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CHEMICAL AND ^{13}C N.M.R. STUDIES ON TWO ARABINANS FROM THE INNER BARK OF YOUNG STEMS OF *Rosa glauca*

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ABSTRACT

Two water-soluble arabinan fractions have been isolated from the inner bark of *Rosa glauca* stems. Methylation analysis and periodate oxidation revealed that the two polysaccharides have similar, highly branched structures consisting of α-L-arabinofuranose residues substituted (1→2), (1→3), and (1→5). The two arabinans differ in their degree of polymerization: 100 and 34, respectively. ^{13}C-N.m.r. spectroscopy has been used to distinguish between glycosidically substituted and free C-5 groups in the polymer, as well as to assign the methoxyl groups at positions 2, 3, and 5 of the methylated arabinans. The different types of linkages could also be identified. The results obtained by chemical and n.m.r.-spectroscopic methods were in good agreement.

INTRODUCTION

Pectic polysaccharides, which are characteristic of primary plant cell-walls, probably play an important function in the process of wall elongation. The presence of arabinans in the inner phloem of rapidly elongating stems of the young *Rosa* shoots may be considered as a property of phloem tissues or of any plant cell-wall in a phase of active growth.

Several strains of *Rosa glauca* tissues cultured *in vitro* have been isolated in this laboratory[1] from the inner bark of one-year-old stems of *Rosa glauca*. One of these strains has been maintained as a suspension culture for several years[2], and its pectic polysaccharides have been studied. We now report the structures of two pure arabinans isolated from the inner bark of the plant and which have not thus far been characterized in the corresponding suspension cultures. Recently, the structure of the water-soluble arabinan from the bark of white willow[3] was compared to that from the wood of maritime pine[4] and from the bark of aspen[5]. The chemical data obtained for the two arabinans from the inner bark of *Rosa glauca* are discussed here and correlated with the results obtained from their ^{13}C-n.m.r. spectra.

RESULTS AND DISCUSSION

Extractive-free bark was subjected to hot-water extraction and the soluble polysaccharides were fractionated. Graded precipitations with 60 and 70% ethanol, and acetone, according to Roudier[4], afforded three fractions containing mainly arabinose, together with xylose, galactose, glucose, and galacturonic acid, but none of them corresponded to a pure arabinan. In a second attempt, the method of fractionation according to Aspinall *et al.*[6] was applied. Treatment with 7% aqueous copper acetate gave an insoluble copper-complex corresponding to an acidic polysaccharide containing rhamnose, arabinose, galactose, and galacturonic acid. From the supernatant, two pure arabinan fractions soluble in 70% aqueous ethanol were obtained. Fraction I, precipitated with acetone, and fraction II, soluble in acetone, gave 93 and 100% of arabinose, respectively, on acid hydrolysis (see Experimental and Table I).

TABLE I

FRACTIONATION OF THE SOLUBLE COPPER COMPLEX FROM THE INNER BARK OF *Rosa glauca*

Fraction	*Yield*[a] (%)	*Protein* (%)	$[\alpha]_D$ (*degrees*)	*Molar ratios of monosaccharides*						*Uronic acids*
				Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	
H_2O extract				3.7	55.5	2.0	6.0	12.3	20.5	13.7
70% Ethanol	8.6	15		1.3	54	3.2	3.1	22.1	16.3	16.6
80% Ethanol	2.3	2		—	39.2	3.8	25.6	9.4	22	7.3
Arabinan I	8.5	0.7	−58.3	—	93.0	—	3.0	1.0	3.0	—
Arabinan II	1.5	2.7	−73.6	—	100	—	—	—	—	—

[a] In percent of total, water-soluble polysaccharides.

Chemical study. — For structural analysis, arabinans I and II were per methylated. Arabinan I was fully methylated in one step by the Hakomori procedure[7], whereas arabinan II by the same treatment was separated into two fractions, II_A soluble in chloroform and II_B soluble in water.

Permethylation of II_B was achieved with one Purdie[8] methylation. Analytical results obtained by g.l.c. and g.l.c.–m.s. of the hydrolysates are given in Table II and show that the sub-fractions II_A and II_B are similar.

