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MODE OF CLEAVAGE OF THE C–C BONDS IN METHYL PENTO- AND HEXO-PYRANOSIDES WITH PERIODATE*

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ABSTRACT

The course of oxidation reactions of three methyl pentopyranosides and five methyl hexopyranosides with periodate was studied by simultaneous determination of the conjugated aldehydes in the products. It was found that the C-3–C-4 bonds in all of these glycosides were cleaved rapidly, but the velocity of subsequent cleavage of the C-2–C-3 bonds depended on the configuration of the substituent groups on the pyranose ring. Equatorial C-1 methoxyl and C-2 hydroxyl groups favored cleavage of the C-2–C-3 bonds more than did the corresponding axial groups, whereas the equatorial C-5 hydroxymethyl group suppressed C-2–C-3-bond-cleavage. The 4-isomeric glycosides were oxidized at the same rate, without regard to the configuration at C-4.

INTRODUCTION

There have been numerous mechanistic studies of periodate oxidation of carbohydrates, mainly based on measurement of periodate consumption. However, the detailed mechanism of oxidation has remained unsolved in many instances, as determination of the periodate consumed by carbohydrates gives only the gross amount of 1,2-diol groups oxidized, and does not give information on the degree of oxidation of each 1,2-diol group when two or more such groups are present. We have recently developed a simple method for simultaneous determination of the conjugated aldehydes in products of oxidation[1b]. This procedure, involving direct, quantitative conversion of the conjugated aldehydes into the corresponding dithioacetals and subsequent gas-chromatographic determination of these dithioacetals, makes it possible to determine the degree of oxidative cleavage of individual C–C bonds. On the basis of product-analysis by this method, this paper discusses the mode of oxidation of methyl pento- and hexo-pyranosides. These glycosides are important, as they may be regarded as models for non-reducing terminal monosaccharide groups in oligo- and poly-saccharides.

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*Periodate Oxidation Analysis of Carbohydrates. Part VIII. For Part VII, see ref. 1a.
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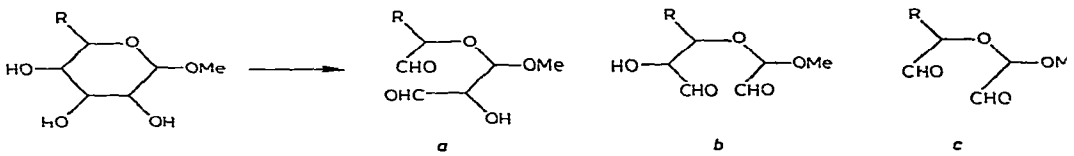
TABLE I

POSSIBLE TYPES OF DIALDEHYDES OBTAINABLE BY OXIDATION OF METHYL PENTO- AND HEXO-PYRANOSIDES



R = H or CH_2OH

Intramolecular hemiacetal and hemiacetal bondings are neglected in these formulas of dialdehydes.

Glycoside	*Component aldehyde*		
	Type a *dialdehyde*	*Type* b *dialdehyde*	*Type* c *dialdehyde*
Methyl pentopyranoside	2-hydroxymalonaldehyde glycolaldehyde	glyoxal glyceraldehyde	glyoxal glycolaldehyde
Methyl hexopyranoside	2-hydroxymalonaldehyde glyceraldehyde	glyoxal tetrose	glyoxal glyceraldehyde

RESULTS AND DISCUSSION

There are three possible types of dialdehyde fragment, *a*, *b*, and *c*, obtainable by glycol-cleavage oxidation of methyl pento- and hexo-pyranosides. Each is composed of a dicarbonyl component (glyoxal or 2-hydroxymalonaldehyde) and a hydroxyaldehyde (glycolaldehyde, glyceraldehyde, or a tetrose), as shown in Table I. Gas-chromatographic analyses of component aldehydes by the dithioacetal method, however, indicated that dialdehydes of type *b* were absent from the products of oxidation of all glycosides examined, because neither glyceraldehyde (for pentosides) nor tetrose (for hexosides) derivatives were detected in the product mixtures. This observation clearly shows favored cleavage of the C-3–C-4 bond, a finding consistent with earlier work on phenyl β-D-glucopyranoside based upon isolation of the hydrolytic fragments of borohydride-reduced oxidation products[2].

