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Note

Location of the *O*-acetyl group in welan by the reductive-cleavage method

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Welan gum (previously named S-130) is a commercial polysaccharide produced by *Alcaligenes* ATCC 31555. Its structure was originally determined by Jansson *et al.*[1] and later confirmed by O'Neill *et al.*[2] The polysaccharide is composed of pentasaccharide units having the following structure:

→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→
3
↑
1
α-L-Rha*p* or α-L-Man*p*

Of these units, approximately two-thirds contain terminal α-L-rhamnopyranosyl groups, while the remainder contain α-L-mannopyranosyl groups. *O*-Acetyl groups have also been shown to be present; however, their position of substitution along the chain has not been determined. We now report the location of *O*-acetyl groups in welan using the reductive-cleavage technique[3].

RESULTS AND DISCUSSION

Reductive cleavage of Hakomori-methylated welan. — Fully methylated (base-catalyzed) welan (**1**, Scheme 1) was subjected to reductive cleavage in the presence of 5 equiv. of triethylsilane (Et_3SiH) as the reducing agent and a mixture of 5 equiv. of trimethylsilyl methanesulfonate (Me_3SiOMs) and one equiv. of boron trifluoride etherate ($BF_3 \cdot Et_2O$) as the catalyst[4]. The mixture of products was acetylated, and the resulting anhydroalditol acetates were analyzed by gas–liquid chromatography (g.l.c.), the results of which are presented in Fig. 1, upper trace. The numbered peaks 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 known standards. Peak 1 was identified as 1,5-anhydro-2,3,4-tri-*O*-methyl-L-rhamnitol (**7**), and Peak 3 was deter-

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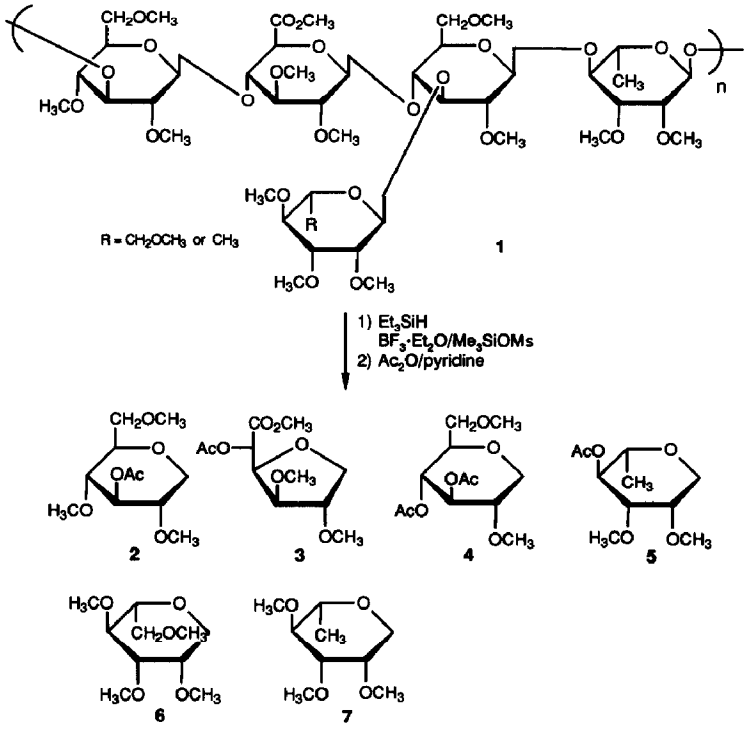


Scheme 1

mined to be 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-L-mannitol (**6**). These compounds arise from the terminal, non-reducing end groups, L-rhamnose and L-mannose, respectively. Peaks 2, 4, and 6 were identified as the three singly linked residues, 4-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-L-rhamnitol (**5**), 3-*O*-acetyl-1,5-anhydro-2,4,6,-tri-*O*-methyl-D-glucitol (**2**), and methyl 2-*O*-acetyl-3,6-anhydro-4,5-di-*O*-methyl-L-gulonate (**3**), respectively. Peak 5 was identified as 3,4-di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol (**4**), arising from the doubly linked hexose residue.

Integration of all peaks and correction for molar response[5,6] gave the mole fraction for each of the products (**2**–**7**) which are displayed in Table I. The experimental values were in close agreement with those expected, with the exception that the ratio of compounds **7** (0.41) to **6** (0.47) was lower than anticipated. However, the combined mole fraction (0.88) for these components suggests some recovery loss. The reason for the low recovery is most likely due to the loss of the volatile per-*O*-methylated rhamnitol component during work-up of the reductive-cleavage mixture.

