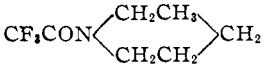
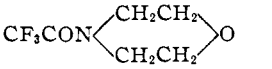
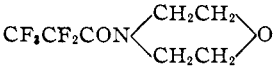
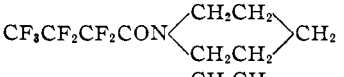
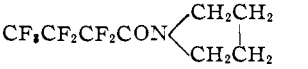
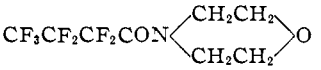
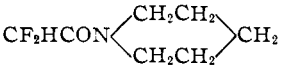
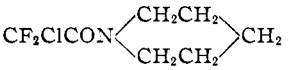
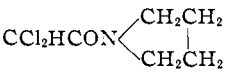
TABLE I

	M.p. or b.p. (mm.), °C.	Yield, %	$n^{25}D$	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Infrared absorption bands in μ[c,d]	
N-Substituted trifluoroacetamides											
$CF_3CON(C_2H_5)_2$[a]	30(2)	60	1.3780	42.60	42.48	5.96	6.03	8.27	8.48	..	..
	44(1)	84	1.4153	46.41	46.46	5.56	5.66	7.73	7.74	5.90	..
	47(1)	87	1.4177	39.38	39.35	4.56	4.40	7.46	7.64	..	..
N-Substituted perfluoropropionamides											
$CF_3CF_2CONHC_4H_9$	48(1)	80	1.3642	38.39	38.37	4.60	4.67	6.38	6.52	..	..
	59(1) 46–47	85	....	36.06	35.99	3.45	3.58	6.00	6.16	..	..
N-Substituted perfluorobutyramides											
$CF_3CF_2CF_2CONHC_4H_9$	56(2)	80	1.3568	35.70	35.88	3.74	3.84	..	..	5.85	6.40
	57(2)	85	1.3846	38.45	38.39	3.58	3.58	4.98	4.99	5.95	..
	65(2)	76	1.3755	35.97	35.92	3.01	3.19	5.24	5.31	5.95	..
	72(2)	89	1.3850	33.94	33.85	2.84	2.94	4.94	4.95	5.95	..
N-Substituted difluoroacetamide											
	66(3)	85	1.4500	51.55	51.36	6.79	6.92	8.58	8.35	..	..
N-Substituted chlorodifluoroacetamide											
	98(10)	90	1.4520	42.55	42.55	5.10	4.92	7.09	7.12	..	..
N-Substituted dichloroacetamide[b]											
	120(2)	75	1.5182	39.59	39.54	4.98	5.06	7.69	7.85	..	..

[a] N,N-Diethyltrifluoroacetamide has been prepared recently by a different method; J. H. Robson and T. Reinhart, THIS JOURNAL, **77**, 498 (1955). [b] Calcd.: Cl, 38.95. Found: Cl, 38.84. [c] Infrared data are included only for those amides not previously reported. [d] *t*-Butyl trifluoroacetate and β-trifluoroethyl trifluoroacetate absorbed at 5.65 μ and 5.55 μ respectively.

esters of trifluoroacetic acid form amides with both primary and secondary amines. In this case it is assumed that the strongly electron-attracting trifluoromethyl group increases the ease of proton transfer so that the reactions proceed by way of the oxonium intermediate with both primary and secondary amines.[2]

Infrared data for compounds used in this investigation which have not been reported previously and the physical data and analyses for all of the new compounds are included in Table I.

Acknowledgment.—The author wishes to thank Dr. Allan R. Day for his interest and support in this study.

Experimental

The esters used in this work, with the exception of *t*-butyl trifluoroacetate, were either purchased or prepared by known methods.

Preparation of *t*-Butyl Trifluoroacetate.—Isobutylene was passed into trifluoroacetic acid (37 g., 0.33 mole) at 50° for one hour. The solution was allowed to stand overnight. More isobutylene was then passed into the solution until no more evolution of heat was noticed. The mixture was fractionally distilled *in vacuo;* yield 75%, b.p. 30° at 60 mm. and 83° at 760 mm., $n^{25}D$ 1.3300.

