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Note

Carbon-13 n.m.r.-spectral study of *Acacia xanthophloea* gum and its degradation products

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INTRODUCTION

^{13}C-N.m.r. spectroscopy has been applied in structural studies on arabinogalactans[1–3]. The present study is concerned with ^{13}C-n.m.r. spectra of the gum from *Acacia xanthophloea* and its degradation products; assignments of signals have been possible on the basis of chemical analysis and from the results of previous workers. Thus ^{13}C-chemical shifts were compared with values reported[4–7] for β-D-galactose, methyl-3-*O*-methyl-β-D-galactopyranoside, methyl α-L-arabinofuranoside, and β-D-glucuronic acid. Analysis of the spectrum of the galactan (polysaccharide **III**) led to the interpretation of the other, more-complex spectra.

EXPERIMENTAL

Origin and purification of gum sample. — Gum from *A. xanthophloea*, Bentham No. 336, was collected in Kenya, East Africa, by Dr. J. O. Kokwaro, Botany Dept., University of Nairobi in August 1976, who also confirmed the classification of this *Acacia* species as a member of Bentham's Series 4 (Gummiferae). Crude gum was purified and examined by standard methods[8].

General experimental methods. — Standard methods for gum analysis were used[8,9]. The solvent systems used in paper chromatography were (v/v): (*a*) 3:18:1:4 AcOH–EtOAc–HCO_2H–H_2O; (*b*) 1:5:3:3 (upper layer) benzene–1-butanol–pyridine–water; (*c*) 10:5:1 EtOH–0.1N H_3PO_4–1-butanol; (*d*) 4:1:5 (upper layer) 1-butanol–EtOH–H_2O. Before using solvent (*c*), papers were dipped in 0.3M NaH_2PO_4 solution and air dried. Optical rotations at equilibrium were measured at room temperature in a Perkin–Elmer polarimeter. G.l.c. was carried out with a Pye 104 chromatograph fitted with a flame-ionisation detector at nitrogen flow-rates of 25 mL/min. The column (200 × 0.3 cm) used was 15% by weight of polyethylene glycol adipate on Universal B (phase-sep) at 170°. Retention times are quoted relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside for the methyl ethers. Molecular weights ($\bar{M}w$) of the

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gum and its degradation products were estimated by light scattering measurements at $27 \pm 0.5°$ with a Sofica photogoniodiffusometer Model 4200. ^{13}C-N.m.r. spectra were recorded with a Varian CFT-20 spectrometer. Data points were accumulated overnight at 36°, a spin rate of 22 r.p.s., and with complete proton decoupling. The spectral width was 4000 Hz and the spectra were calibrated by the addition of 1,4-dioxane to the samples. The polysaccharides (100–200 mg) were dissolved in deuterium oxide (1 mL).

Sugar composition. — The samples (100 mg) were hydrolysed with 0.5M H_2SO_4 for 18 h on a boiling water-bath; after hydrolysis, the solutions were cooled, neutralised ($BaCO_3$), deionised (Amberlite IR-120 resin, H^+ form) and concentrated to syrups on a rotary evaporator at diminished pressure at ~30°. Chromatography was performed on Whatman No. 1 and 3MM papers with the solvent systems described previously[8,9]. The sugar compositions were determined by the phenol–H_2SO_4 method[10].

Partial hydrolysis. — The purified gum (100 mg) was hydrolysed with 0.25M H_2SO_4 for 1 h on a boiling water-bath. The hydrolysate was treated as already described and examined by p.c.

Identification of the aldobiouronic acids. — Purified gum (500 mg) was hydrolysed with 0.5M H_2SO_4 (250 mL) for 8 h at 100°. The hydrolysate was treated as described for sugar analysis and was fractioned on a column (16 × 16 cm) of Duolite A-4 resin in the formate form. After elution of the neutral sugars, elution with 5% formic acid (500 mL) yielded the acidic fraction of the hydrolysate. This was concentrated to a syrup and chromatography was carried out in solvent (*a*). The aldobiouronic acids isolated by p.c. were identified by R_{Gal}, $[\alpha]_D$ and methylation analysis.

Small-scale oligosaccharide methylations[11]. — The oligosaccharide (0.5–2.0 mg) was shaken with MeI (0.2 mL) and Ag_2O (0.2 g) for 18 h at room temperature in the dark. The mixture was filtered and the residue washed with $CHCl_3$. The combined filtrate and washings were concentrated to a syrup. The product was methanolysed and examined by g.l.c. as the methyl ethers.

Methylation of the gum. — The pure gum (442 mg) was methylated[12] to give a product (200 mg), $[\alpha]_D^{20}$ +8.3° (*c* 1.2, $CHCl_3$) (Found: 45.9% OMe) that was subjected to Purdie treatment[13] to give fully methylated derivatives. A portion of the product was methanolysed and examined by g.l.c.

