of anhydrous acetone and 1.73 ml. of concd. sulfuric acid was added. The solution was kept at room temperature 3 hr., and 100 ml. of dry Amberlite IR-45(OH$^-$) anion-exchange resin was added. After filtration, the solution was concentrated under reduced pressure to give a partially crystalline sirup. Crystallization from an ether-hexane solution yielded a product with a m.p. of 55–56°.

A mixture of 2.13 g. of this product (0.019 mole) in 50 ml. of anhydrous chloroform, 20 g. of Drierite,[53] 10 g. of silver oxide, 1 g. of iodine, and glass beads was shaken 30 min. in a dark bottle. To this mixture was added 50 ml. of anhydrous chloroform which contained 7.16 g. of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl bromide (0.02 mole), and the shaking was continued. After 17 hr., the test for ionizable bromine was negative, and the mixture was filtered and concentrated to a sirup; yield 9.8 g., $[\alpha]^{25}_{D}$ + 26° (*c* 1.6, chloroform). Chromatography with irrigant C indicated that there were no unreacted starting materials.

This sirup, 5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) - 2,2′ - *O* - isopropylidene - "α" - D - isosaccharino-1,4-lactone, was dissolved in 80 ml. of 80% glacial acetic acid and heated on a steam plate to remove the isopropylidene group. The rotation became constant after 30 min., and the solution was concentrated under reduced pressure to give a partly acetylated sugar sirup. After addition of 10 ml. of acetic anhydride and 1 g. of fused sodium acetate, the mixture was heated and poured into ice water. The fully acetylated 5-*O*-β-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone was isolated by standard procedures; yield 7.4 g. of thick sirup, $[\alpha]^{25}_{D}$ + 31° (*c* 10.2, chloroform).

To convert the glycosidic linkage of this disaccharide into the *alpha* configuration, 2.5 g. of the hexaacetate was dissolved in 25 ml. of anhydrous chloroform. Three grams of titanium tetrachloride in 35 ml. of anhydrous chloroform was added, and a yellow precipitate formed immediately. This mixture was refluxed 5 hr. in a water bath (65–70°) and then poured into 250 ml. of ice water. The chloroform layer was separated, washed with water, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to a thick sirup; yield 1.6 g., $[\alpha]^{25}_{D}$ + 112° (*c* 3.3, chloroform).

This sirup was dissolved in anhydrous methanol at 0° and an excess of barium methoxide was added. After standing 24 hr. at 0°, the mixture was concentrated under reduced pressure to dryness, suspended in water, and treated with an excess of Amberlite IR-120 (H$^+$) cation-exchange resin. The sirup obtained upon concentration of the resulting solution was purified by removal of trace components by paper chromatography with irrigant A. A sirup was obtained which moved as a single component in irrigant A with R_{ga}[43] and R_l[44] values of 2.9 and 1.1, respectively. It had an R_g value of 3.2 with irrigant B; $[\alpha]^{25}_{D}$ + 106° (*c* 3.8, water). On the basis of its high positive rotation, this lactone is designated 5-*O*-α-D-galactopyranosyl-"α"-D-isosaccharino-1,4-lactone. Hydrolysis produced D-galactose, "α"-D-isosaccharinic acid, and "α"-D-isosaccharino-1,4-lactone.

Synthesis of 5-O-α-D-galactopyranosyl-"β"-D-isosaccharinic acid. The above procedure was repeated starting with 2.13 g. of sirupy methyl 2,2′-*O*-isopropylidene-"β"-D-isosaccharinate. The product obtained was a sirup; $[\alpha]^{25}_{D}$ + 58° (*c* 1.4, water). After autohydrolysis, the solution reached a constant rotation of +37° (based on the original concentration). Investigation of this hydrolyzed solution indicated only D-galactose and "β"-D-isosaccharinic acid. Therefore, this compound has been designated 5-*O*-α-D-galactopyranosyl-"β"-D-isosaccharinic acid.

Acknowledgment. The authors gratefully acknowledge the grant from the Department of Health, Education, and Welfare, which helped to support this work.

LAFAYETTE, IND.

---

[53] A special form of anhydrous calcium sulfate obtainable from the W. A. Hammond Drierite Co., Xenia, Ohio.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

Simplified Preparation of Sophorose (2-*O*-β-D-glucopyranosyl-D-glucose)

BRUCE COXON AND HEWITT G. FLETCHER, JR.