Fig. 1(a) shows a typical example of a gas chromatogram for the mixture obtained by incomplete oxidation of methyl α-D-glucopyranoside. The glyoxal (peak 4) and hydroxymalonaldehyde (peak 5) derivatives are considered to be formed from the type *c* and *a* dialdehydes, respectively, and the D-glyceraldehyde derivative (peak 2) from both types. This inference was further substantiated by the isolation of two dialdehydes by column chromatography of the product. The faster-eluting compound, dialdehyde **1**, which yielded a red pigment on heating with aniline hydrogenphthalate, gave the peaks of the D-glyceraldehyde and glyoxal derivatives on component analysis [Fig. 1(b)]. On the other hand, the peaks of the D-glyceraldehyde and 2-hydroxymalonaldehyde derivatives were observed for the slower-eluting compound, dialdehyde **2**, which turned yellow with the same reagent [Fig.

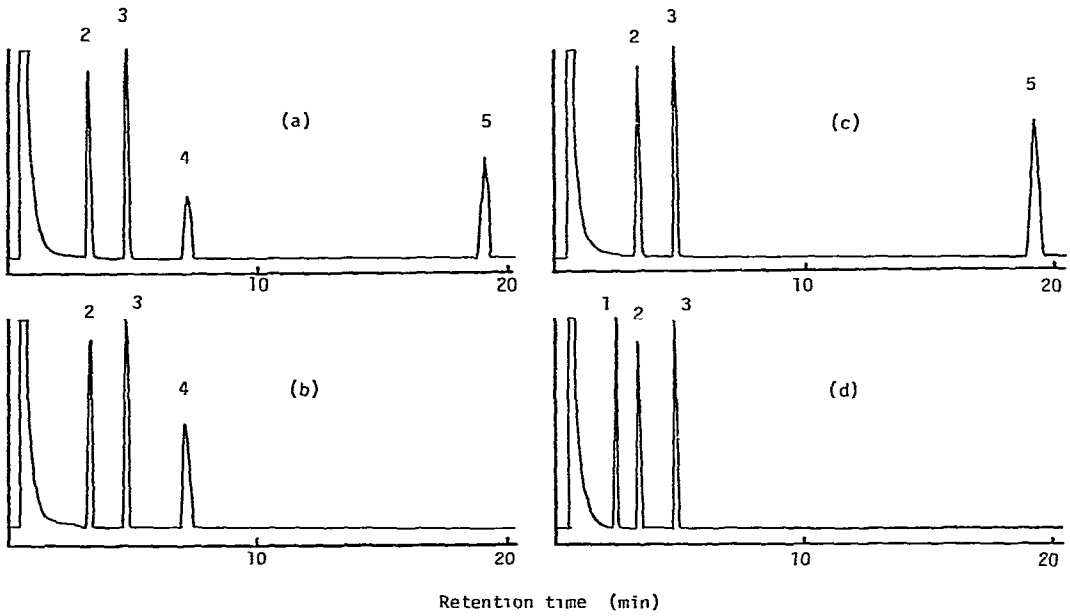


Fig. 1. Gas-chromatographic analyses of the conjugated aldehydes in the following materials by the dithioacetal method. (*a*) the mixture obtained by incomplete oxidation of methyl α-D-glucopyranoside; (*b*) the faster-eluting dialdehyde fraction (dialdehyde **1**) in the column chromatography of (*a*); (*c*) the slower-eluting dialdehyde fraction (dialdehyde **2**) in the column chromatography of (*a*); (*d*) the mixture obtained by reduction of dialdehyde **2** with sodium borohydride. Peak assignment: 1 Me_3Si derivative of glycerol; 2 Me_3Si derivative of D-glyceraldehyde diethyl dithioacetal; 3 Me_3Si derivative of xylitol (internal standard); 4 glyoxal bis(diethyl dithioacetal), 5 Me_3Si derivative of 2-hydroxymalonaldehyde bis(diethyl dithioacetal)

1(c)]. When dialdehyde **2** was reduced with sodium borohydride and then subjected to component analysis, the peak of the 2-hydroxymalonaldehyde vanished and a new peak of trimethylsilylated glycerol arose [Fig. 1(d)]. These observations establish that the former and the latter dialdehydes are of types *c* and *a*, respectively.

