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A QUANTITATIVE METHOD TO DETERMINE THE POINTS OF *O*-(2-HYDROXYPROPYL) SUBSTITUTION IN *O*-(2-HYDROXYPROPYL)-GUAR*

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ABSTRACT

A method is described for locating the *O*-(2-hydroxypropyl) groups in *O*-(2-hydroxypropyl)-substituted guar. Per-*O*-methylation of the *O*-(2-hydroxypropyl)guar yielded guar that was partially *O*-methylated and partially *O*-(2-methoxypropyl)ated. This polymer was hydrolyzed, to afford a mixture of partially *O*-methylated monosaccharides and partially *O*-(2-methoxypropylated), partially *O*-methylated monosaccharides. These monosaccharide derivatives were reduced, and the alditols acetylated, to give a mixture of partially *O*-acetylated, partially *O*-methylated alditols with partially *O*-acetylated, partially *O*-(2-methoxypropyl)ated, partially *O*-methylated alditols. These alditol derivatives were identified by gas–liquid chromatography–mass spectrometry, and quantitated by gas–liquid chromatography.

INTRODUCTION

Guar is a galactomannan isolated from the seeds of guar (*Cyamopis tetragonolobus*). The polysaccharide has a backbone of β-(1→4)-linked D-mannosyl residues, of which ~60 percent are substituted at O-6 with a single α-D-galactosyl group. Guar that is treated with propylene oxide yields *O*-(2-hydroxypropyl)guar (HOPrguar). Methods developed to identify and quantify the positions of substitution in *O*-(2-hydroxypropyl)cellulose[1,2] are not applicable to *O*-(2-hydroxypropyl)guar, and we now present a method for identifying and quantifying the positions of substitution of the *O*-(2-hydroxypropyl) groups in HOPrguar. This method can readily be adapted for a variety of other *O*-(hydroxyalkyl)ated polysaccharides.

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EXPERIMENTAL

HOPrguar having a degree of substitution (d.s.) of 0.4 [mol of *O*-(2-hydroxypropyl) group per mol of hexosyl residue] and HOPrguar having a d.s. of >3 were obtained from Celanese.

Per-*O*-methylation was accomplished by the method of Hakomori[3,4]. Typically, HOPrguar (5 mg) was dried overnight in the presence of phosphorus pentaoxide in a vacuum oven at 40°. The dry HOPrguar was dissolved in freshly distilled dimethyl sulfoxide (1 mL), but, if it did not dissolve quickly, it was kept overnight in an ultrasonic bath, to aid dissolution. 3M Sodium dimethylsulfinyl anion (50 μL), prepared as described[4], was added to the polysaccharide solution, and the mixture was stirred for 1–3 h. Methyl iodide (9.4 μL; equimolar to the sodium dimethylsulfinyl anion) was added, and the solution was stirred for 1 h. The addition of sodium dimethylsulfinyl anion and methyl iodide was repeated twice more in the same way, except that the last addition of methyl iodide consisted of a large excess (50–100 μL). The sample was stirred overnight, and then a stream of filtered air was used to evaporate the excess of methyl iodide. Water (~1.5 mL) was added, and the resulting solution or suspension was dialyzed against distilled water (1 L) for at least 4 h. The liquid outside the dialysis bag was discarded in organic waste, and the per-*O*-methylated HOPrguar inside the dialysis bag was further dialyzed for 18–36 h against running tap-water. The resulting, dimethyl sulfoxide-free material was recovered by lyophilization. The per-*O*-methylated HOPrguar was then hydrolyzed with 2M TFA (0.5 mL) for 2 h at 121°. The resulting, partially *O*-methylated, monosaccharide derivatives were reduced with $NaBD_4$, and the alditols freed of borate, and acetylated as described[5]. The sample was dissolved in dichloromethane (~250 μL), and then decane (~250 μL) was added, to permit injection of the sample into the gas chromatograph.

Gas–liquid chromatography (g.l.c.) was accomplished in a Hewlett–Packard 5880A instrument, and g.l.c.–mass spectrometry (g.l.c.–m.s.) with a Hewlett–Packard 5985B g.l.c.–m.s. system. A DB-1 (J and W) capillary column (15 m × 0.32 mm i.d. for g.l.c.–m.s., and 15 m × 0.26 mm i.d. for g.l.c.) was used for the analyses. The sample (1 μL) was applied to the column by using a split (10:1) injection technique. The temperature program consisted of holding for 2 min at the injection temperature of 150° and then raising the temperature at 2°/min to 240°. For g.l.c.–m.s. analysis (splitless mode), the injection temperature of 100° was held for 2 min, then raised at 30°/min to 150°, and then raised at 2°/min to 240°.

