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Note

Characterization of carbohydrate components of an unusual hydrogel formed by seed coats of *Magonia pubescens* (Tingui)

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Magonia pubescens St. Hil. is a tree that grows in the savannah (*cerrado*) regions of Brazil and is known locally as Tingui or Timbó do Cerrado. Its flat elliptical seeds (8 cm major axis and 5 cm minor axis × 0.3 cm max. thickness) occur in a woody dehiscent fruit (trigonal with 20 cm diameter and 7 cm height), and the seed coat, upon exposure to air with high humidity or on wetting, forms a thick hydrogel which is a germination promoter [1]. Since the gel has the unusual property of being stable at 100 °C, it was of interest to find an explanation in terms of the chemical structures of its components.

A preliminary analysis was carried out on the carbohydrate components of the crude gel after freeze-drying (yield 7.3%, based on seeds; protein 0.25%). Attempts to determine its monosaccharide composition via aqueous acid hydrolysis at 100 °C were unsuccessful, because of its partial insolubility. Presolubilization in cold 72% sulfuric acid, followed by dilution with water and heating [2], gave Rha, Arab, Xyl, Man, Gal, and Glc in a 10:6:45:3:6:29 mol% ratio with the formation of uronic acid-containing oligosaccharides. Under stronger hydrolysis conditions, galacturonic acid was not detected (PC).

Since preferential degradation of pentoses would have occurred in the presolubilization step, dissolution in cold acetic acid–acetic anhydride–sulfuric acid was performed,

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followed by deacetylation and hydrolysis with hot aqueous acid. A more reliable molar ratio of 2:12:67:1:5:13 was obtained, although rhamnose was only partly liberated, probably due to its presence in an aldobiouronic acid.

Methylation analysis was carried out on the freeze-dried material, and the completely *O*-methylated polysaccharide was converted to *O*-methylated alditol acetates deuterated at C-1. Analysis (GC-MS) indicated the presence of non-reducing end-units of Rha*p* (1%), Ara*f* (2%), Xyl*p* (8%), and Gal*p* (3%), with 4-*O*- (47%) and 2,4-di-*O*-substituted units of Xyl*p* (22%), 2-*O*-substituted units of Rha*p* (8%), and 3-*O*-substituted units of Gal*p* (2%). Glc*p* was present as non-reducing end-units (1.8%) and 4-*O*-substituted units (9.2%). Lithium aluminum deuteride reduction of the per-*O*-methylated polysaccharide, followed by conversion of the product to a mixture of *O*-methyl alditol acetates, gave rise to an additional peak of 2,3,4-Me_3-glucitol-1,6-$^2H_3$ acetate (26%), showing the likely presence of non-reducing end-units of Glc*p*A and/or 4-Me-Glc*p*A.

The structures of the uronic acids and associated units were determined following partial acetolysis of freeze-dried hydrogel. After deacetylation, PC showed three oligosaccharide spots with R_{Lact} 0.79, 0.62, and 0.42 (Table 1), whose respective ^{13}C NMR spectra (Table 1) corresponded to those of 4-Me-α-Glc*p*A-(1 → 2)-αβ-Xyl, 4-Me-α-Glc*p*A-(1 → 2)-β-Xyl*p*-(1 → 4)-αβ-Xyl [3], and α-Glc*p*A-(1 → 2)-β-Xyl*p*-(1 → 4)-αβ-Xyl. Another oligosaccharide fraction (R_{Lact} 1.00) appeared after chromatographic enrichment and ^{13}C NMR spectroscopy (Table 1), and methylation analysis showed it to be a mixture of 6-*O*-α-galactopyranosyl- and 6-*O*-α-glucopyranosyl-glucose in a 3:2 molar ratio.

Since the Tingui polysaccharides contain units of glucuronic acid and its 4-*O*-methyl derivative and not galacturonic acid, they are not pectic substances, which function in some plants as part of a drought resistant protective matrix [4,5].