Simultaneous determination of the aldehydes provided more-detailed information on the course of oxidation. As shown in Fig. 2(F) for methyl α-D-glucopyranoside, the amount of glyoxal continued to increase gradually as oxidation proceeded, whereas the amount of 2-hydroxymalonaldehyde decreased after reaching a maximum value at ~30 min, but the total yield of glyoxal and 2-hydroxymalonaldehyde remained approximately equal to the yield of D-glyceraldehyde throughout the course of oxidation. These results indicate initial cleavage of the C-3–C-4 bond and subsequent oxidation of the 2-hydroxymalonaldehyde fragment of the resultant type *a* dialdehyde. This relation was also true for all other glycosides tested, although the rate of formation of glyoxal, and accordingly 2-hydroxymalonaldehyde, varied according to the structure of the glycoside. Without exception, the yields of D-glyceraldehyde from the hexosides (D–H) reached the theoretical value during almost 1 h, indicating rapid scission of the C-3–C-4 bonds. D-Glyceraldehyde is

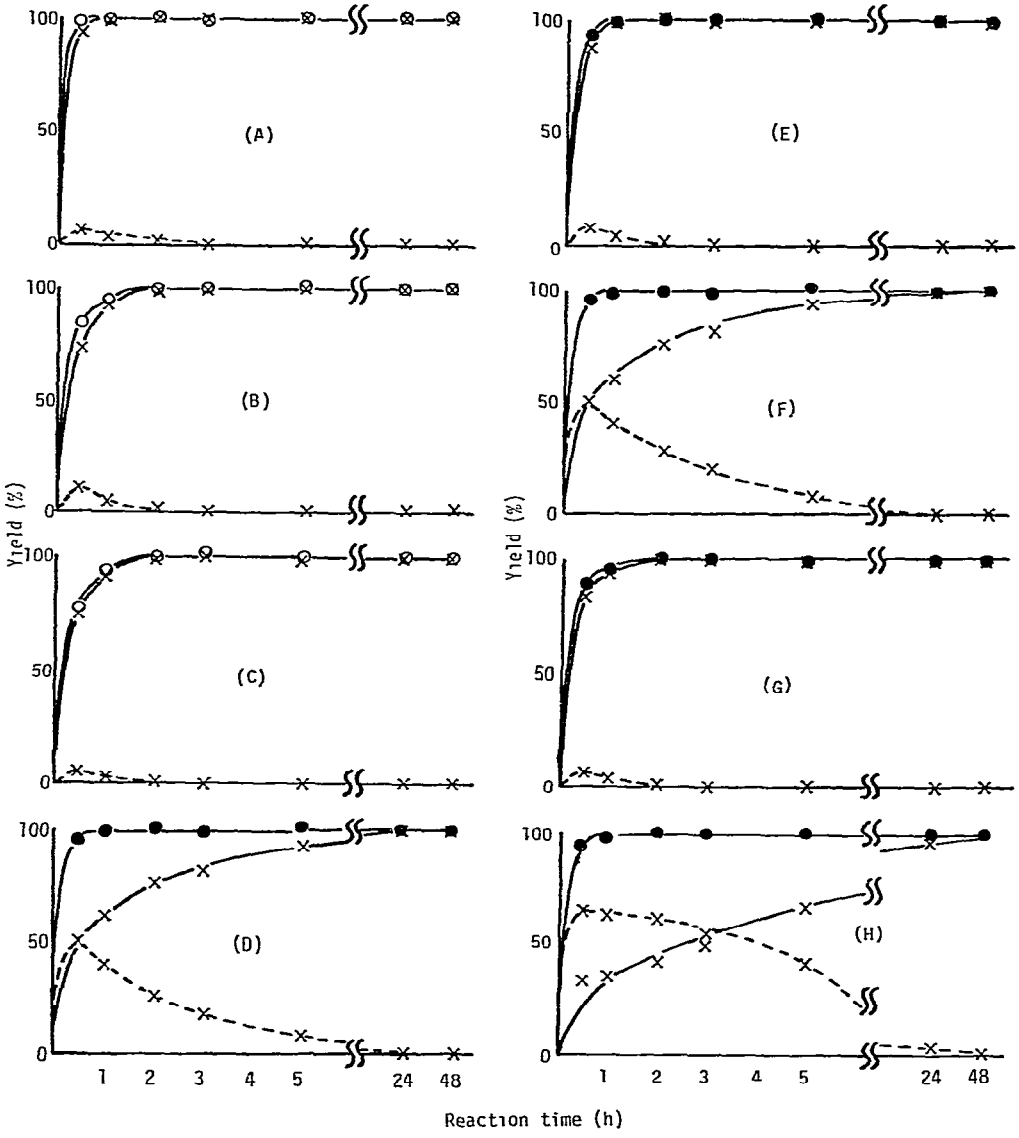


Fig. 2. Course of formation of aldehydes from (*A*) methyl β-L-arabinopyranoside, (*B*) methyl α-D-xylopyranoside, (*C*) methyl β-D-xylopyranoside, (*D*) methyl α-D-galactopyranoside, (*E*) methyl β-D-galactopyranoside, (*F*) methyl α-D-glucopyranoside, (*G*) methyl β-D-glucopyranoside, and (*H*) methyl α-D-mannopyranoside (○——○ glycolaldehyde, ●——● D-glyceraldehyde, ×——× glyoxal, ×---× 2-hydroxymalonaldehyde).

