Synthesis of Tropine-Labeled Atropine III

Synthesis of *N*-Methyl-^{14}C-Tropine and *N*-Methyl-^{14}C-Atropine

By GILBERT C. SCHMIDT, THOMAS E. ELING, and JOHN M. McOWEN

Using a previously described modification of the Robinson condensation, *N*-methyl-^{14}C-tropine, specific gravity 1 mc./mmole, was synthesized from methylamine-^{14}C-HCl in 65 percent of theoretical yield. Subsequent esterification with unlabeled tropic acid gave *N*-methyl-^{14}C-atropine, specific activity 1 mc./mmole, in 45 percent of theoretical yield, based on methylamine. Synthesis of these compounds validates previous studies and firmly establishes a general pathway for labeling the carbon skeleton of tropine with radioactive carbon.

THE HIGH toxicity of atropine dictates that minute doses be administered to test animals and, thereby, limits studies of atropine metabolism. Exclusive use of separations and purifications to trace and locate metabolites is precluded. Selective, isotopic labeling of the alkaloid is essential. Studies of intermediary animal metabolism, plant biogenesis, the mechanisms of pharmacological actions, and pathways that permit certain microorganisms to utilize atropine as a sole carbon and nitrogen source (1–3) depend on the availability of suitably labeled compounds.

An interest in these problems and the associated need for unavailable compounds stimulated a study of methods for selectively labeling tropine and the tropine moiety of atropine. A general, microsynthetic pathway was established (4) and a prototype method for the synthesis of labeled tropine from labeled arabinose was developed (5). The present communication describes the synthesis of *N*-methyl-^{14}C-tropine and *N*-methyl-^{14}C-atropine from labeled methylamine HCl, confirms for the first time Werner's synthesis of these compounds, and establishes that the previously proposed pathways are suitable for use with radioactive carbon.

EXPERIMENTAL

Microsynthetic methods for tropine and atropine are described in detail in the first two papers of this series (4, 5) and were modified only slightly for the synthesis of *N*-methyl-^{14}C-tropine and *N*-methyl-^{14}C-atropine.

Preparation of O-Acetyltropic Acid—Acetyltropic acid was prepared from 395 mg. tropic acid and 0.36 ml. acetyl chloride in the manner previously described (4).[1] The product was dried over $CaCl_2$ in a vacuum desiccator and was converted to *O*-acetyltropic acid chloride at the time of esterification with tropine.[2]

Preparation of Raney Nickel and Check of Activity—Type W-7 Raney Nickel[3] was prepared from 10 Gm. of alloy by the method of Augustine (6).[4] Although the catalyst is active for at least 2 weeks,[5] catalysts used in radiosyntheses were checked for activity on the evening prior to reduction of labeled tropanone.[6]

Using the apparatus and experimental conditions employed in radiosyntheses, 100 mg. unlabeled tropanone was reduced overnight. During early stages of the reduction, hydrogen uptake was checked, and on the following morning a 10-μl. sample of the reaction mixture was spotted on filter paper, air dried, and checked for completness of reduction by spraying with 2,4-dinitrophenylhydrazine solution (7). Tropine was also isolated as the picrate and the melting point of the picrate determined.[7] If reduction was complete, the catalyst was used for the reduction of labeled tropanone.

***N*-Methyl-^{14}C-tropine HCl from *N*-Methyl-^{14}C-Tropanone**—Labeled tropanone, synthesized from the stated quantities of reactants and weighing approximately 100 mg. when dried for 12 hr., was reduced over W-7 Raney nickel by the general method of Van de Kamp (8).[8]

Tropanone was dissolved in 2 ml. absolute ethanol and, while stirring slowly,[9] the system was flushed three times with ultra-pure hydrogen.[10] The gas buret was filled with hydrogen, the system closed, and reduction continued with constant stirring, at room temperature, until the rate of gas uptake