A CP-MAS ^{13}C NMR spectrum of the freeze-dried gel (Fig. 1) showed predominantly carbohydrate components. A C-6 signal of uronic acid appears at δ 175.9 as well as high field signals due to rhamnose (δ 17.6) and *O*-acetyl (δ 21.6) groups. A C-1 signal was present at δ 104.5 with a shoulder at δ 99.2. *O*-Substituted resonances were present at δ 82.3 and 88.6. Small non-carbohydrate signals were observed at δ 33.0, 129.3, and 144.6. Treatment of the freeze-dried gel with 10% sodium hydroxide at 100 °C caused only partial solubilization. The resulting ^{13}C NMR solution spectrum was weak, but contained signals of α-Ara*f*, β-Xyl*p* [6], α-Glc*p*A and/or 4-*O*-Me-α-Glc*p*A, and OAc^- (Table 1).

Acidic xylan and cellulosic oligosaccharide components were isolated from the gel. Two methods were employed (Scheme 1). In one, the gel was initially presolubilized in aqueous cuprammonium hydroxide (cuoxam, Schweizer's reagent), and after processing, insoluble material was isolated. This material was treated with dilute aqueous sodium hydroxide and the remaining insoluble material contained glucose (65%). Repetition of the cuoxam procedure gave insoluble material (3% yield) containing glucose (92%) and xylose (8%). Methylation analysis showed non-reducing end-units (10%) and 4-*O*-substituted units of Glc*p* (81%), as well as 4-*O*-substituted units of Xyl*p* (9%). The linear Glc*p* and linear Xyl*p* structures had β-linkages because of their insolubility, which was confirmed by their susceptibility to cellulase enzymolysis. The average chain-length of the cellulosic oligosaccharides is thus 8–9 units, close to the value of 9–10 for the Glc*p*

Table 1
^{13}C NMR data for oligosaccharides and polysaccharides and assignments

Saccharide	Chemical shifts (δ, ppm) and assignments
Polysaccharide from alkali solubilized gel	C-1: 107.4 (α-Ara*f*), 102.8, 103.4 (β-Xyl*p*), 102.1, 101.3 (?), 98.4 (α-Glc*p*A and/or 4-*O*-Me-α-Glc*p*A); other: 22.0 (OAc^-)
Fraction C, branched-chain acidic xylan structures	C-1's: 103.18 and 103.09 [(1 → 4)-linked β-Xyl*p* main-chain, 23%, 17%, resp.], 102.59 (?, 25%), 105.19, 105.28 [β-Xyl*p*-(1 → 2)-Xyl*p*, 1%, 6%, resp.], 101.6 [β-Xyl*p*-(1 → 2)-β-Xyl*p*]; reducing ends: 93.62 (α-anomer, 2%), 98.0 (β-anomer, 3%), others: 99.1 (C-1: α-Glc*p*A, 4-Me-α-Glc*p*A; 17%), 178.7 (C-6, uronic acids), 61.5 (OMe-4) [a]
Fraction C, cellulosic oligosaccharide structures	C-1's of cellulosic oligosaccharides: reducing ends: 97.5 (β-anomer, 2%), 93.67 (α-anomer, 1%); C-6's (1:3 ratio) of non-reducing ends (62.0), and internal and reducing units (62.4) [a]
Fraction D	C-1's of β-Xyl*p*: 103.25 (20%), 105.2 (8%) (for assignments, see those of Fraction C), based on α-Glc*p*A and 4-*O*-Me derivatives: 99.4 (C-1: plus other signal, 37%), 173.9, 175.4 (C-6), 61.2 (OMe-4); other C-1's not identified: 102.60 (24%), 100.5 (15%), 101.7 (8%) [a]
Oligosaccharides derived via partial acetolysis of gel:	
4-Me-α-Glc*p*A-(1 → 2)-$\alpha\beta$-Xyl, R_{Lact} 0.79	98.5 ($C\text{-}1'_\alpha$), 99.1 ($C\text{-}1'_\beta$), 97.9 ($C\text{-}1_\beta$), 91.2 ($C\text{-}1_\alpha$), 79.8 ($C\text{-}2_\beta$), 77.4 ($C\text{-}2_\alpha$), 61.5 (OMe-4), 178.9 (C-6″)
4-Me-α-Glc*p*A-(1 → 2)-β-Xyl*p*-(1 → 4)-$\alpha\beta$-Xyl, R_{Lact} 0.62	103.2 (C-1′), 99.0 (C-1″), 98.0 ($C\text{-}1_\beta$), 93.5 ($C\text{-}1_\alpha$), 77.8 and 77.9 ($C\text{-}2'_\beta$ and $C\text{-}4_{\alpha\beta}$), 61.5 (OMe-4), 178.9 (C-6″)
α-Glc*p*A-(1 → 2)-β-Xyl*p*-(1 → 4)-$\alpha\beta$-Xyl, R_{Lact} 0.42	103.2 (C-1′), 99.1 ($C\text{-}1''_\beta$), 98.0 ($C\text{-}1_\beta$), 93.5 ($C\text{-}1_\alpha$), 76.5, 77.8, 78.9, 178.7 (C-6″). Very similar to above, but lacking the δ 61.5 (OMe-4) signal
α-Gal*p*-(1 → 6)-$\alpha\beta$-Glc	100.8 (C-1′), 98.7 (C-1, β-anomer), 94.8 (C-1, α-anomer)
α-Glc*p*-(1 → 6)-$\alpha\beta$-Glc	100.2 (C-1′), 98.7 (C-1, β-anomer), 94.8 (C-1, α-anomer)

