonium* plants were injected with ornithine-2-C^{14}, both radioactive hyoscyamine and scopolamine were isolated. If ornithine was incorporated into scopolamine as a single unit through a biogenetic pathway similar to that suggested by Marion (1), the radioactive carbons of the scopolamine would be located at carbons 1 and/or 5 of the tropane ring. To establish if this was the biogenetic pathway, the isolated radioactive scopolamine was converted to hyoscyamine, which was hydrolyzed to tropine and degraded by a previously reported method (1).

Considerable difficulty was encountered in establishing a satisfactory method for the conversion of scopolamine to either hyoscyamine or tropine. Attempts to convert scopolamine to the corresponding episulfide, which could then be desulfurized to yield hyoscyamine, failed. Scopolamine was recovered unchanged when heated with aqueous solutions of thiourea or potassium thiocyanate at temperatures ranging from 25 to 115°.

While the epoxide ring of scopolamine could be

TABLE I.—AGE-ALKALOID RELATIONSHIP OF YOUNG *D. stramonium*

Age, Wk.	Wt. Hyoscyamine Base, mg.	Wt. Scopolamine Base, mg.	Scopolamine Hyoscyamine Ratio, Av.
2	0	6.58	...
	0	6.77	
3	0.76	10.00	17.7
	0.31	6.90	
4	5.26	12.55	2.48
	4.91	13.15	
5	6.38	13.08	2.12
	6.43	14.12	
6	13.20	17.05	1.26
	14.41	17.61	
7	15.05	19.39	1.18
	17.14	18.28	
8	22.82	17.69	0.82
	23.00	19.61	
10	34.01	24.22	0.72
	35.22	25.43	
12	35.81	25.20	0.69
	35.75	24.02	

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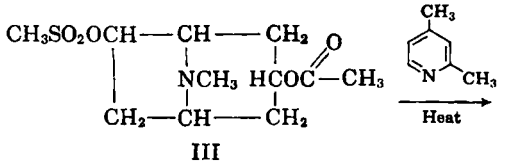
opened by hydrogenation to form 6-hydroxyhyoscyamine, it was not possible to remove the hydroxyl group by reduction of its tosyl derivative. 3-Acetoxytropane (V) was obtained when 3-acetoxy-6-methanesulfonyloxytropane (III) was desulfonated by heating with 2,4-lutidine in a sealed tube and the resulting 3-acetoxy-6,7-dehydrotropane (IV) hydrogenated. Unfortunately, the yield was too low to permit further degradation.



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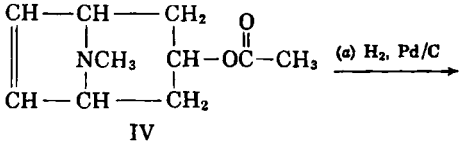

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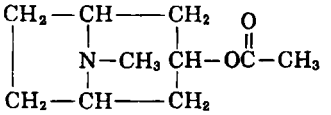


V

Catalytic hydrogenation of scopolamine, followed by hydrolysis, provided the best synthetic route to tropine. This reaction has been reported by Fodor and co-workers (4), who suggested that the hydrogenolysis proceeds through a mechanism similar to that of nickel desulfurization. In view of this hypothesis, the alkaloid was hydrogenated with a large excess of W-28 Raney nickel. After alkaline hydrolysis, a 33% yield of tropine was obtained. Use of a more active catalyst resulted in a lower yield of tropine. When applied to the radioactive alkaloid, this procedure provided a sufficient quantity of tropine for the degradation.

The tropine was then degraded according to a modification of the procedure developed by Marion and co-workers (1). Mild oxidation of tropine (VI) by dilute acidic potassium dichromate (5) afforded tropinone (VII), which upon vigorous chromic acid oxidation yielded *N*-methylsuccinimide (VIII). Reaction of a large excess of phenylmagnesium bromide with the *N*-methylsuccinimide produced approximately equal amounts of 1-methyl-5-hydroxy-5-phenyl-2-pyrrolidone (IX) and 1-methyl-2,5-diphenylpyrrole (X) (6). The latter compound was oxidized to benzoic acid with acid potassium dichromate.

The specific activities of hyoscyamine and its hydrolysis products are shown in Table II, while the data for scopolamine and its degradation products are given in Table III.

The data in Table III indicate that all of the radioactive ornithine incorporated into scopolamine entered the tropane ring. The benzoic acid had essentially the same activity as that of tropine (one-half the specific activity since 2 moles of benzoic acid arose from 1 mole of tropine). This indicates that the No. 2 carbon atom of ornithine produces the bridgehead carbon atoms (No. 1 and No. 5) of the tropane ring. Ornithine-2-C^{14} is thus incorporated into both hyoscyamine and scopolamine.