From the methylation data, it may thus be seen that arabinans I and II have a highly branched structure, as indicated by the amount of 2,3,5-tri-*O*-methylarabinose, and by the proportions of mono-*O*-methylarabinose and arabinose. The presence in arabinan I of 2,3,4-tri-*O*-methylarabinose is indicative of the occurence of a few residues in the pyranose form and has previously been reported for arabinans isolated from different origins[3,5]. When submitted to periodate oxidation, arabinans I and II consumed 0.72 and 0.75 mole respectively, of periodate per pentose residue. This result is in agreement with the theoretical values deduced from methylation analysis, which predicted a periodate consumption of 0.67 and 0.66 mole/mole, respectively.

TABLE II

METHYLATION ANALYSIS OF ARABINANS

Methylated sugar[a]	*Mole %*		
	Arabinan I	*Arabinan II*$_A$	*Arabinan II*$_B$
2,3,5-Me_3-Ara	1.00	1.00	1.00
2,3,4-Me_3-Ara	0.04	—	—
2,5-Me_2-Ara	0.20	0.18	0.13
2,3-Me_2-Ara	0.90	0.47	0.46
2-Me-Ara	0.40	0.31	0.35
3-Me-Ara	0.17	0.08	0.10
L-Arabinose	0.25	0.19	0.20

[a]2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc.

Periodate oxidation followed by reduction and acid hydrolysis gave arabinose and glycerol in the ratios of 0.66 for arabinan I and 0.83 for arabinan II. Traces of erythritol (about 2%) were also present in the hydrolysis products from arabinan I, and probably arose from the glucose and mannose present as impurities in the original fraction.

The negative specific rotations and the rapidity of hydrolysis of the arabinosyl linkages suggest that the arabinose groups are predominantly α-L-arabinofuranose residues.

From all the foregoing chemical and physicochemical data, it may be seen that both arabinans present a highly branched structure constituted of α-L-arabinofuranose residues. They have in common the same proportion of arabinose residues carrying two points of side-branching ($\simeq 8.5\%$) and approximately the same proportion of internal residues carrying only one point of side branching ($\simeq 18\%$). In both polysaccharides, less than 38% of the internal residues are unbranched, α-(1→3)- and α-(1→5)-linked. The main structural difference between arabinan I and II is that the former has about 30% of the unbranched residues substituted at position 5 versus 21% in the latter, whereas their proportion of linkages through position 3 is very similar (6.7 *vs* 8.7%). The overall proportion of unbranched residues in II is thus greater than in arabinan I.

The average degree of polymerization (d.p.) was determined by the ratio of methyl arabinoside to arabinitol (as their acetates) after borohydride reduction followed by hydrolysis and glycosidation. Arabinan I showed a d.p. of 100, whereas Arabinan II had a d.p. of only 34.

N.m.r. studies. — The 250-MHz-^{1}H n.m.r. spectra of the arabinans as their methylated and acetylated derivatives gave very little information. As may be seen in Table III, the resonances of the anomeric protons of the different methyl arabinosides are very close. In the arabinans, the anomeric protons resonate at lower field that those of the model compounds, and it is only possible to conclude from the spectra that the polymers are devoid of α-L-arabinopyranosidic residues.

TABLE III

ASSIGNMENTS OF H-1 SIGNALS IN ^{1}H-N.M.R. SPECTRA OF THE FOUR METHYL ARABINOSIDES AND ARABINAN I

Compound	*H-1*[a]	$J_{1,2}$[b]
Methyl α-L-arabinopyranoside	4.26	7.5
Methyl β-L-arabinopyranoside	4.83	2.8
Methyl α-L-arabinofuranoside	4.91	1.8
Methyl β-L-arabinofuranoside	4.86	4.5
Arabinan I (hydroxylated form)	5–5.4	[c]

[a]Chemical shifts are expressed in p.p.m. relative to T.S.P. as internal standard. [b]In Hz. [c]Multiplet, coupling constants not measurable.

On the other hand, the ^{13}C n.m.r. spectra of the arabinans or their permethylated derivatives gave information in agreement with the results obtained by chemical analysis. The use of a high-resolution spectrometer (CAMECA 250) allows the recording of satisfactory spectra even with the sparingly soluble arabinans. Fig. 1a shows the ^{13}C n.m.r. spectrum of a mixture of the four methyl L-arabinosides[12], and the assignments of the signals are given in Table IV. The discrepancy of shifts of +1.55 p.p.m. as compared to the results of Gorin *et al.*[13] exist because of the fact that different reference systems were used when recording the spectra.

