found to be 8.4 × $10^{-3}$ min.$^{-1}$ and 1.1 × $10^{-2}$ min.$^{-1}$, respectively.

The influence of bile salt on membrane permeability in the goldfish raises the interesting possibility of similar effects on the permeability of the intestinal membranes. The fluid bathing these membranes has significantly higher concentrations of bile salts (in the order of $10^{-2}$ $M$) than the concentrations employed in the present study. This possibility is now under investigation.

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*Keyphrases*

Bile salts—drug uptake, activity potentiation
Taurodeoxycholate, sodium—potentiating effect
Goldfish overturn time—activity analysis
4-Aminoantipyrine uptake—goldfish
Colorimetric analysis—uptake time

# Synthesis of Tropine-Labeled Atropine IV

## Labeling of the 2, 3, and 4 Positions of Atropine from Citric-$^{14}$C Acid

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

**Using a modification of the Robinson condensation, citric-3-$^{14}$C acid, citric-2,4-$^{14}$C acid, and citric-2,3,4-$^{14}$C acid were converted into correspondingly labeled tropine and atropine. The specific activities of the products varied from 0.62 to 1.0 depending on the labeled compound. This is the first reported syntheses of tropine or atropine labeled with $^{14}$C in positions other than the *endo*-methyl group.**

THE PROBLEM of selectively labeling the tropine moiety of atropine remains to be solved. For one reason or another, the biosynthetic compounds (1, 2), tritium-labeled atropine (3), and randomly labeled atropine (4) are inadequate for establishing the metabolic fate of the heterocyclic residue in atropine. Classical, microsynthetic methods for selectively labeling tropine and the tropine moiety of atropine are needed badly.

Excluding the authors' work, only Fodor (5) and Werner (6) have used classical methods to label tropine. In both instances, tropine-*N*-methyl-$^{14}$C and atropine-*N*-methyl-$^{14}$C were synthesized. Fodor's approach would not permit labeling of other positions, but Werner's use of the Robinson condensation is much more versatile. A lack of suitably labeled Robinson intermediates, other than methylamine hydrochloride, apparently prevented Werner from realizing the full potential of the condensation.

Prior to publication of Werner's work, the authors had been studying the Robinson condensation as a tool for labeling the carbon skeleton of tropine. The reaction was studied in detail, a reproducible microcondensation procedure developed, and the microesterification of tropine and tropic acid standardized (7). The overall procedure was checked by the synthesis of tropine-*N*-methyl-$^{14}$C and atropine-*N*-methyl-$^{14}$C, thereby confirming Werner's synthesis of these compounds (8). At the same time, a prototype procedure was developed for the microsynthesis of succindialdehyde and tropine from arabinose (9) which would be used to label the 1,5,6, and 7 positions of atropine.

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This report describes the synthesis of atropine-2,3,4-$^{14}$C, atropine-2,4-$^{14}$C, and atropine-3-$^{14}$C from the correspondingly labeled citric acids. These syntheses are the first successful introductions of $^{14}$C into other than the *endo*-methyl group of either tropine or the heterocyclic moiety of atropine.

## EXPERIMENTAL

Microsynthetic methods for tropine and atropine, described in detail in the first three papers of the series (7–9), were modified only slightly for the synthesis of tropine-$^{14}$C and atropine-$^{14}$C from citric-$^{14}$C acids. In previously reported work, acetone dicarboxylic acid was not a labeled intermediate. The use of this compound as a labeled precursor to atropine required slight modification of the previously described method for handling this intermediate in the Robinson condensation.

The same procedure was used for the synthesis of atropine-2,3,4-$^{14}$C, atropine-2,4-$^{14}$C, and atropine-3-$^{14}$C. To simplify the description of methods, numbers indicating the position of labeling are eliminated. Earlier papers (7–9) should be consulted for additional experimental details.

**Synthesis of Acetone-$^{14}$C Dicarboxylic Acids**—Dry citric-$^{14}$C acid[1] (96 mg. or 0.5 mmole) and unlabeled citric acid (96 mg. or 0.5 mmole) were added while stirring constantly with a thermometer to 0.44 ml. of fuming $H_2SO_4$[2] (20% excess $SO_3$), previously cooled to −8° in an ice-salt bath. The labeled citric acid was added at a rate to maintain the temperature at −5° or lower.

