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 $C_{28}H_{30}O_{11}S_3$: 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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(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.

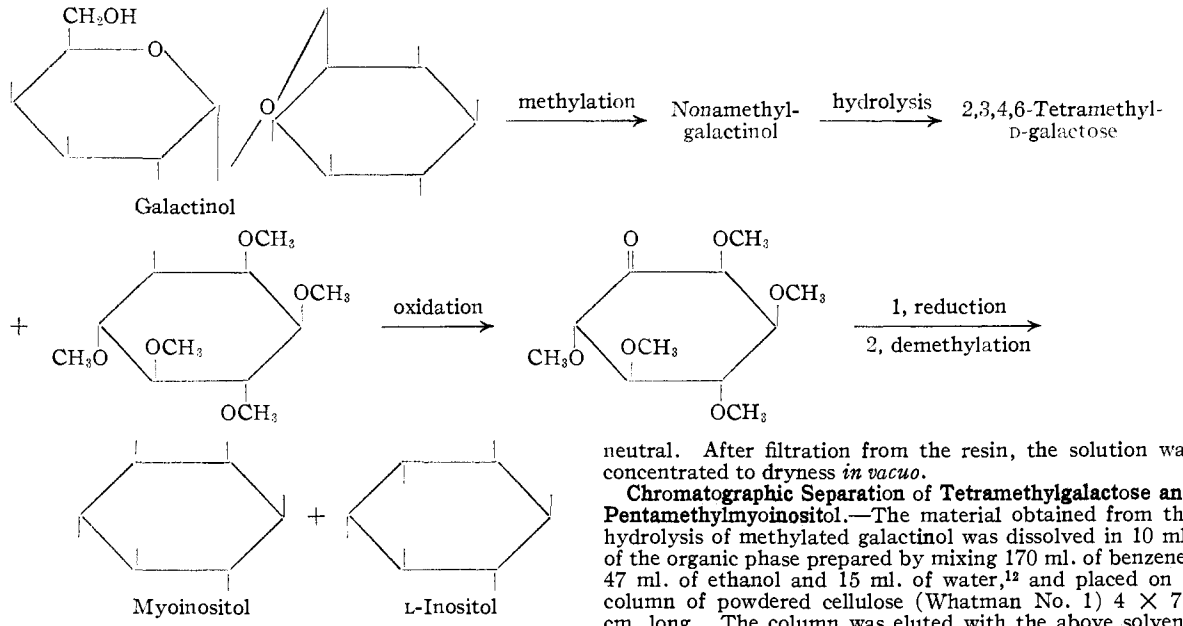
(4) (a) D. W. Woolley, *J. Biol. Chem.*, **147**, 581 (1943); (b) J. Folch, *ibid.*, **146**, 35 (1942); (c) J. Folch and D. W. Woolley, *ibid.*, **142**, 963 (1942); (d) J. Folch, *Federation Proc.*, **6**, 252 (1947).

(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).

tetramethyl-D-galactose confirms the galactopyranose ring form assigned to galactinol by Brown and Serro[6] on the basis of its susceptibility to hydrolysis by melibiase.

The pentamethylmyoinositol obtained has one free hydroxyl group corresponding to the position at which the galactosyl moiety was attached in the original galactinol. The position of this free hydroxyl group was determined by the conversion of the pentamethylmyoinositol to another inositol whose structure is related to myoinositol by inversion at this one position. This conversion was carried out by first oxidizing the free alcohol group in pentamethylmyoinositol to a ketone, then reducing the ketone back to two stereoisomeric alcohols. Demethylation of the reaction products gave a mixture which was separated into myoinositol and L-inositol. The formation of L-inositol (characterized by analysis, melting point and rotation of the free compound as well as of its hexabenzoate) established conclusively that the galactosyl linkage in galactinol is to position *one* of myoinositol. The following scheme illustrates the sequence of reactions carried out



The formation of L-inositol rather than DL-inositol shows that the myoinositol in galactinol is asymmetrically substituted, and that, according to Fletcher,[7] it possesses by virtue of this substitution the D-configuration. Thus, galactinol, the first example of a naturally occurring glycoside of an inositol whose structure has been completely elucidated, is D-1-O-α-D-galactopyranosylmyoinositol.

