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Note

An alternative method for the rapid synthesis of partially O-methylated alditol acetate standards for GC–MS analysis of carbohydrates

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Abstract—Mixtures of partially O-methylated alditol acetate standards (PMAAs) of Glc, Gal, and Man were synthesized rapidly. Methylation of methyl glycosides was carried out in the presence of $BaO/Ba(OH)_2{\cdot}8H_2O$ giving rise to mixtures of partially methylated glycosides (PMGs), whose degree of methylation was monitored by TLC. The batch containing the largest mixture of methyl ethers was converted into partially O-methylated alditol acetate derivatives (PMAAs), via successive hydrolysis, reduction, and acetylation, and then subjected to GC and GC–MS analysis. Detailed data on retention times, TIC, and EIMS are now provided.

Keywords: Partially O-methylated alditol acetates; GC–MS standards; Methylation

Methylation analysis is the most commonly used method for determining the substitution pattern of monosaccharide units in oligo- and polysaccharides or the carbohydrate moieties of glycoconjugates.[1] Permethylation of carbohydrates can be achieved by the methods developed by Haworth,[2] Kuhn et al.,[3] Hakomori,[4] and Ciucanu and Kerek.[5] Among them, the Ciucanu's method is the most frequently used. It yields complete methylation products in only one step. The resulting products are converted into partially O-methylated alditol acetates (PMAAs) via successive hydrolysis, reduction and acetylation. These can then be identified by GC–MS, through correlation of their characteristic retention times (RT) and EI mass spectra with partially O-methylated alditol acetate standards.

Several methods have been described for the synthesis of partially O-methylated alditol acetate standards. Hakomori's method was developed in 1973.[6] In this procedure, methyl iodide is added portionwise in order that the partial methylation might be followed. Since methylation proceeds very quickly, it was rather difficult to control the degree of methylation by the amount of methyl iodide added. Purdie procedure[7] (MeI/Ag_2O) is quite different since it takes 12 h to obtain the highest degree of methylation. The major PMAAs were synthesized, however, 6-O-substitution had not been achieved. Kuhn methylation[6] ($MeI/DMF/Ag_2O$) is more rapid and appears analogous to the Purdie's procedure in forming a large variety of substitution products. Taking into account the toxicity of silver oxide, we turned our attention to a report based on the Kuhn methylation of methyl xyloside in the presence of barium oxide,[6] which proved to be 16 times faster than that the silver oxide method and yielded less complex mixtures of partially methylated glycosides (PMGs). We applied this approach to hexoses, and surprisingly found that the reaction rate could be controlled by the amount of $Ba(OH)_2{\cdot}8H_2O$ added in the initial stage. After optimizing the reaction conditions, we developed a new procedure on the basis of the Kuhn methylation[6] by which almost all possible variously O-methylated methyl glycosides were synthesized in 4 h. The products were then converted to PMAAs for GC–MS analysis. We have now prepared

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partially O-methylated alditol acetate standards of glucose, galactose, and mannose and report their EI mass spectra, TIC, and retention times parameters.

Glc, Gal, and Man were first converted to mixtures of methyl glycosides. Methylation of each product was carried out in the presence of BaO/$Ba(OH)_2{\cdot}8H_2O$. The methylation proceeded gradually. After 30 min, predominance of mono-*O*-methyl ethers was noticed, and 30 min later, di-*O*-methyl derivatives were formed. Higher degrees of methylation were obtained at later stages. Aliquots removed from the methylation mixture after 4 h contained unmethylated compounds, and mono-, di-, tri-, and tetra-*O*-methyl derivatives, which were converted into partially O-methylated alditol acetate derivatives (PMAAs) via successive hydrolysis with trifluoroacetic acid, reduction with $NaBH_4$, and acetylation with acetic anhydride in pyridine. The resulting PMAAs were identified via GC and GC–MS analysis following their EIMS profiles and retention times with reference to previous reports.[1,7,8]