[a] Areas of C-1 signals expressed as percentage of the total.

components in the original gel, and apparently shows a preference for physical association with linear rather than branched β-xylan.

In the second fractionation procedure (Scheme 1), the freeze-dried gel could only be partly dissolved in 10% aqueous potassium hydroxide at 100 °C, the remaining insoluble gel being resistant to further solubilization under the same conditions. The extract was neutralized and dialyzed, which gave a precipitate (Fraction A), leaving most of the polysaccharides in solution (Fraction B).

Fraction A formed a viscous solution in cold 1% aqueous sodium hydroxide. Its structural components were analyzed and found to contain Ara, Xyl, Man, and Glc in a

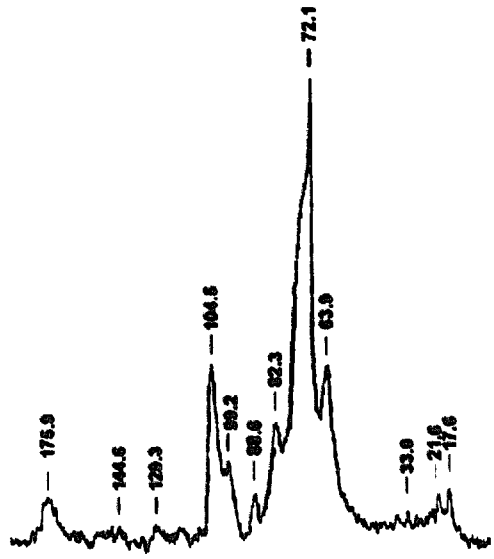
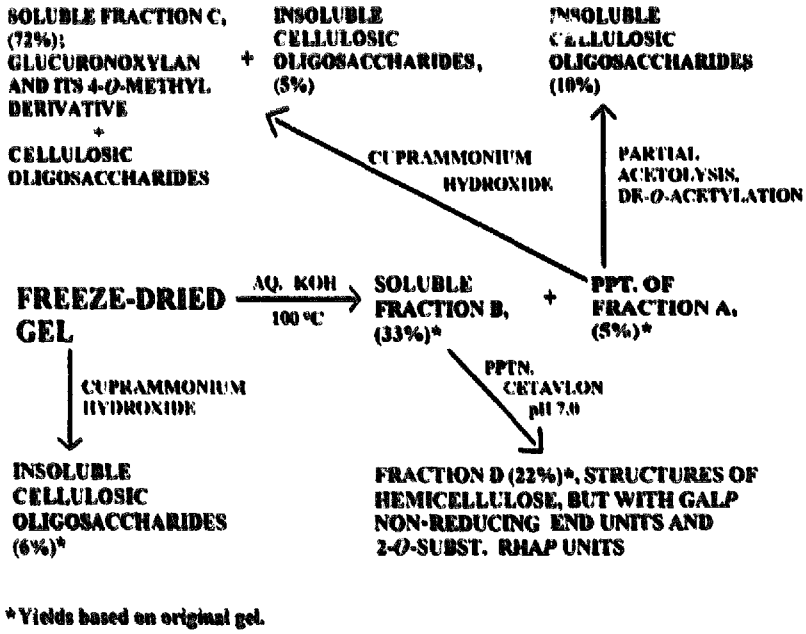


