Carbohydrate Research, 211 (1991) 309–316
Elsevier Science Publishers B.V., Amsterdam

Analysis of a pyruvic acid acetal-containing polysaccharide by the reductive-cleavage method*

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(Received August 9th, 1990; accepted October 23rd, 1990)

ABSTRACT

The applicability of the reductive-cleavage method to the analysis of polysaccharides bearing pyruvic acid acetals has been demonstrated. Direct reductive cleavage of fully methylated gum xanthan yielded the expected products, including 1,5-anhydro-4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-D-mannitol. The latter product was not observed when reductive cleavage was performed subsequent to reduction of ester groups in the fully methylated polysaccharide and mild hydrolysis to remove pyruvic acid acetal substituents. Instead, the latter experiment yielded 1,5-anhydro-2,3-di-*O*-methyl-D-mannitol, establishing the presence in the polysaccharide of terminal (nonreducing) D-mannopyranosyl groups bearing 4,6-*O*-(1-carboxyethylidene) substituents. The products of reductive cleavage were characterized, where appropriate, by comparison of the gas chromatographic retention times and chemical ionization- and electron ionization-mass spectra of their acetates to those of authentic standards. Alternatively, the products of reductive cleavage could be characterized without resort to comparison with authentic standards by analysis of the ^{1}H-n.m.r. spectra of their benzoates, which were obtained in pure form by high-performance liquid chromatography. By either method of product characterization, this two-step procedure of analysis reveals the presence of pyruvic-acetal residues in polysaccharides and establishes both the identity of the sugar residue to which they are attached and their positions of attachment.

INTRODUCTION

In a previous report[1], the applicability of the reductive-cleavage method[2] to the analysis of polysaccharides bearing pyruvic acid acetal (1-carboxyethylidene) substituents was investigated by examining the fate, under reductive-cleavage conditions, of compounds that model the various structural states that might be encountered. Because we envisaged that such analyses might potentially be accomplished directly after (*a*) permethylation, (*b*) sequential permethylation and reduction of ester groups, or (*c*) sequential carboxyl reduction and permethylation, model compounds were chosen that contained 1-methoxycarbonylethylidene, 1-(hydroxymethyl)ethylidene, and 1-(methoxymethyl)ethylidene substituents. From these studies it was established that 1-methoxycarbonylethylidene substituents were quite stable to the usual conditions[2–4] of reductive cleavage but that 1-(hydroxymethyl)ethylidene and 1-(methoxymethyl)

* This investigation was supported by PHS grant number GM34710, awarded by the National Institute of General Medical Sciences, DHHS.
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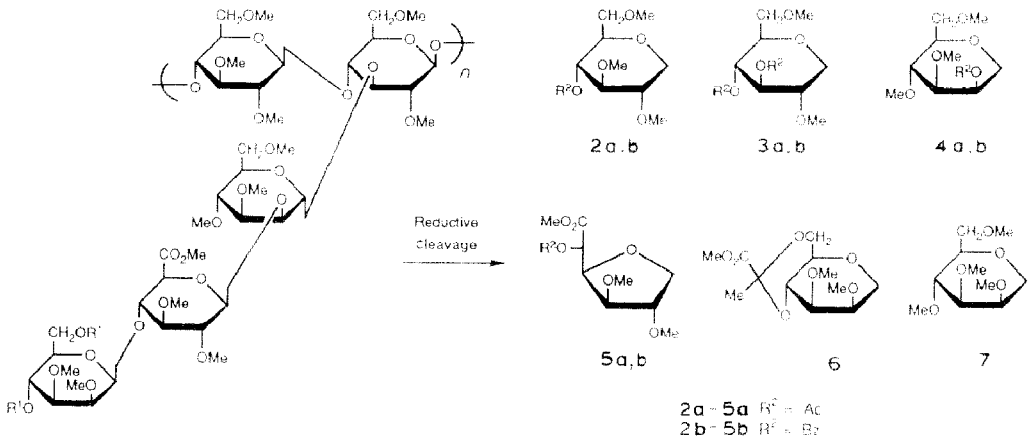
0008-6215/91/$ 03.50

ethylidene substituents were not. Based upon these studies we proposed[1] that the analysis of polysaccharides bearing pyruvic acid acetal substituents could be accomplished by a two-step procedure involving total reductive cleavage of both the fully methylated polysaccharide and its ester-reduced ($LiAlH_4$) and mild acid-treated derivative. As a test of this proposal, the extracellular polysaccharide (gum xanthan) from *Xanthomonas campestris* was analyzed, and the results are described herein.

