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PREVENTION OF OVEROXIDATION IN PERIODATE OXIDATION OF REDUCING OLIGOSACCHARIDES BY THEIR CONVERSION INTO 1,5-ANHYDROALDITOL DERIVATIVES*

SUSUMU HONDA†, KAZUAKI KAKEHI, AND YSOYE KUBONO
Faculty of Pharmaceutical Sciences, Kinki University, Kowakae, Higashi-osaka (Japan)
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ABSTRACT

A procedure for the conversion of reducing oligosaccharides into their 1,5-anhydroalditol derivatives was devised to prevent overoxidation during periodate oxidation. Gas-chromatographic analysis of the aldehydes in the products of complete oxidation of the resultant 1,5-anhydroalditol derivatives by the dithioacetal method[1b] indicated that new types of dialdehyde characteristic of the linkage-types were formed, together with ordinary simple aldehydes. A number of D-gluco-oligosaccharides having various types of interglycosidic linkage were examined by this method. The results were consistent with expectations.

INTRODUCTION

Periodate oxidation is a powerful tool for structural studies of carbohydrates, but there is an annoying problem of overoxidation of reducing carbohydrates that frequently makes interpretation of experimental results difficult. In one extreme example[1b], as much as 76% of the reducing D-glucose residue in gentiobiose was reported to have been oxidized to the glycolaldehyde stage because of hydrolysis of the formic ester group, when the disaccharide was oxidized in a non-buffered, aqueous solution of 0.05M sodium metaperiodate for 24 h at 25°. Several attempts have been made to prevent overoxidation by kinetic control at suitable temperature and pH values[2,3], but it seems impossible to establish general conditions for ideal Malapradian oxidation, that would be generally applicable to a wide variety of reducing carbohydrates.

Recently, we developed a simple and rapid gas-chromatographic method (the dithioacetal method[1b]) for simultaneous determination of the conjugated aldehydes in products of oxidation, and applied this method to the study of interglycosidic linkages in various carbohydrates. For such component analysis, it is desirable to

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†To whom all correspondence should be addressed.

protect the reducing ends by appropriate means and then oxidize the stable derivatized products as completely as possible. This paper describes a simple procedure for conversion of the reducing monosaccharide residues in oligosaccharides into 1,5-anhydroalditols via the acetylated glycosyl bromides. The products of complete oxidation of the 1,5-anhydroalditol derivatives contain aldehydes characteristic of the type of interglycosidic linkage. This paper also describes the results of analysis of these products by the dithioacetal method.

RESULTS AND DISCUSSION

Reducing oligosaccharides may be converted into the corresponding 1,5-anhydroalditol derivatives by sequential acetylation, hydrobrominolysis, and reduction with lithium aluminium hydride[4]. Table I summarizes the stepwise yields of the products under various reaction conditions starting from gentiobiose, which was chosen as the model oligosaccharide.

Of the two acetylating agents tested, acetic anhydride–pyridine was the more effective. More than 80% of the gentiobiose was acetylated in 1 h, and the yield of acetate remained almost constant for an additional h. Therefore, acetylation was performed with 1:1 (v/v) acetic anhydride–pyridine for 1 h at 90°. Hydrolysis of the glycosidic linkage was negligible under these acetylation conditions. Only D-glucose and 1,5-anhydro-D-glucitol were found in the hydrolyzate of the final product of this series of reactions. The absence of 1,4-anhydro-D-glucitol indicates that the reducing end of the acetate was exclusively pyranoid. Furthermore, neither (1→2)- nor (1→3)-linked glucobioses gave 1,4-anhydro-D-glucitol as a product. Formation of the furanoid ring from the (1→4)-linked glucobioses is theoretically impossible.