Anal. Calcd. for $C_6H_9O_2F_3$: C, 42.36; H, 5.33. Found: C, 42.39; H, 5.48.

Preparation of Amides.—In general, the appropriate amine was added gradually to the cooled ester. The reactions were highly exothermic. After standing overnight, the mixtures were fractionally distilled *in vacuo*. In the case of the reactions of *t*-butyl trifluoroacetate with amines, the reaction mixtures were allowed to stand for four days before fractionating.

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(2) The study is being extended to esters having other electron-attracting groups in place of the trihalomethyl group in order to obtain more conclusive evidence for the mechanism involved.

Isolation and Characterization Studies on Muscarine

BY FREDERICK A. KUEHL, JR., NORMAN LEBEL AND JOHN W. RICHTER

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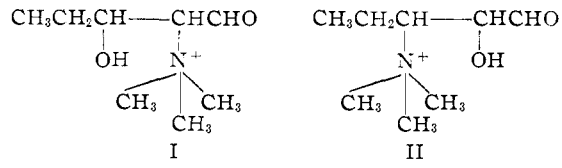
As a result of the work of Kogl, Duisberg and Erxleben,[1] muscarine has been considered to be represented by either structure I or II. These structures were advanced on the basis of analyses

(1) F. Kogl, H. Duisberg and H. Erxleben, *Ann.*, **489**, 156 (1931).

of chloroaurate and Reineckate salt preparations, the apparent presence of an aldehyde group as



I II

indicated by Schiff and Angeli–Rimini tests, and the liberation of trimethylamine and α,β-dihydroxyvaleric acid upon Hofmann degradation of muscarine base. Recently, Eugster and Waser[2] in a communication have taken issue with these results. They obtained muscarine chloride in crystalline form, and from analytical findings on this and other salts have proposed the formula $C_9H_{20}NO_2^+$ instead of the one favored by Kogl, $C_8H_{18}NO_2^+$. Furthermore, they were unable to find any evidence for the presence of an aldehyde group in the molecule, nor did they succeed in isolating trimethylamine or α,β-dihydroxyvaleric acid upon Hofmann degradation of the base. They did, however, verify the quaternary nature of the nitrogen atom of muscarine.

Work independently undertaken in these laboratories has led to the same conclusions as those arrived at by Eugster and Waser.[2] Since all attempts on our part to prepare pure muscarine by fractionation of the Reineckate salts in the manner described by Kogl gave a product grossly contaminated with choline, we investigated other purification methods. Experimental details for the preparation of pure crystalline muscarine chloride and a description of the degradative studies on the pure compound are reported here.

The activity of samples was followed by intravenous toxicity tests in mice, and the homogeneity of the various preparations was evaluated by paper strip analysis. A modified Dragendorff[3] reagent was found to be the most satisfactory method for visualizing the bases on paper strips.

Muscarine chloride was obtained as colorless, extremely hygroscopic crystals, of specific rotation, $[\alpha]^{25}D$ +8.1° (3.5% in ethanol).[4] The chloroaurate salt melted at 116–119°. Kogl's[5] chloroaurate melted at 115–117°, and Eugster and Waser's[3] at 121–121.5°. Analytical data from the two crystalline salts clearly favored the formula $C_9H_{20}NO_2^+$.

Some effort was directed toward the investigation of the nature of the oxygen atoms of muscarine. Muscarine chloride proved to be completely inert to oxidation with periodate, making extremely unlikely any structures containing vicinal hydroxyl groups or adjacent hydroxyl and carbonyl groups. Acetylation of muscarine with acetic anhydride in pyridine gave crystalline acetylmuscarine chloride. An O-acetyl determination on this new compound showed it to be a monoacetyl derivative. That no rearrangement took place during the acetylation was established by the recovery of muscarine upon deacetylation with alkali. Acetylmuscarine chloride was converted to the crystalline chloroplatinate, m.p. 185–192°, which after careful drying was subjected to infrared analysis. Although ester absorption at 5.75 μ was clearly visible, there was no evidence of absorption in the hydroxyl region. It is therefore apparent that muscarine contains only one hydroxyl group. This is in agreement with the report of Kogl, Duisberg and Erxleben,[1] who prepared a monobenzoyl chloroplatinate salt of muscarine.

