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1,6-Anhydro-D-*glycero*-β-D-*ido*-heptopyranose[1,2]

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The sugar D-*glycero*-D-*ido*-heptose (formerly known as D-β-glucoheptose and D-gluco-D-*ido*-heptose) is converted by hot dilute acid to an equilibrium mixture containing about 43% of a non-reducing anhydride. In this respect it behaves like D-idose and D-*ido*-heptulose. The structure of the new crystalline anhydride has been established definitively as 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose. Oxidation of the anhydride by periodate followed by catalytic hydrogenation furnished 2,4-*cis*-5-*cis*-tri-(hydroxymethyl)-1,3-dioxolane (synonym, 2,3-[2-hydroxyethylidene]-erythritol). Isolation of this dioxolane, as its crystalline tri-*p*-nitrobenzoate, from the similar treatment of the epimeric anhydroheptose described earlier has proved conclusively the previously assigned structure of that compound as 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose. Tosylation of the new anhydroheptose has yielded both tri- and tetratosyl derivatives, and a number of their transformation products have been described.

In continuation of our studies on the formation of non-reducing monomeric anhydrosugars by the action of acids on certain aldoses and ketoses, we now wish to describe the first example of an anhydroheptose to be prepared in this way. The subject of our study was D-*glycero*-D-*ido*-heptose (I), the rarer of the two heptoses available through the cyanohydrin synthesis from D-glucose. This sugar, first obtained in sirupy form by Fischer[4] and later by Philippe,[5] was long known as D-β-glucoheptose; at the suggestion of one of us[6] it has been known more recently as D-gluco-D-*ido*-heptose. The sugar was described in crystalline form in 1934 by Isbell.[7] In our preparation of the sugar we have introduced several modifications of procedure and have also verified the general shape of its complex mutarotation curve reported by Isbell.[7]

At the time our researches were begun it was known that D-altrose is converted by mineral acids to an equilibrium mixture containing 57% of the non-reducing 1,6-anhydro-β-D-altropyranose[8]; that D-*altro*-heptulose (commonly called sedoheptulose) is similarly converted to the extent of 80% to an anhydride[9] whose structure we have since established beyond any reasonable doubt as 2,7-anhydro-β-D-*altro*-heptulopyranose[10]; and that, as Sorkin and Reichstein discovered,[11] D-idose similarly shows a 75% conversion to 1,6-anhydro-β-D-idopyranose. Extension of our study of this reaction to D-*glycero*-D-*ido*-heptose (I)[1] then verified our expectation that any sugar with the *ido* configuration for its top four asymmetric carbon atoms would form a monomeric non-reducing anhydride under the influence of acids. Even more recently we have shown that D-*ido*-heptulose is transformed, similarly and in 85% yield, to 2,7-anhydro-β-D-*ido*-heptulopyranose.[12]

Thus, D-*glycero*-D-*ido*-heptose (I) was converted within 24 hours by hot 0.2 *N* hydrochloric acid to an equilibrium mixture containing 43% of non-reducing material. Isolation and crystallization of the latter was accomplished without difficulty and the new compound, melting at 193–199° and showing $[\alpha]^{20}D$ − 74.4° in water, had the composition of an anhydroheptose. Periodate oxidation showed the presence of three contiguous CHOH groups, and successive tosylation, reaction with sodium iodide and hydrogenation to a compound that contained a C-methyl group proved that the anhydroheptose contained also a CH_2OH group. Our new anhydroheptose, therefore, could have only a 1,5:1,6 or 1,2:1,6 combination of rings. To distinguish between these possibilities we noted that the rotation $[\alpha]^{20}D$ − 59.0° of the dialdehyde formed in the periodate oxidation was practically identical with the value −58.7° observed in the similar oxidation of another 1,6-anhydroheptose. Montgomery, Richtmyer and Hudson,[13] in their studies on the alkaline degradation of phenyl glycosides, had obtained a crystalline anhydroheptose by boiling phenyl D-*glycero*-β-D-*gulo*-heptoside (IV) with aqueous potassium hydroxide. Although they made the reasonable assumption that their anhydride contained the same 1,5-ring that was believed to exist in the original glycoside and accordingly wrote their anhydride as III, they did not rigorously exclude the possibility that it contained instead a 1,2-ring. Since the rotations cited above suggested that the same dialdehyde (presumably V) is formed from each of these two anhydroheptoses, our next problem was to find a method for establishing conclusively the structure of both anhydrides and at the same time to obtain a reference compound that could be used to correlate these structures with those of other anhydroheptoses that might be prepared later.