After the addition was completed, the flask and content were removed from the cooling bath and stirred on a magnetic stirrer at room temperature until gas evolution ceased. The reaction mixture was again cooled to −8° and 1 g. of chipped ice added in small portions so that temperature did not exceed 0°. The resulting pale yellow solution contained acetone dicarboxylic acid-$^{14}$C.

**Preparation of Succindialdehyde**—Succindialdehyde (173 mg. or 2 mmoles) was prepared from 0.34 ml. of 2,5-diethoxytetrahydrofuran as described previously (7).

**Synthesis of Tropanone-$^{14}$C**—To the solution of succindialdehyde (2 mmoles) was added the solution of acetone dicarboxylic-$^{14}$C acid. The round-bottom flask which contained the acetone dicarboxylic acid was washed three times with water and the washings added to the reaction mixture. Saturated aqueous $Na_2HPO_4$ was added to adjust the pH to 4.5–5.0 using a glass electrode. The buffered solution was transferred to a 250-ml. side-arm conical flask and the system purged with nitrogen gas for 10 min. To the buffered reaction mixture, 135 mg. (2 mmoles) of methylamine HCl was added, the reaction volume adjusted to 50 ml., and the system quickly evacuated to remove the gas phase. The reaction vessel was closed and the mixture allowed to remain under partial vacuum for 12 hr. The slight evacuation was repeated and the reaction mixture allowed to remain at room temperature for an additional 12 hr.

Tropanone-$^{14}$C was extracted with 100-ml. portions of petroleum ether, exactly as previously described (7), 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-$^{14}$C, which remained in the flask as a brown oil, was dried *in situ* overnight in a desiccator over $CaCl_2$ and NaOH. The crude product was purified by repeated sublimation at 40° and $1 \times 10^{-4}$ 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 tropanone-$^{14}$C were evaporated to dryness on the flash evaporator in a previously tared 25-ml. flask. The product crystallized quickly on cooling, was dried in a desiccator for 12 hr., and was reduced to tropine.

**Synthesis of Tropine-$^{14}$C HCl**—Labeled tropanone (60–95 mg., 1 mc./mmole), prepared as described above and dried for 12 hr., was reduced over W-7 Raney nickel, by the modified Van de Kamp procedure described in the third paper of this series (8). The product was dried in a desiccator over NaOH pellets and converted to the hydrochloride in the exact manner described previously (7). Tropine-$^{14}$C HCl was stored in 10-ml., tared, round-bottom flasks in a vacuum desiccator over NaOH pellets, until used for esterification.

**Synthesis of Atropine-$^{14}$C**—Acetyltropic acid (150 mg.) was prepared from 395 mg. of tropic acid and 0.36 ml. of acetyl chloride in the manner described previously (7). The product was dried in a vacuum desiccator over $CaCl_2$ for 12 hr. and converted to *o*-acetyltropic acid chloride (7). To the acid chloride was added well-dried tropine-$^{14}$C HCl.[3] Using previously described procedures, the reactants then were esterified to form *o*-acetylatropine-$^{14}$C, the acetylated compound hydrolyzed to the free base, and atropine-$^{14}$C finally isolated. The crude product was dried in a vacuum desiccator over $CaCl_2$, and finally recrystallized from petroleum ether (7).

## RESULTS AND DISCUSSION

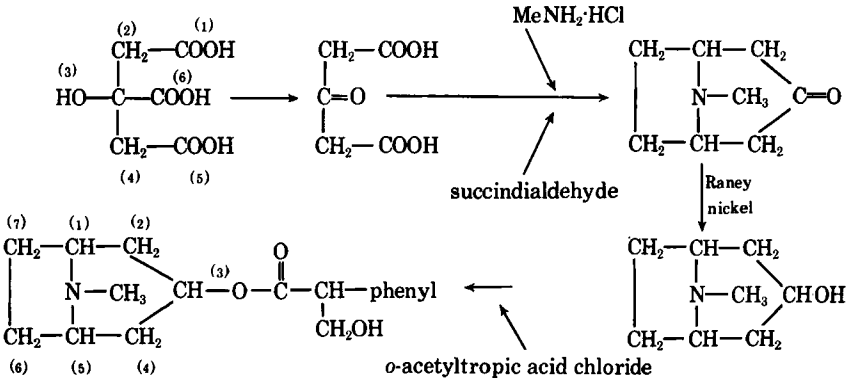
The synthesis of atropine labeled with $^{14}$C in the carbon skeleton of tropine has not been accomplished previously, but recent work has simplified the problem. For the synthesis of tropine, the Robinson condensation has been adapted for radioactive synthesis (6, 8, 9). The Wolffenstein and Mamlock esterification of tropine and tropic acid has been adapted for the use of isotopically labeled intermediates (5, 6, 8, 9). Thus the problem of labeling the tropine moiety of atropine, in positions other than the *endo*-methyl group, has become that of obtaining labeled precursors of succindialdehyde or acetone dicarboxylic acid.

