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Studies of Oligosaccharides. X.[1] Synthesis of Isomaltose and Isomaltotriose by Benzyl Blocking Method

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Isomaltose and isomatoltriose were synthesized unequivocally by condensation of 2-O-benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide (I) with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (II) and with O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (V), respectively, in the presence of mercuric cyanide, followed by the conventional removal of the blocking groups. Concurrent formation of the β-isomers was less than 30% of the corresponding α-linked oligosaccharides, and these concomitants were readily eliminated by column chromatography. The chromatographic behavior of these oligosaccharides are also described.

In our previous paper,[3] we reported the effective synthesis of 1,6-β-linked oligosaccharides of D-glucose series up to the hexose by Königs-Knorr condensation of the preformed oligosaccharide blocks. Our attention is now in the synthesis of 1,6-α-linked oligosaccharides by this block condensation method. There is a problem of neighboring group participation of the C-2 acetyl group in the halides, that facilitates, due to the intermediate formation of cyclic orthoesters, the rear-side attack of oxygenated nucleophiles, producing β-linked oligomers predominantly. Although the use of mercuric cyanide has been recomended to increase the formation proportion of α-linkages, the yields of α-linked products has remained still low even with the elaborate selection of the solvents.[4] Of a few attempts to diminish the neighboring effect by replacing the C-2 acetyl group with other substituent groups, we have noticed the usefulness of benzyl grouping,[5] since the benzyl derivatives are easily available

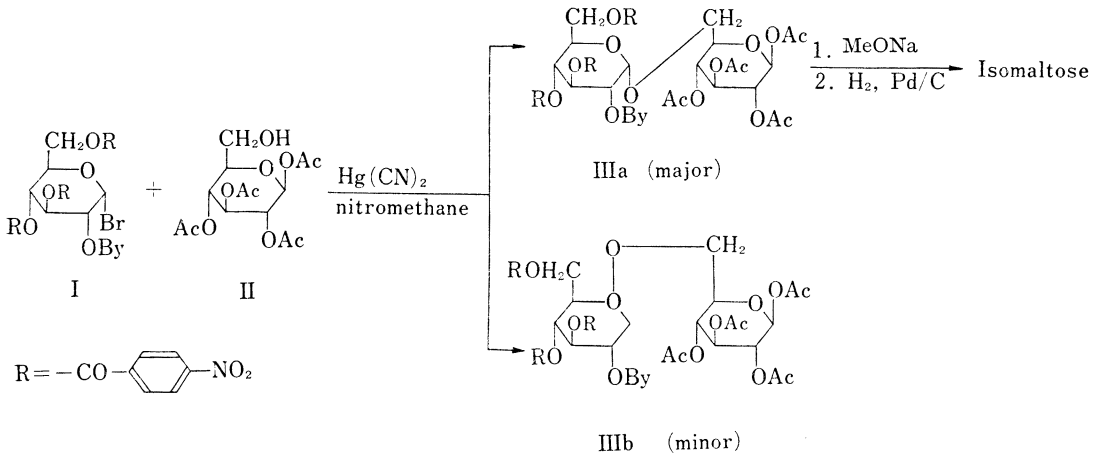

Chart 1

1) Part IX: K. Takiura, S. Honda, T. Endo, and K. Kakehi, *Chem. Pharm. Bull.* (Tokyo), **20**, 438 (1972).
2) Location: *6-1-1, Toneyama, Toyonaka, Osaka.*
3) K. Takiura, S. Honda, T. Endo, and K. Kakehi, *Chem. Pharm. Bull.*, (Tokyo) **20**, 438 (1972)
4) K. Matsuda, *Nippon Nogeikagaku Kaishi*, **33**, 714 (1959).
5) T. Ishikawa and H.G. Fletcher, *J. Org. Chem.*, **34**, 563 (1969).

and this transient blocking group is readily removable by catalytic hydrogenation. The synthesis of O-α-D-glucopyranosyl-(1→3)- and O-α-D-glucopyranosyl-(1→6)-D-galactoses[6] and O-α-L-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-D-glucose[7] by this benzyl group blocking method was reported by Flowers. The present paper describes the synthesis of isomaltose and isomaltotriose, fundamental oligomers leading to glycogen and dextran, as a preliminary study of the systematic synthesis of 1,6-linked oligosaccharides.