Catalytic hydrogenation of the dialdehyde with Raney nickel was selected for this purpose and the resulting product from each of the anhydroheptoses yielded the same crystalline, optically inactive tri-*p*-nitrobenzoate. This established not only the identity of the dialdehydes produced from the

(1) Presented in part before the Division of Sugar Chemistry at the Chicago Meeting of the American Chemical Society, September 5, 1950.

(2) The naming of the compounds described in this paper follows the Rules of Carbohydrate Nomenclature that were approved by the Council of the American Chemical Society, March 17, 1953, and published in *Chem. Eng. News*, **31**, 1776 (1953).

(3) Deceased, December 27, 1952.

(4) E. Fischer, *Ann.*, **270**, 64 (1892).

(5) L.-H. Philippe, *Ann. chim. phys.*, [8] **26**, 289 (1912).

(6) C. S. Hudson, THIS JOURNAL, **60**, 1537 (1938).

(7) H. S. Isbell, *ibid.*, **56**, 2789 (1934).

(8) N. K. Richtmyer and C. S. Hudson, *ibid.*, **57**, 1716 (1935); **61**, 214 (1939); **62**, 961 (1940).

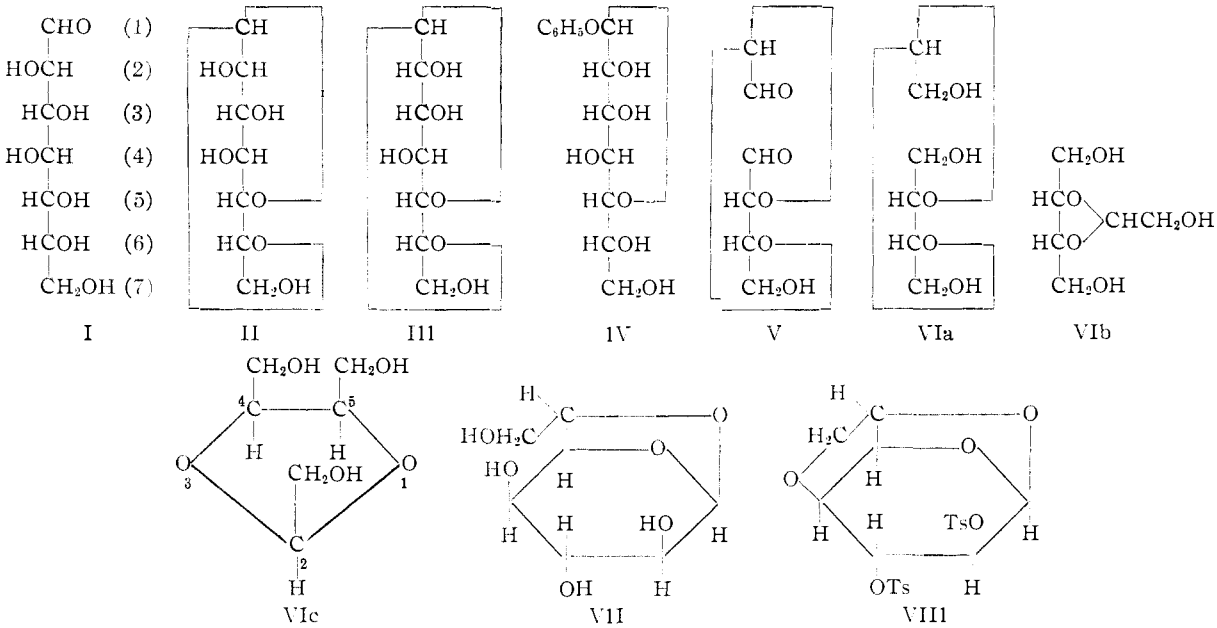
(9) F. B. LaForge and C. S. Hudson, *J. Biol. Chem.*, **30**, 61 (1917).

(10) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **73**, 1876 (1951); **74**, 2200 (1952).

(11) E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **28**, 1 (1945).

(12) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2210 (1952).

(13) R. M. Montgomery, N. K. Richtmyer and C. S. Hudson, *ibid.*, **65**, 1848 (1943).



two anhydroheptoses but also proved that these anhydrides contained the 1,5:1,6 combination of rings since the product formed from a 1,2:1,6 structure would be optically active. Therefore our new anhydroheptose must be 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose (II) and the structure of the older anhydroheptose was reaffirmed as that of the epimeric 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose (III). Because periodate oxidation of these anhydroheptoses has destroyed all isomerism originally due to the asymmetry of carbon atoms 2, 3 and 4, the dialdehyde (V) should be obtainable not only from the epimeric anhydrides II and III but also from similar anhydrides (however they may subsequently be prepared) derived from each of the two epimeric D-mannoheptoses, D-alloheptoses and D-altroheptoses. Furthermore, the catalytic hydrogenation of the dialdehyde (V) to the trihydric alcohol (VIa) and the preparation of its readily crystallizable tribenzoate or tri-*p*-nitrobenzoate should furnish a convenient and conclusive method of identifying the dialdehyde and thus proving the structure of the anhydroheptose.

