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Note

The formation of inter-residue, hemiacetal bonds during the oxidation of methyl β-lactoside*

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It is well known that intra-residue, hemiacetal bonds are formed during the periodate oxidation of the methyl glycosides of D-glucose and other monosaccharides[1,2]. These bonds occur between the 6-hydroxyl group and the aldehyde group generated at C-2 of the oxidized carbohydrate residues. It has been proposed that hemiacetal bonds are formed during the periodate oxidation of glycans, and that such bonds account for the incomplete oxidation of the glycans[3–5]. That hemiacetal bonds are, indeed, formed during periodate oxidation of glycans has been verified by analytical data from experiments on the methylation of oxidized glycans followed by analysis by gas–liquid chromatography (g.l.c.) and mass spectrometry (m.s.)[6,7]. In these experiments, it was shown that partially methylated alditol acetates, which could arise only from residues having protected hydroxyl groups, were produced from the oxidized glycans. The protection of the hydroxyl groups of the non-oxidized residues was attributed to hemiacetal bonds that were formed between an aldehyde group of an oxidized residue and a hydroxyl group of an adjacent, not-yet-oxidized residue[7]. Such bonds are inter-residue in nature, in contrast to the intra-residue bonds that occur in oxidized methyl glycosides.

We have now found that inter-residue, hemiacetal bonds are also formed during the periodate oxidation of glycosides of oligosaccharides. The glycoside employed in our studies was methyl β-lactoside, and the oxidations and analyses were performed by standard methods. Hemiacetal bonds are, therefore, formed not only during periodate oxidation of methyl hexosides and glycans but also during the oxidation of oligosaccharides having substituted hemiacetal hydroxyl groups.

The results of the methylation experiments on the native and modified methyl

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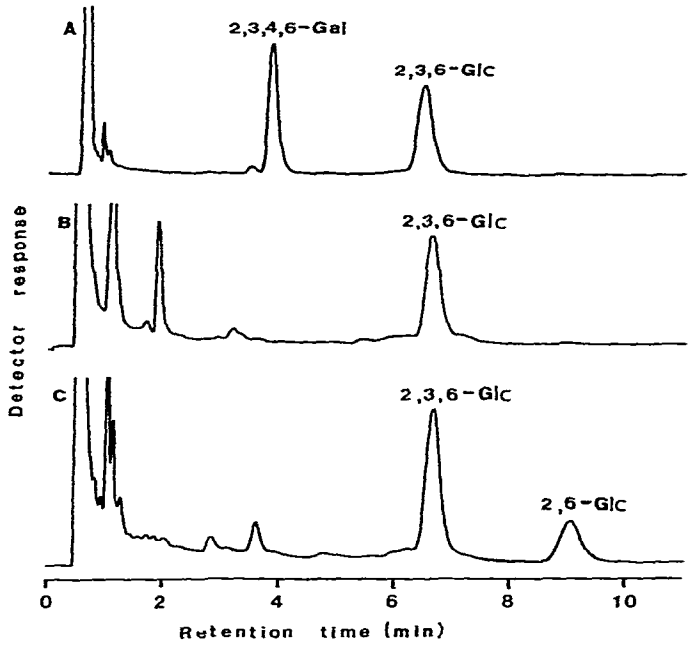

Fig. 1. A photograph of gas–liquid chromatographic patterns of the partially methylated alditol acetates from methyl β-lactoside (A), the periodate-oxidized and borohydride-reduced methyl β-lactoside (B), and the periodate-oxidized methyl β-lactoside (C). [Key: 2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 2,3,6-Glc = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol, and 2,6-Glc = 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol.]

β-lactoside are shown by the g.l.c. patterns reproduced in Fig. 1. These g.l.c. patterns were obtained by analysis of the partially methylated alditol acetates derived from the completely methylated methyl β-lactoside, the oxidized methyl β-lactoside, and the oxidized and subsequently reduced methyl β-lactoside. The procedures that were utilized have been described[8,9]. Fig. 1A shows that, in the reaction sequence, methyl β-lactoside was converted into 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol. These derivatives were separated by g.l.c., and identified from retention times and m.s. data. The two derivatives were present in equimolar concentrations.

Fig. 1C shows that methyl β-lactoside which was oxidized by periodate and the product subjected directly to methylation yielded two alditol derivatives on g.l.c. analysis, namely, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol and 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol. From the areas of the peaks of this pattern, the ratio of the two derivatives was estimated to be 2 : 1. The trimethyl derivative in this reaction mixture arises from D-glucose residues in the β-lactoside that were not oxidized, even though an excess of periodate was employed. Possible reasons for the resistance of these residues to oxidation are discussed later. The dimethyl derivative arises from D-glucose residues in which the 3-hydroxyl group was protected by hemiacetal bonds.