Citric acid is a logical precursor of acetone di-

[1] Citric-2,3,4-$^{14}$C; 2,4-$^{14}$C; and 3-$^{14}$C acids, each having a specific activity of 2 mc./mmole, were prepared by Mallinckrodt Nuclear, Orlando, Fla. Citric-$^{14}$C acids were stored in a desiccator before use.

[2] A 5-ml. round-bottom flask was used routinely.

[3] The yields of tropine-2,3,4-$^{14}$C and tropine-2,3,4-$^{14}$C HCl obtained by reduction of tropanone-2,3,4-$^{14}$C were lower than anticipated. To ensure that sufficient material was present for esterification to atropine-2,3,4-$^{14}$C, unlabeled tropine HCl was added to increase the combined total weight of labeled and unlabeled tropine HCl to 124 mg. (See *Results and Discussion* for a further explanation.)




*Scheme I*

carboxylic acid because high yields of the desired product can be obtained by the method of Adams *et al.* (10). Until recently, suitably labeled citric acids were not available. Citric acid must be labeled in positions other than the carboxyl groups because these are lost in conversion to acetone dicarboxylic acid and subsequent condensation to tropanone. For labeling the tropine ring, citric acid must be labeled in one or more of positions 2, 3, and 4. Of the potentially useful citric acids, citric-3-$^{14}$C, citric-2,4-$^{14}$C, and citric-2,3,4-$^{14}$C acid are now available commercially.

Using the commercially available citric-$^{14}$C acids, the correspondingly labeled atropines were prepared as illustrated in Scheme I.

Using this sequence, tropanone-3-$^{14}$C, tropine-3-$^{14}$C, and atropine-3-$^{14}$C were obtained from citric-3-$^{14}$C acid. Tropanone-2,4-$^{14}$C, tropine-2,4-$^{14}$C, and atropine-2,4-$^{14}$C were synthesized from citric-2,4-$^{14}$C acid. From citric-2,3,4-$^{14}$C acid, tropanone-2,3,4-$^{14}$C, tropine-2,3,4-$^{14}$C, and atropine-2,3,4-$^{14}$C were obtained.

As shown in Table I, the yields of the labeled atropines varied significantly from those predicted on the basis of the authors' previous studies. Previous studies (7, 8) had indicated a 65–70% yield for the formation of tropanone. The deviation from expected yields occurred in this step. In most instances 40–50% yields of labeled tropanone were obtained from labeled citric acids, although an occasional 70% yield was obtained. The critical nature of the citric acid to acetone dicarboxylic acid conversion was not apparent in earlier studies because a known weight of purified keto acid was used in the Robinson condensation. In synthesis, preparation of excess acetone dicarboxylic acid was not practical. Since the quantity of the radioactive acetone dicarboxylic acid from 1 mmole of labeled citric acid was extremely small, no attempt was made to isolate the intermediate, and the entire reaction mixture was used in the Robinson condensation. As a consequence, there are two possible explanations for the observed deviations from expected yields.

Improper labeling or impurity of the labeled citric acids was considered. Using paper chromatography, in a variety of solvent systems, the labeled citric acids were analyzed for impurities. In every instance, an impurity-free starting material was indicated, thus eliminating this potential explanation. That variability of tropanone yields is inherent in the procedure and is not associated with the labeled citric acids is indicated by the following evidence. When unlabeled citric acid was used exactly as described for the labeled compound, tropanone yields were variable and comparable to those from citric-$^{14}$C acid. Furthermore, yields of labeled tropanones were identical whether calculations were based on weight or on radioactivity. This would not have been the case had there been improper labeling or an isotope effect. It was concluded that the use of microquantities of citric acid made much more critical the quantity of fuming $H_2SO_4$ used to convert citric acid to acetone dicarboxylic acid. The authors believe this to be the cause of variability and of deviation from previously predicted tropanone yields.

