sulfide ring compound accompanied by a somewhat lower yield of the final products.

Experimental

Trimethylene sulfide was prepared by a modification of the procedure of Bennet and Hock[4] in an average yield of 45%, b.p. 90–93° (680–685 mm.), $n^{20}D$ 1.5070.

Bis-(3-chloropropyl) Disulfide (I).—A solution of 5 g. (0.0676 mole) of trimethylene sulfide in 25 ml. of chloroform was stirred and externally cooled by a cold water-bath while 2.4 g. (0.0338 mole) of chlorine was passed in as a vapor beneath the surface of the solution. Argon was then bubbled through the mixture for a short time and the solvent was stripped. On distillation and redistillation there was obtained 3.6 g. (48.7%) of a colorless oil, b.p. 113–115° (1 mm.), $n^{20}D$ 1.5450.

Anal.[8] Calcd. for $C_6H_{12}Cl_2S_2$: C, 32.87; H, 5.52; S, 29.25. Found: C, 33.03; H, 5.22; S, 29.57.

A solid derivative was prepared by refluxing the above product with excess piperidine in ethanol, followed by water-washing to remove unreacted piperidine and ethanol, drying the product, and then dissolving it in absolute ethanol and bubbling dry hydrogen chloride through the solution. Ether was added to precipitate the dihydrochloride of II. Recrystallization from an ethanol–ether mixture gave white crystals, melting with decomposition at 210–212°.

Anal. Calcd. for $C_{16}H_{34}Cl_2N_2S_2$: Cl, 18.21; S, 16.46. Found: Cl, 18.03; S, 16.30.

Bis-(3-bromopropyl) Disulfide.—The reaction between trimethylene sulfide and bromine was carried out as in the preparation of I described above except that a solution of bromine in chloroform was added dropwise. Attempts to distil the crude product resulted in decomposition. A sulfur analysis was made on the *crude* product.

Anal. Calcd. for $C_6H_{12}Br_2S_2$: S, 20.81. Found: S, 22.03.

Reaction of the crude product with piperidine followed by dry hydrogen chloride as described for I gave a dihydrochloride identical with that prepared from I, m.p. 210–212° with decomposition. A mixed melting point of the two dihydrochlorides showed no depression.

3-Chloro-1-propanesulfenyl Chloride (III).—A solution of 3.83 g. (0.054 mole) of chlorine in 10 ml. of chloroform to which a few crystals of hydroquinone had been added was chilled in a Dry Ice–acetone-bath. To this a solution of 4 g. (0.054 mole) of trimethylene sulfide in 40 ml. of chloroform, also precooled in a Dry Ice–acetone-bath, was added dropwise over a period of 10 minutes. The reaction mixture was allowed to warm to room temperature, argon was bubbled through it for a short time, and the solvent was stripped. Distillation gave about 3 ml. of orange liquid of extremely strong and irritating odor, b.p. 48–55° (3.5 mm.), and a mixture of higher-boiling lighter-colored liquids of no definite boiling range. The orange liquid was redistilled and yielded 2.3 g. (30%) of product, b.p. 51–53° (4 mm.), $n^{20}D$ 1.5190. This compound III was not analyzed directly, but was immediately combined with trimethylene sulfide (1.2 g.) in chloroform to form the previously prepared bis-(3-chloropropyl) disulfide in a yield of 1.9 g. (54%), b.p. 112–115° (1 mm.), $n^{20}D$ 1.5456. Reaction of this compound with piperidine followed by dry hydrogen chloride gave the same derivative, bis-(3-piperidinopropyl) disulfide dihydrochloride, previously prepared from I.

3-Chloro-1-propanesulfonyl Chloride.—To 80 ml. of a saturated, externally cooled solution of chlorine in 75% acetic acid was added dropwise with stirring 5.8 g. (0.0784 mole) of trimethylene sulfide, and chlorine was introduced below the surface at such a rate that an excess was always present. Addition of chlorine was continued for five minutes after all the sulfide had been added. Air was then blown through the reaction mixture to remove excess chlorine. The mixture was diluted with 400 ml. of water, extracted with ether and the combined extracts were dried over sodium sulfate. Distillation gave 10 g. (72%) of colorless liquid, b.p. 82–85° (1 mm.), $n^{22}D$ 1.4890. The reported physical constants[6] for 3-chloro-1-propanesulfonyl chloride are b.p. 117–118° (15 mm.), $n^{22}D$ 1.4900. The sulfonamide derivative was prepared and found to have a melting point of 62–63°. This is in agreement with the previously reported melting point of 63° for 3-chloro-1-propanesulfonamide.[7]

(8) Microanalyses for carbon and hydrogen were performed by the Clark Microanalytical Laboratory, Urbana, Ill. Chlorine and sulfur analyses were performed by the authors.

