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Note

A convenient method for the preparation of all of the partially methylated derivatives of methyl α-D-mannopyranoside and α-D-galactopyranoside*

TOMOHIRO MEGA, ATSUSHI NISHIKAWA, AND TOKUJI IKENAKA
Department of Chemistry, Osaka University College of Science, Toyonaka, Osaka 560 (Japan)
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Methylation analysis is still one of the most important methods for the structure analysis of oligosaccharides[1]. Gas chromatography–mass spectrometry, as well as ^{13}C- and ^{1}H-n.m.r. spectroscopy, is commonly used for identification of partially methylated monosaccharides. This requires numerous standard methyl derivatives, which are not easy to prepare by conventional techniques of synthesis. In this paper, we described a convenient method for preparing all of the partially methylated derivatives of methyl α-D-mannopyranoside and α-D-galactopyranoside by partial methylation of the methyl glycosides, followed by separation of the derivatives by high-pressure, liquid chromatography.

EXPERIMENTAL

Materials. — Methyl α-D-mannopyranoside and methyl 6-*O*-trityl-α-D-mannopyranoside were synthesized by methods described earlier[2,3]. Methyl α-D-galactopyranoside and sodium borodeuteride were purchased from Wako Pure Chemical Industries, Ltd., Osaka. All organic solvents were purified by distillation.

High-presure, liquid chromatography (h.p.l.c.) — High-pressure, liquid chromatography was performed with a chromatograph (Hitachi, model 638), equipped with a u.v.-monitor (Hitachi 635-M) for detection of trityl and acetyl derivatives, and with an r.i.-detector (Shodex RI SE-11) for other sugar derivatives. The derivatives were separated in a stainless-steel column (7.5 × 300 mm), packed with TSK-Gel LS-410 (10 μm), at room temperature, with aqueous methanol or aqueous acetonitrile as eluent. A solution (150–350 μL) containing 20–50 mg of sample was applied to the column per experiment.

Gas–liquid chromatography–mass spectrometry analysis. — A partially

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methylated sugar (~1 mg) was hydrolyzed with 90% formic acid (0.5 mL) for 2 h at 100° and then the solution was evaporated. The residue was again hydrolyzed with M hydrochloric acid (0.5 mL) overnight at 100°. The solution was evaporated *in vacuo*, and the residue reduced with sodium borodeuteride (0.1 mL of sodium borodeuteride–10 μM sodium hydroxide in deuterium oxide) overnight at 37°. After being desalted by passage through a Dowex 50 column, the reaction mixture was evaporated, and the residue dried by repeated additions and evaporations of methanol. The residue was peracetylated with pyridine–acetic anhydride for 2 h at 100°, and analyzed with a mass spectrometer (JEOL model 01-SG) coupled to a gas–liquid chromatograph (JGC-20K) equipped with a glass column (0.3 × 100 cm), packed with 2% OV-17 on Uniport HP (60–80 mesh), at an ionization potential of 28 eV.

Gas–liquid chromatography of partially methylated sugars and their alditol acetates was performed with a gas–liquid chromatograph (Shimazu model GC-4A), equipped with a flame-ionization detector. The separation of the derivatives was carried out in a glass column (0.4 × 200 cm) packed with Tabsorb (Regis Chemical Co.), at a temperature between 150 and 210° for each sample.

Preparation of a mixture of partially methylated methyl 6-O-trityl-α-D-mannopyranosides (TM). — Methylsulfinyl carbanion (17 mmol, corresponding to 0.6 equiv. of total hydroxyl groups) was added to a solution of methyl 6-*O*-trityl α-D-mannopyranoside (4.1 g) in dimethyl sulfoxide (30 mL), and the mixture stirred for 7 h under nitrogen, at room temperature. Methyl iodide (2.5 g) was added, and the reaction was continued overnight. After concentration of the reaction mixture under reduced pressure, the products (TM) were extracted with chloroform, and used for h.p.l.c. without further purification.

Preparation of a mixture of partially methylated methyl 6-O-methyl-α-D-mannopyranosides (MM). — Dimethyl sulfate (100 mmol) and 30% sodium hydroxide (15 mL) were added dropwise to an aqueous solution of methyl α-D-mannopyranoside (51 mmol) under stirring. The mixture was kept overnight, and the salts were removed by passage through columns of Dowex 50 (H^+ 125 g), Dowex 1 (OH^-, 200 g), and Dowex 50 (H^+), successively. The effluent was evaporated and the residue dried in a desiccator in the presence of phosphorus pentaoxide. The residue was tritylated with chlorotriphenylmethane (14 g) in pyridine (90 mL) overnight at 37°. The reaction mixture was concentrated and extracted with benzene–water to remove the 6-*O*-trityl derivatives. The aqueous phase was evaporated, dried, and tritylated again to remove the remaining OH-6 free derivatives. The aqueous phase (MM) was concentrated and used for h.p.l.c.

