of BHA or tocopherol while the formers were more effective than the latter in the case of the active oxygen method.

In conclusion, the hemin-catalyzed oxygen uptake method using Gilson respirometer is facile and gives quick results. The experimental procedure is simple, and as many as 20 samples can be evaluated simultaneously without difficulty. In the experiment to examine the antioxidant activity of tocopherol, the result obtained by this method indicated almost the same tendency as that by the active oxygen method. On the other hand, when the activity values of different type of antioxidants were compared, their effectiveness was significantly different, depending upon whether the AOM or the oxygen uptake method was used. However, it may be reasonable that the relative effectiveness of antioxidants sometimes varies with the substrate. Anyway, in order to determine the antioxidant activity of a new substance, testing by several different evaluation methods seems necessary. The present method has a possibility to detect antioxidant activity of some substances which are inactive by other evaluation methods. The method is also useful as a screening test not only for the antioxidant activity of water-soluble substances but also for that of oil-soluble substances.

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Syntheses of O-α- and O-β-D-Galactopyranosyl-(1→6)-O-[α-D-glucopyranosyl-(1→4)]-D-glucopyranoses

TAI GI CHUNG and SETSUZO TEJIMA

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

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The title branched trisaccharides were synthesized from 1,2,2′,3,3′,4′,6′-hepta-O-acetyl-β-maltose (**1**). A modified Koenigs-Knorr condensation of **1** with 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride or 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide afforded a protected trisaccharide having α-D- or β-D-galactosidic linkage at the C-6 hydroxyl group in maltose, respectively. The yield was 37 or 52% from **1**. The configuration of new galactosidic linkages in the trisaccharides was confirmed by comparison of the molecular rotation of their corresponding undecaacetates with the value of calculation. Deacetylation of the undecaacetates afforded the title trisaccharides.

Keywords——trisaccharide synthesis; branched trisaccharide; maltose; D-galactose; modified Koenigs-Knorr reaction; molecular rotation

We reported previously about syntheses of new reducing trisaccharides having α-D- and β-D-(1→6)-galactosidic linkages on the C-6′ hydroxyl group of maltose.[2] As further extension of studies on syntheses of oligosaccharides having D-galactose,[3] we synthesized now the title new branched reducing trisaccharides from maltose derivative. A few trisaccharide syntheses binding monosaccharide to the C-6 hydroxyl group of maltose have been reported: Klemer[4] synthesized 6-O-β-D-glucopyranosylmaltose, which was later synthesized from different starting material.[5]

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Our synthetic route bases on condensation of 1,2,2′,3,3′,4′,6′-hepta-O-acetyl-β-maltose (**1**)[6] with protected α-D-galactosyl halides by a modified Koenigs-Knorr reaction and sequential removal of the protecting groups. Namely, a mixture of **1** and 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl chloride (**2**)[7] was refluxed in benzene in the presence of mercuric cyanide and Drierite. After purification through a column of silica gel, the protected trisaccharide (**3**) was isolated in 37% yield from **1**. Debenzylation and sequential acetylation gave the undeca-acetate (**4**) in 89% yield. The nuclear magnetic resonance (NMR) spectrum of **4** indicated the presence of eleven acetyl groups and the signal due to the C-1 proton of the reducing terminus. The α-D-galactosidic configuration of new linkage in **4** was confirmed by comparison of the molecular rotation of **4** with the value of calculation (see Table I).

To synthesize the latter title trisaccharide, a mixture of **1** and 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (**6**)[8] was stirred in nitromethane in the presence of the same catalysts as for **3**. The condensation product was acetylated and the trisaccharide undeca-acetate (**7**) was separated from side products (fully acetylated galactose and maltose) through a column chromatography of silica gel. The yield was 52% from **1**. The NMR spectrum indicated the assigned constitution. The β-D-galactosidic configuration of the new linkage was confirmed by comparison of the molecular rotation of **7** with the value of calculation (see Table I).

In maltose or methyl β-maltoside, the C-6 hydroxyl group is less reactive than that of the C-6′ position owing to the sterically hindered environment.[9] Thus, the yields of **3** (37%) and **7** (52%) were low.