RESULTS AND DISCUSSION

Reductive cleavage of per-*O*-methylated xanthan (**1a**, Scheme 1) was carried out



1a R^1 = Me or 

1b R^1 = Me or H

Scheme 1

in the presence of 5 equiv. of triethylsilane (Et_3SiH), 1 equiv. of boron trifluoride etherate ($BF_3{\cdot}Et_2O$), and 5 equiv. of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) as previously described[4]. A portion of the mixture of products was acetylated, and the resulting anhydroalditol acetates were analyzed by gas–liquid chromatography (g.l.c.). Peaks 1–5 (Fig. 1, upper) were identified by comparison of their g.l.c. retention times and chemical-ionization (c.i.-) and electron-ionization (e.i.-) mass spectra to those of authentic standards. Peak 1 was identified as 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-mannitol (**7**) (ref. 5), arising from terminal (nonreducing) D-mannopyranosyl groups, and Peak 2 was identified as 2-*O*-acetyl-1,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (**4a**) (ref. 5), arising from 2-O linked D-mannopyranosyl residues. Peaks 3 and 4 were identified as 4-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (**2a**) (ref. 6), and 3,4-di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol (**3a**) (ref. 7), arising from the 4-O linked and 3,4-di-O linked D-glucopyranosyl residues of the backbone, respectively.

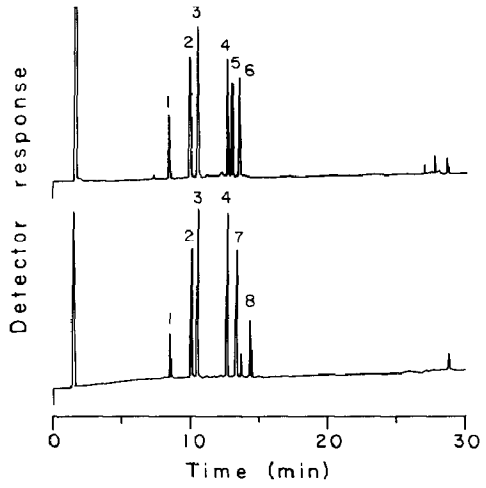


Fig. 1. Gas-liquid chromatograms of the anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated xanthan (upper) and its reduced ($LiAlH_4$) and mild acid-treated derivative (lower). The numbered peaks were identified as follows: 1, **7**; 2, **4a**; 3, **2a**; 4, **3a**; 5, **5a**; 6, **6**; 7, **8a**; and 8, **9a**.

Peak 5 was identified as methyl 2-*O*-acetyl-3,6-anhydro-4,5-di-*O*-methyl-L-gulonate (**5a**) (ref. 8), arising from 4-O linked D-glucopyranosyluronic residues; the product **5a** was a furan anhydroalditol, as expected, since 4-O linked D-glucopyranosyluronic residues are known[8] to undergo ring contraction during reductive cleavage.

The remaining component, Peak 6 (Fig. 1, upper), was found by c.i.-m.s. to have a molecular weight of 276, as would be expected for the pyruvic-acetal-containing anhydro-D-mannitol residue **6** or, indeed, any di-*O*-acetyl-di-*O*-methyl anhydrohexitol. That Peak 6 was indeed 1,5-anhydro-4,6-*O*-[(*S*)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-D-mannitol (**6**) was evident from the fact that its g.l.c. retention time did not change upon acetylation; its structure was verified by isolation and characterization (*vide infra*).