For the synthesis of PMAAs standards, the reaction rate of methylation of glycosides in the early stage should be crucial. It is noteworthy that the amount of $Ba(OH)_2{\cdot}8H_2O$ added could control the rate of methylation of glycosides. The reaction rate increased with the increase in the amount of $Ba(OH)_2{\cdot}8H_2O$. In the early report on the methylation of methyl xyloside,[6] the reaction was completed in 60 min, probably due to the presence of an excess of $Ba(OH)_2{\cdot}8H_2O$. We modified this procedure and were able to control the rate of the reaction. The progress of the methylation was therefore much easier to control and the procedure was greatly simplified. Almost all the substitution patterns necessary for the analysis of Glc, Gal, and Man substitution in naturally occurring polysaccharides or glycoconjugates were prepared in the present procedure, including the acetates of 6-*O*-methyl-Glc-ol, 6-*O*-methyl-Gal-ol, 6-*O*-methyl-Man-ol, 5-*O*-methyl-Glc-ol, 3,5-di-*O*-methyl-Glc-ol, 2,5-di-*O*-methyl-Glc-ol, 4,6-di-*O*-methyl-Glc-ol, 2,5,6-tri-*O*-methyl-Glc-ol, 2,4,6-tri-*O*-methyl-Glc-ol, and 3,5,6-tri-*O*-methyl-Gal-ol. These are tabulated in Table 1, with their relative proportion calculated on the basis of their peak areas in GC. As can be seen in Figure 1, PMAAs are well separated in GC–MS using a DB-5ms capillary column and a temperature programming of 140 °C (3 min) −250 °C (40 min) at 2 °C/min. Some pairs of methylated derivatives (e.g., 2- and 5-*O*-methylhexitol, 3- and 4-*O*-methylhexitol, 2,4-di-*O*-methylhexitol and 3,5-di-*O*-methylhexitol) gave alditols with the same substitution pattern (see Table 1) due to symmetry reasons. They can be distinguished by using $NaBD_4$ instead of $NaBH_4$ as the reducing agent, which introduces deuterium at C-1.[9]

Thus, the new procedure utilizing BaO, $Ba(OH)_2{\cdot}8H_2O$, methyl iodide, and DMF should be an efficient, useful, and very practical method for the simultaneous synthesis of partially O-methylated alditol acetate standards (PMAAs) for the structural analysis of oligo- and polysaccharides.

1. Experimental

1.1. Preparation of methyl glycosides

Gal, Glc, and Man (120 mg) were each refluxed in 2% MeOH–HCl at 70 °C for 12 h. The soln was subsequently neutralized with an excess of powdered NaH-

Table 1. Partially O-methylated alditol acetates obtained via $Ba(OH)_2$/MeI methylation of methyl glycosides of Glc, Gal, and Man for 4 h

O-Methylated alditol acetates[a]	t_R[b]	%[c]	O-Methylated alditol acetates[a]	t_R[b]	%[c]	O-Methylated alditol acetates[a]	t_R[b]	%[c]
2,3,4,6-Me_4-Glc	17.8	1.9	2-Me-Glc	30.5	14.2	2,4-/3,5-Me_2-Gal	29.1	7.5
2,3,5,6-Me_4-Glc	18.0	0.5	3-Me-Glc	31.2	8.3	2-/5-Me-Gal	30.7	15.0
3,4,6-Me_3-Glc	21.7	2.4	4-Me-Glc	31.7	2.5	3-/4-Me-Gal	32.1	7.4
2,4,6-Me_3-Glc	22.0	5.1	Glc hexaacetate	33.3	9.2	Gal hexaacetate	33.8	26.1
2,3,4-Me_3-Glc	22.1	tr[d]	2,3,5,6-Me_4-Gal	18.2	0.4	2,3,4,6-Me_4-Man	17.9	29.5
2,3,6-Me_3-Glc	22.5	7.6	2,3,4,6-Me_4-Gal	18.8	1.0	2,3,6-Me_3-Man	22.2	3.5
2,5,6-Me_3-Glc	22.8	tr	2,5,6-Me_3-Gal	21.9	1.0	2,3,4-Me_3-Man	23.3	1.5
2,5-Me_2-Glc	23.5	0.8	2,3,6-Me_3-Gal	22.2	8.1	2,4,6-Me_3-Man	25.5	3.4
2,6-Me_2-Glc	25.5	10.2	3,5,6-Me_3-Gal	22.5	0.5	3,6-Me_2-Man	26.2	tr
4,6-Me_2-Glc	26.0	2.1	2,4,6-Me_3-Gal	22.9	1.5	2,3-Me_2-Man	27.2	10.4
3,6-Me_2-Glc	26.1	8	2,3,5-Me_3-Gal	24.4	0.5	3,4-Me_2-Man	27.6	tr
3,5-Me_2-Glc	26.5	tr	2,3,4-Me_3-Gal	24.7	3.7	6-Me-Man	27.9	2.4
2,3-Me_2-Glc	27.5	11	2,6-Me_2-Gal	25.4	6.7	2,4-/3,5-Me_2-Man	28.3	2.8
3,4-Me_2-Glc	27.6	tr	3,6-Me_2-Gal	26.3	2.8	2-/5-Me-Man	30.1	21.8
2,4-Me_2-Glc	27.7	7.0	2,3-Me_2-Gal	27.8	10.6	3-/4-Me-Man	31.3	8.2
6-Me-Glc	28.4	7.8	6-Me-Gal	28.4	5.3	Man hexaacetate	32.9	16.6
5-Me-Glc	29.0	1.6	3,4-Me_2-Gal	28.9	1.9			

[a] DB-5ms.
[b] Retention time in min.
[c] Relative percentage.
[d] Trace.

