[CONTRIBUTION FROM THE HOWE LABORATORY OF OPHTHALMOLOGY, HARVARD MEDICAL SCHOOL]

Synthetic Preparation of 2-Chloro-2′-hydroxydiethyl Sulfide, Reaction with Cysteine and Valine, and Measurement of Reaction Rate in Aqueous Media[1]

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Hydrolytic loss of one chlorine from 2,2′-dichloroethyl sulfide (henceforth referred to as H) yields a substance which readily hydrolyzes further to a mixture of thiodiglycol and sulfonium salts. While this intermediate substance can be obtained in very small yields by chloroform extraction of a partially hydrolyzed aqueous solution of H which has been freed of residual H by washing with cyclohexane, kerosene or petroleum ether, the amount of 2-chloro-2′-hydroxydiethyl sulfide (henceforth referred to as Semi-H) obtainable in this way is small on account of the poor solubility of H in water and the rapid, continuous hydrolysis of the product.

Attempts to synthesize Semi-H have been unsuccessful until the present when information on the solubility and stability characteristics obtained from a study of material isolated from hydrolyzing H solutions has permitted development of a convenient procedure for the synthetic preparation of Semi-H, facilitating further study of its reaction rates in water and biological media, as well as preparation of condensation products with amino acids.

Experimental

Preparation of Semi-H.—Forty-eight grams of thionyl chloride dissolved in 120 ml. of chloroform was added with moderate stirring at room temperature to 96 g. of thiodiglycol (Kromfax Solvent, Carbide and Carbon Chemicals Corporation) dissolved in 210 ml. of chloroform. The time for making the addition was about four minutes. Thionyl chloride was added beneath the surface to minimize its decomposition by the water which was formed. The stirring was stopped after the last of the thionyl chloride had been added to allow the water and chloroform layers to separate. The chloroform was withdrawn, dried with anhydrous sodium sulfate and evaporated under reduced pressure until all of the solvent had been removed. To eliminate the H which was formed, the residual liquid (25–30 ml.) was extracted four times with 40-ml. portions of equal parts cyclohexane and petroleum ether. Any residual cyclohexane and petroleum ether was removed by evaporation under reduced pressure. The remaining sirupy liquid was dissolved in 400 ml. of ethyl ether and extracted rapidly three times with 50-ml. portions of water. The latter procedure in addition to removing sulfonium salts removed thiodiglycol and possibly some contaminants from the Kromfax Solvent. The ether solution was dried with anhydrous sodium sulfate. The yield was 16.8 g.

Owing to the fact that undiluted Semi-H readily polymerizes by sulfonium salt formation, it is desirable to keep the compound in ether solution in the refrigerator and to remove the ether by vacuum evaporation only just before use. In ether or isopropyl alcohol solution sulfonium salt formation, as detected by formation of a precipitate with silver nitrate in absolute methyl alcohol, is inhibited.

Anal.—A sample of Semi-H prepared following this procedure by Max Bergmann was found by him to have the following composition[2]: Calcd. for C_4H_9OSCl: C, 34.2; H, 6.4; S, 22.8; Cl, 25.3. Found: C, 34.1; H, 6.4; S, 22.6; Cl, 25.4.

Preparation of S-2-(2-Hydroxyethylthio)-ethyl Cysteine Hydrochloride.—Eight grams of cysteine hydrochloride (Merck) was dissolved in 20 ml. of water at room temperature. Nitrogen was bubbled through the solutions and 8 ml. of 10 *N* sodium hydroxide added to make the mixture pink to phenolphthalein. 10.8 g. of Semi-H in 275 ml. of ether was added in 25-ml. lots during about two hours and sufficient 10 *N* sodium hydroxide added to maintain the phenolphthalein pink (7.8 ml. was required). The reaction mixture was kept at 20–30° by means of a heat lamp. When the reaction was complete the solution was shaken with activated carbon which was then removed by filtration. The filtrate was adjusted to *p*H 5.5 by addition of concentrated hydrochloric acid. After cooling in the refrigerator overnight, the precipitate which formed was filtered off and washed with 10–15 ml. of ethyl alcohol. The dry powder (5.7 g.) was mixed thoroughly with 5 ml. of concentrated hydrochloric acid and dissolved in 75 ml. of boiling absolute ethyl alcohol. After filtering, 450 ml. of ether was added and the solution refrigerated to yield 5.2 g. of glistening white precipitate, m. p. 126–128°. After dissolving in 99% isopropyl alcohol and precipitating with ether the m. p. was 128.5–129°. The nitroprusside test for sulfhydryl and disulfide groups was negative. The ninhydrin test was strongly positive.

