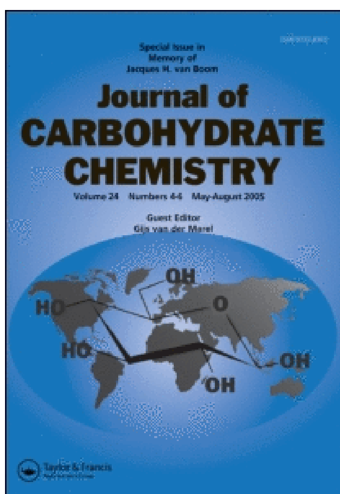
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Isolation and Structure Characterization of a (4-*O*-Methyl-d-glucurono)-d-xylan from the Skin of *Opuntia ficus-indica* Prickly Pear Fruits

Youssef Habibi[a,b]; Mostafa Mahrouz[b]; Michel R. Vignon[a]
[a] Centre de Recherches sur les Macromolécules Végétales, C.N.R.S., and Université Joseph Fourier, Grenoble cedex 9, France [b] Unité de Chimie Agro-alimentaire (LCOA), Département de Chimie, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco

Online publication date: 07 August 2003

To cite this Article Habibi, Youssef , Mahrouz, Mostafa and Vignon, Michel R.(2003) 'Isolation and Structure Characterization of a (4-*O*-Methyl-d-glucurono)-d-xylan from the Skin of *Opuntia ficus-indica* Prickly Pear Fruits', Journal of Carbohydrate Chemistry, 22: 5, 331 — 337
To link to this Article: DOI: 10.1081/CAR-120023476
URL: http://dx.doi.org/10.1081/CAR-120023476

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF CARBOHYDRATE CHEMISTRY
Vol. 22, No. 5, pp. 331–337, 2003

Isolation and Structure Characterization of a (4-*O*-Methyl-D-glucurono)-D-xylan from the Skin of *Opuntia ficus-indica* Prickly Pear Fruits

Youssef Habibi,[1,2] Mostafa Mahrouz,[2,*] and Michel R. Vignon[1,*]

[1]Centre de Recherches sur les Macromolécules Végétales,
C.N.R.S., and Université Joseph Fourier, France
[2]Unité de Chimie Agro-alimentaire (LCOA), Département de Chimie, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco

Downloaded At: 07:04 23 January 2011

ABSTRACT

A slightly water soluble (4-*O*-methyl-D-glucurono)-D-xylan was isolated from the skin of *Opuntia ficus-indica* (OFI) fruits by alkaline extraction, followed by ethanol precipitation and ion-exchange chromatography. The structure of this xylan was determined by sugar determination coupled with a ^{1}H and ^{13}C NMR spectroscopy analysis. The xylan consisted of a linear (1→4)-β-D-xylopyranosyl backbone decorated with 4-*O*-methyl-α-D-glucopyranosyluronic acid groups linked to the C-2 of the xylopyranosyl residues, in the ratio of one uronic acid for six neutral sugar units.

Key Words: *Opuntia ficus-indica*; Prickly pear skin; 4-*O*-methyl-glucuronoxylan; Ion-exchange chromatography; ^{1}H and ^{13}C NMR spectroscopy.

*Correspondence: Mostafa Mahrouz, Unité de Chimie Agro-alimentaire (LCOA), Département de Chimie, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390, 40001, Marrakech, Morocco; E-mail: mahrouz@ucam.ac.ma. Michel R. Vignon, Centre de Recherches sur les Macromolécules Végétales, C.N.R.S., and Université Joseph Fourier, BP 53, 38041 Grenoble cedex 9, France; E-mail: michel.vignon@cermav.cnrs.fr.

