0.09 *M* magnesium sulfate concentration. The 23% of the total yolk protein that precipitates contains 80% of the phosphoprotein phosphorus. Phosvitin is then extracted from the precipitate with 0.4 *M* ammonium sulfate at *p*H 4; ethyl ether is added to coagulate non-phosvitin components. A typical preparation contained 11.9% nitrogen, 9.7% phosphorus (molar ratio N/P = 2.72). The phosphorus is present as mono-esterified orthophosphate.

Amino acid analyses indicated (in equivalents per $10^4$ g.): total β-hydroxy-α-amino acids, 33, and serine, 28 (not corrected for destruction during hydrolysis), compared to 31 moles of phosphorus. There is approximately one phosphoserine residue for each two amino acid residues. Basic amino acids account for nearly one-third of the remaining amino acids.

Osmotic pressure measurements indicated a molecular weight of about 21,000. The best preparations were homogeneous in the ultracentrifuge but showed appreciable boundary spreading on electrophoresis. Inhomogeneity was also indicated by the presence of small amounts (less than 1 g. equivalent per 21,000 g.) of tyrosine, methionine and tryptophan.

A pronounced aggregating effect of magnesium ions was shown by both osmotic pressure and ultracentrifuge measurements; in 0.05 *M* magnesium chloride, the molecular weight found was 38,000 and 39,000, respectively, by the two methods.

Phosvitin was dephosphorylated by citrus phosphatases but not by a bone phosphatase preparation. Alkaline dephosphorylation caused an increase in ultraviolet absorption indicative of the formation of dehydroalanyl residues. Trypsin and pepsin acted more slowly on phosvitin than on casein.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY AND CHEMOTHERAPY, EXPERIMENTAL BIOLOGY AND MEDICINE INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

# 1,5-Anhydrolactitol and 1,5-Anhydromaltitol

BY HEWITT G. FLETCHER, JR., LEONORE H. KOEHLER[1] AND C. S. HUDSON

A previous publication[2] from this Laboratory described the synthesis of 1,5-anhydro-4-(β-D-glucopyranosyl)-D-glucitol (synonym, 1,5-anhydrocellobiitol) and 1,5-anhydro-6-(β-D-glucopyranosyl)-D-glucitol (synonym, 1,5-anhydrogentiobiitol) through the reductive desulfurization of the appropriate aryl 1-thioglycosides with Raney nickel. The present communication describes the extension of this synthetic method to the lactose and maltose series which was undertaken in order to obtain data for certain generalizations regarding the relationships between rotatory power and configuration among the various 1,5-anhydroglycitols and certain related compounds.[3]

Several well-characterized 1-thioglycosides suitable for the present purpose have been reported in the literature for the lactose and maltose series. Purves described phenyl 1-thio-β-lactopyranoside and its heptaacetate[4] as well as phenyl 1-thio-β-maltopyranoside heptaacetate (III).[5] Haskins, Hann and Hudson[6] prepared by the procedure of Purves, among other 1-thioglycosides, 2′-naphthyl 1-thio-β-lactopyranoside (I) and its heptaacetate as well as 2′-naphthyl 1-thio-β-maltopyranoside heptaacetate.

The reductive desulfurization by Raney nickel of 2′-naphthyl 1-thio-β-lactopyranoside (I) has now been carried out to give crystalline 1,5-anhydro-4-(β-D-galactopyranosyl)-D-glucitol (II), which may be assigned the alternative name of 1,5-anhydrolactitol. The specific rotation of this substance in water, $[\alpha]^{20}D$, proved to be +49.4°. Comparison of the molecular rotations of β-cellobiopyranose, β-lactopyranose and 1,5-anhydrocellobiitol listed in Table I shows that 1,5-anhydrolactitol would be expected to be dextrorotatory. Calculation based upon the isorotation hypothesis affords a numerical value of (7040 + 9550) ÷ 326 = +50.9°.

TABLE I

COMPARISON OF SOME MOLECULAR ROTATIONS IN THE CELLOBIOSE, LACTOSE AND MALTOSE SERIES

| | Mol. wt. | $[\alpha]_D$ ($H_2O$) | $[M]_D$ | Difference |
|---|---|---|---|---|
| β-Lactopyranose | 342 | +34.9° | +11,900 | |
| | | | | + 7,040 |
| β-Cellobiopyranose | 342 | +14.2° | + 4,860 | |
| 1,5-Anhydrolactitol | 326 | +49.4° | +16,100 | |
| | | | | + 6,550 |
| 1,5-Anhydrocellobiitol | 326 | +29.3° | + 9,550 | |
| | | ($CHCl_3$) | | |
| Methyl β-maltopyranoside heptaacetate | 651 | +53.5° | +34,800 | |
| | | | | +51,500 |
| Methyl β-cellobiopyranoside heptaacetate | 651 | −25.7° | −16,700 | |
| 1,5-Anhydromaltitol heptaacetate | 621 | +82.0° | +50,900 | |
| | | | | +48,400 |
| 1,5-Anhydrocellobiitol heptaacetate | 621 | + 4.0° | + 2,500 | |

(1) Present address: Cedar Crest College, Allentown, Pa.

