[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

# A New Synthesis of 2,4-Di-*O*-methyl-D-glucose[2]

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Partial methylation of phenyl 6-*O*-trityl-β-D-glucopyranoside followed by detritylation gave phenyl 2,4-di-*O*-methyl-β-D-glucoside in 15% yield. Hydrolysis of the glycoside gave crystalline 2,4-di-*O*-methyl-D-glucose identified through conversion to the known β-methyl glycoside. For characterizing small amounts of the reducing sugar the N-*p*-nitrophenyl- and N-*p*-bromophenyl-2,4-di-*O*-methyl-D-glucosylamines are advantageous derivatives.

In recent methylation studies NRRL B-512 dextran[3] was shown to contain 1,3-linkages at the branch points, hydrolysis of the trimethyl dextran resulting in isolation of a small amount of 2,4-di-*O*-methyl-D-glucose. At the time our methylation studies were undertaken, this dimethyl ether of D-glucose was unknown. The anomeric methyl glycosides, difficultly accessible from existing methods of preparation, were the only known characterizing derivatives. Their value for identifying small amounts of 2,4-di-*O*-methyl-D-glucose appeared limited by the low yields obtainable from the reducing sugar. The investigation of more suitable derivatives would require an adequate supply of 2,4-di-*O*-methyl-D-glucose. This could be provided most practically through an improved synthesis.

2,4-Di-*O*-methyl-D-glucose was first obtained as an unknown methyl di-*O*-methyl-D-glucoside, a fortuitous by-product in the synthesis of 2,3,4-tri-*O*-methyl-D-glucose.[4] The identification of this crystalline by-product as methyl 2,4-di-*O*-methyl-α-D-glucoside was accomplished later by Adams, Reeves and Goebel[5] who effected the first direct synthesis of a 2,4-di-*O*-methyl-D-glucoside. Their product, methyl 2,4-di-*O*-methyl-β-D-glucoside, gave, on interconversion, the corresponding α-methyl anomer which proved to be identical with the earlier preparation.

The first synthesis of methyl 2,4-di-*O*-methyl-β-D-glucoside stimulated subsequent publication of a number of alternative methods for its preparation.[6,7] Unfortunately, most of these syntheses, all of which are based on the blocking of the 3- and 6-positions of methyl β-D-glucoside, have the disadvantage of complexity and all have given low yields. Consequently there has been little opportunity to prepare and study the reducing sugar itself. In the present work, our aim was to devise a practical method for preparing a 2,4-di-*O*-methyl-D-glucoside. Acid hydrolysis would give 2,4-di-*O*-methyl-D-glucose, the product of primary interest.

It appeared to us that a simple solution to the synthesis of a 2,4-di-*O*-methyl-D-glucoside might be afforded through a partial methylation approach. Thus, the successful use of phenyl β-D-glucopyranoside by Richtmyer for the preparation of 2,4,6-tri-*O*-methyl-D-glucose[8] suggested that the 6-*O*-trityl derivative of this glycoside might serve equally well for the preparation of phenyl 2,4-di-*O*-methyl-β-D-glucoside. This proved to be the case.

Thus phenyl β-D-glucopyranoside, readily obtainable[9] from its easily prepared tetra-*O*-acetate,[10] gave on tritylation an almost quantitative yield of the 6-*O*-trityl derivative. Partial methylation of this substance by the Purdie method afforded, upon detritylation, crystalline phenyl 2,4-di-*O*-methyl-β-D-glucoside in 15% yield. Hydrolysis of the glycoside gave in good yield (82%) the crystalline reducing sugar, 2,4-di-*O*-methyl-D-glucose, identified by conversion to the known β-methyl glycoside.[5,6]

Following the initial report of the preparation of crystalline 2,4-di-*O*-methyl-D-glucose,[2] Bell and Manners[11] obtained this di-*O*-methyl-D-glucose from trityl-laminarin. Since their synthesis did not yield a pure product directly, isolation of the reducing sugar as the β-methyl glycoside (yield about 7% based on trityl-laminarin) was required. Hydrolysis gave crystalline 2,4-di-*O*-methyl-D-glucose having a melting point and a specific rotation which agreed closely with the values originally reported by us.[2]