No carbonyl group could be detected in the infrared spectrum of either the crystalline chloride or of the chloroaurate salt. Other attempts to detect an aldehyde group in muscarine included tests with Schiff reagent, Tollens reagent, Fehling solution, the malachite green test,[6] and *p*-phenylenediamine test,[7] the Angeli–Rimini test[8] and the azobenzenephenylhydrazine sulfonic acid test.[9] All were negative. Further evidence for the absence of an aldehyde group in muscarine was shown by its resistance to catalytic reduction and oxidation. Oxidation attempts using mercuric oxide under conditions normally utilized in the oxidation of sugars resulted only in the isolation of unreacted starting material.

It seems evident that the second oxygen atom of muscarine is neither hydroxylic nor carbonyl in nature. The inertness of the second oxygen atom might suggest an ether structure. It does not appear to be a methyl ether, however, since the Zeisel methoxyl determination gave a negative result.

Hofmann degradations of muscarine base under a variety of conditions including those described by Kogl and co-workers[1] did not give appreciable amounts of trimethylamine, nor could any acidic substances be detected, only unchanged muscarine. This also supports the work of Eugster and Waser,[2] rather than that of the earlier investigators.[1]

The failure of synthetic aldehydes having structures I and II to exhibit muscarine activity[5] has led other workers[10] to the belief that muscarine might possess an alkoxytrimethylammonium group because of the biological activity of synthetic salts of this type. This, however, is not supported by the stability of muscarine free base to hydrolysis. Even after drastic acid or alkaline hydrolysis no evidence for the formation of aldehydes could be obtained.

Experimental

Toxicity Determinations.—Aqueous solutions of weighed samples were injected into the tail veins of mice. The lethal dose (LD_{100}) is reported for a 20-g. mouse.

Extraction Procedure.—Freshly gathered specimens[11] (50 lb.) of *Amanita muscaria* (red variety) were covered with ethanol and whipped to a finely divided suspension with an efficient stirrer. The insoluble material was collected on a filter cloth and the cake was thoroughly pressed. The fil-

(2) C. H. Eugster and P. G. Waser, *Experientia*, **10**, 198 (1954).

(3) H. Bregoff, E. Roberts and E. Deliwicke, *J. Biol. Chem.*, **205**, 565 (1953).

(4) Kogl found $[\alpha]^{20}D$ +1.57° in water.

(5) F. Kogl and H. Veldstra, *Ann.*, **552**, 36 (1942).

(6) F. Feigl, "Qualitative Analyses by Spot Tests," 3rd Edition, Elsevier Publishing Co., New York, N. Y., 1946, p. 339.

(7) Ref. 6, p. 345.

(8) Ref. 6, p. 346.

(9) Ref. 6, p. 341.

(10) E. F. Rogers, D. Bovet, V. G. Gong and G. B. Marine-Bettolo, *Experientia*, **9**, 260 (1953).

(11) The authors are indebted to Miss Jean MacGregor and Mrs. M. L. Kelly who were kind enough to procure this material in the environs of Mendocino City, California.