RESULTS AND DISCUSSION

When HOPrguar (d.s. 0.4) was methylated, it yielded polymeric HOPrguar that was partially *O*-methylated and partially *O*-(2-methoxypropyl)ated. The polymer was hydrolyzed to a mixture of partially *O*-methylated (galactose plus mannose) with partially *O*-(2-methoxypropyl)ated, partially *O*-methylated (galac-

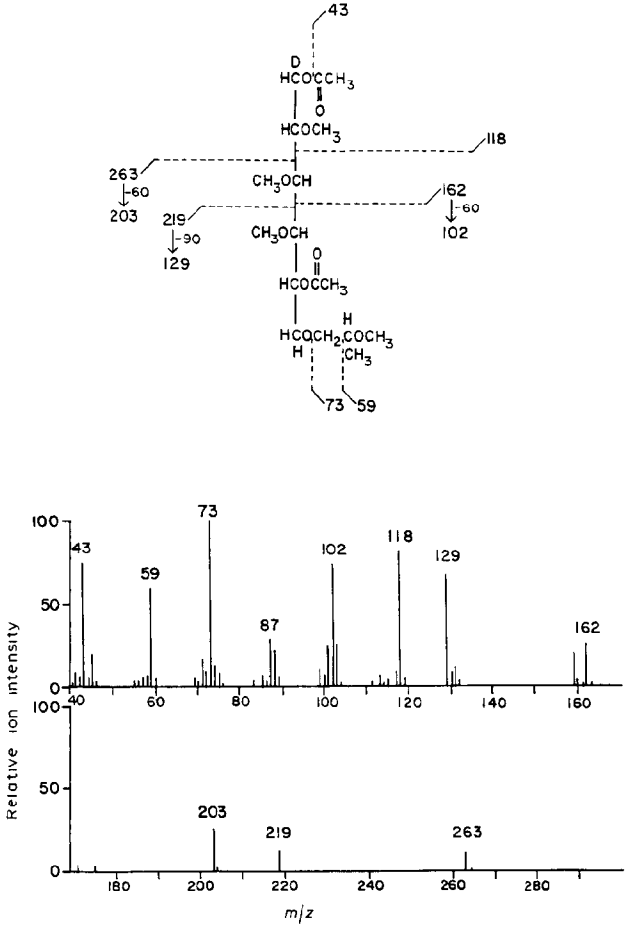

Fig. 1. The e.i.-mass spectrum of 1,5-di-*O*-acetyl-6-*O*-(2-methoxypropyl)-2,3,4-tri-*O*-methylgalactitol, and the origins of its more-abundant fragment-ions.

tose plus mannose). These monosaccharide derivatives were reduced with $NaBD_4$, and the alditols acetylated. The resulting mixture of galactitol and mannitol that were partially *O*-acetylated and partially *O*-methylated, and galactitol and mannitol that were partially *O*-acetylated, partially *O*-(2-methoxypropyl)ated, and partially *O*-methylated was analyzed by g.l.c.–m.s. The positions of substitution by the *O*-methyl, the *O*-(2-methoxypropyl), and the *O*-acetyl groups on each alditol were located by means of known electron impact (e.i.)-m.s. fragmentation-pathways of partially *O*-acetylated, partially *O*-alkylated alditols[6]. The e.i.-mass spectrum of 1,5-di-*O*-acetyl-6-*O*-(2-methoxypropyl)-2,3,4-tri-*O*-methylgalactitol and the origin of its major fragment-ions are illustrated in Fig. 1.

The alditols resulting from (terminal) galactosyl groups and linear and branched mannosyl residues could be readily distinguished from each other by the