DOI: 10.1081/CAR-120023476 0732-8303 (Print); 1532-2327 (Online)
Copyright © 2003 by Marcel Dekker, Inc.
www.dekker.com

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

INTRODUCTION

Xylans are the most abundant noncellulosic polysaccharides in the plant kingdom. They constitute the major hemicelluloses in the primary cell walls of monocots and are found in smaller amounts in the primary cell walls of dicots, where they exist in many different compositions and structures.[1] They are also found as an important component in the hardwood secondary wall.[2,3]

From a structural point of view, xylans form a family of polysaccharides consisting of linear β (1→4) linked D-xylosyl backbones, with various side groups or chains attached to the *O*-2 and/or *O*-3 of the xylosyl residues. These side chains mainly consist of α-D-glucuronic acid, 4-*O*-methyl-α-D-glucuronic acid and some neutral sugars units (α-L-arabinofuranose, α-D-xylopyranose or α-D-galactopyranose). Among the common side groups are acetyl groups, phenolic acids, ferulic and coumaric acids.[4]

The structural features of various xylan-type polysaccharides depend on their botanical origin, together with their mode of isolation. In glucuronoxylan, the relative ratio of xylose to 4-*O*-methyl-α-D-glucuronic acid can vary respectively from 2:1 in quince slime mold[5,6] to 65:1 in the pericarp of seeds of *Opuntia ficus-indica* (OFI).[7] Relatively pure xylans and 4-*O*-methyl-α-D-glucuronoxylans may be used in various industrial and non-industrial applications. They have even been reported to inhibit the growth rate of tumors, probably in relation to the indirect stimulation of the non-specific immunological host defense.[8,9]

Because of the high adaptation of the cactus to the harsh desert environment and its different utilizations, its fruit is an important and abundant potential raw material for the Moroccan industry. Efforts are currently under way to develop the fruit production and to increase its introduction into various food products. In this context, the polysaccharides from OFI have attracted our attention. This paper deals with the structure elucidation of a 4-*O*-methyl-β-D-glucuronoxylan isolated from the skin of the fruits of this cactus.

RESULTS AND DISCUSSION

Fruits of OFI were peeled, and the skin tissues were observed by scanning electron microscopy (SEM) as shown in Figure 1. This image obtained after critical point drying revealed the organization of the different types of cells. From the outside to the inside of the fruit, we can observe thin walled epidermal cells, two or three layers of chollenchyma and parenchyma cells. We can also observe mineral inclusions (calcium oxalate crystals). The skins were disencrusted by two extractions with hot water, two extractions with ammonium oxalate and one extraction with hot dilute HCl.

Under these conditions the various cells lost most of their noncellulosic constituents. The various cells occurred then as cell-ghosts that correspond essentially to the flattened microfibrillar envelopes of the cellulose microfibrils within the wall of the corresponding cells. This disencrusted material was treated with a 2% sodium hydroxide solution and after precipitation in ethanol a crude hemicellulose fraction (CHF) was obtained. This CHF was purified by ion-exchange chromatography and the fraction eluted with phosphate buffer was subsequently characterized by gel permeation chromatography, sugar analysis, ^{1}H and ^{13}C NMR spectroscopy.

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

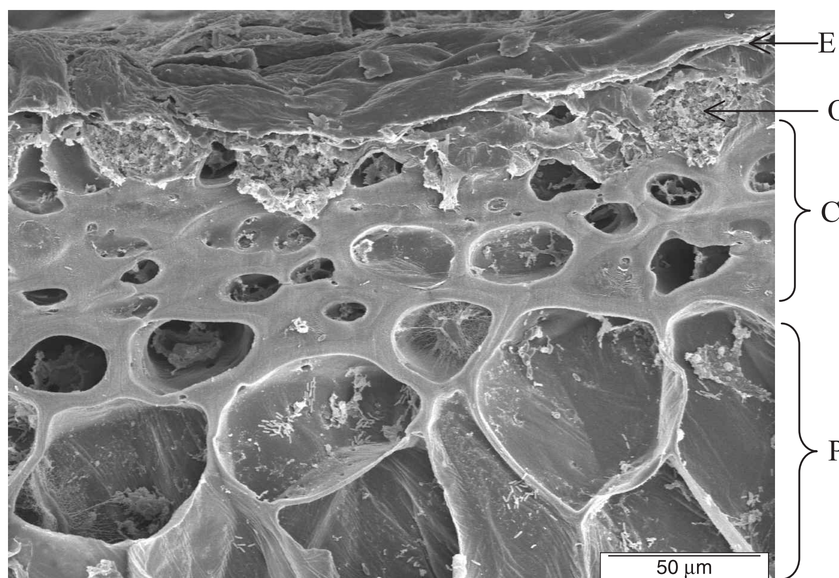


Figure 1. SEM of a cross-section of the skin of *Opuntia ficus-indica* fruit. E: Epidermi cells; O: Calcium oxalate crystals; C: Collenchyma cells; P: Parenchyma cells.

