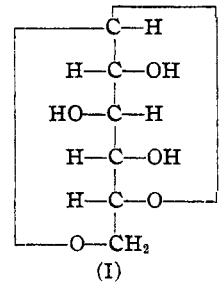
[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

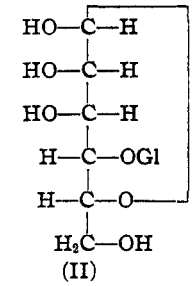
Synthesis of Cellobiose

BY W. T. HASKINS, RAYMOND M. HANN AND C. S. HUDSON

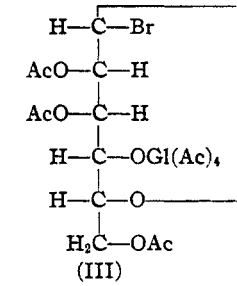
In 1933, Freudenberg and Nagai[1] condensed levoglucosan (I) with acetobromo-D-glucose and obtained a mixture of sirupy condensation products. The sirup, which was presumed to contain 4-[2,3,4,6-tetraacetyl-β-D-glucopyranosido]-D-glucosan <1,5>β<1,6> as one of its possible constituents, was allowed to stand in 50% sulfuric acid for twenty hours at 20°, since kinetic studies had indicated that such conditions would allow rupture of the β<1,6> ring with a minimum hydrolysis of the cellobiose linkage. The resulting product, after removal of the sulfuric acid, was acetylated and it yielded a crystalline compound which gave combustion analyses for a disaccharide octaacetate. A mixed melting point, with authentic cellobiose octaacetate (presumably the α-form), showed the value of 225° (*i. e.*, no depression); the yield (0.25 g.) was only 1.9% based on the acetobromo-D-glucose employed. The lowness of the yield is not surprising when it is considered that a molecule of the type of levoglucosan, which contains three secondary hydroxyl groups, could yield a mixture of several possible condensation products with acetobromoglucose under the experimental conditions employed. With full recognition of the importance of Freudenberg and Nagai's synthesis of cellobiose and of the fact that it is a total synthesis, there still remains the problem of obtaining a structurally definitive synthesis of the disaccharide. Although no derivative of levoglucosan has ever been described in which only the secondary hydroxyl group at carbon atom four would be available for a structurally definitive synthesis of cellobiose, attention has been directed[2] to the possibility of utilizing 2,3-isopropylidene-D-mannosan <1,5>β<1,6> for the synthesis of disaccharides which are linked through carbon atom four of D-mannose, and we have subsequently used this substance in a structurally definitive synthesis of epi-cellobiose (II) (4-[β-D-glucopyranosido]-D-mannose).[3] In the present paper we describe the conversion of the latter substance into its epimer, cellobiose (VI) (4-[β-D-glucopyranosido]-D-glucose).[4] The octaacetate of epi-cellobiose, upon treatment with hydrogen bromide in glacial acetic acid, gave the acetobromo-epi-cellobiose (III) described by Brauns.[5] The latter compound was reduced with zinc and acetic acid to form crystalline cellobial hexaacetate (IV), which was identical with the well known compound obtained from acetobromocellobiose. The cellobial hexaacetate was oxidized with perbenzoic acid in moist ether solution, since the work of Levene and Tipson[6] indicates that oxidation by this reagent of glycals containing a substituent on carbon atom three leads mainly to products in which the introduced hydroxyl group on carbon atom two is in the *trans* position to that on carbon atom three; such a course of reaction should lead, in the present case,