TABLE IV

ASSIGNMENTS OF SIGNALS IN ^{13}C-N.M.R. SPECTRA OF THE FOUR METHYL ARABINOSIDES AND OF THE HYDROXYLATED ARABINAN (CHEMICAL SHIFTS ARE EXPRESSED IN p.p.m. RELATIVE TO T.S.P. AS INTERNAL STANDARD)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*OCH_3-1*
Methyl α-L-arabinopyranoside	106.51 (160)[a]	73.24	74.91	70.6	68.36 (143.5); ((3.05–4.5))[b]	59.3
Methyl β-L-arabinopyranoside	102.46 (169)	70.88	71.37	71.46	65.03 (143.5); ((3.05–4.5))	57.7
Methyl α-L-arabinofuranoside	110.96 (172.4)	83.43 (150)	79.16 (148)	86.32 (149)	63.86 (143.5); ((6.10))	57.36
Methyl β-L-arabinofuranoside	104.77 (174) 110.35 (174)	79.15 (148)	77.43	84.59	65.76 (143.5); ((10.7)) 69.80 (143.5); ((6))	59.84
Arabinan I (hydroxylated form)	109.90 (172.5) 109.21 (175)				69.45 (142–143.5); ((4.5)) 69.15 64.06 (142–143.5)	

[a]Values (in Hz) in parentheses represent the $^1J(^{13}C,H)$ values. [b]Double parentheses indicate the long-range carbon–proton, spin–spin coupling constant.

