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Oxidation of oligoglycosyl alditols during methylation catalyzed by sodium hydroxide and iodomethane in methyl sulfoxide

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ABSTRACT

Certain artifacts in the glycosyl-linkage analysis of oligoglycosyl alditols were shown to be caused by oxidation of the alditol moieties when *O*-methylation was catalyzed by NaOH in Me_2SO. The partially methylated alditol acetates derived from 1-[^{2}H]-cellobiitol that was *O*-methylated by this method were analyzed in detail to confirm this conclusion.

INTRODUCTION

Methylation analysis is a widely used method to determine the sites of substitution and ring forms of the glycosidic residues of complex carbohydrates.[1–4] This technique utilizes gas chromatography–electron impact mass spectrometry (g.c.–e.i.m.s.) analysis of partially methylated alditol acetate (PMAA) derivatives of the glycosyl residues of complex carbohydrates. PMAAs are prepared using the following reaction sequence. The complex carbohydrate is per-*O*-methylated and then hydrolyzed in aqueous acid. The partially methylated glycoses released by acid treatment are reduced with borodeuteride to form the corresponding alditols which are then *O*-acetylated. The positions of *O*-acetyl groups in these alditols correspond to oxygen-bearing carbons that were protected from *O*-methylation by glycosylation or ring closure in the original complex carbohydrate.

The introduction by Hakomori[3] of methylsulfinyl methanide in methyl sulfoxide (Me_2SO) as a powerful catalyst for *O*-methylation of carbohydrates has made glycosyl-linkage analysis by *O*-methylation routine. More recently, a procedure that utilizes MeI and solid NaOH in Me_2SO for *O*-methylating complex carbohydrates was introduced by Ciucanu and Kerek[4]. This new procedure is rapid and simple, as it is not necessary to first prepare methylsulfinyl methanide.

Techniques which are currently available for glycosyl-linkage analysis often result in non-quantitative recovery of PMAA derivatives due to undermethylation, chemical degradation, and incomplete cleavage of glycosyl-linkages during hydrolysis. It is especially difficult to obtain quantitative yields of the derivatives of the alditol

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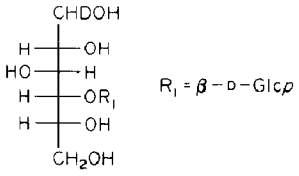
residues of oligoglycosyl alditols. We report here that significant oxidation of these alditols occurs when the NaOH/MeI/Me_2SO *O*-methylation procedure of Ciucanu and Kerek[4] is used. The oxidized alditol is converted into a methyl glycoside during the methylation reaction.