Size exclusion gel permeation chromatography of CHF on a biogel P6 column showed that the sample was eluted in the void volume, indicating an average molecular weight of 2000–3000. A hydrolyzate of CHF revealed that xylose was the major component with a small amount of glucose (xylose to glucose ratio of 97:3, molar composition of Xyl:Glc 48:1). The uronic acid content was estimated by a colorimetric method to be 26.6% (w/w), which corresponded to a molar ratio of xylose to uronic acid, respectively, of 6.7:1.

The structure and the molar ratio of xylose to uronic acid were also investigated by NMR spectroscopy. The ^{13}C NMR spectrum of the sample recorded in D_2O (Figure 2), showed five main signals at δ 102.39 (C-1), 73.45 (C-2), 74.47 (C-3), 77.17 (C-4) and 63.74 (C-5) ppm, corresponding to (1 → 4) linked β-D-Xyl residues. Other less intense signals observed at δ 177.16, 98.30, 72.12, 77.56, 83.05, 73.10 and 60.31 ppm, are characteristic respectively of C-6, C-1, C-2, C-3, C-4, C-5 and the methoxyl group carbon of a 4-*O*-methyl-α-D-glucuronic acid residue. Signals at δ 101.97, 77.42, 72.95, 76.88, 63.59 ppm are characteristic of C-1, C-2, C-3, C-4, C-5, respectively, of β-D-Xyl units substituted with 4-*O*-methyl-α-D-GlcA. All the data reported in Table 1 are in good agreement with the structures of (4-*O*-methyl-α-D-glucurono)-β-D-xylans have been described in a number of plants.[6,10–14] The average integration of all signals for different sugar residues groups, in ^{13}C quantitative spectra, revealed a molar ratio of xylose to 4-*O*-methyl-α-D-glucuronic acid, respectively, of 5.8:1.

Examination of the proton spectrum (Figure 3) of the xylan showed the relative simplicity of the structure as exhibited by: 1) major signals at δ 4.38 (H-1), 4.00 (H-5eq), 3.69 (H-4), 3.47 (H-3), 3.29 (H-5ax) and 3.21 ppm (H-2), corresponding to non-substituted β-D-Xyl residues; 2) minor signals at δ 5.16 (H-1), 4.18 (H-5), 3.67 (H-3),

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

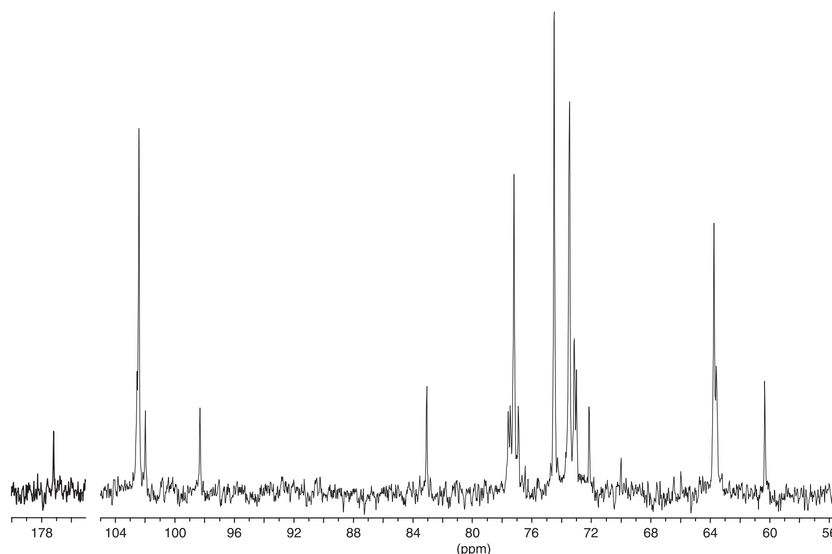


Figure 2. ^{13}C NMR spectrum of (4-*O*-methyl-D-glucurono)-D-xylan from skins of *Opuntia ficus-indica* in D_2O at 333 K