Deacetylation of **4** or **7** afforded the title trisaccharide (**5** or **8**, respectively). The product **8** was crystallized from methanol, and **5** was isolated as a hygroscopic amorphous powder.

Table I. Molecular Rotations of Compounds (**4** and **7**) Compared to the Sum of the Molecular Rotations of Constituents

Compound	$[\alpha]_D$[a]	Mol. wt.	$[M]_D$ degree $\times 10^{-2}$
1,2,2′,3,3′,4′,6′-Hepta-O-acetyl-β-maltose (**1**)	+66°	636	+419.7
Methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside[b]	+133.3°	362	+482.5
Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside[b]	−14.5°	362	−52.4
Compound **4**[c]	+114.1°	975	+1112.5
1+methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside			+902.2
Compound **7**[d]	+46.2°	966	+446.3
1+methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside			+367.3

a) Optical rotations determined in chloroform.
b) J. Swiderski and A. Temeriusz, *Carbohyd. Res.*, **3**, 225 (1966).
c) O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)]-1,2,3-tri-O-acetyl-β-D-glucopyranose hemihydrate.
d) O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)]-1,2,3-tri-O-acetyl-β-D-glucopyranose.

Experimental

Instruments used in the experimental section and the conditions for chromatography were same as reported before,[2] unless otherwise indicated. Optical rotations were measured with an automatic digital

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polarimeter, Model PM-201, Union Giken Co. Ltd., in a 0.5 dm tube. Infrared (IR) spectra were measured with a Jasco Model IRA-2 spectrometer. Thin-layer chromatography (TLC) was performed with solvent combination (v/v): (A), CH_2Cl_2–acetone (6: 1); (B), 70% 2-PrOH–AcOEt (2: 1); (C), benzene–AcOEt (5: 1). Paper partition chromatography (PPC) was performed with BuOH–pyridine–H_2O (6: 4: 3, v/v) and detection was effected with alkaline silver nitrate.[10]

1,2,2′,3,3′,4′,6′-Hepta-O-acetyl-β-maltose (1)——The product was prepared as reported previously.[6] IR ν_{max}^{KBr} cm^{-1}: 3510 (OH). NMR ($CDCl_3$) δ: 2.02, 2.03, 2.04, 2.11 (21H, all s, 7 OAc), 2.49 (1H, br. s, exchangeable with D_2O), 5.77 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc). TLC: *Rf* 0.41 (solvent A).

O-(2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl)-(1→6)-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)]-1,2,3-tri-O-acetyl-β-D-glucopyranose (3)——To a solution of **1** (700 mg, 1.1 mmol) and **2**[7] (800 mg, 1.43 mmol) in dry benzene (20 ml), mercuric cyanide (950 mg, 3.76 mmol) and Drierite (500 mg) were added. The mixture was refluxed for 24 hr with mechanical stirring under exclusion of light and moisture. After removal of insoluble material by filtration, the filtrate was washed with satd. $NaHCO_3$ and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in benzene and was purified through a column of silica gel with benzene–AcOEt (5: 1) as eluent. Removal of the solvent from the combined fraction having a single spot on TLC (solvent C) gave **3** (470 mg, 37%) as an amorphous powder, $[\alpha]_D^{22}$ +55.8° (c=1.22, $CHCl_3$). NMR ($CDCl_3$) δ: 2.00, 2.01, 2.02 (21H, all s, 7 OAc), 5.66 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 7.20—7.49 (20H, m, aromatic protons). TLC: *Rf* 0.11 (solvent C). *Anal.* Calcd. for $C_{60}H_{70}O_{23}$: C, 62.16; H, 6.08. Found: C, 61.87; H, 5.97.

