formational changes, it is dangerous to make any generalization regarding relative shielding effects of these functional groups on the vinylic protons in these systems. This could best be accomplished on mono-substituted compounds.

EXPERIMENTAL

The NMR spectra were determined with a Varian A-60 and/or HR-60 spectrometer. Decoupling was achieved by frequency sweep, double resonance procedure with a Varian DA-60IL spectrometer.

Compounds I and II have been reported in a previous paper (1) as were compounds V–VIII (2).

endo - 5 - Amino - exo - 6 - (p - chlorophenyl)-bicyclo[2.2.2]oct-2-ene Hydrobromide.—The amine was obtained in 48% yield by the reduction of the corresponding nitro compound I with iron in acetic acid by the method of Kornblum and co-workers (5) and as previously described for analogous compounds (6). The hydrobromide salt was formed by bubbling HBr gas into a ligroin solution of the amine. The salt was recrystallized from a mixture of benzene and ethanol, m.p. 247–250° (Kofler micro hot stage).

Anal.—Calcd. for $C_{14}H_{17}BrClN$: C, 53.44; H, 5.45. Found: C, 53.03; H, 5.38.

REFERENCES

(1) Roll, D. B., Nist, B. J., and Huitric, A. C., *Tetrahedron*, **20**, 2851(1964).
(2) Roll, D. B., and Huitric, A. C., *J. Pharm. Sci.*, **54**, 1110(1965).
(3) Johnson, C. E., Jr., and Bovey, F. A., *J. Chem. Phys.*, **29**, 1012(1958).
(4) Huitric, A. C., and Trager, W. F., *J. Org. Chem.*, **27**, 1926(1962).
(5) Kornblum, N., Gurowitz, W. D., Larson, H. O., and Hardies, D. E., *J. Am. Chem. Soc.*, **82**, 3099(1960).
(6) Trager, W. F., Vincenzi, F. F., and Huitric, A. C., *J. Org. Chem.*, **27**, 3006(1962).

Synthesis of Tropine-Labeled Atropine I

Micro Methods for the Synthesis of Tropine and for Its Esterification with Tropic Acid

By GILBERT C. SCHMIDT, THOMAS E. ELING*, and JOHN C. DRACH†

Yields for each step in the esterification of tropine and tropic acid have been determined with micro and semimicro quantities of reactants. In a similar way, the Robinson condensation has been studied. Data are compared with the findings of other investigators. In the esterification of micro quantities of tropine and tropic acid, 70–75 per cent of theoretical atropine yields are obtained routinely. Based on any intermediate in the Robinson condensation, micro quantities of reactants routinely produce 67–72 per cent of theoretical tropine yields. Predicted yields of 47–54 per cent atropine from Robinson intermediates were confirmed by synthesis of atropine from each of the Robinson reactants. The procedures are designed for the synthesis of labeled tropine and tropine-labeled atropine from labeled arabinose.

THE ESTABLISHMENT of metabolic pathways in tropine metabolism awaits the development of synthetic methods for selectively labeling the tropine ring. The fate of this heterocycle, free or as a structural component of atropine and related compounds, is essentially unknown because suitably ring-labeled compounds are not available in amounts adequate for animal studies.

Tritium-labeled atropine (1) and randomly-^{14}C-labeled atropine (2) are of limited usefulness because the labeling is not specific. Biosynthetic atropines (3, 4) are suitably labeled, and could be used to determine the metabolic fate of the tropine moiety, but complexities of preparation have limited their availability and prevented their use in animal studies. Fodor *et al.* (5) and Werner *et al.* (6) prepared *N*-methyl-^{14}C-tropine, then esterified it with unlabeled tropic acid to obtain tropine-labeled atropine. Subsequent metabolic studies (7), although not extensive, clearly indicate the need to label tropine and atropine in other positions. During studies of atropine toxicity, this need became very apparent to the authors.

The extreme toxicity of atropine and related compounds is an added complication, because low doses must be used for *in vivo* studies, and minor metabolites are correspondingly difficult to detect. Compounds of reasonably high specific

Received September 16, 1966, from the Children's Hospital, the Division of Pharmacology, Department of Pediatrics, and the Department of Biological Chemistry, Medical College of Alabama, University of Alabama, Birmingham. 35233.
Accepted for publication October 19, 1966.
Abstracted in part from a thesis submitted by Thomas E. Eling to the Graduate School, University of Alabama, Birmingham, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported by research grant GM-13844 (formerly grant GM-08447) from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and research grant AFOSR 87-65,66 from the U. S. Department of the Air Force, Washington, D. C.
* National Institutes of Health Predoctoral Fellow.
† Present address: Research Division, Parke-Davis and Co. Ann Arbor, Mich.

activity are required, the use of unlabeled carriers in synthetic procedures is not practical, and a requirement for microsynthetic methods is imposed. For selective labeling of tropine, versatile microsynthetic methods are not available. Furthermore, micro procedures for the esterification of tropine and tropic acid have been used infrequently, by very few investigators (5, 6, 8), and many details essential for good reproducibility have not been published.