There were no difficulties in the reduction of labeled tropanones to tropine or in the esterification to labeled atropine. The decreased yield of tropine-2,3,4-$^{14}$C, obtained by reduction of tropanone-2,3,4-$^{14}$C (see Table I), was due to a known, mechan-

TABLE I—LABELED COMPOUNDS SYNTHESIZED FROM COMMERCIALLY AVAILABLE LABELED CITRIC-$^{14}$C ACIDS

| Labeled Compd. | Predicted Yield, % | 3-Labeled Obs. Yield, % | 3-Labeled S.A. mc./mmole | 2,4-Labeled Obs. Yield, % | 2,4-Labeled S.A. mc./mmole | 2,3,4-Labeled Obs. Yield, % | 2,3,4-Labeled S.A. mc./mmole |
|---|---|---|---|---|---|---|---|
| Acetone dicarboxylic acid | 95[a] | — | — | — | — | — | — |
| Tropanone | 65–70 | 44 | 1.0 | 68 | 1.0 | 54 | 1.0 |
| Tropine | 64–69 | 43 | 1.0 | 65 | 1.0 | 41 | 1.0 |
| Atropine | 43–49 | 26 | 1.0 | 34 | 1.0 | 25[b] | 0.62 |

[a] Predicted yields of ACDA based on isolation, using semimicro amounts of citric acid, unlabeled (7). The intermediate was not isolated when the ACDA was labeled. [b] Yield is based on isotope rather than on weight.

ical loss rather than to the procedure. Since this was the first compound made, unlabeled tropine HCl, sufficient to increase the total weight to the 124-mg. weight normally obtained, was added to the tropine-2,3,4-$^{14}$C HCl. For this reason the specific activity of the product was correspondingly decreased to 0.62 mc./mmole. In subsequent experiments, the labeled compounds were not diluted.

The radiochemical purity of the resulting labeled atropines was established by paper and thin-layer chromatography as described previously (8). Each of the labeled atropines contained an impurity that chromatographed in all systems as tropine. The impurity, varying from 2 to 6% of total radioactivity (depending on labeled product), could be removed by a second recrystallization from petroleum ether. The yields reported in Table I have been corrected for the impurity and accurately reflect the yields of the products shown.

## SUMMARY AND CONCLUSIONS

Using the microsynthetic procedures described previously (7, 9), three different tropine-labeled atropines were prepared. From citric-3-$^{14}$C acid, tropanone-3-$^{14}$C (44%), and atropine-3-$^{14}$C (26%) were synthesized with a specific activity of 1 mc./mmole. From citric-2,4-$^{14}$C acid was prepared tropanone (68%), tropine (65%), and atropine (34%) having specific activity of 1 mc./mmole and correspondingly labeled. From citric-2,3,4-$^{14}$C acid was prepared correspondingly tropanone (54%), tropine (53%), and atropine (25%).

The labeled atropine contains a tropine impurity that varied from 2% to 6% of the total radioactivity. This impurity could be removed by recrystallization from petroleum ether.

This report described the first synthesis, by other than biosynthetic method, of tropine and atropine selectively labeled with $^{14}$C in the tropine ring.

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*Keyphrases*

Tropine-labeled atropine—synthesis
Atropine—citric-$^{14}$C acid labeling of 2, 3, 4 positions
Paper chromatography—analysis, radiochemical purity
TLC—analysis, radiochemical purity

# Correlation Between *In Vitro* and *In Vivo* Disintegration Times of Enteric-Coated Tablets

By SØREN RASMUSSEN

**Investigations were made with enteric-coated tablets on the correlation between the first appearance of a substance in the plasma or the urine and the thickness of the vernix layer, measured by the *in vitro* disintegration time. The content of the tablets is quinine hydrochloride, sodium sulfanilate, or sodium *p*-aminosalicylate. The content of each tablet was either 50 or 500 mg. It was shown that within preparations of the same content a proportionality exists between the *in vivo* and the *in vitro* disintegration times. Between series of preparations with different content the factor of proportionality may differ significantly.**

NUMEROUS PAPERS have been published on the correlation between *in vitro* tests and the therapeutic effectiveness of pharmaceutical preparations. The principal aim of such investigations is to establish a predictability of biological properties from simple *in vitro* tests.

With oral preparations of different types, much important work has been done by Levy and his collaborators (1). As to the dissolution rate, it was possible in some cases to show a correlation between the results of *in vitro* and *in vivo* determinations. In such cases it should be possible to predict from *in vitro* tests

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