Preparation and examination of degraded gum A. — Purified gum (6.0 g) was hydrolysed with 5mM H_2SO_4 (325.5 mL) for 96 h at 100°. After cooling, neutralisation with $BaCO_3$, and filtration, the solution was deionised with Amberlite IR-120 (H^+) resin, concentrated and dialysed against distilled water for 24 h and then against running tap-water for a further 48 h and freeze-dried. The dialysate was concentrated and chromatographed in solvents (*a*) and (*b*). Degraded gum A was hydrolysed with 0.25M and 0.5M H_2SO_4 under the conditions given previously[9]. Degraded gum A (270 mg) was methylated to give a product (118 mg), $[\alpha]_D^{20}$ −29° (*c* 1, $CHCl_3$) (Found: 46.5% OMe).

Preparation and examination of degraded gum B. — Degraded gum A (1.7 g) was dissolved in water (50 mL) and 0.5M $NaIO_4$ (50 mL) was added. After 72 h in darkness at room temperature the reaction was stopped by the addition of ethylene glycol (2 mL).

The solution was treated as described previously[9]. Degraded gum B was studied by acid hydrolysis followed by p.c.

Smith-degradation studies. — A series of three sequential Smith degradations was performed with the pure gum as starting material (42.3 g) to obtain polysaccharide **I** (14 g). Polysaccharide **I** (11.0 g) gave polysaccharide **II** (1.7 g), and polysaccharide **II** (1.7 g) gave polysaccharide **III** (88.7 mg). The preparation of polysaccharides **I** and **II** was repeated in order to check the yields and to obtain more material for examination. The experimental conditions for these degradations were, in general, as described previously[9].

RESULTS AND DISCUSSION

The polysaccharide gum from *Acacia xanthophloea* contains five different sugars and has a high molecular weight (Table I). This polymer, in deuterium oxide, gave a complex ^{13}C-n.m.r. spectrum (Fig. 1) having four well-defined regions, in agreement with previous results[2]. The first region shows a peak at 60.9 p.p.m. from the methoxyl group in 4-*O*-methyl-α-D-glucuronic acid[14]. There is a prominent peak at 61.4 p.p.m. from the hydroxylated C-6 of galactose[15] and C-5 of α-L-arabinofuranose residues[3,16], as observed in the spectrum of the original gum of *Acacia senegal*[2]. The second region has peaks between 67 and 77 p.p.m. that are the resonances of the hydroxylated ring-carbon atoms. The large peak at 67.4 p.p.m. is due to 1,4-dioxane, added as the internal reference. The group of peaks centred at 83 p.p.m. is due mainly to glycosidically linked carbon atoms. The resonances of the anomeric carbon atoms lie in the 100–110 p.p.m. region and show the presence of at least five different types of linkage. The peaks at 97.76 and 98.72 p.p.m. are due to terminal reducing residues[16] and the peak at 100.67 p.p.m. is probably attributable to an α linkage[14]. The group of peaks centred on 104.3 p.p.m. is certainly due to β linkages[17]. The specific rotation of the polysaccharides (Table I) show that the gum is mainly β-linked[1]. The peaks at 108 and 110 p.p.m. are due to furanoside residues, as reported previously[1,18].

The ^{13}C-n.m.r. spectrum (Fig. 2) of degraded gum A, obtained by mild acid hydrolysis of *A. xanthoploea* gum, was simpler than that of the original gum because of removal of the acid-labile arabinosyl residues (Table I). The four main regions defined previously for the whole gum (Fig. 1) are still present. The peaks at 58.85 and 59.79 p.p.m. are assigned to the methoxyl groups of 4-*O*-methyl-α-D-glucuronic acid and C-6 of galactose, respectively. The peak at 65.3 p.p.m. is certainly due to glycosidically linked C-6 of galactose[19], shifted downfield by 5.5 p.p.m. relative to the unsubstituted of C-6 of galactose. The resonances in the range 67–77 p.p.m. for hydroxylated ring-carbon atoms, and the group centred at 83 p.p.m., assigned to glycosidically linked carbon atoms are simplified. The number of anomeric-carbon peaks is decreased in the 98–103 p.p.m. range; the majority of these appear to come from β-linked galactose. Interestingly the peaks in the range 105–110 p.p.m. in the spectrum of the whole gum (Fig. 1) do not appear in this spectrum. They must be due to linked arabinose, as most of these residues in the original gum were removed in the preparation of degraded gum A (Table I).

TABLE I

Data for *A. xanthoploea* gum and its degradation products

Polysaccharide	$[\alpha]_D^t$	*Constituent sugars (%)*					*Mol. wt. (× 10^3)*
		Gal	*Ara*	*Rha*	*GlcA*	*4MeGlcA*	
Original gum	+35	57	22	6	8.8	6.2	860
Degraded gum A	+18	69	4	8	13.0	6.0	320
Degraded gum B	−5.5	100					n.d.[a]
Polysaccharide **I**	−18	70	26		3.3	0.7	210
Polysaccharide **II**	−7	90	10				33
Polysaccharide **III**	−17	100					n.d.[a]

[a] Not determined.

