Available online at www.sciencedirect.com
SCIENCE DIRECT®
CARBOHYDRATE RESEARCH
ELSEVIER
Carbohydrate Research 338 (2003) 1609–1612
www.elsevier.com/locate/carres

Note

An alkali-soluble heteroxylan from seeds of *Phoenix dactylifera* L.

Omar Ishurd,[a] Yousef Ali,[b] Wanxing Wei,[a] Fatma Bashir,[b] Amna Ali,[b] Ahmed Ashour,[c] Yuanjiang Pan[a,*]

[a] *Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China*
[b] *IRC Libyan. Organization., Tripoli, Libya*
[c] *Department of Food Science, College of Agriculture, Alftateh University, Tripoli, Libya*

Received 10 December 2002; accepted 18 April 2003

Abstract

Alkali-soluble polysaccharides, isolated from the seeds of dates, have been investigated using methylation and partial hydrolysis studies. The polysaccharides are shown to contain D-xylose and 4-*O*-methyl-D-glucuronic acid in a molar ratio of 5:1. An aldobiouronic acid from hemicellulose was characterized, and investigation revealed that the hemicellulose consists of a polymer of (1→4)-linked D-xylopranosyl residues having branches of D-xylopyranosyl and 4-*O*-methyl-α-D-glucopyranosyluronic acid.

Keywords: Alkali-soluble polysaccharide; Date seed; *Phoenix dactylifera* L.

The date palm, (*Phoenix dactylifera* L.), is the major fruit tree in most Arabian countries. During the pre-oil period, the date palm was the main source of food, together with camel's milk and fish.[1] Dates are an economically important crop for many populations in North Africa, where the date palm also has an environmental impact in a desert climate.[2] Flesh of the dates has always played an essential role in the diet of the local inhabitants of the Arab countries, leaving a large quantity of seeds as a waste product of dates.[3] Much work has been done on the composition of dates and influence of the stage of maturity.[4–7] However, in spite of this extensive exploitation of the date, little information has been published about the alkali-soluble polysaccharides from dates, and these reports indicate only the general structural features.[8,9] In our previous papers, we reported gluco- and galactomannan from seeds of dates.[10,11] In this paper, we wish to report the results of investigations conducted on one of the hemicellulose fractions isolated from fibers of the seeds of dry dates, variety 'Aple'.

The extractive-free fibers contained 9.6% lignin, which was removed by two successive treatments of the material with sodium chlorite. The product was successively extracted with hot water, followed by 4 and 9% NaOH solutions. The three hemicellulose fractions and residual, cellulose-rich material were obtained in yields of 0.5, 20.4, 11.5 and 68%, respectively, on basis of the dry weight of the holocellulose. The results of the analysis of these fractions are shown in Table 1.

Fraction II, containing xylose, 4-*O*-methylglucuronic acid, arabinose, and traces of galactose, glucose, and mannose, were studied in detail. It was purified through formation of its copper complex.[12] On hydrolysis, the purified material yielded xylose as the only neutral sugar, and 4-*O*-methylglucuronic acid and an aldobiouronic acid as the acidic sugars. The uronic acid in the purified hemicellulose was reduced completely, and on hydrolysis, the product yielded xylose and 4-*O*-methylglucose in a molar ratio of 5:1. These two products were isolated from a large batch of reduced, hemicellulose hydrolyzate, and study of their specific rotation (+20° and +59° in water, respectively) indicated that both had the D configuration.

Partial hydrolysis of the pure hemicellulose with 0.2 M sulphuric acid for 11 h at 100 °C yielded several oligosaccharides from which an acidic oligosaccharide ($[\alpha]_D^{23}$ +110° and equivalent weight 332) yielded three products that on paper chromatography had the same

* Corresponding author. Tel./fax: +86-571-87951264.
E-mail address: panyuanjiang01@yahoo.com (Y. Pan).

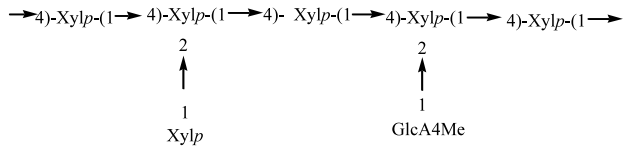
0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(03)00202-7

Table 1
Result of preliminary analysis of different fractions of hemicelluloses from seed of dates