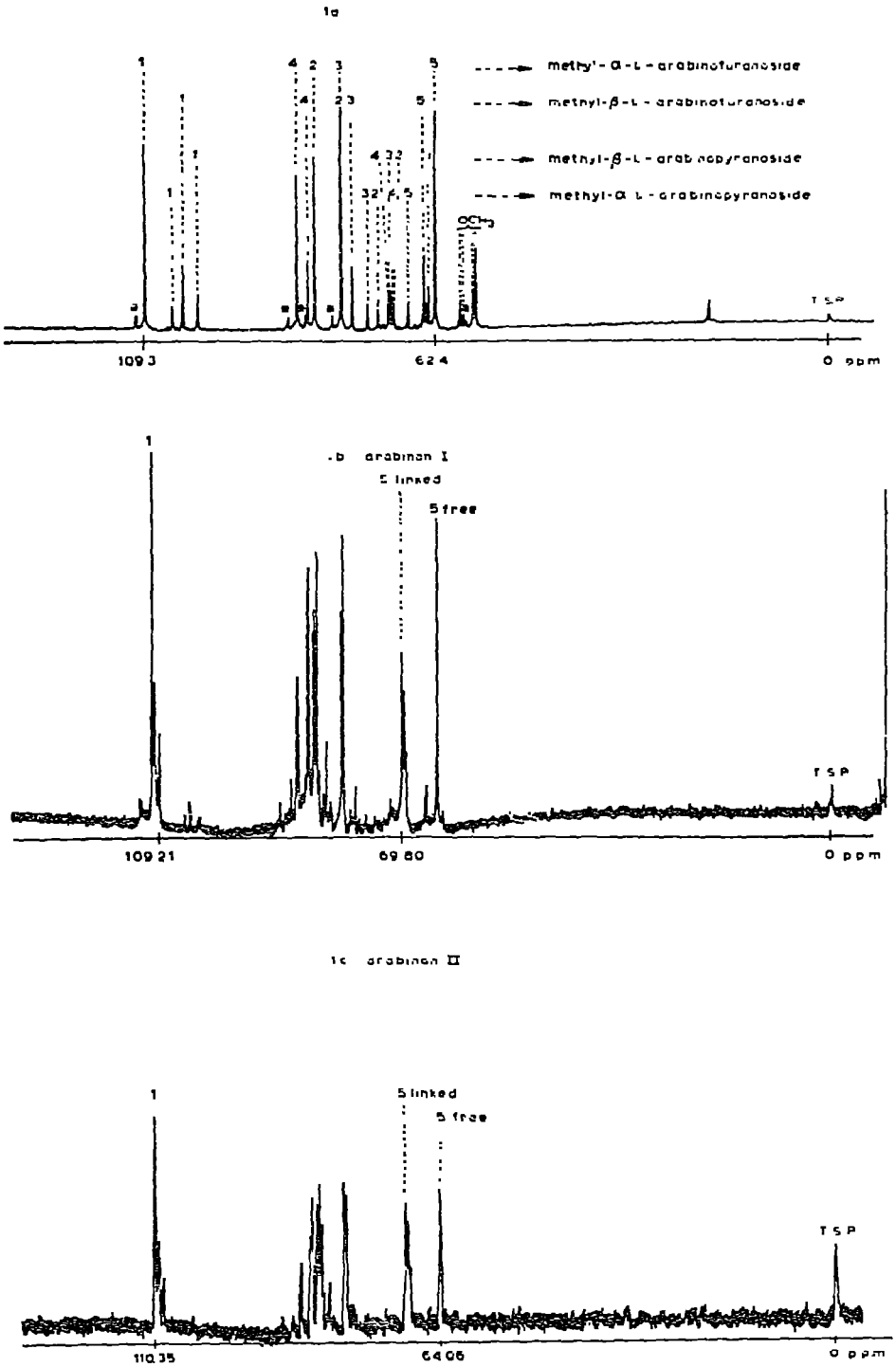

Fig. 1. (a) ^{13}C-n.m.r. spectrum of a mixture of the four methyl L-arabinosides (for assignments see Table IV); (b) ^{13}C-n.m.r. spectrum of arabinan I; (c) ^{13}C-n.m.r. spectrum of arabinan II.

In the ^{13}C spectrum of arabinan I (Fig. 1b), in addition to the 15 to 20 intense signals corresponding to the α-L-arabinosyl residues, the presence of signals of low intensity may be observed. Some of these signals are also found in the spectrum of the methyl arabinosides obtained from arabinose of commercial origin (Prolabo), (denoted by an asterisk in Fig. 1a), and probably correspond to hexose impurities, as arabinan I is contaminated with galactose, glucose, and mannose (1, 3, and 3%, respectively, Table I). Indeed, the foregoing signals were absent from the ^{13}C n.m.r. spectrum of arabinan II (Fig. 1c), which contains only arabinose residues (Table I). The absence of signals in the region 70-79 p.p.m. is thus a proof of purity for arabinofuranans. The signals appearing between 79 and 87 p.p.m., corresponding to the resonances of C-2, C-3, and C-4 of the arabinosyl residues, could not be assigned at present. The signals resonating at higher field (64.06 and 69.5 p.p.m.) are of particular interest as they are well separated. An off-resonance experiment showed that they are signals of primary alcohol groups, as they gave triplets. This result demonstrates that they correspond to C-5 of the arabinose residues, to free CH_2OH groups for the signal at 64.06 p.p.m., and to glycosidically substituted CH_2-O-groups for the signals centered at 69.5 p.p.m. Because of the different spin-lattice relaxation times (T_1) and nuclear Overhauser enhancements of linked and non-linked carbon atoms, a quantitative estimation of ^{13}C resonance areas of the C-5 carbons was not reliable. This difficulty was overcome by recording the spectrum under appropriate conditions. The protons were irradiated during the acquisition time (0.6 sec) followed by a delay of 5 sec without irradiation[14,15].

With the foregoing conditions, the relative proportions of the glycosidically linked O-5 and free O-5 groups for arabinan I were shown to be 61.8 and 38.2%, respectively. This result compares well with the data (59 and 40.4%, respectively) obtained by methylation analysis.

It is noteworthy that the free C-5 atoms exhibit a single signal at δ 64.06 p.p.m., whereas the glycosidically bound C-5 atoms exhibit a group of 3 signals at δ 69.80, 69.45, and 69.15 p.p.m., having the integrated values of 21, 32.4, and 8.4% of the total C-5 areas, respectively.