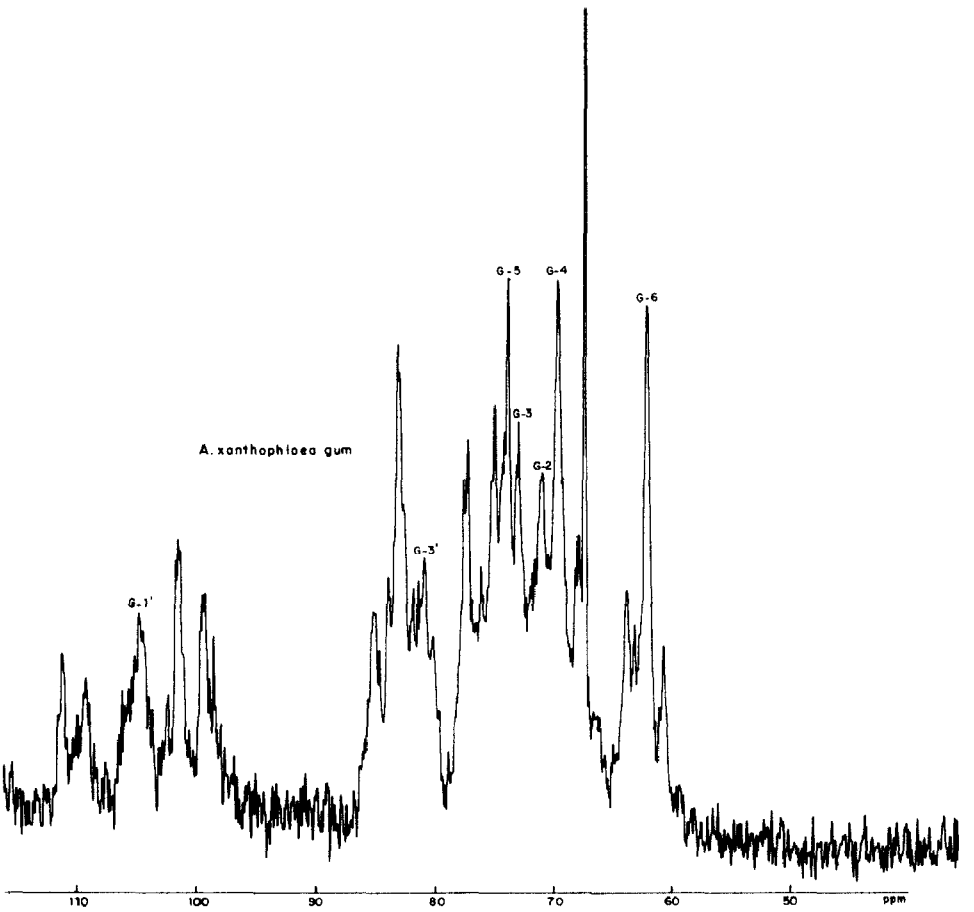

Fig. 1. ^{13}C-N.m.r. spectrum (D_2O) of *A. xanthoploea* gum (G = Gal G' = linked Gal).

TABLE II

Methylation analysis of *A. xanthoploea* gum and its degradation products

Methyl ethers[a]	*Original gum*	*Degraded gum A*	*Polysaccharides* I	II	*Linkage*
2,3,4-Rha	8	1	10		Rha*p*-(1→
2,3,5-Ara	8	0.5	trace		Ara*f*-(1→
2,3,4-Ara	2				Ara*p*-(1→
3,5-Ara	5	25	26	29	→2)-Ara*f*-(1→
2,5-Ara	46	2	8	trace	→3)-Ara*f*-(1→
3,4-Ara	8				→2)-Ara*p*-(1→
2,3,4,6-Gal	7	1	5	trace	Gal*p*-(1→
2,3,6-Gal	1	4			→4)-Gal*p*-(1→
2,4,6-Gal	trace	10	26	62	→3)-Gal*p*-(1→
2,3,4-Gal	trace	27	17	9	→6)-Gal*p*-(1→
2,4-Gal	trace	16	trace	trace	3,6)Gal*p*-(1→
2,3,4-GlcA[b]	12	10	8		GlcA*p*-(1→

[a] Relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. [b] As methyl ester glycoside.