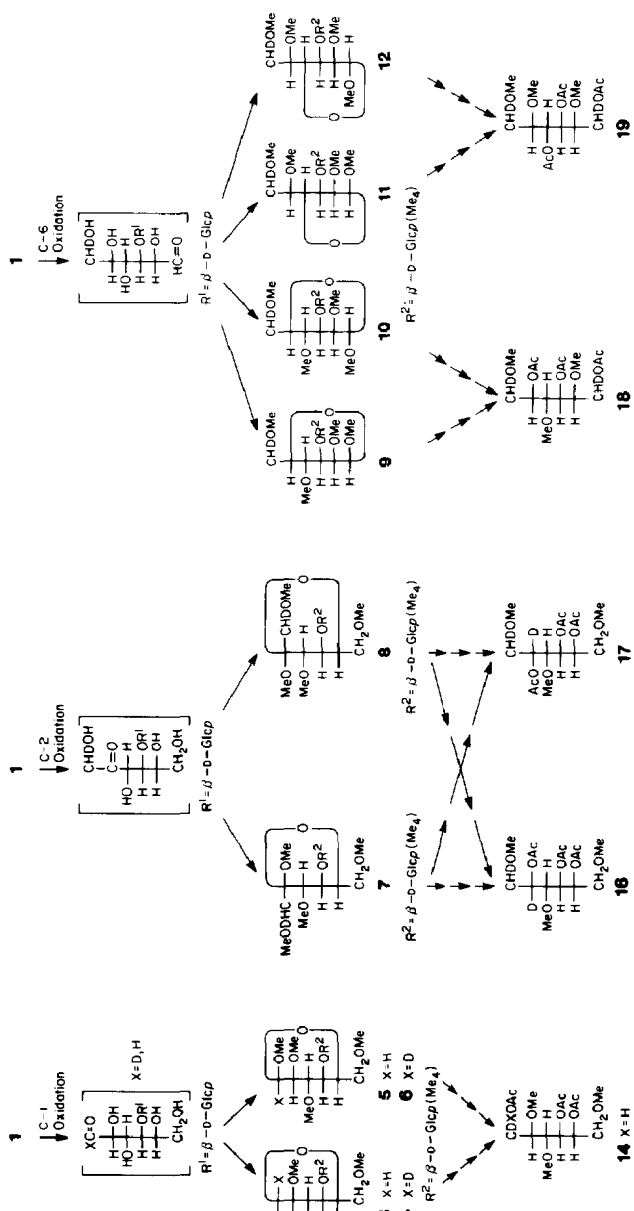
RESULTS

The structures of oligoglycosyl alditols derived from plant cell-wall xyloglucans were examined in a recent study[5] by various chemical and spectroscopic techniques. The 4-linked glucose moiety present at the reducing end of each xyloglucan oligosaccharide was converted into a 4-linked glucitol by reduction with borohydride, and the resulting oligoglycosyl glucitols were subjected to glycosyl-linkage analysis using the *O*-methylation procedure of Ciucanu and Kerek[4]. The methylated products were hydrolyzed, reduced, and acetylated to form PMAAs. In addition to the expected derivatives, small amounts of PMAA derivatives that did not correspond to any known constituent of the highly purified oligoglycosyl alditols were detected by g.c.–e.i.m.s. The unexpected derivatives included 2,4,6-tri-*O*-methyl-hexitol triacetate which normally is produced when a 3-linked hexopyranosyl residue is present in the complex carbohydrate. When oligosaccharides that had not previously been converted into oligoglycosyl alditols were analyzed by the same procedure, the 2,4,6-tri-*O*-methyl-hexitol triacetate derivative was not detected. These results led to the realization that the anomalous 2,4,6-tri-*O*-methyl-hexitol triacetate might be derived from a 3-linked-L-gulopyranosyl intermediate formed by oxidation of C-6 of the 4-linked glucitol residue during *O*-methylation of the oligoglycosyl alditol. This hypothesis has now been tested using 1-[^{2}H]-cellobiitol (**1**) as a model compound.



1

Cellobiose was reduced with $NaBD_4$ to form **1**, which was per-*O*-methylated[4] using NaOH and MeI in Me_2SO. The methylated products were analyzed by g.l.c.–e.i.m.s. (Fig. 1B)[6]. The most abundant product (Scheme 1) was **2** (T_R12.3 min., aA_1 *m/z* 219, aA_2 *m/z* 187, bJ_1 *m/z* 296, bJ_2 *m/z* 236). Less abundant products having longer g.l.c. retention times were also detected (Fig. 1B). Nonreducing end fragment ions of these less abundant products (aA_1 *m/z* 219, aA_2 *m/z* 187) indicated that they all contained a terminal tetra-*O*-methyl-hexosyl residue. Reducing end-fragment ions of the less abundant products (Fig. 1B) indicated they were methyl glycosides (Scheme 1) with either a single deuterium (*e.g.*, compounds **4** and **6–12**, bJ_1 *m/z* 280, bJ_2 *m/z* 220) or no deuterium (*e.g.*, compounds **3** and **5**, bJ_1 *m/z* 279, bJ_2 *m/z* 219). In contrast, methylation of **1** by the procedure of Hakomori did not result in the formation of significant amounts of methyl glycosides (Fig. 1A).