The authors concluded that labeling atropine in the tropine moiety would depend on increased reproducibility of published esterification procedures (5–9) and on adaptation of the Robinson condensation for use with micro quantities of reactants. A semimicro, prototype method evolved from quantitated studies of these two reaction sequences. From similar studies of the prototype, there resulted a reproducible microsynthesis of tropine and atropine. Detailed microsyntheses of atropine and tropine, employing 100–200 mg. of reactants, are reported. Results of both the micro and semimicro methods are compared with observations of other investigators. The feasibility of this approach to tropine and atropine labeling is established. Establishment of these pathways, and the availability of a microsynthesis of succindialdehyde from arabinose (10), provide the first feasible approach to labeling tropine in other than the *N*-methyl position.

EXPERIMENTAL

Preparation of O-Acetyltropic Acid Chloride—The method of Wolffenstein and Mamlock (9) was modified for use with micro quantities of reactants. For the synthesis of *O*-acetyltropic acid, 120 mg. tropic acid and 0.12 ml. acetyl chloride[1] were introduced into a 10-ml. round-bottom flask,[2] the flask was stoppered, and the reaction mixture allowed to stand for 12–15 hr. at room temperature. The resulting mass was heated at 100° for 7 min., and the excess acetyl chloride removed at 50°, *in vacuo*, using a flash evaporator.[3] Crystallization of the oily product, which otherwise required several days, was initiated by seeding with a crystal of pure *O*-acetyltropic acid.[4] When stored for 2–4 hr., in a vacuum desiccator, over calcium chloride, the product crystallized as a solid mass which was immediately converted to the acid chloride. To the *O*-acetyltropic acid, contained in the original reaction vessel and usually weighing 148 mg.,[1] was added 0.66 ml. thionyl chloride. The reaction vessel was fitted immediately with a calcium chloride drying tube and the reaction mixture heated, on an oil bath, at 50°, for 1 hr., during which period the mass liquefied and gas evolved. The drying tube was removed, the flask was fitted with a short-path vacuum distillation apparatus, and the excess thionyl chloride removed, at 70°, *in vacuo*.[5] The last traces of thionyl chloride were removed by the addition and *in vacuo* distillation of six 0.5-ml. portions of anhydrous benzene.[6] The product, a lemon-yellow oil having a strong and disagreeable odor, was reacted immediately with tropine HCl.[7]

Preparation of Tropine HCl—To 100 mg. dry tropine,[8] contained in the 10-ml. flask in which it had been prepared, was added approximately 5 ml. anhydrous ether. Tropine HCl[9] precipitated instantaneously when anhydrous HCl was passed carefully into the ether solution. The flow of gas was interrupted immediately, ether was removed quickly, *in vacuo*, and the crystalline product stored immediately,[10] within the reaction vessel, in a vacuum desiccator, over fresh NaOH pellets, until used for esterification.[11]

Esterification of O-Acetyltropic Acid Chloride and Tropine Hydrochloride—Thoroughly dried tropine HCl, 124 mg., was added to 160 mg. freshly prepared *O*-acetyltropic acid chloride, still contained in the original reaction vessel.[9–11] While stirring frequently, the reaction mixture was heated for 20–25 min., under anhydrous condition, in an oil bath that had been adjusted previously to 100° The resulting *O*-acetyltropine HCl was cooled to room temperature, then treated *in situ*[12] with 0.66 ml. water and maintained at 50° for 24 hr. The reaction mixture was cooled quickly to 0° and the atropine precipitated by careful, dropwise addition of 6–8 drops of saturated, aqueous NaOH. While maintaining the temperature at 0°, the aqueous

[1] In some micro experiments, 395 mg. tropic acid and 0.36 ml. acetyl chloride were used. After recrystallization, 148 mg. *O*-acetyltropic acid was removed, transferred to a thoroughly dried 10-ml. flask, then converted to the acid chloride as described here.

[2] Micro-Ware, Vineland, N. J.

[3] Rinco Instrument Co., Greenville, Ill.

[4] If the reaction mixture is not seeded, crystallization is delayed and atropine is contaminated with an unidentified, low-melting impurity. This difficulty is avoided by rapid crystallization and immediate conversion of the product to the acid chloride.

[5] Anhydrous conditions are essential and a dry ice–acetone trap must be placed between the distillation apparatus and the vacuum source.

[6] Unless all traces of thionyl chloride are removed, yields in the subsequent esterification are prohibitively low. The benzene must be anhydrous and the total time to effect all additions and removals should not exceed 15 min.

[7] The product is extremely sensitive to moisture and must be reacted immediately with tropine HCl. Prior to beginning the synthesis of *O*-acetyltropic acid chloride, tropine was prepared, converted to the HCl, and stored in a vacuum desiccator for 24 hr.

[8] Tropine was prepared within a day or two of use and was stored over NaOH pellets until converted to the HCl. Longer period of storage results in significant decomposition of tropine, which can be purified by vacuum distillation.

