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Note

The ^{13}C-n.m.r. spectra of (1→6)-α-D-galactosyl-(1→4)-β-D-mannans

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Grasdalen and Painter[1] have assigned the ^{13}C resonances of the galactosyl and mannosyl residues in galactomannans from locust-bean gum (LBG), guar gum, and clover seeds and, from the mannosyl C-4 chemical-shifts, were able to describe the sequencing and d.s. on the mannosyl residues as influenced by the substitution on the nearest residues glycosidically linked through position 4.

We now draw attention to the variations associated with locust-bean gum, guar gum, and fenugreek gum. The gums were analysed after partial, acid hydrolysis that was mild enough not to affect the structures as observed by n.m.r. spectroscopy; the small changes in mannose–galactose ratios are shown in Table I. This method is used for the routine analysis of galactomannans in foodstuffs and for identifying galactomannans in the presence of carrageenans. We confirm the results of Grasdalen

TABLE I

MANNOSE–GALACTOSE RATIOS OF GALACTOMANNANS BEFORE AND AFTER HYDROLYSIS

Sample	*State*	*Mannose–galactose*
Fenugreek	Before hydrolysis	51/49
Fenugreek	Hydrolysed	53/47
Guar gum (Vidocreme A)	Before hydrolysis	60/40
Guar gum (Vidocreme B)	Hydrolysed	64/36
LBG (Cold-water soluble)	Before hydrolysis	78/22
LBG (Cold-water soluble)	Hydrolysed	78/22
LBG (RL200)	Before hydrolysis	80/20
LBG (RL200)	Hydrolysed	79/21
LBG (Hot-water soluble)	Before hydrolysis	81/19
LBG (Hot-water soluble)	Hydrolysed	84/16
Guar, enzyme modified	Before hydrolysis	69/31
Guar, enzyme modified	Hydrolysed	68/32
LBG, enzyme modified	Before hydrolysis	82/18
LBG, enzyme modified	Hydrolysed	82/18

TABLE II

ASSIGNMENTS OF PEAKS IN ^{13}C-N.M.R. SPECTRA OF GALACTOMANNANS

Type of unit	*Chemical shift*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Galactose[b]	95.0	71.1	71.9	72.0	73.1	63.8
Galactose in galactomannan	101.5	71.2	72.0	72.2	73.8	63.9
(Δ p.p.m.)	(+6.5)	(+0.1)	(+0.1)	(+0.2)	(+0.7)	(+0.1)
β-D-Mannose[b]	96.3	73.9	75.7	69.3	78.8	63.7
Unsubstituted mannose in galactomannan	102.8	72.8	74.2	79.1[c] (79.35)	77.7	63.3
(Δ p.p.m. from mannose)	(+6.5)	(−1.1)	(−1.5)	(+9.8)	(−1.1)	(−0.4)
6-*O*-Substituted β-D-mannose in galactomannan	102.7	72.6	74.1	79.6[c] (79.35)	76.0	69.2
(Δ p.p.m. from mannose)	(+6.4)	(−1.3)	(−1.6)	(+10.3)	(−2.8)	(+5.5)
(Δ p.p.m. from unsubstituted mannose)	(−0.1)	(−0.2)	(−0.1)	(+0.5)	(−1.7)	(+5.9)

[a]In p.p.m.; internal DSS reference. For correction to Me_4Si scales, see Experimental. [b]Standard spectrum assignments from ref. 3. [c]The 79.35 p.p.m. resonance is due to M→*M*G and MG→*M*.

TABLE III

FENUGREEK GUM: GALACTOSYL SUBSTITUTION CALCULATED FROM THE MANNOSYL C-4 RESONANCES

Diads[1,4]	*Mannose units*[a]	*Fenugreek gum Observed (n.m.r.) abundance (%)*	*Chemical shift (p.p.m.)*
F_{11}	→4(MG)1→4(*M*G)1→	87 ±2	79.6
F_{12}	→4(MG)1→4(*M*)1→)	13 ±2	79.35
F_{21}	→4(M)1→4(*M*G)1→)		
F_{22}	→4(M)1→4(*M*)1→	0	79.1

[a]G = Gal; M = Man.

TABLE IV

COMPARISON OF MAN/GAL RATIOS

	From G.l.c. (alditol acetates)	*Present n.m.r. results*	*From oxidation results*[4]
Guar Vidocreme, β-hydrolysed	64/36	65/35	64/36
LBG RL200, hydrolysed	79/21	80/20	81/19
Fenugreek, hydrolysed	53/47	52/48	—

and Painter and their interpretation of the splitting of the mannosyl C-4 resonances[1].

The chemical shift values (Table II) (referred to DSS) are, on average, 0.3 p.p.m. lower than those reported by Grasdalen and Painter[1] [referred to sodium 3-(trimethylsilyl)-propionate-d_4], but the assignments are the same, with the exception of the C-2 and C-3 galactosyl resonances which were inferred from the later work of Gorin and Mazurek[2].

The results for fenugreek gum (Table III) fit in with the assignments of Grasdalen and Painter[1] for the mannosyl C-4 resonances, which are correlated with the diads F_{11} (MG—*M*G), F_{12} (MG—*M*) = F_{21} (M—*M*G), and F_{22} (M—*M*). Fenugreek gum, after hydrolysis, has 93% of the mannosyl residues substituted with galactose.