Integration of all peaks and correction for molar response[9,10] gave the molar ratio of each product (**2a**–**5a**, **6**, and **7**) derived from per-*O*-methylated xanthan (Table I). As is evident, the experimental values were in good agreement with those expected, with the exception that **3a** and **5a** were derived in lower-than-expected proportions. The reason

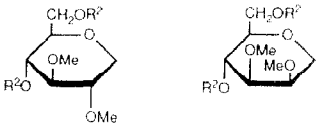
TABLE I

Molar ratios of products (compounds **2a**–**9a**) derived by reductive cleavage of per-*O*-methylated xanthan **1a** and its reduced ($LiAlH_4$) and mild acid-treated derivative **1b**

Polysaccharide	*Molar ratio*							
	2a	**3a**	**4a**	**5a**	**6**	**7**	**8a**	**9a**
1a	1.0	0.76	0.98	0.59	0.44	0.47	—	—
1b	1.0	0.90	0.75	—	—	0.24	0.62	0.27

for the low proportion of **3a** is not known, but similar results in other experiments[11] suggest that the loss occurs during work-up of the reductive-cleavage reaction mixture. Similarly, the low proportion of product **5a** derived from 4-O linked D-glucopyranosyluronic residues has been previously noted[8]. It should also be noted that the combined molar ratio (0.91) of products **6** and **7** arising from terminal (nonreducing) D-mannopyranosyl and pyruvic-acetal-containing D-mannopyranosyl groups of the side chains was in good agreement with the expected value. In addition, products **6** and **7** were derived in approximately equimolar proportions, as expected[12].

The foregoing experiment established the presence in the polysaccharide of a non-reducing terminal hexose group containing acetal-linked pyruvic acid, but it did not establish the identity of the parent hexose or the positions of substitution of the 1-carboxyethylidene acetal. The strategy employed in the second experiment was to selectively remove the pyruvic acid acetal from the fully methylated polysaccharide, then again perform reductive cleavage so as to identify the newly-produced, partially methylated anhydrohexitol acetate. The fully methylated polysaccharide was therefore subjected to reduction with $LiAlH_4$ (ref. 13), and the newly formed 1-(hydroxymethyl) ethylidene acetal was hydrolyzed. The resultant polysaccharide **1b** (see Scheme 1) was then subjected to reductive cleavage as before, and the products were analyzed by g.l.c. (see Fig. 1, lower) and g.l.c.–m.s. As is evident in Fig. 1, reduction of fully methylated xanthan **1a** and mild hydrolysis to remove pyruvic acid acetal substituents resulted in the disappearance of Peak 5 (compound **5a**) and Peak 6 (compound **6**) and the appearance of two new peaks (Peaks 7 and 8) in the chromatogram. Based upon a comparison of their g.l.c. retention times and e.i.- and c.i.-mass spectra with those of authentic standards, Peak 7 was identified as 4,6-di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-glucitol (**8a**) (ref. 8), and Peak 8 was identified as 4,6-di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-mannitol (**9a**) (ref. 14). Peak 7 (compound **8a**) obviously arose from 4-O linked D-glucopyranosyluronic residues in the polysaccharide, and Peak 8 (compound **9a**) arose from 4,6-*O*-(1-carboxyethylidene)-D-mannopyranosyl groups. Integration of Peaks 1–4, 7, and 8 and correction for molar response gave the molar ratios listed in Table I. As is evident in Table I, the molar ratios of **2a** (1.0) and **3a** (0.90), derived, respectively, from the 4-O linked and 3,4-di-O linked D-glucopyranosyl residues of the backbone, were close to those expected, but the proportions of **4a** (0.75), **8a** (0.62), and **7** + **9a** (0.51) were significantly lower than expected. The fact that all side-chain residues gave lower than expected molar ratios suggests that, in part, some cleavage of the side-chain from the backbone occurred during the mild acid hydrolysis step to remove the reduced pyruvic acid acetal substituent. The proportions of these products were not critical to the analysis, however.



8a R^2 = Ac
b R^2 = Bz

9a R^2 = Ac
b R^2 = Bz

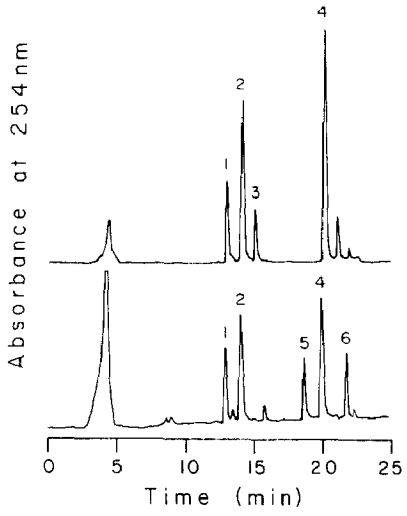


Fig. 2. High-performance liquid chromatograms of the anhydroalditol benzoates derived by reductive cleavage of per-*O*-methylated xanthan (upper) and its reduced ($LiAlH_4$), and mild acid-treated derivative (lower). The numbered peaks were identified as follows: 1, **4b**; 2, **2b**; 3, **5b**; 4, **3b**; 5, **9b**; and 6, **8b**.