TABLE I

OPTIMIZATION OF DERIVATIZATION CONDITIONS[a]

Chemical process	*Reagent*	*Solvent*	*Reaction temperature (°)*	*Reaction time (h)*	*Yield (%)*
Acetylation	Ac_2O	pyridine	90	1	82
			90	2	81
	Ac_2O–NaOAc		90	1	55
			90	2	78
Hydro-brominolysis	HBr	$CHCl_3$-AcOH	0	1	105
Reduction	$LiAlH_4$	1,4-dioxane	25	1	10
		ether	25	1	79
		oxolane	25	1	79
		1,2-dimethoxyethane	25	1	78

[a]For the reaction: gentiobiose —acetylation→ gentiobiose octaacetate —hydrobrominolysis→ acetylated gentiobiosyl bromide —reduction→ 1,5-anhydro-gentiobiitol

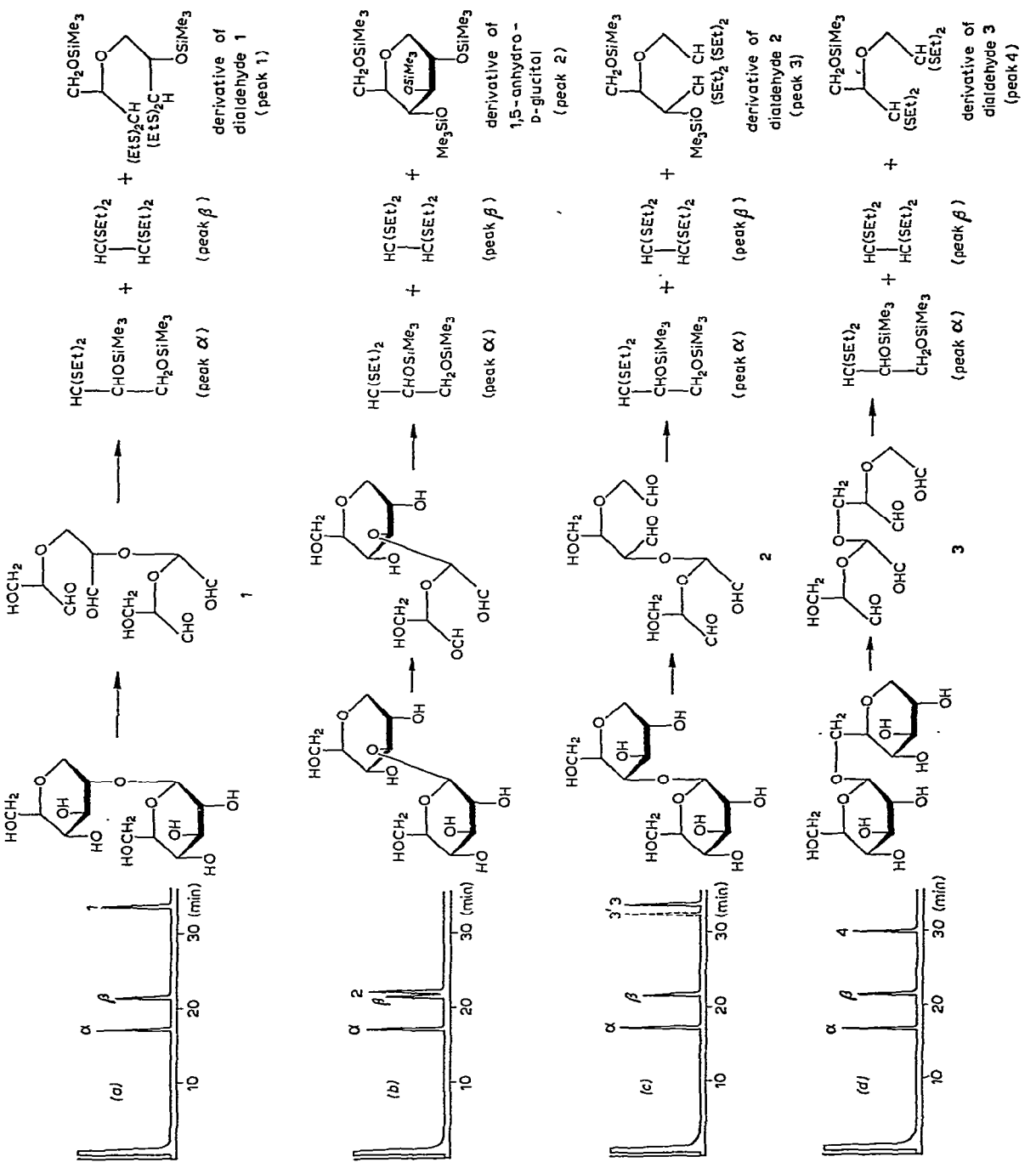

Fig. 1. Gas-chromatographic analysis of the products of periodate oxidation of D-glucopyranosyl-1,5-anhydro-D-glucitols. (*a*) 1,5-Anydrosophoritol; (*b*) 1,5-anhydrolaminarabiitol; (*c*) 1,5-anhydrocellobiitol; and (*d*) 1,5-anhydrogentiobiitol. Peak assignments are shown in the scheme. Peak 3' is of the dimethyl acetal of the diethyl dithioacetal of dialdehyde 2.

TABLE II

DETERMINATION OF THE TYPES OF GLYCOSIDIC LINKAGE IN VARIOUS GLUCOBIOSES BY THEIR CONVERSION INTO 1,5-ANHYDRO-D-GLUCITOL DERIVATIVES, FOLLOWED BY OXIDATION

Glucobiose	*Type of linkage*	*Aldehydes from the non-reducing* D-*glucose residue*		*Aldehydes from the reducing* D-*glucose residue*	
		D-*Glyceraldehyde*	*Glyoxal*		
Sophorose	1→2	1	0.9	dialdehyde **1**	0.9
Laminarabiose	1→3	1	1.0	1,5-anhydro-D-glucitol	0.9
Cellobiose	1→4	1	1.0	dialdehyde **2**	0.8
Gentiobiose	1→6	1	1.0	dialdehyde **3**	1.0

Conventional hydrobrominolysis of gentiobiose octaacetate with hydrogen bromide in cold acetic acid gave acetylated gentiobiosyl bromide in almost nearly quantitative yield with only a little hydrolytic cleavage of the glycosidic linkage.

