Synthesis of 3-O-β-D-Glucopyranosyl-D-mannose

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3-O-β-D-Glucopyranosyl-D-mannose has been unequivocally synthesized using the Kochetkov orthoester glycoside method. Its properties do not correspond to those of a disaccharide previously isolated from Asparagus racemosus and which has been reported to be the title compound.

Landge and Bose¹ have recently described the isolation from Asparagus racemosus of a glucosylmannose to which the structure of 3-O-β-D-glucopyranosyl-D-mannose was assigned, based on the results of acid hydrolysis, oxidation of the reducing end of the disaccharide followed by acid hydrolysis, alkaline degradation and periodate oxidation. The anomeric configuration was deduced from the optical rotation. The synthesis of a 3-glucosylmannose has previously been reported by Gakhokidze and Gvelukashvili through the alkaline isomerization of a 3-glucosylglucose.² The m.p. and optical rotation ([α]D +27.9°) of the 3-glucosylmannose correspond closely to those described by Landge and Bose.

![Chemical Structures](image-url)

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The aglycone, benzyl 2-O-benzyl-4,6-O-benzylidene-α-d-mannopyranoside (I), was made as follows. Benzyl α-d-mannopyranoside⁵ was converted into the 4,6-O-benzylidene derivative by brief treatment with excess benzaldehyde and formic acid as described by Buchanan and Schwarz for the corresponding methyl mannoside.⁶ The yield of crystalline material was 36 %. Partial benzylolation of the 4,6-O-benzylidene derivative with 1 mol benzyl bromide and silver oxide in dimethyl formamide⁵ gave a mixture from which the 2-O-benzyl ether (I) crystallized out in a yield of 22 %. The constitution of I follows from its elemental analysis and from examination of a hydrolysate of I (0.25 M aqueous sulphuric acid at 100° overnight) by paper electrophoresis in geraninate buffer⁶ which revealed the presence of 2-O-substituted mannoside in addition to a smaller amount of mannoside presumably produced by partial hydrolysis of the benzyl ether groups.⁷ The constitution of I was further demonstrated by NMR on the acetate of I which showed, inter alia, δ 4.90, 1 H, doublet, J = 2 Hz, anomeric proton, which collapsed to a singlet by decoupling irradiation at δ 3.85, 1 H; the latter signal therefore was given by H-2. H-3 (acetoxylated carbon) gave a signal at δ 5.32, J₃,₅ = 3 Hz, J₂,₃ = 9 Hz. The coupling constants (equatorial-axial and axial-axial) as well as the shift (downfield from H-1) are in accordance with the acetoxyl group being situated at C-3. 3,4,6-Tri-O-acetyl-1,2-O-methylthioacetetyl-α-d-glucopyranoside⁸ was condensed with I to yield the substituted disaccharide (II) which, without purification, was deacetylated to yield impure III which was purified by solvent partition to get rid of monosaccharide units. A small aliquot of III was hydrogenated in order to remove benzyl and benzylidene groups, converted into the corresponding glucosyl alditol by sodium borohydride reduction, permethylated,⁹ and examined by GLC-MS.¹⁰ The product gave two peaks in a ratio of 1:4, the slower-moving component predominating. The retention times for both components were of the expected magnitude for hexosyl hexitol nonamethyl ethers. Both components, apart from minor differences in peak intensities, gave the same mass spectra with the expected mass fragmentation. The disaccharide derivative III therefore was contaminated with a minor proportion of methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(α-d-glucopyranosyl)-α-d-mannopyranoside. This α-linked disaccharide was removed from the major, β-linked isomer III by chromatography of the peracetylated mixture on silicic acid. Two fractions were obtained, one containing pure III acetate and one containing a mixture of III acetate and the corresponding α-linked isomer; the ratio of α-linked/β-linked isomer was 2:1 in this fraction. The pure disaccharide IV was obtained from purified III acetate (II) by deacetylation followed by catalytic hydrogenation. A product, analyzing correctly for IV and with m.p. 195–197°, [α]D − 10.5° (5 min) − 26.2° (final value, water) was obtained. The Asparagus racemosus disaccharide¹ has m.p. 164–165° and [α]D + 28.5° (ethanol) while the disaccharide prepared by Gakhokidze has m.p. 165° and [α]D + 27.9° (water).² The higher m.p. of our compound and the difference in optical rotation is noteworthy. Only final optical rotations are given in the two previous reports. The low solubility of our compound in ethanol precluded the measurement of the optical rotation in this solvent. The present findings make the assignment of the structure of...
3-\(\alpha\)-\(\beta\)-d-glucopyranosyl-d-mannose to the *Asparagus racemosus* disaccharide
doubtful.

The constitution of the disaccharide IV was confirmed as follows: Acid
hydrolysis and sugar analysis\(^{11}\) gave glucose and mannose in a ratio of 1:1.
The disaccharide was homogeneous on paper chromatography. NMR on the
product of borohydride reduction followed by trimethylsilylation\(^{12}\) revealed
a coupling constant of 6.5 Hz (\(\delta\) 4.60 ppm) for the anomeric glucopyranose
proton, in accordance with the expected \(\beta\)-configuration. These values are
identical to those given by Hellerqvist and co-workers\(^{12}\) for the same trimethyl-
silyl glucosyl alditol obtained by an alternative route.