The two-step procedure just described indeed established both the identity of the sugar residue bearing the pyruvic acid acetal and the positions of substitution of the pyruvic acid acetal. However, the analysis as presented was based upon a comparison of the g.l.c. retention times, and e.i.- and c.i.-mass spectra of the products with those of authentic standards. Recognizing that such comparisons may not always be feasible, the validity of this method of analysis of polysaccharides containing acetal-linked pyruvic acid was also demonstrated by isolation and direct characterization of the products by ^{1}H-n.m.r. spectroscopy[15]. Shown in Fig. 2 are the chromatograms obtained when the benzoylated reductive cleavage products derived from per-*O*-methylated xanthan (upper) and its reduced ($LiAlH_4$) and mild-treated derivative (lower) were subjected to high-performance liquid chromatography. The individual peaks were collected, solvent was removed by evaporation under vacuum, and the ^{1}H-n.m.r. spectra of the samples were acquired without further purification. The identities of the components and the positions of substitution of benzoyl and methyl groups were established based upon a straightforward analysis of the multiplicities and coupling constants of the ring-proton resonances[15]. Peaks 1–3 (Fig. 2) were identified as mono-*O*-benzoyl derivatives, namely 1,5-anhydro-2-*O*-benzoyl-3,4,6-tri-*O*-methyl-D-mannitol (**4b**), 1,5-anhydro-4-*O*-benzoyl-2,3,6-tri-*O*-methyl-D-glucitol (**2b**) (ref. 15), and methyl 3,6-anhydro-2-*O*-benzoyl-4,5-di-*O*-methyl-L-gulonate (**5b**), respectively. Peaks 4–6 (Fig. 2) were identified as di-*O*-benzoyl derivatives, namely 1,5-anhydro-3,4-di-*O*-benzoyl-2,6-di-*O*-methyl-D-glucitol (**3b**), 1,5-anhydro-4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-D-mannitol (**9b**), and 1,5-anhydro-4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-D-glucitol (**8b**), respectively. As expected, methyl 3,6-anhydro-2-*O*-benzoyl-4,5-di-*O*-methyl-L-gulonate (**5b**) was present (Peak 3; Fig. 2, upper) in the product mixture derived from

fully methylated xanthan (**1a**), but it was absent (Fig. 2, lower) in the product mixture derived from its reduced and mild acid-treated derivative (**1b**). In place of compound **5b**, the product mixture derived from **1b** contained a new component (**8b**; Peak 6, Fig. 2, lower) originating from the 4-O linked D-glucopyranosyluronic residue, as expected. Importantly, the product mixture derived from **1b** also contained another component (Peak 5, Fig. 2, lower) not seen in the product mixture derived from fully methylated xanthan **1a**, and its identification as 1,5-anhydro-4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-D-mannitol (**9b**) unequivocally establishes that the pyruvic acid acetal substituent is linked to the O-4 and O-6 positions of a terminal, nonreducing D-mannopyranosyl group.

Obviously, products **6** and **7** which lack a benzoyl group were not detected by high-performance liquid chromatography (Fig. 2) since the effluent was monitored for absorbance at 254 nm. The pyruvic-acetal-containing anhydro-D-mannitol derivative **6** was, however, isolated in a second experiment (see Experimental section) and characterized by ^{1}H-n.m.r. spectroscopy. The ^{1}H-n.m.r. spectrum of **6** displayed singlets for an equatorial[14,16] acetalic methyl group (δ 1.55), two *O*-methyl groups (δ 3.45, 3.54), and a methyl ester (δ 3.82), in addition to the expected number of ring hydrogen resonances. Importantly, the H-1e resonance was observed as a doublet of doublets (J 2.0, 12.8 Hz) which is characteristic[5,15] for 1,5-anhydro-D-mannitol derivatives. Thus, the spectrum of **6** is fully in accord with its structure, demonstrating that the behavior during reductive cleavage, of the 4,6-*O*-(1-carboxyethylidene)-D-mannopyranosyl group was that to be expected based upon the prior studies with model compounds.