TABLE II.—HYOSCYAMINE HYDROBROMIDE AND HYDROLYSIS PRODUCTS

Compd.	Specific Activity, c.p.m./mM × 10^{4a}
Hyoscyamine hydrobromide	5.10
Tropic acid	Traces
Atropic acid	Traces
Tropine picrate	5.11

[a] All specific activities have been corrected for efficiency and self-absorption.

TABLE III.—SCOPOLAMINE HYDROBROMIDE AND DEGRADATION PRODUCTS

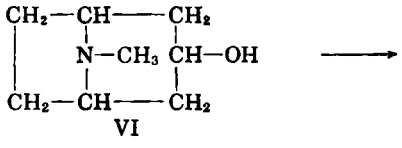
Compd.	Specific Activity, c.p.m./mM × 10^{4a}
Scopolamine hydrobromide	2.16
Atropic acid	Inactive
Tropic acid	Inactive
3,6-Tropanediol	1.88
Tropine picrate	2.01
N-Methylsuccinimide	1.80
1-Methyl-2,5-diphenylpyrrole	1.76 (after correction)[b]
Benzoic acid	0.88 (after correction)[b]

[a] All specific activities have been corrected for efficiency and self-absorption. [b] These are specific activities corrected for dilution with inactive *N*-methylsuccinimide.

EXPERIMENTAL

General Procedure for Extraction and Separation of Alkaloids from D. Stramonium

Extraction of the Alkaloids.—The method suggested by Evans and Partridge (7) for the extraction of the alkaloids of *D. stramonium* was employed in the isolation of hyoscyamine and scopolamine.



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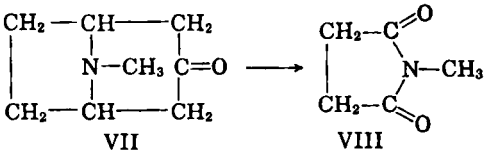
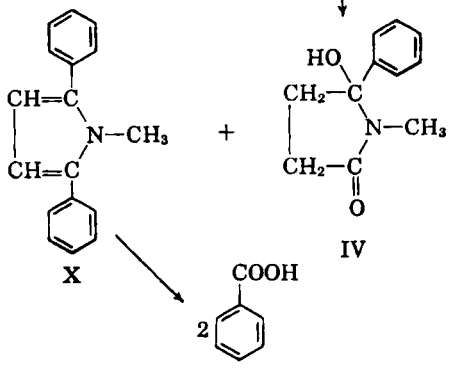


VII VIII



Separation of the Alkaloids by Column Chromatography.—The hyoscyamine and scopolamine were separated by column chromatography using a Celite column. The procedure described by Schill and Agren (8) was employed. To determine the quantity of alkaloid present, the residue from each fraction was separately dissolved in 4 ml. of hydrochloric acid. Gold chloride solution (0.2 *M*) was added to the acidic solution to precipitate the aurichloride salts of the alkaloids. After cooling in the refrigerator for 1 day, the aurichlorides were collected by suction filtration, washed with cold water, dried, and weighed. The corresponding weights of the free bases were calculated from the weights of the aurichloride salts.

The weights of hyoscyamine and scopolamine in samples of *D. stramonium* which vary in age from 2 to 12 weeks are presented in Table I.

Isolation of the Radioactive Alkaloids

Administration of DL-Ornithine-2-C^{14} to D. stramonium Plants.—From 27-week-old *D. stramonium* plants, the 10 most vigorous plants of equal size were selected for the administration of ornithine-2-C^{14}. The flower pods were just beginning to emerge at this time. A solution was prepared containing 16.9 mg. of DL-ornithine hydrochloride-2-C^{14} with a specific activity of 1.0 mc./m*M* and a total activity of 0.1 mc. or 2.22×10^8 c.p.m. in 1 ml. of water. This solution was then injected into the plants by employing the following technique.

While holding the plant in a horizontal position, 1 drop of the active solution was placed at the base of the petiole of a large leaf. With a small sewing needle, a prick was made at the site of the water droplet. As soon as the solution had been absorbed into the plant, another drop was placed at the same spot. Several pin pricks were made in the same area to speed the uptake of the solution. From 50 to 100 μl. of the solution was allowed to be absorbed in each area. After the radioactive solution was absorbed, an additional 2–3 drops of water was allowed to be taken up to ensure that none of the ornithine was left clinging on the outside of the petiole. Using several leaves, a total of 0.1 ml. of the radioactive solution was injected into each plant.

One week after the administration of the radioactive ornithine, the plants were harvested. The leaves, roots, and stems were separated and dried in a circulating hot air oven at 50–55° for 2 days. They were then ground to a No. 40 powder.