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[1] M.p. 87–88°, yield 468 mg.
[2] The product must be thoroughly dry. Storage for 4 days under previously described conditions usually is adequate and storage for 3 months causes no decrease in atropine yields.
[3] Type W-2 catalyst was used in earlier work. Reduction is more rapid and less difficulties are encountered with the W-7 catalyst.
[4] When prepared as described and stored under 250 ml. ethanol, the suspension contains 28 mg. catalyst per ml.
[5] Stored in the refrigerator in bottles filled with absolute ethanol.
[6] A check of catalyst activity under experimental conditions circumvents many difficulties and prevents losses of radioactive intermediates. Traces of impurities of hydrogen quickly poison the catalyst. The gas must be purchased in highly purified form, or it must be purified before use.
[7] Yields should be practically quantitative. M.p. picrate 295°.
[8] Van de Kamp did not indicate the grade of Raney nickel used. Although W-2 catalyst was used in earlier work, the W-7 grade was more satisfactory.
[9] A glass-coated magnetic stirring bar was used.
[10] Ultra-pure hydrogen, E. H. Sargent and Co. Use of ultra-pure hydrogen is virtually mandatory. Although commercial hydrogen of ordinary purity may be purified by passing through concentrated H_2SO_4, results are still erratic.

asymptotically approached zero. A 2-μl. aliquot of the reaction mixture was spotted on filter paper, air dried, and tested with 2,4-dinitrophenylhydrazine solution. If a negative test was not obtained,[11] the reaction mixture was filtered through a fine-porosity filter and the catalyst washed with approximately 10 ml. absolute ethanol. Filtrate and washings were concentrated to approximately 2 ml.[12] and the reduction repeated with fresh catalyst. Finally, the catalyst was removed by filtration and all solvent was removed by use of the flash evaporator. The product was stored in a desiccator, without vacuum, over NaOH pellets,[13] until converted to the hydrochloride.

After suitable storage, *N*-methyl-^{14}C-tropine[14] was converted to the hydrochloride, in the exact manner described previously (4). The hydrochloride[15] was stored in the 10-ml. reaction vessel, in a vacuum desiccator, over NaOH pellets, until used for esterification.

Synthesis of *N*-Methyl-^{14}C-Atropine from O-Acetyltropic Acid and *N*-Methyl-^{14}C-Tropine HCl—To the *O*-acetyltropic acid chloride obtained from 150-mg. *O*-acetyltropic acid (4)[16] was added the thoroughly dried *N*-methyl-^{14}C-tropine HCl produced from the quantities of reactants used in the Robinson condensation.[17] The reactants were esterified to form *O*-acetyl-*N*-methyl-^{14}C-atropine, the acetylated compound was hydrolyzed to the alkaloidal free base, and *N*-methyl-^{14}C-atropine finally isolated, exactly as described for the unlabeled alkaloid (4). The product was dried immediately in a vacuum desiccator over $CaCl_2$. The dry product was dissolved in 75 ml. anhydrous petroleum ether, the solution was concentrated to approximately 10 ml., and labeled atropine precipitated by cooling in the deep freezer. Petroleum ether was decanted from the crystals,[18] the product recrystallized from petroleum ether, then dried in a desiccator over $CaCl_2$.[19]

Synthesis of *N*-Methyl-^{14}C-Tropanone—Labeled tropanone was prepared from methylamine-^{14}C-HCl, succindialdehyde, and acetone dicarboxylic acid, according to previously described procedures (4, 5). The earlier papers should be consulted for essential experimental details.

Acetone dicarboxylic acid was prepared from 1-Gm. citric acid, collected after the final cooling to −8°, and immediately used in the Robinson condensation. During collection of acetone dicarboxylic acid, succindialdehyde was prepared. To 5 μl. of concentrated HCl and 5 ml. water, contained in a 10-ml. beaker, was added 0.34 ml. of 2,5-diethoxytetrahydrofuran. The mixture was hydrolyzed by stirring, at room temperature for 10 min. The resulting dialdehyde[20] was used immediately in the Robinson condensation.[21]

To the solution of succindialdehyde was added 40% of the moist acetone dicarboxylic acid from 1 Gm. of citric acid. Saturated, aqueous Na_2HPO_4 was added to adjust the pH to 4.5–5.0, using a glass electrode. The buffered mixture was transferred to a previously assembled, 250-ml., conical, side-arm flask and the system purged with nitrogen for 10 min. To the buffered mixture was added 5 ml. water, containing 33.8 mg. of methylamine-^{14}C-HCl[22] and 33.8 mg. unlabeled methylamine HCl. The volume was adjusted to 50 ml. and the system quickly evacuated to remove most of the gas phase.[23] The system was closed and the mixture allowed to stand at room temperature for 12 hr. The slight evacuation was repeated and, after standing at room temperature for an additional 12 hr., the solution was saturated with Na_2CO_3.