The Man/Gal ratios calculated from the n.m.r. data (Table IV) compare very favourably with parallel g.l.c./alditol acetate determinations on the same partially hydrolysed samples of galactomannan and with figures calculated from the results of Painter *et al.*[3] for guar and LBG.

Because of the high viscosity of concentrated solutions of unhydrolysed galactomannans, it was very difficult to obtain good spectra. However, the spectra were easily distinguishable one from another and bore a close resemblance to the spectra of the partially hydrolysed gums. Close inspection indicated that the galactose peaks

were sometimes more intense in the spectra of the unhydrolysed gums, which may be due to changes in relaxation time T_1 since chemical analysis showed that very little loss of galactose had occurred (Table I).

The spectra of the unhydrolysed galactomannans indicated that, although there is close similarity in structure between, for example, two samples of guar gum, there are also differences. This is also true of locust-bean gums and tara gums. The tara gums gave spectra more similar to those of LBG than to those of guar gum, which is consistent with the Man/Gal ratios of these polysaccharides.

The ^{13}C-n.m.r. spectra of enzyme-modified guar gum and hydrolysed, enzyme-modified guar gum were very similar, both to each other and to the starting guar gum.

Enzymic hydrolysis of locust-bean gum (Genugum RL 200) was more successful and gave a product having a Man/Gal ratio of 82:18. The ^{13}C-n.m.r. spectrum of this material was almost identical with that of HWS-LBG (Man/Gal ratio, 84:16). There were no obvious differences in the spectra in the region 78–79 p.p.m. and no conclusions could be drawn concerning changes in proportions of blocks of mannosyl residues substituted with galactose.

LBG and guar gum are ubiquitous food additives. Their synergistic behaviour with other polysaccharides, *e.g.*, agar, carrageenan, and xanthan gum, greatly increases the interest in these materials. The reliable identification of these galactomannans entails the measurement of the Man/Gal ratio, which is usually achieved by g.l.c. of the derived alditol acetates[4]. Spectroscopy (*e.g.*, i.r.) has been unsatisfactory, because of the close similarity between the structures of the two gums. ^{13}C-N.m.r. spectroscopy provides a rapid method for the identification of these materials; in addition, they may be confidently identified in the presence of other polysaccharides, *e.g.*, agar and carrageenan, which also yield galactose on acid hydrolysis.

Mixtures of carrageenan with LBG and guar gum have been analysed successfully by ^{13}C-n.m.r. spectroscopy using the anomeric peaks at $\sim$100 p.p.m. Methods using borax and quaternary ammonium halides for the selective separation of carrageenans and galactomannans have been found to be unreliable due to the inefficient separation normally achieved.

EXPERIMENTAL

Materials. — Guar gums were samples of Vidocreme A and B (Unipectin), and were purified by dissolution, filtration, and freeze-drying. Locust-bean gum (LBG) was also purified before use. A sample of commercial LBG of high quality, Genugum RL200, was provided by Copenhagen Pectin Factory. Hot-water soluble (HWS) and cold-water soluble (CWS) samples of LBG were obtained from CECA and used without further purification. Fenugreek gum was prepared from fenugreek beans by grinding and extracting with cold water followed by the usual purification steps. Enzymically modified LBG and guar gum were kindly donated by Dr. M. Hustler (recently of Royal Holloway College).

Partial hydrolysis. — A solution of galactomannan (0.5 g) in distilled water (50 ml) (pH adjusted to 2–2.5 with dilute hydrochloric acid) was heated at 98° for 1 h, cooled to room temperature, and neutralised to pH 7 with 2M sodium hydroxide. The product was dialysed at room temperature for 3 days against distilled water. Sodium 3-trimethylsilyl-1-propanesulfonate (DSS, 20 mg) and D_2O (5 ml) were added, and the volume was reduced to 5 ml by rotary evaporation.

Partial hydrolysis of the polysaccharides improved the quality of the spectra through increased solubility and mobility and, therefore, increased signal-to-noise.

The mannose–galactose ratios of the starting polysaccharides are shown in Table I. These figures were obtained by hydrolysis, and g.l.c. of alditol acetates[4]. It was expected that partial hydrolysis of the galactomannans would preferentially cleave the side-chain galactose and thus the materials remaining would contain less galactose than the starting material. Mannose–galactose ratios of the galactomannans after partial hydrolysis are also shown in Table I.

N.m.r. spectroscopy. — The natural abundance, ^{13}C-{H}-n.m.r. spectra were recorded on a Bruker WP-200 spectrometer operating at 50.3 MHz in the F.t. mode. Parameters: spectral width, 10,000 Hz (200 p.p.m.); acquisition time, 0.4 s; flip angle, 50°; F.t. length of 8 k. The samples were run at 70° to reduce viscosity-broadening of the resonances, and using a spinning 15-mm tube. An accumulation of 20,000 transients produced a usable spectrum. However, the spectra were usually recorded overnight (140,000 transients). The chemical shifts are given relative to that of the highest field peak of internal DSS. Correction factors: relative to external Me_4Si, −1.8 p.p.m.; relative to 5% Me_4Si in chloroform, −2.5 p.p.m.

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