The fact that only residues **1** and **2** (Fig. 2) have their C-5 atoms free, and thus correspond to the signal at 64.06 p.p.m., indicates that the glycosidic substitution at C-3 of **2** has no influence on the chemical shift of C-5. The situation is more complex for C-5 atoms engaged in glycosidic linkages, as four residues of this type may be found in the polysaccharides (residues **3**, **4**, **5**, and **6**, Fig. 2), and only three signals are seen. It is thus not possible to assign these signals without reference to model compounds, nor can it be stated whether the influence depends on the substitution at C-2 and/or C-3 of the residue, or on the neighbouring residues attached to C-5 and having several possible positions of substitution.

As for the three signals exhibited in the anomeric region of the spectrum of the hydroxylated polymers, it was not possible to assign them to the differently linked anomeric carbon atoms. However the values of the chemical shifts of these carbon atoms (109–110 p.p.m.) show that the polymers consist of α-L-arabinofuranose

residues (see Table IV). Confirmation of the furanosidic nature of the arabinose residues was obtained by the coupling constants $^1J(^{13}C\text{-}1, {}^1H\text{-}1)$, as measured by the gated-decoupling method (Table IV), although it was not possible with this technique to decide between the α or β anomeric configuration for arabinosyl residues as both have similar values.

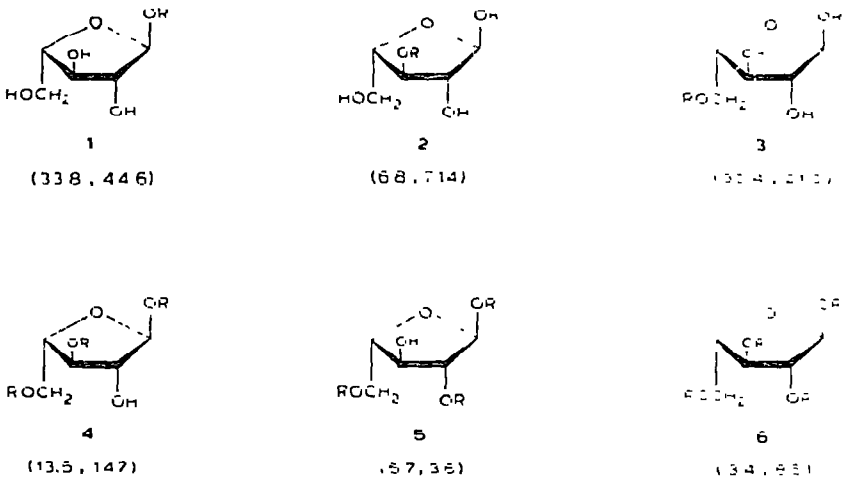

Fig. 2. Different linkages occuring in the two arabinans R = arabinofuranosyl. Values in parentheses give the relative percentages for arabinans I and II, respectively (calculated from Table II).

Additional information was obtained from the ^{13}C-n.m.r. spectra of the methylated arabinans. First, the spectra exhibited three signals in the O–CH_3 region (the integration of which was performed as described already) which, together with methylation-analysis data, allowed assignment of the following resonances: the signal at δ 59.33 p.p.m. (18.4%) corresponds to O–CH_3 at position 5, the signal at δ 57.97 p.p.m. (43.2%) to O–CH_3 at position 2, and the signal δ 57.50 p.p.m. (38.4%) to O–CH_3 at position 3. It can thus be deduced that the methyl carbon atoms are insensitive to, or affected only a little by the nature of the glycosidic linkages, as they resonate at precise frequencies depending on their position on the sugar ring. A second result was given by the groups of signals at δ 66.40 and 72.60 p.p.m. the integral of which allowed (with reference to the methylation data) their assignment to the glycosidically linked C-5 and methylated C-5 (non-glycosidically linked) atoms, respectively. The spectra of the methylated arabinans also showed three sharp anomeric-signals.

As three types of linkages ($1\rightarrow2$, $1\rightarrow3$, and $1\rightarrow5$) exist in the arabinans, the anomeric signals were assigned according to the methylation results. Methylation analysis and ^{13}C-n.m.r. spectroscopy gave the respective following percentages: for $1\rightarrow2$ linkages, 14 *vs* 12.1%; for $1\rightarrow3$ linkages, 28 *vs* 26.1%, and for $1\rightarrow5$ linkages, 57 *vs* 61.7%. The attribution of the C-1 signals are thus δ 107.05 p.p.m., corresponding

to (1→2)-α-arabinofuranosyl linkages: δ 10.6.10 p.p.m. to (1→5)-α-arabinofuranosyl linkages, and δ 105.08 p.p.m. to (1→3)-α-arabinofuranosyl linkages.