Reductive cleavage of neutral methylated welan. — A second experiment was performed to determine the identity of the sugar residue bearing the acetate and to locate its position of substitution. In this experiment, methylation of welan was carried out under neutral conditions as described by Prehm[7]. The *O*-acetyl group remained intact (as judged by n.m.r. spectroscopy), and the permethylated polysaccharide containing its native acetate group was subjected to reductive cleavage with Et_3SiH and

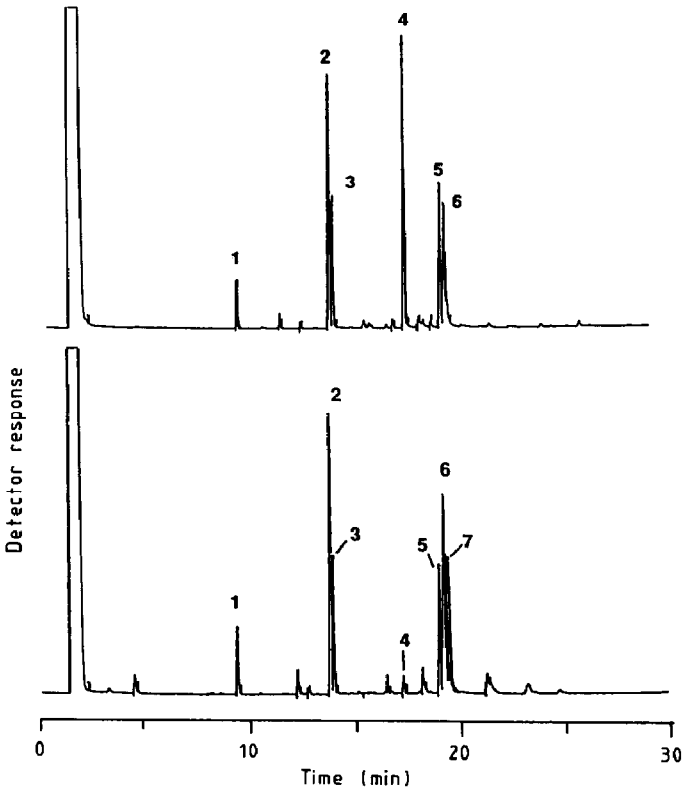


Fig. 1. Gas–liquid chromatograms of the anhydroalditol acetates derived by reductive cleavage of per-*O*-methylated (base-catalyzed) welan (upper) and per-*O*-methylated (neutral conditions) welan (lower). The numbered peaks were identified as follows: (1) **7**; (2) **5**; (3) **6**; (4) **2**; (5) **4**; (6) **3**; (7) **8**.

TABLE I

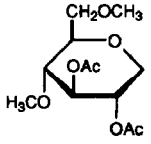
Mole fractions of products derived by reductive cleavage of per-*O*-methylated welan[a]

Compound	*Mole %*	
	A	*B*
1,5-Anhydro-2,3,4-tri-*O*-methyl-L-rhamnitol (**7**)	0.41	0.60
1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-L-mannitol (**6**)	0.47	0.40
4-*O*-Acetyl-1,5-anhydro-2,3-di-*O*-methyl-L-rhamnitol (**5**)	1.00	1.00
3-*O*-Acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol (**2**)	0.75	0.07
Methyl 2-*O*-acetyl-3,6-anhydro-4,5-di-*O*-methyl-L-gulonate (**3**)	0.65	0.74
3,4-Di-*O*-acetyl-1,5-anhydro-2,6-di-*O*-methyl-D-glucitol (**4**)	0.60	0.45
2,3-Di-*O*-acetyl-1,5-anhydro-4,6-di-*O*-methyl-D-glucitol (**8**)	—	0.62

[a] Key: A, methylated (base-catalyzed) welan reductively cleaved using Et_3SiH, ME_3SiOMs and $BF_3 \cdot Et_2O$; B, methylated (neutral conditions) welan reductively cleaved using Et_3SiH and $CF_3SOSiMe_3$.

trimethylsilyl trifluoromethanesulfonate ($CF_3SO_3SiMe_3$) and acetylation *in situ*[8]. Model studies[9] indicated the stability of *O*-acetyl groups to $CF_3SOSiMe_3$-catalyzed reductive cleavage ($BF_3 \cdot Et_2O$–ME_3SiOMs-catalyzed reductive cleavage slowly reduces acetate esters to ethyl ethers), but no precedent existed for direct analysis of polysaccharides containing such a group.