Fig. 1. CP-MAS ^{13}C NMR spectrum of freeze-dried gel from seeds of Tingui.

mol% ratio of 5:78:2:15. Methylation analysis indicated the presence of Xyl*p* as non-reducing end-units (9%), 4-*O*-substituted units (38%), and 2,4-di-*O*-substituted units (28%), non-reducing end-units (1.2%) and 4-*O*-substituted units of Glc*p* (12.8%), non-reducing ends of Ara*p* (2%), and 4-*O*- and/or 5-*O*-substituted units of Ara*f* (2%). The per-*O*-methylated polysaccharide was reduced with lithium aluminum deuteride and converted to a mixture of *O*-methyl alditol acetates which contained 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-glucitol-$^2H_1$ (17%), corresponding to non-reducing end-units of



Scheme 1.

Glc*p*A and/or 4-Me-Glc*p*A. The CP-MAS ^{13}C NMR spectrum of Fraction A contained signals broader than those of the gel component (Fig. 1), and only one signal was recognizable, namely that of C-1 at δ 104.8 which arose from Xyl*p* and Glc*p* units having the β-configuration, but whose broadness would obscure any signal in the δ 99.0 region.

The presence of cellulosic oligosaccharides in Fraction A was found following successive partial acetolysis and deacetylation, which gave rise to rhamnose, arabinose, and xylose in a molar ratio of 1:7:92 and acidic oligosaccharides with R_{Lact} 0.79, 0.62, and 0.42. On dissolution in water, a precipitate remained (Scheme 1), which contained glucose (95%), as did a precipitate (6% yield) which was formed from the mother liquor upon addition of 1:1 v/v acetone–ethanol, indicating that the xylose-containing polysaccharide was acetolyzed preferentially. Both precipitates contained Glc*p* units that were 4-*O*-substituted with 10 and 12% of non-reducing end-units, respectively (methylation data).

Fraction A was solubilized in Schweizer's reagent and the solution was processed as above (Scheme 1), giving a precipitate of cellulosic oligosaccharides which was characterized by methylation analysis (12% of non-reducing end-units) and lysis with cellulase. The supernatant (Fraction C), upon hydrolysis, gave xylose, glucose, mannose, and galactose in a 80:18:1:1 molar ratio. Its ^{13}C NMR spectrum (Table 1) contained a complex C-1 region, but some signals could be assigned to Xyl*p* of a 1 → 4-linked main-chain with substitution at O-2 with Xyl*p* [7] and to reducing ends. A C-1 signal of α-Glc*p*A and 4-Me-α-Glc*p*A units was present as were those of OMe-4 and C-6. Signals of cellulosic oligosaccharides could only be detected with certainty for C-1 reducing ends and C-6 [8]. As the solution of Fraction C had been treated with acidic ion-exchange resin prior to NMR examination, a limited autohydrolysis could have taken place, most likely with the acidic xylan component, because reducing end-unit signals of β-Xyl*p* were present.

Acidic Fraction D, consisting of a water-soluble hetero-polysaccharide(s), was isolated and partly characterized. It contained an acidic xylose-containing component similar to that of Fraction A. Fraction D was isolated from soluble Fraction B by treatment with hexadecyltrimethyl-ammonium bromide (Cetavlon), being precipitated at pH 7.0 as the major constituent (Scheme 1). Much smaller fractions were obtained via precipitation at pH 8.5 and 12.0, in the presence of borax, and from the final mother liquor.

Fraction D gave an aqueous solution, which was relatively non-viscous when compared to that of Fraction A. It contained Rha, Arab, Xyl, Man, Gal, Glc (GC-MS), and uronic acid (colorimetry) in a 16:2:38:3:11:2:28 mol% ratio. This composition differed from those of the other smaller Cetavlon fractions. Partial acetolysis of Fraction D gave rise to acidic oligosaccharides with R_{Lact} 0.79, 0.62, and 0.42, similar to those obtained from freeze-dried gel.