1,4-Dioxane, ether, oxolane, and 1,2-dimethoxyethane were evaluated as solvents for reduction of the bromide with lithium aluminum hydride. 1,4-Dioxane gave a low yield of 1,5-anhydro-gentiobiitol because both the reagent and the sugar derivative were sparingly soluble in this solvent. The yields of 1,5-anhydro-gentiobiitol in ether, oxolane, and 1,2-dimethoxyethane were approximately the same. However, ether is immiscible with water and forms two layers during processing, so that g.l.c. analyses were not reproducible. Oxolane was undesirable, as it is a poor solvent for the glycosyl bromide. 1,2-Dimethoxyethane proved the best solvent for the reduction, giving 79% of 1,5-anhydrogentiobiitol after reduction for 1 h. Losses were probably due to adsorption of part of the product on the surface of the precipitate of bulky aluminum hydroxide, but the precision of the analysis was excellent.

Fig. 1 shows the gas chromatograms of the dithioacetal derivatives of the products of periodate oxidation of the 1,5-anhydro-*O*-D-glucopyranosyl-D-glucitols obtained from various β-linked glucobioses. The molar proportions of products are given in Table II. The peaks (α and β) at the retention times(T_R) of 0.89 and 1.10 relative to trimethylsilylated xylitol, are common to all these glucosyl anhydroglucitols, and are assigned to 1,1-bis(ethylthio)-2,3-bis(trimethylsilyloxy)propane and 1,1,2,2-tetrakis(ethylthio)ethane, respectively, which arise from the D-glucose residue. Peaks 1, 3, and 4 were assigned to the trimethylsilylated diethyl dithioacetals of the dialdehydes formed from the anhydroglucitol residue. Dialdehydes **1** and **2** are positional isomers, and their peaks (1 and 3, respectively) are indistinguishable from each other (T_R 1.73). However, the dialdehyde **2** from 1,5-anhydrocellobiitol [(1→4)-linkage] was readily acetalated (presumably at O-2 and O-4 of the D-erythrose moiety) by treatment with acidified 1,1-dimethoxyethane, and the acetalated diethyl dithioacetal formed gave rise to peak 3′ at shorter retention-time (T_R 1.69), whereas the dial-

