ELSEVIER

Carbohydrate Research 260 (1994) 259–270

CARBOHYDRATE RESEARCH

# Structural investigation of *Ceratozamia spinosa* mucilage

Gaspare Barone, Maria Michela Corsaro *, Cristina De Castro, Rosa Lanzetta, Lorenzo Mangoni, Michelangelo Parrilli

*Dipartimento di Chimica Organica et Biologica, Università di Napoli Federico II, Via Mezzocannone 16, 80134 Napoli, Italy*

(Received June 18th, 1993; accepted February 21st, 1994)

**Abstract**

The polysaccharide fraction from *Ceratozamia spinosa* appears to be made up mainly by a chemically homogeneous polysaccharide but with a wide range of molecular weight. By NMR and chemical degradative methods, it is shown to consist essentially of a backbone of alternate →4)-β-D-Glc*p*A-(1→ and →2)-α-D-Man*p*-(1→ units. On the 4 position of the latter, β-D-Glc*p*A residues are linked. End units of α-L-Ara*f*, β-D-Xyl*p*, α-L-Rha*p*, and α-L-3-OMe-Rha*p* are linked to C-3 and/or C-4 positions of β-D-Glc*p*A residues.

## 1. Introduction

Recently, we have undertaken a systematic investigation of the polysaccharide fraction from mucilage of Cycads and our first report [1] described the structural features of the main acidic polysaccharide from the mucilage of *Encephalartos friderici guilielmi*. We now report data on the structure of the polysaccharide fraction from the mucilage of *Ceratozamia spinosa* species.

## 2. Experimental

*Origin and purification of the polysaccharide.*—A sample of mucilage (800 mg), collected from a specimen of *Ceratozamia spinosa* cultivated in the Botanical Garden of Naples, was pulverised and treated by stirring in water (700 mL) at room temperature. The dispersion obtained was centrifuged for 30 min at 10000

* Corresponding author.


*SSDI* 0008-6215(94)00076-R

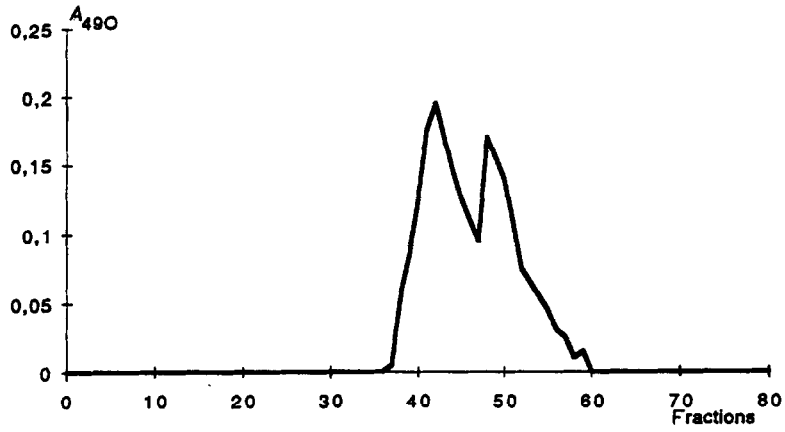



Fig. 1. Bio-Gel A 1.5m chromatography (50 mM NaOAc, pH 5.2; column, 1.0×110 cm; flow rate, 9 mL/h) of the polysaccharide fraction.

rpm and 20°C. The supernatant liquid was filtered and freeze-dried. The carbohydrate nature of the residue (600 mg) was tested by the phenol–$H_2SO_4$ assay [2].

Since it was shown to contain uronic acid by the *m*-hydroxybiphenyl test [3], it was chromatographed on Q-Sepharose eluting first at pH 7.5 with 1600 mL of 20 mM Tris–HCl and then with 1800 mL of a linear gradient up to 800 mM. Based on colorimetric tests for total sugars, the appropriate fractions were pooled to give only one fraction, which showed a negligible absorption at 280 nm.

The latter fraction was further chromatographed on Bio-Gel A 1.5m (50 mM NaOAc, pH 5.2) to give, on the basis of the colorimetric phenol test, two very close peaks (Fig. 1). As methanolysis of fractions taken across peaks gave a similar molar ratio of monosaccharides, the structural investigation was performed on the whole polysaccharide fraction ($[\alpha]_D - 30.1°$) without further purification. Apparent molecular weights of 675000 and 350000 were estimated for the two peaks by calibration of the above column with dextran standards.