CONCLUSION

The fact that two structurally similar, but not identical, arabinans have been isolated from the bark of *Rosa glauca* is another demonstration of the heterogeneity of polysaccharides present in plants[9–11], which could originate from the great diversity in the cell types constitutive of phloem tissues. Structural features of these polysaccharides may thus only be regarded as the average character of a family of polymers resulting from a more-or-less wide spectrum of related entities. That is interpretation would explain why slightly different fractions may be obtained during the process of isolation under slightly different conditions.

The chemical investigation allowed the characterization of the different types of linkages existing in the arabinans, and established the main structural similarities and differences between arabinan I and arabinan II. Most of the information obtained by chemical analysis was confirmed by n.m.r. spectroscopy.

It may be noted that ^{13}C-n.m.r. spectroscopy has brought more information to the structural investigation of the polysaccharides than did ^{1}H-n.m.r. spectroscopy[20,21]. With ^{13}C-n.m.r. spectra, it is possible to differentiate the glycosidically linked, primary alcoholic groups (C-5 in the arabinose residues) from the corresponding free ones.

The configuration of the anomeric linkage may be identified, as well as the percentage and positions of the hydroxyl groups accessible to methylation, that is, the average number of each type of linkages existing in the polymer. All of this information is essential for characterizing a polysaccharide, and could be of great help when comparing polysaccharides belonging to a same family, but differing in their structure.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Roussel et Jouan Quick polarimeter at 20°. For g.l.c., a Packard–Becker 417 instrument fitted with a flame-ionisation detector was used. Separations were performed on glass columns (200 × 0.15 cm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 185° (for alditol acetates) or 150° (for partially methylated alditol acetates). G.l.c.–m.s. was performed with a Girdel 3000-MS 30 AEI instrument fitted with a column containing 3% of OV-225 on Chromosorb W kept for 4 min at 120° and then heated at 1°/min and holding at 200°. Unless otherwise stated[16] all hydrolyses were carried out according to Saeman; protein[17] contents were determined using the method of Lowry; and uronic acids were estimated by the carbazole method[18].

Isolation of the arabinans. — Powdered inner bark of *Rosa glauca* was extracted under reflux twice with 80% ethanol for 20 min and then with 1:2 ethanol–benzene

for 20 h, yielding 93% of dry material. The tissues (200 g) were treated for 3 h with water at 90°, pH 5.5. The operation was repeated 4 times. Freeze drying of the extracts gave 19 g (9.5%) of polysaccharides.

Purification according to Roudier[4]. Ethanol was added gradually to a 0.2% solution of the mixture of polysaccharides (4 g) in water whereupon a distinct precipitation end-point was observed at 4°. Two precipitates were thus obtained for concentrations of ethanol of 60 and 70% respectively. A third fraction was also collected by addition of acetone (4 volumes) to the supernatant. All three fractions contained arabinose, xylose, galactose, glucose, and galacturonic acid.

Purification according to Aspinall[6]. To an aqueous solution of the polysaccharide mixture (8 g in 4 litres), a 7% solution of copper acetate (0.8 litre) was added, which, after decantation overnight at 4°, yielded an insoluble copper-complex. After centrifugation, the supernatant was fractionated successively by ethanol and acetone precipitation. Ethanol precipitation followed by decomplexation with 1% ethanol-hydrogen chloride gave two fractions, at 70% (0.691 g; 8.6%) and 80% (0.186 g; 2.3%) concentrations of ethanol. Precipitation of components in the supernatant by acetone gave a third fraction (0.680 g; 8.5%, Arabinan I). The mother liquor was lyophilized and gave a residue (0.124 g; 1.5% Arabinan II). The respective compositions of the aforementioned fractions are given in Table I.