Scheme 1

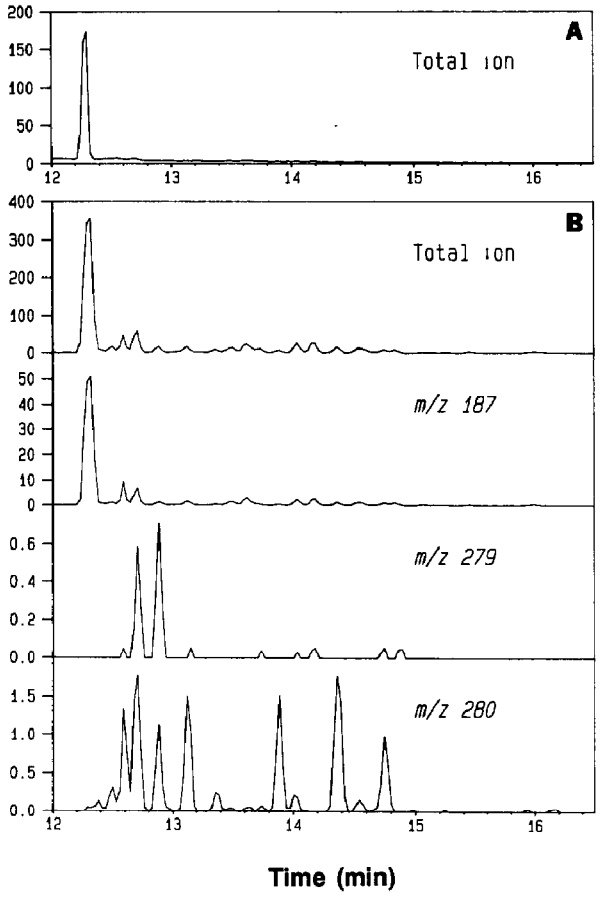


Fig. 1. G.l.c.–e.i.m.s. of the products formed by methylation of 1-[^{2}H]-cellobiitol by A) the method of Hakomori[3] and B) the method of Ciucanu and Kerek[4]. The expected product was eluted at 12.3 min. The bA_2 ion (*m/z* 187) is diagnostic of non-reducing terminal tetra-*O*-methyl-D-glucopyranose residues. The aJ_1 ion (*m/z* 279) is diagnostic of methyl hexoside residues, and the aJ_1 ion (*m/z* 280) is diagnostic of singly deuterated methyl hexoside residues.

Monomers released by acid hydrolysis of the mixture of products obtained by methylation of **1** by the method of Ciucanu and Kerek were reduced with $NaBD_4$ and *O*-acetylated. The resulting PMAAs were examined by g.l.c.–e.i.m.s. The two largest peaks in the ion chromatogram corresponded to the expected products (**13** and 1,5-di-*O*-acetyl-1-deuterio-2,3,4,6-tetra-*O*-methyl-D-glucitol, not illustrated). Minor products were also observed (Table I), of which several appeared to be derived from methyl glycosides formed during the methylation reaction. These included **14** and **15** (Fig. 2A), which coeluted, **16** (Fig. 2B), its 2-epimer **17**, **18** (Fig. 2C), and **19** (Fig. 2D). Other products (Table I) were attributed to undermethylation. In contrast, the PMAAs derived from the products obtained by methylation of **1** by the procedure of Hakomori did not include any derivatives that could be attributed to oxidation.

The recovery of dideuterio PMAAs after hydrolysis, reduction, and acetylation of the products formed from **1** when methylated by the method of Ciucanu and Kerek

TABLE I

Partially methylated glucitol acetates recovered after methylation of 1-deuterio-cellobiitol (**1**) by the method of Ciucanu and Kerek (ref. 4)