replaced by glycolaldehyde for the pentosides (A–C), but glycolaldehyde was also observed to be formed rapidly. In contrast, the degree of cleavage of the C-2–C-3 bond, which may be estimated from the yield of glyoxal, increased rather slowly, and the velocity of its cleavage differed greatly according to the stereochemistry of the methoxyl, hydroxyl, and hydroxymethyl groups in these glycosides.

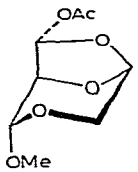
TABLE II

C-2–C-3 TO C-3–C-4 CLEAVAGE RATIOS FOR THE OXIDATION OF METHYL PENTO- AND HEXO-PYRANOSIDES

Glycoside	$\frac{C\text{-}2\text{–}C\text{-}3}{C\text{-}3\text{–}C\text{-}4}$ *Cleavage ratio*						
	0.5 h	1 h	2 h	3 h	5 h	24 h	48 h
Methyl β-L-arabinopyranoside	0.88	0.94	0 99	0 98	1.00	1.00	—
Methyl α-D-xylopyranoside	0 88	0 93	0.99	0.99	1.00	1 00	—
Methyl β-D-xylopyranoside	0.95	0.97	0.99	0 99	1.00	1.01	—
Methyl α-D-galactopyranoside	0.50	0.61	0.74	0 81	0 89	1.00	—
Methyl β-D-galactopyranoside	0.93	0 97	0 98	0 99	0.99	1 00	—
Methyl α-D-glucopyranoside	0.51	0 60	0.74	0 82	0 88	1 00	—
Methyl β-D-glucopyranoside	0.93	0.98	0 99	0 99	1.00	1 00	—
Methyl α-D-mannopyranoside	0.33	0.36	0.41	0 48	0.60	0.95	0.99