Methylation analysis of Fraction D showed a complex structure with non-reducing end-units of Rha*p* (2%), Xyl*p* (10%), Man*p* (3%), and Gal*p* (9%), 2-*O*- (12%) and 2,3-di-*O*-substituted units of Rha*p* (4%), 4-*O*- (28%) and 2,4-di-*O*-substituted Xyl*p* residues (27%), and 3-*O*-substituted Gal*p* (4%) and 4-*O*-substituted Glc*p* units (2%). Lithium aluminum deuteride reduction of per-*O*-methylated polysaccharide gave rise to

acetates of 3-Me-xylitol and 2,3,4-Me_3-glucitol-1,6-$^2H_3$ in equal amounts, showing the presence of at least 22% of Glc*p*A and/or 4-Me-Glc*p*A non-reducing end-units.

The ^{13}C NMR solution spectrum of Fraction D (Table 1) was compared to that of water-soluble material derived from Fraction A in terms of signals and their relative intensities. The same β-Xyl*p* C-1 signals were present in a similar ratio, as well as the α-Glc*p*A signal at δ 99.4, which appeared to be superimposed on another one, because of its high percentage area of 37, greater than that of 28% of uronic acid determined colorimetrically (the 4-*O*-Me signal, and that of uronic acid at δ 174.9, were also present). However, the presence of the other component(s) was shown by a relatively small C-6 signal at δ 173.5 and two other C-1 signals. Fraction D thus contains, along with the hemicellulose component of Fraction A, a polysaccharide which contains a different structure associated with uronic acid, non-reducing end-units of Gal*p*, and 2-*O*-substituted units of Rha*p*.

In summary, the most significant component of Tingui gel is Fraction A, which consists of cellulosic oligosaccharides with an average chain-length of 7–10 units associated with branched acidic lightly acetylated β-xylan, whose (1 → 4)-linked main-chain is unsubstituted (28%) and partly substituted at O-2, having non-reducing end-units of β-Xyl*p*-(1 → 2)- (9%), 4-Me-Glc*p*A-(1 → 2)-, and Glc*p*A-(1 → 2)- (21%). This association appears analogous to the physical association of a linear xylan on cellulose powder via hydrogen bonds, a process that is only partly reversible [9].

As a heat-stable gel, the Tingui component appears unique. However, quince (*Cydonia oblongata*; family *Rosaceae*) seeds form a water-soluble mucilage with related chemical structures, namely a soluble partly *O*-acetylated branched (4-*O*-methyl-glucurono)-xylan [10] containing cellulose microfibrils [11].

1. Experimental

Extraction of seeds of Tingui.—Twenty-four seeds (160 g), collected in the region of Brasília-DF, Brazil, were treated with H_2O (5 L) for 48 h, and the resulting surface gel was removed by teasing through tightly closed fingers. This process was repeated after immersion for a further 48 h and the combined gels were then passed through coarse cloth to remove seed fragments, and then freeze-dried (11.7 g; 7.3% yield).

Monosaccharide composition of polysaccharides.—Portions (1 mg) of freeze-dried gel, Fraction A, and cellulosic oligosaccharide-rich fractions obtained via partial acetolysis of the gel, were each dissolved in Ac_2O–AcOH–H_2SO_4 (0.11 mL; 10:10:1 v/v) for 18 h at 25 °C. The products were deacetylated with $CHCl_3$–methanolic NaOMe and were treated with 2 M CF_3CO_2H for 8 h at 100 °C. The hydrolyzates were evaporated to dryness, reduced with $NaBH_4$ and then acetylated with Ac_2O–pyridine at 100 °C.

Water-soluble fractions were hydrolyzed with 2 M CF_3CO_2H and derivatized similarly. The products were examined by GC-MS using a capillary column of OV-225 (30 m × 0.25 mm i.d.), held at 50 °C during injection, then programmed at 40 °C/min to 220 °C (constant temp).

