2 ml. of ether cooling to −4° and adding 0.5 g. of ethyl chlorocarbonate. The solid (m.p. 94.6–97°) decomposed upon standing. Mixed melting point determinations showed it to be identical with the derivative prepared from the reduction of fumaric acid.

Reduction of Fumaric Acid.—In a manner similar to the above, 0.2 mole of fumaric acid was reduced with 0.5 mole of lithium aluminum hydride to 2-butene-1,4-diol in 78% yield. Titration of a sample of the product with bromine in chloroform indicated that the unsaturated diol was 98.1% pure.

Reduction of acrylic acid was also carried out as in the previously described reactions. The yield of allyl alcohol (68.3%) was obtained from 0.283 mole of acid and 0.35 mole of the hydride. The derivative of the alcohol (m.p. 67.8–68.6°) prepared by treatment with phenyl isocyanate was shown by mixed melting point determination to be identical with an authentic sample.

Reduction of Propiolic Acid.—The reduction of the triple bond acid was carried out as described above, using 0.285 mole of acid and 0.35 mole of lithium aluminum hydride. Allyl alcohol was produced in 85% yield as an azeotrope boiling at 78–81°. Mixed melting point determinations of the phenylurethan derivative showed no depression when mixed with an authentic sample.

DEPARTMENT OF CHEMICAL ENGINEERING
SCHOOL OF MINES AND METALLURGY
UNIVERSITY OF MISSOURI
ROLLA, MISSOURI

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The Rate of Reaction of Cyclopentyl Chloride with Potassium Iodide in Acetone[1]

BY F. G. BORDWELL AND GLENN D. COOPER

In a recent investigation of the reduction of sulfones by lithium aluminum hydride,[2] it was observed that five-membered ring sulfones are reduced at a much faster rate than open-chain sulfones. One possible formulation of this reaction is displacement of the oxygen by attack of aluminohydride ion (AlH_4^-) on sulfur. It, therefore, seemed worthwhile to determine whether or not cyclopentyl halides showed enhanced reactivity as compared to open-chain secondary halides in displacement reactions. The remarkable inertness of cyclohexyl halides and the current interest in the effect of ring size on halide reactivity[3] afforded additional incentives.

Cyclopentyl bromide reacts readily with sodium iodide in acetone,[4a] and with a variety of other nucleophilic reagents,[4b] but the rates of these reactions have not been measured. Accordingly, the rate of reaction of cyclopentyl chloride with potassium iodide in acetone at 58.5° was determined; $k_{58.5°} = 7.0 \times 10^{-3}$ liter mole^{-1} hr.$^{-1}$. The rates of this reaction for five methylalkylcarbinyl chlorides were reported by Conant and Hussey[5]; the k values obtained at 60° ranged between 3.4×10^{-3} and 17.2×10^{-3}.[6] It is apparent that the rate of reaction of cyclopentyl chloride with potassium iodide in acetone is comparable with that of open-chain secondary chlorides.

Experimental

The rates were measured by the method of Conant and Kirner.[7] Cyclopentyl chloride was prepared from cyclopentanol and concentrated hydrochloric acid; b.p. 111° (750 mm.), $n^{20}D$ 1.4500; literature[4a] b.p. 111–112°, $n^{25}D$ 1.4485.

Rate at $t = 58.5 \pm 0.05°$

Time, hr.	Reacted, %	$k \times 10^3$
72	9.6	7.1
120	16.1	7.0
236	27.2	6.8
	Average	7.0

The solution was only very faintly colored by iodine after 236 hours.

The rate of reaction of isopropyl chloride, measured for comparison, was $k_{58.5°} = 2.8 \times 10^{-3}$; Conant and Hussey[5,6] obtained $k_{60°} = 3.4 \times 10^{-3}$.

(1) This investigation was supported by the Office of Naval Research under Contract No. N7onr-45007.

(2) Bordwell and McKellin, THIS JOURNAL, **73**, 2251 (1951).

(3) Brown, Fletcher and Johannesen, *ibid.*, **73**, 212 (1951).

(4) (a) Rogers and Roberts, *ibid.*, **68**, 843 (1946); (b) Loevenich, Utsch, Moldrickx and Schaefer, *Ber.*, **62B**, 3084 (1929).

(5) Conant and Hussey, THIS JOURNAL, **47**, 476 (1925).

(6) These are values of k. The values reported by Conant and Hussey are for $0.4343k$.

(7) Conant and Kirner, THIS JOURNAL, **46**, 232 (1924).