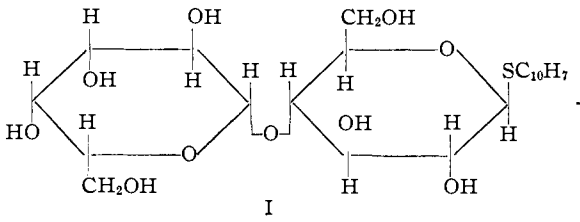
(2) H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **70**, 310 (1948).

(3) H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, **71**, 3682 (1949).

(4) C. B. Purves, *ibid.*, **51**, 3619 (1929).

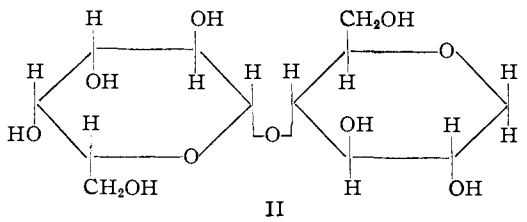
(5) C. B. Purves, *ibid.*, **51**, 3631 (1929).

(6) W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **69**, 1668 (1947).



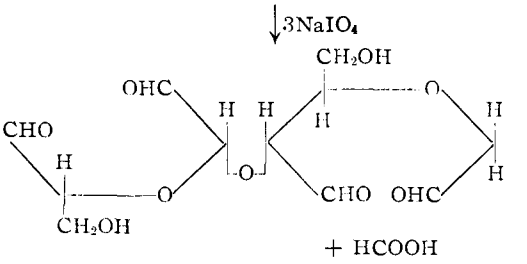
I

2′-Naphthyl 1-thio-β-lactopyranoside

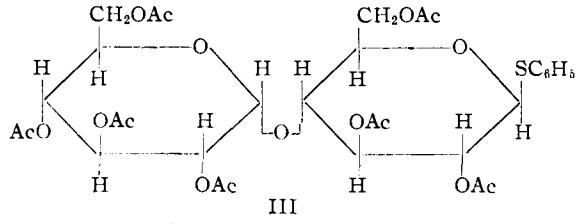


II

1,5-Anhydro-4-(β-D-galactopyranosyl)-D-glucitol

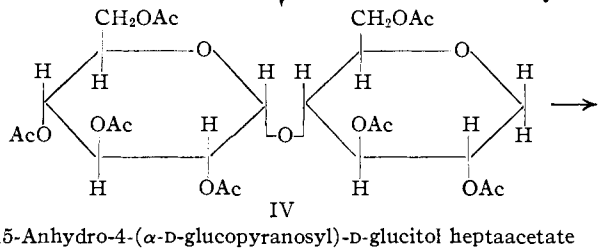


+ HCOOH



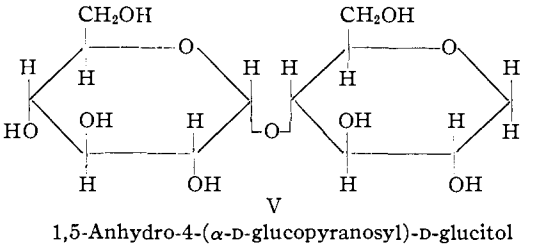
III

Phenyl 1-thio-β-maltopyranoside heptaacetate



IV

1,5-Anhydro-4-(α-D-glucopyranosyl)-D-glucitol heptaacetate



V

1,5-Anhydro-4-(α-D-glucopyranosyl)-D-glucitol

Repeated attempts to prepare the heptaacetate of 1,5-anhydrolactitol in crystalline condition through the reductive desulfurization of both phenyl 1-thio-β-lactopyranoside heptaacetate and 2′-naphthyl 1-thio-β-lactopyranoside heptaacetate as well as through the direct acetylation of the anhydride itself gave only amorphous products.