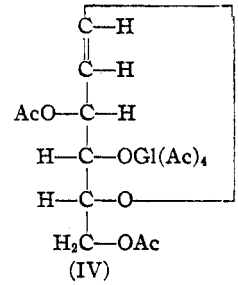
(I)
Levoglucosan



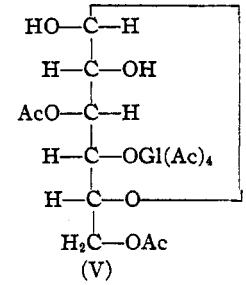
(II)
Epi-cellobiose



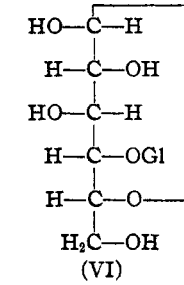
(III)
Acetobromo-epi-cellobiose



(IV)
Hexaacetyl cellobial



(V)
Hexaacetyl cellobiose



(VI)
Cellobiose

(1) Freudenberg and Nagai, *Ber.*, **66**, 27 (1933).
(2) Knauf, Hann and Hudson, THIS JOURNAL, **63**, 1447 (1941).
(3) Haskins, Hann and Hudson, *ibid.*, **63**, 1724 (1941).
(4) Haworth, Long and Plant, *J. Chem. Soc.*, 2809 (1927).
(5) Brauns, THIS JOURNAL, **48**, 2776 (1926).
(6) Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931).

to the cellobiose configuration rather than to that of epi-cellobiose. The sirupy oxidation product, which was, presumably, cellobiose hexaacetate (V) principally, was acetylated and it yielded a mixture of crystalline α- and β-cellobiose octaacetates. The acetates upon deacetylation with barium methylate formed crystalline cellobiose (VI) which was identified by its melting point, initial and final rotations and mutarotation rate, as identical with authentic cellobiose. The yield was 35% based on the epi-cellobiose octaacetate employed.[6a]

The D-mannosan $<1,5>\beta<1,6>$ which was used in these syntheses of epi-cellobiose and cellobiose was prepared by the pyrolysis of vegetable ivory, a complex natural product. Recently this mannosan has been synthesized in this Laboratory[7] by the action of alkali on β-phenyl-D-mannopyranoside, which in turn was made from D-mannose; the disaccharide syntheses accordingly represent total ones, *i. e.*, they are finally referable solely to inorganic substances through Emil Fischer's total syntheses of mannose and glucose. It is obvious that the method of synthesis of epi-cellobiose and cellobiose is applicable in principle to the synthesis of similarly constituted compound sugars of longer chain length, and one can visualize, at least in imagination, an approach by this method to the laboratory synthesis of cellulose, according to present views of the structure of this polysaccharide.

We express our appreciation to Dr. A. T. Ness for performing the microchemical analyses in connection with this work.

Experimental

Acetobromo-epi-cellobiose (III).—This compound was prepared by the directions of Pacsu[8] for obtaining acetobromocellobiose. A suspension of 10 g. of synthetic epi-cellobiose octaacetate[3] in 20 cc. of glacial acetic acid was heated on the steam-bath for a few minutes and the resulting partial solution was cooled to 20°; 5 cc. of acetic anhydride and 40 g. of a 30% solution of hydrobromic acid in glacial acetic acid were added and the reaction mixture was allowed to stand at 5° for eighteen hours. The solution was poured upon crushed ice and the reaction product was extracted with chloroform in the usual manner; the washed and dried chloroform solution was concentrated *in vacuo* to a sirup and upon addition of 25 cc. of ether, acetobromo-epi-cellobiose crystallized in the form of long needles. The yield was 8.5 g. (82%); the pure compound[9] melted at 168–169° (cor.) and rotated +78.0° (*c*, 0.9) in chloroform. Brauns[5] recorded a melting point of 168–169° and a specific rotation $[\alpha]^{20}D$ of +77.9°, and Haworth and co-workers[10] a melting point of 169° and a specific rotation $[\alpha]^{20}D$ of +77° for acetobromo-epi-cellobiose.

Anal. Calcd. for $C_{26}H_{35}O_{17}Br$: Br, 11.4. Found: Br, 11.5.