Fraction	Yield [a] (g)	$[\alpha]_D^{23}$ in NaOH	Approximate mol% [b]					
			Xyl	Uronic acid	Ara	Man	Gal	Glc
1	0.23	−20	58.1	10.8	5.2	7.3	8.4	10.2
2	9.18	−64	78.4	15.4	4.2	trace	trace	trace
3	5.12	−61	72.2	12.1	4.4	2.8	2.8	5.0
Purified fraction								
2		−72	82.0	16.8	trace			

[a] On dry weight basis of the hemicelluloses.
[b] The carboxyl-reduced hemicelluloses were analyzed, after hydrolysis, by GLC.

mobility as those of the unreacted oligosaccharide, 4-*O*-methylglucuronic acid, and xylose. The values of the equivalent weight and specific rotation indicated that the oligosaccharide might be 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. To check this out, the aldobiouronic acid was converted in to its methyl ester methyl glycoside, and this derivative was reduced with lithium aluminum hydride in oxolane. This carboxyl-reduced aldobiouronic acid and the original aldobiouronic acid were then methylated by Kuhn's procedure. The methylated aldobiouronic acid was reduced with lithium aluminum hydride. These two products were then separately hydrolyzed, and the hydrolyzates were both studied by GLC and by isolating the products by resolution on paper chromatography. The results, shown in Table 2, together with those discussed earlier, proved that the oligosaccharide was indeed 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. The pure hemicellulose was now methylated (that it was fully methylated was shown by the absence of OH bands in the IR spectrum). The product was first hydrolyzed with 85% formic acid, and after removing the formic acid, with 0.5 M sulphuric acid. This acid was then neutralized with $BaCO_3$. Part of the hydrolyzate was converted into alditol acetates, and the mixture was analyzed by GLC. The rest of the hydrolyzate was sequentially passed through Dowex-50W X-8 (H^+) and through Dowex 1-X4 (HCO_3^-) resins. The eluate and washings were combined and evaporated to a syrup. The mixture was resolved on paper chromatography, and the three methylated sugars were characterized (see Table 3). The acidic fraction was eluted from the column of Dowex-1 X-4 with 0.5 M sulphuric acid, and the eluate was made neutral and decationized, and the product was converted into the methyl ester methyl glycoside, which was then reduced with lithium aluminum hydride. The product was hydrolyzed, and the individual sugars were characterized after isolation from paper chromatography. The results are given in Table 3. It was possible to now assign the structure of the average repeating unit of the fiber hemicellulose as **1**.



1

That is, it proved to be a polymer of (1→4)-linked D-xylopyranosyl residues having branches at O-2 consisting of 4-*O*-methyl-α-D-glucopyranosyluronic acid and D-xylopyranosyl groups. For every five D-xylopyranosyl residues in the main chain, there is one uronic acid group, and for approx 22 such D-xylopyranosyl residues, there is one D-xylopyranosyl group. The $[\alpha]_D$ value of the polysaccharide also indicated the presence of β

Table 2
Methyl ethers of sugars from (A) the hydrolyzate of reduced, methylated aldobiouronic acid, and (B) the hydrolyzate methylated, reduced aldobiouronic acid

Sugars [a]	T [b]	Mol%		Properties of isolated sugars		
		A	B	$[\alpha]_D^{23}$ in water	Derivative	Mp of derivatives
2,3,4,6-Glc	1.00	53		+82	anilide	135
2,3,4-Glc	2.23		51	+68.4	anilide	144
3,4-Xyl	1.2	47	49	+19	anilide	116

[a] 2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, etc. Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucose as unity, in column (b).

Table 3
Methyl, ethers of sugars from the hydrolyzate of methylated hemicellulose for seed of dates

Sugars	T^b	Approximate mol%	Properties of isolated sugars		
			$[\alpha]_D^{23}$ in water	Derivative	Mp of derivatives
2,3,4-Xyl	0.55	4	+20	anilide	96
2,3-Xyl	1.20	74	+25	anilide	121
3-Xyl	2.17	3	+15.5	anilide	135
Me-Aldo acid		19	+145		
2,3,4-Glc	2.23		+68	anilide	145
3-Xyl	2.17		+14.5	anilide	133