The gas–liquid chromatogram of the products (Fig. 1, lower trace) was similar to the chromatogram from the previous experiment (Fig. 1, upper trace), except that Peak 4 was significantly reduced and a new component was present. This new component (Peak 7) was identified as 2,3-di-*O*-acetyl-1,5-anhydro-4,6-di-*O*-methyl-D-glucitol (**8**). The presence of this anhydroalditol establishes that the *O*-acetyl group present in welan is located on the 2-position of the 3-linked glucosyl residue.



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Integration of all peaks and correction for molar response[5,6] gave the mole fraction for each of the products which are listed in Table I. The mole fraction for 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-glucitol (**2**) was 0.07; therefore, this experiment suggests approximately 93% of the 3-linked glucosyl residues are substituted. This number may be a slight overestimation due to experimental recovery losses evident by the molar ratios of compounds **2** and **8** (0.69 combined). The high acetate content, however, was verified independently by ^{1}H-n.m.r. spectroscopy of the native polysaccharide. The integral area of the acetyl group (δ 2.01) relative to the methyl group (δ 1.19) of the non-terminal L-rhamnosyl residue indicated approximately 85% *O*-acetyl substitution.

The two-step procedure described in this paper establishes both the identity of the acetylated sugar residue and the position of substitution of its acetate group. The first experiment was performed on the fully methylated (base-catalyzed) polysaccharide, thereby removing the native acetate group. Total reductive cleavage followed by acetylation identified the individual sugar components present and established their position of linkage. A second experiment was performed on the neutral methylated polysaccharide. Under these conditions, the native acetate group remained intact. Reductive cleavage with $CF_3SO_3SiMe_3$ as the catalyst and acetylation *in situ* identified a new component in the product mixture. This new component was verified as a 2,3-di-*O*-acetyl-anhydroglucitol, which established that the acetate present in welan is located on the 2-position of the 3-linked D-glucopyranosyl residue. Determination of the location of the *O*-acetyl substituent will serve to support concurrent X-ray diffraction studies[10] on the secondary structure of the polysaccharide.

EXPERIMENTAL

General. — Modified Hakomori[11] methylation was carried out as described by Blakeny and Stone[12] using lithium methylsulfinyl carbanion[13] as the base. Neutral methylation was performed as described by Prehm[7], with the exception that the ratio of 2,6-di-*tert*-butylpyridine to methyl triflate was doubled. Fully methylated polysaccharides were extracted into dichloromethane, then purified by chromatography on a column (15 × 350 mm) of Sephadex LH-20 in 2:1 (v/v) dichloromethane–methanol. Fractions testing positive in the phenol–sulfuric acid assay[14] were combined and used for further analysis. Reductive cleavage using a combination of ME_3SiOMs and $BF_3 \cdot Et_2O$ as the catalyst was performed as described by Jun and Gray[4]. The reaction was allowed to proceed for 2 h, and the products were isolated and acetylated in the usual way[4]. Reductive cleavage using $CF_3SO_3SiMe_3$ as the catalyst was performed, as described by Rolf *et al.*[8] for 3 h, followed by acetylation *in situ*. Gas–liquid chromatography was performed on a Hewlett–Packard Model 5830A gas–liquid chromatograph equipped with a Hewlett–Packard Model 18850A integrator, a flame-ionization detector, and a J & W Scientific DB-5 fused-silica capillary column (30 m × 0.53 mm i.d.). The temperature of the column was held for 2 min at 110° and then programmed to 250° at 5°/min. G.l.c.–m.s. analyses were performed on a VG Analytical Ltd. Model VG 7070-HF high-resolution, double-focusing mass spectrometer. Column effluents were analyzed by c.i.m.s. with ammonia as the reagent gas, and by e.i.m.s. The e.i. mass spectra for all the acetylated anhydroalditols in the glucitol and mannitol series have been previously reported[15]. Acetylated anhydroalditols in the rhamnitol series will be reported shortly[16]. ^{1}H-N.m.r. spectroscopy of the native (Me_2SO-d_6) and permethylated polysaccharide ($CDCl_3$) was performed on a Varian Gemini 300 n.m.r. spectrometer.

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