[9] Formation of the HCl by dissolving tropine in methanolic HCl and removing the solvent *in vacuo* was satisfactory at the semimicro level but gave inadequate yields at the micro level.

[10] Because tropine HCl is extremely hygroscopic, very rigid precautions to exclude moisture must be observed if a crystalline product is to be obtained.

[11] Tropine HCl and *O*-acetyltropic acid were always esterified in the reaction vessel that contained the acyl halide. Prior to esterification, this vessel was fitted with a standard taper T-tube, the side arm of which carried a calcium chloride tube, and the vertical arm of which contained a stirring rod secured in place with rubber tubing. This arrangement assured exclusion of moisture and allowed for manual stirring during the esterification.

[12] Experience has shown that, under the conditions stated, esterification is complete. During heating, the originally plastic mass liquefies, then becomes extremely viscous when cooled. When treated with water and stirred, it gradually dissolves to form a tan solution. If the esterification mixture is heated longer or more vigorously than stated, a brown to dark brown solution results when the product is treated with water. Under the latter conditions, yields are reduced from 10–30%.

phase was removed by careful decantation, the product washed with three 1-ml. portions of ice water, and the washed product seeded with a crystal of pure atropine.[13]

The crystalline, atropine free base was dried immediately in a vacuum desiccator over $CaCl_2$. When dry, the product was dissolved in 75 ml. anhydrous petroleum ether,[14] the solution was concentrated to approximately 10 ml., and purified atropine precipitated by cooling in the deep-freezer. Petroleum ether was carefully decanted from the crystals and the product dried for 12 hr. in a desiccator over $CaCl_2$ before converting to atropine sulfate. The dried, purified atropine base, usually 70% of theoretical yields, was dissolved in acetone and the calculated amount of 2 *N* H_2SO_4 in acetone was added. Atropine sulfate precipitated immediately. When cooled in a deep-freezer to insure complete precipitation, then washed with cold acetone and dried, the collected crystals represented 95–98% conversion to the sulfate.

Preparation of Acetone Dicarboxylic Acid—Acetone dicarboxylic acid was prepared from citric acid by modification of the general scheme outlined by Adams *et al.* (14).

For microsynthesis, 2.2 ml. fuming H_2SO_4 (20% excess SO_3), contained in a 100-ml. beaker, was cooled to −8° in a salt and ice bath. Using a thermometer as a stirring rod, 1.0 Gm. of citric acid was added, with constant stirring during approximately 15 min., at a rate slow enough to maintain the temperature at −5° or lower throughout the addition. The beaker and contents were removed from the cooling bath and stirred on a magnetic stirrer, at room temperature, for about 1 hr., until the evolution of carbon monoxide ceased. The reaction mixture was cooled again to −8°, and 4 Gm. chipped ice was added, carefully and in small portions, so the temperature never exceeded 0°. After the addition of all the ice, the reaction was cooled again to −8°, the product was collected on a sintered-glass filter, and was used at once.[15]

Preparation of Succindialdehyde—Succindialdehyde was prepared *in situ* by hydrolysis of 2,5-diethoxytetrahydrofuran,[16] by a simplified modification of the procedure of Fakstorp (15). To 2 ml. water and 5 μl. concentrated HCl, contained in a 50-ml. beaker, was added 0.17 ml. diethoxytetrahydrofuran.[17] The mixture was stirred at room temperature for 10 min., then used immediately for the synthesis of tropanone.[18]

Synthesis of Tropanone—After preliminary experiments the following "standard" procedure for the synthesis of micro quantities of tropanone was adopted. When molecular ratios were altered, the weight of methylamine hydrochloride was the reference point because this reactant can be weighed easily. Correspondingly more succindialdehyde was made available by simple proportionate changes during its preparation, and the amount of acetone dicarboxylic acid was altered by the use of a greater proportion of the moist material.

To the solution of succindialdehyde, prepared as described above and representing 1 mmole dialdehyde, was added 1 mmole acetone dicarboxylic acid, freshly prepared and still in the moist state.[19] Saturated aqueous Na_2HPO_4 was added to adjust the pH to 4.5–5.0, the solution was transferred to a 250-ml. side arm conical flask, and the system purged with nitrogen for 10 min. After the addition and dissolution of 1 mmole solid methylamine HCl, the system was quickly evacuated to remove most of the gas phase, then closed and allowed to remain under partial vacuum, at room temperature, for 12 hr. The slight evacuation was then repeated and the reaction mixture allowed to remain at room temperature for an additional 12 hr.