EXPERIMENTAL

General. – ^{1}H-N.m.r. spectra were recorded with an IBM NR-300 or IBM NR-200 AF n.m.r. spectrometer for solutions in $CDCl_3$ and were referenced to internal tetramethylsilane ($CHCl_3$ resonance at δ 7.26). Gas–liquid chromatography (g.l.c.) was performed in a Hewlett–Packard Model 5890A gas–liquid chromatograph equipped with a Hewlett–Packard Model 3392A integrator, a flame-ionization detector, and a J. & W. Scientific DB-5 fused-silica capillary column (0.25 mm × 30 m; film thickness 0.25 μm). The temperature of the column was held for 2 min at 110°, and then programmed to 300° at 6°/min. G.l.c.–m.s. analyses were performed using a VG Analytical Ltd. Model VG 7070E-HF high-resolution, double-focusing mass spectrometer. High-performance liquid chromatography (h.p.l.c.) was performed using a Beckman model 338 System Gold chromatograph. Chromatography was performed on a column (9.2 mm × 25 cm) of DuPont Zorbax ODS, eluted with a 20-min gradient (curve 0, model 166 Programmable Detector Module) from 40% acetonitrile in water to 80% acetonitrile in water at a flow rate of 3 mL/min. Medium-pressure liquid chromatography (m.p.l.c.) was carried out in an instrument consisting of a Rheodyne 7125 injector, an Eldex model B-100-S4 pump, a Scientific Systems model LP-21 pulse dampener, a Waters Associates differential refractometer, and a ChiraTech Scientific Instruments, Inc. ultraviolet absorption monitor. Chromatography was conducted on a glass column (0.8 × 25 cm) packed with 40Å silica gel (35–70 mesh) from E. Merck. The column was eluted with 2:1 (v/v) hexane–ethyl acetate.

Methylation of gum xanthan (Sigma Chemical Co.) was performed by a modification of the procedure of Hakomori[17]. To the lyophilized polysaccharide (150 mg) in Me_2SO (30 mL) was added 30 mL of 1.4M lithium methylsulfinyl carbanion[18], and the solution was stirred at room temperature for 4 h. Cold (0–4°) methyl iodide (2.6 mL) was then added with external cooling, and the mixture was stirred for 18 h. The reaction mixture was then diluted with an equal volume of water, and the solution was dialyzed against running water overnight. The contents of the dialysis bag were extracted five times with an equal volume of dichloromethane, and the organic extracts were dried over Na_2SO_4 then concentrated to leave the per-*O*-methylated polysaccharide (110 mg) as a clear film. Reductive cleavages were carried out as previously described[4], and the products were either acetylated[9] or benzoylated. Benzoylations were carried out overnight under nitrogen using approximately 1.2 equiv. of benzoyl chloride per hydroxyl goup in a five-fold excess of pyridine. The reaction mixtures were worked up by direct passage through a small column of silica gel, and 3:1 (v/v) hexane–ethyl acetate was used to elute the benzoylated products.

Reduction of ester groups in the fully methylated polysaccharide was accomplished with $LiAlH_4$ in tetrahydrofuran as previously described[13]. Subsequent hydrolysis of the newly formed 1-(hydroxymethyl)ethylidene group was accomplished by treatment with 2M acetic acid for 2 h at 80°. The reaction mixture was concentrated to dryness under vacuum, and volatiles in the residue were coevaporated 3 times with toluene. ^{1}H-N.m.r. spectroscopy of the product verified that complete removal of the 1-methoxycarbonylethylidene substituent had been achieved.