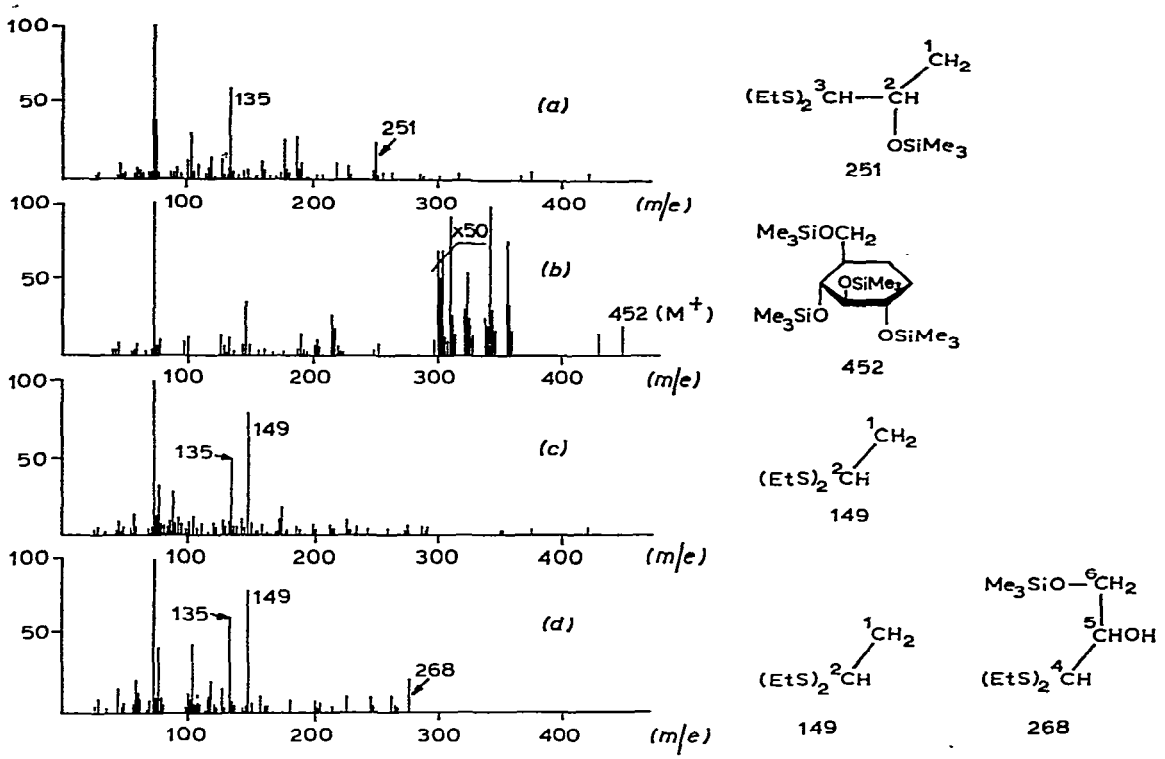

Fig. 2. Mass spectra of the compounds of peaks 1, 2, 3, and 4. (*a*) peak 1, (*b*) peak 2, (*c*) peak 3, (*d*) peak 4.

dehyde **1** from 1,5-anhydrosophoritol [(1→2)linkage] was immune to acetalation, so that its peak remained at the same retention time. The dialdehyde **3** from 1,5-anhydrogentiobiitol [(1→6)linkage] has fewer carbon atoms than dialdehydes **1** and **2**, and peak 4 of its derivative was observed at T_R 1.54, well separated from the other peaks. With 1,5-anhydrolaminarabiitol [(1→3)-linkage], the anhydroglucitol residue is not attacked by periodate, and trimethylsilylated 1,5-anhydro-D-glucitol was detected at T_R 1.13 as peak 2.