Experimental[8]

Methylation of Galactinol.—Four grams of galactinol dihydrate was methylated with dimethyl sulfate and 40% sodium hydroxide.[9] After one methylation only 0.3 g. of chloroform-extractable sirup was obtained, and it was necessary to concentrate the aqueous phase to dryness, extract with ethanol, combine the concentrated ethanol extract with the chloroform-extractable substance and remethylate. After three such treatments the product could be extracted from the methylation mixture with chloroform to give 3.86 g. of sirup (50.3% OCH_3). The sirup was then given two treatments with methyl iodide and silver oxide,[10] and upon reisolation yielded a sirup which crystallized from pentane in clusters of fine needles. A first crop of 1.77 g. melted at 82.5–89°. Repeated recrystallization from pentane failed to improve the methoxyl content or melting point. Further methylation of 1.7 g. was carried out with sodium and methyl iodide in ether solution,[11] and yielded on crystallization from pentane 1.5 g. of long needles with m.p. 96–97.5°. After recrystallization from pentane the m.p. was 96.5–98°, and the substance showed $[\alpha]D$ +119° (*c* 2, water).

Anal. Calcd. for $C_{12}H_{13}O_2(OCH_3)_9$ (468): C, 53.9; H, 8.6; OCH_3, 59.5. Found: C, 54.1; H, 8.6; OCH_3, 59.0.

On further methylation with methyl iodide and sodium in ether of the mother liquors from the original crystallization, an additional 0.9 g. of nonamethyl galactinol was obtained.

Hydrolysis of Methylated Galactinol.—Hydrolysis of 1.02 g. of nonamethylgalactinol was carried out under reflux in 50 ml. of 0.5 *N* hydrochloric acid to constant rotation (αD +1.94, 1 dm.) (12 hours). Portions of Amberlite IR-4B were then stirred into the hydrolysate until the solution was neutral. After filtration from the resin, the solution was concentrated to dryness *in vacuo*.

Chromatographic Separation of Tetramethylgalactose and Pentamethylmyoinositol.—The material obtained from the hydrolysis of methylated galactinol was dissolved in 10 ml. of the organic phase prepared by mixing 170 ml. of benzene, 47 ml. of ethanol and 15 ml. of water,[12] and placed on a column of powdered cellulose (Whatman No. 1) 4 × 75 cm. long. The column was eluted with the above solvent and the eluate was collected in 6-ml. fractions.[13] The tubes of eluate were concentrated on a steam-bath. Tubes 56–63 on evayoration gave a crystalline residue, tube 64 gave a sirup which crystallized overnight, tubes 65–75 yielded a sirup. Paper chromatographic examination and spraying with aniline oxalate showed that the sirupy material corresponded to a tetramethylhexose.[14] Small amounts were also present in tubes 62, 63 and in tubes 75–80. Tubes 64–80 were combined and rechromatographed; tubes 55–58 of the second run deposited a crystalline material, tube 59 crystallized after addition and evaporation of acetone, and tubes 60–72 contained the sirupy tetramethylhexose.

Pentamethylmyoinositol.—The material from tubes 53–61 of the first chromatographic separation was recrystallized from hot pentane to give 206 mg., m.p. 114–117°.

(7) H. G. Fletcher, L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(8) All melting points were taken in capillaries. Microanalyses by Microchemical Laboratory, University of California, and by Dr. A. Elek, Los Angeles, California.

(9) W. N. Haworth, E. L. Hirst and D. A. Ruell, *J. Chem. Soc.*, 3125 (1932).

(10) T. Purdie and J. C. Irvine, *ibid.*, **83**, 1021 (1903).

(11) K. Freudenberg and R. Hixon, *Ber.*, **56**, 2119 (1923).

(12) This solvent mixture was recommended by Dr. W. H. Wadman.

(13) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(14) The methylated myoinositol did not react with any spray which was tried.

It was then dissolved in ethanol, treated with Darco G-60, filtered, concentrated to dryness and recrystallized from hot pentane to give 194 mg. with m.p. 117–117.5° and $[\alpha]_D$ −4° (*c* 2.5, water).

Anal. Calcd. for $C_6H_7O(OCH_3)_5$ (250): C, 52.8; H, 8.8; OCH_3, 62.0. Found: C, 53.1; H, 8.7; OCH_3, 61.6.

Additional pentamethylmyoinositol was obtained by reworking the mother liquors. In subsequent preparations it was separated from the hydrolysis mixture by direct crystallization from pentane, although this procedure gave a rather poor yield.