Fig. 1B shows that, on methylation and analysis, methyl β-lactoside that has

been oxidized by periodate and subsequently reduced by borohydride yields only the trimethyl derivative of D-glucose. The absence of the dimethyl derivative shows that the 3-hydroxyl group was no longer protected in the oxidized and reduced material, as the hemiacetal bonds would be cleaved and reduced during reductions of the oxidized compound. The presence of the trimethyl derivative of D-glucose (see Fig. 1B) shows that some D-glucose residues of the oxidized and reduced β-lactoside are resistant to oxidation, even though these residues possess unsubstituted glycol groups. Derivatives of fragments from the oxidized, and the oxidized and reduced, β-lactoside (see Fig. 1B and C) were detectable in the g.l.c. pattern, but the nature of these derivatives was not investigated.

The data from the experiments on the methylation of the native, the oxidized, and the oxidized and reduced methyl β-lactoside are interpreted as follows. First, all of the D-galactosyl groups of the β-lactoside were oxidized by periodate. Second, many of the D-glucose residues were not oxidized. Third, the presence of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol (see Fig. 1C) shows that the 3-hydroxyl groups of some of the D-glucose residues were protected and, accordingly, not methylated. The protection of these D-glucose residues was due to hemiacetal bonds that were formed between an aldehyde group of an oxidized D-galactosyl group and the 3-hydroxyl group of a non-oxidized, D-glucose residue.

The identification of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol in the methylation products from the oxidized material (see Fig. 1C) and from the oxidized and reduced compound (see Fig. 1B) shows that some of the D-glucose residues of the β-lactoside were not oxidized, even though such residues were not protected by hemiacetal bonds. Earlier work[10,11] has shown that D-glucose residues in the furanose form, or having bulky substituents adjacent to the glycol group, are resistant to oxidation by periodate. This resistance has been attributed to steric hindrance that interferes with the formation of the periodate–glycol complex[10,11]. Steric hindrance may be due to a trans arrangement of the hydroxyl groups in the glycol unit, or to an equatorial orientation of the substituent at C-4 of the residue being oxidized.

In methyl β-lactoside, the 2- and 3-hydroxyl groups of the D-glucose residue are in the trans arrangement and the D-galactosyl group is equatorially attached to O-4 of the D-glucose residue. It is, therefore, possible that a combination of these structural features accounts for the nonreactivity of these D-glucose residues to periodate oxidation. Calculations from the data in Fig. 1 show that steric effects protect two-thirds of the D-glucose residues, and hemiacetal bonds protect one-third of the D-glucose residues of the oxidized compound. These results establish that inter-residue bonds are, indeed, formed in appreciable proportion during the periodate oxidation of methyl β-lactoside. Whereas the formation of intra-residue, hemiacetal bonds in periodate-oxidized methyl hexosides[1], and of inter-residue, hemiacetal bonds in oxidized glycans[7] has previously been shown to occur, the formation of such bonds during periodate oxidation of oligosaccharide derivatives has not heretofore been recorded.

EXPERIMENTAL

Materials. — Methyl β-lactoside was synthesized from hepta-*O*-acetyl-lactosyl bromide and methyl alcohol, in the presence of silver carbonate. The product was deacetylated with sodium methoxide, and the free glycoside was purified by chromatography on cellulose, and crystallized from ethanol. A sample of authentic methyl β-lactoside was kindly provided by Dr. P. A. J. Gorin, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada. Methylation of methyl β-lactoside, followed by analyses by g.l.c. and m.s., was performed as described in a later section. In the reaction mixture, two derivatives, namely, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol (see Fig. 1A) were identified. Integration of the peak areas in the pattern (Fig. 1), and appropriate calculations, showed that these two derivatives were produced in equimolar amounts. M.s. data and retention times in g.l.c. analysis of the derivatives are recorded in a later section.

A reference standard of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol was prepared from methyl 2,6-di-*O*-methyl-3,4-di-*O*-(*N*-phenylcarbamoyl)-α-D-glucopyranoside; an authentic sample[12] was kindly provided by Dr. R. E. Reeves, Biochemistry Department, College of Medicine, Louisiana State University, New Orleans, LA. The derivative (10 mg) was refluxed in 0.4M barium hydroxide for 2 h; the excess of barium hydroxide was precipitated as barium carbonate by bubbling carbon dioxide through the reaction mixture, and the suspension was filtered. The filtrate (containing methyl 2,6-di-*O*-methyl-α-D-glucopyranoside) was then evaporated to dryness under diminished pressure. The residue was extracted with ethyl alcohol, and the alcohol was removed from the extract in a stream of nitrogen. The α-D-glucoside was hydrolyzed initially in 90% formic acid and then in 50mM sulfuric acid for 16 h; the acid was neutralized, and the product was reduced with sodium borohydride. The resulting alditol was then acetylated with acetic anhydride–pyridine, and the tetraacetate was subjected to g.l.c. and m.s. analyses as described in a later section.