Condensation of 2-O-benzylated bromide I with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (II) was effected using mercuric cyanide in nitromethane. Fractionation of the product on a silca gel column afforded a pure isomaltose derivative IIIa in a yield of 32%. Concurrent production of the isomeric gentiobiose derivative IIIb was in a yield of only 6%. The proton magnetic resonance (PMR) spectrum of IIIa indicated the presence of 12 acetyl protons, 17 aromatic protons (5 for the benzyl group and 12 for the *p*-nitrobenzoyl groups), 2 methylene protons of the benzyl group and 14 ring protons. These PMR data accorded with the structure shown in Chart 1. Deacylation of IIIa in methanolic sodium methoxide, followed by the removal of the benzyl group by catalytic hydrogenation, yielded pure isomaltose in a yield of 56%. Although attempts to check the homogeneity by thin-layer chromatography, paper chromatography and paper electrophoresis were unsuccessful because of ill-separation, we found a suitable condition of liquid chromatography on an anion exchanger column for this analytical purpose. The isomaltose obtained by this method gave a single peak at a retention volume of 142 ml, while the samples prepared according to Lindberg's[8] and Wolfrom, *et al.*s'[9] methods gave a relatively intense peak of concomitant gentiobiose at 162 ml, together with a main peak of isomaltose.

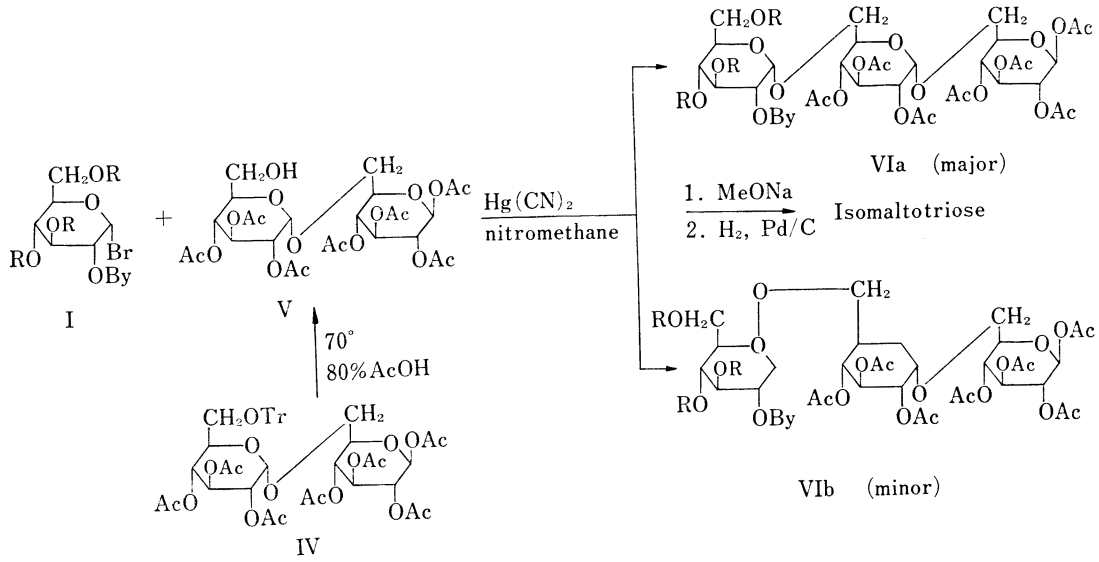

Chart 2

On this basis, we extended our work to the synthesis of isomaltotriose as shown in Chart 2. The partially blocked disaccharide derivative (V) required as one of the reactants, was conveniently prepared by a modification of the literature.[10] By conducting the detrityl-