Compound	T_R[2]	*Substituents Sites*[a]			*Formation*[c]	*Norm Mole%*[d]
		O-Methyl	O-*Acetyl*	*Deuterium*		
11	11.12	1,2,3,5,6	4	1	Expected	29.0
–[e,f]	13.83	1,3,5,6	2,4	1	UM(C-2,Glcol)	0.2
–[e,f]	13.83	1,2,5,6	3,4	1	UM(C-3,Glcol)	0.2
–[e,f]	13.91	1,2,3,6	4,5	1	UM(C-5,Glcol)	0.3
–[f]	14.62	2,3,4,6	1,5	1	Expected	53.3
–[f]	14.89	1,2,3,5	4,6	1	UM(C-6,Glcol)	0.3
–[f]	15.44	2,3,5,6	1,4	1	UM(C-1,Glcol)	0.6
17[g]	16.31	1,3,6	2,4,5	1,2	Ox(C-2,Glcol)[h]	0.9
–[g]	16.78	1,3,6	2,4,5	1,2	Ox(C-2,Glcol)[h]	0.9
–[f]	17.50	2,4,6	1,3,5	1	UM(C-3,T-Glc)	1.3
–[f]	17.80	3,4,6	1,2,5	1	UM(C-2,T-Glc)	0.9
19	18.04	1,2,5	3,4,6	1,6	Ox(C-6,Glcol)[h]	1.3
18	18.30	1,3,5	2,4,6	1,6	Ox(C-6,Glcol)[i]	5.1
–[f]	18.82	2,3,4	1,5,6	1	UM(C-6,T-Glc)	0.5
14/15	19.12	2,3,6	1,4,5	1/1,1[j]	Ox(C-1,Glcol)[i]	5.1

[a] The carbon atoms are numbered by considering the final PMAAs as D-glucitol (or D-mannitol) derivitives, even if the derivative was formed *via* a D-gulose intermediate. That is, the carbon bearing the deuterium that was introduced before methylation is defined as C-1. [b] Retention time on a 30-m SP2330 column. [c] UM indicates undermethylation at the position of the residue indicated in parentheses; Ox indicates oxidation at the position of the residue indicated in parentheses. [d] Values given for a typical analysis. [e] Tentative identification only, due to incomplete separation during g.l.c.–e.i.m.s. analysis. [f] Derivative detected but not illustrated. [g] Both D-*manno* and D-*gluco* derivatives formed by hydrolysis and reduction of the D-fructofuranosyl intermediate were recovered. [h] These PMAAs were formed by hydrolysis, $NaBD_4$ reduction, and acetylation of the methyl furanoside formed after oxidation at the carbon indicated. [i] As in [h] except *via* the methyl pyranoside intermediate. [j] Contains a mixture of the 1-deuterio and 1,1-dideuterio alditols, indicating that this PMAA was formed predominantly via oxidation. A small amount may have formed by undermethylation at C-4 of the T-Glc residue.

but not when methylated by the method of Hakomori, confirmed that the method of Ciucanu and Kerek results in the partial oxidation of **1**. The position of deuterium atoms and *O*-methyl substituents in the PMAAs obtained when methylation was performed using the conditions of Ciucanu and Kerek (Fig. 2, Table I) indicated that the oxidized alditol was trapped as a methyl glycoside during *O*-methylation. Thus the formation of anomolous tri-*O*-methyl-hexitol triacetate derivatives during methylation analysis of xyloglucan oligoglycosyl alditols can be explained by oxidation of the glucitol moiety during the methylation reaction.

The chemical mechanism of the described oxidation was not investigated. However, Johnson and Phillips showed[7] that a dimethylmethoxysulfonium salt prepared by reacting Me_2SO with MeI reacts with alkoxides to form the corresponding carbonyl compound (*i.e.*, acetaldehyde from sodium ethoxide and acetone from sodium 2-propoxide). It is possible that similar reactions occur with carbohydrate alkoxides and

