established by conversion through D- and L-di-isopropylidene-*aldehydo*-arabinose to enantiomorphous, crystalline, 1-C-cyclohexylarabitols. These substances have all been related to 3,4:5,6-di-isopropylidene-D-mannitol of known structure.

The position of the isopropylidene group in 4,5-isopropylidene-L-arabinose diethyl mercaptal has been established by lead tetraacetate oxidation.

1-C-Cyclohexyl-1,2,3,4-tetraacetyl-5-trityl-D-arabitol has been prepared.

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1-Thiosorbitol

BY M. W. FARLOW, MADISON HUNT,[1] C. M. LANGKAMMERER, WILBUR A. LAZIER,[2] W. J. PEPPEL[3] AND F. K. SIGNAIGO[4]

The deactivation or poisoning of hydrogenation catalysts by even small amounts of sulfur, hydrogen sulfide, or sulfur-containing organic compounds is a familiar phenomenon of hydrogenation chemistry. Accordingly, the discovery in this laboratory[5] that catalysts, such as cobalt polysulfide, function effectively in the conversion of aldehydes, ketones, and nitriles to thiols by hydrogenation in the presence of sulfur or hydrogen sulfide represents an important advance in this field.

Among the aldehydes and ketones to which this reaction can be applied, sugars are of especial interest since their hydrogenation in the presence of hydrogen sulfide has made available for study a variety of new polyhydroxyalkane monothiols. This paper describes the preparation, properties, and more interesting chemical reactions of 1-thiosorbitol[6] which is derived from D-glucose.

The preparation of thiosorbitol from D-glucose by hydrogenation in the presence of sulfur can be represented by the equations

$$H_2 + S \xrightarrow{\text{catalyst}} H_2S$$

$$CH_2OH\overset{\frown O \frown}{CH(CHOH)_3CHOH} + H_2S \longrightarrow \left[CH_2OH\overset{\frown O \frown}{CH(CHOH)_3CHSH}\right] + H_2O$$

$$\left[CH_2OH\overset{\frown O \frown}{CH(CHOH)_3CHSH}\right] + H_2 \xrightarrow{\text{catalyst}} CH_2OH(CHOH)_4CH_2SH$$

This mechanism is supported by the following facts: (1) thioketones and thioaldehydes readily hydrogenate to thiols under the conditions used here; (2) aldehydes and ketones have not undergone hydrogenation to alcohols under the conditions and with the catalysts used here; and (3) alcohols and hydrogen sulfide have not yielded thiols under these conditions. The reactions indicated have been carried out conveniently in pressure equipment using an aqueous reaction medium, free sulfur, and commercial dextrose. At 125–150° and a hydrogen pressure of 1000–1500 lb./sq. in. the reaction is complete in three to four hours. There is obtained a good yield of crude thiosorbitol sirup from which highly purified thiosorbitol can be isolated by several procedures. The preferred method for the isolation of thiosorbitol involves preparation and separation of the cuprous salt which is suspended in ethanol and treated with hydrogen sulfide to regenerate the free thiol. The aqueous solution is evaporated to dryness, and white crystalline 1-thiosorbitol, m. p. 92–93°, is recovered in 25–30% over-all yields by crystallization at low temperatures from alcohol. Nearly pure varieties of thiosorbitol can be obtained by direct crystallization of concentrated crude sirup from ethanol or by oxidation to the corresponding disulfide, which is recrystallized and subsequently cleaved by catalytic reduction in the presence of sulfactive catalysts. Crude thiosorbitol sirup contains organic sulfur compounds which are not thiols. Some of these products are thought to be the result of side reactions involving thioacetal formation or dehydration of thiosorbitol to cyclic sulfides. Low molecular weight cleavage products are also present in crude sirup. Removal of these prior to the above purification procedure is best accomplished by steam distillation or by extracting the aqueous reaction medium with an immiscible organic solvent.

1-Thiosorbitol is a white, crystalline, water-soluble compound showing the reactions characteristic of aliphatic mercaptans and of polyhydric alcohols. For example, oxidation with iodine in hot absolute alcohol gives the corresponding disulfide in excellent yields. The hexaacetate can be prepared by treatment of thiosorbitol with fused sodium acetate and acetic anhydride at 100°. The corresponding benzoate was obtained as a sirup.

Reaction of 1-thiosorbitol in alkaline dioxane with *n*-dodecyl bromide yields *n*-dodecyl 2,3,4,5,6-pentahydroxyhexyl sulfide.