6) H.M. Flowers, *Carbohyd. Res.*, **18**, 211 (1971).
7) H.M. Flowers, *Carbohyd. Res.*, **18**, 219 (1971).
8) B. Lindberg, *Acta Chem. Scand.*, **3**, 1350 (1949).
9) M.L. Wolfrom, A. O. Pittet, and I. C. Gillan, *Proc. Natl. Acad. Sci. U. S.*, **47**, 700 (1961).
10) N. Roy and C.P.J. Glaudemans, *J. Org. Chem.*, **33**, 1559 (1968).

ation of O-(2,3,4-tri-O-acetyl-6-O-trityl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (IV) in 80% acetic acid at 70°, the incidental cleavage of the glycosidic bond was minimized, and syrupy V, which is the smallest block in the block condensation of this series, was obtained in a yield of 72%. Condensation of I with V in a similar manner produced a mixture of two products, which was separated into its components, VIa and VIb, by fractionation on a silica gel column. The PMR spectra of both compounds presented 21 acetyl protons, 17 aromatic protons (5 for the benzyl group and 12 for the benzoyl groups), 2 methylene protons of the benzyl group and 21 ring protons, indicating the formation of the trisaccharides shown in Chart 2. Deacylation, followed by hydrolysis of the product in 1N hydrochloric acid yielded the mixtures of 2-O-benzyl-D-glucose and D-glucose, in both cases. Gas chromatographic determination indicated that the molar proportions of 2-O-benzyl-D-glucose to D-glucose were 1: 2 for both compounds. This is again a structural proof that VIa and VIb were the triose derivatives. VIa was deacylated by the conventional method and hydrogenated in the presence of palladium on charcoal as a catalyst giving the corresponding triose in amorphous state in 83% yield. This triose had a strongly dextrorotatory optical rotation and its specific rotation (+139°) accorded with the authentic data of isomaltotriose.[11] The formation of an α-linkage was further proved by negative action of β-glucosidase. Thus, we have succeeded in the first unequivocal chemical synthesis of this doubly α-linked trisaccharide. On the other hand, deblocking of VIb gave the corresponding triose, which had a specific rotation (+71.9°) lower than that of isomaltotriose, and was hydrolyzed by β-glucosidase to D-glucose and isomaltose. Consequently, it became obvious that VIb was β-D-glucosyl-isomaltose. Although paper chromatographic behavior of these two isomeric trioses and the doubly β-linked isomer, gentiotriose, was almost indistinguishable each other, liquid chromatography gave a fairly good separation of these trioses. Acetyl migration of the C-4 acetyl group to C-6 hydroxyl group, that was encountered in the synthesis of gentio-oligosaccharides,[3] suggests a possible contamination with 1,4-linked oligomers. Methylation analysis of these trioses, however, eliminated this possibility, since both trioses gave only 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucose. This is a reliable evidence that both trioses were 1, 6-linked.

Experimental[12]

Chromatography——Thin-layer chromatography (TLC) was performed on glass plates (20 × 5 cm) coated with Wakogel B-5 using a solvent system, benzene–ethyl acetate (7: 3), otherwise mentioned. Spots were visualized by spraying with concentrated sulfuric acid. Ascending paper chromatography (PC) was carried out, except for methylation analysis, on Whatman No. 1 filter paper using a solvent system, *n*-butanol–pyridine–water (6: 4: 3). For methylation analysis, descending paper chromatography was performed using *n*-butanol–ethanol–water–ammonia (40: 10: 49: 1) at 25°. R_g and R_{tmg} denote the relative mobilities to D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose, respectively. Detection was effected with alkaline silver nitrate[13] or aniline hydrogen phthalate.[14] Gas–liquid chromatography (GLC) was performed with a Shimazu GC–1C instrument equipped with a hydrogen flame ionization detector. A stainless steel column containing 3% SE-30 was used at 210°, and the carrier gas (N_2) was regulated at a flow rate of 60 ml/min. Liquid chromatography (LC) was performed with a JEOL-3BC apparatus. A glass column (0.8φ × 15 cm) packed with JEOL LC–R–3 resin was used at 55°, and the column was eluted with pH 9.0 (88 ml), followed by pH 9.6 borate buffer, at a flow rate of 0.49 ml/min. Samples were applied in pH 9.6 buffer solution, and the effluent colored with the orcinol — sulfuric acid reagent was submitted automatically to spectrophotometric analysis at 440 nm.