*General.*—The $^1H$ and $^{13}C$ NMR spectra were obtained at 400 and 100 MHz, respectively, with a Bruker AM 400 spectrometer equipped with a dual probe, in the FT mode at 70°C, if not otherwise indicated; $^{13}C$ and $^1H$ chemical shifts were measured using 1,4-dioxane (67.4 ppm) and sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$, respectively, as internal standards. The DEPT experiment was performed using a polarisation transfer pulse of 135° and a delay adjusted to an average C,H coupling of 160 Hz. The standard Bruker software was used for the heteronuclear H,C COSY experiment (XHCORR) under the following conditions: the time domain in $f_2$ was 1K; 64 spectra were collected with 1280 scans; the spectral width was 8000 Hz in the $f_2$ domain and 1200 Hz in $f_1$; delays were optimised for $^1J_{C,H} = 160$ Hz. Fourier transformation was performed with a shifted sine-bell function in both dimensions. H,H COSY and NOESY experiments were performed with standard COSY-45 and NOESYPH pulse sequences under the

following conditions: the time domain in $f_1$ was 512w and in $f_2$ 1K; spectral width = 1200 Hz. For the NOESY experiment, the best results were obtained with a mixing time of 800 ms, the FID matrix was processed with a trapezoidal shifted function. Optical rotations were determined on a Perkin–Elmer 141 polarimeter. HPLC was performed with a Varian 5020 instrument, using a Waters R 401 differential refractometer as the detector. TLC was carried out on Silica Gel $F_{254}$ (Merck) and PC on Whatman paper. GLC was performed with a Carlo Erba instrument equipped with a flame-ionisation detector, and GLC–MS with a Hewlett–Packard 5890 instrument. Mass spectra were recorded with a VG ZAB HF instrument equipped with a FAB source.

Samples of each polysaccharide were hydrolysed with 2 M $CF_3CO_2H$ [4] according to the following procedure: the sample was kept at 120° C for up to 5 h and, each hour, the content of neutral sugars was analysed as alditol acetates; the molar ratios of the sugars were evaluated by using *myo*-inositol as internal standard. The maximum values for each monosaccharide, taken at the appropriate time, are reported in the Tables.

Methanolysis was performed and analysed as reported [1]. Carboxyl reduction of polysaccharides was carried out as described [1].

Samples of polysaccharide were methylated as described [1] and worked up according to ref 5. The crude reaction product was filtered on a $C_{18}$ SEP-PAK cartridge (Waters), previously washed with EtOH (20 mL), MeCN (2 mL), and water (10 mL). The fractions were eluted with water (50 mL), 4:1 water–MeCN (8 mL), MeCN (2 mL), and EtOH (6 mL). The last two fractions were pooled and evaporated to give the methylated polysaccharide, which was carboxyl-reduced with 1 M $LiEt_3BD$ in THF and, after desalting on Dowex 50W-X8 ($H^+$), hydrolysed with 2 M $CF_3CO_2H$. The partially methylated products in the hydrolysates were reduced with $NaBD_4$, acetylated, and analysed by GLC–MS and GLC [1].

*Autohydrolysis of the native polysaccharide.*—A solution of the polysaccharide (10 mg) in water was stored at 95°C for 20 h, then dialysed (Spectrapor 4 cellulose tube with a cut-off of 12 000–14 000 Da). Both the contents of the dialysis tube and the dialysate were freeze-dried. The residue from the inside contents (8 mg) was chromatographed on Bio-Gel A 1.5m, giving only one peak by the phenol test. This was submitted to $^{13}C$ NMR analysis. The residue from the dialysate (2 mg) was submitted to TLC (4:1 2-propanol–water) for monosaccharide analysis. Only Rha, Ara, and Xyl were detected.

*Isolation of the "core" by acid hydrolysis of the native polysaccharide fraction.*—The polysaccharide fraction (100 mg) was treated with 0.1 M $CF_3CO_2H$ (25 mL) at 110°C for 5 h. The $CF_3CO_2H$ was removed by evaporation with 2-propanol and the dry residue was chromatographed on Bio-Gel P2 (BioRad), using MilliQ-water as the eluent. Fractions were combined on the basis of TLC analysis (4:1 2-propanol–water), giving 3-OMe-Rha, Rha, Xyl, and Ara, in addition to a fraction (48 mg) eluted within the exclusion volume. The latter was chromatographed on Bio-Gel P60 (50 mM NaOAc, pH 5.2), to give a symmetrical peak (phenol test). The pooled fractions were dialysed and freeze-dried to give a residue (40 mg) which was submitted to $^1H$ NMR, $^{13}C$ NMR, and chemical analysis. An apparent

molecular weight of $2.1 \times 10^4$ for the "core" was estimated by calibration of the column with dextran standards.