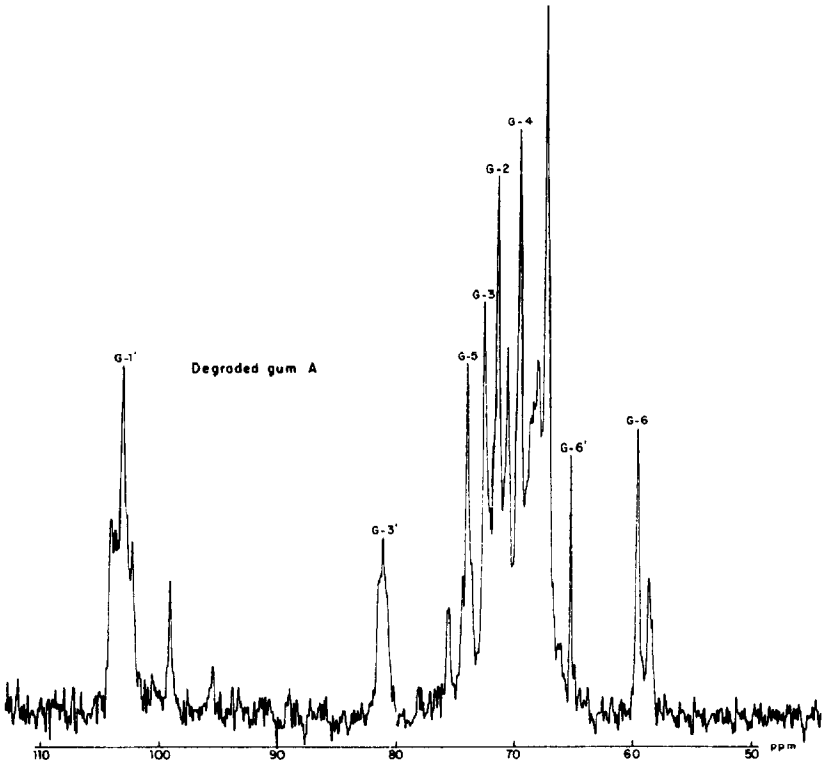

Fig. 2. ^{13}C-N.m.r. spectrum (D_2O) of degraded gum A obtained by mild acid hydrolysis of original gum (G = Gal G′ = linked Gal).

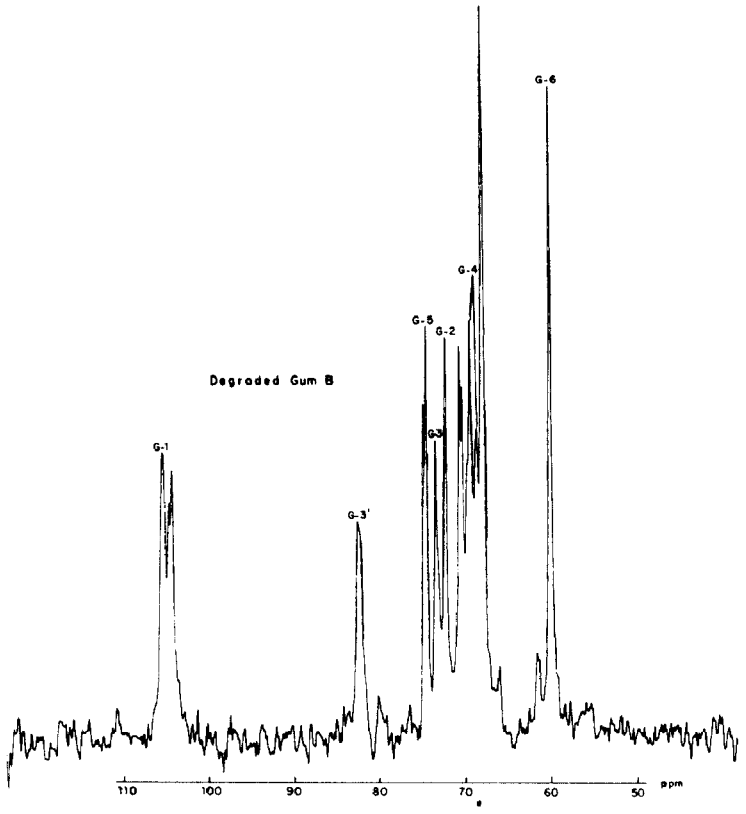

Fig. 3. ^{13}C-N.m.r. spectrum (D_2O) of degraded gum B obtained by drastic oxidation of degraded gum A (G = Gal G′ = linked Gal).

Degraded gum B, obtained by drastic oxidation of degraded gum A, affords a spectrum (Fig. 3) simpler than the previous ones. This was expected because of degraded gum B is basically a residual galactan after all of the periodate-vulnerable sugar residues have been removed; 3-*O*-methyl-β-D-galactopyranoside provided a basis for assigning the individual signals attributed to the galactan (see Table III). This interpretation is in agreement with the presence of a simple chain of β-(1→3)-linked galactose residues, as demonstrated by chemical methods.

Succesive Smith degradations of *A. xanthophloea* gum produced polysaccharides **I–III**, whose chemical compositions are shown in Table I. The spectra of these polysaccharides (Figs. 4–6), are, as expected, simpler than that of the original gum, because vulnerable residues in the original structure have been removed. The spectrum of polysaccharide **I** (Fig. 4) shows the four well-defined regions described previously. The first region has a prominent peak for C-6 of galactose and C-5 of α-L-arabinofuranose, as observed in the spectrum of the original gum (Fig. 1). The peak that occurred at 60.86 p.p.m. in the original spectrum was absent. The methoxyl content of polysaccharide **I** was very low (Table I). The resonances in the 67–77 p.p.m. region, due to hydroxylated ring-carbon atoms, are still complex. The 80–85 p.p.m. region includes the peaks of