3.49 (H-2), 3.37 (OCH_3), 3.16 ppm (H-4), corresponding to 4-*O*-methyl-α-D-GlcA acid residues, and at δ 4.53 (H-1), 4.03 (H-5eq), 3.71 (H-4), 3.55 (H-3), 3.44 (H-2) and 3.33 ppm (H-5ax) assigned to β-D-Xyl units substituted with 4-*O*-methyl-α-D-GlcA. The proton spectrum also showed three doublets in the anomeric region at 4.38 ppm [(1 → 4)-β-D-Xyl*p*], 4.53 ppm [(1 → 4)-β-D-Xyl*p*-2-*O*-Glc*p*A] and 5.16 ppm (4-*O*-methyl-α-D-Glc*p*A) with an intensity ratio of 5:1:1, respectively. The proton NMR data confirmed the presence of six xylose units per uronic acid residue.

Table 1. Chemical shift data[a] for glycosyl residues of (4-*O*-methyl-D-glucurono)-D-xylan from the skin of *Opuntia ficus-indica* prickly pear fruits.

Glycosyl residues		Assignment				
		1	2	3	4	5
(1→4)-β-D-Xyl*p*	^{1}H	4.38	3.21	3.47	3.69	H_{eq}: 4.00 H_{ax}: 3.29
	^{13}C	102.39	73.45	74.47	77.17	63.74
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.53	3.44	3.55	3.71	H_{eq}: 4.03 H_{ax}: 3.33
	^{13}C	101.97	77.42	72.95	76.88	63.59
4-*O*-Me-α-D-Glc*p*A	^{1}H	5.16	3.49	3.67	3.16	4.18 (OCH_3: 3.37)
	^{13}C	98.30	72.12	77.56	83.05	73.10 (OCH_3: 60.31) (C-6: 177.16)

[a]In ppm relative to the signal of internal acetone in deuterium oxide, at 2.1 ppm (^{1}H, 338 K) or at 31.5 ppm (^{13}C, 333 K).

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

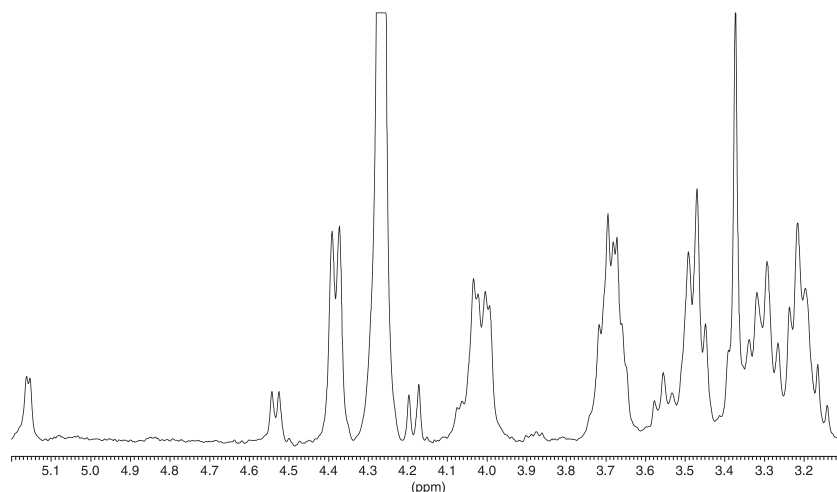


Figure 3. ^{1}H NMR spectrum of (4-*O*-methyl-D-glucurono)-D-xylan from skins of *Opuntia ficus-indica* in D_2O at 338 K.

This acidic xylan-containing fraction was shown by sugar analysis and NMR spectroscopy to contain a backbone of $(1 \rightarrow 4)$-linked β-D-xylopyranosyl residues substituted with 4-*O*-methyl-α-D-glucuronic acid at position C-2. Our results showed that, on average, the (4-*O*-methyl-D-glucurono)-D-xylan from the skin of *O. ficus-indica* fruits consisted of one 4-*O*-methyl-α-D-glucuronic acid for seven D-xylosyl residue. This structure is, therefore, very similar to that of the (4-*O*-methyl-D-glucurono)-D-xylan isolated from sugar beet parenchyma cells.[14] We postulate that the (4-*O*-methyl-D-glucurono)-D-xylan in the skin tissues of the OFI fruits is strongly hydrogen bonded to the cellulose microfibrils within parenchyma cell walls. The 2% sodium hydroxide treatment is required to break the hydrogen bonding interactions and solubilize the (4-*O*-methyl-D-glucurono)-D-xylan, but at the same time the presence of *O*-acetyl groups, if they are present, cannot be estimated, as they are saponified under these alkaline conditions.