Cellobial Hexaacetate from Acetobromo-epi-cellobiose.—To a cooled solution (10°) of 6.5 g. of acetobromo-epi-cellobiose in 100 cc. of 90% acetic acid 40 g. of zinc dust and 1 drop of 0.5% solution of chloroplatinic acid in 50% acetic acid were added and the reaction mixture was stirred vigorously for four hours. The zinc was removed by filtration and the filtrate poured into 1 liter of ice water; the crystallization of the cellobial hexaacetate was allowed to proceed for sixteen hours at 5°; the product (3.6 g., yield 69%) was recrystallized by solution in 3 parts of chloroform and the addition of 7 parts of petroleum ether. The compound melted at 135–136° (cor.) and showed a specific rotation of −20.1° (*c*, 0.9) in chloroform, in agreement with recorded values[10] of 137° and −20°, respectively. A mixed melting point with authentic cellobial hexaacetate (from acetobromo-cellobiose) showed no depression.

Anal. Calcd. for $C_{24}H_{32}O_{15}$: C, 51.42; H, 5.75; CH_3CO, 46.1. Found: C, 51.34; H, 5.58; CH_3CO, 46.0.

α- and β-Cellobiose Octaacetate from Cellobial Hexaacetate.—A solution of 5.0 g. of cellobial hexaacetate in 30 cc. of ethyl acetate was agitated for twenty hours at 25° with 30 cc. of a 0.3545 *M* ether solution of perbenzoic acid (1.19 molecular equivalents) and 5 cc. of water. Titration of a 1 cc. subsample with 0.1 *N* thiosulfate indicated that 0.92 molecular equivalent of perbenzoic acid had been consumed; a test with bromine water indicated the absence of cellobial hexaacetate. The reaction mixture was neutralized by shaking with a saturated solution of sodium bicarbonate and the aqueous layer was separated and extracted with chloroform; the combined ether and chloroform extracts were dried and concentrated *in vacuo* to a thick sirup. The sirup, which presumably contained much cellobiose hexaacetate, was dissolved in 10 cc. of pyridine and, after the addition of 10 cc. of acetic anhydride, the acetylation mixture was allowed to stand at room temperature for twenty-four hours and was then poured upon 200 g. of crushed ice; the crystalline product which precipitated (4.6 g.) was recrystallized from 30 parts of alcohol and it yielded 3.8 g. (63%) of needles (m. p. 180–185°), presumably a mixture of α- and β-cellobiose octaacetates since upon deacetylation, as described in the following paragraph, it gave a quantitative yield of cellobiose.

Anal. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.47; H, 5.53.

(6a) It has just come to our attention that Helferich and Bredreck [*Ber.*, **64**, 2411 (1931)] have reported a synthesis of heptaacetyl-β-methylcellobioside by coupling acetobromoglucose with 2,3,6-triacetyl-β-methylglucoside in a yield which they describe as minute ("winzig"). Insufficient material was obtained for a combustion analysis, but their synthetic product (m. p. 188.5°) upon admixture with authentic heptaacetyl-β-methylcellobioside (m. p. 192°) melted at 189°. (Note added April 27, 1942.)

(7) Montgomery, Richtmyer and Hudson, unpublished results.

(8) Pacsu, THIS JOURNAL, **52**, 2571 (1930).

(9) All of the crystalline compounds described in the experimental part were recrystallized to constant melting point and specific rotation, $[\alpha]^{20}D$; *c* is the concentration in grams in 100 cc. of solution; the tube length was 4 dm.

(10) Haworth, Hirst, Streight, Thomas and Webb, *J. Chem. Soc.*, 2636 (1930).

Cellobiose from the Mixed α- and β-Cellobiose Octaacetates.—A suspension of 3.0 g. of the finely powdered cellobiose octaacetates in 150 cc. of methyl alcohol was deacetylated by barium methylate in the usual manner, The disaccharide (1.5 g., quantitative) was obtained in the form of prisms which melted with decomposition at 225–226° (cor.); a mixed melting point determination with authentic cellobiose showed no depression; an aqueous solution of the substance showed initial and final rotations of +16.2° and +34.9° (*c*, 1.0), respectively, in water, with a mutarotation rate of 0.0043 at 20°. Hudson and Yanovsky[11] reported an initial rotation of +16°, a final rotation of +35° and a mutarotation rate of 0.0047 for cellobiose. The over-all yield of cellobiose from epi-cellobiose octaacetate was 35%.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.18; H, 6.53.