Periodate oxidation. — A solution of methyl β-lactoside (35 mg) in water (17.5 mL) was mixed with 0.04M sodium periodate (17.5 mL). Calculations from the foregoing data showed that the periodate concentration was several times in excess of that required for complete oxidation of the methyl β-lactoside. The reaction was allowed to proceed for 48 h at 4° in the dark. The concentration of periodate was monitored periodically by the absorbance[13] at 223 nm, and these values showed that the periodate concentration dropped to a constant value in 36 h. Calculations from these data showed that two equivalents of periodate were used per mole of methyl β-lactoside. At this point, the excess of periodate was decomposed by addition of ethylene glycol (0.1 mL). The inorganic compounds and the oxidized methyl β-lactoside were separated by chromatography on a column (110 × 1.3 cm) of Bio-Gel P2, elution of the compounds being effected with water, and 0.2-mL samples of the eluate being collected. Aliquots of the samples were analyzed for iodate, and for carbohydrate by the phenol–sulfuric acid method[14]: both types of compound yield a

color complex with the phenol–sulfuric acid reagent. Standards were also subjected to Bio-Gel chromatography, in order to establish the chromatographic behavior of the oxidized β-lactoside. It was found that the oxidized β-lactoside was eluted in fractions 40–50, and the iodate in fractions 55–70. Fractions 40–50 were combined, and lyophilized. The yield of oxidized β-lactoside was 25 mg.

To a solution of the oxidized β-lactoside (10 mg) in water (5 mL) was added sodium borohydride (5 mg), and reduction was allowed to proceed for 16 h at room temperature. At the end of this time, the base was neutralized with 0.5M HCl, and the borate was removed by several evaporations with methanol. The oxidized and reduced glycoside was then lyophilized to dryness.

Methylation analysis. — Samples (2–5 mg) of the native, the oxidized, and the oxidized and subsequently reduced methyl β-lactoside were subjected to methylation, and then to g.l.c. and m.s. analyses. The methylation was performed by the Hakomori method[15], and the analyses by the method of Lindberg and associates[8]. Details of the procedures employed in our laboratory have been published[9]. Gas-liquid chromatography was performed in a Varian 1400 unit in a column of 3% of OV-225 on Supelcoport (80–100 mesh; Supelco, Bellefonte, PA). The g.l.c. patterns for *O*-methylalditol acetates from the three samples are reproduced in Fig. 1. The retention time for 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol was assigned a value of 1.00, and the retention times for the other derivatives relative to this compound were 1.12 for 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 2.00 for 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol, and 2.74 for 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol.

Mass spectrometry. — Mass-spectral data were obtained with a DuPont 21-490 mass spectrometer as described previously[9]. The following fragments characteristic of partially methylated derivatives of hexitol acetates were obtained for the products of methylation analysis on methyl β-lactoside, oxidized methyl β-lactoside, and oxidized and subsequently reduced methyl β-lactoside: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol 45 (80), 117 (100), 161 (70), and 205 (10); 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol 45 (30), 117 (100), and 233 (30); 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylhexitol 45 (40) and 117 (100). The figures in parentheses denote relative intensities, with the most-prominent fragment, generally 117, being assigned a value of 100. Reference 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylhexitol yielded a single peak in g.l.c. analysis, with a retention time of 2.74. The mass-spectral data for this reference compound were identical to those just listed for this derivative.

REFERENCES

1 C. D. Hurd, P. J. Baker, Jr., R. P. Holysz, and W. H. Saunders, Jr., *J. Org. Chem.*, 18 (1953) 186–191.
2 J. E. Cadotte, G. G. S. Dutton, I. J. Goldstein, B. A. Lewis, F. Smith, and J. W. van Cleve, *J. Am. Chem. Soc.*, 79 (1957) 691–695.
3 J. H. Sloneker, D. G. Orentas, C. A. Knutson, P. R. Watson, and A. Jeanes, *Can. J. Chem.*, 46 (1968) 3353–3361.
4 T. J. Painter and B. Larsen, *Acta Chem. Scand.*, 24 (1970) 813–833.
5 M. F. Ishak and T. J. Painter, *Carbohydr. Res.*, 32 (1974) 227–237.

6 J. H. PAZUR AND L. S. FORSBERG, *Carbohydr. Res.*, 58 (1977) 222–226.
7 L. S. FORSBERG AND J. H. PAZUR, *Carbohydr. Res.*, 75 (1979) 129–140.
8 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
9 J. H. PAZUR, D. J. DROPKIN, K. L. DREHER, L. S. FORSBERG, AND C. S. LOWMAN, *Arch. Biochem. Biophys.*, 176 (1976) 257–266.
10 R. J. DIMLER, H. A. DAVIS, AND G. E. HILBERT, *J. Am. Chem. Soc.*, 68 (1946) 1377–1380.
11 E. F. GARNER, I. J. GOLDSTEIN, R. MONTGOMERY, AND F. SMITH, *J. Am. Chem. Soc.*, 80 (1958) 1206–1208.
12 R. E. REEVES, *J. Am. Chem. Soc.*, 70 (1948) 259–260.
13 R. D. GUTHRIE, *Methods Carbohydr. Chem.*, 1 (1962) 435–441.
14 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
15 S.-I. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.