*Isolation of oligosaccharides by acid hydrolysis of the "core".*—A sample of the "core" (10 mg) was treated with 2 M $CF_2CO_2H$ at 110°C for 80 min. After the usual work up, the residue was chromatographed on Bio-Gel P2, using water as the eluent. The eluted fractions were combined on the basis of TLC (4:1 2-propanol–water) into two main fractions.

The less polar fraction (3 mg) was a mixture of disaccharides. It showed five anomeric signals in the $^1H$ NMR spectrum at $\delta$ 5.37 (d, 1.95 Hz), 5.27 (d, 1.95 Hz), and 5.03 (d, 0.98 Hz), 4.97 (d, 0.98 Hz), which were assigned to the $\alpha$- and $\beta$-anomeric protons of two reducing mannose residues, in agreement with their disappearance after $NaBH_4$ reduction to the alditol, and at $\delta$ 4.59 (very strong doublet, 7.8 Hz). In the $^1H$ NMR spectrum of the reduced fraction, only two anomeric signals at 4.67 and 4.62 ppm appeared as doublets with a large coupling constant (7.8 Hz), in accordance with $\beta$-D-glucopyranosyluronic units [6]. These data suggested that the fraction was a mixture of two disaccharides, each one composed of reducing mannose glycosylated by a $\beta$-D-glucopyranosyluronic unit.

The $^{13}C$ NMR spectrum (30°C) of the unreduced fraction showed anomeric signals at 102.8 and 102.1 ppm for the $\beta$-D-glucopyranosyluronic units, and at 95.7, 94.2, and 92.6 ppm, assignable to the reducing mannose residues. In particular, the latter chemical shift indicated a mannose glycosylated at the 2 position [7]. Therefore, the mixture was composed of two disaccharides of mannose glycosylated by $\beta$-Glc*p*A at O-2 and, on the basis of methylation analysis of the native polysaccharide fraction, at O-4. This was confirmed by GLC–MS analysis of the methylated alditols, which revealed 4- and 2-linked mannitol in addition to the terminal uronic units.

The other fraction appeared as a mixture by descending paper chromatography (4:1:5 butanol–acetic acid–water). A pure oligosaccharide (2 mg) was isolated by chromatography on 3 MM Whatman paper in the above conditions. The $^{13}C$ NMR (30°C) spectrum showed three anomeric signals, two at 102.7 and 101.7 ppm, whose intensities appeared almost identical, and a third at 92.1 ppm, assignable to the C-1 of a reducing mannose glycosylated at O-2 [7]. The $^1H$ NMR spectrum showed three anomeric signals at $\delta$ 5.32 (d, 1.95 Hz), 4.97 (d, 0.98 Hz), and 4.58 (d, 7.8 Hz) ppm, assignable to the $\alpha$- and $\beta$-anomeric protons of a reducing mannose and to H-1 of a $\beta$-D-glucopyranosyluronic unit, respectively. The other anomeric signals, buried in the HOD signal at 70°C, appeared at 4.51 (d, 7.8 Hz) ppm in the $^1H$ NMR spectrum at 30°C. All these data suggested a trisaccharide structure, as confirmed by the positive-ion FABMS spectrum of its permethylated (1-$^2H$)alditol derivative, which showed a pseudomolecular peak $MH^+$ at *m/z* 704 and, as fragmentation, only a very strong peak at *m/z* 472, in agreement with the loss of a glucopyranosyluronic unit (232 Da). Furthermore, GLC–MS analysis of the methylated alditol acetates revealed only 2,4-linked mannopyranosyl and terminal uronic units. Therefore, the structure 2-*O*-($\beta$-D-glucopyranosyluronic acid)-[4-*O*-($\beta$-D-glucopyranosyluronic acid)]-D-mannose was assigned to the trisaccharide.