CHEMICAL LABORATORY
NORTHWESTERN UNIVERSITY
EVANSTON, ILLINOIS

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Fluoroacetylcholine Bromide and Some Other Choline Ester Salts

BY THOMAS R. BLOHM

The striking differences between the parasympathomimetic activities of acetylcholine and its chloro-[1] and bromo-substitution[2] products could be due either to the electronegative character of these halogens, or the size of their atoms compared to hydrogen. Since fluorine more nearly approaches hydrogen in size while retaining high electronegativity, it was thought to be of interest to prepare fluoroacetylcholine. Interest in this compound also stems from the ability of fluoroacetic acid to block the Krebs cycle.[3]

In addition to fluoroacetylcholine bromide, the syntheses of trichloroacetylcholine perchlorate, dichloroacetylcholine perchlorate, fluoroacetylcholine perchlorate and β-bromoethyl fluoroacetate are described.

Preliminary pharmacological data on rabbits indicate that these choline esters are much less active than acetylcholine in their parasympathomimetic effects when given by the intravenous route.

Experimental

Fluoroacetyl Chloride.—This intermediate was prepared by the method of Gryskiewicz-Trochimowski, Sporzynski and Wnuk[4] using fluoroacetic acid, benzotrichloride and zinc chloride. This method was found to be definitely superior to methods using phosphorus pentachloride, inasmuch as preparations made with this reagent were found to contain phosphate—yielding contaminants even after careful fractionation. Fluoroacetic acid was prepared from the sodium salt (90%, Monsanto) and 100% H_2SO_4, similarly to the procedure of Saunders and Stacey.[5]

β-Bromoethyl Fluoroacetate.—Five grams (0.040 mole) of freshly distilled ethylene bromohydrin was dissolved in 25 ml. of dry benzene and added to 5.0 g. (0.052 mole) of fluoroacetyl chloride, also dissolved in 25 ml. of dry benzene in a 100-ml. round-bottomed flask. The mixture was refluxed on a boiling water-bath for one hour; the benzene

(1) R. R. Renshaw and J. C. Ware, THIS JOURNAL, **47**, 2989 (1925).

(2) D. Glick, *J. Biol. Chem.*, **130**, 530 (1939).

(3) G. R. Bartlett and E. S. G. Barron, *ibid.*, **170**, 67 (1947); G. Kalnitsky and E. S. G. Barron, *Arch. Biochem.*, **19**, 75 (1948); W. B. Elliott and G. Kalnitsky, *J. Biol. Chem.*, **186**, 487 (1950).

(4) Gryskiewicz-Trochimowski, Sporzynski and Wnuk, *Rec. trav. chim.*, **66**, 413 (1947).

(5) B. C. Saunders and G. V. Stacey, *J. Chem. Soc.*, **58**, 1777 (1948).

and excess acid chloride were then removed under reduced pressure, and the ester distilled *in vacuo*. A colorless or pale yellow liquid was obtained at 115–122° at 75 mm.; 6.70 g. (90.6%) was obtained. The product had a faint ester odor combined with a slight pungency which may have been due to retention of traces of the acid chloride.

Anal. Calcd. for $C_4H_6O_2FBr$: Br, 43.21; F, 10.27. Found: Br, 43.08; F, 9.86.

Fluoroacetylcholine Bromide.—A 15 × 120 mm. Pyrex test-tube was marked to contain 2.4 ml. (1.6 g., 0.027 mole) of trimethylamine. The amine was allowed to distil into the tube, which was cooled in an ice–salt-bath, until the mark was reached, when the tube was sealed off, a slender, fragile neck being left on the tube. This tube was then dried and placed inside a larger Pyrex tube which already contained 6.0 g. (0.032 mole) of β-bromoethyl fluoroacetate dissolved in 20 ml. of dry benzene. The large tube was then sealed off, and the small tube was broken inside the large one by careful manipulation. After 48 hours at room temperature the crystalline mass was collected and washed with two 20-ml. portions of boiling anhydrous acetone. The crystals (colorless needles) were quickly transferred to a vacuum desiccator for removal of the remaining acetone, after which a beaker containing P_2O_5 was placed in the desiccator. The compound melts at 124° (uncor.) and is very hygroscopic. 5.3 g. was obtained, 80.5%.

Fluoroacetylcholine bromide may be recrystallized from absolute alcohol. It gives the ester test of Hestrin.[6]

Anal. Calcd. for $C_7H_{15}O_2NFBr$: F, 7.78; Br, 30.5. Found: F, 7.50; Br, 30.6.

Trichloroacetylcholine Perchlorate.—13.95 g. (0.10 mole) of choline chloride was placed in a clean, dry erlenmeyer flask and 33.9 g. (0.12 mole) of trichloroacetyl chloride was added. The mixture was stirred by hand until it had become hard and appeared fairly dry. It was kept in an ice-bath all the while. The solid mass was dissolved in 20 ml. of ice-cold absolute ethanol and 15 ml. of 70% $HClO_4$ was added to this solution. A crystalline precipitate formed immediately. This was filtered off under suction and washed with cold absolute alcohol. The filtrate was placed in the ice-box and the precipitate (m.p. 184–192°) dissolved in anhydrous acetone and precipitated with dry ether. This raised its melting point to 192–197° (uncor.), which value remained constant upon further recrystallization. Meanwhile the filtrate deposited crystals of choline perchlorate (m.p. 273°).