After 24 hr., the system was opened and the solution saturated with K_2CO_3, added in small portions, while stirring constantly and cooling the reaction mixture in a salt–ice bath. The alkaline solution was cooled to −5°, extracted with 100 ml. petroleum ether (boiling range 30–80°),[20] and the organic layer removed and reserved. The cooling, saturation, and extraction of the aqueous phase were repeated 6 times, the reaction vessel and separator were washed with petroleum ether, and the combined extracts dried over anhydrous Na_2SO_4. Portions were transferred to a sublimation flask,[21] solvent was removed *in vacuo* at 40° on the flash evaporator, and the Na_2SO_4 finally washed with anhydrous petroleum ether. After the *in vacuo* removal of this final solvent aliquot, crude oily brown tropanone remained in the sublimation flask. The product, contained in the sublimation flask was dried overnight in a desiccator over NaOH and $CaCl_2$.[22]

After drying, the product was purified by sublimation. A cold finger filled with dry ice and acetone was inserted into the sublimation flask containing the crude product. Tropanone was sublimed at 40°

[13] Atropine free base precipitated as a brownish, very viscous oil that adhered to the sides of the reaction vessel. The aqueous phase and washings were easily removed by careful decantation. The product quickly crystallized when maintained at 0° and seeded with pure atropine.

[14] Boiling range 30–60°.

[15] In semimicro experiments it was found that a very carefully washed product may be stored in a desiccator for 4 or 5 days, but that washing and recrystallization are accompanied by significant losses of product. Losses may be minimized by using ice-cold anhydrous ethyl acetate. If traces of sulfuric acid remain, there is significant decomposition in 2–3 hr. For calculation of yields the product was washed with ice-cold, anhydrous ethyl acetate and dried in a desiccator for 2 days. A yield of 95% and a product melting at 135° were obtained. When recrystallized from ethyl acetate, acetone dicarboxylic acid melted at 138°. To prevent losses during the synthesis of tropine, acetone dicarboxylic acid was neither washed nor recrystallized, but was used immediately in the Robinson condensation.

[16] Beacon Chemical Industries, Inc., Cambridge, Mass.

[17] In semimicro experiments, 1 vol. diethoxytetrahydrofuran, 16 vol. water, and 0.02 vol. concentrated HCl was mixed, then stirred until the translucent mixture became crystal clear

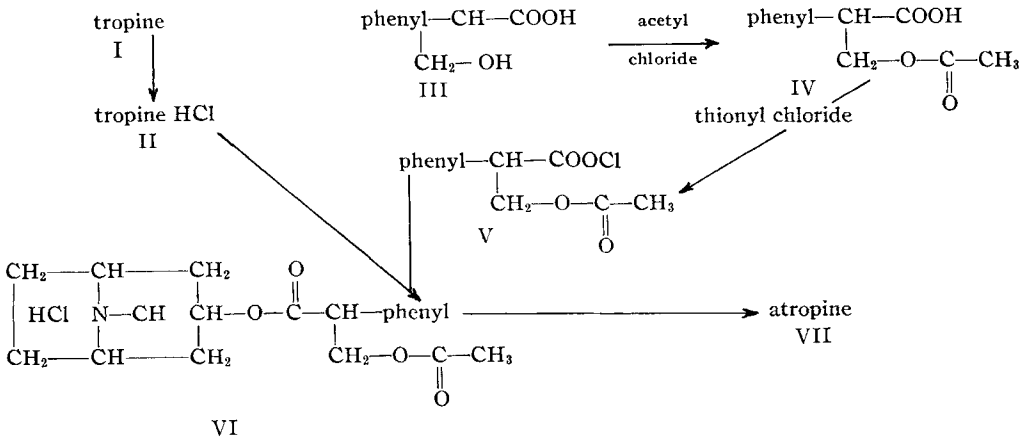
[18] To determine the extent of hydrolysis as a function of time, aliquots were treated with 2,4-dinitrophenylhydrazine, as described by Shriner *et al.* (16). A quantitative yield of the bis-dinitrophenylhydrazone, m.p. 270–275°, without recrystallization, was obtained after 5 min. in micro experiments. At the semimicro level, yields of 95–100% were always obtained when the opaque reaction mixture had become crystal clear.

[19] Twenty per cent of the moist weight of the product resulting from 1 Gm. citric acid corresponds to 1 mmole of acetone dicarboxylic acid.

[20] Allied Chemical Corp. The solvent must have a boiling range of 30–80 or extraction is incomplete and there are appreciable decreases in tropanone yield. Diethyl ether and a continuous extractor were used in semimicro experiments, but difficulties were encountered at the micro level with this solvent. For this reason, petroleum ether and manual extraction were used in micro experiments.

[21] This flask had a 250-ml. capacity and was of the design described by Werner (6).

[22] Care must be exercised in removing the solvent, and the use of a vacuum desiccator is inadvisable, because the low vapor pressure of tropanone results in losses on undue exposure to vacuum.