*Smith degradation of the native polysaccharide fraction.*—A sample of the native

Table 1
Molar ratios of the monosaccharides obtained by hydrolysis and methanolysis of native and carboxyl-reduced polysaccharide fractions

| Monosaccharide | Native | | Carboxyl-reduced Hydrolysis |
|---|---|---|---|
| | Hydrolysis | Methanolysis | |
| 3-OMe-L-Rha | 1.0 | 1.0 | 1.0 |
| L-Ara | 2.0 | 2.0 | 1.9 |
| L-Rha | 4.9 | 4.8 | 4.6 |
| D-Xyl | 6.8 | 6.2 | 6.3 |
| D-Man | 4.8 | 7.0 | 5.6 |
| D-GlcA (D-Glc) | | 13.0 | (9.6) |

polysaccharide (50 mg) was subjected to Smith degradation [8]. The crude $CF_3CO_2H$ hydrolysate was dialysed against water, using a Spectrapor 6 cellulose tube with a cut-off of 1000 Da. The inside material was freeze-dried (11 mg) and chromatographed on Bio-Gel P60 (50 mM NaOAc, pH 5.2). The chromatographic profile, by phenol assay, showed two peaks; the later one (8 mg) appeared homogeneous when rechromatographed on the same column.

*Determination of the absolute configuration of the monosaccharides.*—The mixture of monosaccharides (Ara, 3-OMe-Rha, Rha, Xyl) liberated together with the "core" by partial hydrolysis was separated by chromatography on Bio-Gel P2 and by HPLC on a column (30 cm × 7.8 mm i.d.) of Aminex HPX-87H (BioRad), eluting with 3 mM $H_2SO_4$. Mannose and glucuronic acid were obtained by total acid hydrolysis of the "core" and separated by HPLC as above.

The configuration of each monosaccharide was established by polarimetry.

## 3. Results and discussion

The qualitative and quantitative monosaccharide composition (Table 1) of the polysaccharide fraction of *Ceratozamia spinosa* was obtained by acid hydrolysis and methanolysis of the native polysaccharide and substantiated by acid hydrolysis of the carboxyl-reduced polysaccharide. The data reported in Table 1 showed incomplete acid hydrolysis for mannose and glucose, after 5 h, while methanolysis data appeared to be more quantitative [9].

Table 2
Molar ratios of methylated monosaccharides from the hydrolysis of the native polysaccharide fraction

| | | | |
|---|---|---|---|
| 2,3,5-Tri-OMe-Ara*f* | 1.0 | 2-OMe-Glc*p*A | 3.4 |
| 2,3,4-Tri-OMe-Rha*p* | 2.7 | 3,6-Di-OMe-Man*p* | 3.3 |
| 2,3,4-Tri-OMe-Xyl*p* | 3.3 | 6-OMe-Man*p* | 0.2 |
| 2,3-Di-OMe-Glc*p*A | 2.8 | | |

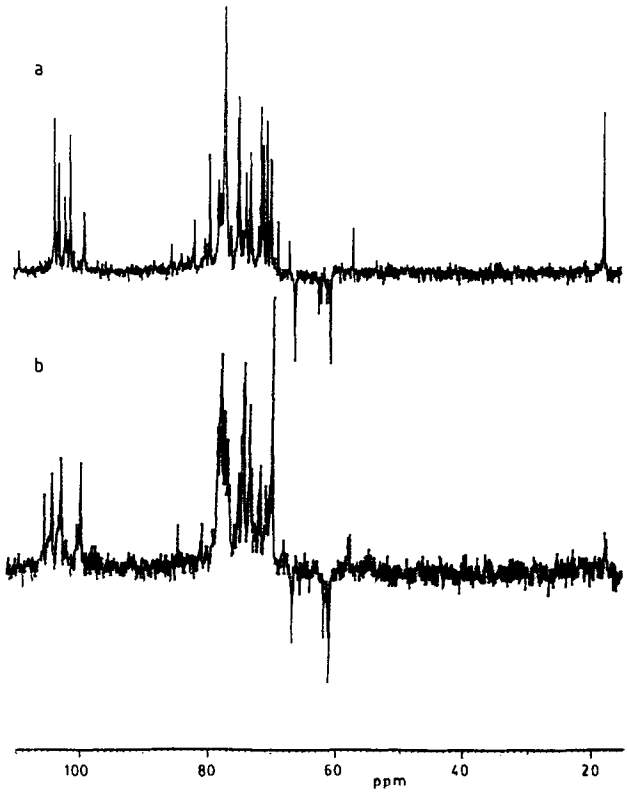



Fig. 2. (a) DEPT spectrum of the native polysaccharide; (b) DEPT spectrum of the autohydrolysis product, at 30°C.