Table II gives the ratios of cleavage of the C-2–C-3 bond to that of the C-3–C-4 bond during the course of oxidation. It may be observed that the secondary cleavage at the C-2–C-3 bond is complete after 24 h for all of the glycosides except for the α-D-mannoside, which required a further 24 h for complete oxidation. All of these glycosides of naturally occurring monosaccharides have the equatorial C-3 hydroxyl group as a common feature, and are considered to exist in the $^4C_1$(D) conformation in aqueous solutions. Therefore, the configurational effect of substituent groups attached to the pyranose ring on the cleavage of the C-2–C-3 bond may be evaluated by comparing the C-2–C-3 to C-3–C-4 cleavage ratios between suitable pairs of glycosides. Thus, comparison of the cleavage ratios between the anomeric pairs of D-galactosides and D-glucosides indicates that equatorial orientation of the 1-methoxyl group is more advantageous for cleavage of the C-2–C-3 bond than the axial orientation. The periodate uptake, as measured by iodometry[3], of α- and β-D-glucosides after oxidation for 30 min was 1.58 and 1.67 mol per mol of glycoside, respectively. Each value was in good agreement with the value predicted from the results in Fig. 2 as the sum of D-glyceraldehyde and glyoxal (1.5 and 1.6 mol, respectively). It was suggested, on the basis of the analysis of periodate consumption, that the equatorial phenoxyl group in phenyl β-D-glucopyranoside hindered cleavage of the C-2–C-3 bond much more than the axial one in the α anomer[2]. We are not able to explain this diversity by the difference in electron availability and/or bulkiness between these substituent groups, as each of the anomers of these glucosides is considered to exist in the $^4C_1$(D) conformation in aqueous media[4,5] and produce dialdehydes having the same ring-structure. Although the effect of the 1-methoxyl group on the cleavage of the C-2–C-3 bonds in 4-substituted methyl glycosides was reported to be smaller than that of the phenoxyl group but in the same direction[6], the mechanism of oxidation of these isolated 1,2-diols should be quite different from that of the 1,2,3-triols discussed in the present paper.

The equatorial 2-hydroxyl group is considered to be more favorable than the axial one, as cleavage of the α-D-glucoside is faster than that of the α-D-mannoside. The periodate consumption of the α-D-mannoside after 30 min was 1.39 mol (predicted value, 1.3 mol), which was obviously smaller than that for the α-D-glucoside. The preference of the diequatorial (*trans*) disposition of the C-2 and C-3 hydroxyl groups to the axial–equatorial (*cis*) one is incompatible with the general rule of glycol scission that *cis*-diols are more readily oxidized than *trans*-diols[2,6–11], but it should be noted that the second attack of periodate does occur on the dialdehydes of type *a* that result from C-3–C-4 cleavage of these glycosides. These dialdehydes are considered to form cyclic structures by intramolecular hemiacetal bonding, as neither aldehydic protons nor carbon atoms were detected in their n.m.r. spectra. A bridged, 1,4-dioxane ring was proposed for the dialdehyde obtained by incomplete oxidation of the β-L-arabinoside in dimethyl sulfoxide[12]. This structure was later confirmed to be 7(S)-acetoxy-2(S)-methoxy-1(S)-3,6,8-trioxabicyclo[3.2.1]octane **A** (after acetylation of the oxidized compound)[13] and contains neither α-glycol nor α-hydroxyaldehyde groups, and does not consume a further ion of periodate in such a non-aqueous solvent as dimethyl sulfoxide. In aqueous solution, however, an equilibrium



A

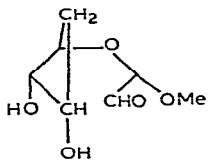
between this cyclic structure and its dissociated form is considered to exist, and the latter form may be oxidized further to give the dialdehyde of type *c*. A similar cyclic structure may be considered for the dialdehydes of type *a* formed from the foregoing hexosides, although the primary hydroxyl group might also participate in ring formation. The cyclic forms of the type *a* dialdehydes resulting from oxidation of the α-D-glucoside and α-D-mannoside should have the same ring skeleton, but differ sterically in the orientation of the methoxyl and hydroxymethyl groups. This difference may influence the relative ease of dissociation of these cyclic forms. If dissociation is the rate-determining step and further oxidation of the dissociated form proceeds rapidly, the difference of cleavage velocities between these two glycosides may be rationalized.