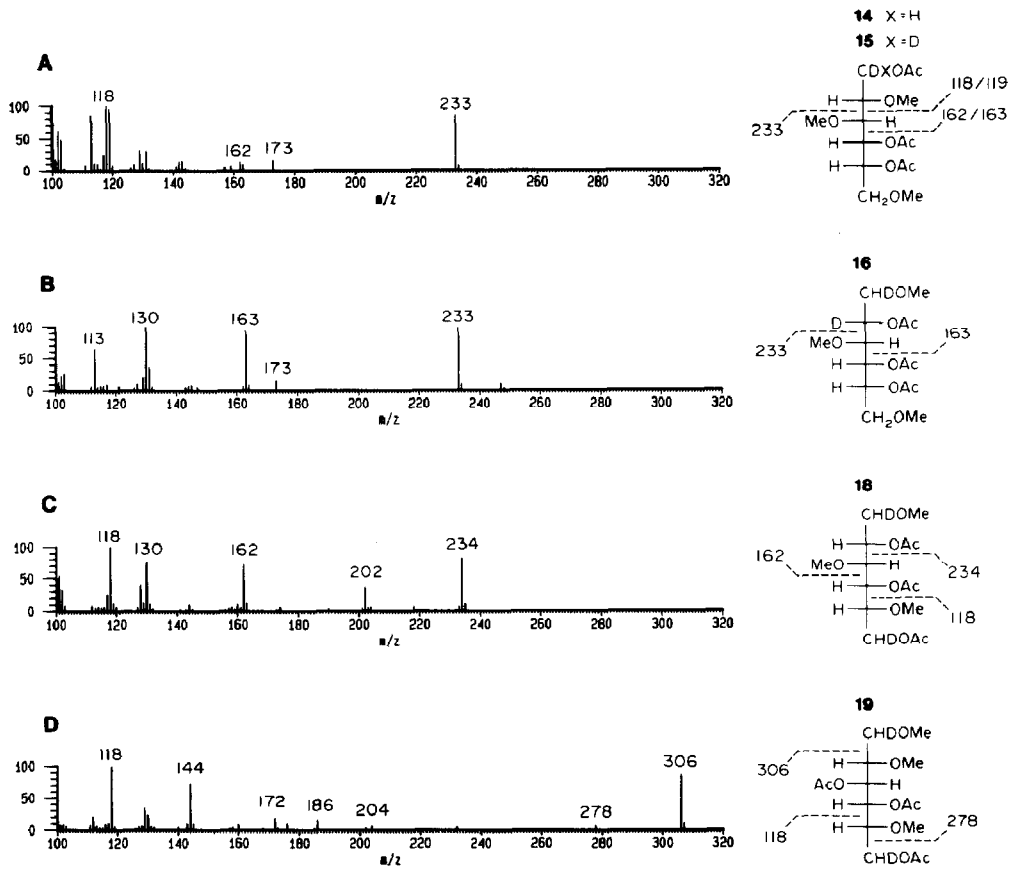


Fig. 2. Electron impact mass spectra of several PMAAs formed by hydrolysis, $NaBD_4$ reduction, and *O*-acetylation of the products recovered after methylation of 1-[^{2}H]-cellobiitol using NaOH/MeI in Me_2SO. The ion at *m/z* 130 in the spectrum of **17** was formed by β-elimination of deuteriomethanol[2] from the primary fragment at *m/z* 163.

dimethylmethoxysulfonium iodide formed when carbohydrates are dissolved in Me_2SO containing MeI and solid NaOH. Under the methylation conditions of Ciucanu and Kerek, very little soluble hydroxide anion is present,[4] as NaOH is only slightly soluble in Me_2SO, while alkoxides formed by deprotonation of the carbohydrate are present in relatively high concentrations. The concentration of carbohydrate alkoxides is apparently high enough to react with dimethylmethoxysulfonium iodide to form the oxidized product. Conversely, under the methylation reaction conditions of Hakomori, the presence of high concentrations of methylsulfinyl methanide would catalyze the conversion of any dimethylmethoxysulfonium salts present to formaldehyde and methyl sulfide[7] before reaction with a carbohydrate alkoxide could occur. Thus, oxidation is not observed during Hakomori methylation.

It is interesting, but not surprising, that derivatives formed *via* oxidization of the alditol moiety of cellobiitol **1** were detected after hydrolysis, reduction, and acetylation,

but derivatives formed by oxidation of the terminal, non-reducing glucopyranosyl residue were not detected. Carbonyl compounds formed by oxidation of the alditol moiety can be trapped as cyclic ketals or acetals (*i.e.*, methyl glycosides), while those formed by oxidation of the already cyclic glucopyranosyl residue cannot form a cyclic acetal or ketal at the newly oxidized carbon. Thus, ketones and aldehydes that may have formed by oxidation of the glucopyranosyl residue would be susceptible to base-catalyzed degradation[8], and would not be detected.

