[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Epimerization in the Synthesis of 2,4,6-Tri-*O*-methyl-D-mannose[1]

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2,4,6-Tri-*O*-methyl-D-mannose monohydrate, m.p. 64°, $[\alpha]$D +17° (water), has been synthesized from 2,4,6-tri-*O*-methyl-D-glucose by epimerization with dilute alkali.

The glucomannan obtained from urediospores of *Puccinia graminis tritici*, the organism causing stem rust of wheat, yielded upon methylation followed by hydrolysis, among other products, a crystalline tri-*O*-methyl-D-mannose monohydrate, m.p. 64°, $[\alpha]^{25}$D +17° (water).[2] That this trimethyl sugar was a derivative of D-mannose was shown by the fact that methylation of its anilide with silver oxide and methyl iodide[3] afforded 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannopyranosylamine.

In Table I are recorded the known tri-*O*-methyl derivatives of D-mannose. The fact that the glucomannan was relatively stable to dilute sulfuric acid, coupled with the observation that the anilide of the tri-*O*-methyl-D-mannose gave upon methylation the characteristic 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannopyranosylamine, ruled out the 2,3,5-tri-*O*-methyl isomer. The unknown crystalline trimethyl-D-mannose failed to induce crystallization of 2,3,4-tri-*O*- and 2,3,6-tri-*O*-methyl-D-mannose, both of which are liquids, and when mixed with crystalline 3,4,6-tri-*O*-methyl-D-mannose there was a depression of the melting point.

TABLE I

TRI-*O*-METHYL DERIVATIVES OF D-MANNOSE

Tri-*O*-methyl-D-mannose	M.p., °C.	$[\alpha]$D (water)	Ref.
2,3,4-	Liquid	+2°	4
2,3,6-	Liquid	+6°	5
2,3,5-	Liquid	+28° (0.1 *N* H_2SO_4)	6
2,4,6- (α-anomer)	90	+21° → +14°	7
2,4,6- (β-anomer)	104–107	−5.7° → 19°	7
3,4,6- (α-anomer)	101–102	+21° → +8.2°	8
Unknown tri-*O*-methyl-	64	+17° (no mutarotation)	2

There remained, therefore, only the 2,4,6-tri-*O*-methyl derivative and it was tentatively concluded that the unknown tri-*O*-methyl-D-mannose was the 2,4,6-tri-*O*-methyl isomer even though the unknown compound had a different melting point and rotation from the 2,4,6-tri-*O*-methyl-D-mannose previously isolated.[7] Inasmuch as no crystalline 2,4,6-tri-*O*-methyl-D-mannose was available for comparison purposes, a synthesis of it was sought.

This was accomplished by the following series of reactions: D-glucose → 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose → 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose → methyl 3-*O*-benzyl-D-glucopyranoside → methyl 3-*O*-benzyl-2,4,6-tri-*O*-methyl-D-glucopyranoside → methyl 2,-4,6-tri-*O*-methyl-D-glucopyranoside → 2,4,6-tri-*O*-methyl-D-glucose → 2,4,6-tri-*O*-methyl-D-mannose.

The transformation of 2,4,6-tri-*O*-methyl-D-glucose into the corresponding D-mannose derivative was effected by epimerization with dilute alkali[9] and the resulting mixture separated by partition chromatography on a cellulose–hydrocellulose column.[10] The synthetic 2,4,6-tri-*O*-methyl-D-mannose readily crystallized as a monohydrate which proved to be identical with the tri-*O*-methyl-D-mannose obtained from the methylated urediospore glucomannan. Crystallization from ether, the solvent used previously[7] for crystallizing the 2,4,6-tri-*O*-methyl-D-mannose from methylated yeast mannan, gave the same crystalline form of the monohydrate. The discrepancy between the properties of the 2,4,6-tri-*O*-methyl-D-mannose, prepared from the methylated urediospore glucomannan and by synthesis, and the 2,4,6-tri-*O*-methyl-D-mannose reported previously[7] is not understood but it is perhaps possible that the compound reported herein is a mixture of anomeric forms since it does not show mutarotation in water whereas the previous α- and β-forms of 2,4,6-tri-*O*-methyl-D-mannose do[7] (see Table I).

The above synthesis of 2,4,6-tri-*O*-methyl-D-mannose from the corresponding D-glucose derivative indicates that epimerization may be of general preparative value for the synthesis of certain methylated sugars, providing the hydroxyl group at position two is blocked by a methyl group to prevent ketose formation. When used in conjunction with chromatography, the above reaction is useful for the identification of an unknown methylated aldose sugar if its epimer is known.