As a demonstration of our statement that the expected trihydric alcohol VIa from these 1,6-anhydroheptopyranoses would be optically inactive, we have written it also in the form VIb, in which it is clearly recognizable as a symmetrical acetal of *meso*-erythritol, and again in the form VIc, in which it appears as a symmetrically trisubstituted 1,3-dioxolane.[14] Hydrolysis of the acetal VIb should occur readily and with the production of erythritol; actually, in the hydrogenation of our dialdehyde obtained from the anhydro-D-*glycero*-D-*gulo*-heptose some hydrolysis appears to have occurred spontaneously (perhaps because of a slight acidity at the 100° temperature), for on benzoylating the product we obtained not only the tribenzoate of the trihydric alcohol (VI) but also the known tetrabenzoate of erythritol. Had our anhydrides possessed the already disproved 1,2:1,6 system of rings, we should have obtained not erythritol but glycerol on hydrolysis.

The reaction of our 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose (II, VII) with excess *p*-toluenesulfonyl chloride in pyridine yielded about 20% of a crystalline tritosyl compound and about 70% of a sirupy tetratosyl derivative; the latter was converted by standard reactions to crystalline iodo and deoxy derivatives, as described in the Experimental part. The tritosyl derivative, however, was of especial interest, for it recalled the earlier experiences in this Laboratory[13] with the epimeric 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose (III) which, with excess *p*-toluenesulfonyl chloride, had furnished a 76% yield of a crystalline tritosyl derivative. At that time it was suggested that a tosyl group entering first on the primary alcohol group at C_7 effectively hindered subsequent tosylation at C_4 even though acetylation at C_4 could still be accomplished. While other and more important factors, including the conformation of the pyranoid ring, may play a part in directing the course of this reaction, the net result appears to be the same. By analogy we have assumed that similar, though perhaps not such strong, steric influences are involved here (for we were able to obtain a large proportion of the product as the tetratosyl derivative) and that our new tritosyl derivative has its groups also in the 2-, 3- and 7-positions. By the same sequence of reactions that were employed before, namely, acetylation at C_4, replacement of the tosyloxy group at C_7 by an iodine atom, and deacetylation, we have converted this tritosyl derivative into a new dianhydro derivative that is undoubtedly 1,6:4,7-dianhydro-2,3-di-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose (VIII). Elimination of sodium *p*-toluenesulfonate from this last-named compound by reaction with sodium methoxide in chloroform appeared to be successful,

(14) It is possible to write an alternative *meso* formula for this dioxolane by reversing the positions of the H and CH_2OH on C_2, but only the compound pictured could be derived from an anhydroheptose whose rings are fixed in the positions shown by the Haworth-type projection formula VII.

but characterization of the expected 1,6:2,3:4,7-trianhydroheptopyranose failed because of the scarcity of material at that point.

Experimental

Isolation of Cadmium D-*glycero*-D-*ido*-Heptonate Cadmium Bromide from Mother Liquors of D-*glycero*-D-*gulo*-Heptonolactone.—The mother liquors that remained from earlier preparations of D-*glycero*-D-*gulo*-heptonolactone in this Laboratory had been obtained by a cyanohydrin synthesis from D-glucose either using sodium cyanide and calcium chloride as described by Hudson, Hartley and Purves[15] or by a subsequent modification using sodium cyanide alone.[16] From these preparations much of the D-*glycero*-D-*gulo*-heptonolactone had been separated in crystalline form but further fractionation to isolate the crystalline D-*glycero*-D-*ido*-heptonolactone was discouragingly slow and tedious. Separation of the epimeric acids through their brucine salts, as had been effected by earlier workers, was not considered to be satisfactory in view of the similar properties of those salts[17] and consequent losses during their fractionation. Accordingly, we decided to take advantage of the knowledge that cadmium salts of acids with xylonic and idonic configurations form readily crystalline double salts with cadmium bromide and that cadmium D-*glycero*-D-*ido*-heptonate cadmium bromide had already been prepared by Miss Olive Hartley and described in an earlier publication from this Laboratory.[18]

