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Note

The location of α-D-galactopyranose residues in gum arabic

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Gum arabic and exudate gums from other *Acacia* species[1] contain D-galactopyranose residues in different locations. The β-D-galactan core consists of relatively uniform sub-units[2] of ~13 (1→3)-linked residues to which other β-D-galactopyranose residues are attached in side-chains. Other sugar residues, notably those of L-arabinose, D-glucuronic acid, and L-rhamnose, are in outer chains, together with those of α-D-galactopyranose, as shown by the isolation of 3-*O*-α-D-galactopyranosyl-L-arabinose on controlled partial acid hydrolysis[3]. It has been assumed without direct evidence that these latter residues occupy terminal positions in the polysaccharide structure. Information on this aspect of structure has been sought in a comparison of the parent polysaccharide with that obtained after treatment with coffee-bean α-D-galactosidase (α-D-galactoside galactohydrolase; EC 3.2.1.22).

The enzyme preparation[4] of defined anomeric specificity liberated only galactose (~8%) from the gum and the enzyme-degraded polysaccharide was isolated and shown to be otherwise unaltered in sugar composition (Table I). By virtue of the α-D-galactopyranose residues, major fractions of gum arabic are bound to columns of the α-D-galactopyranose-specific lectin from *Griffonia simplicifolia*[5], but none of the enzyme-degraded gum was retained on such a column*, thus pointing to the removal of these sugar residues as non-reducing end-groups. Methylation analyses (Table II) of the parent gum and the enzyme-degraded polysaccharide showed substantial decrease, but not complete removal, of galactopyranose end-groups, a corresponding decrease in the proportion of 3-linked arabinofuranose units, and a comparable increase in terminal arabinofuranose units as the newly exposed end-groups. The main structural change resulting from α-D-galactosidase action may be summarized as:

$$\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-L-Ara}f\text{-}(1\text{-} \rightarrow \text{D-Gal} + \text{L-Ara}f\text{-}(1\rightarrow$$

Samples of the parent and enzyme-degraded polysaccharide were partially hydrolyzed under conditions known to generate disaccharides from the cleavage of

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TABLE I

SUGAR COMPOSITION OF NATIVE AND ENZYME-DEGRADED GUM ARABIC

Polysaccharide sample	*Sugar composition (%)*			
	Rha	*Ara*	*Gal*	*Uronic acid*
Gum arabic	13	28	37	17
Enzyme-degraded gum[a]	14	28	30	16

[a]Relative proportions are normalized with respect to arabinose, assuming no change in the proportions of this sugar constituent.

TABLE II

METHYLATION ANALYSIS OF NEUTRAL SUGAR COMPONENTS OF GUM ARABIC AND ENZYME-DEGRADED GUM

Sugar	*Relative proportions of derived alditol acetates*[a]		
	T[b]	*Gum*	*Enzyme-degraded gum*
2,3,4-Me_3 Rha[c]	0.44	11	11
2,3,5-Me_3 Ara[c]	0.44	17	28
2,3,4-Me_3 Ara	0.55	2	2
2,5-Me_2 Ara	0.87	11	3
2,3,4,6-Me_4 Gal	1.10	9	2
2,4,6-Me_3 Gal	1.95	2	3
2,3,4-Me_3 Gal	3.04	2	2
2,6-Me_2 Gal	4.83	2	3
2,4-Me_2 Gal	5.10	17	18
2-Me Gal	6.02	11	12

[a]Values calculated on the basis allowing for 17% of uronic acid residues and assuming no change in the proportions of terminal rhamnose and internal galactose residues. [b]Retention times of partially methylated alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on column D at 180°. [c]The relative proportions of these two derivatives were estimated separately on column C (130°, 10 min, 5°/min to 180°, and hold).