Extraction of the Radioactive Alkaloids.—The 31.7 Gm. of combined plant powder was thoroughly moistened with 20 ml. of water and allowed to macerate for 1 hour. The moistened drug was triturated with 6.5 Gm. of calcium hydroxide and extracted with 300 ml. of ether for 6 hours. The alkaloids were then obtained from the ether extract as previously described (7).

Column Chromatographic Separation of the Alkaloids.—The hyoscyamine and scopolamine were separated on a Celite column moistened with 1.0 *N* hydrochloric acid in the manner previously described (8). To determine the purity of each fraction, the solvents were evaporated and the residues dissolved in 1 ml. of chloroform. Five microliters of each solution was chromatographed on paper according to the method of Drey and Foster (9). The developed chromatograms showed only one spot from each solution, an indication that the fractions were pure (Table IV).

TABLE IV.— CHROMATOGRAPHICALLY SEPARATED ALKALOIDS

	Alkaloid Wt., mg.	Specific Activity, c.p.m./m*M* × 10^6	% Incorporation
Hyoscyamine hydrochloride	29.00	8.2	0.37
Scopolamine	39.25	9.8	0.44

Preparation of the Hydrobromide Salts of the Alkaloids.—To the hyoscyamine and scopolamine fractions was added 25 ml. of chloroform saturated with hydrogen bromide gas, and the solvent was removed *in vacuo*. Inert hyoscyamine hydrobromide (318.0 mg.) and scopolamine hydrobromide (515.4 mg.) were added to their respective radioactive fractions and the salts recrystallized twice from absolute ethanol-ether to obtain a constant specific activity.

Degradation of the Radioactive Alkaloids

Saponification of Hyoscyamine Hydrobromide.—The 0.238 Gm. of recrystallized radioactive hyoscyamine hydrobromide was diluted with 1.757 Gm. of inactive alkaloid, and the mixture was saponified by refluxing for 45 minutes with 25 ml. of 10% sodium hydroxide solution. The aqueous solution was continuously extracted with ether for 20 hours.

After drying over anhydrous magnesium sulfate, the ether extract was evaporated to a light yellow syrup which crystallized after standing for several hours in a vacuum desiccator. The yield of light yellow crystals of tropine was 0.69 Gm. (90.8%), m.p. 62.5–63.5°.

The aqueous solution recovered from the ether extraction was acidified with 15 ml. of 6 *N* hydrochloric acid to precipitate the atropic acid. After cooling in an ice bath, the white solid was collected by suction filtration, washed with water, and dried. The weight of atropic acid was 0.34 Gm. (42.5%), m.p. 105–106°.

The aqueous mother liquor from the atropic acid was extracted with three 25-ml. portions of ether. The ether extracts were dried over anhydrous magnesium sulfate and evaporated to yield a light green oil which crystallized upon scratching. The yield of tropic acid was 0.42 Gm. (47%), m.p. 96–105°. After recrystallization from ether-petroleum ether (b.p. 30–60°), the melting point was 115–116°.

Hydrogenation of Scopolamine Hydrobromide.—The 0.385 Gm. of recrystallized radioactive scopolamine hydrobromide, after dilution with 10.612 Gm. of inactive alkaloid (26.8 mmoles), was dissolved in 100 ml. of water and hydrogenated for 4 days at room temperature and atmospheric pressure in a glass microhydrogenation apparatus. Eighteen grams of commercial W-28 Raney nickel (Raney Catalyst Co.), washed free of excess alkali, was used as the catalyst.

The catalyst was removed by suction filtration and thoroughly washed with water. The filtrate was concentrated to approximately 25 ml. and refluxed for 90 minutes with 30 ml. of 20% sodium hydroxide solution. The alkaline solution was continuously extracted with ether for 2 days. The

white crystals that had collected in the ether extract were removed by suction filtration, washed with ether, and dried. The yield of 3,6-tropanediol was 2.45 Gm. (54.6%), m.p. 177–179°.

The ether filtrate was evaporated to a yellow syrup which was placed in a vacuum desiccator to induce crystallization. The weight of tropine was 1.35 Gm. (33.6%), m.p. 59–61°.

The alkaline solution from the ether extraction was acidified with concentrated hydrochloric acid to precipitate atropic acid which was collected by suction filtration, washed with water, and dried; yield, 2.48 Gm. (58.5%), m.p. 97–101°. After recrystallization from ethanol-water, it melted at 105–106°.

The filtrate from the atropic acid isolation was extracted with four 25-ml. portions of ether which, after drying over magnesium sulfate, was evaporated to dryness. The light yellow oil crystallized to furnish 1.14 Gm. (24.0%) of tropic acid which, after recrystallization from ether-petroleum ether (30–60°), melted at 115–116°.