The available mother liquors were first freed from metallic ions, if any were present, by passage through a column of Amberlite IR-120 ion-exchange resin. A small aliquot of the combined solutions and washings was titrated with sodium hydroxide (to a permanent pink color with phenolphthalein) to obtain an estimate of the amount of heptonic acids and lactones present. Then a portion of the main solution was converted to mixed cadmium salts by boiling for 15 minutes with a slight excess of cadmium carbonate. Undissolved solid was removed by filtration, and to the filtrate was added aqueous cadmium bromide in slight excess over that required to form the double salt on the assumption that the heptonic acids and lactones consisted entirely of the D-*glycero*-D-*ido* epimer. The resulting solution was concentrated *in vacuo* to dryness and the solid residue was recrystallized by dissolving it in 15 parts of boiling water and adding an equal volume of hot 95% ethanol. The amount of double salt thus obtained enabled us to calculate the amount of cadmium bromide it contained, and in subsequent preparations it was necessary to add only 10% more than this theoretical quantity of cadmium bromide to obtain satisfactory yields of product. The cadmium double salt, recrystallized twice from aqueous alcohol and dried in the air at room temperature, appeared to be anhydrous rather than a monohydrate as reported earlier.[18]

Anal. Calcd. for $C_{14}H_{26}CdO_{16}\cdot CdBr_2$: C, 20.14; H, 3.14; Cd, 26.92. Calcd. for $C_{14}H_{26}CdO_{16}\cdot CdBr_2\cdot H_2O$: C, 19.71; H, 3.31; Cd, 26.36. Found: C, 20.14; H, 3.06; Cd, 27.09.

Transformation of the Double Salt to D-*glycero*-D-*ido*-Heptono-1,4-lactone.—Each 50-g. portion of once-recrystallized cadmium D-*glycero*-D-*ido*-heptonate cadmium bromide was dissolved in 1 liter of water by heating the mixture nearly to boiling; 25 g. of powdered silver carbonate was added and the hot mixture was shaken or stirred vigorously for about 30 minutes to complete the reaction. Solids were removed by filtration through a buchner funnel precoated with a layer of Filter-Cel and the remaining cadmium and silver ions were precipitated with hydrogen sulfide. At this point a smoothly rubbed suspension of 3 g. of bentonite in a small amount of water was added to facilitate the coagulation of colloidal sulfides and the mixture was filtered through a buchner funnel precoated with a mixture of Filter-Cel and activated carbon. The colorless solution was concentrated *in vacuo* to a thin sirup that was transferred to an evaporating dish and final concentration effected on the steam-bath by a current of air. Lactonization was promoted by further heating, usually overnight, and by stirring the crystallizing mass occasionally with portions of ethanol. The crude lactone thus obtained was recrystallized by dissolving it in 2.5 parts of boiling methyl cellosolve, adding a small amount of activated carbon, filtering, and allowing the crystals to separate at room temperature overnight. The product was filtered and washed with ethanol. Although only about half of the lactone was recovered in this recrystallization, the procedure (avoiding chilling) furnished a readily filterable product, and by subsequent concentration of the mother liquors the total yield of crystalline lactone became practically quantitative.

Our recrystallized lactone (from absolute ethanol) melted at 151–152° in agreement with Fischer[4] and with Deulofeu and Deferrari,[19] rather than with the 161–162° value reported by Philippe.[5] In aqueous solution (*c* 2) our lactone showed $[\alpha]^{20}D$ −76.8° (2.5 minutes) changing to −67.2° (1.5 hours; constant 24 hours). Fischer reported $[\alpha]^{20}D$ −79.2° (about 20 minutes) changing to −67.7° (24 hours, constant; *c* 10). Philippe recorded initial values of $[\alpha]D$ −82.1° (*c* 10) and −78.0° after three minutes, for two different specimens, with identical values of −68.2° being reached after six hours; an additional rotation of −67.9° after 24 hours was reported for the first specimen.

Reduction of D-*glycero*-D-*ido*-Heptono-1,4-lactone and Preparation of D-*glycero*-D-*ido*-Heptose Dibenzyl Mercaptal.—Each 50-g. portion of lactone was reduced with 1800 g. of 2.5% sodium amalgam in the usual manner. After concentration of the neutralized solution, most of the sodium sulfate was precipitated by the addition of 85% ethanol and the remainder of the ionizable material was removed by passage through columns of Amberlite IR-120 and Duolite A-4 ion-exchange resins. Because concentration of the aqueous solutions thus prepared yielded sugar sirups that often contained considerable amounts of D-*glycero*-D-*ido*-heptitol,[20] purification of the sugar was effected through its dibenzyl mercaptal. In a typical run, 22 g. of an impure sirup was dissolved in 44 ml. of concentrated hydrochloric acid, 22 ml. of benzyl mercaptan was added, and the mixture was shaken at 20° for 20 hours during which period the product crystallized spontaneously. Two recrystallizations from 20 parts of hot ethanol furnished 15 g. of D-*glycero*-D-*ido*-heptose dibenzyl mercaptal as shiny white hexagonal platelets with m.p. 130–131° and $[\alpha]^{20}D$ +71.4° in pyridine (*c* 2.6).