11) J.R. Turvey and W.J. Whelan, *Biochemistry*, **67**,49 (1957).
12) Melting points were determined on a hot stage using a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were measured in a 1 dm tube. PMR spectra were measured at 60 MHz on a Hitachi R-20A spectrometer, and chemical shifts are expressed on the τ-scale in ppm for deuteriochloroform solutions with tetramethylsilane as the internal standard. All evaporations were carried out below 40° under diminished pressure.
13) W.E. Trevelyan, D.P. Procter, and J. S. Harrison, *Nature*, **166**, 444 (1950).
14) S.M. Partridge, *Nature*, **164**, 443 (1949).

2-O-Benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide (I)——This bromide was prepared by a slight modification of the literature;[5] 2-O-benzyl-D-glucose (2.0 g, 7.4 mmoles) and *p*-nitrobenzoyl chloride (7.4 g, 30 mmoles) were added to pyridine (50 ml) at 10—15° and the mixture was allowed to stand overnight. The reaction mixture was poured into a cold aqueous sodium bicarbonate solution (1 liter) to give a crystalline precipitate. The precipitate was collected and fractionated on a silica gel column (Wakogel C-200, 200 g) using chloroform as an eluant. 2-O-Benzyl-1,3,4,6-tetra-O-*p*-nitrobenzoyl-α-D-glucopyranose and its β-isomer were obtained in yields of 67% and 13%, respectively. The α-anomer (1.0 g, 1.2 mmoles) was dissolved in a saturated solution of HBr in dichloromethane (50 ml) at room temperature. After 1.5 hr, the precipitate of *p*-nitrobenzoic acid was removed by filtration, and the filtrate was washed with ice-water, followed by aqueous Na_2SO_4, and evaporated to dryness. The residual syrup was purified on a silica gel column (Wakogel C-200, 20 g) using dichloromethane as an eluant. Recrystallization of the product from dichloromethane–hexane afforded 2-O-benzyl-3,4,6-tri-O-α-D-glucopyranosyl bromide (I, 0.7 g, 67%) as needles, mp (decomp.) 155—156°; $[\alpha]_D^{25}$ +75.5° (c=1.0, dichloromethane). Its PMR spectrum indicated a doublet of H-1 ($J_{1,2}$=4.0 Hz) centered at 3.49. *Anal.* Calcd. for $C_{34}H_{26}O_{14}N_3Br$: C, 52.32; H, 3.36; N, 5.38; Br, 10.24. Found: C, 52.25; H, 3.36; N, 5.24; Br, 10.15. Although the β-anomer of the bromide was reported to be obtained in a considerable yield in the literature, we could not find it in the mother liquor at all.

O-(2-O-benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (IIIa)——1,2,3,4-Tetra-O-acetyl-β-D-glucopyranose (II, 212.4 mg, 0.6 mmoles) was dissolved in anhydrous nitromethane (8 ml), and to this solution were added pulverized Drierite (500 mg) and mercuric cyanide (151.2 mg, 0.6 mmoles). After stirring the mixture for 30 min, the bromide I (300 mg, 0.4 mmoles) was added. Stirring was continued at room temperature for 5 days, and the reaction mixture was filtered. The filtrate was washed with water, followed by aqueous sodium bicarbonate solution. The solution was evaporated to dryness and the residual syrup was fractionated on a silica gel column (Wakogel C-200, 30 g) using benzene–ethyl acetate (9: 1) as an eluant. Compound IIIa (130 mg, 32%) was obtained as amorphous powder, $[\alpha]_D^{22}$ +85.5° (c=1.1, chloroform). *Anal.* Calcd. for $C_{48}H_{45}O_{21}N_3$: C, 55.01; H, 4.33; N, 4.01. Found: C, 55.35; H, 4.28; N, 3.85. PMR: 3.99 (H-1, doublet, $J_{1,2}$=8.7 Hz); 7.99 (12 acetyl protons); 2.85 (5 benzyl ring protons); 1.83 (12 benzoyl protons).