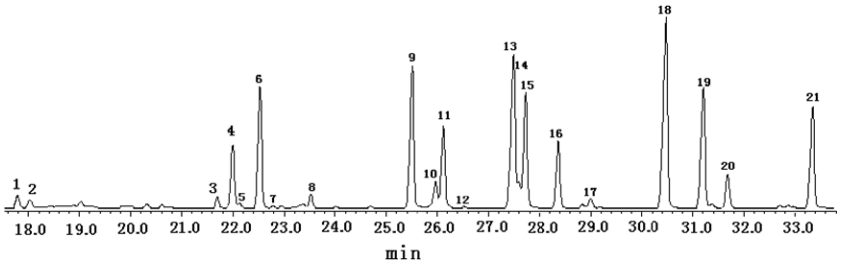

Figure 1. Total ion chromatogram from GC–MS analysis of PMAAs of Glc: (1) 2,3,4,6-Me_4-Glc; (2) 2,3,5,6-Me_4-Glc; (3) 3,4,6-Me_3-Glc; (4) 2,4,6-Me_3-Glc; (5) 2,3,4-Me_3-Glc; (6) 2,3,6-Me_3-Glc; (7) 2,5,6-Me_3-Glc; (8) 2,5-Me_2-Glc; (9) 2,6-Me_2-Glc; (10) 4,6-Me_2-Glc; (11) 3,6-Me_2-Glc; (12) 3,5-Me_2-Glc; (13) 2,3-Me_2-Glc; (14) 3,4-Me_2-Glc; (15) 2,4-Me_2-Glc; (16) 6-Me-Glc; (17) 5-Me-Glc; (18) 2-Me-Glc; (19) 3-Me-Glc; (20) 4-Me-Glc; (21) Glc hexaacetate.

CO_3, filtrated, and evaporated to dryness. The residue was then dissolved in MeOH (20 mL) for further use.

1.2. Methylation of methyl glycosides

The soln (10 mL) of methyl glycoside was concentrated to dryness by rotary evaporation under diminished pressure. The residue was then dissolved in DMF (2 mL), barium oxide (200 mg), $Ba(OH)_2{\cdot}8H_2O$ (10 mg) and methyl iodide (1 mL) were added and the suspension was vigourously stirred using a vibrational shaker in the dark to give partially methylated glycosides (PMGs). Aliquots (300 μL) of each PMGs were removed at 30 min intervals, poured into MeOH, filtered, and assayed by TLC (3:0.6 $CHCl_3$–MeOH) to monitor the degree of methylation using the orcinol–sulfuric acid staining reagent. The portion that presented a maximum number of spots on the silica gel plate was retained for the next step.

1.3. Preparation of PMAAs

The batch containing the more complex PMGs mixture was evaporated, and the residue was then subjected to acid hydrolysis with 2 M TFA for 2 h at 100 °C. MeOH (3 × 3 mL) was added and the solns were evaporated to dryness. The hydrolyzates were reduced with $NaBH_4$ (25 mg) in water (1 mL) and 27% ammonia (10 μL) for 3 h at room temperature. Then the solns were treated with glacial AcOH to destroy the excess of reducing agent and concentrated to dryness. The residue was co-distilled with 2 mL of MeOH at 50 °C, repeating this step thrice. Anhydrous pyridine (2 mL) and Ac_2O (2 mL) were added and the reaction left overnight to give partially O-methylated alditol acetate derivatives (PMAAs). The mixtures were dried. Chloroform (1 mL) and water (1 mL) were added to the sample, followed by vortexing. The organic phase was washed two times with 1 mL portions of water. The dried sample was dissolved in $CHCl_3$ and passed through a silica gel column. The fractions containing PMAAs were eluted with 8:1 $CHCl_3$–MeOH and collected for GC and GC–MS analysis.

1.4. GC and GC–MS analysis of PMAAs

1.4.1. GC. Each mixture was examined by GC, before GC–MS analysis, for the separation and quantification of the partially methylated alditol acetates. The PMAAs were applied to a DB-5ms capillary column, with the same programmed temperature as that for GC–MS.

1.4.2. GC–MS. GC–MS analysis of the PMAAs was carried out on a Shimadzu QP2010 GC–MS, equipped with a DB-5ms capillary column (30.0 m × 0.25 mm × 0.25 μm) using a temperature programming of 140 °C (3 min) −250 °C (40 min) at 2 °C/min. Helium was used as the carrier gas.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.05.028.

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