Monosaccharide configurations, established by polarimetry, were L for Ara, Rha, and 3-OMe-Rha; and D for Xyl, Man, and GlcA.

The methylation data (Table 2) of the native polysaccharide indicated a highly branched structure where 2,4-substituted Man*p* and 3,4-substituted Glc*p*A are the main branching points. Trace of 2,3,4-linked Man*p* were found too. Ara*f*, Xyl*p*, Rha*p*, and 3-OMe-Rha*p* make up the nonreducing end units.

The $^{13}C$ NMR spectrum of the polysaccharide fraction confirmed the chemical degradative data. Actually, the presence of uronic acid, 6-deoxy sugar, and *O*-methyl sugar was inferred from carboxyl signals at $\delta$ 175.7 and 174.4, and from methyl signals at 17.6 and 56.9 ppm [10], respectively. In addition, the DEPT spectrum (Fig. 2a) clearly showed signals at 109.2, 85.2, and 81.7 ppm assignable to nonreducing terminal $\alpha$-arabinofuranosyl units [10], besides five other anomeric main signals at 103.5, 102.9, 101.6, 101.0, and 98.8 ppm, and methyleneoxy signals at 65.8 and 60.3 ppm assignable to C-5 of $\beta$-xylopyranose and C-6 of mannopyranose residues [10], respectively.

Table 3
Correlations among anomeric signals of the native polysaccharide fraction from 2D NMR, one-bond H,C-COSY

| Proton | Carbon | Assignment |
|---|---|---|
| 5.43 [a] | 109.2 [a] | Ara*f* |
| 5.39 br s | 98.8 | Man*p* |
| 4.83 (d, 7.6 Hz) | 103.5 | Xyl*p* |
| 4.78 br s | 101.0 | Rha*p* |
| 4.55 (d, 7.8) | 102.9 | Glc*p*A |
| 4.49 (d, 6.8) | 101.6 | Glc*p*A |

[a] This correlation was not observed in the 2D H,C-COSY.

The $^{1}H$ NMR spectrum displayed six anomeric signals at 5.43 br s, 5.39 br s, 4.83 (d, 7.6 Hz), 4.78 br s, 4.55 (d, 7.8 Hz), and 4.49 (d, 6.8 Hz) ppm in addition to a methyl doublet at 1.27 (6.6 Hz) ppm. A 2D NMR one-bond H,C COSY experiment allowed us to establish the correlations (Table 3) among the anomeric signals. The assignments of the signals in Table 3 were inferred from $^{1}H$ and $^{13}C$ NMR data of the native polysaccharide, and the autohydrolysis and "core" products (see below).

The $^{13}C$ NMR spectrum of the homogeneous contents of the dialysis tube of the autohydrolysis product showed (Fig. 2b), when compared to that of the native polysaccharide fraction, the absence of all signals of α-L-arabinofuranosyl units and a strong decrease in the relevant signals at 103.5, 101.0, 65.8, and 17.6 ppm, indicating the loss of most of the rhamnopyranose and of part of the xylopyranose during the autohydrolysis. This was confirmed by the presence of arabinose, xylose, and rhamnose in the dialysate. These results led us to assign the signals at 103.5 and 101.0 ppm to xylopyranose and rhamnopyranose, respectively.

In addition, the enhancement of the low-field signal at 83.1 ppm suggested glycosylation at O-3 of a glucopyranosyluronic unit, on the grounds of the glycosylation shift [11], in particular by xylopyranosyl and/or 3-OMe-rhamnopyranosyl units, whose signals still appeared in the spectrum.

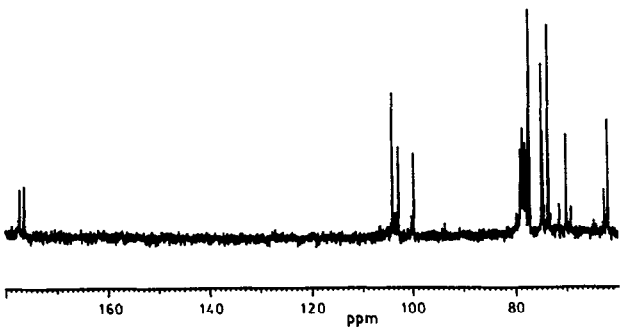



Fig. 3. $^{13}C$ NMR spectrum of the "core" at 85°C, pH 7.0.