EXPERIMENTAL

Materials. Fresh fruits of OFI were collected in November 2000 from a pilot plantation in the vicinity of Marrakech (Morocco).

General methods. Uronic acid content was determined according to the Blumenkrantz and Asboe-Hansen method.[15] Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates,[16] using a Packard and Becker 417 instrument coupled to a Hewlett-Packard 3380 A integrator. Glass columns (3 mm × 2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh), or 3% OV 17 on the same support were used.

Microscopy. For SEM analysis, small cubes were cut out from fresh skin of OFI, fixed with glutaraldehyde and dried under critical point conditions in a Polaron Critical

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Point Dryer operated with liquid CO_2. Before observation, the samples were sputtered with gold palladium alloy in a JEOL JFC sputterer. The observations were made with a JEOL JMS-6100 SEM operating at an accelerating voltage ranging from 5 to 8 kV and in secondary electron mode.

Isolation of a (4-*O*-methyl-D-glucurono)-D-xylan. The fruits were carefully hand-peeled, and the peels (with a thickness of about 3–4 mm) were cut into small pieces and dried in a ventilated oven adjusted to 50°C. After drying, 100 gms of dried peel were ground for a few minutes in a domestic coffee grinder and sieved. Fats, waxes and oils were removed by refluxing in a Soxhlet apparatus during 24 h with 38:62 toluene-ethanol. The pectic polysaccharides were extracted sequentially with hot water (2×2 h at 60°C), aq 0.5% ammonium oxalate (2×2 h at 60°C), and 0.05N HCl solution (2×1 h at 80°C). The residue was then extracted with a 2% NaOH solution at 80°C (2×2 h), and the resulting two filtrates were pooled, neutralized to pH ≈ 7 with 20% AcOH solution, and precipitated with 4 volumes of ethanol. The precipitate was collected by centrifugation, and washed with ethanol-water (19:1). A solution of the resulting precipitate in water was exhaustively dialysed against distilled water (2×24 h), and freeze-dried afterwards to give 2.58 g of crude hemicelluloses (CHF) which corresponded to a yield of 2.4% on the basis of the dry weight of starting material.

A sample of CHF (0.8 g) was suspended in 100 mL of 0.05 M phosphate buffer (pH = 6.3) and loaded onto a column of DEAE-Trisacryl M (phosphate form), which was eluted sequentially with phosphate buffer and then with a NaCl gradient (0.125–1 M) in the same buffer. The buffer eluted fraction was then dialysed against distilled water and freeze-dried to give a polysaccharide fraction with a yield of 30%, on the basis of the dry weight of CHF (0.8% on the basis of the dry weight of skins, or 0.03% on a dry basis of the whole fruit), which is composed of D-xylose and 4-*O*-methyl-D-glucuronic acid.

Gel permeation chromatography. The buffer eluted fraction was analyzed by size-exclusion chromatography on a polyacrylamide Biogel P6 column (4–100 cm), eluted with 0.05 M sodium nitrate solution at 80 ml/h. The salts were removed by dialysis and the solution freeze-dried.

NMR spectroscopy. ^{1}H experiments were recorded on a Varian Plus 400 spectrometer (operating frequency of 400.13 MHz). Samples were examined as solutions in D_2O (2 mg in 1 mL) at 338°K in 5 mm o.d. tubes. ^{13}C NMR experiments were obtained with an AC 300 Bruker spectrometer (operating frequency: 75.468 MHz). Samples were examined as solutions in D_2O (20 mg in 0.75 mL) at 333°K in 5 mm o.d. tubes [internal acetone ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si]. Quantitative ^{13}C spectra were recorded using the INVGATE Brucker sequence, with 90° pulse length (6.5 μs), relaxation delay of 1.7 s, 8000 data points, 0.54 s acquisition time, and 30000 scans.