*1,5-Anhydro-3,4-di-*O*-benzoyl-2,6-di-*O*-methyl-*D*-glucitol* (**3b**). — Per-*O*-methylated gum xanthan **1a** was reductively cleaved for 3 h in the presence of 5 equiv. of Et_3SiH, 1 equiv. of $BF_3{\cdot}Et_2O$, and 5 equiv. of Me_3SiOSO_2Me as previously described[4], and the products were benzoylated. Compound **3b** was isolated from the mixture of products as described in Fig. 2. For **3b**: ^{1}H-n.m.r.: δ 3.34 (s, 3 H, MeO), 3.39 (s, 3 H, MeO), 3.34–3.80 (complex, 5 H, H-1a, 2, 4, 6, 6′), 4.28 (dd, 1 H, *J* 5.2, 11.2 Hz, H-1e), 5.35 (t, 1 H, *J* 9.5 Hz, H-4), 5.56 (t, 1 H, *J* 9.5 Hz, H-3), 7.32–7.40 (m, 4 H, *m*-Ar), 7.47–7.52 (m, 2 H, *p*-Ar), and 7.90–7.97 (m, 4 H, *O*-Ar).

*1,5-Anhydro-2-*O*-benzoyl-3,4,6-tri-*O*-methyl-*D*-mannitol* (**4b**). — Compound **4b** was isolated as described for **3b**. For **4b**: ^{1}H-n.m.r.: δ 3.36–3.69 (complex, 6 H, H-1a, 3, 4, 5, 6, 6′), 3.45 (s, 6 H, 2 MeO), 3.56 (s, 3 H, MeO), 4.15 (dd, 1 H, *J* 2.2, 13.0 Hz, H-1e), 5.56 (m, 1 H, H-2), 7.41–7.48 (m, 2 H, *m*-Ar), 7.54–7.63 (m, 1 H, *p*-Ar), and 8.05–8.10 (m, 2 H, *O*-Ar).

*Methyl 3,6-anhydro-2-*O*-benzoyl-4,5-di-*O*-methyl-*L*-gulonate* (**5b**). — Compound **5b** was isolated as described for **3b**. For **5b**: ^{1}H-n.m.r.: δ 3.34, 3.40 (two s, 6 H, 2 MeO), 3.80 (s, 3 H, MeO_2C), 3.85 (dd, 1 H, *J* 2.0, 9.7 Hz, H-6), 3.89–3.95 (complex, 2 H, H-4,5), 4.13 (dd, 1 H, *J* 4.4, 9.7 Hz, H-6′), 4.46 (dd, 1 H, *J* 4.1, 8.9 Hz, H-3), 5.38 (d, 1 H, *J* 8.9 Hz, H-2), 7.44–7.48 (m, 2 H, *m*-Ar), 7.57–7.59 (m, 1 H, *p*-Ar), and 8.04–8.08 (m, 2 H, *O*-Ar).

*1,5-Anhydro-4,6-*O*-[(S)-1-methoxycarbonylethylidene]-2,3-di-*O*-methyl-*D*-mannitol* (**6**). — Compound **6** was isolated by m.p.l.c. from the product mixture resulting from reductive cleavage (5 equiv. Et_3SiH and 5 equiv. $BF_3{\cdot}Et_2O$ per equiv. of acetal, 6 h) of

permethylated gum xanthan. Fractions were collected and analyzed for the presence of **6** by g.l.c. (see Fig. 1). For **6**: ^{1}H-n.m.r.: δ 1.55 (s, 3 H, acetal Me), 3.24 (dt, 1 H, J 5.1, 10.7 Hz, H-5), 3.36–3.44 (complex, 2 H, H-1a, 3), 3.45, 3.54 (two s, 6 H, 2 MeO), 3.67 (m, 1 H, H-2), 3.82 (s, 3 H, MeO_2C), 3.71–3.84 (complex, 2 H, H-4, 6a), 4.02 (dd, 1 H, J 5.1, 10.7 Hz, H-6e), and 4.15 (dd, 1 H, J 2.0, 12.8 Hz, H-1e); g.l.c.–e.i.-m.s. (NH_3, positive): m/z 125 (37), 143 (82), 175 (65), 217 (100), 277 (2), and 294 (65); g.l.c.–e.i.-m.s.: m/z 43 (100), 45 (19), 58 (13), 69 (44), 75 (10), 111 (26), 117 (29), 125 (32), and 217 (19).