Anal. Calcd. for $C_{21}H_{28}O_6S_2$: C, 57.25; H, 6.41; S, 14.55. Found: C, 57.41; H, 6.45; S, 14.84.

D-*glycero*-D-*ido*-Heptose (I).—Regeneration of the sugar from its dibenzyl mercaptal was achieved by stirring 41.5 g. of the latter with 52.2 g. (2.04 molecular equivalents) of mercuric chloride and 93 g. of washed cadmium carbonate in 1500 ml. of water for three hours at 55°. The solution was filtered and the chloride ions were removed by shaking with silver carbonate. An attempt to precipitate the metallic ions with hydrogen sulfide resulted in a colloidal solution that was clarified eventually through the addition of about 20 g. of zinc oxide, 80 ml. of glacial acetic acid and 20 g. of bentonite. The filtered solution was then deionized completely by passage through columns of Amberlite IR-120 and Duolite A-4; it seems probable, however, that deionization in the first place would have been a much better procedure. The solution was concentrated *in vacuo* to a thick

(15) C. S. Hudson, O. Hartley and C. B. Purves, THIS JOURNAL, **56**, 1248 (1934).

(16) C. S. Hudson, *ibid.*, **73**, 4498 (1951).

(17) *Cf.* R. M. Hann and C. S. Hudson, *ibid.*, **56**, 1390 (1934).

(18) Reference 13, page 1852, footnote 17.

(19) V. Deulofeu and J. O. Deferrari, *J. Org. Chem.*, **17**, 1087 (1952).

(20) Recent experiments have shown that we can probably eliminate most of the heptitol production by carrying out the sodium amalgam reduction in a solution buffered with oxalate as suggested by Isbell [H. S. Isbell, U. S. Patent 2,632,005, March 17, 1953; see also H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952)]. Our results indicated that *p*H 3 is about the optimum for the reduction of D-*glycero*-D-*ido*-heptono-1,4-lactone. This degree of acidity was established by adding 25.5 g. of oxalic acid dihydrate and 50 g. of sodium oxalate to 1 liter of water containing 10 g. of the lactone, and maintained by adding 6.85 g. of crystalline oxalic acid immediately before the addition of each successive 50-g. portion of 2.5% granular sodium amalgam. The results indicated that the amalgam is used more efficiently when it is added in portions rather than all at once. In one reduction, using this procedure and a total of 500 g. of amalgam, the production of heptose was virtually quantitative as estimated by the Hagedorn–Jensen–Hanes ferricyanide titration method, and the product, after elimination of salts by precipitation and deionization in the usual manner, crystallized spontaneously and extensively with no evidence of the presence of the interfering heptitol.

sirup that was redissolved in 50 ml. of ethanol and placed in a desiccator. Overnight, crystallization began spontaneously and a total of 11.0 g. of the desired heptose separated slowly as slender prisms melting at 119–124°, a value that was unchanged by recrystallization from 12 parts of methanol; Isbell[7] reported m.p. 121°. The rotation of our D-*glycero*-D-*ido*-heptose in water (*c* 4) was $[\alpha]^{20}D$ −2.4°, −6.4° (minimum) and −0.17° after 2.1 minutes, 15 minutes and 24 hours (constant), respectively, in good agreement with Isbell's values of −1.4°, −2.7°, −5.93° (minimum) and −0.13° after 1.3 minutes, 3.7 minutes, 18 minutes and 24 hours, respectively.

1,6-Anhydro-D-*glycero*-β-D-*ido*-heptopyranose (II, VII).—A preliminary experiment showed that a solution of D-*glycero*-D-*ido*-heptose became more levorotatory when it was heated with dilute acid, and a constant $[\alpha]^{20}D$ value of −32°, calculated as an anhydroheptose, was reached in about 24 hours. Accordingly, 10.1 g. of that sugar in 325 ml. of 0.2 *N* hydrochloric acid was heated in a boiling water-bath for 34 hours. The brownish solution was decolorized with activated carbon, and to the cooled filtrate was added 25 g. of calcium carbonate and 2 ml. of bromine, dropwise, with shaking. After standing overnight, the solution was filtered, deionized and concentrated *in vacuo* to a thick sirup. When the sirup was dissolved in 35 ml. of hot methanol and then cooled, the product crystallized readily in clusters of small rods. The 2.1 g. of 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose thus obtained was recrystallized from 30 parts of hot methanol; it then melted at 193–199° and showed $[\alpha]^{20}D$ −74.4° in water (*c* 0.6). On the basis of this rotation and the constant value of −32° reached in the preliminary experiment, it appears that D-*glycero*-D-*ido*-heptose is transformed in dilute acid to an equilibrium mixture containing about 43% of the anhydroheptose.