As a by-product, the isomeric O-(2-O-benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (IIIb) was obtained in a yield of 6%, $[\alpha]_D^{19}$ −21° (c=1.1, chloroform).

O-α-D-Glucopyranosyl-(1→6)-D-glucose (Isomaltose)——Compound (IIIa) (100 mg) was stirred in 0.2 N methanolic sodium methoxide (5 ml). Immediately after dissolution, O-(2-O-benzyl-α-D-glucopyranosyl)-(1→6)-D-glucose was precipitated as amorphous solid, which was dissolved, after standing at room temperature for 2 hr, by addition of a small amount of water. The solution was decationized by stirring with Amberlite IR-120 (H^+ form), and evaporated to dryness. *p*-Nitrobenzoic acid separated was removed by extraction with ether. The aqueous layer was evaporated to dryness, and the residual syrup was dissolved in 80% acetic acid (20 ml). This solution was hydrogenated under the catalyst of 10% Pd-C (20 mg) at 5 atm for 18 hr. Isomaltose (15 mg, 56%) was obtained as a syrup which gave a single spot on PC (Rg 0.56) and a single peak on LC (retention volume, 142 ml), $[\alpha]_D^{22}$ +120° (c=1.2, water). *Anal.* Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 41.98; H, 6.53.

O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (V)——This compound was prepared by a modification of the method described in the literature.[10] O-(2,3,4-Tri-O-acetyl-6-O-trityl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (IV, 8.0 g) was suspended in 80% acetic acid (120 ml) at 70° and stirred for 1.5 hr, during wihch time the tritylate was dissolved, and triphenyl carbinol precipitated gradually. The reaction mixture was extracted with chloroform (500 ml) and the exttact was washed three times with water (200 ml). The solvent was evaporated to dryness and the residual syrup was purified by silica gel column chromatography (Wakogel C-200, 150 g) using benzene–ethyl acetate (1: 1) as an eluant. Compound V (5.0 g, 72%) was obtained as a colorless syrup, $[\alpha]_D^{19}$ +118° (c=2.0, chloroform). *Ancl.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 49.15; H, 5.63. PMR: 3.76 (H-1, doublet, $J_{1,2}$=7.5 Hz); 6.87 (OH).

O-(2-O-Benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-α-D-glucopyranosyl)-, and O-(2-O-benzyl-3,4,6-tri-O-*p*-nitrobenzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-α-D-glucopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose, (VIa) and (VIb)——Compound V (6.0 g, 9 mmoles) was dissolved in anhydrous nitromethane (100 ml), and to this solution were added pulverized Drierite (3.7 g) and mercuric cyanide (3.0 g, 1.2 mmoles). After stirring the mixture for 2 hr, the bromide I (7.0 g, 0.9 mmoles) was added. Stirring was continued at room temperature for 5 days, until the spot of the bromide on TLC disappeared. The reaction mixture was filtered, and the filtrate was washed with water, followed by aqueous sodium bicarbonate solution. After evaporation of the solvent to dryness, the residual syrup was fractionated on a silica gel column (Wakogel C-200, 150 g) using benzene–ethyl acetate (7: 3) as an eluant. The compounds, VIa and VIb were separated as amorphous powder in yields of 61% and 17%, respectively.

VIa: $[\alpha]_D^{17}$ +82.4° (c=1.27, chloroform). *Anal.* Calcd. for $C_{60}H_{61}O_{23}N_3$: C, 53.93; H, 4.60; N, 3.15. Found: C, 53.60; H, 4.48; N, 3.12. PMR: 3.70 (H-1, doublet, $J_{1,2}$=7.0 Hz); 7.99 (21 acetyl protons); 2.83 (5 benzyl ring protons); 1.83 (12 benzoyl protons).

VIb: $[\alpha]_D^{12}$ +46.5° (c=1.29, chloroform). *Anal.* Calcd. for $C_{60}H_{61}O_{23}N_3$: C, 53.93; H, 4.60; N, 3.15. Found: C, 53.62; H, 4.58; N, 2.97. PMR: 3.74 (H-1, doublet, $J_{1,2}$=7.0 Hz); 8.00 (21 acetyl protons); 2.81 (5 benzyl ring protons); 1.85 (12 benzoyl protons).