Tropanone was extracted with 100-ml. portions of petroleum ether, exactly as previously described (4), except that extraction was continued until significant amounts of radioactivity no longer were removed. The petroleum ether extracts were dried over Na_2SO_4, transferred to a 250-ml. sublimation flask, and the solvent removed, *in vacuo*, on the flash evaporator, at 40°. Tropanone, which remained in the flask as a brown oil, was dried *in situ*, in a desiccator, over $CaCl_2$ and NaOH, overnight. The crude product was purified by repeated sublimation at 40° and 1×10^{-4} mm. Hg. After each sublimation, the product was washed from the cold finger with small amounts of absolute ethanol. Sublimation was repeated until the washings no longer contained significant amounts of radioactivity. The combined, alcoholic solutions of *N*-methyl-^{14}C-tropanone were evaporated to dryness in the flash evaporator, in a previously tared 25-ml. flask. The product[24] crystallized quickly on cooling, was dried in a desiccator for 12 hr., then reduced to tropine.

RESULTS AND DISCUSSION

The synthesis of tropine, labeled with ^{14}C in the carbon skeleton, and of atropine labeled in the tropine moiety, has remained an unsolved problem. For synthesis of the heterocycle, the Robinson condensation (9, 10) is inherently versatile and has been proven adaptable for microsynthesis (4, 11). In a similar way, the Wolffenstein and Mamlock esterification has been adapted for microsynthesis (4, 11, and 12) and has been proven adequate for the

[11] Disappearance of the 2,4-dinitrophenylhydrazine color was a more reliable index of complete reduction than was hydrogen uptake. In no instance was a negative color accompanied by unexpectedly low tropine yields. At times, theoretical or greater than theoretical hydrogen uptake was accompanied by a positive 2,4-dinitrophenylhydraline test. Repetition of the reduction gave a negative color and the expected tropine yields.

[12] Losses may occur by volatilization. Evaporation of the solvent must be done carefully.

[13] The product must be dry before it is converted to the hydrochloride, yet drying must not be unduly prolonged because losses occur by volatilization. Crystallization usually occurs within 12 hr. and storage for an additional 24 hr. usually is adequate for drying. A maximum storage of 2 days, without vacuum, is recommended.

[14] Yield 93.8 mg., specific activity 1 mc./mmole, m.p. 63–65°.

[15] Yield 119 mg., specific activity 1 mc./mmole, m.p. 280° with decomposition.

[16] Requirements for freshly prepared acyl halide, removal of all traces of thionyl chloride, and exclusion of moisture must be re-emphasized.

[17] Labeled tropine HCl from the stated amounts of methylamine HCl, acetone dicarboxylic acid and succindialdehyde weighed 115 mg., specific activity 1 mc./mmole, m.p. 280°, when corrected for tropanone contamination.

[18] At this point, the product usually contains small amounts of tropine. In no instance did tropine represent more than 8% of total counts, equivalent to 4% of product weight.

[19] Yield 130 mg. (45% based on methylamine HCl), specific activity 1 mc./mmole, m.p. 114–116°, after recrystallization from petroleum ether.

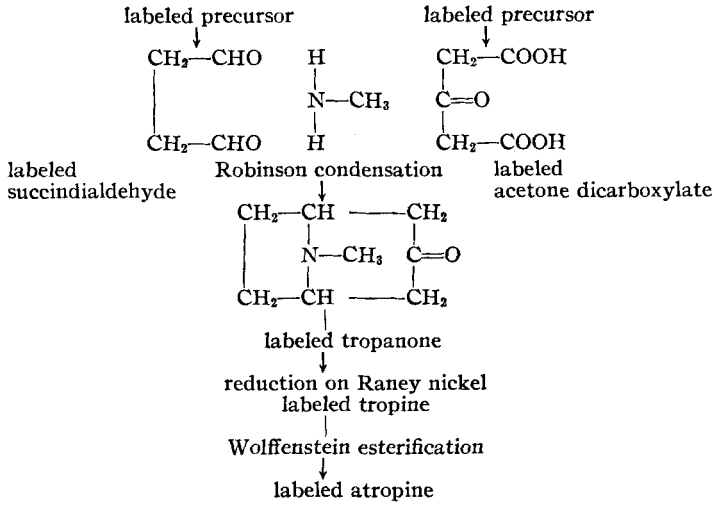
[20] This quantity of diethoxytetrahydrofuran produces 2 mmole (173 mg.) of succindialdehyde.