trate was concentrated under reduced pressure at a temperature below 40° to a volume of about 5 liters. The fats were extracted from this concentrate with two 4-l. portions of ether. The aqueous layer was concentrated *in vacuo* to a thick, dark brown sirup. This sirup was shaken vigorously with a liter of ethanol. The alcohol was decanted, and a small amount of water added to the residue to reform a thick sirup. This extraction was repeated three times with one-liter portions of ethanol. The alcoholic layers were combined and stored in a cold room. This solution contained 90 mg. of solids/ml. and exhibited intravenous toxicity at 12.5 mg./20-g. mouse. One-liter portions of this solution were concentrated again to a sirup and extracted with 250 ml. of ethanol. The solution was decanted and concentrated *in vacuo*. The thick sirup from the second alcohol extraction was dissolved in 200 ml. of water containing 10 g. of Darco and stirred under an atmosphere of nitrogen. After removal of the charcoal, the yellow filtrate was lyophilized. The sticky residue, weighing 25–30 g., was found to be toxic at 3.0 mg./mouse.[12]

Ion Exchange on IRC-50 Resin.—A solution of 25 g. of the second alcoholic extract in 100 ml. of water was percolated through a column (1.8 × 65 cm.) containing 180 ml. of Amberlite IRC-50 resin in the hydrogen form. The column was washed with water and eluted with 0.1 *N* acetic acid at a rate of 1–2 ml./min. Fractions of 100-ml. volume were collected and assayed. The active fractions, which were found in the range between 700 and 1500 ml. of eluate, exhibited toxicity at 30 to 250 μg./mouse. The product active at 30–100 μg./mouse was dissolved in water and treated with Darco under an atmosphere of nitrogen. After filtration, the solution was adjusted to *p*H 3 with dilute hydrochloric acid and lyophilized. The residue was extracted with absolute ethanol. The yellow oil remaining after concentration *in vacuo* was dissolved in 10 ml. of water and passed again through the column of Amberlite IRC-50 (H^+). The column was washed with water and eluted with 0.1 *N* acetic acid. Successive 100-ml. fractions were collected, and the product, showing toxicity of 15–40 μg./mouse, was found in fractions 2–8. The less toxic products (100–250 μg./mouse) were purified separately with IRC-50 resin to afford material toxic at 20–40 μg./mouse. The overall recovery of activity was 70%.

Paper Strip Analysis.—The strips were run by the chromatocoil technique,[13] using the following solvent mixtures: 4 parts of *n*-butanol plus 1 part of dioxane, saturated with water; *n*-butanol, acetic acid and water in ratios of 4:1:5 (upper phase); methyl ethyl ketone, water and ethyl cellosolve in ratios of 300:706:15; and 95% ethanol and concentrated aqueous ammonia in a ratio of 19:1. In these systems the R_f values for muscarine chloride are, respectively, 0.35, 0.49, 0.19 and 0.50; for choline chloride 0.20, 0.11, 0.11 and 0.44.

Partition Chromatography.—The solvent system used was 4:5:1 *n*-butanol, water and glacial acetic acid. To a well-stirred mixture of 30 g. of Super-Cel and 200 ml. of upper phase, 30 ml. of the lower phase was slowly added. The equilibrated Super-Cel was slurried into a column (1.5 o.d. × 84 cm.). A mixture of 100 mg. of crude muscarine from ion exchange (toxic at 15–25 μg.), 0.5 ml. of water, 0.4 ml. of *n*-butanol and 0.1 ml. of glacial acetic acid and 0.5 g. of Super-Cel was equilibrated with shaking and then slurried with the aid of an excess of the upper phase into the top of the column. The upper phase of the solvent system was passed through the column at a rate of 10 ml./hour. The first 120 ml. of cluate was discarded and then 20-ml. fractions were collected. Each fraction was diluted with water and lyophilized. The residue was dissolved in 3 ml. of 0.02 *N* hydrochloric acid and lyophilized again. Muscarine exhibiting a toxicity of 7–10 μg./mouse was found in the eluates from 160–220 ml. Muscarine chloride was crystallized from a solution in absolute ethanol by treatment with Darco, followed by the addition of acetone. Stout prisms formed on recrystallization from ethanol–acetone mixtures.

Anal. Calcd. for $C_9H_{20}NO_2Cl$: C, 51.54; H, 9.61. Found: C, 50.94; H, 9.79.

Muscarine Chloroaurate.—This salt was prepared from the crystalline chloride. Muscarine chloroaurate melted at 116–119°.