Scheme I

at 1 × 10^{-4} mm. mercury,[23] for 10 min. The vacuum was disconnected, dry ice and acetone were removed, the cold finger was removed from the flask, and the accumulated tropanone was washed into a 150-ml. beaker using small portions of absolute ethanol. The operation was repeated 2 times, using 10 and 15-min. sublimation periods. The combined ethanol solutions of sublimed tropanone were evaporated to dryness, on the micro flash evaporator, in a previously weighed 25-ml. flask. The product crystallized quickly on cooling, and was dried in a desiccator overnight.[22,24]

Reduction of Tropanone to Tropine—To the tropanone (approximately 100 mg. from 67.5 mg. methylamine HCl), contained in the 25-ml. flask in which it had been dried, was added 5 ml. absolute ethanol, a magnetic stirring bar, and 0.5 ml. of a suspension of Raney nickel in absolute ethanol.[25] The flask was placed in a water bath, attached to a gas buret, and the reaction mixture continuously stirred while flushing the system seven times by alternate admission and expulsion of hydrogen. Finally, the system was filled with hydrogen, closed, and continuously stirred until reduction was complete.[26]

To remove Raney nickel, the ethanol solution was filtered through a sintered-glass filter into a 25-ml. round-bottom flask, the original flask and nickel were washed repeatedly with small portions of absolute ethanol, and most of the solvent removed on the flash evaporator. When the volume of ethanol had been reduced to approximately 10 ml., the solution was transferred to a tared 10-ml. flat-bottom flask, evaporated to dryness, and both flask and contents dried in a vacuum desiccator[27] before conversion to the hydrochloride.

[23] Two stage vacuum pump, Welch Scientific Co., Chicago, Ill.

[24] Tropanone yield was determined from the weight of this product, m.p. 42°.

[25] To prepare Raney nickel, 2.5 Gm. of alloy (Raney Nickel Co., Chattanooga, Tenn.) was heated at 50° for 15 min. with a solution of 3.2 Gm. NaOH in 12.5 ml. water. The product was washed with water, ethanol, and absolute ethanol, then suspended in absolute ethanol. The final suspension represented 35 mg./ml. of Raney nickel.

[26] When reduction was complete, a 1-μl. aliquot gave a negative test when spotted on filter paper and treated with 2,4-dinitrophenylhydrazine solution. It is sometimes necessary to add additional aliquots of Raney nickel to effect complete reduction.

[27] Conversion to the hydrochloride is carried on in this vessel. The product is white, crystalline, m.p. 63–65°, and adequately pure for conversion to the HCl without recrystallization. Picrate darkens at 275° and melts at 295°.

RESULTS AND DISCUSSION

For the synthesis of tropine-labeled atropine, the two key steps are synthesis of labeled tropine and esterification of the labeled heterocycle with unlabeled tropic acid. Good reproducible yields with micro quantities of reactants must be obtainable with each of these steps for the synthesis of tropine-labeled atropine to be feasible.

Microesterifications of tropine and tropic acid have been done by very few investigators (5, 6, 8). All reported procedures are modifications of the Wolffenstein and Mamlock method (9) and involve conversion of tropine (I) to the hydrochloride (II), conversion of tropic acid (III) to *O*-acetyltropic acid chloride (V) *via* the intermediate acetyl compound (IV), esterification of II and V to form *O*-acetylatropine HCl (VI), and mild, selective hydrolysis to the free alkaloid (VII). (Scheme I.)

Although 63–70% yields have been reported for the microesterification of tropine and tropic acid, there have been no detailed studies of the method. Lack of reproducibility was disclosed in semimicro studies and was re-encountered at the micro level. Werner's data (6) indicate a similar difficulty. He obtained 70% yields of tropine-labeled atropine after a single esterification, but isolation of unreacted tropic acid and two esterifications were required to obtain a 63% yield of tropic acid-labeled atropine. Because of these inconsistencies, the authors quantitatively studied each step in the reaction sequence. Results of these studies are compared in Table I with summarized observations of other investigators.

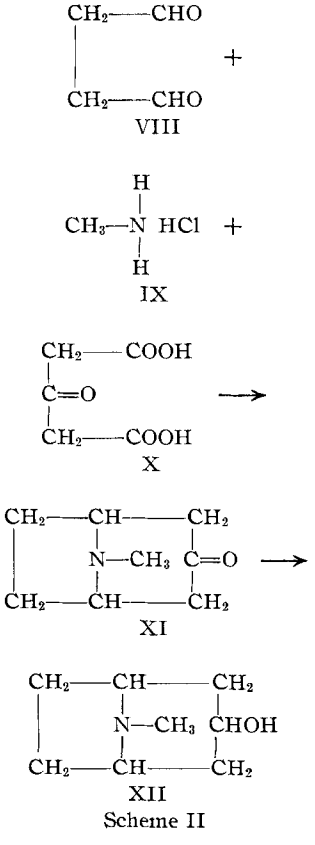
From the studies summarized in Table I, there evolved a reproducible microesterification of tropine and tropic acid. In contrast to others, equimolar quantities of tropine and tropic acid are used, and the method is equally adaptable for labeling atropine in either of these component moieties. The problem of synthesizing tropine-labeled atropine is thereby reduced to that of labeling the heterocycle. The need is for a microsynthesis of tropine that is adaptable for the introduction of appropriately labeled intermediates.