Mass spectra also served for identification of the peaks (Fig. 2). Peak 2 gave the molecular ion at *m/e* 452. The other peaks did not give molecular ions, but each of them showed characteristic fragment(s) (251 for peak 1; 149 for peak 3; 149 and 268 for peak 4), together with the common peak of $^{+}CH(SEt)_2$ at *m/e* 135. The structures proposed for these fragments are shown in Fig. 2.

Thus, the positions of attachment of the interglycosidic linkages in these β-linked 1,5-anhydro-D-*O*-glucopyranosyl-D-glucitols could be determined readily by analyzing the aldehydes in the products of their oxidation. Similarly, two 1,5-anhydro-*O*-hexopyranosyl-D-glucitols (A and B), isolated previously in our laboratory from the roots of *Polygala senega* and given the structures *O*-α-D-galactopyranosyl-(1→2)-1,5-anhydro-D-glucitol and *O*-β-D-glucopyranosyl-(1→6)-1,5-anhydro-D-gluci-

TABLE III

ANALYSIS[a] OF THE INTERGLYCOSIDIC LINKAGES IN VARIOUS GLUCO-OLIGOSACCHARIDES BY THEIR CONVERSION INTO 1,5-ANHYDRO-D-GLUCITOL DERIVATIVES, FOLLOWED BY OXIDATION

Oligosaccharide	*Aldehydes from the non-reducing terminal and interior* D-*glucose residues*			*Aldehydes from the reducing* D-*glucose residue*
	D-*Glycer-aldehyde*	D-*Erythrose*	*Glyoxal*	
Laminaratriose	1 (1)	0.0 (0)	1.0 (1)	1,5-anhydrolaminarabiitol detected (1)
Cellotriose	1 (1)	0.9 (1)	2.0 (2)	dialdehyde **2** 1.0 (1)
Cellotetraose	1 (1)	1.9 (2)	2.9 (3)	dialdehyde **2** 0.9 (1)
Gentiotriose	2 (2)	0.0 (0)	2.0 (2)	dialdehyde **3** 0.9 (1)
Gentiotetraose	3 (3)	0.0 (0)	3.0 (3)	dialdehyde **3** 0.9 (1)

[a]The numbers in parentheses are theoretical values.

tol (1,5-anhydrogentiobiitol), respectively[5], were examined by this method. As expected, compound A yielded the D-glyceraldehyde and glyoxal derivatives and the compound of peak 1, all in equimolar proportions. Compound B gave equimolar amounts of the D-glyceraldehyde and glyoxal derivatives and the compound of peak 4; that is, the same aldehyde distribution as observed for the synthetic 1,5-anhydrogentiobiitol. Although previous studies by the (2,4-dinitrophenyl)hydrazone method[6] had indicated that compound B gave a value of approximately 3 for the D-glyceraldehyde–glyoxal molar ratio, D-glyceraldehyde might have been overestimated because of overlap of the chromatographic zone of its hydrazone and that of the bis(hydrazone) of the dialdehyde from the anhydroglucitol residue. On the other hand, the dialdehyde from the anhydroglucitol residue of compound A might give a bis(hydrazone) having a mobility almost identical with that of the hydrazone of glycolaldehyde, which would result in overestimation of the glycolaldehyde content, as reported in the previous paper[6]. In these earlier studies, the dialdehyde compounds from the anhydroglucitol residue were presumed to be hydrolyzed at the O-1–C-5 bond (as numbered for the original sugars) to give the hydrazones of their component aldehydes when treated with the hydrazine hydrochloride, but this is unlikely to be so, considering the results obtained by the dithioacetal method.