The characterization of 2,4-di-*O*-methyl-D-glucose, which does not crystallize readily even when pure, has been effected through formation of the β-methyl glycoside.[11,12] Unfortunately, the best method available for the preparation of this glycoside from the reducing sugar is tedious[13] and has afforded a rather low yield.[11] For the identification of the small amounts of 2,4-di-*O*-methyl-D-glucose which are encountered occasionally in methylation studies of certain types of polysaccharides[3,14] we have found the N-aryl-2,4-di-*O*-methyl-D-glycosylamines, produced by interaction of the reducing sugar with *p*-bromo- or *p*-nitroaniline, to be superior derivatives. These crystalline substances are prepared readily and in high

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(2) Presented at the 122nd Meeting of the American Chemical Society, Atlantic City, N. J., September 15, 1952.

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yield. Moreover, their formation from chromatographically separated 2,4-di-*O*-methyl-D-glucose fractions is not hindered by the occasional presence, in such fractions, of certain non-carbohydrate impurities which originate with the partitioning solvent.[3]

## Experimental

Evaporations were performed under reduced pressure unless otherwise stated. Melting points were determined with a Fisher–Johns melting point apparatus.[15] For paper chromatograms Whatman No. 1 filter paper, methyl ethyl ketone–water azeotrope as partitioning solvent, and *p*-dimethylaminoaniline spray reagent[16] were used.

**Phenyl 6-*O*-Trityl-β-D-glucopyranoside.**—To a solution of anhydrous phenyl β-D-glucopyranoside (25.7 g., 0.10 mole) in anhydrous pyridine (125 ml.) was added trityl chloride (30.7 g., 0.11 mole) and the resulting mixture, protected from the moisture of the air, was heated at 95° (bath) for 4 hours.

The cooled reaction mixture was poured in a thin stream with vigorous stirring into ice–water slush (500 ml.) which contained an excess of sodium bicarbonate. The precipitated sirup was extracted with chloroform, the extract dried over anhydrous sodium sulfate and evaporated (bath temperature slowly raised to 60°) to give a thick sirup essentially pyridine-free. A chloroform (300 ml.) solution of the sirup was diluted gradually with Skelly C[15] (heptane, 800 ml.). Precipitated flocs were dissolved by warming and the solution slowly distilled (bath, 65–70°) at slightly less than atmospheric pressure in order to selectively remove the chloroform. After 500 ml. of distillate had been collected, additional Skelly C (200 ml.) was added to the distilling flask which was then brought to room temperature by cooling under the water tap. Decantation of the supernatant liquid followed by evacuation of the sirupy residue gave phenyl 6-*O*-trityl-β-D-glucopyranoside, a frothy amorphous solid weighing 48.8 g. (yield 98%). Analysis indicated that this substance was essentially pure, although it could not be induced to crystallize; $[\alpha]^{25}D$ −20.6° (acetone, *c* 4).

*Anal.* Calcd. for $C_{31}H_{30}O_6$: C, 74.7; H, 6.1. Found: C, 74.2; H, 6.3.

**Methylation of Phenyl 6-*O*-Trityl-β-D-glucopyranoside.**—To a refluxing solution (bath, 50°) of phenyl 6-*O*-trityl-β-D-glucopyranoside (47.1 g.) in methyl iodide (150 ml.) was added freshly prepared silver oxide (58 g.) in 10 portions over a 10-hour period with frequent agitation of the suspension. After standing overnight the reaction mixture was diluted with acetone, filtered and the silver compounds washed thoroughly with boiling acetone. Evaporation of solvent from the combined filtrate and washings gave a red sirup.[17]

**Detritylation.**—A chloroform (200 ml.) solution (0°) of the above sirup was saturated rapidly with anhydrous hydrogen chloride. After 30–40 minutes at 0°, the solution was neutralized by adding a large excess of barium carbonate together with a little water. Dilution with 95% ethanol (600 ml.) gave a homogeneous solution from which barium salts were removed by filtration.