2,3,4-Xyl = 2,3,4-tri-*O*-methyl-D-xylose, etc. Me-aldobiouronic acid = 3-*O*-methyl-2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

linkages.[12] The anomeric proton singlet at δ 5.17 (d, J 7.4 Hz) in the ^{1}H NMR spectrum and δ 111.7 for C-1 in the ^{13}C NMR spectrum confirm that the sugar residues are linked β-glycosidically, which agrees with presence of an IR band 890 cm^{-1}.[12–14] The ^{13}C NMR spectrum of the hemicellulose showed several resonances corresponding to polysaccharide unit carbon: δ 111.7 for C-1; δ 83.5 for C-2, C-3 and C-5; δ 73.5 for C-6. The ^{13}C NMR measurements indicate that the monomeric side chains of the L-arabinofuranosyl residues are linked to C-3 of the 4-*O*-methyl-D-glucopyranosyluronic acid groups, which are attached to C-2 of D-xylopyranosyl units. From the results of both chemical analyses and ^{13}C NMR measurements, the hemicellulose extracted from the seed of dates was shown to be essentially a (1→4)-linked β-D-xylan with a 4-*O*-methyl-α-D-glucopyranosyluronic acid group attached at C-2.

1. Experimental

1.1. General

Solvents were evaporated under diminished pressure at 50 °C (bath). Optical rotations were determined with a Perkin–Elmer 141 polarimeter. Ascending PC was performed on Whatman No.1 and 3 MM papers, using A, 8:2:1 EtOAc–Py–water; B, 2-butanol–water–azeotrop; C, 9:2:2 EtOAc–AcOH–water; and D, 40:11:19 1-butanol–EtOH–water. Sugars were detected by alkaline silver nitrate and aniline oxalate. Thin-layer chromatography (TLC) was performed on Silica Gel 60 (E. Merck), using solvents B and C, and detection was with anisaldehyde sulphuric acid and 2% aq sulphosalicylic acid. IR spectra were recorded with a Beckman Acculab 10 instrument using a mixture KBr. NMR spectra were recorded on a Bruker 500 instrument. For ^{1}H NMR spectroscopy at 70 °C, the sample (10 mg) was repeatedly dissolved in D_2O (5 × 5 mL), and the solution was lyophilised. The final, freeze-dried sample was dissolved in 1 mL of 99.99% D_2O. For ^{13}C NMR spectroscopy at 50 °C, the sample was dissolved in D_2O. GLC was conducted on Hewlett–Packard Model 419 and a Hewlett–Packard Model 5713 gas chromatographs, each equipped with flame-ionization detector. Resolutions were performed on glass columns (1.83 m × 6 mm) containing: (a) 1.3% of ECNSS-M on Gas Chrom Q (100–200 mesh); and 2.3% of OV-225 on GC-Q (100–200 mesh) at 190 °C for alditiol acetate sugars; and (b) 1% of OV-225 on Gas Chrom Q (80–100 mesh) at 170 °C for alditiol acetates of partially methylated sugars.

1.2. Plant material

Dry dates (2 kg) of the Aple variety were used in this study. Fruits uniform in shape, size, and color were obtained from South Libya at weekly intervals from July 15 to August 15, 2000. Seeds of dry dates were ground after removing the flesh and calyx. The maturity of the date fruits selected for this study is in Tamar stage (full ripeness), as it contains a larger amount of polysaccharides than fruit from the green and yellow stages.[15]

1.3. Isolation and fractionation of the polysaccharide

Date seeds (500 g) were fed through a mill, which was not fitted with a screen. After several repetitions of this process practically all the seeds were broken in to several pieces. The split seeds (250 g) were extracted with 1:2 (v/v) ethanol–benzene and dried. The extractive-free material (50 g) was delignified by treatment with aq NaCl (0.7%) in 0.1 M AcONa–AcOH buffer, pH 4.0 (1:150 fiber–liquor) for 5 h at 75 °C, and the fibrous material was recovered by filtration, and dried. The whole process was then repeated once. The resulting holocellulose (45 g) was successively extracted with hot water for 4 h, at 100 °C, then with 4 and 9% NaOH in each case for 6 h at room temperature (rt) under an N_2 atmosphere. The alkaline solutions were made neutral with AcOH, and the hemicellulose in each fraction was

isolated by precipitation with EtOH and subsequent centrifugation. Fraction II (9.2 g), which was obtained in relatively large amounts, was used for future study. This fraction was purified through its copper complex by treatment with Fehling solution, dissociation of the complex with acid, and isolation by repeated precipitation with EtOH (yield 7.1 g)

1.4. Hydrolysis

For isolation of the aldobiouronic acid, the hemicellulose was partially hydrolyzed with 0.2 M sulfuric acid for 6 h at 100 °C. Oligosaccharides were hydrolyzed with M sulphuric acid for 6 h. The methylated polysaccharide was hydrolyzed, first by heating with 85% formic acid for 2 h at 100 °C. The formic acid was then removed by co-evaporation with water and second hydrolyzing with 0.5 M sulphuric acid was carried out for 16 h at 100 °C.