Summary

Synthetic epi-cellobiose octaacetate, prepared by the action of an acid-acetylating mixture on the condensation product of 2,3-isopropylidene-D-mannosan<1,5>β<1,6> with acetobromo-glucose, has been converted to the known cellobial hexaacetate by customary procedures. Cellobial hexaacetate, upon oxidation with perbenzoic acid, follows the rule proposed by Levene and Tipson, and adds the introduced hydroxyl at carbon two mainly in a *trans* position to the acetylated hydroxyl group on carbon three; the resulting reaction product is apparently chiefly cellobiose hexaacetate because, upon acetylation, it yields a mixture of crystalline α- and β-cellobiose octaacetates in 63% yield. Crystalline cellobiose was obtained in quantitative yield by deacetylation of the octaacetate mixture. The over-all yield of cellobiose from epi-cellobiose octaacetate was 35%. The results constitute the structurally definitive syntheses of epi-cellobiose and cellobiose from D-mannose and D-glucose; they are also total syntheses in the sense that they are finally referable solely to inorganic substances through Emil Fischer's total syntheses of the two hexoses.

(11) Hudson and Yanovsky, THIS JOURNAL, **39**, 1035 (1917).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BANTING INSTITUTE, UNIVERSITY OF TORONTO]

l-Glycidol

BY JOHN C. SOWDEN AND HERMANN O. L. FISCHER

The preparation of optically-active glycidol ("epihydrin alcohol," 2,3-epoxypropanol-1) was first reported by Abderhalden and Eichwald.[1] These authors obtained the enantiomorphic glycidols by a series of reactions from 1-amino-2,3-dibromopropane after having subjected this amine to resolution with *d*-tartaric acid. The glycidols were then employed, by reaction with fatty acids, to prepare the first examples of optically-active α-monoglycerides. Moreover, by the addition of ammonia to the glycidols, a reaction previously studied by L. Knorr and E. Knorr[2] for racemic glycidol, Abderhalden and Eichwald obtained the optically active forms of 1-aminopropanediol-2,3. These amino alcohols, in turn, were used to prepare enantiomorphic α,β-diglycerides and triglycerides. It is evident that the degree of optical purity of the α-monoglycerides, α,β-diglycerides and triglycerides thus obtained is limited by the degree of optical purity of the enantiomorphic glycidols employed for their preparation.

(1) E. Abderhalden and E. Eichwald, *Ber.*, **47**, 1856, 2880 (1914); **48**, 1847 (1915).

(2) L. Knorr and E. Knorr, *ibid.*, **32**, 750 (1899).

Previous publications from this Laboratory have described the use of the natural asymmetry of the mannitols for the preparation of *d*(+)-acetone-glycerol[3] and *l*(−)-acetone-glycerol.[4] These enantiomorphic acetone-glycerols were then employed to prepare optically active α-monoglycerides,[5] α,β-diglycerides,[6] triglycerides,[5,6] the α-glycerophosphoric acids,[7] and α-ethers of glycerol.[8]

The same general method has now been used in the preparation and proof of optical purity of *l*-glycidol[9] and *l*-1-aminopropanediol-2,3.[9]

Starting with *d*(+)-acetone-glycerol, the reactions involved are illustrated in Fig. 1. It can be

(3) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 463 (1939).

(4) E. Baer and H. O. L. Fischer, THIS JOURNAL, **61**, 761 (1939).

(5) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 475 (1939).

(6) J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **63**, 3244 (1941).

(7) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 491 (1939); **135**, 321 (1940).

(8) E. Baer and H. O. L. Fischer, *ibid.*, **140**, 397 (1941).

(9) The prefix *l*- here designates the optical relationship of the compounds to *l*-glyceraldehyde. Thus the terminal carbon atom to which the free hydroxyl group is bound in *l*-glycidol and *l*-1-aminopropanediol-2,3 is considered to be the potential aldehydic carbon atom of the glyceraldehyde, *e. g.*, *l*-glyceraldehyde, to which these compounds are related.