The structure of the 1,5-anhydro-4-(β-D-galactopyranosyl)-D-glucitol (II) was confirmed by oxidation with periodate. As was to be expected, the substance consumed on a molar basis three moles of periodate with the concomitant formation of one mole of formic acid. As a further confirmation of its structure, the anhydride was hydrolyzed with aqueous acid and the products identified as 1,5-anhydro-D-glucitol (polygalitol) and D-galactose, the latter isolated as its α-methyl-α-phenylhydrazone.

Phenyl 1-thio-β-maltopyranoside heptaacetate (III) was reductively desulfurized with Raney nickel to yield crystalline 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol heptaacetate (IV) (1,5-anhydromaltitol heptaacetate) rotating +82° in chloroform. A calculation similar to that shown above for 1,5-anhydrolactitol but based upon the molecular rotations of methyl β-cellobiopyranoside heptaacetate, methyl β-maltopyranoside heptaacetate and 1,5-anhydrocellobiitol heptaacetate (Table I) leads to the prediction of a value of +87° for 1,5-anhydromaltitol heptaacetate through the isorotation hypothesis.

Catalytic deacetylation of 1,5-anhydromaltitol heptaacetate afforded the free 1,5-anhydromaltitol (V), (synonym, 1,5-anhydro-4-[α-D-glucopyranosyl]-D-glucitol), which could not be induced to crystallize. Confirmation of its structure as a derivative of polygalitol was obtained through acid hydrolysis to 1,5-anhydro-D-glucitol (*i. e.*, polygalitol) and D-glucose, identification of the latter being made through the phenylosazone and the particularly convenient D-glucose phenylosotriazole.

**Acknowledgment.**—We are indebted to Mrs. Margaret M. Ledyard for microanalyses and to Dr. W. T. Haskins for samples of 2′-naphthyl 1-thioglycosides. One of us (L. H. K.) held a Fellowship from the Corn Industries Research Foundation while engaged in this research.

## Experimental[7]

**1,5-Anhydro-4-(β-D-galactopyranosyl)-D-glucitol (II).**—Two grams of pure 2′-naphthyl 1-thio-β-lactopyranoside, prepared according to the method of Haskins, Hann and Hudson,[6] was dissolved in 200 ml. of 70% aqueous alcohol, treated with approximately 25 g. of freshly prepared Raney nickel and boiled gently under reflux for one hour. The supernatant solution was then removed, the nickel washed successively with three 100-ml. portions of boiling water and the combined solution and washings concentrated *in vacuo* at 55° (bath) to a volume of approximately 2 ml.

(7) Melting points were measured with a calibrated Anschütz type thermometer completely immersed in the bath liquid. Rotations are specific rotations for sodium light at 20°; concentration is expressed in g. of substance per 100 ml. of solution.

On scratching with a glass rod at room temperature, hard, clear prismatic crystals formed; these, combined with two additional crops obtained from the mother liquor by the addition of alcohol, amounted to 850 mg. (63%). Two recrystallizations from water brought the product to a rotation of +49.4° in water (*c*, 2.512) which was unchanged by further recrystallization. 1,5-Anhydro-4-(β-D-galactopyranosyl)-D-glucitol melts with decomposition, the melting point range varying with the rate of heating. When a sample in a capillary tube was plunged into a bath whose temperature of 226° was rising uniformly at the rate of 3° per minute, a melting point of 233–237° was obtained.

1,5-Anhydrolactitol is insoluble in methanol, dioxane, methyl cellosolve and methyl ethyl ketone, difficultly soluble in boiling glacial acetic acid and readily soluble in hot water.

*Anal.* Calcd. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.20; H, 6.82.

One gram of the anhydride was hydrolyzed by boiling for two hours in 2.5 ml. of 0.39 *N* sulfuric acid. After quantitative removal of the acid and reconcentration to a volume of 3 ml. the hydrolyzate was treated with 0.4 g. of α-methyl-α-phenylhydrazine. After three hours at room temperature 100 mg. of crystalline material was removed by filtration. Recrystallization from 30% ethanol gave colorless leaflets melting at 191–192° either alone or in admixture with an authentic sample of the α-methyl-α-phenylhydrazone of D-galactose. The remainder of the hydrolyzate was heated for several hours on the steam-bath, filtered through decolorizing carbon, and concentrated to a colorless sirup which in methanol solution gave a deposit of 200 mg. (40%) of 1,5-anhydro-D-glucitol melting at 142°. Mixed with authentic polygalitol the material melted at 142–143°. Thus the synthetic 1,5-anhydro-4-(β-D-galactopyranosyl)-D-glucitol was shown to be a derivative of polygalitol.