Table 4
Molar ratios of methylated monosaccharides from the methylated native "core" and carboxyl-reduced "core"

| Monosaccharide | Native | Carboxyl-reduced |
|---|---|---|
| 2,3,4-Tri-OMe-Glc*p*A | 1.0 | |
| 2,3,4,6-Tetra-OMe-Glc*p* | | 1.2 |
| 2,3-Di-OMe-Glc*p*A | 1.2 | |
| 2,3,6-Tri-OMe-Glc*p* | | 1.1 |
| 3,6-Di-OMe-Man*p* | 1.0 | 1.0 |

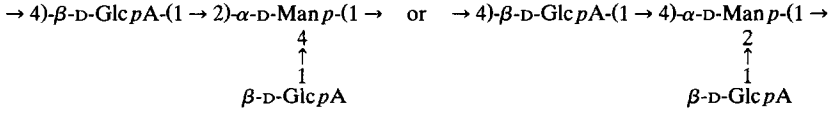
The $^{13}C$ NMR spectrum of the "core" (Fig. 3) was diagnostic for the backbone of the polysaccharide fraction. It showed three anomeric signals at 103.5, 102.2, and 99.2 ppm, and two carboxyl signals at 176.3 and 175.6 ppm, in accordance with acid hydrolysis data, which indicated a 1:2 mannose–glucuronic acid ratio.

A coupled DEPT experiment of the "core" led us to read a $^1J_{C,H}$ of 164 Hz for both signals at 102.7 and 101.3 ppm and of 174 Hz for that at 98.7 ppm, indicating a $\beta$- and $\alpha$-anomeric configuration [12], respectively. In agreement with these data was the $^1H$ NMR spectrum which showed three anomeric signals at $\delta$ 5.35 (br s) and two doublets at 4.47 and 4.41 ppm, each with a $^3J_{H,H}$ of 7.8 Hz.

The assignment of the carbon and proton anomeric signals allowed us to establish the anomeric configurations $\alpha$ for Ara*f* and Rha*p*, and $\beta$ for Xyl*p*, Man*p*, and Glc*p*A, on the basis of the $^{13}C$ chemical shift [10] and the $^3J_{H,H}$ and $^1J_{C,H}$ [12] values. The configuration of 3-OMe-Rha*p*, whose $^1H$ and $^{13}C$ anomeric signals were not identified (maybe because of very low intensity), was assumed to be $\alpha$ since this is, to our knowledge, the only anomeric configuration found so far in plant polysaccharides [13].

Further structural details on the "core" structure were obtained from methylation analysis of both the native and carboxyl-reduced "core" (Table 4).

These data, in addition to the finding of 2-*O*-($\beta$-D-glucopyranosyluronic acid)-D-mannose, 4-*O*-($\beta$-D-glucopyranosyluronic acid)-D-mannose, and 2-*O*-($\beta$-D-glucopyranosyluronic acid)-[4-*O*-($\beta$-D-glucopyranosyluronic acid)]-D-mannose oligosaccharides in the mild acid hydrolysate, suggested the following two possible repeating substructures for the backbone of the polysaccharide fraction:




**1** **2**

In order to choose between **1** and **2**, a NOESY experiment was performed after the assignment of "core" protons by H,H-COSY and one-bond H,C-COSY experiments. These 2D NMR experiments were carried out at 85°C, to improve the $^1H$ resolution, and at pH 7.

The comparison of $^{13}C$ chemical shifts of the "core" with those of the corresponding methyl glycosides, taken as reference substances (Table 5), showed the low-field shifts of carbon 4I, besides those of 2M and 4M, indicating I as the uronic

Table 5
H,H-COSY, one-bond H,C-COSY, and NOESY data for the native "core" at 85°C and pH 7 (low-field glycosylation shift)