Perhaps the most unusual property of 1-thiosorbitol is its ability to form water soluble salts with a

(1) Present address, Jackson Laboratory, E. I. du Pont de Nemours & Co., Deepwater, N. J.

(2) Present address, Chas. Pfizer and Co., Brooklyn, N. Y.

(3) Present address, Jefferson Chemical Co., Inc., N. Y., N. Y.

(4) Present address, Rayon Department, Technical Division, E. I. du Pont de Nemours & Co., Buffalo, N. Y.

(5) Signaigo, U. S. 2,230,390, Feb. 4, 1941; Farlow and Signaigo, U. S. 2,402,613, June 25, 1946.

(6) Lazier and Signaigo, U. S. 2,402,640, June 25, 1946.

variety of heavy metals.[7] For example, aqueous 1-thiosorbitol solutions dissolve silver chloride readily with the liberation of hydrochloric acid. Similarly, 1-thiosorbitol forms soluble salts with Cu^+, Cu^{++}, Fe^{++}, Pb^{++}, Hg^{++}, Sn^{++}, Ni^{++}, and Zn^{++} ions. The ability of thiosorbitol to retain these heavy metals in solution in the presence of the usual precipitating negative ions indicates a very low degree of ionization of the thiosorbitol heavy metal derivative. Hydrogen sulfide, however, generally precipitates the metals from thiosorbitol solutions. The preparation of the cuprous salt is described in connection with the purification of 1-thiosorbitol.

The general process described in the experimental part for the conversion of D-glucose to 1-thiosorbitol has been applied successfully to other sugars such as sucrose, maltose, D-fructose and soluble starches. In these cases, the products were sirups from which pure crystalline polyhydroxyalkane thiols have not been isolated.

Experimental

Preparation of Cobalt Sulfide Catalysts.—To 1500 ml. of water in a vessel of 2-liter capacity provided with a stirrer was added 240 g. of $Na_2S \cdot 9H_2O$ and 64 g. of sulfur and the whole was stirred until the sulfur was dissolved. The filtered solution was added over a ten to fifteen-minute period to a solution of 242 g. of $CoCl_2 \cdot 6H_2O$ in 1700 ml. of water contained in a vessel of 4–5 liter capacity equipped with a large paddle stirrer. After the addition, stirring was continued for another half hour. The catalyst was collected by suction filtration and washed with water on the funnel until the filtrate was colorless. The 750–1000 g. of hard paste obtained contains approximately 150 g. of cobalt polysulfide (CoS_3) and is 15–20% solids. If it is not to be used at once, it should be stored out of contact with air.

Preparation of 1-Thiosorbitol.—A 3-gallon, stainless steel, horizontal autoclave equipped with stirrer was charged with 1500 g. of D-glucose, 800 g. of sulfur, 2500 g. of water and 1000 g. of 15% cobalt polysulfide catalyst paste prepared as described above. The autoclave was then sealed and hydrogen introduced from high-pressure storage tanks until the total pressure was 1000 lb./sq. in. The autoclave and contents were heated to a temperature of 125° and maintained at this temperature. The pressure dropped as a result of the reaction of hydrogen with sulfur and additional hydrogen was forced into the autoclave to maintain a pressure of 1000 lb./sq. in. The temperature was then raised to 150° and the pressure was raised to 1500 lb./sq. in. These conditions were maintained for a period of three hours at the end of which time absorption of hydrogen had practically ceased. Excess hydrogen and hydrogen sulfide were vented from the cooled autoclave and then the product was rinsed out with 700 ml. of water. The reaction mixture was filtered to remove catalyst and then evaporated to one-half its original volume at 60° and at 40 mm. pressure. If all the water is removed, 1430 g. of a sirup containing about 13% thiol sulfur and 15% total sulfur is obtained. The viscous liquid remaining from the evaporation was diluted with 2000 g. of water and converted into a solution of the cuprous salt by adding 563 g. of powdered cuprous oxide with stirring at a temperature of 55°. The reaction mixture was kept under an atmosphere of nitrogen during this and subsequent operations. The solution of the cuprous salt was added slowly with vigorous stirring to 13 liters of methanol to precipitate the cuprous salt, which was then separated by filtration and washed twice on the filter with 1500-ml. portions of methanol. The copper salt was suspended in 2400 g. of 90% ethanol in a 3-gallon stainless steel autoclave and treated with hydrogen sulfide under 500 lb./sq. in. pressure until no further pressure drop was observed. The contents of the autoclave were rinsed out with 400 g. of absolute alcohol. The cuprous sulfide was removed by filtration, and the filtrate was treated with 20 g. of carbon black and refiltered. The alcohol solution was evaporated to dryness at 50° and 28 mm. pressure. Seven hundred milliliters of absolute alcohol was added and the evaporation procedure repeated to complete removal of water. The thiosorbitol residue was dissolved in 700 ml. of warm absolute alcohol and filtered. The solution was cooled first to room temperature and finally was kept at 5° overnight. The crystalline 1-thiosorbitol which separated was filtered and washed with cold absolute alcohol and finally with ethyl ether. After drying *in vacuo*, the resulting white crystalline non-hygroscopic material, m. p. 92–93°, weighed 427 g. (27% yield based on *d*-glucose). By titration with standard iodine solution it was found to contain thiol groups corresponding to a purity of 96.2% 1-thiosorbitol.