The classical approach to tropine synthesis is the method of Robinson (11), as modified by Schopf and Lehman (12). Succindialdehyde (VIII), methylamine HCl (IX), and acetone dicarboxylic acid (X) are condensed under essentially physiological condi-

TABLE I—COMPARISON OF OBSERVED AND REPORTED DATA FOR THE ESTERIFICATION OF TROPINE AND TROPIC ACID

	Micro[a]	Semimicro[b]	Werner[c]	Werner[d]	Fodor[e]	Mamlock[f]
Wt. of tropic acid used	120 mg.	1.5 Gm.	395 mg.	...	...	3.0 Gm.
Yield of *O*-acetyltropic acid	148 mg. (100%)	1.81 Gm. (100%)	495 mg. (100%)	...	...	...
M.p.[g] *O*-acetyltropic acid	87–88°	89–90°	...	...	...	...
Wt. *O*-acetyltropic acid used	148 mg.	1.81 Gm.	495 mg.	130 mg.	...	...
Yield *O*-acetyltropic acid chloride	160 mg. (99%)	1.95 Gm. (99%)	540 mg. (100%)	...	...	...
Wt. tropine used[h]	100 mg.	1.27 Gm.	...	98.8 mg.	620 mg.	...
Yield tropine HCl	124 mg. (99%)	1.56 Gm. (98%)	...	124.3 mg. (101%)	260 mg. (84.4%)	...
M.p. tropine HCl	280°	280°	...	200	...	...
Wt. tropine HCl used	124 mg.	1.56 Gm.	505 mg.	124.3 mg.	263 mg.	3.2 Gm.
Wt. *O*-acetyltropic acid chloride used	160 mg.	1.95 Gm.	540 mg.	141 mg.	320 mg.	...
Yield of atropine[i] free base	144 mg. (70%)	1.95 Gm. (75%)	430.9 mg. (63%)	142 mg. (70%)	120 mg. (63%)	(80%)
M.p.[j] atropine free base	114–116°	116–117°	...	115°	108°	115°
Mixed m.p. atropine free base	114–116°	114–116°	...	...	...	...
M.p. atropine picrate	174–175°	174–175°	...	...	...	...
M.p.[k] atropine sulfate	191°	191°	189°	189°	...	...

[a] Micro procedure described in this communication. [b] Semimicro procedure described in this communication. [c] Micro method of Werner *et al.* (6) for labeling tropic acid moiety. [d] Micro method of Werner *et al.* for labeling the tropine moiety (6). [e] Fodor *et al.* (5). [f] Wolffenstein and Mamlock (9). [g] All melting points determined with Fisher-Johns melting point apparatus. [h] Fodor used tropine picrate and converted to the HCl with an ion exchange resin. [i] Yields based on tropine except for Werner's tropic acid labeling, which was based on radioactive tropic acid and represents the sum of two esterifications. [j] Melting point reported by Fodor is for hyoscyamine. [k] "Merck Index," 7th ed., Merck & Co., Inc., Rahway, N. J., p. 111, reports melting points for the sulfate from 190–194°.



Scheme II

tions to yield tropanone (XI), which then is reduced to tropine (XII). (Scheme II.)

Although the scheme is inherently versatile and therefore potentially suited for radiosynthesis, there have been surprisingly few studies of the reaction (11–13) and no reference to its use at the micro level.[28] In Table II the results of semimicro and microsyntheses, developed during the studies described in this communication, are compared with the findings of other investigators.

To determine the effect of hydrogen-ion concentration and potential losses from decreasing weights of reactants, concentration of all reactants was fixed at 0.20 *M*, while decreasing the weight of methylamine HCl from 33.0 Gm. to 1.69 Gm. at each of four hydrogen-ion levels within the range pH 4.0–pH 10.5. Neither decreasing weights nor varying hydrogen-ion level had significant effects on tropanone yields. The weight of methylamine HCl was then fixed at 1.69 Gm., equimolar concentrations of reactants were used, and concentration was varied from 0.01–0.20 *M*, at each of the four hydrogen-ion levels previously employed. There were no significant differences in tropanone yields, thereby validating arbitrary choices of both concentration and hydrogen-ion level, within the ranges studied. In comparison, Keagle and Hartung (13) reported no significant differences in tropanone yields within the concentration range 0.04–0.16 *M* and the pH range pH 5.0–pH 11.0. Agreement with Keagle and Hartung's findings is apparent.

There is general agreement with Schopf and

[28] Werner's microsynthesis of *N*-methyl-labeled tropine and atropine were published just prior to completion of this investigation.