Sugar analysis of the fraction containing the mixture of IV and the cor-
responding \(\alpha\)-glucosyl isomer obtained by hydrogenation of the fraction con-
taining III and the corresponding \(\alpha\)-glucosyl isomer described above also gave
mixture and mannose in a ratio 1:1. NMR on the borohydride-reduced and
trimethylsilylated product\(^{11}\) gave, \textit{inter alia}, one doublet with the same
chemical shift and coupling constant as those described above for the \(\beta\)-
anomer from IV and a major doublet, \(\delta\) 4.68, \(J_{1,2} = 3.0\) Hz in agreement with
the postulated \(\alpha\)-configuration at the glucose. The mixture of IV and the cor-
responding \(\alpha\)-glucosyl isomer after borohydride reduction was also subjected
to methylation and the mixture examined by GLC-MS.\(^{9}\) Both peaks again
gave the fragmentation pattern expected for reduced and methylated IV.
This is expected since both reduced, methylated IV and the corresponding
\(\alpha\)-anomer apart from possible small differences in relative peak intensities will
give the same mass fragmentation.

The orthoester glycoside synthesis developed by Kochetkov and co-
workers is believed to possess a high degree of stereospecificity and to produce
glycosides with 1,2-\textit{trans}-configuration.\(^{8}\) Interestingly, this method when first
presented by Lemieux and Morgan was reported to give \(\alpha\)-glycosides.\(^{13}\) When
we, in an effort to increase the yield of the disaccharide derivative III, raised
the concentration of mercuric bromide about ten-fold, a disaccharide mixture
containing IV and the corresponding \(\alpha\)-linked disaccharide was produced, in
which the latter predominated. This variation in anomeric composition in the
orthoester glycoside synthesis with reaction conditions is currently under in-
vestigation.

**EXPERIMENTAL**

Concentrations were performed at reduced pressure. Melting points are corrected.
Optical rotations were determined at room temperature (20 – 22\(^{\circ}\)) using a Perkin-Elmer
141 polarimeter. NMR spectra (CDCl\(_3\)) were recorded with a Varian A-60 A spectrometer
with a V-6058 unit for decoupling experiments. Tetramethylsilane was used as internal
reference and chemical shifts (\(\delta\)) measured in ppm downfield from this reference. TLC was
performed on silica gel (Merck). Sulphuric acid was used as spray reagent. Paper chro-
matography was performed on Whatman No. 1 paper, solvent ethyl acetate-acetic acid-
water 3:1:1. GLC-MS was run on a Perkin-Elmer 270 instrument. Spectra were recorded
at a manifold temperature of 220\(^{\circ}\), an ionization potential of 70 eV, an ionization current
of 80 \(\mu\)A and a temperature at the ion source of 80\(^{\circ}\).

*Benzy1 4,6-O-benzylidene-\(\alpha\)-D-mannopyranoside.* Benzy1 \(\alpha\)-D-mannopyranoside \(^{4}\) (85.5 g)
was dissolved in formic acid (650 ml) during 1.5 min. Benzaldehyde (650 ml) was added.
After 5 min at room temperature the solution was poured with vigorous stirring into
a mixture of light petroleum (40 – 60\(^{\circ}\)) (4.5 l) and water (4.5 l) containing 1 500 g potassium

carbonate. The resulting crystals were filtered off, washed with light petroleum and re-crystallized from ethanol to yield 40.5 g m.p. 148°-151°, [α]D +76° (c 0.4, chloroform). (Found: C 67.1; H 6.19; O 26.8. C12H14O4 requires: C 67.0; H 6.06; O 26.8.)

Benzyl 2-O-benzyl-4,6-O-benzylidene-α-D-mannopyranoside (1). Benzyl 4,6-O-benzylidene-α-D-mannopyranoside (36 g) in dimethyl formamide (360 ml) was stirred with benzyl bromide (17.4 g) and silver oxide (60 g) at room temperature in the dark during 48 h. The mixture was diluted with chloroform, filtered through kieselguhr and concentrated at a bath temperature of 80°. The mixture was dissolved in ethanol and allowed to stand. The resulting crystals were recrystallized from ethanol to yield 9.9 g m.p. 97°-99°, [α]D +39° (c 0.3, chloroform). (Found: C 72.3; H 6.18; O 21.4. C20H24O4 requires: C 72.5; H 6.29; O 21.4.)

3-O-β-D-Glucopyranosyl-D-mannose. 3,4,6-Tri-O-acetyl-1,2-O-methylthioacetyl-α-D-glucopyranose has been described as an amorphous substance, [α]D +34° (CHCl₃). In the present work it was obtained as described by Kochetkov and co-workers, but in crystalline form, m.p. 49°-53°, [α]D +36° (c 2.7, chloroform). (Found: C 49.6; H 5.08; O 44.0. Calc for C₃₄H₃₆O₁₆: C 49.7; H 6.12; O 44.2.) The orthoester (3.44 g) and I (4.25 g) were dissolved in nitromethane (40 ml). The solvent was distilled off at constant volume by the continuous addition of fresh nitromethane during 5.5 h. Mercuromethane (128 mg) was added and the mixture refluxed overnight. The mixture was filtered, the filtrate was concentrated and the material deacetylated with 1.67% ammoniacal methanol overnight. The resulting mixture was separated by partitioning between water—benzene/hexane 5:6 which removed the aglycone. The aqueous phase was extracted with chloroform which extracted the disaccharide derivative, leaving the product arising from unreacted orthoester in the aqueous phase. The weight of the deacetylated disaccharide fraction containing mainly III was 3.0 g corresponding to 51% yield in the coupling step.

Since III was contaminated with the corresponding α-glucosyl anomer (see general part above) the product III was recrystallized and the resulting derivative III chromatographed on silica gel (solvent ethyl ether—hexane 2:1). The pure III obtained (2.01 g) was deacetylated as described above and then hydrogenated in ethanol with 5% palladium on carbon to yield crystalline IV (700 mg) m.p. 195°-197° (α)D -10.5° (5 min) → -26.3° (final value, c 2.0, water). (Found: C 41.9; H 6.50; C₁₁H₁₄O₁₁ requires: C 42.1; H 6.48.)

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