Anal. Calcd. for $C_7H_{12}O_6$: C, 43.75; H, 6.30. Found: C, 43.65; H, 6.41.

Oxidation of 1,6-Anhydro-D-*glycero*-β-D-*ido*-heptopyranose with Sodium Metaperiodate.—To an aqueous solution of 0.1001 g. of the pure anhydroheptose was added 5 ml. of 0.475 *M* aqueous sodium metaperiodate, and the solution was diluted exactly to 25 ml. with water. In the course of ten days at 20° the $[\alpha]^{20}D$ value dropped to −59.0° (constant), calculated as the expected dialdehyde $C_6H_8O_5$ (V); this is in agreement with the rotation −58.7° obtained previously[13] in the oxidation of the epimeric 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose (III). Analysis of the solution showed the formation of 1.05 molar equivalents of formic acid and the consumption of 1.8 molar equivalents of oxidant. The test for formaldehyde with the dimedon reagent was negative.

Transformation of the Epimeric 1,6-Anhydro-D-*glycero*-β-D-*ido*- and 1,6-Anhydro-D-*glycero*-β-D-*gulo*-heptopyranoses to 2,4-*cis*-5-*cis*-Tri-(hydroxymethyl)-1,3-dioxolane (= 2,3-[2-hydroxyethylidene]-erythritol) (VIa, b, c).—A 3.6-g. sample of 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose was oxidized with excess sodium metaperiodate to the dialdehyde V; the solution was partially freed from iodate and periodate by precipitation with barium chloride and the filtrate was neutralized with aqueous sodium bicarbonate. Concentration of this solution *in vacuo* to a small volume, extraction of the residue with 70 ml. of methanol and filtration from the crystalline salts yielded a solution of the dialdehyde nearly free of inorganic material. The solution was diluted with an equal volume of water, 3 g. of Raney nickel added, and the mixture shaken under hydrogen at 3100 p.s.i. for 22 hours at 100°. The cooled and filtered solution was optically inactive and did not reduce Fehling solution. It was concentrated to a sirup that was dried by the alternate addition of benzene and evaporation of the solvent on the steam-bath in a stream of dry air. The resulting sirup was dissolved in 75 ml. of dry pyridine and 15 g. of freshly prepared *p*-nitrobenzoyl chloride added in four portions. After 22 hours at room temperature the mixture was poured over cracked ice, whereupon a partly crystalline mass of material separated. From this was obtained, after several recrystallizations from pyridine, 200 mg. of tiny, light-tan, prismatic crystals that were dried for analysis four hours at 100° and 5 mm. pressure. The product, **2,4-*cis*-5-*cis*-tri-*p*-nitrobenzoxy-1,3-dioxolane**, melted at 165–170°.

In similar fashion, 5.0 g. of 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose[13] was oxidized with excess periodate, the resulting dialdehyde was hydrogenated, and a portion of the product from the latter operation (corresponding to 1.2 g. of the original anhydroheptose) was treated with *p*-nitrobenzoyl chloride and pyridine. There was thus obtained, after recrystallization from pyridine and subsequent drying at 100° *in vacuo*, 0.7 g. of 2,4-*cis*-5-*cis*-tri-*p*-nitrobenzoxy-1,3-dioxolane as tiny, light-tan, prismatic crystals melting at 168–170°. A mixture of this substance with the tri-*p*-nitrobenzoate derived from the epimeric anhydroheptose showed no depression in melting point.

Anal. Calcd. for $C_{27}H_{21}N_3O_{14}$: C, 53.03; H, 3.46; N, 6.87. Found: (from D-*glycero*-D-*ido* compound) C, 53.21; H, 3.62; (from D-*glycero*-D-*gulo* compound) C, 53.14; H, 3.60; N, 6.88.

The remainder of the oxidized and subsequently hydrogenated material, corresponding to 3.8 g. of the original 1,6-anhydro-D-*glycero*-β-D-*gulo*-heptopyranose, was treated with benzoyl chloride and pyridine in the usual manner. The product was found to be a mixture that was separated readily by fractional crystallization from benzene by the addition of *n*-hexane. The less-soluble compound was identified as erythritol tetrabenzoate through its m.p. 189–190° and mixed m.p. with an authentic specimen. The more-soluble compound separated as clusters of clear, prismatic needles melting at 126–127°; analysis indicated it to be **2,4-*cis*-5-*cis*-tribenzoxy-1,3-dioxolane**.

Anal. Calcd. for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 68.24; H, 5.12.