The filtrate was diluted with water (2,000 ml.) and distilled (bath, 50°) until all chloroform and ethanol had been removed. The aqueous solution remaining was filtered (Solution 1), thus separating the gummy triphenylmethanol. The latter was dissolved in acetone (1,000 ml.), the solution diluted with water (1,000 ml.) and just enough additional acetone added (*ca.* 900 ml.) to redissolve the precipitate produced by the water. The acetone then was removed slowly by distillation. The resulting aqueous solution, containing finely divided triphenylmethanol in suspension, was digested on the steam-bath, allowed to stand overnight and filtered (Solution 2).

**Isolation of Phenyl 2,4-Di-*O*-methyl-β-D-glucoside.**—Solutions 1 and 2 were combined and evaporated to dryness, residual moisture being removed by distilling with benzene. Thorough extraction of the resulting residue with boiling chloroform and evaporation of the extracts gave a dark-colored solid (*ca.* 16 g.). Treatment of a methanol (500 ml.) solution of this product with charcoal (15 g.) removed most of the colored impurities. The solid subsequently obtained by evaporation of solvent was extracted thoroughly with boiling Skelly C (*ca.* four 200-ml. portions required), each boiling extract being quickly separated by decantation. The combined extracts on evaporation of solvent yielded a white crystalline product which was extracted again as before with boiling Skelly C.[18] Evaporation of the extracts and treatment of the resulting product in hot benzene (1,000 ml.) solution with charcoal (10 g.) gave, after removal of charcoal and evaporation of solvent, a white crystalline residue (*ca.* 10 g.). On dissolving the crystals in the smallest volume of boiling benzene and allowing to cool, phenyl 2,4-di-*O*-methyl-β-D-glucoside rapidly crystallized. It was purified by 5–8 recrystallizations (1 hour required for each) in the same way. The mother liquors from the recrystallizations were combined, solvent removed by evaporation and the residue recrystallized several times, as before, to yield a second crop. In all, five crops were obtained by repetition of this procedure, three-fourths of the total product being represented by the first two crops; yield (5 crops), 4.02 g. or 14.9% of the theoretical amount based on phenyl 6-*O*-trityl-β-D-glucopyranoside, m.p. 166.5–167.5°, $[\alpha]^{25}D$ −54.5° (acetone, *c* 3).

*Anal.* Calcd. for $C_{14}H_{20}O_6$: C, 59.1; H, 7.1; $OCH_3$, 21.8. Found: C, 59.2; H, 6.9; $OCH_3$, 21.9.

**2,4-Di-*O*-methyl-β-D-glucose.**—Phenyl 2,4-di-*O*-methyl-β-D-glucoside (1.367 g.) was dissolved in 0.5 *N* sulfuric acid (100 ml.) and heated at 95° (bath) until the optical rotation became constant (6 hours). Neutralization of the hydrolyzate with barium carbonate followed by filtration (the filter cake was washed with methanol) and evaporation of solvent (bath, 50°) gave a sirup from which residual moisture was removed by distilling with methanol–benzene. To the anhydrous sirup dissolved in a little absolute methanol (3 ml.) was added benzene (*ca.* 30 ml.), to produce turbidity, and a seed crystal. In 10–14 days, crystallization was complete; yield (2 crops) 0.822 g. or 82% of the theoretical amount. The melting point varied depending upon the speed of heating. On slow heating, m.p. 124–129° was obtained; rapid heating gave m.p. 128–130° (with softening). When the crystals were dissolved in water the shift in optical rotation, $[\alpha]^{25}D$ +37.3° (5 min.) → +76.5° (equil.) (water, *c* 1.6), indicated that the sugar had crystallized in the β-anomeric form.

These constants for crystalline 2,4-di-*O*-methyl-D-glucose agree well with those given by Bell and Manners[11] who reported the melting point of their preparation to vary from 125 to 129°. They gave $[\alpha]^{25}D$ +43.3° (4 min.) → +73.7° (equil.) ($H_2O$, *c* 2.8), thus indicating that they also obtained the β-anomeric form.