Anal. Calcd. for $C_9H_{20}NO_2 \cdot AuCl_4$: C, 21.06; H, 3.92. Calcd. for $C_8H_{18}NO_2 \cdot AuCl_4$: C, 19.24; H, 3.63. Found: C, 20.98; H, 3.41.

Muscarine Reineckate.—This salt was prepared in the usual manner. No satisfactory analytical data could be obtained on this salt despite repeated recrystallizations from aqueous acetone.

Anal. Calcd. for $C_9H_{20}NO_2 \cdot [Cr(NH_3)_2(SCN)_4]$: C, 31.69; H, 5.32; N, 19.90. Calcd. for $C_8H_{18}NO_2 \cdot [Cr(NH_3)_2(SCN)_4]$: C, 30.11; H, 5.05; N, 20.48. Found: C, 32.50; N, 21.42.

Acetylmuscarine Chloride.—A suspension of 8.2 mg. of muscarine chloride in 0.8 ml. of pyridine and 0.4 ml. of acetic anhydride was agitated at room temperature for 18 hours, during which time the muscarine went into solution. The product was then precipitated by the addition of 10 volumes of ether. The precipitate was washed with ether and dissolved in hot ethyl acetate to which a few drops of ethanol had been added. As the solution cooled, white hygroscopic plates were deposited. This acetyl derivative is soluble in ethanol, acetone and chloroform. An R_f value of 0.35 was found for acetylmuscarine chloride upon paper strip analysis (Whatman No. 1 paper) using the solvent system: 300 ml. of methyl ethyl ketone, 706 ml. of water and 15 ml. of ethyl cellosolve. An O-acetyl determination was carried out by the method of Kunz and Hudson.[14]

Anal. Calcd. for $C_9H_{19}NO_2Cl \cdot Ac$: acetyl, 17.08. Found: acetyl, 17.44.

Hofmann Degradation of Muscarine. A.—A solution of 2.5 mg. of muscarine chloride in 1.0 ml. of water was shaken with 26 mg. of silver oxide for 3 hours and allowed to stand overnight. The excess silver oxide and silver chloride were removed by filtration. The filtrate and the water washings were distilled through a micro apparatus through which a stream of nitrogen was allowed to pass. The distillate was collected in a cooled trap containing 5.0 ml. of 0.1 *N* hydrochloric acid. The distillation was continued until the residue in the flask was concentrated almost to dryness. This residue was acidified with hydrochloric acid and dissolved in methanol. After concentration to dryness, 3.8 mg. of an oil resulted which on crystallization from acetone–ethanol yielded muscarine chloride. No evidence of any volatile amine could be detected by examination of the collection trap.

B.—In a second experiment 14.9 mg. of muscarine chloride was converted to muscarine hydroxide by percolation through Dowex 1 resin in the hydroxyl form. The distillation was carried out in the manner described above except that the residue was dry distilled at 130–140° for one hour. The residue in the distillation flask was shown by paper strip analysis to be muscarine.

Acknowledgment.—The authors are indebted to Mr. Robert Walker for infrared analysis, Mr. R. N. Boos for microanalyses, and Mr. Charles Butz of the Merck Institute for Therapeutic Research for the toxicity studies. The authors are also indebted to Dr. Alfred C. Haven, Jr., and to Mr. Edward Acton for their assistance in some of the early work on this problem.

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(12) The isolation procedure to this point is a modification of that described by H. King, *J. Chem. Soc.*, 1743 (1922).

(13) V. Schwartz, *Chemistry and Industry*, 102 (1953).

(14) Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926).

The Synthesis of 9α-Substituted-Δ⁴-androstene-11β-ol-3,17-diones

BY ROBERT H. LENHARD AND SEYMOUR BERNSTEIN

RECEIVED SEPTEMBER 2, 1955

Fried and co-workers[1] recently have announced certain important observations on the biological activities of the 9α-halogenated derivatives of a number of 11-oxygenated steroids. One of the out-

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954); J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1069 (1955).