1,6-Anhydro-2,3,7-tri-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—A solution of 2.94 g. of the anhydro-D-*glycero*-D-*ido*-heptose and 18 g. of *p*-toluenesulfonyl chloride in 25 ml. of dry pyridine was allowed to stand for one week at room temperature. The mixture was decomposed with ice-water, the product extracted with chloroform, and that solution washed, dried and concentrated in the usual manner. The residual sirup was dissolved in a small amount of chloroform and diluted with pentane to a point just short of cloudiness. Crystallization yielded 1.9 g. (19%) of the tritosyl derivative as clusters of short rods; the rest of the material was found to contain the tetratosyl derivative, and will be described below. The tritosyl derivative upon recrystallization from 20 parts of hot methanol formed long, silky fibers that melted at 147–149° dec. and showed $[\alpha]^{20}D$ −51.4° in chloroform (*c* 2).

Anal. Calcd. for $C_{28}H_{30}O_{12}S_3$: C, 51.36; H, 4.62; S, 14.69. Found: C, 51.28; H, 4.58; S, 14.68.

4-*O*-Acetyl-1,6-anhydro-2,3,7-tri-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—To 0.28 g. of the tritosylanhydroheptose dissolved in 10 ml. of dry pyridine was added 5 ml. of acetic anhydride. After 24 hours at room temperature the mixture was poured over ice, the product extracted with chloroform and the chloroform extract washed, dried and concentrated. The addition of pentane produced 0.27 g. of very small needles. Recrystallized from 250 parts of hot methanol, the substance formed long, silky fibers melting at 151–152° and showing $[\alpha]^{20}D$ −10.9° in chloroform (*c* 0.8).

Anal. Calcd. for $C_{30}H_{32}O_{13}S_3$: S, 13.80. Found: S, 13.48.

4-*O*-Acetyl-1,6-anhydro-7-deoxy-7-iodo-2,3-di-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—A solution of 115 mg. of the tritosylacetylanhydroheptose and 200 mg. of sodium iodide in 20 ml. of acetone was heated in a sealed tube for nine hours at 100°. After filtration, the dropwise addition of water to the acetone solution produced the spontaneous crystallization of small rods to the extent of 108 mg. The m.p. 206–207° was unchanged by recrystallization from aqueous acetone; the $[\alpha]^{20}D$ value was −95.1° in chloroform (*c* 0.6).

Anal. Calcd. for $C_{23}H_{25}IO_{10}S_2$: C, 42.34; H, 3.86; I, 19.45; S, 9.83; CH_3CO, 6.60. Found: C, 42.45; H, 3.97; I, 19.49; S, 9.91; CH_3CO, 6.57.

4-*O*-Acetyl-1,6-anhydro-7-deoxy-2,3-di-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—A suspension of 159 mg. of the iodo compound just described and 1 g. of Raney nickel in 100 ml. of methanol containing 0.1 ml. of diethylamine was shaken for two hours under a slight positive pressure of hydrogen. The compound dissolved during the course of the hydrogenation. The solution was filtered and evaporated to dryness in a current of air, needle-like crystals appearing spontaneously during the concentration. The product was

washed with water and dried; weight 117 mg. Upon recrystallization from 10 ml. of warm methanol it separated as squat prisms that melted at 124–126° and showed $[\alpha]^{20}D$ −50.0° in chloroform (*c* 1).

Anal. Calcd. for $C_{23}H_{26}O_{10}S_2$: C, 52.46; H, 4.98; CH_3 (to C), 5.71. Found: C, 52.27; H, 5.01; CH_3 (to C), 5.76.

1,6:4,7-Dianhydro-2,3-di-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose (VIII).—To a cold solution of 588 mg. of 4-*O*-acetyl-1,6-anhydro-7-deoxy-7-iodo-2,3-di-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose in 170 ml. of a mixture of chloroform and methanol (7:10) was added 5 ml. of a 3% sodium methoxide solution. The solution was kept at 10° for two days, then carbon dioxide was bubbled through it to decompose any remaining sodium methoxide, and the solvents were volatilized at room temperature by a current of air. The residual solid was washed with water, filtered and dried; weight 430 mg. The dianhydro compound thus isolated was recrystallized from 30 parts of methanol from which it separated as stout needles with m.p. 94–95° and $[\alpha]^{20}D$ −8.5° in chloroform (*c* 1).

Anal. Calcd. for $C_{21}H_{22}O_9S_2$: C, 52.27; H, 4.60. Found: C, 52.32; H, 4.76.