For fractions richer in cellulosic oligosaccharides (see below), obtained following one and two treatments with Schweizer's reagent, it was necessary to carry out an initial

partial solubilization and lysis step in Ac_2O–AcOH–H_2SO_4 for 20 h at 55 °C, followed by de-*O*-acetylation, in order for the carbohydrates to become soluble in the aqueous hydrolysis medium.

Methylation analysis of polysaccharides and cellulosic oligosaccharides.—Polysaccharides (50 mg) and oligosaccharides (5 mg) were each methylated by the method of Haworth [12], being dissolved in 30% aq NaOH containing a trace of $NaBH_4$ prior to methylation. The initial Haworth procedure was required for the freeze-dried gel, Fraction A, and cellulosic oligosaccharides in order to render the products soluble in the reaction medium of Kerek and Ciacanu [13]. Methylation was completed using the procedure of Kuhn et al. [14]. The *O*-methylated products were refluxed in 3% MeOH–HCl for 3 h and were hydrolyzed with M H_2SO_4 at 100 °C for 18 h. After $NaBH_4$ reduction and acetylation, the resulting mixtures were examined by GC-MS on OV-225 as above and on DB-210, the latter with a program of 50 °C (40 °C/min) to 190 °C (constant temp). This resolved the alditol acetates of 2-Me-Xyl, 3-Me-Xyl, and 2,3,6-Me_3-Glc.

^{13}C NMR spectroscopy.—Solution spectra were obtained at 75 MHz and 33 °C in D_2O or, in the case of freeze-dried gel, ^2H_2O containing 10% NaO^2H_2. Chemical shifts are expressed in δ ppm, based on a Me_4Si standard ($\delta = 0$) determined in a separate experiment. For CP-MAS ^{13}C experiments, samples were rotated at 3.5 KHz; contact time, 1000 μs; pulse interval, 3 s; spectral width, −50 to 250 ppm.

Determination of uronic acid.—Determinations were carried out using the carbazole method [15].

Partial acetolysis of hydrogel and isolation and characterization of resulting oligosaccharides.—The freeze-dried hydrogel (3.5 g) was treated with Ac_2O–AcOH–H_2SO_4 (210 mL; 10:10:1 v/v) for 5 h at 25 °C (the temperature was lower than 30–40 °C used by Hess and Dziengel [16] for preparation of oligosaccharides from cellulose), and the mixture was added to ice–water. After 3 h the mixture was extracted with $CHCl_3$, and the organic layer was washed twice with H_2O and evaporated to dryness (yield, 2.71 g). The residue was dissolved in $CHCl_3$ (50 mL) to which 0.1 M NaOMe in MeOH (5 mL) was added. After 2 h, the mixture was acidified (AcOH), evaporated to dryness and the residue was treated with an aqueous suspension of Amberlite IR120 (H^+ form). Removal of the resin and evaporation gave a mixture, which contained (according to PC using 1-BuOH–pyridine–H_2O (5:3:3 v/v/v) as solvent and developed with $AgNO_3$–H_2O–acetone (dip)), monosaccharides and oligosaccharides with R_{Lact} 0.62, 0.79, and 0.42.

The mixture was fractionated on a column of 1:1 w/w powdered active charcoal (Reagen, Quimibras Industrias Químicas, Brazil) and diatomaceous earth (terra de infusórios, Reagen). Monosaccharides were eluted with H_2O and oligosaccharides with 30% aq EtOH (348 mg) and then 50% aq EtOH (97 mg). The last two fractions were combined and fractionated on a Whatman No. 3 filter paper using the 1-BuOH–pyridine–H_2O solvent with three days elution. Fractions with R_{Lact} 0.79 (80 mg), 0.62 (47 mg), and 0.42 (30 mg) were isolated. The first two gave xylose and 4-*O*-methylglucuronic acid (PC) on hydrolysis with 4 M TFA, 100 °C for 4 h, and the other glucuronolactone and xylose. ^{13}C NMR spectral data are recorded in Table 1. Also isolated was a component (15 mg) having R_{Lact} 1.00, which on hydrolysis with 2 M

TFA, 100 °C, 8 h, gave galactose and glucose (3:7), and upon acid hydrolysis of $NaBH_4$-reduced material, glucose and galactose (*p*-anisidine hydrochloride-PC). Methylation analysis showed the presence of 6-*O*-galactopyranosyl- and 6-*O*-glucopyranosyl-glucose. Its ^{13}C NMR signals (Table 1) were consistent with α-glycosidic linkages.