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Alkyl Esters of Isodehydroacetic Acid

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This note will serve to record the preparation and properties of some previously undescribed alkyl esters of isodehydroacetic acid. The esters were obtained by reaction of isodehydroacetic acid chloride with the alcohol. The acid chloride was prepared from the acid and thionyl chloride and the acid was obtained as previously described.[1] Since the acid chloride has not been previously characterized, details of its preparation and supporting analytical data are included. The methyl, *n*- and isopropyl, butyl, isoamyl and cetyl esters described in Table I have been obtained from the corresponding alcohols. The procedure used in their preparation is similar to that described for the *n*-propyl ester in the Experimental section. With redistilled, commercial 2-ethylhexyl alcohol a liquid product was obtained from which crystals separated on standing. This product did not give analytical values in accord with theory.

Since this procedure has not been previously used for the preparation of esters of isodehydroacetic acid, we have prepared the known methyl ester from the acid chloride and converted it to the known 3-bromo derivative. Both have melting points in agreement with those previously recorded for these compounds as prepared by other methods.

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Experimental[2]

Isodehydroacetic Acid Chloride.—Eighteen ml. (0.25 mole) of thionyl chloride was added to 25 g. (0.15 mole) of isodehydroacetic acid in a 125-ml. claisen flask. The mixture was heated under reflux for 10–20 minutes. The gaseous products and excess thionyl chloride were removed under vacuum. The residue was distilled to give 22–25.0 g. (79–90% of the theoretical amount) of isodehydroacetic acid chloride, b.p. 145–150° at 15 mm.; reported[3] b.p. 138–140° at 12 mm. The product solidifies on cooling, m.p. 52°.

Anal. Calcd. for $C_8H_7O_3Cl$: Cl, 19.00. Found: Cl, 18.88.

***n*-Propyl Isodehydroacetate.**—The acid chloride, prepared as above, was melted and portions (4.9–5.6 g.) were poured into 1 × 4 inch test-tubes. Such a sample of acid chloride (5.6 g., 0.03 mole) was melted under a reflux condenser on a water-bath and to it was added an excess (3.24 g., 0.054 mole) of dried *n*-propyl alcohol. The solution was refluxed 10–15 minutes and fractionated to give 5.0 g. (79% of the theoretical amount) of *n*-propyl isodehydroacetate, b.p. 166° at 8 mm.), $n^{23.5}D$ 1.5093.

(1) R. H. Wiley and N. R. Smith, THIS JOURNAL, 73, 3531 (1951).

(2) Analyses by Clark Microanalytical Laboratory and Micro-Tech Laboratory.

(3) H. M. Basal, *et al.*, U. S. Patent 2,364,304, December, 1944.

TABLE I

ALKYL ESTERS OF ISODEHYDROACETIC ACID

Alkyl	Yield, %	B.p., °C.	Mm.	$n^{t}D$	t, °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl	99	67[a]	....	....	....	...	...	...	...
n-Propyl	79	116	8	1.5093	23.5	62.85	62.64	6.67	6.80
i-Propyl	85	158	8	1.5050	24	62.85	62.74	6.67	6.80
n-Butyl	89	167	7	1.5063	23.5	64.22	64.27	7.15	7.37
i-Amyl	68	177	8	1.5032	23.8	65.47	65.20	7.56	7.62
Cetyl	71.5	55[a]	....	....	....	73.42	73.33	10.41	10.50

[a] Melting point.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.85; H, 6.67. Found: C, 62.64; H, 6.80.

Methyl isodehydroacetate was prepared from 1.0 g. (0.054 mole) of the acid chloride and 5 ml. of absolute methanol. Evaporation gave *ca.* 1.0 g. of the ester, m.p. 67°; reported m.p. 67–67.5°.[4] The product was converted to the 3-bromo derivative by reaction with an equivalent amount of bromine in carbon tetrachloride; m.p. 133–134°; reported m.p. 135°.[5]

(4) R. Anschutz, P. Bendix and W. Kerp, *Ann.*, **259**, 156 (1890).
(5) E. Buchner and H. Schröder, *Ber.*, **35**, 790 (1908).