arabinofuranosyl linkages. The identities of two disaccharides, 3-*O*-α-D-galactopyranosyl-L-arabinose and 3-*O*-β-L-arabinopyranosyl-L-arabinose[6], were indicated by t.l.c. and confirmed by g.l.c.–m.s. of the derived permethylated disaccharide alditols. With the latter disaccharide as internal reference, the results showed that, although formed in diminished amounts, the first-mentioned disaccharide was still formed on partial hydrolysis of the enzyme-treated gum. This observation indicates that enzymic hydrolysis had not liberated all the α-D-galactopyranose residues in the gum. Two possibilities may be considered: (*a*) that not all of the α-D-galactopyranose end-groups are accessible to the enzyme or to the binding sites of the lectin; and (*b*) that some of these sugar residues occupy non-terminal positions in the polysaccharide and are masked by glycosyl substituents, e.g. arabinofuranosyl,

which are readily liberated on mild acid hydrolysis. An attempt to distinguish between these possibilities by the detection of derivatives of 3-*O*-α-D-galactopyranosyl-L-arabinose formed on controlled acid hydrolysis of the methylated gum samples was unsuccessful.

EXPERIMENTAL

Unless otherwise stated, general methods are those described in the accompanying paper[7]. The gum sample was that used in earlier investigations from this laboratory[8].

Coffee-bean α-D-*galactosidase.* — The enzyme preparation was prepared from Santos powdered green coffee beans by the method of Helferich and Vorsatz[4]. Enzyme activity was assayed by its action on *p*-nitrophenyl α-D-galactopyranoside with liberation of galactose measured by the method of Nelson and Somogyi[9], and release of *p*-nitrophenol. Anomeric specificity was checked by liberation of galactose from methyl α-D-galactopyranoside and carob galactomannan, and by lack of action on methyl β-D-galactopyranoside and larch arabinogalactan.

α-D-*Galactosidase-degraded gum arabic.* — Gum arabic (200 mg) in water (20 mL) was digested with a suspension of α-D-galactosidase (20 mg) for 24 h. An aliquot portion was withdrawn and reducing-sugar estimation[9] showed liberation of 6% of galactose. The remaining suspension was dialyzed against distilled water to remove liberated galactose, a further quantity (10 mg) of enzyme was added, and digestion for a further 24 h resulted in the liberation of an additional 2% of galactose. The suspension was boiled for 5 min to denature/inactivate the enzyme. Acetone (3 vol.) was added to the filtered mixture, and the precipitate was dissolved in water and freeze-dried to give enzyme-degraded polysaccharide (120 mg), $[\alpha]_D$ −39.5° (*c* 1.3, water). The results of compositional analysis (individual neutral sugars and uronic acid) for the parent and enzyme-degraded gum samples are given in Table I.

Methylation. — Methylations of polysaccharides by the Hakomori procedure and subsequent methylated sugar analyses were performed as described by Jansson *et al.*[10], and the results are reported in Table II.

Partial hydrolysis. — Gum arabic (200 mg) in 0.1M trifluoroacetic acid was boiled under reflux for 1.5 h. Partially degraded polysaccharide was precipitated by the addition of acetone (4 vol) to the cooled solution. The supernatant solution was concentrated under diminished pressure to remove acid, and the resulting syrup was fractionated successively by molecular-sieve chromatography on Biogel P-2 and adsorption chromatography on charcoal–Celite with elution with water containing 10% of ethanol to give a fraction rich in two disaccharides with the paper chromatographic mobilities of 3-*O*-α-D-galactopyranosyl-L-arabinose and 3-*O*-β-L-arabinopyranosyl-L-arabinose. Supporting evidence for the identities of these disaccharides after conversion into permethylated disaccharide alditols by reduction followed by methylation was obtained by g.l.c.–m.s. of these derivatives on column

F (200°, 2°/min to 220° and hold). The mass spectra showed characteristic fragment-ions at (i) *m/z* 219, 191, 187, 159, and 89, and (ii) *m/z* 191, 175, 159, 143, and 89, respectively. Samples of gum and enzyme-degraded gum were partially hydrolyzed under the same conditions and direct treatment of the hydrolyzates with sodium borohydride followed by methylation gave mixtures of products that were amenable to direct examination by g.l.c.–m.s. The two methylated disaccharide alditols were detected in samples from gum and enzyme-degraded gum, but the relative proportion of the 3-*O*-α-D-galactopyranosyl-L-arabinose derivative from the degraded gum was approximately one quarter of that from the native gum.

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