Extraction of freeze-dried gel with Schweizer's reagent and isolation of cellulosic oligosaccharides.—The freeze-dried gel (0.85 g) was dissolved in Schweizer's reagent [17] (250 mL) containing $NaBH_4$ (10 mg). After 18 h, the solution was centrifuged to remove a small quantity of brown material and the solvent was partially evaporated to remove excess ammonium hydroxide. The solution was acidified (AcOH) and dialyzed, giving a precipitate (0.38 g) containing rhamnose, xylose, mannose, galactose, and glucose in a mol% ratio of 22:16:12:14:36. This precipitate was suspended in 1% aq NaOH (5 mL) with stirring for 30 min, which resulted in partial dissolution, and the insoluble material (51 mg) was isolated and resubmitted to the above procedure, giving 26 mg of insoluble material.

Extraction of freeze-dried gel with hot aqueous alkali.—The freeze-dried gel (1.10 g) was stirred in water (140 mL) containing $NaBH_4$ (10 mg) and KOH (14 g) and the mixture was maintained at 100 °C for 16 h. Insoluble gel was removed by centrifugation and the solution neutralized (AcOH), dialyzed, and left overnight at 4 °C. A precipitate formed (Fraction A; 60 mg) and its mother liquor was freeze-dried to give water-soluble polysaccharides (Fraction B; 0.36 g).

Preparation of soluble material from Fraction A using Schweizer's reagent.—Fraction A (175 mg) was dissolved in Schweizer's reagent and processed in a manner similar to the freeze-dried gel. After one treatment the precipitate (9 mg) which formed after dialysis was removed, leaving a supernatant which was treated with Amberlite IR120 (H^+ form), filtered, and the filtrate freeze-dried to give Fraction C (126 mg).

Cetavlon fractionation of soluble polysaccharides.—Fraction B was dissolved in H_2O (70 mL), to which was added Cetavlon (2.0 g) in H_2O (70 mL), both solutions being previously adjusted to pH 7.0. After 4 days, the fine resulting precipitate was isolated by centrifugation at 15,000 rpm (rotor 23 cm diameter at base), dissolved in aq AcOH, insoluble material removed and polysaccharide precipitated with excess EtOH (yield of Fraction D, 218 mg). The Cetavlon supernatant was added to 5% borax in H_2O (80 mL), both solutions being previously adjusted to pH 8.5. After 4 days the precipitate was centrifuged off at 15,000 rpm and the supernatant adjusted to pH 12.0 with aq NaOH to give a more rapidly formed coarser precipitate, which was centrifuged at 3000 rpm (rotor 28 cm diameter at base). The pH 8.5 precipitate was a polysaccharide (20 mg) containing rhamnose, arabinose, xylose, mannose, galactose, and glucose in a mol% ratio of 5:9:70:3:12:1. The precipitate obtained at pH 12.0 (12 mg) had a ratio of 2:19:62:2:14:1, while that precipitated from the supernatant after neutralization (AcOH), dialysis, and freeze-drying (40 mg) had a ratio of 12:8:32:0:10:38.

Partial acetolysis of Fraction A.—Fraction A (195 mg) was treated with Ac_2O–AcOH–H_2SO_4 (5.5 mL; 10:10:1 v/v) for 12 h, and worked up as described above to give a mixture of acetates (241 mg) which were de-*O*-acetylated to the free sugars (molar ratio of rhamnose, arabinose, and xylose, 1:7:92). The mixture was dissolved in H_2O (5 mL) which was frozen and then thawed at 4 °C, resulting in the formation of a

precipitate which was isolated (8 mg). The supernatant was evaporated to 1 mL and acetone–EtOH (10 mL, 1:1 v/v) was added, which gave rise to a precipitate (yield, 11 mg). The aq and acetone–EtOH precipitates both contained arabinose, xylose, galactose, and glucose in a molar ratio of 2:2:1:95. PC examination of the supernatant of the precipitates was performed.

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