Trichloroacetylcholine perchlorate is a colorless crystalline compound, solubility in water slightly less than 10 mg. per ml., hydrolyzed quite rapidly compared to acetylcholine, and undergoing very rapid alcoholysis in hot absolute alcohol. It is soluble in boiling acetone and may be recrystallized therefrom. It is not hygroscopic.

Anal. Calcd. for $C_7H_{12}O_6NCl_4$: Cl, 40.2; N, 4.01. Found: Cl, 40.6; N, 3.92.

Dichloroacetylcholine Perchlorate.—This was prepared by the method given above for trichloroacetylcholine perchlorate. The compound was obtained as colorless needles, m.p. 126° (uncor.), non-hygroscopic.

Anal. Calcd. for $C_7H_{14}O_6NCl_3$: Cl, 33.8; N, 4.45. Found: Cl, 34.0; N, 4.50.

Fluoroacetylcholine Perchlorate.—This was prepared from the bromide: colorless, non-hygroscopic needles, m.p. 89° (uncor.), insoluble in absolute alcohol, very soluble in acetone.

Anal. Calcd. for $C_7H_{15}O_6NFCl$: Cl, 13.5. Found: Cl, 13.7.

(6) S. Hestrin, *J. Biol. Chem.*, **180**, 247 (1949).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF TEXAS SCHOOL OF DENTISTRY
HOUSTON 4, TEXAS

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The Exchange Stability of Cobalt in Vitamin B_{12}

BY RICHARD N. BOOS, CHARLES ROSENBLUM AND DAVID T. WOODBURY

The characterization of vitamin B_{12} (cyanocobalamin) as a cobalt coördination complex[1] was based originally on the firmness of the cobalt binding, the apparent trivalency of this element in the compound, and its slight conductance in aqueous solution. This has been confirmed more recently by measurements of magnetic susceptibility.[2,3,4]

An indication as to the stability of the complex, and the nature of the bonds linking the complexed groups to the cobalt atom, can be obtained from the rate of exchange[5] of cobalt ions in a solution of vitamin B_{12} and radioactive cobaltous sulfate or vice versa. Fantes, Page, Parker and Smith[6] have observed no exchange of cobalt (from radioactive cobaltous sulfate) after 2 hours of admixture in 0.1 *N* acid or alkali, and 1 hour in neutral boiling aqueous solution. In view of the known slow deactivation of vitamin B_{12} under such conditions,[1,6] it is conceivable that, despite the negative results of the short time experiments, prolonged contact of vitamin and cobaltous salt would result in a detectable exchange of cobalt with the cobalt of the vitamin proper or in its gradually formed degradation products. We have, therefore, performed similar experiments in 0.01 *N* H_2SO_4, in 0.01 *N* NaOH and in distilled water (*p*H 5.5–6), but have extended the study for about three months at room temperature and for about two weeks at 55°.

As outlined above, the exchange experiments would fail to reveal irreversible decomposition of vitamin B_{12} with formation of ionic cobalt. This possibility was investigated by the use of radioactive (cobalt 60) vitamin B_{12}[7] mixed with normal cobaltous sulfate. A single experiment on the effect of light, which converts[8,9] vitamin B_{12} to other cobalamins,[10] was also carried out.

The exchange experiments were performed by adding a known amount of cobaltous sulfate (equivalent to 4.5–5 μg. cobalt) to an aqueous solution of vitamin B_{12} of equivalent cobalt content in ground glass-stoppered cylinders of Pyrex glass, and stored in the dark at room temperature (≃22°) and 55° for various periods of time. Parallel mixtures of cobaltous (60) sulfate[11] with non-radioactive vitamin B_{12}, and inactive cobalt with radioactive (Co^{60}) vitamin B_{12}, were prepared. Total volumes per experiment amounted to 11 ml., and total radioactivity was 3000–3400 c.p.m.; measured in 1 inch diameter stainless steel planchets with a thin window GM counter system with a 6% counting efficiency. Cylinders containing only the radioactive cobalt salt solution, or simply

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(8) G. E. Boxer and J. C. Rickards, *Arch. Biochem.*, **30**, 382 (1951).

(9) W. L. C. Veer, J. H. Edelhausen, H. G. Wijmenga and J. Lens, *Biochim. et Biophys. Acta*, **6**, 225 (1950).

(10) E. A. Kaczka, D. E. Wolf, F. A. Kuehl, Jr., and K. Folkers *Science*, **112**, 354 (1950).

(11) Purchased from Tracerlab, Inc., on allocation from the Isotopes Division, U. S. Atomic Energy Commission.