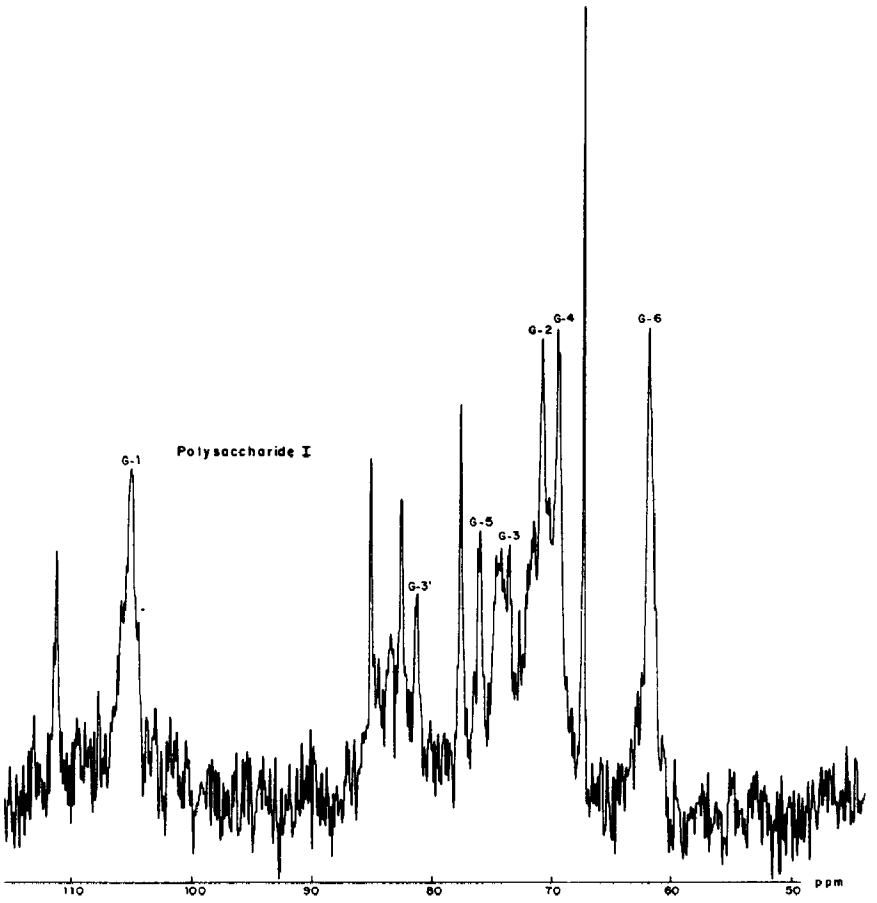

Fig. 4. ^{13}C-N.m.r. spectrum (D_2O) of polysaccharide **I** obtained by one Smith degradation of original gum (G = Gal G′ = linked Gal).

glycosidically linked carbon atoms and C-4 of α-L-arabinofuranose residues. The peaks of the anomeric carbon atoms are attributable to β-linked galactose and α-linked L-arabinofuranose. The interpretation of the ^{13}C-n.m.r. spectrum of polysaccharide **II** (Fig. 5, Table III), indicates that the structure consists of a framework of β-(1→3)-linked galactose residues with some substitution at their C-6 position, as demonstrated by chemical methods (Table II). The peaks at 102.20 and 102.83 p.p.m. are attributed to C-1 of galactose linked to O-6 and O-3 of galactose[3], respectively. The spectrum of polysaccharide **III**, Fig. 6, is very similar to that of degraded gum B (Fig. 3). This is not surprising as both are basically chains of β-(1→3)-linked galactose residues. These results are in agreement with those published for *A. senegal* gum[2], based on spin–lattice relaxation studies.

Comparison of ^{13}C-n.m.r. data of galactose in *A. xanthoploea* gum and its degradation products (Table III), shows downfield displacements of the resonances of those ring carbon atoms involved in interglycosidic linkages. For the C-3 resonance of

