Polysaccharides in the Antipsoriatic Mahonia Extract: Structure of a (1→4)-β-D-Glucan

[a]A. KARDOŠOVÁ, [a]J. ALFÖLDI, [a]E. MACHOVÁ, and [b]D. KOŠŤÁLOVÁ

[a]*Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava*

[b]*Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, SK-832 32 Bratislava*

Received 24 October 2000

From the aqueous-ethanolic antipsoriatic mahonia extract a mixture of low-molecular-mass polysaccharides was isolated by ethanol precipitation of the undialyzable portion. They were composed of neutral sugar components with dominating glucose. Ion-exchange and gel-permeation chromatography was employed to obtain the dominant polysaccharide component which was proved by methylation analysis and NMR spectrometric measurements to be a linear (1→4)-β-D-glucan.

Mahonia aquifolium (Pursh) Nutt. from the *Berberidaceae* family has been used for a long time in homeopathy as an organotropic drug for treatment in inflammatory, scaling dermatoses and now is available also as a topical antipsoriatic drug [1]. The active principle of the mahonia tincture were thought to be the alkaloids [2, 3] contained in the extract but some experiments led to the suggestion that there are probably other components positively influencing the immune mechanisms of human leucocytes. We have proved in the extract the presence of both sugars and ethanol-precipitable carbohydrates [4]. As in our preliminary tests not only the extract itself but also the isolated crude polysaccharide showed immunomodulating activity on human monocytes (unpublished results), it was desirable to characterize this natural product as closely as possible.

The present work describes the isolation and fractionation of the crude polysaccharide from the mahonia tincture and provides the results on structure identification of the dominant component of the largest polysaccharide fraction.

EXPERIMENTAL

The plant mahonia was gathered in the garden of the Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic in 1997 and the voucher specimen is deposited at the Herbarium of the Faculty of Pharmacy. The anion-exchanger DEAE-Ostsorb (bead cellulose) was purchased from Tessek Ltd. (Prague, Czech Republic), DEAE-Sephadex A-50 from Pharmacia (Sweden), Bio-Gel P-2 from Bio-Rad (USA), and Sep-pak C_{18} cartridges from Waters Associates (USA). All chemicals used were of anal. grade.

Concentrations were performed under diminished pressure at a bath temperature not exceeding 45 °C. Free-boundary electrophoresis of polysaccharide solution (10 mg cm^{-3}) was performed in 0.05 M sodium tetraborate buffer (pH 9.3) with Zeiss 35 apparatus at 150 V and 8 mA for 30 min. Optical rotation of the glucan (1 cm^3 cell) was measured at (20 ± 1) °C with a Perkin—Elmer Model 141 polarimeter. The number average relative molecular mass M_r was determined osmometrically at 30 °C using a Knauer pressure osmometer. High-pressure gel-permeation chromatography (HPGPC) of polysaccharide fractions was performed using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) equipped with two Labio Prague Biospher GM 300 and 1000 exclusion columns (8 mm × 250 mm) and using aqueous 0.1 M-$NaNO_3$ as solvent and eluent (0.4 cm^3 min^{-1}). The eluate was monitored by RI detector. A set of pullulan standards P-5, P-10, P-20, and P-50 (Shodex Standard, P-82, Macherey—Nagel, Germany) was used for calibration of the columns. A computing procedure [5] based on linear effective calibration curve was applied to obtain the molecular-mass distribution. The infrared spectrum of the methylated polysaccharide was recorded with a Nicolet Magna 750 spectrometer.

Descending paper chromatography was performed on Whatman No. 1 paper in the solvent systems S_1 EtOAc—pyridine—water (φ_r = 8:2:1) and S_2 EtOAc—AcOH—formic acid—water (φ_r = 18:3:1:4), the sugars being detected with anilinium hydrogen phthalate. Carbohydrates were determined by the phenol—sulfuric acid assay [6]. Total hydrolysis of polysaccharides was effected with 2 M-TFA at 120 °C for 2 h. The sugars in the hydrolyzate were converted to alditol trifluoroacetates and analyzed by gas chromatography on a Hewlett—Packard 5890 Series II chromatograph equipped with a PAS-1701 column

(0.32 mm × 25 m) at the temperature program of 110—125 (2 °C min^{-1})—165 °C (20 °C min^{-1}) and flow rate of hydrogen 20 cm^3 min^{-1}. Partial acid hydrolysis of the glucan was effected with 1 M-TFA at 100 °C for 1 h and the disaccharide was separated from the hydrolyzate on the column (2.5 cm × 130 cm) of Bio-Gel P-2.

GC-MS analysis of partially methylated alditol acetates of glucose was performed on a FINNIGAN MAT SSQ 710 spectrometer equipped with an SP 2330 column (0.25 mm × 30 m) at 80—240 °C (6 °C min^{-1}), 70 eV, 200 μA, and ion-source temperature 150 °C.