Preparation of mixtures of partially methylated methyl 6-O-trityl-α-D-galactopyranosides (TG) and partially methylated methyl 6-O-methyl-α-D-galactopyranosides (MG). — Methylsulfinyl carbanion (24 mmol) was added to a solution of methyl α-D-galactopyranoside (10.3 mmol) in dimethyl sulfoxide (9 mL). The solution was stirred for 4 h, and then methyl iodide (5 g) was added and the reaction was continued overnight at room temperature. The mixture was concentrated, and the residue dissolved in 50% aqueous methanol (30 mL) and passed through columns of Dowex

50 (H^+), Dowex 1 (OH^-), Dowex 50 (H^+), and Dowex 1 (OH^-), successively. The effluent was evaporated and dried in the presence of phosphorus pentaoxide. The residue was tritylated with chlorotriphenylmethane (3.2 g) in pyridine (18 mL) overnight at 37°. The mixture was concentrated and extracted with ether–water. The aqueous phase was evaporated, and the residue tritylated again. The final aqueous phase was a mixture of partially *O*-methylated methyl 6-*O*-methyl-α-D-galactosides (MG). The two organic phases from the tritylation were used as a mixture of partially methylated methyl 6-*O*-trityl-α-D-galactopyranosides (TG) for h.p.l.c., after removal of crystalline triphenylmethanol produced by concentration.

RESULTS AND DISCUSSION

The use of ion-exchange resins effectively removed the salts (or reagents) from the mixtures obtained by both methylation methods (Haworth and Hakomori). The 6-*O*-trityl and 6-*O*-methyl derivatives were easily separated by extraction of the methylation mixtures with ether (or benzene)–water, almost all of the 6-methyl ether derivatives (including the tetramethyl ether) were kept in the aqueous phase.

Partially methylated methyl 6-O-trityl-α-D-mannopyranosides. — The sample (TM) was chromatographed on h.p.l.c. with 7:3 (v/v) acetonitrile–water as solvent and monitoring at 220 nm, to give nine peaks (Fig. 1-A). The purity of each fraction was tested on h.p.l.c. with 7:3 (v/v) methanol–water. Fractions TM-III and TM-V gave each two fractions (III-1 and III-2, and V-1 and V-2, respectively) (Fig. 1-B and 1-C). Eight methylated sugars were expected in the TM mixture if no side-reaction had taken place during methylation, but eleven fractions were obtained on h.p.l.c. Their structures were confirmed by g.l.c. and g.l.c.–m.s.[4]. Fractions I and IX were unknown substances, and V-1 was triphenylmethanol. The other fractions were found to contain each one sugar component. The results are reported in Table I.

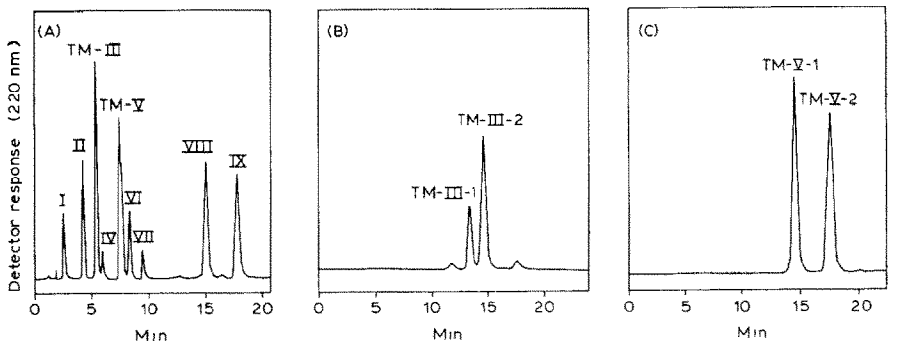

Fig. 1. H.p.l.c. of partially methylated methyl 6-*O*-trityl-α-D-mannopyranosides. (A) TM mixture in 70% acetonitrile: II, methyl 6-*O*-trityl-α-D-mannopyranoside; III-1, 3-methyl; III-2, 2-methyl; IV, 4-methyl; V-2, 2,3-dimethyl; VI, 2,4-dimethyl; VII, 3,4-dimethyl; and VIII, 2,3,4-trimethyl ether; V-1, triphenylmethanol; and I and IX, unknown; (B) Fraction III from A, in 70% methanol; and (C) Fraction V from A in 70% methanol.