[21] Ideally, collection of acetone dicarboxylic acid and hydrolysis of diethoxytetrahydrofuran should be completed at the same time.

[22] Mallinckrodt Nuclear (formerly Nuclear Research Chemicals), Orlando, Florida. Specific activity 2 mc./mmole.

[23] Flask evacuated to the point at which the liquid in it bubbles slightly but does not boil vigorously.

[24] Yield 94.3 mg., needles, m.p. 41–43°, specific activity 1 mc./mmole.



Scheme I

esterification of tropine and tropic acid. It is clear, from the reaction sequence (Scheme I), that labeled precursors of succindialdehyde or acetone dicarboxylic acid become logical instruments for labeling the carbon skeleton of tropine.

Several studies with unlabeled compounds support the feasibility of this scheme, but the use of labeled intermediates has been limited and published findings remain unconfirmed. For convenience of discussion, the esterification and condensation steps may be considered as separate entities.

After a single esterification, Werner (11) obtained 70% of theoretical yields for the esterification of *N*-methyl-^{14}C-tropine with unlabeled tropic acid. This is in agreement with yields obtained in the microesterification of unlabeled intermediates (4), but other observations indicate a potential lack of reproducibility. Fodor (13) also esterified *N*-methyl-^{14}C-tropine with unlabeled tropic acid. In this instance, yields were "slightly less than 60%" and the author did not comment on the extent of contamination. Werner obtained similar yields for the esterification of unlabeled tropine with labeled tropic acid, and reported that two esterifications were necessary. These discrepancies indicated a potential lack of reproducibility, possibly associated with the use of ^{14}C-labeled intermediates. To clarify this point and to establish for future studies reproducibility of the esterification, the authors synthesized *N*-methyl-^{14}C-atropine from *N*-methyl-^{14}C-tropine and unlabeled tropic acid. The results indicate that the use of labeled tropine has little or no effect on the esterification. It is clear, from the data in Table I, that synthesis of the labeled heterocycle is the major obstacle encountered in the synthesis of tropine-labeled atropine.

Excluding biosynthetic methods, syntheses of ^{14}C-labeled tropine and correspondingly labeled atropine have been confined to labeling the methyl group attached to the endo nitrogen bridge. Two general approaches have been through the Robinson condensation, employed by the authors and by Werner, and through methylation of nortropine or norhyoscyamine, employed by Fodor (13). Comparative yields are shown in Table I. Fodor's approach does not permit labeling of other positions n the tropine moiety and yields are prohibitively low. The authors' findings with unlabeled intermediates, and Werner's findings with methylamine-^{14}C implied that use of the Robinson condensation would be a far more efficient approach. The data shown in Table I confirm, for the first time, Werner's synthesis of *N*-methyl-^{14}C-tropine and *N*-methyl-^{14}C-atropine. The near identity of results obtained with labeled and unlabeled intermediates indicates no isotope effect on either the condensation or the esterification. These observations firmly establish the Robinson condensation as an instrument for labeling tropine and thereby open the way for introducing radioactive carbon into other ring positions.

Identity of the products was established by melting points, and by paper[25] and thin-layer[26] chromatography in a variety of solvent systems. Paper and thin-layer chromatograms of *N*-methyl-^{14}C-tropine demonstrated traces of an impurity, which represented less than 3% of the total radioactivity and chromatographed as tropanone in all solvent systems used. This impurity disappeared in subsequent steps and no attempt was made to further purify the tropine[27] or more positively identify the impurity. On precipitation from petroleum ether, *N*-methyl-^{14}C-atropine contained an impurity that chromatographed as tropine[28] in all solvent systems used. The impurity represented 7.6% of total radioactivity, equivalent to 3.8% of product weight, and disappeared after a single recrystallization from petroleum ether. Representative paper and thin-layer chromatograms of the final, recrystallized product are shown in Fig. 1.

[25] Paper chromatograms by the ascending technique, Whatman No. 1 paper, using butanol–acetic acid–water (344:56:130). Spots were located by cutting inch-wide strips into 1-cm. segments and counting individually in a Packard Tri-carb liquid scintillation counter. Counting done in toluene, using POP and dimethyl POPOP.