Examination by paper chromatography, involving comparison with reference specimens of the other methylated glucoses simultaneously produced in this synthesis, revealed that the crystalline sugar was chromatographically pure. It was readily soluble in water, methanol and ethanol, less soluble in chloroform, sparingly soluble in anisole (hot) and ethylene dichloride (hot) and insoluble in benzene and petroleum ether.

*Anal.* Calcd. for $C_8H_{16}O_6$: C, 46.2; H, 7.7; $OCH_3$, 29.8. Found: C, 46.2; H, 7.6; $OCH_3$, 29.7.

**Methyl 2,4-Di-*O*-methyl-β-D-glucoside.**—Attempts to prepare this substance by refluxing either the reducing sugar or the β-phenyl glycoside with methanolic hydrogen chloride until the optical rotation was constant invariably produced a sirupy mixture which yielded little or no crystalline product. Consequently, methyl 2,4-di-*O*-methyl-β-D-glucoside was prepared from the reducing sugar (0.397 g.) by Oldham's[18] method. We found it advantageous to modify this procedure by saponifying the benzoylated β-methyl glyco-

(15) The mention in this article of firm names or commercial products does not constitute an endorsement of such firms or products by the U. S. Department of Agriculture.

(16) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

(17) A second methylation of this sirup gave a product which, upon detritylation, yielded a mixture containing considerable tri-*O*-methyl derivative and from which no phenyl 2,4-di-*O*-methyl-β-D-glucoside could be isolated.

(18) An insoluble dark-colored sirup remaining after each of these extractions reduced Fehling solution and appeared, from paper chromatography, to be essentially a mixture of mono- and di-*O*-methyl-D-glucoses. Failure to remove these reducing sugars at this point made it impossible later to isolate phenyl 2,4-di-*O*-methyl-β-D-glucoside in pure form.

side with a methanolic solution of barium hydroxide.[19] Treatment of the crude product in benzene (100 ml.) with charcoal (2 g.) followed by removal of charcoal and solvent gave a sirup which crystallized spontaneously. When recrystallized from the smallest volume of benzene–Skelly C (1:1), pure methyl 2,4-di-*O*-methyl-β-D-glucoside was obtained; yield 0.115 g. (27% of the theoretical amount), m.p. 124°, $[\alpha]^{25}D$ −16° (acetone, *c* 1). A mixed melting point with an authentic specimen of methyl 2,4-di-*O*-methyl-β-D-glucoside prepared by Reeves[5] gave no depression.

*Anal.* Calcd. for $C_9H_{18}O_6$: C, 48.7; H, 8.2; $OCH_3$, 41.9. Found: C, 48.9; H, 8.2; $OCH_3$, 41.7.

**N-*p*-Nitrophenyl-2,4-di-*O*-methyl-D-glucosylamine.**—To a solution of 2,4-di-*O*-methyl-D-glucose (0.112 g.) in absolute ethanol (3 ml.) was added *p*-nitroaniline (0.108 g., 50% excess) and 1 drop of glacial acetic acid. During 1 hour of refluxing the crystalline product separated. From the cooled reaction mixture was obtained, by filtration and washing of the product with a little absolute ethanol, N-*p*-nitrophenyl-2,4-di-*O*-methyl-D-glucosylamine (0.161 g., or 91% of the theoretical amount). Recrystallized from anhydrous ethyl acetate (150 ml.) it had m.p. 250–251° dec. If heated slowly the crystals had a lower melting point. $[\alpha]^{25}D$ −252° (10 min.) → −268° (equil.) (pyridine, *c* 0.5).

(19) P. Brigl and R. Schinle, *Ber.*, **67**, 756 (1934).

*Anal.* Calcd. for $C_{14}H_{20}O_7N_2$: C, 51.2; H, 6.1; N, 8.5; $OCH_3$, 18.9. Found: C, 51.4; H, 6.1; N, 8.6; $OCH_3$, 19.0.

