Carbohydrate Research, 233 (1992) 265–269
Elsevier Science Publishers B.V., Amsterdam

Note

A linear (1 → 5)-linked α-L-arabinofuranan from the seeds of guapuruvu (*Schizolobium parahybum*)

Selma F. Zawadzki-Baggio, Maria-Rita Sierakowski, João B.C. Corrêa [†] and Fany Reicher

Department of Biochemistry, PO Box 19046, Universidade Federal do Paraná, 81.531, Curitiba (Brazil)

(Received November 11th, 1991; accepted February 17th, 1992)

Seeds of many plants of the family Leguminosae have been studied extensively because of their galactomannans, which can be commercially important[1]. Other minor component polysaccharides, such as arabinans, have not received the same attention. They have been isolated from the cell walls of roots[2,3], seeds[4–6], pollen tubes[9], bark[10,11], and fruits[12] of various species, generally being associated with pectic material[13].

The few α-L-arabinofuranans so far analyzed are branched, with (1 → 5) links between the main chain residues, which are partially substituted at O-2 and/or O-3 with single or two-unit side chains. Although a linear (1 → 5)-linked α-L-arabinofuranan has been reported in processed apple juice, it may have been formed from a more complex polymer by the action of degradative enzymes[14].

We now describe the isolation and structural analysis of a linear arabinan from the seeds of *Schizolobium parahybum*, known as guapuruvu in Brazil.

EXPERIMENTAL

General methods.—Polysaccharides were hydrolyzed with M trifluoroacetic acid (4 h, 100°). Hydrolyzates were reduced with sodium borohydride, then acetylated in 1:1 pyridine–acetic anhydride (16 h, room temperature). The resulting alditol acetates were analyzed by GLC, with a model 2440 Varian chromatograph operating at 180°, on columns packed with 3% OV 225 or ECNSS (1.5 mm i.d. × 200 cm, Gas Chrom Q support). The carrier gas was nitrogen (40 mL/min). The methylated polysaccharides were hydrolyzed with aq 72% H_2SO_4 (1 h, 0–4°) and

Correspondence to: Professor F. Reicher, Department of Biochemistry, PO Box 19046, Universidade Federal do Paraná, 81.531, Curitiba, Brazil.

[†] Deceased January 9th, 1992.

thereafter water was added to a final acid concentration of 0.5 M (5 h, 100°). The solution was neutralized ($BaCO_3$) and the products converted into *O*-methyl alditol acetates that were analyzed by GLC–MS with a model 3980 Hewlett–Packard chromatograph equipped with an HP1 capillary column (0.2 mm i.d. × 30 m) linked to an HP 5988 A mass spectrometer unit (electron impact, 70 eV). Injections were carried out at 150° and the column was programmed to increase at 4°/min to 250°, then hold.

^{13}C NMR spectroscopy was performed with a Bruker AC-300 spectrometer at 75 MHz in the Fourier-transform mode, with complete proton decoupling at 30°, using 5-mm tubes and $(CD_3)_2SO$ as solvent. The spectral width was 200 ppm. Chemical shifts are expressed in ppm relative to the resonance of external DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate). Optical rotation was measured with a Perkin–Elmer model 141 polarimeter in 5% NaOH, *c* 0.3. Total carbohydrate was assayed by the phenol–H_2SO_4 method[15] and *O*-acetyl by the hydroxylamine method[16].

Polysaccharide source.—Seeds of *S. parahybum* were collected on the campus of the Universidade do Rio dos Sinos, São Leopoldo, Rio Grande do Sul.

Polysaccharide isolation.—For this purpose, two methods were used: (*A*) Whole seeds were crushed and extracted with 2:1 benzene—EtOH in a Soxhlet apparatus for 16 h, the residual mass being twice refluxed (4 h per cycle) with 4:1 MeOH–water[17,18]. The residue was successively extracted with water at 4, 25, and 50°, and with 2 M NaOH in the presence of $NaBH_4$ for 16 h at room temperature. Hemicellulose A was precipitated by acidification of the alkali extract to pH 5 with 50% acetic acid, and hemicellulose B was isolated from the supernatant by precipitation with excess EtOH[19]. (*B*) Whole seeds were boiled in water for 30 min and kept at room temperature until swelling took place, then endosperm, seed coat, and embryo were separated. The endosperm was submitted to extractions as described above. The seed coat was milled, treated with 2:1 benzene–EtOH in a Soxhlet, and submitted to aqueous extractions (fractions SC-I and SC-II). The residue was divided into two parts, one being treated with 2 M NaOH to yield hemicellulose A (fraction SC-III) and hemicellulose B (fraction SC-IV). The second part was extracted with Me_2SO at 36° for 7 days, yielding fraction SC-V.

Graded acid hydrolysis of SC-III.—Polysaccharide SC-III was hydrolyzed with 0.5 M H_2SO_4 at 100°, in sealed glass tubes; with M trifluoracetic acid (TFA); and with 0.01 M TFA. Reducing sugars were determined by the method of Somogyi and Nelson[20,21].