1,6-Anhydro-7-deoxy-7-iodo-2,3,4-tri-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—The tosylation of 1,6-anhydro-D-*glycero*-β-D-*ido*-heptopyranose, as described above, yielded 19% of crystalline tritosyl derivative. The mother liquor was concentrated to 8.4 g. of a sirup that was presumed to consist principally of the expected tetratosyl derivative. Accordingly, 6.4 g. of the dried sirup and 8.5 g. of sodium iodide in 100 ml. of acetone were heated in a sealed ampoule for 15 hours at 100°. The solution was filtered to remove 1.46 g. (95%) of sodium *p*-toluenesulfonate and diluted with water to precipitate the product as an oil. The oil was separated and washed with water by decantation. When allowed to stand under methanol overnight it crystallized, yielding 3.2 g. of solid material. The iodo compound was recrystallized from 80 parts of methanol, forming feathery needles melting at 141–142° and showing $[\alpha]^{20}D$ −32.0° in chloroform (*c* 0.9).

Anal. Calcd. for $C_{28}H_{29}IO_{11}S_3$: C, 43.98; H, 3.82; I, 16.60; S, 12.58. Found: C, 43.88; H, 3.77; I, 16.48; S, 12.53.

1,6-Anhydro-7-deoxy-2,3,4-tri-*O*-*p*-tolylsulfonyl-D-*glycero*-β-D-*ido*-heptopyranose.—A 1-g. sample of the preceding iodo compound in 150 ml. of methanol containing 0.3 ml. of diethylamine was shaken with 1 g. of Raney nickel for two hours at room temperature under a slight positive pressure of hydrogen. The absorption of hydrogen appeared to stop at the end of the first half hour. The solution was filtered to remove the catalyst and the solvent was evaporated in a stream of air. The product was induced to crystallize from methanol, separating as clusters of needles that melted at 130–131° and showed $[\alpha]^{20}D$ −37.0° in chloroform (*c* 0.6). The yield was 0.3 g.

Anal. Calcd. for $C_{24}H_{30}O_{11}S_3$: C, 52.65; H, 4.73; CH_3 (to C), 2.35. Found: C, 52.74; H, 4.90; CH_3 (to C), 2.49.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA]

On the Structure of Galactinol

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Galactinol, an α-D-galactoside of myoinositol, has been shown by methylation studies to be D-1-O-α-D-galactopyranosyl myoinositol.

Myoinositol (also called *meso*-inositol, see ref. 7) occurs widely in nature in both a free and bound form.[2] The latter form, which may represent as much as 90% of the total, has a varied composition, although the phosphate esters are certainly a major component.[3] The bound form may be freed completely by strong acid hydrolysis, and partly by the action of phosphatases and glycosidases. Structural studies on inositol containing phosphatides indicate that the myoinositol is chemically fixed in these substances through glycosidic bonds, phosphate ester links, and possibly esterification with carboxylic acids.[4,5]

Brown and Serro[6] have recently described a glycoside of myoinositol which they found to occur free in the juice of the sugar beet, and which they isolated in a pure crystalline form. From hydrolysis studies and characterization of the hydrolytic products, Brown and Serro[6] concluded that the substance is an α-D-galactoside of myoinositol. It is quite possible that this substance represents one of the building units present in the phosphoinositides; in fact, Woolley has isolated from soya bean phosphatide by partial hydrolysis a substance which was characterized as a galactoside of myoinositol.[4a] For this reason it is of immediate interest that the complete structure of the compound should be elucidated, particularly with respect to the point of attachment of the galactosidic linkage to the myoinositol. With receipt of a generous sample of galactinol kindly supplied by Dr. Brown, we attacked this problem. The results of this investigation are reported herein.

Galactinol, by exhaustive methylation, has been converted to the crystalline nonamethyl ether. Following hydrolysis of the methylated galactinol two products were isolated; 2,3,4,6-tetramethyl-D-galactose as the anilide, and a crystalline pentamethyl ether of myoinositol. Isolation of 2,3,4,6-

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(2) J. Scherer, *Ann.*, **73**, 322 (1850); E. V. Eastcott, *J. Phys. Chem.*, **32**, 1094 (1928); R. J. Anderson and E. G. Roberts, *J. Biol. Chem.*, **89**, 599 (1930); D. W. Woolley, *ibid.*, **139**, 29 (1941).

(3) E. Bartow and W. W. Walker, *Ind. Eng. Chem.*, **30**, 300 (1938); P. Fleury and P. Balatre, "Les Inositols," Masson et Cie., Paris, 1947.

(4) (a) D. W. Woolley, *J. Biol. Chem.*, **147**, 581 (1943); (b) J. Folch, *ibid.*, **146**, 35 (1942); (c) J. Folch and D. W. Woolley, *ibid.*, **142**, 963 (1942); (d) J. Folch, *Federation Proc.*, **6**, 252 (1947).

(5) H. Wittcoff, "The Phosphatides," Reinhold Publ. Corp., New York, N. Y., 1951, p. 35.

(6) R. J. Brown and R. F. Serro, THIS JOURNAL, **75**, 1040 (1953).