Received December 27, 1960

Condensation of methyl 4,6-*O*-benzylidene-α-D-glucoside with tetra-*O*-acetyl-α-D-glucopyranosyl bromide gives a sophorose derivative from which the substituting groups may be removed (through successive acetolysis and deacetylation) to give crystalline sophorose in an over-all yield of 30%. This comparatively simple two-step synthesis makes this hitherto rare disaccharide relatively accessible.

Owing to the widespread biological and medical uses to which D-glucose is put, the discovery of sophorose (III), 2-*O*-β-D-glucopyranosyl-D-glucose, in typical "pure" commercial samples of D-glucose[1] focuses new interest on this little-known disaccharide. This interest is enhanced by the discovery of the remarkable activity shown by the sugar in stimulating cellulase production by a strain of *Trichoderma viride*[1] and by the fact that it is a representative of the theoretically interesting but comparatively rare 2-*O*-glycosylaldoses.

The history of sophorose is unusual in that this disaccharide was synthesized prior to its discovery in nature. In 1928 Freudenberg, Toepffer and Anderson[2] described the condensation of methyl 4,6-*O*-benzylidene-α-D-glucoside (I) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Some years later Freudenberg and Soff[3] succeeded in converting the product of this condensation (II) into a disaccharide which they showed to be a 2-*O*-β-D-glucopyranosyl-D-glucose (III); the process involved will

---

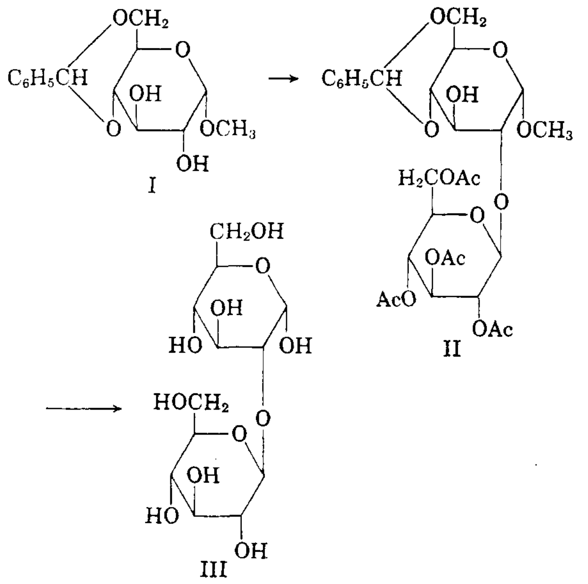
[1] M. Mandels and E. T. Reese, *Biochem. Biophys. Research Communs.*, 1, 338 (1959).

[2] K. Freudenberg, H. Toepffer, and C. C. Andersen, *Ber.*, 61, 1750 (1928).

[3] K. Freudenberg and K. Soff, *Ber.*, 69, 1245 (1936).

be described briefly later in this paper. In 1941 Gakhokidze[4] synthesized sophorose through the condensation of 1,3,4,6-tetra-*O*-acetyl-D-glucose with tetra-*O*-acetyl-α-D-glucopyranosyl bromide.

Sophorose and some of its derivatives have been reported in a variety of materials. In 1937 Rabaté and Dussy[5] found that mild acid hydrolysis of the glycoside sophoraflavonoloside, obtained from the green berries of *Sophora japonica* L., afforded a disaccharide which they showed to be 2-*O*-β-D-glucopyranosyl-D-glucose and which they named "sophorose." In subsequent publications Freudenberg and his co-workers[6,7] established the identity of sophorose with their synthetic material.



Sophorose has been detected among the products formed when D-glucose is treated with acid[8]; doubtless its presence in commercial D-glucose is due to reversion of D-glucose during the acid hydrolysis of starch. Peat, Whelan, and Hinson[9] detected sophorose after treating a concentrated D-glucose solution with almond emulsin while Barker *et al.*[10] showed it to be among the products produced from a mixture of cellobiose and D-glucose by a strain of *Aspergillus niger*. The substance has also been found in cultures of *Aspergillus nidulans* and various *Acetobacter* species grown in defined, glucose-containing media.[11] Vis and Fletcher[12] have demonstrated that the very sweet ester-glycoside stevioside from *Stevia rebaudiana* (*Bertoni*) contains a sophorose residue.