The rate of cleavage of the C-2–C-3 bond is almost independent of the orientation of the C-4 hydroxyl group. This is reasonable, as both D-galactosides and D-glucosides having the same anomeric configuration should yield the same dialdehydes (of type *a*) by initial C-3–C-4 cleavage.

The presence of the equatorial 5-hydroxymethyl group is significant. With both the β-L-arabinoside–α-D-galactoside and α-D-xyloside–α-D-glucoside pairs, which have the same configurations at C-1–C-4, the C-2–C-3 bonds in the pentosides were oxidized faster than those in the hexosides. The uptake of periodate by the α-D-xyloside after 30 min (1.68 mol; predicted value, 1.6 mol), was only slightly higher

than that for the α-D-glucoside (1.58 mol). The predicted value for the α-D-xyloside may be calculated as the sum of glycolaldehyde and glyoxal. The slower cleavage of the C-3–C-4 bond in the α-D-xyloside seems to offset the faster oxidation of the C-2–C-3 bond. However, there was a distinct difference of periodate consumption after 1 h, namely 1.92 mol for the α-D-xyloside and 1.75 mol for the α-D-glucoside. These values agreed well with the predicted values (1.9 and 1.7 mol, respectively).

For the oxidation of methyl glycosides with lead tetraacetate in acetic acid, greater reactivity of *cis*-diols has been demonstrated[14]. For instance, the α-D-mannoside was reported to be oxidized much faster than the α-D-glucoside, as measured by uptake of lead tetraacetate, although unreliability of this analytical technique through contamination by non-specific oxidation has been pointed out. Detailed analysis of the oxidation products by the dithioacetal method will provide more-reliable information on the mechanism of oxidation. As no D-erythrose was detected in the product of oxidation of the α-D-mannoside[15] by lead tetraacetate, a hypothesis was



B

proposed that the dialdehyde of type *b*, formed predominantly following initial attack on the C-2–C-3 bond by lead tetraacetate, equilibrates to give a reactive hemiacetal B that is quickly oxidized by second attack by the oxidant, yielding the dialdehyde of type *c*[16]. This is not likely for the periodate oxidation of this glycoside, because, were this the case, such a large amount of 2-hydroxymalonaldehyde as compared with glyoxal should not have been produced. Furthermore, the absence of D-glyceraldehyde in the products of periodate oxidation of pentosides cannot be explained by this mechanism, as the dialdehydes of type *b* from pentosides do not give such hemiacetal structures.

The foregoing observations elucidate the order of cleavage of individual C–C bonds and the steric effect of substituent groups in these glycosides. This information is useful for the optimization of conditions for periodate oxidation of oligo- and poly-saccharides.

EXPERIMENTAL

Materials. — Methyl α-D-glucopyranoside was obtained from Wako Pure Chemical Industries, Ltd. (Doshomachi, Osaka), and recrystallized twice from methanol before use. All of the other glycosides were purchased from Sigma Chemical Co. (St. Louis, Missouri), and used without purification. All reagents for oxidation and component analysis were of the highest grade commercially available.

Isolation of the dialdehydes **1** *and* **2**. — Methyl α-D-glucopyranoside (970 mg,

5.00 mmol) and sodium metaperiodate (3.21 g, 15.0 mmol) were dissolved in water (1 liter) and the solution was kept for 45 min in the dark. The solution was deionized by stirring it with Amberlite IR-120 (H^+ form) and Amberlite IRA-410 (HCO_3^- form), and evaporated to dryness. The residual syrup gave two spots in t.l.c. (Merck TLC Plate Silica Gel 60, 10:1 chloroform–methanol), when the plate was sprayed with aniline hydrogenphthalate and heated in an oven. The upper (red) and lower (yellow) spots had R_F 0.54 and 0.41, respectively. The syrup was fractionated on a column of silica gel (Wako Gel C-200, 50 g) with 10:1 chloroform–methanol. The fractions eluted in 80–130 ml and 150–210 ml were evaporated to dryness to give dialdehyde **1** (175 mg) and dialdehyde **2** (550 mg), respectively, in pure state. Analysis of the component aldehydes was performed by the dithioacetal method (see later), and the results are given in Fig. 1.