**Sodium Metaperiodate Oxidation of 1,5-Anhydro-4-(β-D-galactopyranosyl)-D-glucitol.**—The anhydride (98.1 mg.) was oxidized in aqueous solution with sodium metaperiodate, using the technique of Jackson and Hudson.[8] After twenty-six hours at room temperature, analysis showed the consumption on a molar basis of 2.98 moles of oxidant and the formation of 1.02 moles of formic acid.

**1,5-Anhydro-4-(α-D-glucopyranosyl)-D-glucitol Heptaacetate, IV.**—Ten grams of phenyl 1-thio-β-maltoside heptaacetate, prepared according to the method of Purves,[5] melting at 87–92° and showing a rotation of +48.7° in chloroform was suspended in 100 ml. of absolute alcohol and treated with approximately 40 g. of freshly prepared Raney nickel. The suspension was boiled for one hour and the supernatant liquor then decanted while still hot, the nickel being washed repeatedly, first with hot absolute alcohol and then with boiling acetone. Concentration of the combined decantate and washings *in vacuo* at 60–70° (bath) led to the spontaneous formation of fine, needle-like crystals amounting to 6.66 g. (78%) and melting at 127–128°. Recrystallized once from a mixture of 1.8 parts acetone and 2.3 parts of ether and then thrice from ten parts of alcohol, the substance melted at 133–134° and rotated +82.0° in chloroform (*c*, 3.4). 1,5-Anhydro-4-(α-D-glucopyranosyl)-D-glucitol heptaacetate is insoluble in water and pentane, sparingly soluble in ether and cold alcohol and readily soluble in acetone and in hot alcohol.

*Anal.* Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.35; H, 6.03.

(8) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

**1,5-Anhydro-4-(α-D-glucopyranosyl)-D-glucitol, V.**—The catalytic deacetylation of 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol heptaacetate with barium methylate in the conventional manner gave a sirup which could not be induced to crystallize. On reacetylation, a sample of the amorphous material gave the original heptaacetate identified by melting point and mixed melting point, demonstrating that the catalytic deacetylation had produced no unforeseen change in the anhydride.

A portion (1.2112 g.) of the amorphous 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol which had been dried *in vacuo* at 45° was hydrolyzed by boiling in 3.0 ml. of 0.39 *N* sulfuric acid for two hours. One-half of the resulting solution was treated with phenylhydrazine in the usual manner to yield 60 mg. of D-glucose phenylosazone, identified by its melting point and by its inability to depress the melting point of authentic D-glucose phenylosazone. By way of confirmation the D-glucose phenylosazone was converted to D-glucose phenylosotriazole by the procedure of Hann and Hudson.[9] The 4.6 mg. of D-glucose phenylosotriazole thus obtained melted at 196–197° either alone or in admixture with authentic D-glucose phenylosotriazole. The second half of the hydrolysis solution was freed of sulfuric acid and then subjected for two days to the action of baker's yeast to remove the D-glucose. After filtration through decolorizing carbon and removal of solvent there was obtained a colorless sirup which, when diluted with methanol, gave 100 mg. (33%) of clear prismatic crystals melting at 142–143°. Mixed with authentic polygalitol these showed the same melting point.

The specific rotation of 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol in water was obtained by quantitative deacetylation of its pure heptaacetate in the following manner. The heptaacetate (0.6919 g.) was dissolved in a mixture of 2 ml. of chloroform and 10 ml. of methanol and treated with 0.3 ml. of approximately 1.3 *N* sodium methylate solution. After two days at room temperature the solvent was removed with a gentle stream of filtered air and the residual colorless sirup diluted with water to 25.00 ml. From the rotation of this solution a specific rotation of +132° (*c*, 1.455) for 1,5-anhydromaltitol was obtained.

## Summary

1,5-Anhydro-4-(β-D-galactopyranosyl)-D-glucitol has been obtained through the reductive desulfurization with Raney nickel of 2′-naphthyl 1-thio-β-lactopyranoside. Its structure has been confirmed by periodate oxidation and by hydrolysis to 1,5-anhydro-D-glucitol (*i. e.*, polygalitol) and D-galactose.

Phenyl 1-thio-β-maltopyranoside heptaacetate has been reductively desulfurized with Raney nickel to give crystalline 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol heptaacetate. Catalytic deacetylation of this heptaacetate gave amorphous 1,5-anhydro-4-(α-D-glucopyranosyl)-D-glucitol the structure of which was confirmed by hydrolysis to 1,5-anhydro-D-glucitol and D-glucose.

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(9) R. M. Hann and C. S. Hudson, *ibid.*, **66**, 735 (1944).