TABLE I

RELATIVE YIELDS OF PARTIALLY METHYLATED METHYL α-D-MANNOPYRANOSIDES IN MIXTURES TM AND MM

Mixture TM	*Fraction*	*Relative yield*[a] (%)
Methyl *O*-methyl-6-*O*-trityl-α-D-mannopyranoside		
None	II	9
3-	III-1	12
2-	III-2	20
4-	IV	3
2,3-di-	V-2	16
2,4-di-	VI	9
3,4-di-	VII	5
2,3,4-tri-	VIII	26

Mixture MM	*Relative yield* (%)
Methyl *O*-methyl-α-D-mannopyranoside	
6-	—[b]
2,6-di-	31
4,6-di-	18
3,6-di-	23
2,4,6-tri-	14
2,3,6-tri-	11
3,4,6-tri-	3
2,3,4,6-tetra-	

[a]Calculated from peak areas in Fig. 1 for mixture TM and in Fig. 2 for mixture MM. [b]This value is excluded.

Partially methylated methyl 6-O-methyl-α-D-mannopyranosides. — The mixture MM was fractionated first with 8% acetonitrile. As the separation was not complete (Fig. 2-A), all peaks were rechromatographed with 13% acetonitrile as solvent (Fig. 2-B). Fraction MM-III gave four fractions (methyl 2,4,6-tri-*O*-methyl-, 2,3,4-tri-*O*-methyl-, 3,4,6-tri-*O*-methyl-, and 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside).

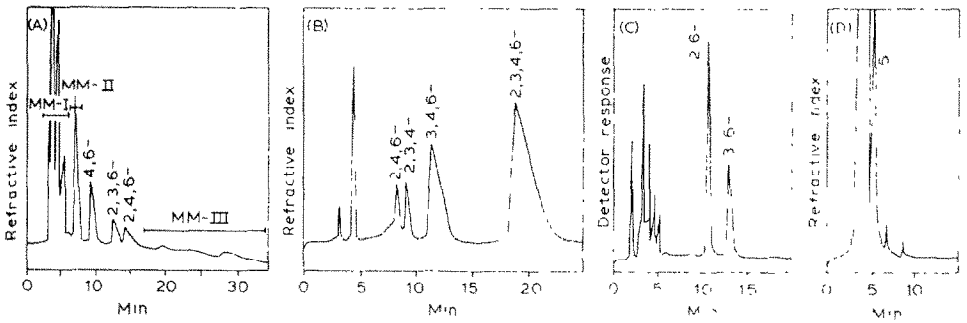

Fig. 2. H.p.l.c. of partially methylated methyl 6-*O*-methyl-α-D-mannopyranosides. (A) MM mixture in 8% acetonitrile; (B) Fraction III from A in 13% acetonitrile; (C) peracetylated Fraction II from A in 40% methanol; and (D) Fraction I from A in 20% methanol.

The presence of a small amount of 2,3,4-trimethyl ether indicated that the tritylation of the OH-6-free sugar had not been complete. Fraction MM-II included two methyl ethers (methyl 2,6-di-*O*-methyl- and methyl 3,6-di-*O*-methyl-α-D-mannopyranoside), which could be separated by h.p.l.c. of their peracetyl derivatives with 40% methanol as the mobile phase (Fig. 2-C). Methyl 6-*O*-methyl-α-D-mannopyranoside was isolated from Fraction MM-I with 20% methanol as the solvent (Fig. 2-D). The eight expected 6-methyl ethers were obtained from Fraction MM. The yields could not be calculated, because the peak of methyl 6-*O*-methyl-α-D-mannopyranoside overlapped other peaks of unknown substances, and a part of the methylated sugars might have been extracted into the organic solvent. The structures of the methylated sugars were determined by g.l.c.–m.s. of the alditol acetates[4], and the ratios of the yields are reported in Table I.

Handa and Montgomery[5] were unable to obtain the 2,3- and 4,6-di-methyl ethers from partially methylated mixtures prepared by the Haworth, Kuhn, or Hakomori method. They speculated that these two dimethyl sugars could not be detected in the methylation mixtures because of low yields, or of being rapidly changed into trimethyl derivatives, if they were produced. However, both compounds were obtained in considerable proportions in the present experiment. The difference between Handa and Montgomery's conditions[5] and the present ones probable resides in the technique of separation of the glycosides, as g.l.c. and t.l.c., which might not separate all six dimethyl derivatives, were mainly used in the earlier experiments[5].