| Assignment | Carbon | Proton | $^1$H NOE | $^{13}$C references [a] |
|---|---|---|---|---|
| 1E [b] | 103.5 | 4.50 d (7.8 Hz) | 4M; 3M; E5; E3; E2 | 104.3 |
| 2E | 74.2 | 3.38 | | 73.8 |
| 3E | 76.8 | 3.54 | | 76.5 |
| 4E | 73.0 | 3.52 | | 72.4 |
| 5E | 76.6 | 3.73 | | 75.5 |
| 6E | 177.2 [c] | | | |
| 1I [d] | 102.2 | 4.46 d (7.8 Hz) | 2M; 1M; 4I; 3I | 104.3 |
| 2I | 73.8 | 3.40 | | 73.8 |
| 3I | 77.7 | 3.65 | | 76.5 |
| 4I | 78.3 (5.9) | 3.75 | | 72.4 |
| 5I | 76.6 | 3.73 | | 75.5 |
| 6I | 176.4 [c] | | | |
| 1M [e] | 99.2 | 5.40 br s | 2M; 1I; 4I | 101.9 |
| 2M | 77.9 (6.7) | 4.20 | | 71.2 |
| 3M | 69.4 | 3.95 | | 71.8 |
| 4M | 77.4 (9.4) | 3.90 | | 68.0 |
| 5M | 72.8 | 3.83 | | 73.7 |
| 6M | 61.2 | 3.85 | | 61.4 |

[a] Data for methyl α-D-mannopyranoside and methyl β-D-glucopyranosiduronic acid from ref 10. [b] End uronic acid unit. [c] Interchangeable values. [d] Inner uronic acid residue. [e] Mannose residue.

inner unit, i.e., the 4-substituted one. Owing to the spatial proximity between the anomeric proton of the glycosyl residue and the proton on the carbon atom to which it is linked, NOE relaxation is expected and may be used to identify the atoms involved in an interglycosidic linkage. In this respect, the NOE contact established (Table 5) between H-1 of I and H-2 of M and that between H-1 of E and H-4 of M, in addition to that between H-1 of M and H-4 of I, were in agreement with the "core" structure **1**. Further NOE contacts were measured both for internal protons and as a consequence of spin diffusion effects.

On the skeleton **1**, the nonreducing end units of Ara*f*, Rha*p*, Xyl*p*, and 3-OMe-Rha*p*, in accordance with methylation data of the native polysaccharide

Table 6
Molar ratios of methylated monosaccharides from methylation of the Smith-degraded native polysaccharide

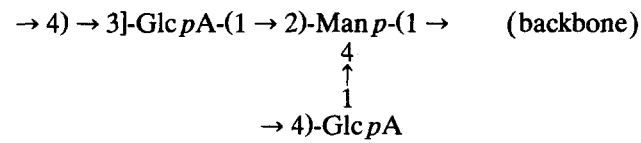
| | | | |
|---|---|---|---|
| 2,3,4-Tri-OMe-Rha*p* | 1.0 | 6-OMe-Man*p* | 0.8 |
| 3,4,6-Tri-OMe-Man*p* | 2.2 | 2,3,4-Tri-OMe-Glc*p*A | 3.3. |
| 2,3,6-Tri-OMe-Man*p* | 1.5 | 2-OMe-Glc*p*A | 1.1 |
| 3,6-Di-OMe-Man*p* | 1.6 | 2,3-Di-OMe-Glc*p*A | 2.3 |

fraction, should be linked at 3 and 4 positions of Glc*p*A units. To this purpose, we submitted the native polysaccharide fraction to Smith degradation followed by permethylation and acid hydrolysis of the degraded polysaccharide (Table 6).

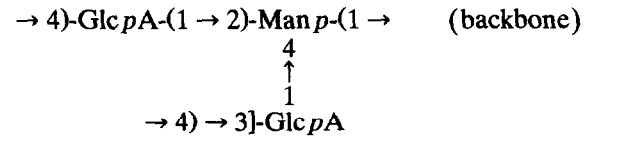
The rhamnose found in the crude reaction product must be the periodate-resistant 3-OMe-Rha*p*, and it is probably linked to the 3 position of the inner Glc*p*A, being in a 1:1 ratio with 3,4-substituted Glc*p*A, in agreement with the spectral data for the autohydrolysis product. Furthermore, the following structural details can be inferred from the Smith-degradation data.