Table III shows further results obtained for higher oligomers of D-glucose. Qualitative analysis of the products derived from the anhydroglucitol residue unequivocally establishes the position of the glycosidic linkage attached to the reducing D-glucose residue. Thus, the detection of peak 3 from the products of oxidation of cello-oligosaccharides, and therefore the presence of dialdehyde **2** in these products, indicates that the 4-position of the reducing D-glucose residue is glycosylated by the neighboring D-glucose residue. Similarly, the detection of peak 4 from the products of oxidation of gentio-oligosaccharides, and thus the presence of dialdehyde **3** in the products, is indicative of the (1→6)linkage between the reducing D-glucose residue

and the neighboring D-glucose residue. The molar proportions of such simple aldehydes as D-glyceraldehyde, D-erythrose, and glyoxal indicate the positions of attachment of other glycosidic linkages. The anhydroglucitol and the interior D-glucose residues in the 1,5-anhydroalditol derivative of laminaratriose, a homogeneously (1→3)-linked glucotriose, were not attacked by the oxidant, so that this portion was detected as the slowest-eluted peak of trimethylsilylated 1,5-anhydrolaminarabiitol. The results in Table III indicate that all of the distributions of aldehydes obtained for these oligomers were consistent with their structures.

EXPERIMENTAL

Materials. — Reagents used were of the highest grade commercially available. Solvents were dehydrated and distilled before use. Sophorose was prepared by the Koenigs–Knorr condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 1,3,4,6-tetra-*O*-acetyl-α-D-glucose, followed by deacetylation[7]. The samples of gentiooligosaccharides were synthesized in our laboratory[8]. Crystalline gentiobiose octaacetate and acetylated gentiobiosyl bromide were intermediates in these syntheses. The samples of laminara-oligosaccharides were prepared by fractionation of an acetolyzate of pachyman on a column of silica gel, followed by deacetylation[9]. The samples of cello-oligosaccharides were similarly prepared from cotton wool. Authentic specimens of 1,4- (ref. 10) and 1,5- (ref. 4) anhydro-D-glucitols were prepared by published methods. The samples of 1,5-anhydro-sophoritol, -laminarabiitol, -cellobiitol, and -gentiobiitol were obtained by hydrobrominolysis of the crystalline peracetates of the corresponding disaccharides, with subsequent reduction by lithium aluminum hydride as described for 1,5-anhydro-D-glucitol[4].

Apparatus. — Gas chromatography was performed on a Shimadzu 4BMPF instrument equipped with a hydrogen flame-ionization detector. A glass column (0.3 cm i.d., 2 m long) packed with 3% silicone OV-1 on Chromosorb W was used, and the carrier gas (nitrogen) was regulated at a flow rate of 50 mL/min throughout the work. For analysis of the hydrolyzates of 1,5-anhydroalditol derivatives, the column was maintained at 180°. For analysis of the conjugated aldehydes in the products of periodate oxidation, however, a temperature gradient of 100→250° (5°/min) was initiated immediately after injection of a sample. Peaks were integrated by a Shimadzu ITG 2A integrator. Gas chromatography–mass spectrometry was performed on a Shimadzu LKB 9000B spectrometer, and the conditions for the column were similar to those already described. The temperature of the ion source was 270°, and the mass spectra were obtained at 70 eV. The data were plotted as bar graphs by a data-transmission system equipped with a Shimadzu GC-MS 300D computer.

Optimization of conditions of individual derivatization reactions. — As direct determination of gentiobiose octaacetate (for acetylation), hepta-*O*-acetylgentiobiosyl bromide (for hydrobrominolysis), and 1,5-anhydrogentiobiitol (for reduction)

was difficult, the reaction conditions for individual processes were optimized as follows.