**N-*p*-Bromophenyl-2,4-di-*O*-methyl-D-glucosylamine.**—To a solution of 2,4-di-*O*-methyl-D-glucose (0.104 g.) in absolute ethanol (3 ml.) was added *p*-bromoaniline (0.130 g., 50% excess) and the mixture was refluxed for 4 hours. The product which crystallized during the refluxing was separated from the cooled reaction mixture by filtration and was washed with a little ethanol; yield 0.162 g. or 90% of the theoretical amount. Recrystallized from anhydrous ethyl acetate (200 ml.), pure N-*p*-bromophenyl-2,4-di-*O*-methyl-D-glucosylamine was obtained, m.p. 243–244° (sl. dec.), $[\alpha]^{25}D$ −147° (pyridine, *c* 0.5). No mutarotation was observed.

*Anal.* Calcd. for $C_{14}H_{20}O_5NBr$: C, 46.4; H, 5.6; N, 3.9; Br, 22.1; $OCH_3$, 17.1. Found: C, 46.4; H, 5.8; N, 3.9; Br, 22.3; $OCH_3$, 17.7.

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[CONTRIBUTION FROM THE BEN MAY LABORATORY FOR CANCER RESEARCH, UNIVERSITY OF CHICAGO]

# Enzymatic Preparation of Two Ketohexose 1-Phosphates

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Propionaldehyde and 3-methoxypropionaldehyde each undergo enzymatic aldol condensation with dihydroxyacetone phosphate in muscle extracts containing aldolase and triose phosphate isomerase. The products, presumably 5,6-dideoxy-D-fructose 1-phosphate and 5-deoxy-6-*O*-methyl-D-fructose 1-phosphate, were isolated in good yield, thus extending the list of ketoses and ketose 1-phosphates which may be easily prepared enzymatically.

Aldolase catalyzes the reversible reaction

D-glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇌ D-fructose 1,6-diphosphate (1)

It has been found[2–4] that a variety of aldehydes may replace glyceraldehyde 3-phosphate in this reaction; however, dihydroxyacetone phosphate is a specific reaction partner. When "foreign" aldehydes react enzymatically with dihydroxyacetone phosphate corresponding ketose 1-phosphates are formed; for instance, D-fructose 1-phosphate is formed from D-glyceraldehyde[2,3] and D-xylulose 1-phosphate is formed from glycolaldehyde.[4]

Dialyzed rabbit muscle extracts contain, in addition to aldolase, triose phosphate isomerase, catalyzing the reaction

D-glyceraldehyde 3-phosphate ⇌ dihydroxyacetone phosphate (2)

In such extracts the readily available D-fructose 1,6-diphosphate may be used as starting material for the relatively simple enzymatic preparation of new ketose 1-phosphates, in the presence of a large excess of a "foreign" aldehyde. In this way, D-fructose 1-phosphate,[2,3] L-sorbose 1-phosphate,[2,3] 5-deoxyxylulose 1-phosphate,[2] and D-xylulose 1-phosphate[5] have been prepared. With a specific aldolase of liver, erythrulose 1-phosphate has also been prepared.[5] Extracts of peas contain phosphatases in addition to aldolase and isomerase and with such preparations Jones and colleagues have prepared a series of free (dephosphorylated) ketoses.[6] In all enzymatic aldolization products which have been studied to this end, the configuration of hydroxyl groups at carbon atoms 3 and 4 has been found to be D-*threo* (*trans*-glycol). These products are thus homologs of D-xylulose, and D-fructose or L-sorbose, etc. The enzymatic formation of the L-*threo* or D- or L-*erythro* configurations by aldolase has not been observed, although D-tagatose 1,6-diphosphate (an *erythro* form) is very slowly cleaved by aldolase.[3]

In this paper the enzymatic preparation of 5,6-dideoxyhexulose 1-phosphate and 5-deoxy-6-*O*-methylhexulose 1-phosphate from the corresponding aldehydes (propionaldehyde and 3-methoxypropionaldehyde) is described. The naming of the products is non-committal with respect to configuration at carbons 3 and 4, which was not directly determined. However, it is probable that the products consist largely if not entirely of 5,6-dideoxy-D-fructose 1-phosphate and 5-deoxy-6-*O*-methyl-D-

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