The difficulties involved in preparing large quantities of 1,3,4,6-tetra-*O*-acetyl-D-glucose for Gakhokidze's synthesis[4] induced us to re-examine the procedure described by Freudenberg.[6,7] The latter author reported the following sequence: methyl 4,6-*O*-benzylidene-α-D-glucoside (I) → methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β- D - glucosyl)-α-D-glucoside (II) → methyl α-sophoroside heptaacetate → sophorose octaacetate (not isolated) → 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)-α-D-glucosyl bromide → β-sophorose octaacetate → α-sophorose (III). The yield in the last step was not reported but, assuming that it was quantitative, the over-all yield from methyl 4,6-*O*-benzylidene-α-D-glucoside (I) was 4.6%. In the course of the present investigation it has been found that the yield in the first step of the synthesis may be increased from the 11% reported by Freudenberg to 40%. Furthermore, the product of this condensation, methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)-α- D - glucoside (II), may be subjected directly to successive acetolysis and deacetylation to give crystalline sophorose of high purity in 76% yield. Since methyl 4,6-*O*-benzylidene-α-D-glucoside is readily preparable[13,2] from commercially available methyl α-D-glucopyranoside in 70% yield, this relatively simple, two-step process, with an over-all yield of 30%, appears to be of the most practical for the preparation of substantial quantities of this interesting disaccharide.

The ease with which steviolbioside heptaacetate is cleaved by hydrogen bromide in glacial acetic acid to form the relatively insoluble 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)-α-D-glucosyl bromide ("α-acetobromosophorose")[12] suggests that, despite the negative finding of Freudenberg,[2] methyl α-sophoroside heptaacetate may also give this halide when treated in such a manner. In the course of the present research this suggestion was confirmed. In the experiment reported here the yield of the bromide was comparatively low; no attempt to maximize this yield was made.

---

[4] A. M. Gakhokidze, *J. Gen. Chem.* (*U.S.S.R.*), **11**, 117 (1941); *Chem. Abstr.*, **35**, 5467 (1941).

[5] J. Rabaté and J. Dussy, *Compt. rend.*, **205**, 1431 (1937); *Bull. soc. chim. biol.*, **20**, 467 (1938); J. Rabaté, *Bull. soc. chim. France*, **7**, 565 (1940). NOTE ADDED IN PROOF: M. J. Clancy [*J. Chem. Soc.*, 4213 (1960)] has recently described an improved preparation of sophorose from the pods of *Sophora japonica* L. as well as some new derivatives of this sugar.

[6] K. Freudenberg and H. Knauber, *Naturwissenschaften*, **34**, 344 (1947).

[7] K. Freudenberg, H. Knauber, and F. Cramer, *Chem. Ber.*, **84**, 144 (1951).

[8] A. Thompson, K. Anno, M. L. Wolfrom, and M. Inatome, *J. Am. Chem. Soc.*, **76**, 1309 (1954).

[9] S. Peat, W. J. Whelan, and K. A. Hinson, *Nature*, **170**, 1056 (1952).

[10] S. A. Barker, E. J. Bourne, G. C. Hewitt, and M. Stacey, *J. Chem. Soc.*, 3734 (1955).

[11] A. W. Khan, D. N. Pellegrino, and T. K. Walker, *Nature*, **183**, 682 (1959); T. K. Walker, D. N. Pellegrino, and A. W. Khan, *Arch. Biochem. Biophys.*, **83**, 161 (1959).

[12] E. Vis and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **78**, 4709 (1956).

[13] N. K. Richtmyer in *Methods in Carbohydrate Chemistry*, M. L. Wolfrom and R. L. Whistler, eds., Academic Press Inc., New York, in press.

EXPERIMENTAL[14]

*Methyl 4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-β-*D*-glucosyl)-α-*D*-glucoside* (II). A mixture of 11.5 g. of methyl 4,6-*O*-benzylidene-α-D-glucoside, 11.5 g. of silver carbonate, 30 g. of Drierite and 142 ml. of dry methylene chloride, protected from atmospheric moisture, was stirred for 0.5 hr. Iodine (3 g.) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (20.9 g., 1.25 molar equivalents) were added and the mixture stirred for 19 hr. at room temperature. A test for soluble bromide ion was then negative. The reaction mixture was filtered through a thin layer of decolorizing carbon and Celite and concentrated *in vacuo* to a thick sirup which was dissolved in 60 ml. of warm 2-ethoxyethanol. Crystallization ensued and, after 1 hr. at room temperature and 3 hr. at −5°, the product was removed and washed successively with cold 2-ethoxyethanol and absolute ethanol: 10.1 g. (40% based on the substituted glucoside), m.p. 225–227°, $[\alpha]^{20}_{D}$ +40.6° in chloroform (*c* 1.3). For the succeeding step this material was sufficiently pure. After two recrystallizations from 2-ethoxyethanol, a sample of the product melted at 227–228° and showed $[\alpha]^{20}_{D}$ +42.4° in chloroform. Further recrystallization failed to change these values. Freudenberg, Toepffer, and Anderson[2] reported m.p. 232° and $[\alpha]^{21}_{D}$ +47° (chloroform).