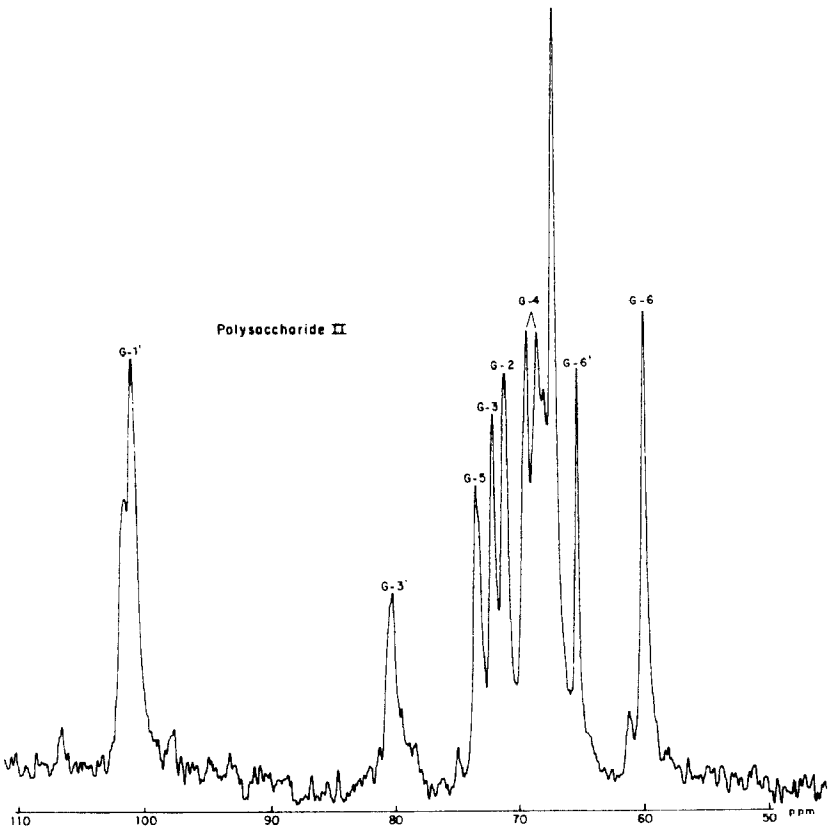

Fig. 5. ^{13}C-N.m.r. spectrum (D_2O) of polysaccharide **II** obtained by Smith degradation of polysaccharide **I** (G = Gal G′ = linked Gal).

the galactan (polysaccharide **III**, degraded gum B) it was 7.97 p.p.m., that is, very close to the 8.2 p.p.m. observed on 3-*O*-substitution of β-D-galactopyranoside[15,16] (Table III). The downfield displacement of the C-6 resonance in degraded gum A and polysaccharide **II** was 5.53 p.p.m., close to the 5.8 p.p.m. observed on 6-*O*-substitution of D-glucose[4,19]. Dearabinosylation enhanced the signals due to the galactan and resolved the signal at 65.3 p.p.m. (Table III). This fact has been reported for other highly substituted arabinogalactans[2,3]. The resonances in the spectra of degraded gum B and polysaccharide **III**, comparable to those obtained for *A. senegal gum*[2], appear as doublets, probably because of the environments shown in Fig. 7.

The resonances of the arabinofuranose residues (Table IV), were assigned on the basis of the spectrum of methyl α-L-arabinofuranoside and by previous results[3,16]. Loss of arabinofuranosyl residues accompanying the preparation of polysaccharide **I** led to the loss of the peaks at 82.66, 82.82, and 108.21 p.p.m. assigned to linked C-3 and C-1 of α-L-arabinofuranose. This provides evidence that these residues were (1→3)-linked[20] in the original gum. On the other hand, the peaks at 110.17 and 110.34 p.p.m. in the spectrum of the original gum, which appear at 110.06 and 110.28 p.p.m. in the spectrum

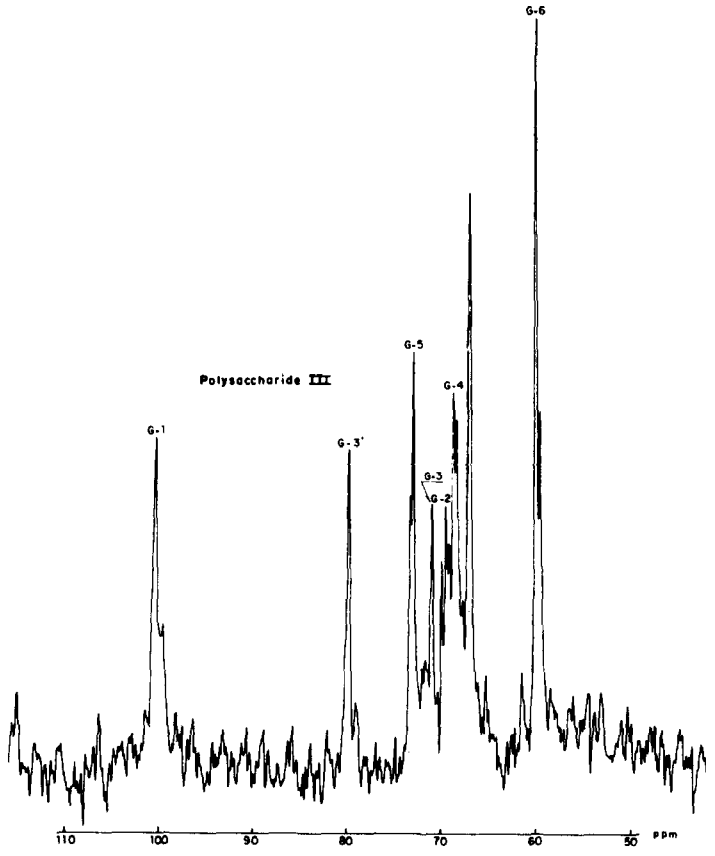

Fig. 6. ^{13}C-N.m.r. spectrum (D_2O) of polysaccharide **III** (galactan) obtained by Smith degradation of polysaccharide **II** (G = Gal G′ = linked Gal).