Partially methylated methyl 6-O-*trityl*-α-D-*galactopyranosides.* — Separation of the mixture TG into each derivative with 70% acetonitrile gave five peaks (Fig. 3-A). Fractions II and III were further fractionated by h.p.l.c. (70% methanol) into three monomethyl and three dimethyl ethers, respectively (Fig. 3-B and 3-C). Structures for the components of these peaks were assigned by g.l.c.–m.s. of the alditol acetates[4], and the results are reported in Table II.

Partially methylated methyl 6-O-*methyl*-α-D-*galactopyranosides.* — Eight methyl sugars were separated from the mixture MG by h.p.l.c. with 8% acetonitrile

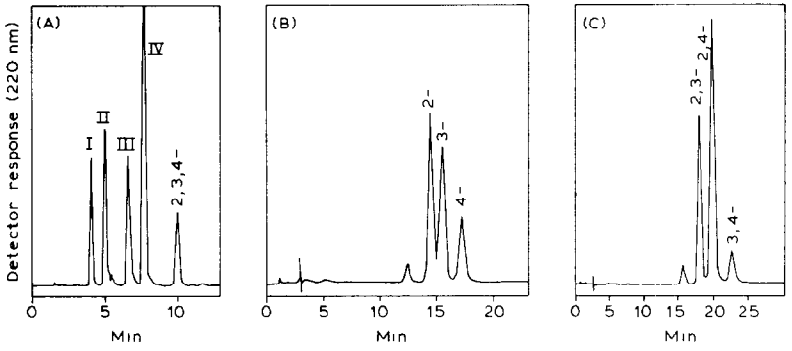

Fig. 3. H.p.l.c. of partially methylated methyl 6-*O*-trityl-α-D-galactopyranosides: (A) TG mixture in 70% acetonitrile (I, starting material; and IV, triphenylmethanol); (B) Fraction TG-II from A in 70% methanol; and (C) Fraction TG-III from A in 70% methanol.

TABLE II

RELATIVE YIELDS OF PARTIALLY METHYLATED METHYL α-D-GALACTOPYRANOSIDES IN MIXTURES TG AND MG

Mixture TG	*Relative yield*[a] (%)
Methyl *O*-methyl-6-*O*-trityl-α-D-galactopyranoside	
None	11
2-	11
3-	10
4-	6
2,3-di-	12
2,4-di-	20
3,4-di-	4
2,3,4-tri-	26

Mixture MG	*Relative yield* (%)
Methyl *O*-methyl-α-D-galactopyranoside	
6-	—[b]
2,6-di-	10
3,6-di-	5
4,6-di-	2
2,3,6-tri-	17
2,4,6-tri-	17
3,4,6-tri-	3
2,3,4,6-tetra-	46

[a]Calculated from peak areas in Fig. 3 for mixture TG, and in Fig. 4 for mixture MG. [b]This value is excluded.

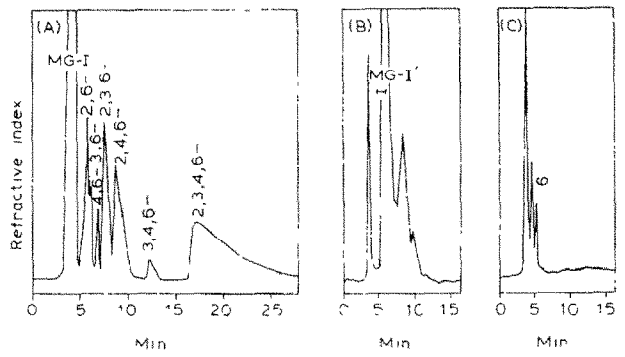

Fig. 4. H.p.l.c. of partially methylated methyl 6-*O*-methyl-α-D-galactopyranosides: (A) MG mixture in 8% acetonitrile; (B) Fraction I from A in 20% methanol; and (C) Fraction I′ from B in 10% methanol.

(Fig. 4-A). Each fraction was further purified by rechromatography. Separation of the 3,6- and 2,6-di-methyl ethers was performed with 8% methanol (data not shown). Methyl 6-*O*-methyl-α-D-galactopyranoside was isolated from the first fraction (Peak MG-I of Fig. 4-A) by chromatography with 20% and then with 10% methanol (Fig. 4-B and 4-C). The relative yields of the eight methyl sugars are reported in Table II, except for the 6-methyl ethers. Contamination with sugars having a free OH-6 was not observed.

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