Gel filtration of polysaccharide fractions SC-III and SC-V.—A Sephacryl S-300 chromatographic column (1.1 × 46.7 cm) was calibrated with standard dextrans (molecular mass range 266, 72, 40, and 17 kDa; Sigma Chemicals), and the void volume was determined with Blue Dextran. Polysaccharide samples (0.5 mL; 2 mg/mL) were applied and eluted with 50 mM NaOH or 7 M urea, fractions (1 mL) being collected and carbohydrate absorbance (phenol–H_2SO_4) being monitored.

Smith degradation of SC-III.—A sample of SC-III (50 mg) was dissolved in 0.5 M NaOH (5 mL), and acetic acid was added to pH 7, followed by $NaIO_4$ to a final concentration of 50 mM. At the conclusion of the oxidation (7 days) the product was reduced with $NaBH_4$ and dialyzed. An aliquot was removed, and the remainder was immediately treated again with $NaIO_4$ and later reduced with $NaBH_4$. Samples of material subjected to one and two cycles of oxidation–reduction were hydrolyzed and reduced, and the products analyzed by GLC as alditol acetates.

Methylation analysis of SC-III and SC-V.—Arabinan fractions SC-III and SC-V were each methylated according to Ciucanu and Kerek[22]. The procedure was repeated twice and the per-*O*-methylated polysaccharides hydrolyzed and analyzed by GLC and GLC–MS of the derived partly *O*-methylated alditol acetates.

RESULTS AND DISCUSSION

Extractive-free seeds were subjected to aqueous extractions at progressively increasing temperatures. Most of the soluble polysaccharide (~ 20%) was liberated on cold aqueous extraction and found to be a galactomannan having a mannose to galactose ratio of 3.0:1. In hemicelluloses A and B arabinose and xylose were also present.

The isolated embryo, composed mainly of proteins[23], was discarded and the endosperm was submitted to the same extraction procedure as the whole seeds. Galactomannan was found as the main component of the aqueous extracts (~ 60%). Arabinose was also present in the alkali extract of the endosperm, indicating the presence of an arabinan.

The monosaccharide compositions of the seed-coat fractions are shown in Table I. The only monosaccharide component of SC-III and SC-V was arabinose. *O*-Acetyl groups, which would have been preserved in the Me_2SO extract[24], were not found. As the ^{13}C NMR, methylation, and gel-filtration data for the two fractions were identical, they were considered to be the same polymer.

TABLE I

Extraction of polysaccharide from the seed coat of *S. parahybum*

Fraction	Extractant	Yield (%) [a]	Monosaccharide composition [b] (mol %)			
			Ara	Xyl	Man	Gal
SC-I	Water, 4°	6.1	16	6	50	28
SC-II	Water, room temperature	3.3	40	4	34	22
SC-III	2 M NaOH, r.t.	1.1	100			
SC-IV	2 M NaOH, r.t.	2.1	21	44	14	20
SC-V	Me_2SO, 36°	0.8	100			

[a] Weight percent of the lipid-free seed coat. [b] Determined by GLC of the derived alditol acetates.

The arabinan was readily hydrolyzed with 0.5 M H_2SO_4 at 100°, as described for other arabinans[8,12]. However, in contrast with the findings in the cited cases, the liberated reducing sugar began to show signs of degradation after 30 min. The polysaccharide was also completely hydrolyzed in 1 h at 100° with 0.01 M TFA[25], and when 1 M TFA was used no reducing sugar degradation took place after 5 h. The high negative specific rotation ($[\alpha]_D^{20}$ −133°) of the arabinan, coupled with its rapid rate of hydrolysis with acid, suggested the presence of α-L-arabinofuranosyl units.

The arabinan was homogeneous on gel-column chromatography over Sephacryl S-300, showing a molecular mass of ~ 18000. Methylation analysis yielded 2,3-di-*O*-methyl- and 2,3,5-tri-*O*-methyl-arabinose in a molar ratio of 99:1, indicating a linear structure for the polysaccharide. Periodate oxidation followed by reduction and acid hydrolysis gave glycerol and arabinose in a molar ratio of 97:3. However, when the polyol was reoxidized with periodate and reduced, acid hydrolysis gave rise to glycerol only, as expected. Interunit hemiacetal formation must have taken place during the initial periodate treatments inhibiting further oxidation. The ^{13}C NMR spectrum of the arabinan (Fig. 1) consisted of 5 signals, at δ 107.9 (C-1), 81.5 and 81.7 (C-2, C-4), 77.4 (C-3), and 66.9 (C-5), corresponding to those of a linear (1 → 5)-linked α-arabinofuranan[3,11,14,26]. The lines for C-2 and C-4 are nearly superposed; by comparison with methyl α-L-arabinofuranoside[27] C-4 suffers an upfield β shift of 3 ppm due to substitution at C-5. Very small signals (< 1%) visible in an amplified spectrum at δ 61.3, 64.1, and 102.0 probably arise from C-4 and C-5 of nonreducing-end units and C-1 of the reducing ends, respectively.