NMR spectra were recorded at 25 °C on an FT NMR Bruker Avance DPX 300 spectrometer (300.13 MHz) equipped with a gradient enhanced spectroscopy kit (GRASP) for generation of z-gradient up to 50 × 10^{-4} cm^{-1} in 5 mm inverse probe. The sample was dissolved in D_2O and chemical shifts of the signals were referenced to external acetone (δ = 2.225 and 31.07 for ^1H and ^{13}C, respectively). The data matrice for HSQC [7] experiment was processed with squared sine function, using Bruker software XWIN-NMR version 1.3.

Methylation analysis was performed on 50 mg glucan sample applying the method of *Ciucanu* and *Kerek* [8] to give a fully methylated product, as evidenced by the absence of IR absorption for hydroxyl. The methylated sample was recovered using a Sep-pak C_{18} cartridge by the procedure of *Waeghe et al.* [9]. The product was then converted into partially methylated alditol acetates and subjected to linkage analysis by GC-MS [10].

Isolation of the Glucan

The extract of *Mahonia aquifolium* (Pursh) Nutt., prepared after [3] by maceration of dry stems with 62 % aqueous ethanol, was exhaustively dialyzed against distilled water (constant conductivity) and the undialyzable residue was batch-wise purified on a DEAE-Ostsorb ion-exchanger (carbonate form) by elution with water and 0.5 M ammonium carbonate solution. The water eluate was precipitated with 4 volumes of ethanol to give a brownish crude polysaccharide (P). This product (200 mg) was fractionated on a column (4.0 cm × 120 cm) of DEAE-Sephadex A-50 (chloride form) by irrigation with water (fractions A, B, C) and 0.5 M-NaCl solution (fraction D). The eluates were dialyzed and freeze-dried to give A (40 mg), B (35 mg), C (25 mg), and D (75 mg). The largest fraction D was subjected to gel filtration on a column (2.5 cm × 130 cm) of Bio-Gel P-2 to give a homogeneous polysaccharide which on total hydrolysis afforded only glucose.

RESULTS AND DISCUSSION

From the undialyzable portion of the antipsoriatic mahonia extract after its partial depigmentation a brownish crude polysaccharide (P) was isolated by ethanol precipitation in 0.02 % yield. Its sugar composition (Table 1) pointed to heterogeneity of the product, therefore it was fractionated by ion-exchange chromatography to four fractions differing in mole proportions of the neutral sugar components (Table 1). Uronic acids were not detected in any fraction. The distribution pattern of fractions A, B, and D on HPGPC chromatograms showed low-molecular-mass polysaccharides (3 090—21 900) of low polydispersity (1.02—1.17) (Table 2). The data for fraction C are omitted because of low solubility of the compound. As seen from Table 1, D-glucose was the prevalent component practically in all fractions. Its high proportion (48.0 %) in the largest fraction suggested the presence of a glucan-type polysaccharide and, therefore, this fraction was further purified

Table 2. Relative Molecular-Mass Distribution Pattern on HPGPC Chromatograms of the Polysaccharide Fractions Isolated from the Mahonia Extract

Fraction	M_n	M_m/M_n	Area/%
A	4 300	1.02	6.70
	21 900	1.17	93.30
B	3 090	1.02	12.30
	13 500	1.11	87.70
D	4 400	1.02	32.83
	9 600	1.12	67.17

Table 1. Sugar Composition of the Polysaccharide Fractions from the Mahonia Extract

Fraction	x_i/mole %					
	D-Glc	D-Gal	D-Man	L-Ara	D-Xyl	L-Rha
P	48.9	20.9	6.0	12.6	8.9	2.5
A	17.8	20.3	5.0	50.4	2.4	4.0
B	42.7	24.0	5.7	20.7	5.0	1.8
C	47.6	15.8	8.5	8.2	14.7	5.1
D	48.0	26.8	3.1	4.0	16.0	2.0