(*a*) Terminal Glc*p*A (3.3) indicated the presence in the stubs of: →4)→3]-Glc*p*A-(1→

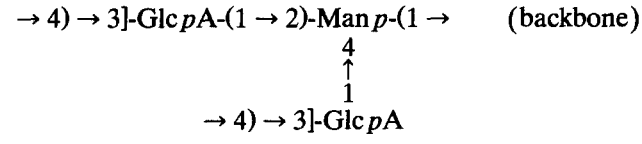
(*b*) 2-Linked Man*p* (2.2) indicated:



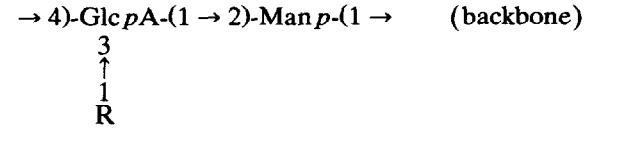

(*c*) 4-Linked Man*p* (1.5) indicated:




(*d*) 2,4-Linked Man*p* (1.6) indicated:



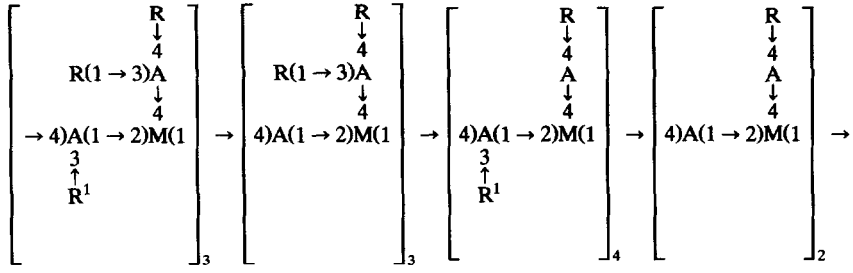

(*e*) 4-Linked Glc*p*A (2.3) indicated:




R = Xyl*p*, Rha*p*, Ara*f* (no 3-OMe-Rha*p*)

The presence of mannose residues carrying periodate-resistant substituents (0.8) was also inferred, but their frequency should be low as indicated by the methylation data of the native polysaccharide.

The foregoing structural features can be summarised in the following average structural fragment, irrespective of the sequence of branches along the backbone:



$R^1$ = Rha*p*, Ara*f*, 3-OMe-Rha*p*; R = Rha*p*, Ara*f*, Xyl*p*

The repeating fragment 4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → of the backbone of this structure, first discovered in the gum exudates of *Anogeissus* spp. (Combretaceae; Myrtales) [13], is common to other gums [14] and has recently been found in another Cycad species [15,16]. Therefore, it is possible to suggest a chemotaxonomic relationship between *Encephalartos* and *Ceratozamia*, two of the eleven known genera of Cycads [17]. However, for *Encephalartos*, a plant endemic to Africa, a mannose-free fraction has also been isolated [1] from the mucilage, while for *Ceratozamia*, a plant endemic to the American continent, this fraction, in our knowledge, does not occur.

## Acknowledgments

We thank Professor G.S. Gigliano (Botanical Garden of the University Federico II of Naples) for a kind gift of mucilage, the Centro di Metodologie Chimico-Fisiche of the University Federico II of Naples for the NMR spectra, and M.U.R.S.T. and C.N.R., Rome, for financial support.

## References

[1] M. Adinolfi, M.M. Corsaro, L. Mangoni, M. Parrilli, and E. Poerio, *Carbohydr. Res.*, 222 (1991) 215–221.
[2] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[3] M. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 28 (1973) 484–489.
[4] P. Albersheim, D.J. Nevins, P.D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
[5] W.S. York, A.G. Darvill, M. McNeil, T.T. Stevenson, and P. Albersheim, *Methods Enzymol.*, 118 (1986) 3–40.
[6] M. Mori and K. Kato, *Carbohydr. Res.*, 91 (1981) 49–58.
[7] A. Kardosova and J. Rosik, *Chem. Pap.*, 40 (1986) 89–94.
[8] J. Defaye and E. Wong, *Carbohydr. Res.*, 150 (1986) 221–231.
[9] G.A. De Ruiter, H.A. Schols, A.G.J. Voragen, and F.M. Rombouts, *Anal. Biochem.*, 207 (1992) 176–185.
[10] K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.

[11] K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, (1976) 179–182.
[12] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
[13] G.O. Aspinall in G.O. Aspinall (Ed.), *The Polysaccharides*, Vol. 2, Academic, New York, 1983, pp 97–193.
[14] S.C. Churms and A.M. Stephen, *Carbohydr. Res.*, 167 (1987) 239–255.
[15] D.C. Vogt and A.M. Stephen, *Carbohydr. Res.*, 238 (1993) 249–260.
[16] D.C. Vogt and A.M. Stephen, *Carbohydr. Res.*, 241 (1993) 217–226.
[17] D.W. Stevenson, *Mem. N. Y. Bot. Gard.*, 57 (1990) 8–55.