Since the first step of arabinan extraction was enzyme inactivation, and also the mild method of Me_2SO extraction could be used to obtain the polysaccharide, probably the arabinan is a true component of the guapuruvu seed coat, and not a degradation product of a more complex material.

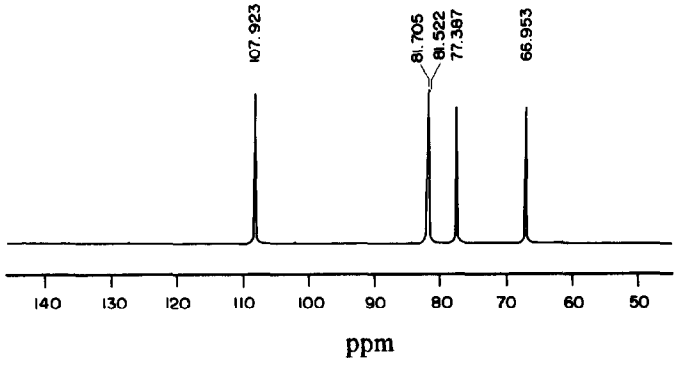

Fig. 1. ^{13}C NMR spectrum of the arabinan from the seeds of *S. parahybum*, 75 MHz, $(CD_3)_2SO$, 30°.

ACKNOWLEDGMENTS

The authors thank Dr. P.A.J. Gorin for helpful discussions and for the provision of 2,3-di-*O*- and 3,4-di-*O*-methylarabinose standards, Dr. J.L.M.S. Ganter and C. Bosso (CERMAV-Grenoble) for ^{13}C NMR spectra, Father C. Steffen for collecting the seeds, and CNPq and UFPR for financial support.

REFERENCES

1 I.C.M. Dea, in M. Yalpani (Ed.), *Industrial Polysaccharides*, Elsevier, Amsterdam, 1987, pp. 207–216.
2 J.-P. Joseleau, G. Chambat, and M. Lanvers, *Carbohydr. Res.*, 122 (1983) 107–113.
3 P. Capek, R. Toman, A. Kardošová, and J. Rosík, *Carbohydr. Res.*, 117 (1983) 133–140.
4 G. Muralikrishna and R.N. Tharanathan, *Food Chem.*, 22 (1986) 245–250.
5 O. Larm, O. Theander, and P. Aman, *Acta Chem. Scand., Ser. B*, 29 (1975) 1011–1014.
6 I.R. Siddiqui and P.J. Wood, *Carbohydr. Res.*, 36 (1974) 35–44.
7 R.N. Tharanathan, U.R. Bhat, G.M. Krishna, and S.V. Paramahans, *Phytochemistry*, 24 (1985) 2722–2723.
8 S. Ukai, C. Hara, T. Kiho, and K. Hirose, *Chem. Pharm. Bull.*, 26 (1978) 1729–1736.
9 A.L. Rae, P.J. Harris, A. Bacic, and A.E. Clarke, *Planta*, 166 (1985) 128–133.
10 Š. Karácsonyi, R. Toman, F. Janeček, and M. Kubačková, *Carbohydr. Res.*, 44 (1975) 285–290.
11 J.P. Joseleau, G. Chambat, M. Vignon, and F. Barnoud, *Carbohydr. Res.*, 58 (1977) 165–175.
12 M. Tomoda, M. Takahashi, and S. Nakatsuka, *Chem. Pharm. Bull*, 21(4) (1973) 707–711.
13 B.J.H. Stevens and R.R. Selvendran, *Phytochemistry*, 19 (1980) 559–561.
14 S.C. Churms, E.H. Merrifield, A.M. Stephen, D.R. Walwyn, A. Polson, K.J. van der Merwe, H.S.C. Spies, and N. Costa, *Carbohydr. Res.*, 113 (1983) 339–344.
15 M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
16 F. Downs and W. Pigman, *Methods Carbohydr. Chem.*, 7 (1967) 241–243.
17 J. Ganter, J. Corrêa, F. Reicher, A. Heyraud, and M. Rinaudo, *Plant Physiol. Biochem. (Paris)*, 29 (1991) 139–146.
18 F. Reicher, S.C.S. Leitner, M.R. Sierakowski, J.D. Fontana, and J.B.C. Corrêa, *Appl. Biochem. Biotechnol.*, 28/29 (1991) 353–361.
19 R.L. Whistler and M.S. Feather, *Methods Carbohydr. Chem.*, 5 (1965) 144–145; 353–361.
20 M.A. Somogyi, *J. Biol. Chem.*, 160 (1945) 61–68.
21 N.A. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
22 I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
23 M.N. Mazzini and A.S. Cerezo, *J. Sci. Food Agric.*, 30 (1979) 881–891.
24 F. Reicher, P.A.J. Gorin, M.R. Sierakowski, and J.B.C. Corrêa, *Carbohydr. Res.*, 193 (1989) 23–31.
25 M.R. Sierakowski, P.A.J. Gorin, F. Reicher, and J.B.C. Corrêa, *Phytochemistry*, 26 (1987) 1709–1713.
26 N.R. Swamy and P.V. Salimath, *Phytochemistry*, 30 (1991) 263–265.
27 P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.