TABLE II—COMPARISON OF OBSERVED AND REPORTED YIELDS FOR THE SYNTHESIS OF TROPANONE BY THE MODIFIED ROBINSON CONDENSATION

Method	Wt. of Amine HCl	Molarity Amine HCl	Molecular Ratios of Amine–Aldehyde–Acid	Initial[a] pH	Tropanone,[b] Yield, %	Identification[c]
						Distils 113–115°, 25 mm.
	1.69 Gm.	0.01 *M*	1:1:1	4.0	71	Distils 72–74°, 10 mm.
Semimicro[d]	to	to	1:1:1	4.5	75	M.p. free base 41–42°
	33.8 Gm.	0.20 *M*	1:1:1	7.0	73	M.p. picrate, 220°
			1:1:1	10.5	69	M.p. oxime, 111°
						M.p. dipiperonylidene derivative, 214°
	67.5 mg.	0.01 *M*	1:1:1	5.0	70	
	67.5 mg.	0.02 *M*	1:1:1	5.0	70	
	67.5 mg.	0.01 *M*	1:2:2	5.0	65	M.p. free base, 42°
Micro[d]	67.5 mg.	0.02 *M*	1:2:2	5.0	71	M.p. picrate, 220°
	135 mg.	0.02 *M*	2:1:2	5.0	68	M.p. oxime, 111°
	135 mg.	0.04 *M*	2:1:2	5.0	72	M.p. dipiperonylidene
	135 mg.	0.02 *M*	2:2:1	5.0	67	derivative, 214°
	135 mg.	0.04 *M*	2:2:1	5.0	69	I.R. analysis
Werner (6)	67.5 mg.	0.008 *M*	1:2:3	5.6	70	M.p. free base, 42°
			4:2:5	3.0	47	
			4:2:5	5.0	54	
			4:2:5	7.0	65	
			4:2:5	9.0	66	
Schopf and Lehman	...	0.04 *M*	4:2:5	11.0	86	M.p. picrate, 220°
(12)			4:2:5	13.0	3	
			2:1:1	3.0	68	
			2:1:1	5.0	83	
			2:1:1	7.0	78	
			2:1:1	9.0	61	
			2:1:1	11.0	64	
			2:1:1	13.0	5	
	2.7 Gm.	0.04 *M*	2:1:2	5.2	58	
	2.7 Gm.	0.04 *M*	2:1:2	7.1	62.4	
Keagle and Hartung	2.7 Gm.	0.04 *M*	2:1:2	7.1	62.4	M.p. picrate, 220°
(13)	2.7 Gm.	0.04 *M*	2:1:2	11.02	60.3	M.p. dipiperonylidene
	2.7 Gm.	0.08 *M*	2:1:2	11.02	60.3	derivative, 214°
	21.6 Gm.	0.16 *M*	2:1:2	...	60–65	

[a] Hydrogen-ion concentration measured with Corning model 7 pH meter. [b] Yields for micro and semimicro procedures and for Werner's method are as the free base, after purification by sublimation. Other yields are as the picrate. [c] Dipiperonylidene derivative and picrates prepared as described by Keagle and Hartung (13). Melting points reported in this communication were determined with a Fisher-Johns melting point apparatus. For infrared analysis, samples were compared with authentic samples of tropine and tropinone as picrates using a Perkin-Elmer model 221 I.R. spectrophotometer. [d] Semimicro and micro procedures described in this communication.

Lehman (12), but their data indicate a possible interdependence of pH optima and ratios of reactants. When both amine and keto acid were in excess, tropanone yields were appreciably higher at pH 11.0, but when only the amine was in excess, yields were appreciably higher at pH 5.0. Because of the narrow pH range studied, our data do not completely exclude an interdependence. Results obtained in semimicro experiments, with varying ratios of reactants at pH 5.0 and pH 7.0, showed no dependence of tropanone yield on molecular ratios. These findings were confirmed in microsyntheses at pH 5.0, as shown in Table II. All data reported here were obtained when diethoxytetrahydrofuran was used as the source of succindialdehyde, while both Schopf and Lehman and Keagle and Hartung prepared the aldehyde from pyrrole *via* the intermediary succinaldoxime. The furan derivative is a far superior aldehyde source. Based on experience with both pyrrole and diethoxytetrahydrofuran, the authors concluded that Schopf and Lehman's data probably reflect a lack of reproducibility arising from the use of pyrrole.

During adaptation and modification of the prototype procedure, previously described experiments were repeated on a more limited scale. The data, which are not presented here, showed that flexibility of pH was no different when microquantities of reactants were used. An initial pH of 5.0 was chosen arbitrarily because carbon dioxide evolution is readily apparent and is a reliable, visual indication that the condensation is progressing properly. Because carbon dioxide is totally or partially bound, this guide is not available at hydrogen-ion levels near and above neutrality.

Changes in buffer composition were considered for possible effects on tropanone yields. The micro procedure described here was compared in parallel experiments with Werner's method (6). Identical 70% yields of pure tropanone were obtained, thereby confirming Werner's work and indicating that acetate offered no advantage over phosphate. Phosphate was then compared with citrate at different hydrogen-ion levels and different reaction times. Maximum yields of 50% pure tropanone were isolated when citrate buffers were used. The authors

could not confirm the claims of Hungarian workers (17) that the use of citrate buffers results in tropanone yields of 90% or more and a decrease in reaction time to 6 hr. The intent was to isolate pure tropanone in maximum yields, and the variables introduced by their use of the reineckate as a criterion for completeness of condensation was not considered. For our purposes, phosphate and acetate were equally effective and both were superior to citrate. Phosphate was chosen on the basis of more extensive experience with this system.