ACKNOWLEDGMENTS

We acknowledge the financial help of the Comité Mixte Franco-Marocain (action intégrée 236/SVS/00), and also thank Miss C. Fraschini and Mrs D. Dupeyre for her help in NMR experiments and Scanning Electron Microscopy, respectively.

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

REFERENCES

1. Stephen, A.M. Other plant polysaccharides. In *The Polysaccharides*; Aspinall, G.O., Ed.; Academic Press, Inc.: Orlando, 1983; 98–193.
2. Aspinall, G.O.; Hirst, E.L.; Mahomed, R.S. Hemicellulose A of beechwood (*Fagus sylvatica*). J. Chem. Soc. **1954**, 1734–1738.
3. Teleman, A.; Lundqvist, J.; Tjerneld, F.; Stålbrand, H.; Dahlman, O. Characterization of acetylated 4-*O*-methylglucuronoxylan isolated from aspen employing ^{1}H and ^{13}C NMR spectroscopy. Carbohydr. Res. **2000**, *329*, 807–815.
4. Wilkie, K.C.B. The hemicelluloses of grasses and cereals. Adv. Carbohydr. Chem. Biochem. **1979**, *36* (1), 215–264.
5. Lindberg, B.; Mosihuzzaman, M.; Nahar, N.; Abeysekera, R.M.; Brown, R.G.; Willison, J.H.M. An unusual (4-*O*-methyl-D-glucurono)-D-xylan isolated from the mucilage of seeds of the quince tree (*Cydonia oblonga*). Carbohydr. Res. **1990**, *207*, 307–310.
6. Kardosova, A.; Matulova, M.; Malovikova, A. (4-*O*-methyl-D-glucurono)-D-xylan from *Rudbeckia fulgida*, var. *sullivantii* (*Boynton et Beadle*). Carbohydr. Res. **1998**, *308*, 99–105.
7. Habibi, Y.; Mahrouz, M.; Vignon, M.R. Isolation and structure of D-xylans from pericarp seeds of *Opuntia ficus-indica* prickly pear fruits. Carbohydr. Res. **2002**, *337*, 1593–1598.
8. Hashi, M.; Takeshita, T. Studies on antitumor activity of wood hemicelluloses. I Antitumor effect of 4-*O*-methylglucuronoxylan on solid tumor in mice. Agric. Biol. Chem. **1979**, *43* (5), 951–959.
9. Hashi, M.; Takeshita, T. Studies on antitumor activity of wood hemicelluloses. II The host mediated antitumor effect of 4-*O*-methylglucuronoxylan. Agric. Biol. Chem. **1979**, *43* (5), 961–967.
10. Kohn, R.; Hromadkova, Z.; Ebringerova, A.; Toman, R. Distribution pattern of uronic acid units in 4-*O*-methyl-D-glucurono-D-xylan of beech (*Fagus sylvatica L.*). Collect. Czechoslov. Chem. Commun. **1986**, *51*, 2243–2249.
11. Swamy, N.R.; Salimath, P.V. Structural features of acidic xylans isolated from red gram (*Cajanus Cajan*) husk. Carbohydr. Res. **1990**, *197*, 327–337.
12. Bazus, A.; Rigal, L.; Gaset, A.; Fontaine, T.; Wieruszeski, J.M.; Fournet, B. Isolation and characterization of hemicelluloses from sunflower hulls. Carbohydr. Res. **1993**, *243*, 323–332.
13. Vignon, M.R.; Gey, C. Isolation, ^{1}H and ^{13}C NMR studies of (4-*O*-methyl-D-glucurono)-D-xylans from luffa fruit fibres, jute bast fibres and mucilage of quince tree seeds. Carbohydr. Res. **1998**, *307*, 107–111.
14. Dinand, E.; Vignon, M.R. Isolation and NMR characterization of a (4-*O*-methyl-D-glucurono)-D-xylan from sugar beet pulp. Carbohydr. Res. **2001**, *330*, 285–288.
15. Blumenkrantz, P.N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. Anal. Biochem. **1973**, *54*, 484–489.
16. Selvendran, R.R.; March, J.F.; Ring, S.G. Determination of aldoses and uronic acid content of vegetable fibre. Anal. Biochem. **1979**, *96*, 282–292.

Received July 24, 2002
Accepted April 18, 2003

Downloaded At: 07:04 23 January 2011

Copyright © 2003 by Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016