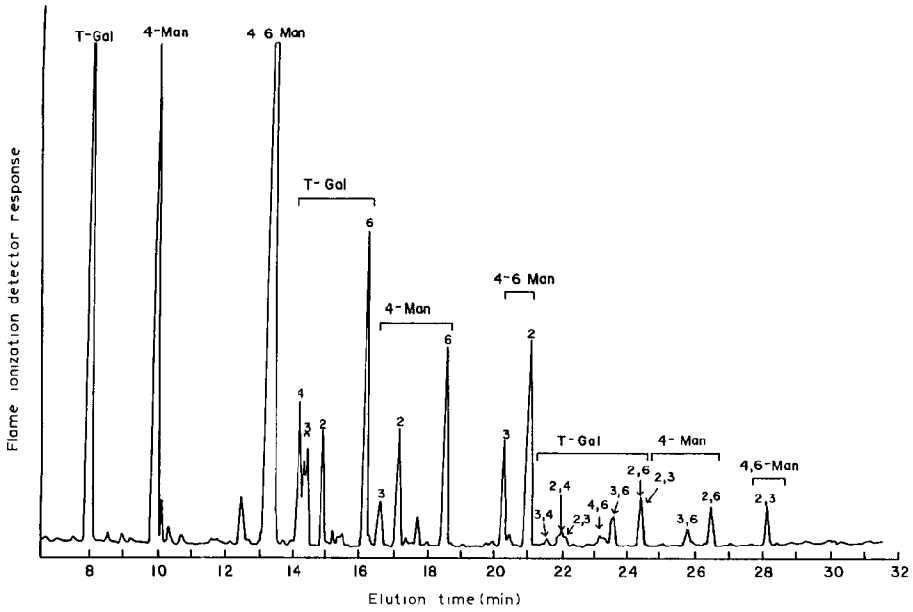

Fig. 2. Gas–liquid chromatogram of the mixture of partially *O*-acetylated, partially *O*-methylated alditols and partially *O*-acetylated, partially *O*-(2-methoxypropyl)ated, partially *O*-methylated alditols prepared from *O*-(2-hydroxypropyl)guar (d.s. 0.4). The chromatography was performed as described in the Methods section. The numbers above the peaks refer to the positions of *O*-(2-methoxypropyl) substitution. The components were identified from their e.i.-mass spectra obtained during g.l.c.–m.s. analysis.

number of their *O*-acetyl groups and the known, glycosyl-linkage composition of guar. The di-*O*-acetylhexitols were necessarily derived from the galactosyl groups, the tri-*O*-acetylhexitols from the linear, 4-linked mannosyl residues, and the tetra-*O*-acetylhexitols from the branched, 4,6-linked mannosyl residues. Thus, all of the partially *O*-acetylated, partially *O*-(2-methoxypropyl)ated, partially *O*-methylated hexitol derivatives were readily identified. A gas–liquid chromatographic separation of these derivatives is depicted in Fig. 2.

When guar is derivatized by reaction with propylene oxide to afford *O*-(2-hydroxypropyl)guar, two stereoisomers should be formed at any given position of substitution, because of the chiral center in the (2-hydroxypropyl) group. Thus, each pair of derivatives that results from the methylation, hydrolysis, reduction, and acetylation of *O*-(2-hydroxypropyl)guar should be separable by gas–liquid chromatography. In the case of 1,5-di-*O*-acetyl-3-*O*-(2-methoxypropyl)-2,4,6-tri-*O*-methylgalactitol [the derivative resulting from (2-hydroxypropyl)ation at O-3 of a galactosyl group], the g.l.c. did partially separate the two isomers (see Fig. 2). In some cases, the presence of the two (very similar) derivatives led to peak broadening [see the di-*O*-(2-methoxypropyl)galactitol derivatives in Fig. 2]. However, in most instances, the two derivatives co-chromatographed, that is, were eluted as a single peak (see Fig. 2).

TABLE I

MOLAR RESPONSE FACTORS FOR THE DERIVATIVES PRODUCED BY THE ANALYSIS OF *O*-(2-HYDROXYPROPYL)GUAR

Compound	*Response factor*[a]
T-Gal[b]	0.70
4-Man[c]	0.74
4,6-Man[d]	0.80
T-Gal mono-HOPr[e]	0.90
T-Gal di-HOPr	1.1
4-Man mono-HOPr	0.94
4-Man di-HOPr	1.1
4,6-Man mono-HOPr	1.0
4,6-Man di-HOPr	1.2

[a]To calculate the relative mol percent of each compound, the integrated area for each derivative was divided by the response factor. The resulting values were normalized to 100 mol percent. [b]T-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol. [c]4-Man = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylmannitol. [d]4,6-Man = 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylmannitol. [e]T-Gal mono HOPr = T-Gal in which one *O*-methyl group was replaced by an *O*-(2-methoxypropyl) group. The names of other derivatives are abbreviated in an analogous manner.