Tropinone.—A solution of 1.33 Gm. (9.45 mmoles) of tropine in 4 ml. of 25% sulfuric acid was cooled in an ice-bath. To this solution was added dropwise with constant swirling, 16 ml. of a solution composed of 100 ml. of 2 *M* potassium dichromate and 43 ml. of concentrated sulfuric acid (equivalent to 1.1 Gm. of potassium dichromate). The dark solution, kept cold in an ice bath, was treated with sufficient 20% sodium hydroxide solution to neutralize the sulfuric acid and dissolve the precipitate of chromium hydroxide. The solution was saturated with anhydrous potassium carbonate and extracted with ten 25-ml. portions of ether. The organic extracts were dried over anhydrous magnesium sulfate and the solvent evaporated to yield an oil which crystallized upon scratching. The yield of white crystalline tropinone was 1.08 Gm. (82.5%), m.p. 39–41°. [Lit. 41–42° (10).]

Oxidation of Tropinone.—To a cooled solution of 1.05 Gm. (7.6 mmoles) of tropinone in 15 ml. of 25% sulfuric acid was slowly added a solution of 7.0 Gm. of chromic acid in 20 ml. of 25% sulfuric acid. The resulting solution was heated in an oil bath at 140° for 4 hours. The acidic solution was poured over 30 Gm. of ice and the excess chromic acid decomposed by the addition of 5 Gm. of sodium bisulfite. The aqueous solution was continuously extracted with thiophene-free benzene for 2 days.

The benzene extract was dried over anhydrous magnesium sulfate, and the solvent was then evaporated at 30° by means of a flash evaporator. The residue, which consisted of white crystals in a yellow gum, was washed with several small portions of cold ether to remove the yellow oil and to yield 70.5 mg. (8.2%) of light brown crystals of *N*-methylsuccinimide, m.p. 64–66°.

1-Methyl-2,5-diphenylpyrrole.—To 1.5 Gm. (58 mmoles) of dried magnesium turnings was added 8 ml. (76 mmoles) of redistilled bromobenzene and 30 ml. of anhydrous ether. The reaction was protected from the atmosphere by means of soda-lime and calcium chloride. After the reaction had been initiated, an additional 15 ml. of ether was added, and the mixture was stirred until all of the magnesium had reacted.

A solution of 57.0 mg. (0.505 mmoles) of radioactive *N*-methylsuccinimide and 108.8 mg. (0.95 mmoles) of inactive *N*-methylsuccinimide in 40 ml. of dry benzene was prepared. This solution was added dropwise over a period of 30 minutes to the well-stirred Grignard solution. Stirring was continued at room temperature for an additional 22 hours.

The reaction mixture was poured over 50 Gm. of ice containing 3 ml. of sulfuric acid. This mixture was transferred to a separator, thoroughly mixed, and the layers separated. The aqueous layer was extracted with eight 30-ml. portions of ether and then set aside. The ether extracts were combined with the original organic layer, and the solvent was then removed *in vacuo*. The residue was steam distilled to remove excess bromobenzene and biphenyl.

The residue remaining after the steam distillation was cooled in an ice bath, the excess water decanted, and the brown tar dissolved in 15 ml. of absolute ethanol. The ethanol solution was then placed in the refrigerator for 2 days, during which time a crystalline product formed. The light tan crystals were collected and dried to yield 28.44 mg. (7.8%) of 1-methyl-2,5-diphenylpyrrole, m.p. 192–195°. [Lit. 192–196° (1).]

A solid which had crystallized after a few days from the aqueous layer was collected by suction filtration and dried. The weight of solid was 22.47 mg. (7.6%) and melted at 137–139°. [Lit. 139–141° (6).] The infrared spectrum was identical to 1-methyl-5-phenyl-5-hydroxy-2-pyrrolidone.

Oxidation of 1-Methyl-2,5-diphenylpyrrole to Benzoic Acid.—A mixture of 28.4 mg. (0.12 mmoles) of 1-methyl-2,5-diphenylpyrrole, 0.5 Gm. of potassium dichromate, and 25 ml. of 20% sulfuric acid was refluxed for 3 hours, during which time it was occasionally shaken. The small amount of unreacted starting material, removed by filtration, was again refluxed with 0.25 Gm. of fresh potassium dichromate in 15 ml. of 20% sulfuric acid for 2 hours. The solutions were combined, cooled, and the excess dichromate was decomposed with sodium bisulfite. The aqueous solution was then continuously extracted with ether for 18 hours.

The ether extract was dried over anhydrous magnesium sulfate, then evaporated to dryness *in vacuo* below 20° to afford 23.88 mg. of a light tan solid melting at 102–107°. The impure benzoic acid was recrystallized from water to yield 5.40 mg. (18.3%) of white benzoic acid melting at 119–120°. Evaporation of the mother liquor produced 6.03 mg. (20.4%) of additional impure benzoic acid, m.p. 110–113°.

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