O-(2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)]-1,2,3-tri-O-acetyl-β-D-glucopyranose (4)——Compound **3** (305 mg, 0.26 mmol) in MeOH (10 ml) was hydrogenated over Pd catalyst at room temperature under atmospheric pressure until absorption of H_2 ceased: the Pd catalyst was prepared[11] from $PdCl_2$ (200 mg). After removal of the catalyst by filtration, the filtrate was evaporated to dryness (210 mg), which was acetylated with Ac_2O (5 ml) and pyridine (5 ml) at room temperature overnight. Evaporation by repeated co-distillation with EtOH and toluene gave an amorphous powder. Purification through a column of silica gel with CH_2Cl_2–acetone (10: 1) as eluent gave an amorphous powder (227 mg, 89%), which crystallized from EtOH. Recrystallization from EtOH gave short needles (170 mg, 66%), mp 148—149°, $[\alpha]_D^{22}$ +114.1° (c=0.65, $CHCl_3$). NMR ($CDCl_3$) δ: 1.99, 2.02, 2.06, 2.10, 2.13, 2.17 (33H, all s, 11 OAc), 5.70 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc). TLC: *Rf* 0.51 (solvent A). *Anal.* Calcd. for $C_{40}H_{54}O_{27}\cdot 1/2H_2O$: C, 49.18; H, 5.77. Found: C, 49.05; H, 5.61.

O-α-D-Galactopyranosyl-(1→6)-O-[α-D-glucopyranosyl-(1→4)]-D-glucopyranose (5)——To a solution of **4** (110 mg, 0.11 mmol) in dry MeOH (5 ml) was added 0.1 N methanolic NaOMe (0.2 ml). The mixture was stirred for 3 hr at room temperature: complete deacetylation was monitored by TLC (solvent B). The solution was neutralized with dry Amberlite IR-120 (H^+) resin, and filtered. Evaporation of the filtrate gave **5** (54 mg, 94%), as a hygroscopic amorphous powder, $[\alpha]_D^{21}$ +155° (c=1.76, MeOH). The product reduces Fehling's solution. TLC: *Rf* 0.22 (solvent B). PPC: *Rf* 0.16. *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot H_2O$: C, 41.38; H, 6.55. Found: C, 41.60; H, 6.66. A mixture of **5** (10 mg) and 5% H_2SO_4 (5 ml) was heated at 95—100° for 6 hr, and neutralized with $BaCO_3$. The filtrate was concentrated to a thin sirup, in which glucose (*Rf* 0.41) and galactose (*Rf* 0.37) were identified by PPC.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)]-1,2,3-tri-O-acetyl-β-D-glucopyranose (7)——To a solution of **1** (2 g, 3.1 mmol) and **6**[8] (2.5 g, 6.1 mmol) in dry nitromethane (40 ml), mercuric cyanide (4 g, 15.8 mmol) and Drierite (4 g) were added. The mixture was stirred, with exclusion of light and moisture, at room temperature for 24 hr, and filtered. The filtrate was evaporated to a sirup, which was acetylated with Ac_2O (20 ml) and pyridine (20 ml). After storage at room temperature overnight, the mixture was poured into ice-H_2O, and extracted with CH_2Cl_2. The combined extracts were successively washed with H_2O, dil. H_2SO_4, H_2O, satd. $NaHCO_3$, and H_2O, dried (Na_2SO_4), and evaporated to dryness. Purification through a column of silica gel with CH_2Cl_2-acetone (10: 1) as eluent afforded an amorphous powder (1.58 g, 52%), which crystallized from EtOH–H_2O (1: 1). The product had a mp 175—176°, $[\alpha]_D^{21}$ +46.2° (c=2.38, $CHCl_3$). NMR ($CDCl_3$) δ: 1.97, 2.01, 2.04, 2.08, 2.11, 2.16 (33H, all s, 11 OAc), 5.72 (1H, d, $J_{1,2}$=7.5 Hz, H-1, β-Glc). TLC: *Rf* 0.49 (solvent A). *Anal.* Calcd. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.62. Found: C, 49.48; H, 5.34.

O-β-D-Galactopyranosyl-(1→6)-O-[α-D-glucopyranosyl-(1→4)]-D-glucopyranose (8)——Deacetylation of **7** (170 mg, 0.17 mmol) as for **4** yielded **8** (83 mg, 94%) as an amorphous powder which crystallized from MeOH, mp 168—170°, $[\alpha]_D^{22}$ +91° (c=0.99, H_2O). The product reduces Fehling's solution. TLC: *Rf* 0.20 (solvent B). PPC: *Rf* 0.16. *Anal.* Calcd. for $C_{18}H_{32}O_{16}\cdot 1/2H_2O$: C, 42.11; H, 6.48. Found: C, 42.11; H, 6.77. After acid hydrolysis, glucose and galactose were identified by PPC.

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