Further studies with the microsynthetic method, shown in Table II, were limited on the basis of probable conditions in radiosyntheses. Variations of methylamine weight from 67.5–135 mg., concentrations from 0.01–0.04 *M*, and molecular ratios within the probable experimental range had no significant effect on tropanone yields. Similarly, the use of an excess of one or more reactants had no effect. These observations confirm, for the micro modification, the results obtained with the semimicro prototype; reproducibility is good, there is a flexibility in actual quantities and molecular ratios of reactants, and there is no requirement for an excess of any particular intermediate.

The micro condensation was developed as an instrument for tropine labeling and is well suited for this purpose. It is flexible and reproducible. Equimolar ratios, or any desired proportion of reactants, can be used without affecting yields. There is no requirement for added carriers and specific activity of the product is limited only by the specific activities of the intermediates.

CONCLUSIONS

The esterification of tropine and tropic acid has been studied in detail, using both semimicro and microquantities of reactants. Yields for each step in the sequence have been determined and have been compared with the findings of other investigators. From these studies there evolved a micro procedure for esterifying tropine and tropic acid. Equimolar quantities of reactants are employed and the method is equally suited for labeling either of the component moieties of atropine. With proper attention to detail, esterification is unusually reproducible and yields of 70–75% atropine free base are obtained routinely.

In a similar way, the Robinson condensation has been studied. There has evolved a reproducible micro method for condensing equimolar quantities of methylamine HCl, acetone dicarboxylic acid, and succinic dialdehyde. There exists an ideal flexibility of molecular ratios, concentrations of reactants, and hydrogen-ion levels. The method is ideally suited for labeling tropine through any or all intermediates of the Robinson condensation, and yields of 67–72% tropine are obtained routinely.

Based on studies of the esterification and the condensation, yields of 47–54% atropine free base should result from any precursor of the Robinson condensation. This prediction has been confirmed by synthesis of atropine from each of the three compounds. This study, and Werner's work, clearly establish the feasibility of using the Robinson condensation to label tropine. The feasibility of esterifying labeled tropine with unlabeled tropic acid has been established. Results of this study, and a preliminary report describing a prototype synthesis of succindialdehyde from arabinose (10), make available for the first time a synthetic method for labeling the carbon skeleton of the tropine moiety. Details of the synthesis will be published in the second paper of this series.

REFERENCES

(1) Schmidt, H. L., and Werner, G., *Ann.*, **656**, 149 (1962).
(2) Geiling, K. M. K., *Med. Ann. District Columbia*, **201**, 197(1951).
(3) Leete, E., Marion, L., and Spenser, I. D., *Can. J. Chem.*, **32**, 1116(1964), Leete, E., Marion, L., and Spenser, I. D., *Nature*, **174**, 650(1954).
(4) Kaczkowski, J., Schutte, H. R., and Mothes, K., *Naturwiss.*, **47**, 304(1960).
(5) Fodor, G., and Janszo, G., Otvos, L., and Benfi, D., *Ber.*, **93**, 2681(1960).
(6) Werner, G., Schmidt, H. L., and Kassner, E., *Ann.*, **644**, 109(1961).
(7) Werner, G., and Schmidt, H. L., *Naturwiss.*, **46**, 626(1959).
(8) Murray, A., and Williams, D. L., "Organic Synthesis with Isotopes," part I, Interscience Publishers, Inc., New York, N. Y., 1958, p. 550.
(9) Wolffenstein, R., and Mamlock, L., *Ber.*, **41**, 723 (1908).
(10) Schmidt, G. C., Drach, J., and Eling, T. E., Abstracts from the American Association for the Advancement of Science, Montreal, Quebec, Canada, December 30, 1964.
(11) Robinson, R., *J. Chem. Soc.*, **111**, 762(1917).
(12) Schopf, A., and Lehman, G., *Ann.*, **518**, 5(1935).
(13) Keagle, A. C., and Hartung, W. H., *J. Am. Chem. Soc.*, **68**, 1608(1946).
(14) Adams, R., Chiles, M. M., and Rasswoller, G. F., "Organic Synthesis," coll. vol. I, John Wiley & Sons, Inc., New York, N. Y., 1946, p. 10.
(15) Fakstrop, J., Raleigh, D., and Schnupp, L., *J. Am. Chem. Soc.*, **72**, 869(1950).
(16) Shriner, R. L., Fuson, R. L., and Curton, D. Y., "The Systemic Identification of Organic Compounds," 5th ed., John Wiley & Sons, Inc., New York, N. Y., 1964, p. 253.
(17) Simonyi, G., and Takar, O., *Acta Chim. Acad. Sci. Hung.*, **6**, 365(1955).