[26] Thin-layer chromatograms on Eastman Silica Gel G sheets, using chloroform, diethylamine, acetone, 25% aqueous ammonia (50:10:35:5). Strips were cut and counted as described for paper chromatograms. For both paper and thin-layer chromatograms, samples were adjusted with HCl to pH 4.5 before application to the chromatograms.

[27] If tropine were the desired end product, tropanone could be removed by repeating the reduction or by fractional sublimation of the impure tropine.

[28] Preliminary studies with labeled tropine have shown that it is not metabolized by mice. Thus the product can be used after a single crystallization from petroleum ether, the trace of tropine serving as an internal standard for locating tropine on chromatograms.

TABLE I—COMPARISON OF OBSERVED AND REPORTED YIELDS[a] FOR THE SYNTHESIS OF *N*-METHYL-^{14}C-TROPINE AND *N*-METHYL-^{14}C-ATROPINE

Ref.	Tropanone		Tropine[c]		Atropine[d]	
	Yield	S.A.[b]	Yield	S.A.[b]	Yield	S.A.[b]
Schmidt *et al.*[e] (4)	70%	...	69%	...	48%	...
This communication	65%	1	65%	1	45%	1
Werner *et al.* (11)	70%	5	70%	5	49%	5
Fodor *et al.*[f] (13)	...	...	44%	0.22	25%	0.21

[a] All yields based on methylamine, except where indicated otherwise. [b] Specific activity is millicuries per millimole. [c] Tropine yields for this communication are corrected for 3% tropanone impurity. [d] Atropine yields after recrystallization from petroleum ether to remove approximately 4% tropine impurity. [e] Microsynthesis using unlabeled intermediates. [f] Fodor did not synthesize tropanone, but methylated tropine with methyliodide-^{14}C.

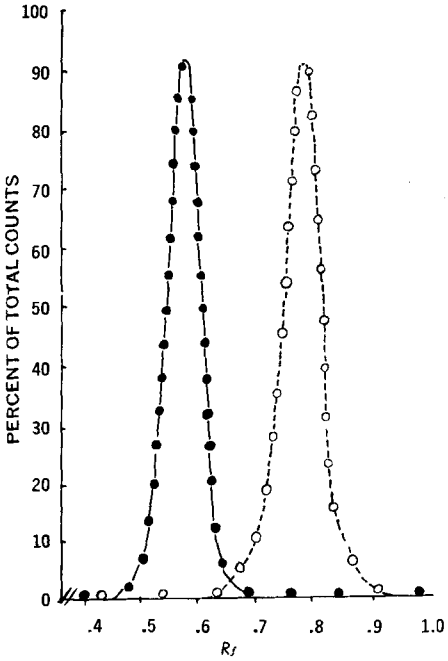


Fig. 1—Representative paper and thin-layer chromatograms of N*-methyl-*^{14}*C-atropine after recrystallization* ——●——●——, *thin layer:* ---O---O---, *paper.*

CONCLUSIONS

Using a previously described, micro adaptation of the Robinson condensation, *N*-methyl-^{14}C-tropine was synthesized from methylamine-^{14}C-HCl in 65% of theoretical yield. The product contained less than 3% of an impurity that chromatographed as unreduced tropanone. The impurity was lost in subsequent steps and no attempt was made to further purify the product or more positively identify the impurity.

Labeled tropine was converted to *N*-methyl-^{14}C-atropine in 45% of theoretical yield, based on methylamine ^{14}C-HCl. When crystallized from petroleum ether, the product contained approximately 4% tropine, representing approximately 8% of the total radioactivity. The impurity was removed by a single recrystallization from petroleum ether.

Werner's synthesis of *N*-methyl-^{14}C-tropine and *N*-methyl-^{14}C-atropine is confirmed for the first time. A comparison of present findings, Werner's data, and previous observations with unlabeled Robinson intermediates indicates that no unusual difficulties are encountered in the use of labeled compounds. Accordingly, a general pathway for labeling the carbon skeleton of tropine is firmly established. Labeled succindialdehyde or labeled acetone dicarboxylic acid become logical precursors of labeled tropine. As indicated previously (5), the use of commercially available pentoses-^{14}C would permit labeling of the ring carbons that arise from succindialdehyde. No other method for labeling the carbon skeleton of tropine has been published.

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Keyphrases

Atropine—tropine labeled
N-Methyl-^{14}C-tropine—synthesis
N-Methyl-^{14}C-atropine—synthesis
Paper chromatography—identity
TLC—identity