Firstly, hepta-*O*-acetylgentiobiosyl bromide (0.1–1 μmol) was dissolved in 100 μL of one of the solvents, given in Table I, that had been saturated with lithium aluminum hydride, and the mixture was kept for 1 h. Water (400 μL) was cautiously added to the mixture with cooling, the mixture was deionized by passage through a small column of Amberlite CG-120 (H^+ form, 0.5 mL) and CG-400 (OAc^- form, 0.5 mL) resins, and the column was washed with water (30 mL). The combined eluate and the washings were evaporated, and the residual syrup was dissolved in 0.5M sulfuric acid (200 μL) in a small ampoule, which was flushed with nitrogen, shielded, and heated for 3 h on a boiling-water bath. The ampoule was opened, the solution was deionized in a similar manner, and the combined eluate and the washings were evaporated. The syrupy residue was dissolved in pyridine (50 μL) containing D-mannitol (internal standard), and trimethylsilylated with hexamethyldisilazane (100 μL) and chlorotrimethylsilane (50 μL) for 30 min at 50°. The mixture was centrifuged and the supernatant (1 μL) was analyzed by gas chromatography. The retention time and molar response factor of trimethylsilylated 1,5-anhydro-D-glucitol relative to trimethylsilylated D-mannitol were 0.64 and 0.68, respectively. The relative retention time for trimethylsilylated 1,4-anhydro-D-glucitol was 0.39. Trimethylsilylated D-glucose gave two peaks, at T_R 0.80 and 1.18, and its total molar response factor (m.r.f.) was 0.73. The yield of 1,5-anhydrogentiobiitol may be regarded as that of 1,5-anhydro-D-glucitol, as both hydrolysis and trimethylsilylation are considered to proceed almost quantitatively under these conditions. The molar amounts of D-glucose and 1,5-anhydro-D-glucitol were approximately the same in all instances.

Secondly, gentiobiose octaacetate (0.1–1 μmol) was dissolved in cold acetic acid (150 μL) saturated with hydrogen bromide, and the solution was kept for 1 h at 0°. Chloroform (200 μL) and ice–water (300 μL) were added, and the mixture was shaken vigorously. The aqueous layer was discarded, and the chloroform layer was washed three times with ice–water (300 μL), dried with anhydrous sodium sulfate (100 mg), and evaporated to dryness. The residual syrup was then reduced with lithium aluminum hydride in 1,2-dimethoxyethane, the product was hydrolyzed, and the hydrolyzate was analyzed by gas chromatography after trimethylsilylation in the same manner as already described. The yield of 1,5-anhydro-D-glucitol (87%) was slightly greater than that of D-glucose found (83%), but D-glucitol was not detected. Accordingly, it seems that a part of gentiobiose octaacetate was split during hydrobrominolysis and the resultant D-glucose acetates were completely converted into tetra-*O*-acetyl-α-D-glucosyl bromide, which would yield the excess of 1,5-anhydro-D-glucitol. The yield of hepta-*O*-acetylgentiobiosyl bromide may be regarded as the yield of D-glucose divided by the yield of the reduction process, namely 79% (Table I), and the degree of hydrolytic cleavage of the glycosidic linkage is estimated to be approximately one half of the difference in the yields between 1,5-anhydro-D-glucitol and D-glucose. The observed values were 105 and 2%, respectively.

Finally gentiobiose (0.1–1 μmol) was acetylated with 200 μL of one of the

acetylating agents given in Table I. The mixture was cooled, chloroform (200 μL) and water (300 μL) were added, and the mixture was processed as described for the product of hydrobrominolysis. The residual syrup obtained was subjected to sequential hydrobrominolysis, reduction with lithium aluminum hydride in 1,2-dimethoxyethane, hydrolysis, and trimethylsilylation, and the final product was analyzed by gas chromatography as already described. The yields of gentiobiose octaacetate were calculated as the yields of D-glucose divided by the product of the yields of the hydrobrominolysis and reduction processes, namely 105 and 79%. The degree of hydrolysis of the glycosidic linkage during these three sequential reactions, estimated as described for the product of hydrobrominolysis, was 2% for both examples given in Table I. As this value was identical with that already described for the hydrolysis during hydrobrominolysis, the degree of the hydrolysis during acetylation is considered to be negligibly small.