1.5. Methylation analysis

The pure hemicellulose (3 g) was first acetylated with Py and Ac_2O in formamide, with stirring at rt. The product (2.95 g) was dissolved in oxolane (60 mL), and then methylated with dimethyl sulphate (50 mL) and NaOH (50 g) according to the procedures of Hamilton and Kircher[16] and Carson and Maclay.[17] The product was further methylated by the Purdie[18] method, a fully methylated product showing no OH absorption band in the IR spectrum; yield 2.1 g, $[\alpha]_D^{23}$ −61° (*c* 1, $CHCl_3$). The brownish, glassy material (2 g) was dissolved in $CHCl_3$ (25 mL) and fractionated by gradual addition of petroleum ether giving three fractions (0.31, 4.1 and 0.2 g). The second fraction was used for subsequent analysis. This methylated hemicellulose was hydrolyzed, first with 85% formic acid for 2 h at 100 °C, and then with 0.5 M sulphuric acid for 16 h at 100 °C. Neutral, partially methylated sugars were analyzed as their alditol acetates by GLC using column (b). For identification of the different methylated sugars, these were resolved on paper using solvent C, and then by preparing suitable derivatives as described earlier.

1.6. Preparation of carboxyl-reduced hemicelluloses

1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulphonate (1 g) was added to stirred dispersion of the hemicellulose (50 mg) in water (50 mL), and the pH of the solution was maintained at pH 4.75 by dropwise addition of 0.01 M HCl. After 2 h, 2 M aq $NaBH_4$ (80 mL) was added dropwise during 45 min, with the pH being kept at pH 7.0 by concurrent addition of 4 M HCl. After stirring for 1 h, the solution was dialyzed against distilled water for 24 h, and the dialyzate was then lyophilized. The whole process was repeated once.

Acknowledgements

One of us, Dr O. Ishurd (Libya), gratefully acknowledges the postdoctoral fellowship awarded by Zhejiang University, Hangzhou, PR China. The work was supported by the Industrial Research Center, Tripoli, Libya.

References

1. Ahmed, I. A; Robinson, R. K. *Food Chem.* **1999**, *66*, 307–312.
2. Elarosi, H.; Muasa, A. E. A.; Jaheen, N. *Proceedings of the first Symposium on the Date Palm in Saudi Arabia*, King Faisal University: Al-Hassa, 1983; pp. 68–379.
3. Shinwari, M. A. *Encyclopedia of Food Science, Food Technology and Nutrition*; Academic Press: London, 1992; pp 1300–1305.
4. Al-Hooti, S.; Sidhu, J. S.; Qabazard, H. *Arab Gulf J. Sci. Res.* **1995**, *13*, 553–563.
5. Ahmed, I. A.; Ahmed, A. W. K.; Robinson, R. K. *Food Chem.* **1995**, *54*, 305–309.
6. Myhara, R. M.; Karkalas, J.; Taylor, M. S. *J. Agric. Food Sci.* **1999**, *79*, 1345–1350.
7. Ishurd, O.; Sun, C.; Xiao, P.; Ashour, A. *Carbohydr. Res.* **2002**, *337*, 135–1328.
8. Jindal, V. K.; Mukherjee, S. *Indian J. Chem.* **1970**, *8*, 417–419.
9. Jindal, V. K.; Mukherjee, S. *Indian J. Chem.* **1971**, *9*, 207–208.
10. Ishurd, O.; Zahid, M.; Ahmad, V. U.; Pan, Y. *J. Agric. Food Chem.* **2001**, *49*, 3772–3774.
11. Ishurd, O.; Zahid, M.; Zhou, H.; Pan, Y. *Carbohydr. Res.* **2001**, *333*, 297–301.
12. Sun, R.; Mark, J.; Banks, W. *Ind. Crops Prod.* **1998**, *7*, 121–128.
13. Park, H.; Park, J.; Okmoon, J. *Phytochemistry* **1999**, *51*, 147–151.
14. Paresh, C.; Gupta, D.; Subodh, K. S.; Day, A. *Carbohydr. Res.* **1976**, *48*, 73–80.
15. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1966**, 828–829.
16. Hamilton, J. K.; Kircher, W. H. *J. Am. Chem. Soc.* **1958**, *80*, 4703–4709.
17. Carson, J. F.; Maclay, W. D. *J. Am. Chem. Soc.* **1946**, *68*, 1015–1017.
18. Purdie, T.; Irvine, J. C. *J. Chem. Soc.* **1903**, *83*, 1021–1022.