Anal. Calcd. for $C_{28}H_{36}O_{15}$(612.57): C, 54.89; H, 5.92. Found: C, 55.13; H, 5.97.

*2-O-β-*D*-Glucopyranosyl-α-*D*-glucose* (*α-sophorose,* III). A suspension of methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)-α-D-glucoside (9.09 g.) in acetic anhydride (20 ml.) was stirred with a solution (20 ml.) of 4% concd. sulfuric acid in acetic anhydride (v./v.). Solution of the crystals took place in 10 min., with the development of a deep red color which slowly turned to a pale green as the mixture stood at 25° for 4 hr.[15] The mixture was then poured onto a mixture of ice and solid sodium bicarbonate and the product extracted with chloroform (200 ml., 3 × 50 ml.). The combined extracts were washed twice with water and concentrated *in vacuo* to a pale yellow sirup which was dried by repeated distillation (*in vacuo*) with absolute ethanol and finally *in vacuo* over phosphorus pentoxide. The dried sirup was then dissolved in warm methanol (150 ml.), the solution cooled to room temperature, and 9 ml. of 1.5*N* barium methoxide added. A gelatinous white solid was immediately precipitated. After standing at room temperature for 4 hr. the strongly alkaline suspension was diluted with methanol and deionized by passage through columns of IR-120 (H) (2.5 × 23 cm.) and IR-45 (2 × 26 cm.), the resins being washed with 50% aqueous methanol. The eluate was concentrated *in vacuo* to a volume of *ca.* 150 ml. and then extracted with chloroform (3 × 50 ml.). The combined chloroform extracts were washed with water and the combined aqueous solutions concentrated to a colorless sirup which was crystallized from warm aqueous ethanol–ether. The disaccharide was dried *in vacuo* at 40°: 4.08 g. (76%, calculated as the monohydrate), m.p. 185–188°. Paper chromatography in butanol-pyridine-water (10:3:3, v./v), using *p*-anisidine hydrochloride spray, showed that the product was very slightly contaminated with glucose. One recrystallization from aqueous acetic acid or from aqueous ethanol–ether removed this impurity, giving needles melting at 188–190° (foaming), $R_{glucose}$ 0.50 in the above-mentioned system. After drying *in vacuo* at 60° over phosphorus pentoxide for 1.5 hr. the anhydrous sophorose showed $[\alpha]^{2}_{D}$ +33.4 (extrap.) → +21.2° (22 hr.) in water (*c* 4.58). Freudenberg and Soff[3] recorded $[\alpha]^{20}_{D}$ +34.5 → +19.9° (water, *c* 4).

*3,4,6-Tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-β-*D*-glucosyl)-α-*D*-glucosyl bromide.* Methyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucosyl)-α-D-glucoside[3] (533 mg.) was dissolved in 10 ml. of glacial acetic acid in a partially filled, all-glass, 2-dm. polarimeter tube. A steam of hydrogen bromide was directed on the surface of the solution for 1 min. and the mixture left at 20°. After 2 hr. the rotation of the solution, α_D = +5.33°, was the same as before the addition of the acid. The mixture was, therefore, heated at 45° for 2 hr. and then left at 20° for 2.5 days: α_D = +10.8°. Methylene chloride, ice, and water were added and the organic layer washed successively with water (4 × 200 ml.) and cold aqueous sodium bicarbonate. Moisture was removed with magnesium sulfate and the solution concentrated to a pale-yellow sirup which crystallized as clusters of needles on trituration with ether. Washed successively with ether and pentane and dried, the bromide (102 mg., 18%) melted at 178–185°. Recrystallized from methylene chloride–ether and then from methylene chloride–pentane, it melted at 190–191° and showed $[\alpha]^{20}_{D}$ +97.4° in chloroform (*c* 0.41). Freudenberg and Soff[3] reported m.p. 194° (d.) and $[\alpha]^{20}_{D}$ +95.6° (chloroform) for this compound.

Acknowledgment. We are indebted to Mr. Harry W. Diehl for assistance in some of the preparations.

BETHESDA 14, MD.

---

[14] Melting points are corrected.

[15] Such color changes are characteristic of benzaldehyde and of benzylidene acetals in such acetolysis mixtures.