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Studies on Pituitary Adrenocorticotropin. III. Differentiation of Three Active Types on XE-97 Resin

BY W. F. WHITE AND W. L. FIERCE

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By the use of the cation exchange resin Amberlite XE-97,[1] three active ACTH types have been differentiated in hog pituitary extracts. Two active types have been separated from the highly purified fractions of acid- and pepsin-treated extracts described in a previous publication.[2] A third active type has been found in highly potent oxycellulose eluates made from hog pituitaries[3] and in other non-hydrolyzed preparations.

Figure 1 shows a typical fractionation of an acid- and pepsin-hydrolyzed sample which had previously been purified by means of a chromatopile. The XE-97 bed[4] was equilibrated with 0.1 *M* sodium carbonate–bicarbonate buffer at *p*H 8.5 before application of the sample as a solution in the same buffer. The portion passing directly through the column at *p*H 8.5 (fraction I) was physiologically inert. However, successive substitution of *p*H 9.25 and 11.25 buffers eluted two additional fractions (II and III, Fig. 1) which showed activities in the range of 100 to 150 u./mg. of peptide.[5]

(1) Rohm & Haas Co., Washington Square, Philadelphia 5, Pa.
(2) W. F. White, W. L. Fierce and J. B. Lesh, *Proc. Soc. Exptl. Biol. Med.*, **78**, 616 (1951).
(3) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).
(4) The resin was prepared as follows: The material as supplied by the manufacturer was stirred three or four times with water, each time allowing the suspension to settle four or five minutes and pouring off the fines. The washed resin was then cycled three times batchwise using *N* sodium hydroxide and *N* hydrochloric acid. After thorough washing with water, the resin was stored in the acid form either wet or dry until use.
(5) Fractions were assayed by the Munson modification of the adrenal ascorbic depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinol.*, **42**, 379 (1948). This technique has provisional USP approval. The samples were administered intravenously. All activities are expressed as USP units.

In other runs the range between *p*H 8.5 and 11.25 has been investigated in small increments without revealing additional components. The non-identity of fractions II and III has been proved by rerunning them individually through the same type of column, under which condition the integrities of the fractions were maintained.

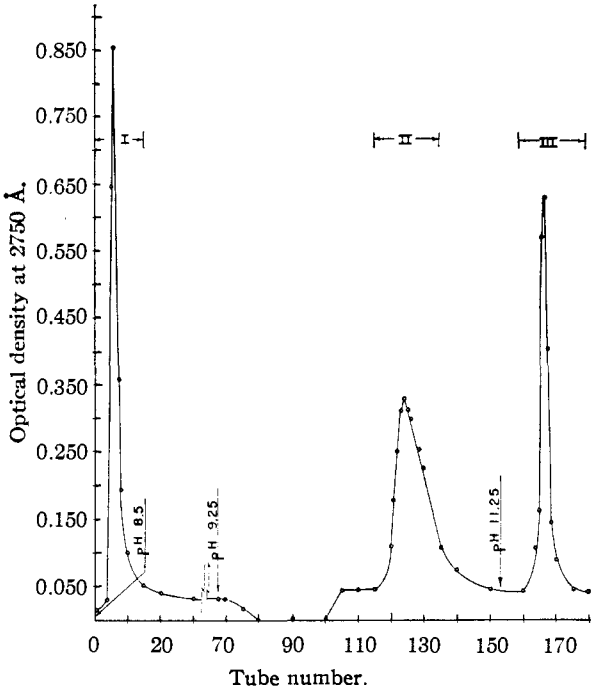

Fig. 1.—Chromatography of a highly purified acid- and pepsin-treated ACTH preparation on Amberlite XE-97 resin. The sample (10.5 mg. at 65 u./mg.) in 1 ml. *p*H 8.5 buffer was applied to a column 15 cm. high in a tube 0.9 cm. in diameter. Rate of flow was 0.5 ml./min. Volume collected per tube was 1 ml. The distribution of ultraviolet absorption in the fractions indicated was: I, 25%; II, 28%; III, 24%. The distribution of activity was: I, < 2%; II, 64%; III, 19%.

A much different result was obtained when highly active unhydrolyzed ACTH preparations were subjected to the XE-97 procedure. Here almost all of the ultraviolet absorbing material was eluted at *p*H 8.5 and the activity appeared to be associated with a minor component poorly separated from the major peak.[6] Very little ultraviolet absorbing material and no activity appeared either at *p*H 9.25 or *p*H 11.25. Increasing the height of the

(6) Dixon, *et al.*, *Nature*, **168**, 1044 (1951), noted a similar behavior with a crude unhydrolyzed preparation. However, their conditions of *p*H and buffer composition were different.