Anal. Calcd. for $C_6H_{14}O_5S$: C, 36.3; H, 7.12. Found: C, 36.7, 36.7; H, 7.2, 7.2.

1-Thiosorbitol is readily soluble in water, pyridine, ethylene glycol, and formamide. It is insoluble in benzene, petroleum ether, carbon tetrachloride and carbon disulfide. At 20°, 100 ml. of absolute ethanol, dioxane, ethyl ether, trichloroethylene and acetone, respectively, dissolve 1.7 g., 1.2 g., 0.016 g., 0.016 g. and 0.010 g. of 1-thiosorbitol. 1-Thiosorbitol has a specific rotation in water of $[\alpha]^{27}D$ −1.9 at 2% concentration and 27° in a tube 40 cm. in length.

1-Thiosorbitol Disulfide.—Ten grams of 1-thiosorbitol dissolved in 50 ml. of hot absolute alcohol was treated with alcoholic iodine until the iodine color persisted. The solution was then filtered and cooled overnight to allow the product to separate. Recrystallized from alcohol, eight grams of disulfide (80% yield), m. p. 128–130°, was obtained.

Anal. Calcd. for $C_{12}H_{26}O_5S_2$: C, 36.6; H, 6.7. Found: C, 36.7; H, 6.9.

The decaacetate of thiosorbitol disulfide was prepared by treatment with fused sodium acetate and acetic anhydride, m. p. 125–130°.

Anal. Calcd. for $C_{32}H_{46}O_{20}S_2$: S, 7.87. Found: S, 7.82.

1-Thiosorbitol Hexaacetate.—Two grams of thiosorbitol and 1 g. of fused sodium acetate were treated with 10 ml. of acetic anhydride and heated at 90–100° for three hours. The product was poured into water and washed by decantation several times. The solid product purified by recrystallization from aqueous alcohol melted at 87–89°.

Anal. Calcd. for $C_{18}H_{26}O_{11}S$: C, 48.0; H, 5.8. Found: C, 48.4; H, 6.0.

The corresponding benzoate obtained by the reaction of benzoyl chloride with 1-thiosorbitol in pyridine could not be induced to crystallize.

S-Dodecyl-1-thiosorbitol.—Twenty grams of 1-thiosorbitol was dissolved in 50 ml. of water and 4 g. of sodium hydroxide added. To this was added 25 g. of dodecyl bromide in 50 ml. of dioxane and the mixture heated under reflux for two hours. The solid which separated on cooling was washed with water, dioxane and ether. Thirty-one grams (84%) was obtained, m. p. 107°.

Anal. Calcd. for $C_{18}H_{38}O_5S$: C, 58.95; H, 10.48; S, 8.73. Found: C, 58.12; H, 10.37; S, 8.45.

Summary

1-Thiosorbitol has been prepared by the hydrogenation of D-glucose in the presence of sulfur. Methods for the isolation and purification of this new polyhydroxyalkane thiol have been described.

(7) Peppel and Signaigo, U. S. 2,410,844, November 12, 1946.

1-Thiosorbitol has been found to undergo normal mercaptan reactions such as oxidation to the disulfide, acylation, and etherification with alkyl halides. In addition, it has been observed to form water-soluble salts with a variety of heavy metals.