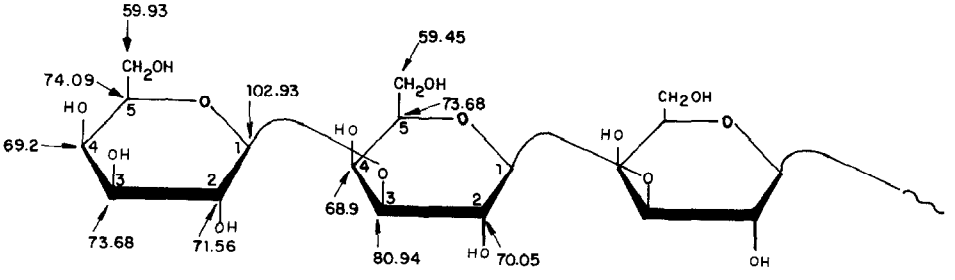

Fig. 7. The galactan backbone of *A. xanthoploea* gum.

TABLE III

Comparison of ^{13}C-n.m.r. data[a] of galactose in *A. xanthoploea* gum and its degradation products

Model compounds	*Polymer*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
β-D-Galactopyranose[b]		97.30	72.90	73.80	69.70	76.0	62.0
Methyl 3-*O*-methyl-β-D-galactopyranoside[b]		103.90	69.80	(82.00)	69.20	75.10	61.20
	Original gum	97.76 103.51 103.84	70.89	72.84 (80.65)	69.60	73.72	62.29
	Degraded gum A	102.13 103.11	71.37	72.47 (80.87)	69.53	73.90	59.79 (65.33)
	Degraded gum B	102.13 103.16	69.79 70.09 71.56	72.67 (81.35)	68.19 68.91	73.74 74.01	60.01
	Polysaccharide **I**	104.14	70.73	73.43 (80.95)	69.34 69.47	75.84	62.06
	Polysaccharide **II**	102.20 102.83	71.45	72.45 (80.81) (80.99)	68.09 68.68 69.54	73.87	59.80
	Polysaccharide **III**	102.93	70.05	71.56 (80.94)	68.90 69.22	73.68 74.09	59.45 59.93

[a] Values relative to the signal of 1,4-dioxane (67.4 p.p.m. relative to the signal of Me_4Si). [b] Ref. 16. Figures in parentheses are assigned to C-3 and C-6 linked galactose residues.

TABLE IV

^{13}C-N.m.r. data[a] of α-L-arabinofuranose in original gum and polysaccharide **I** of *A. xanthoploea*

Model compounds	*Polymer*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*
Methyl L-arabinofuranoside[b]		110.90	82.30	76.50	84.90	62.00
	Original gum	108.21 110.17 110.34	82.35 (84.61)[d,e]	(82.66)[c] (82.82)[c]	84.84[d]	62.90
	Polysaccharide **I**	110.06 110.28	82.21 (84.75)[e]	77.40	84.75	62.90

[a] Values relative to the signal of 1,4-dioxane (67.4 p.p.m. relative of the signal of Me_4Si). [b] Ref. 16. [c] Ref. 20. [d] Assignments might have to be reversed. [e] Ref. 22, figures in parentheses are assigned to 2- and 3-linked α-L-arabinofuranose residues.

TABLE V

^{13}C-N.m.r. data[a] of uronic acid residues in original gum and degraded gum of *A. xanthoploea* gum

Model compounds	*Polymer*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*4-OMe*
β-D-Glucuronic acid[b]		97.4	75.5	77.1	73.3	77.5	177.5	
4-*O*-methyl-α-D-glucuronic acid[c]		99.7	72.2	73.3	82.7	70.8		61.1
	Original gum	100.67[d] 104.19 104.44	74.80[d]	73.72[d] 77.05	82.66[d] 73.72	77.4		60.86[d]
	Degraded gum A	101.42[d] 103.11	74.29[d]	73.54[d]	81.20[d] 73.54			58.85[d]

[a] Values to the signal of 1,4-dioxane (67.4 p.p.m. relative to the signal of Me_4Si. [b] Ref. 5. [c] Ref. 14. [d] Signals due to 4-*O*-methyl-α-D-glucuronic acid.