*1,5-Anhydro-4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-*D*-glucitol* (**8b**). – Modified gum xanthan (**1b**, 7 mg) was reductively cleaved for 3 h in the presence of 5 equiv. Et_3SiH, 1 equiv. of $BF_3{\cdot}Et_2O$, and 5 equiv. of Me_3SiOSO_2Me, and the products were isolated in the usual way[4]. Compound **8b** was isolated from the benzoylated product mixture by h.p.l.c. (see Fig. 2, lower). For **8b**: ^{1}H-n.m.r.: δ 3.24 (t, 1 H, J 11.0 Hz, H-1a), 3.38–3.48 (complex, 2 H, H-2,3), 3.51 (s, 6 H, 2 MeO), 3.74 (ddd, 1 H, J 2.7, 5.5, 9.6 Hz, H-5), 4.16 (dd, 1 H, J 4.4, 11.0 Hz, H-1e), 4.29 (dd, 1 H, J 5.5, 12.2 Hz, H-6), 4.54 (dd, 1 H, J 2.7, 12.2 Hz, H-6'), 5.28 (t, 1 H, J 9.6 Hz, H-4), 7.35–7.61 (complex, 6 H, *m*-, *p*-Ar), and 7.96–8.07 (m, 4 H, *o*-Ar).

*1,5-Anhydro-4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-*D*-mannitol* (**9b**). – Compound **9b** was isolated as described for **8b**. For **9b**: ^{1}H-n.m.r.: δ 3.43–3.51 (m, 1 H, H-1a), 3.43, 3.51 (two s, 6 H, 2 MeO), 3.56 (dd, 1 H, J 3.2, 9.3 Hz, H-3), 3.77 (m, 1 H, H-2), 3.82 (ddd, 1 H, J 3.0, 6.0, 9.3 Hz, H-5), 4.25 (dd, 1 H, J 2.7, 12.8 Hz, H-1e), 4.37 (dd, 1 H, J 6.0, 12.1 Hz, H-6), 4.55 (dd, 1 H, J 3.0, 12.1 Hz, H-6'), 5.61 (t, 1 H, J 9.3 Hz, H-4), 7.32–7.56 (complex, 6 H, *m*-, *p*-Ar), and 7.99–8.06 (m, 4 H, *o*-Ar).

REFERENCES

1 S. G. Zeller and G. R. Gray, *Carbohydr. Res.*, 198 (1990) 285–303.
2 D. Rolf and G. R. Gray, *J. Am. Chem. Soc.*, 104 (1982) 3539–3541.
3 D. Rolf, J. A. Bennek, and G. R. Gray, *J. Carbohydr. Chem.*, 2 (1983) 373–383.
4 J.-G. Jun and G. R. Gray, *Carbohydr. Res.*, 163 (1987) 247–261.
5 J. U. Bowie and G. R. Gray, *Carbohydr. Res.*, 129 (1984) 87–97.
6 D. Rolf, J. A. Bennek, and G. R. Gray, *Carbohydr. Res.*, 137 (1985) 183–196.
7 D. Rolf and G. R. Gray, *Carbohydr. Res.*, 152 (1986) 343–349.
8 S. A. Vodonik and G. R. Gray, *Carbohydr. Res.*, 175 (1988) 93–102.
9 J. U. Bowie, P. V. Trescony, and G. R. Gray, *Carbohydr. Res.*, 125 (1984) 301–307.
10 D. P. Sweet, R. H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.
11 S. A. Vodonik and G. R. Gray, *Carbohydr. Res.*, 172 (1988) 255–266.
12 P.-E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 45 (1975) 275–282.
13 B. Lindberg, *Methods Enzymol.*, 28 (1972) 178–195.
14 G. R. Gray, *Methods Enzymol.*, 193 (1990) 323–337.
15 C. K. Lee and G. R. Gray, *J. Am. Chem. Soc.*, 110 (1988) 1292–1293.
16 P. J. Garegg, B. Lindberg, and I. Kavarnström, *Carbohydr. Res.*, 77 (1979) 71–78.
17 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
18 A. J. D'Ambra, M. J. Rice, S. G. Zeller, P. R. Gruber, and G. R. Gray, *Carbohydr. Res.*, 177 (1988) 111–116.