Although oxidation of (cyclic) glycosyl residues does not directly give rise to artifactual derivatives, base-catalyzed degradation[8] of these residues would uncover hydroxyl groups on adjacent residues, and thus indirectly give rise to PMAA derivatives that do not correspond to any structure in the original complex carbohydrate. We have in fact observed such artifacts in our studies of xyloglucan oligosaccharides. The amount of 4-linked glucopyranosyl residues was overestimated during glycosyl-linkage analysis of a hexadecaglycosyl alditol[9] when the method of Ciucanu and Kerek[4] was used. This was demonstrated to be an artifact by rigorously determining the structure of the oligoglycosyl alditol by both spectroscopic and enzymic methods[9]. We now can attribute the unexpectedly high recovery of the 2,3,6-tri-*O*-methyl-D-glucitol acetate (corresponding to 4-linked D-glucopyranosyl residues) to the following reaction sequences: (*i*) oxidation of C-1 of the 4-linked glucitol moiety, followed by formation of the methyl glycoside, and (*ii*) oxidation of xylosyl residues attached to C-6 of 4,6-linked glycopyranosyl residues, followed by degradation[8] of the oxidized xylosyl residues, thus converting 4,6-linked glucopyranosyl residues to 4-linked glucopyranosyl residues.

The results described above indicate that oxidative side-reactions can occur when the NaOH/MeI/Me_2SO method is used to prepare methylated derivatives of complex carbohydrates. These side-reactions can lead to the formation of anomalous derivatives upon formation of PMAAs. The results obtained may thus be in error, making confirmation by other analytical techniques necessary. Nevertheless, the ease with which this reaction can be carried out may in many cases outweigh this undesirable aspect.

EXPERIMENTAL

1-[^2H]-Cellobiitol. — Cellobiose (Sigma Chemical Co., 5 mg) was reduced with $NaBD_4$ (Aldrich Chemical Co., 5 mg/mL in 2.0 mL of 0.5M NH_4OH, 1h), quenched with glacial HOAc, and passed through Dowex-50 X 2 [H^+] (Aldrich Chemical Co., 5 mL). Residual borate was removed by coevaporation with acidified methanol[10]. The 250 MHz ^1H-n.m.r. spectrum of reduced product **1** contained no signals corresponding to the starting material, and included only one anomeric proton signal (d, δ 4.57, $J_{1,2}$ 7.8 Hz), confirming that complete reduction of the disaccharide was achieved. The negative-ion f.a.b.-mass spectrum of the reduced product **1** using 1-amino-2,3-dihydroxypropane as a matrix[5] included an abundant $[M - H]^-$ ion at *m/z* 344. The $[M - H]^-$ (*m/z* 341) ion corresponding to the starting material was not detected above background.

Chemical Analysis. — *O*-Methylation was accomplished as noted according to either Ciucanu and Kerek[4] or Hakomori[3], except that the Hakomori methylation was catalyzed by potassium methylsulfinyl methanide[2] rather than by sodium methylsulfinyl methanide. G.l.c.–e.i.m.s. of the methylated products was performed with a Hewlett-Packard (HP) mass selective detector attached to an HP model 5890 gas chromatograph fitted with a DB-1 (J&W Scientific) fused-silica capillary column. The g.l.c. oven temperature was maintained at 80° for 2 min after sample injection, increased to 260° at 30°/min, and maintained at 260°.

PMAAs were prepared from the methylated products as described[11]. G.l.c.–e.i.m.s. of the PMAAs was performed as described above, except that an SP-2330 fused-silica capillary column (Supelco) was used and the g.l.c. oven temperature was maintained at 80° for 2 min after sample injection, increased to 170° at 30°/min, then increased to 240° at 4°/min. Quantitation of PMAAs was performed in a separate experiment by integration of the flame-ionization detector signal corrected for the molar response of the various PMAAs[12].

ACKNOWLEDGMENTS

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