The relative content of each of the partially *O*-acetylated, partially *O*-(2-methoxypropyl)ated, partially *O*-methylated alditols was determined by g.l.c. The molar response-factors, calculated by the method of Sweet *et al.*[7], are given in Table I. Three identical samples of *O*-(2-hydroxypropyl)ated guar (d.s. 0.4) were derivatized and analyzed. The results are presented in Table II.

The reaction of guar with propylene oxide might have resulted in the substitution of one *O*-(2-hydroxypropyl) group by a second *O*-(2-hydroxypropyl) group, leading to an alditol substituted with two hydroxypropyl groups at a single position. Such a derivative would yield e.i.-m.s. fragment-ions at *m/z* 131 analogous to the fragment-ions at *m/z* 73 formed from positions substituted with a single group (see Fig. 1). No such fragment-ions were detected in the analysis of *O*-(2-hydroxypropyl)guar (d.s. = 0.4), but, to be certain that no such derivatives went undetected, a guar that was highly substituted with 2-hydroxypropyl groups (d.s. >3), and that therefore possessed hydroxypropyl groups substituted with hydroxypropyl groups, was analyzed. E.i.-m.s. analysis of this highly substituted *O*-(2-hydroxypropyl)guar did, in fact, yield fragment ions at *m/z* 131, indicating the presence of alditol derivatives containing 2-hydroxypropyl groups attached to the oxygen atoms of other 2-hydroxypropyl groups. Thus, derivatives resulting from residues possessing these types of doubly substituted position can be detected, but are not present in *O*-(2-hydroxypropyl)guar having d.s. 0.4.

In conclusion, a method for determining the positions of the *O*-(2-hydroxypropyl) groups in *O*-(2-hydroxypropyl)guar was developed. This method can be readily applied to other carbohydrate polymers substituted with *O*-(hydroxyalkyl)

TABLE II

THE MOL % OF EACH *O*-(2-HYDROXYPROPYL)-SUBSTITUTED GLYCOSYL RESIDUE IN *O*-(2-HYDROXYPROPYL)GUAR[a]

Glycosyl residue	*Mol %*		
	Sample 1	*Sample 2*	*Sample 3*
No substitution			
T-Gal	17	19	17
4-Man	17	15	14
4,6-Man	30	32	30
Monosubstitution			
T-Gal-4-HOPr[b]	0.8	0.6	1.3
T-Gal-3-HOPr	3.4	1.6	2.9
T-Gal-2-HOPr	2.2	2.3	2.1
T-Gal-6-HOPr	6.6	7.1	6.7
4-Man-3-HOPr	1.7	1.7	1.7
4-Man-2-HOPr	3.0	3.2	3.1
4-Man-6-HOPr	5.0	5.4	5.1
4,6-Man-3-HOPr	2.5	2.9	3.1
4,6-Man-2-HOPr	5.7	6.2	6.2
Disubstitution			
T-Gal-2,4-HOPr			
+ T-Gal-3,4-HOPr[c]	0.2	0.2	0.4
T-Gal-2,3-HOPr	0.6	0.6	0.7
T-Gal-4,6-HOPr	0.3	0.4	0.5
T-Gal-3,6-HOPr	0.8	0.9	1.1
T-Gal-2,6-HOPr			
+ 4-Man-2,3-HOPr[c]	1.1	1.2	1.2
4-Man-3,6-HOPr	0.8	0.8	0.4
4-Man-2,6-HOPr	1.1	1.1	1.1
4,6-Man-2,3-HOPr	0.1	0.3	0.1
d.s.[d]	0.40	0.41	0.45

[a]The analysis was conducted on three different samples of the same batch of *O*-(2-hydroxypropyl)guar (d.s. 0.4). [b]T-Gal 4-HOPr = a terminal galactosyl group substituted at O-4 with a 2-methoxypropyl group. The other compounds are identified analogously. [c]These two pairs of compounds co-chromatographed on the g.l.c. column, which was slightly different from that used to generate the chromatogram illustrated in Fig. 2. [d]Degree of substitution (d.s.) was calculated from (d.o. + 2 t.s.)/100, where d.o. = total mol percent of the glycosyl residues substituted with one 2-hydroxypropyl group, and t.s. = total mol percent of the glycosyl residues substituted with two 2-hydroxypropyl groups. The d.s. as stated by the supplier of this guar derivative was 0.4.

groups. For example, the positions of substitution of *O*-(2-hydroxyethyl)ated guar, starch, cellulose, and dextran can be determined.

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