Compound (VIb) (270 mg) was stirred in 0.2 N methanolic sodium methoxide (10 ml). Immediately after dissolution, O-(2-O-benzyl-α-D-glucopyranosyl)-(1→6)-O-(α-D-glucopyranosyl)-(1→6)-D-glucose was precipitated, which was dissolved, after standing at room temperature for 2 hr, by addition of a small amount of water. The solution was decationized by stirring with Amberlite IR-120, and evaporated to dryness. *p*-Nitrobenzoic acid separated was removed by extraction with ether, and the aqueous layer was evaporated to dryness. The deacylated compound (110 mg, 91%) was obtained as amorphous powder, $[\alpha]_D^{19}$ +93° (c= 1.05, water). *Anal.* Calcd. for $C_{25}H_{38}O_{16}$: C, 50.50; H, 6.44. Found: C, 50.44; H, 6.19. For analytical purpose this deacylated compound (3 mg) was hydrolyzed in 1 N HCl on a boiling water bath for 6 hr, followed by evaporation of the solvent. The hydrolysate was trimethylsilylated for GLC. GLC determination indicated that the proportion of peak areas of D-glucose to 2-O-benzyl-D-glucose were 1: 0.43. The deacylated compound (100 mg) was dissolved in 80% acetic acid, and hydrogenated under the catalyst of 10% Pd-C (50 mg) at 25 atm for 48 hr at room temperature. Isomaltotriose (69 mg, 83%) was obtained as amorphous powder and was shown to be homogeneous on PC (R_g 0.22) and gave a single peak on LC (retention volume, 120 ml), $[\alpha]_D^{21}$ +139° (c=0.5, water). *Anal.* Calcd. for $C_{18}H_{32}O_{16}$: C, 40.00; H, 6.71. Found: C, 39.65; H, 6.85. This triose (1 mg) and β-glucosidase (1 mg), Sigma Chemicals Co., Ltd., lot 125B-0100, prepared from almond) were dissolved in 1 ml of 0.05 M acetate buffer (pH 5.0), and the solution was incubated at 40°. PC indicated that this compound was not hydrolyzed at all by β-glucosidase after incubation for 16 hr. Maltose under the same condition was also unchanged after 70 hr.

Methylation[15] of this triose yielded a syrupy substance, which, on subsequent hydrolysis in 5% HCl under reflux for 6 hr, gave two spots (R_{tmg} 0.90, 1.00) on PC. R_{tmg} of authentic 2,3,4-tri-O-methyl-D-glucose, 0.90.

Compound (VIb) (580 mg) was deacylated in a similar manner as in VIa. The deacylated compound (232 mg, 90%) was obtained as amorphous powder, $[\alpha]_D^{23}$ +52.6° (c=1.4, water). *Anal.* Calcd. for $C_{25}H_{38}O_{16}$: C, 50.50; H, 6.44. Found: C, 50.37; H, 6.21. GLC examination of the hydrolysate of this deacylated products also gave the same result as the result in the examination of compound VIa. The deacylated compound (170 mg) was catalytically hydrogenated in a similar manner described and the corresponding triose (137 mg, 95%) was obtained, which was homogeneous on PC (R_g 0.22) and gave a single peak on LC (retention volume, 124 ml), $[\alpha]_D^{22}$ +71.9° (c=1.1, water). *Anal.* Calcd. for $C_{18}H_{32}O_{16}$: C, 40.00; H, 6.71. Found: C, 39.70; H, 6.52. Incubation of the resulting triose with β-glucosidase and PC examination of the hydrolysate in a similar manner described for VIa indicated that this compound was hydrolyzed completely to D-glucose and maltose after 16 hr. Methylation of this triose yielded a syrupy substance which, on subsequent hydrolysis in 5% HCl under reflux for 6 hr, gave two spots (R_g 0.90, 1.00) on PC, corresponding to 2,3,4-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose, respectively.

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15) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).