Conversion of reducing oligosaccharides into their 1,5-anhydroalditol derivatives. — The standard procedure was as follows. A sample of oligosaccharide (0.1–1 μmol) was dissolved in a 1:1 (v/v) mixture (200 μL) of acetic anhydride and pyridine contained in a small test-tube (0.6 cm i.d. × 4.5 cm), and the solution was heated for 1 h at 90°. The mixture was cooled to room temperature, chloroform (200 μL) and water (300 μL) were added, and the mixture was shaken vigorously. The aqueous layer was discarded, and the chloroform layer was washed three times with water (300 μL) and evaporated to a syrup, which was dried overnight in a desiccator under diminished pressure and redissolved in chloroform (50 μL). A solution of acetic acid (150 μL) saturated with hydrogen bromide was added to this solution, and the mixture was kept for 1 h in an ice bath. Chloroform (200 μL) and water (300 μL) were added, and the mixture was processed similarly. The chloroform layer finally obtained was dried with anhydrous sodium sulfate (100 mg), transferred to a second tube of the same size as the first one, and evaporated to a syrup that was dissolved in 1,2-dimethoxyethane (50 μL). A solution (100 μL) of 1,2-dimethoxyethane saturated with lithium aluminum hydride was added to this solution and the mixture was kept for 1 h at 25°. Water (400 μL) was added dropwise with cooling, the mixture was deionized by passing the solution through a small column of Amberlite CG-120 (H^+ form, 0.5 mL) and CG-400 (OAc^- form, 0.5 mL) resins, and the column was washed with water (30 mL). The combined eluate and washings were concentrated to low volume, transferred to a third tube of the same size as the first one, and evaporated.

Oxidation of the 1,5-anhydroalditol derivatives with periodate. — The syrupy 1,5-anhydroalditol derivative obtained by the standard procedure just described was dissolved in 0.05M sodium metaperiodate (200 μL), and the solution was kept for 24 h at 25° in the dark. The solution was deionized by passing it through a small column of Amberlite CG-120 (H^+ form, 0.5 mL) and CG-400 (OAc^- form, 0.5 mL) resins, and the column was washed with water (30 mL). The combined eluate and the washings were evaporated, and the residual syrup was subjected to component analysis by the dithioacetal method.

Simultaneous determination of the conjugated aldehydes in the products of

oxidation of the 1,5-anhydroalditol derivatives. — The procedure was essentially the same as that described in the previous paper[1b]. The residual, syrupy oxidation product was dissolved in a 10:1 (v/v) mixture (20 μL) of ethanethiol and trifluoroacetic acid, and the solution was kept for 30 min at 25°. A solution (50 μL) containing xylitol (internal standard) in pyridine was added to stop the mercaptalation, followed by hexamethyldisilazane (100 μL) and chlorotrimethylsilane (50 μL), and the mixture was kept for 30 min at 50° with occasional shaking. The mixture was centrifuged and the supernatant solution (1 μL) was injected into the g.l.c. column.

For the (1→2)- and (1→4)-linked oligosaccharides, each oxidation product was divided into two equal portions after deionization, and both were evaporated to syrups. Each of them was processed in the same manner until the mercaptalation process was completed. Thereafter, one of these batches was processed as already described, but the other batch was treated as follows. Pyridine (50 μL) was added to stop the mercaptalation, and the mixture was evaporated. The residue was acetalated by adding a 1:1 (v/v) mixture (50 μL) of 1,1-dimethoxyethane and boron trifluoride etherate and keeping the mixture for 2 h at 25°. A solution (50 μL) containing D-xylitol in pyridine was added, followed by hexamethyldisilazane (100 μL) and chlorotrimethylsilane (50 μL), and the mixture was kept for 30 min at 50° with occasional shaking. Gas chromatography of the derivatized product was performed as described for the first batch.

The retention times (T_R) and the molar response factors (m.r.f.) of the aldehyde derivatives relative to trimethylsilylated xylitol were as follows. Trimethylsilylated D-glyceraldehyde diethyl dithioacetal: T_R 0.89, m.r.f. 0.64: trimethylsilylated D-erythrose diethyl dithioacetal: T_R 1.16, m.r.f. 0.72; glyoxal bis(diethyl dithioacetal): T_R 1.10, m.r.f. 0.52; compound of peak 1: T_R 1.73, m.r.f. 0.85; compound of peak 2 (trimethylsilylated 1,5-anhydro-D-glucitol): T_R 1.13, m.r.f. 0.94; compound of peak 3: T_R 1.73, m.r.f. 0.88; compound of peak 4: T_R 1.54, m.r.f. 0.66. The T_R value of trimethylsilylated 1,5-anhydrolaminarabiitol was 2.23.

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