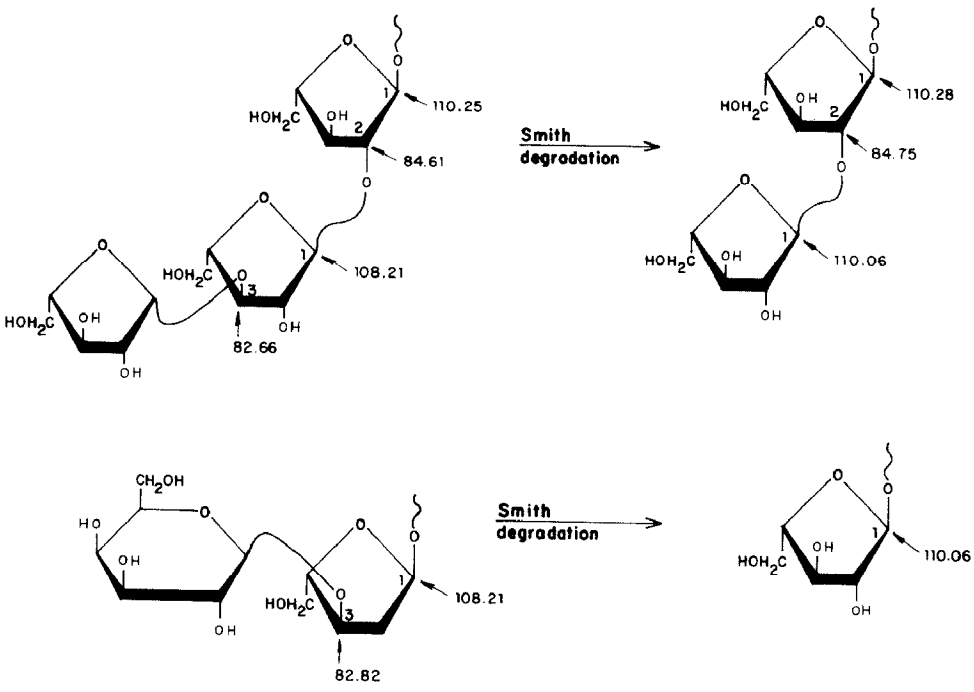

Fig. 8. Arabinose residues present in the side-chain of *A. xanthoploea* gum. The signals at 82.66 and 82.81 p.p.m. may be reversed.

of polysaccharide I (Fig. 4), were attributed to C-1 of α-L-arabinofuranose[1,18,21]. The peaks at 84.61 and 84.75 p.p.m. in the spectra of the original gum and of polysaccharide I were attributed to 2-linked arabinose residues[22]. These 2-*O*-substituted arabinose residues may be at internal positions whereas the 3-*O*-substituted ones, eliminated during preparation of polysaccharide I, must be at external positions. The isolation and characterisation of 3-*O*-β-D-galactopyranosyl-L-arabinose (R_{Gal} 0.70 (*b*); $[\alpha]_D^{20}$ +50°) and 3-*O*-β-L-arabinofuranosyl-L-arabinose (R_{Gal} 1.20 (*a*); $[\alpha]_D^{20}$ +90°) are in agreement with the presence of 3-*O*-substituted arabinose in the original gum. Chemical and spectral evidence suggest that arabinose side-chains are possibly arranged as shown in Fig. 8. It is noteworthy that the resonances of L-arabinopyranose and L-rhamnopyranose residues were not detected because of the relatively low contents of these sugars in *A. xanthoploea* gum (Tables I and II).

The resonances assigned to the glucuronic acid residues (Table V), indicate that the uronic acids present are β-D-glucuronic acid and 4-*O*-methyl-α-D-glucuronic acid in the original gum and degraded gum A. This fact is in agreement with the aldobiouronic acid isolated and identified during structural study of this gum (Fig. 9). The peaks assigned to 4-*O*-methyl-α-D-glucopyranosyluronic acid (Table V), have been reported[14] in related compounds.

These studies have shown that valuable structural information may be obtained

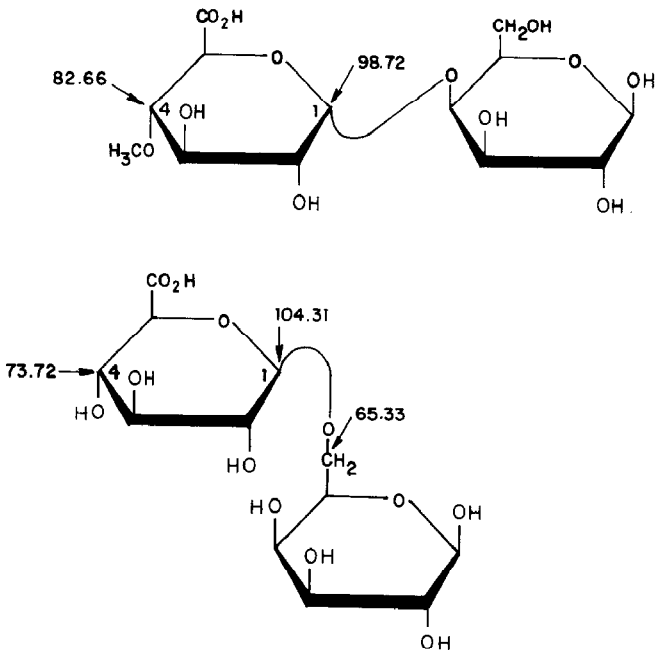

Fig. 9. Aldobiouronic acid residues isolated and identified in *A. xanthoploea* gum.

from ^{13}C-n.m.r. spectra despite the complexity of these acid heteropolysaccharides of high molecular weight.

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