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L-α-Glycerylphosphorylcholine

BY ERICH BAER AND MORRIS KATES[1]

Studies with labelled choline (N^{15}) and radioactive phosphorus (P^{32}) have shown that a rapid metabolic turnover of phospholipids, particularly of the small intestine, liver and kidney, takes place. These observations evoke considerable interest in the role of glycerylphosphorylcholine (G.P.C.) as an intermediary metabolite, since it is highly probable that this diester plays an essential part in the biosynthesis and the turnover of lecithins. Until quite recently (1945) an investigation of the metabolic fate of the diester was difficult because it was not obtainable in sufficient quantity or purity.

Attempts to isolate G.P.C. from biological material have been made frequently. In 1935 Contardi and Ercoli[2] incubated lysolecithin with purified rice bran extracts and observed the formation of a water-soluble organic phosphate. Although this substance was not isolated in pure state, its behavior indicated that it was a glycerylphosphorylcholine. Kahane and Lévy[3] on hydrolysis of egg yolk lecithin with lecithinase B (rat intestine) obtained a choline derivative of glycerophosphoric acid which was soluble in water, methanol, ethanol and insoluble in acetone. Further experimental work strongly suggesting the presence of G.P.C. in commercial preparations of dried beef pancreas,[4] and in tissue of fresh heart muscle of frogs and rabbits[5] has been reported.

Schmidt, Hershman and Thannhauser[6] succeeded in isolating from beef pancreas autolysates *levo*-rotatory G.P.C. in fairly pure form and were able to establish its constitution as that of the choline ester of α-glycerophosphoric acid. Utilization of a biological source, however, does not lend itself readily to the preparation of G.P.C. on a laboratory scale in amounts exceeding a few grams.

During the past ten years much of the work in this Laboratory has been directed toward the synthesis of optically pure enantiomers of asymmetrically substituted glycerol derivatives. In the desire to extend our synthetic endeavours to the field of the phospholipids and in the hope of being able to supply the biochemist with a much needed material, the synthesis of L-α-G.P.C., a substance closely related to the lecithins, was attempted.

In a previous communication[7] it was shown that the optically active α-glycerophosphoric acid isolated from lecithins belongs to the L-series and can be synthesized by phosphorylation of D(+)-acetone glycerol. The use of the latter substance insured simultaneously the position of attachment of the phosphate group and the desired L-configuration of the α-glycerophosphoric acid.[8] It was to be expected that the α-G.P.C. obtained from lecithin would have the same configuration and should be obtainable in a similar manner by esterification of phosphoric acid with D(+)acetone glycerol and choline. The synthesis, especially the phosphorylation step offered, however, a number of technical difficulties which had to be overcome before a procedure could be found which would give consistently satisfactory yields of glycerylphosphorylcholine. The method of synthesis of L-α-G.P.C. which was finally adopted and the steric relationships of the various intermediate compounds are illustrated in the accompanying reaction scheme. After trying numerous phosphorylation procedures it was found that the intermediary acetone glycerylphenylphosphorylcholine chloride (C-Cl) is obtainable in adequate amounts by phosphorylation of D(+)acetone glycerol with phenylphosphoryl dichloride in the presence of quinoline, followed by esterification of the reaction product with choline in the presence of pyridine. The isolation of the choline ester from the reaction mixture was greatly facilitated by the observation that its reineckate, in contrast to the reineckates of pyridine and quinoline, precipitates from an alkaline–aqueous solution and can be separated from the similarly alkali-insoluble reineckates of choline and other choline-containing reaction products by means of its solubility in ethyl acetate. The reineckate of (C) was converted to the corresponding sulfate (C-SO_4/2) before removing the protective phenyl and acetone groups in order to avoid complications introduced

(1) This paper forms part of a thesis which will be submitted by M. Kates to the Department of Chemistry, University of Toronto, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. An account of this work was presented before the Canadian Physiological Society, at the London (Ontario) meeting, October 24–25, 1947.

(2) A. Contardi and A. Ercoli, *Arch. sci. biol.*, **21**, 1 (1935).

(3) E. Kahane and J. Lévy, *Compt. rend.*, **219**, 431 (1944).

(4) E. J. King and M. Aloisi, *Biochem. J.*, **39**, 470 (1945).

(5) G. L. Cantoni and A. W. Bernheimer, *Fed. Proc. Am. Soc. Exp. Biol.* (Part II), Vol. **6**, No. 1, 315 (1947).

(6) G. Schmidt, B. Hershman and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 523 (1945).

(7) E. Baer and H. O. L. Fischer, *ibid.*, **128**, 491 (1939).

(8) An optically active α-monoglyceride is considered as being related to the glyceraldehyde which would be obtained by oxidation of the γ-carbon atom.