Experimental

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose.—1,2:5,6-Di-*O*-isopropylidene-D-glucofuranose (36 g.)[11] was dissolved in liquid ammonia (500 ml.) and sodium (3.3 g.) was added in small pieces with stirring until a permanent blue color resulted. Benzyl chloride (16.3 ml.) was added slowly after which the stirring was continued for 2 hours. The reaction mixture was allowed to stand overnight at room temperature without stirring to allow the ammonia to evaporate. The residue was dissolved in chloroform and the solution was washed well with water to remove sodium chloride and unreacted 1,2:5,6-di-*O*-isopropylidene-D-glucofu-

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ranose. The chloroform solution, after drying with magnesium sulfate, was evaporated to remove the chloroform and most of the remaining benzyl chloride. After removing the last traces of benzyl chloride *in vacuo* (50° and 0.02 mm.) distillation of the sirupy residue gave 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose, a viscous colorless liquid, 21.5 g., b.p. 143°, 0.003 mm., $n^{28}D$ 1.4932, $[\alpha]^{28}D$ −28.3° (in ethanol; *c* 1.3); literature[12,13] $[\alpha]D$ −27° (in ethanol).

3-*O*-Benzyl-D-glucose.—The 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-D-glucofuranose (21.5 g.) was dissolved in methanol (45 ml.) and *N* sulfuric acid (21.5 ml.) was added. After the solution had been kept at 70° for 6 hours the methanol was removed by evaporation *in vacuo* and the solution neutralized—($BaCO_3$), filtered and evaporated to give 3-*O*-benzyl-D-glucose (17 g.). After recrystallization from acetone–ethyl acetate and from acetone, the 3-*O*-benzyl-D-glucose (2.2 g.) had m.p. 138–141°, $[\alpha]^{29}D$ +20.6° → +41.3° (in water, *c* 0.9); literature[14] m.p. 138–141° and $[\alpha]D$ +20.3° → +41.9° (in water).

Methyl 3-*O*-Benzyl-D-glucopyranoside.—The 3-*O*-benzyl-D-glucose (2.2 g.) was dissolved in 0.5% methanolic hydrogen chloride (14 ml.) and heated for 6 hours in a sealed tube in a boiling water-bath. The rotation of the solution, initially $[\alpha]D$ +36.5°, changed in 4 hours to +68° (constant for a further 2 hours). The solution was neutralized with silver carbonate, centrifuged and the supernatant liquid evaporated to give methyl 3-*O*-benzyl-D-glucopyranoside (2.5 g.) as a viscous liquid.

Methylation of Methyl 3-*O*-Benzyl-D-glucopyranoside.—A solution of methyl 3-*O*-benzyl-D-glucopyranoside (2.5 g.) in methyl iodide (35 ml.) containing methanol (5 ml.) was stirred and refluxed for 10 hours in the presence of anhydrous calcium sulfate ("Drierite") (1.0 g.) and silver oxide (2.0 g.). After filtering and washing the solids with acetone, the combined filtrate and washings were evaporated to dryness *in vacuo*. The sirupy product was subjected to five additional methylations in the same way to give methyl 3-*O*-benzyl-2,4,6-tri-*O*-methyl-D-glucopyranoside, b.p. (bath. temp.) 160°, 0.001 mm., $n^{28}D$ 1.4925, $[\alpha]^{28}D$ +70° in ethanol (*c* 1) (yield 2.1 g.); literature[15] $[\alpha]D$ +43.5° (ethanol). *Anal.* Calcd. for $C_{17}H_{26}O_6$: OCH_3, 38.0. Found: OCH_3, 36.4. The difference in rotation can be attributed to the presence of α- and β-anomers of the glycoside in the above preparation.

Methyl 2,4,6-Tri-*O*-methyl-D-glucopyranoside.—To the methyl 3-*O*-benzyl-2,4,6-tri-*O*-methyl-D-glucopyranoside (2.1 g.) in a round-bottom flask, sodium (3.8 g.) was added in small pieces.[16] After the addition of ethanol (10 ml.) the mixture was refluxed. Successive portions of ethanol (10 ml.) were added over a 2-hour period, with continued refluxing, until the sodium was decomposed. After cooling the reaction mixture, water (60 ml.) was added followed by acetic acid until the solution remained only slightly alkaline. The solution was extracted with chloroform (four 30-ml. portions), the chloroform solution was dried with magnesium sulfate and evaporated to give the crude methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside (1.5 g.) as a pale yellow sirup which was purified by distillation: b.p. (bath. temp.) 130–145°, 0.001 mm., $n^{29}D$ 1.4543, $[\alpha]^{28}D$ +90° in chloroform (*c* 1), yield 1.1 g.