Anal. Calcd. for $C_7H_{16}NS_2O_3Cl$: C, 32.17; H, 6.13; N, 5.37; S, 24.58; Cl, 13.6. Found: C, 32.07; H, 6.13; N, 5.06; S, 25.12; Cl, 13.72.

Preparation of N-2-(2-Hydroxyethylthio)-ethyl Valine.—A solution was prepared of 1 g. of *dl*-valine (Lemke) in 7 ml. of water plus 0.4 ml. of 10 *N* sodium hydroxide by warming to 50–70°. Thirty ml. of ether containing 2.3 g. of Semi-H was added in small portions. The reaction flask was kept warm on the hot-plate and nitrogen was bubbled through the reaction mixture to accelerate the evaporation of ether. The necessary quantities of 10 *N* sodium hydroxide were added to maintain phenolphthalein pink. When the reaction was complete, the aqueous solution was shaken with three 7-ml. portions of chloroform. The *p*H was adjusted to 2.7 by addition of 0.8 ml. of concentrated hydrochloric acid and the solution shaken twice with 10-ml. portions of ether. The water was evaporated under vacuum and the residual solid was washed twice with 10 ml. of 99% isopropyl alcohol. Extraction of the solid with 150–175 ml. of boiling isopropyl alcohol and subsequent cooling gave a precipitate of white powder. Reextraction of the undissolved solid with the mother liquor and again cooling gave more of the same precipitate; yield, 0.6 g.; m. p. 209–212° (dec.). Chloride ion and ninhydrin tests were negative. Formol titration was zero.

Anal. Calcd. for $C_9H_{19}NSO_3$: C, 48.9; H, 8.6; N, 6.33; S, 14.48. Found: C, 48.69; H, 8.99; N, 5.92; S, 14.24.

Measurement of Reaction Rates of Semi-H.—Hydrolysis rates of Semi-H in water, sodium chloride solutions and reaction rate in blood were measured by determining the concentration present at intervals after addition of a known amount of Semi-H to give an initial concentration of 50–100 micrograms per ml. The Semi-H concentration was determined in periodic samples of pure water solutions by photoelectric colorimetry of the absorption of 520 mμ by a 5-ml. mixture containing the water sample, 1% 4-(*p*-nitrobenzyl)-pyridine and 55% isopropyl alcohol, to which 0.1 ml. of piperidine was added after sixty minutes at 23°.

(1) The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

(2) Personal communication.

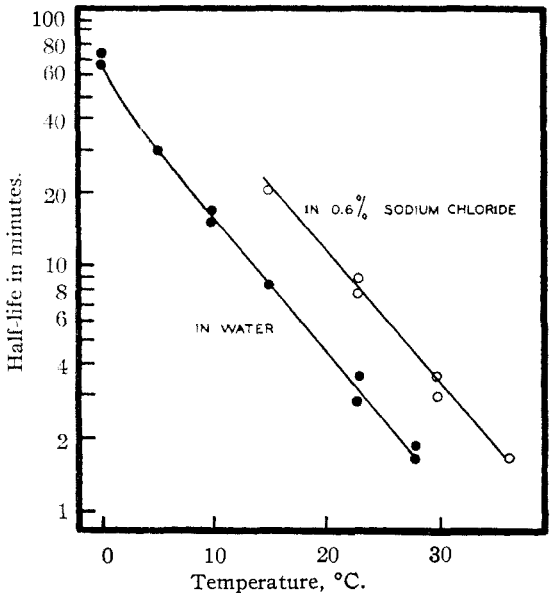

Fig. 1.—Relationship of the half-life of 2-chloro-2′-hydroxydiethyl sulfide to temperature in water and in 0.6% sodium chloride solutions.