Analytical-scale oxidation of glycosides with periodate. — Equal volumes of a 0.01M aqueous solution of the glycoside and 0.1M aqueous sodium metaperiodate were mixed and the resultant solution was kept at 25° in the dark. An aliquot (2.00 ml) was removed after each specified reaction-time, and deionized by passing it through a column of Amberlite CG-120 (H^+ form, 100 mesh, 0.5 ml) and Amberlite CG-400 (OAc^- form, 100 mesh, 0.5 ml), and the column was washed with water (20 ml). The eluate and washings were combined and evaporated. The residual syrup was redissolved in aqueous acetone and the volume was adjusted to 2.00 ml. A 200-μl portion of the solution was evaporated to dryness in a small sample tube (0.5 cm i.d., 5 cm long). The average recovery of dialdehydes, as calculated on the basis of the amounts of glycolaldehyde (for pentosides) and D-glyceraldehyde (for hexosides), after oxidation for 24 h, was 86%.

Simultaneous determination of the conjugated aldehydes in products of oxidation. — This determination was performed as follows, according to the procedure described in the previous paper[1b]. The residual syrup obtained as in the previous experiment, in a sample tube, was dissolved in a freshly prepared, 10:1 mixture (v/v, 20 μl) of ethanethiol and trifluoroacetic acid, and the solution was kept for 30 min at 25°. By this procedure the hydroxyaldehyde components (glycolaldehyde, D-glyceraldehyde, and D-erythrose) and the dicarbonyl components (glyoxal and 2-hydroxymalonaldehyde) in the dialdehydes were converted into their mono(dithioacetals) and bis(dithioacetals), respectively. To the solution was added a pyridine solution (50 μl) of D-xylitol (internal standard, 1 μmol), followed by hexamethyldisilazane (100 μl) and chlorotrimethylsilane (50 μl). The mixture was kept for 30 min at 50° with occasional shaking, and then centrifuged. The clear supernatant (1 μl) was analyzed by gas chromatography with a Shimadzu 4BMPF instrument equipped with a hydrogen flame-ionization detector. The operational conditions were as follows: column, 3% OV-1 on Chromosorb W (2 m, glass); column temperature, 180°; detector temperature, 240°; carrier gas (N_2), 50 ml/min. Peaks were integrated with a Shimadzu ITG-2A integrator. The retention times and molar response-factors of the derivatives of the aldehydes and of the starting glycosides, relative to that of trimethylsilylated xylitol were: trimethylsilylated derivatives of glycolaldehyde diethyl dithioacetal 0.36,

0.464; D-glyceraldehyde diethyl dithioacetal 0.70, 0.641; D-erythrose diethyl dithioacetal 1.74, 0.772; glyoxal bis(diethyl dithioacetal) 1.53, 0.521; 2-hydroxymalonaldehyde bis(diethyl dithioacetal) 4.14, 0.753; methyl β-L-arabinopyranoside 0.51, 0.643; methyl α-D-xylopyranoside 0.78, 0.643; methyl β-D-xylopyranoside 0.82, 0.643; methyl α-D-galactopyranoside 1.43, 0.869; methyl β-D-galactopyranoside 1.63, 0.869; methyl α-D-glucopyranoside 1.78, 0.869; methyl β-D-glucopyranoside 1.98, 0.869; and methyl α-D-mannopyranoside 1.23, 0.869. Each plot in Fig. 2 was corrected by the average recovery of dialdehydes.

Although the peak of trimethylsilylated D-erythrose diethyl dithioacetal was superposed on the peak of trimethylsilylated methyl α-D-glucopyranoside, the area of the peak having a relative retention time of 1.7 was almost identical with that for the mixture obtained by direct trimethylsilylation but without mercaptalation. This result indicates that the amount of D-erythrose was negligible for this glycoside. Overlap of peaks was also observed for trimethylsilylated D-glyceraldehyde diethyl dithioacetal and methyl α-D-xylopyranoside, but the amount of D-glyceraldehyde was considered from a similar experiment to be negligible.

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