2,3,4,6-Tetramethyl-D-galactose.—The sirup from tubes 60–72 of the second chromatographic separation showed $[\alpha]^{20}D$ +110° (*c* 2, water), which compares well with the value reported for 2,3,4,6-tetramethyl D-galactose, $[\alpha]_D$ +117° (water).[14a] This sirup (0.37 g.) was refluxed with 2 ml. of aniline in 20 ml. of ethanol for two hours. Clusters of long needles separated on cooling, and were collected and dried. The product weighed 0.28 g. and had m.p. 196°. When mixed with an authentic sample of 2,3,4,6-tetramethyl-D-galactose anilide, the m.p. showed no depression. The anilide gave $[\alpha]^{22}D$ −78° (*c* 1, acetone) and −141° (*c* 0.5, pyridine) as compared with the reported values −77° (acetone)[15] and −141° (pyridine).[16]

Conversion of Pentamethylmyoinositol to a Mixture of L-Inositol and Myoinositol.—Pentamethylmyoinositol (220 mg.) was dissolved in 15 ml. of glacial acetic acid and to this was added 47 ml. of glacial acetic acid containing 78 mg. of chromic anhydride (33% excess approx.). After standing 3.5 hours at room temperature, the solution was concentrated at reduced pressure and extracted ten times with 5-ml. portions of hot chloroform. The chloroform was removed *in vacuo* from the filtered solution and the residue was extracted with several 10-ml. portions of ethyl acetate, and this extract concentrated to give a blue-green sirup. The sirup was dissolved in 15 ml. of ethyl acetate and hydrogenated at room temperature and pressure, using 300 mg. of 5% platinum-on-charcoal catalyst. The catalyst was removed by filtration, and the solution concentrated *in vacuo* to a crystalline residue. To this was added 5 ml. of hydriodic acid (sp. gr. 1.7) and the solution was refluxed gently for 1.5 hours to effect demethylation, after which it was concentrated at reduced pressure to dryness. Twice water was added and removed *in vacuo*, thereby removing nearly all of the free iodine. The sirupy residue was dissolved in about 75 ml. of water and the solution was stirred one hour with Amberlite IR 100H and IR4B (about 5 g. of each). It was then filtered and the solvent removed *in vacuo*, to give a mixture of myoinositol and L-inositol, in approximately equal amounts. When the reduction was carried out using sodium borohydride in place of catalytic hydrogenation, the product consisted almost entirely of myoinositol, with very little L-inositol, as evidenced by paper chromatography.

Chromatographic Separation of Inositol Mixture.—The above mixture of inositols was dissolved in 3 ml. of water, and enough powdered cellulose was added to make a thick paste. To the paste was added 10 ml. of acetone and additional cellulose to give a thin slurry.[17]

This slurry was placed at the top of a prepared cellulose column and eluted with a mixture of acetone and water (80:20 v./v.).[18] The eluate, collected in 6-ml. fractions, was analyzed by papergrams. The first component appeared in tubes 200–290, and the second (corresponding to myoinositol) in tubes 340–440.

Characterization of L-Inositol.—The combined eluate from tubes 200–290 was concentrated to dryness, and the residue was washed by decantation with 10 ml. of absolute ethanol. The alcohol-insoluble, crystalline material (about 50 mg.) was dissolved in 1 ml. of water and the solution was filtered through No. 50 Whatman paper. The filtrate was diluted with 20 ml. of absolute ethanol and left to crystallize. After several hours at 5°, the crystals were collected, washed with absolute ethanol and dried *in vacuo* at 60°.

The substance melted at 238–240°, and showed $[\alpha]^{22}D$ −69° (*c* 1, water). Reported for L-inositol is the m.p. 246° and $[\alpha]_D$ −65° (water). The melting point of a mixture with authentic L-inositol was not depressed.

Anal. Calcd. for $C_6H_{12}O_6$ (180): C, 40.0; H, 6.7. Found: C, 39.9; H, 6.8.

The hexabenzoate of L-inositol from galactinol showed m.p. 251.5–252.5°, and the m.p. was undepressed on mixing with authentic L-inositol hexabenzoate. The substance showed $[\alpha]_D$ −62.1° (*c* 1.9, ethylene dichloride).

Anal. Calcd. for $C_{48}H_{36}O_{12}$ (804.8): C, 71.6; H, 4.5. Found: C, 71.8; H, 4.4.

Authentic L-inositol hexabenzoate, prepared in a yield of 80% from L-inositol, by the method used by Fletcher and Findlay[19] for the racemate, had m.p. 251.5–252.5° and showed $[\alpha]^{25}D$ −62.4° (*c* 2.4, ethylene dichloride). Literature values of m.p. are 247° for the L-isomer[20] and 253° for the D-isomer.[21]

Anal. Calcd. for $C_{48}H_{36}O_{12}$ (804.8): C, 71.6; H, 4.5. Found: C, 71.6; H, 4.6.

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