Sodium chloride solutions and blood samples were shaken with equal volumes of chloroform which extracted approximately 70% of the Semi-H and avoided introducing interfering chloride ions into the chromogenic reaction mixture. The analytical procedure was then the same as for water except that the reaction mixture was altered to contain 10% chloroform and 45% instead of 55% isopropyl alcohol.

The half-life of Semi-H in heparinized rabbit blood at 25° was found to be 5.8 minutes, and in plasma, 8.2 minutes. The value in 0.6% saline for 25° interpolated from Fig. 1 was 6.5 minutes. Unlike the reaction of H, which is markedly slowed in blood, presumably due to solution and protection in a lipoid phase, the reaction of the more water-soluble Semi-H is not significantly different in blood than in a solution of equivalent salt content.

Summary

A method has been described for the synthetic preparation of 2-chloro-2′-hydroxydiethyl sulfide and for isolation of its condensation products with cysteine and valine. Data are given for the hydrolysis rate of 2-chloro-2′-hydroxydiethyl sulfide at different temperatures in water, 0.6% saline and rabbit blood. Unlike 2,2′-dichloroethyl sulfide, the mono-chloro compound did not persist in blood longer than in equivalent saline.

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Dialkylaminoalkyl Diarylthiolacetates

BY R. O. CLINTON AND U. J. SALVADOR

The recent appearance of a patent[1] describing several dialkylaminoethyl diphenylthiolacetates prompts a description of similar work carried out in these laboratories, prior to the appearance of this patent.

Diethylaminoethyl diphenythiolacetate hydrochloride was prepared in high yield by the condensation of 2-diethylaminoethanethiol with diphenylacetyl chloride in dry benzene. In a similar manner a number of other dialkylaminoalkyl diphenylthiolacetates were prepared in excellent yield. The substitution of 9-fluorenecarboxyl chloride for the diphenylacetyl chloride effected analogous results, but with some diminution of yield due to side reactions of the acid chloride.

This direct reaction of the thiol with the acid chloride offers evident advantage over the somewhat tedious procedure described by the patent,[1] since the intermediates are readily prepared and a single reaction affords a nearly pure, water-soluble product. In certain cases it has proven advantageous to prepare a salt other than the hydrochloride (*e.g.*, because of hygroscopicity); these salts are readily obtained from the easily prepared bases in this series by conducting the condensation of thiol and acid chloride in a mixture of water, base and inert solvent, as described in the experimental part.

(1) U. S. Patent 2,390,555 (Dec. 1945).

The melting point of the diethylaminoethyl diphenylthiolacetate prepared in the present work differed markedly from that given by the patent[1]; this difference has been shown to be due to diethylamine hydrochloride present as an impurity in the compound when prepared by the patent procedure.

Experimental[2]

2-Diethylaminoethyl Diphenylthiolacetate Hydrochloride.—To a stirred, cooled solution of 13.3 g. of 2-diethylaminoethanethiol[3] in 100 ml. of dry benzene was slowly added a solution of 23.05 g. of pure diphenylacetyl chloride[4] in 200 ml. of dry benzene. A white crystalline precipitate appeared immediately. When addition was complete the mixture was heated to boiling for a few minutes, cooled in ice and diluted with an equal volume of Skellysolve B. Filtration and drying of the precipitate gave 31.3 g. of product, m. p. 125.5–127.5°. One recrystallization from benzene–Skellysolve B yielded tiny white needles, m. p. 129.5–130.5°. The patent[1] reports a melting point of 82–85°.[5]

(2) All melting points and boiling points are corrected. We are indebted to Mr. Morris Auerbach and staff for the analyses.

(3) Albertson and Clinton, THIS JOURNAL, **67**, 1222 (1945).

(4) Hellerman, Cohn and Hoen, *ibid.*, **50**, 1725 (1928).

(5) A sample of this compound when prepared by the patent method[1] melted initially at 89–98°. Several crystallizations from benzene-Skellysolve B gave a material melting at 129.5–130.5°; mixed m. p. with the present preparation was 129.5–130.5°. The impurity was shown by isolation and mixed m. p. determination to be diethylamine hydrochloride.