2,4,6-Tri-*O*-methyl-D-glucose.—The methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside (0.533 g.) was hydrolyzed with *N* sulfuric acid (12 ml.) by heating for 16 hours at 95°. The solution, $[\alpha]D$ +71°, was neutralized ($BaCO_3$), and evaporated *in vacuo* to give a sirup which crystallized spontaneously. For purification, the crude 2,4,6-tri-*O*-methyl-D-glucose was dissolved in methyl ethyl ketone:water azeotrope (2.0 ml.) and chromatographed using the cellulose–hydrocellulose column[10] and methyl ethyl ketone:water azeotrope as the irrigating solvent. Two fractions were obtained from the eluate: 2,4,6-tri-*O*-methyl-D-glucose (0.440 g.) which crystallized, and unhydrolyzed sirupy methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside (0.095 g.). After two recrystallizations from ethanol–petroleum ether the 2,4,6-tri-*O*-methyl-D-glucose had m.p. 123–126°, $[\alpha]^{23}D$ +112° (initial value) changing to +70° in methanol (*c* 0.5), $[\alpha]^{23}D$ +91° (initial value) changing to +71° in water (*c* 0.6); yield 0.110 g. These values agree with those reported previously.[15,17–19] *Anal.* Calcd. for $C_9H_{18}O_6$: OCH_3, 41.8. Found: OCH_3, 42.6.

Epimerization of 2,4,6-Tri-*O*-methyl-D-glucose and Isolation of 2,4,6-Tri-*O*-methyl-D-mannose.—To the 2,4,6-tri-*O*-methyl-D-glucose (0.101 g.), 0.035 *N* barium hydroxide solution (20 ml.) was added at room temperature, nitrogen being bubbled through the solution for a few minutes to displace air. The solution was kept at 35° for 8 days[9] in a tightly stoppered container. The rotation changed from $[\alpha]^{24}D$ +69° (initial value) to +30° (after 7 days), and +31° (after 8 days).

The solution was neutralized with 0.1 *N* sulfuric acid, centrifuged and the supernatant liquid evaporated to give a pale yellow sirup which showed $[\alpha]^{27}D$ +43.2° (in water; *c* 2.1) indicating the presence of approximately equal quantities of 2,4,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-mannose.

The sirup (101 mg.) was dissolved in methyl ethyl ketone: water azeotrope (1 ml.) and put on the cellulose–hydrocellulose column.[10] Collection of the eluate with an automatic fraction collector was started at 30 minute intervals giving 18 ml. of eluate per tube and continued for 8.5 hours, after which the collection was continued at 10-minute intervals (6 ml. per tube) for 14.5 hours. Thereafter collections were made for 10 hours at 30-minute intervals. The methylated sugars were located in tubes 36–72 by testing with *p*-anisidine.[20] Tubes 36–46 contained 2,4,6-tri-*O*-methyl-D-mannose (31 mg.) which had an R_f (0.55) identical with that of the unknown tri-*O*-methyl-D-mannose from the methylated urediospore polysaccharide; tubes 47–60 contained a mixture (51 mg.) of 2,4,6-tri-*O*-methyl-D-mannose and 2,4,6-tri-*O*-methyl-D-glucose (R_f 0.48) and tubes 61–72 contained 2,4,6-tri-*O*-methyl-D-glucose (4 mg.) (total recovery, 84.5%).

The 2,4,6-tri-*O*-methyl-D-mannose (31 mg.) crystallized spontaneously as a monohydrate, and, after recrystallization either from ethanol–petroleum ether or from ethyl ether, it had m.p. 62–64°, $[\alpha]^{26}D$ +17° in water (*c* 2.5) without mutarotation. There was no depression of the melting point when mixed with the tri-*O*-methyl sugar obtained from the methylated urediospore polysaccharide which had m.p. 64° and $[\alpha]^{26}D$ +17° (in water, *c* 2.5) and likewise showed no mutarotation.

After keeping over "Drierite" *in vacuo* the crystals of 2,4,6-tri-*O*-methyl-D-mannose liquefied, but upon exposure to the atmosphere for several hours, crystallization again took place. When the crystalline 2,4,6-tri-*O*-methyl-D-mannose was heated at 56° *in vacuo* over phosphorus pentoxide, one molecular proportion of water was lost. *Anal.* Calcd. for $C_9H_{20}O_7$: C, 45.0; H, 8.3; OCH_3, 38.8; H_2O, 7.5. Found (for the tri-*O*-methyl-D-mannose from the methylated urediospore polysaccharide): C, 45.2; H, 8.0; OCH_3, 39.9; H_2O (by loss in weight), 7.5. Found (for the synthetic 2,4,6-tri-*O*-methyl-D-mannose monohydrate: C, 45.3; H, 8.1; H_2O, 7.5.

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