Identification of L-*arabinose.* — The polysaccharides (31 mg) in 2M trifluoroacetic acid (5 ml) were kept for 1 h at 120°. The hydrolysates was evaporated to dryness and the sugars isolated by preparative, paper chromatography (Whatman no. 3, solvent system: 8:2:1 ethyl acetate–pyridine–water). Elution of appropriate strips gave mainly L-arabinose, $[\alpha]_{589}^{20}$ +91 (5 min) → +103.3° (equil., *c* 3.3, water).

Methylation analyses. — These were performed according to Hakomori[7]. Arabinan I (306 mg) was solubilized in freshly distilled methyl sulphoxide (30 ml) and a solution of methylsulphinyl sodium in methyl sulphoxide (5 ml) was added. The suspension was sonicated[22] for 30 min and kept overnight at room temperature in a nitrogen atmosphere with stirring. Methyl iodide (8 ml) was then added over a 3-h period and the resulting clear solution sonicated for 20 min. The solution was poured into water (120 ml), dialysed for 24 h, and extracted with chloroform to give a fully methylated polysaccharide (331 mg) that showed no hydroxyl band in the i.r. The product had $[\alpha]_{589}^{20}$ −116° (*c* 3.2, chloroform).

Arabinan II (63.6 mg), methylated as already described, gave a fraction II_A soluble in chloroform (21 mg) and a fraction II_B soluble in water. Fraction II_B was remethylated by refluxing it in methyl iodide–silver oxide to give a fully methylated product (43.5 mg). Fraction II_A showed $[\alpha]_{589}^{20}$ −91° (*c* 1.1, chloroform) and fraction II_B $[\alpha]_{589}^{20}$ −117° (*c* 1.8, chloroform).

Analytical values for the methylated arabinans are given in Table II.

Periodate oxidation. — Arabinans I and II (15 mg), solubilized in aqueous sodium metaperiodate (0.015M, 20 ml), were kept at room temperature in the dark, the consumption of periodate being spectrophotometrically monitored on aliquots at 223 nm[19]. After 120 h, the consumption of periodate was 0.72 mole per mole of

L-arabinose residues for arabinan I and 0.75 mole/mole for arabinan II. Reduction of the resulting polyaldehydes by sodium borohydride, followed by hydrolysis and g.l.c. analysis, showed glycerol and arabinose, with a trace of erythritol in the case of arabinan I.

Determination of the d.p.$_n$. — The arabinans (10 mg) were reduced with a solution of sodium borohydride for 24 h at room temperature, and after neutralization with 50% acetic acid, deionization with Amberlite IR-120 (H^+) resin and several evaporations with 1% methanolic hydrogen chloride, the reduced polysaccharides were hydrolysed with 0.5M H_2SO_4 (3 ml, 6 h, 100°). The hydrolysed products were converted into methyl glycosides[12] which were then acetylated. The ratio of acetylated arabinitol was determined by g.l.c. on a 3% ECNSS-M column for 6 min at 80°, and programmed at 5°/min to 180°. The d.p.$_n$ was 100 for arabinan I and 34 for arabinan II.

N.m.r. analysis. — The ^{13}C-n.m.r. spectra were obtained with a CAMECA 250 spectrometer. Hydroxylated polysaccharides were in solution in deuterium oxide at 90° (150 mg in 1.5 ml, with tubes of 8-mm o.d.) and chemical shifts in δ values are relative to T.S.P. (sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate) as the internal reference; the chemical-shift values differ by +1.55 p.p.m. from those expressed relative to Me_4Si contained in a coaxial sample tube. Methylated arabinans were in solution in chloroform-*d* at 50° (50 mg in 0.5 ml, with tubes of 5-mm o.d.), and chemical shifts are relative to Me_4Si as internal reference. The pulse width was 10 μsec ($\simeq 70°$) and the acquisition time was 0.6 sec.

Normal ^{13}C spectra were recorded with complete proton-decoupling at 62.86 MHz on a CAMECA spectrometer equipped with Fourier transform (spectral windows of 200 p.p.m., and digitalization into 12 K data points).

Determination of the coupling constants was made with a gated, ^{1}H-decoupler sequence (decoupler on–decoupler off, 70° pulse, acquire data) to retain nuclear Overhauser enhancements. For "off-resonance" experiments, irradiation was effected at the ^{1}H resonance frequency of the T.S.P. or Me_4Si.

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