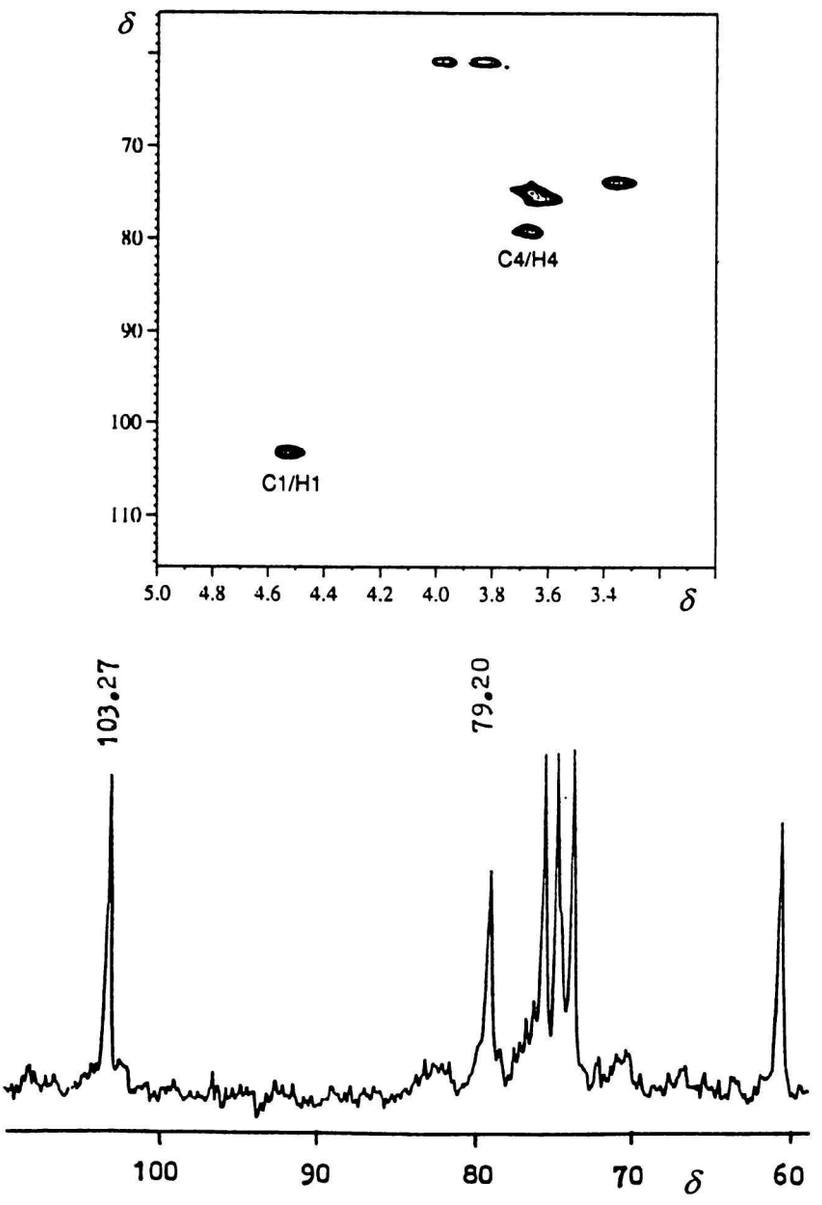


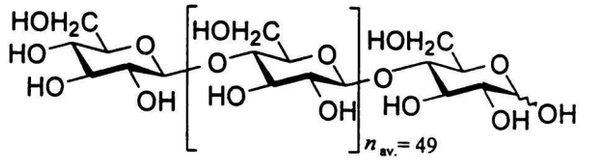
Fig. 1. ^{13}C NMR spectrum (HSQC spectrum inserted) of the (1→4)-β-D-glucan from the extract of *Mahonia aquifolium* (Pursh) Nutt.

by gel-permeation chromatography. The compound eluted from the Bio-Gel P-2 column in the void volume represented a polysaccharide homogeneous by free-boundary electrophoresis and HPGPC, and on total hydrolysis yielded only D-glucose. Its number average relative molecular mass was 8100 and optical rotation −6.7°. The results of methylation analysis, revealing 2,3,6-trimethylglucose and 2,3,4,6-tetramethylglucose derivatives, indicated a linear structure of the polymer with (1→4)-linked glucopyranosyl units. The ratio of trimethyl- to tetramethylglucose was 4.9:0.1, well corresponding with DP = 51 calculated from M_r. Cellobiose, isolated from the partial acid hydrolyzate of the glucan and identified on the basis of comparison of its ^{13}C NMR data (Table 3) with the literature ones on cellooligosaccharides [11, 12], as well as the spectral data of the glucan (Fig. 1) confirmed the above results. The ^{13}C NMR spectrum of the glucan (Fig. 1; HSQC spectrum inserted) displayed 6 signals evidencing the linearity of the polysaccharide. The C-1 signal at δ = 103.27, indicating β-type linkages, and the signal at δ = 79.20, evidencing O-4 substitution, unambiguously proved that the polysaccharide has the following structure

Table 3. ^{13}C NMR Data for Cellobiose Obtained by Partial Acid Hydrolysis of the Glucan

Cellobiose	Chemical shift, δ					
	C-1	C-2	C-3	C-4	C-5	C-6
NRU	103.09	73.72	76.15	70.92	76.15	60.82
RUα	92.63	72.50	73.72	70.49	72.07	60.82
RUβ	96.59	74.69	75.60*	70.49	74.69*	60.82

NRU = nonreducing unit; RU = reducing unit; * = or *vice versa*.



It may be concluded that the antipsoriatic aqueous-ethanolic mahonia extract contains besides detectable amounts of glucose and mannose also a mixture of neutral polysaccharides. The dominant polysaccharide component is a water-soluble, low-molecular-mass (1→4)-β-D-glucan, while the other species, probably heteropolysaccharides, are present in much lower proportions. Tests for immunomodulating activity of the herein studied fraction with dominant glucan component are in progress.

Acknowledgements. This work was supported by the Grant No. 2/7138 from the Slovak Scientific Grant Agency (VEGA).

REFERENCES

1. Galle, K., Müller-Jakic, B., Proebstle, A., Jurcic, K., Bladt, S., and Wagner, H., *Phytomedicine 1*, 59 (1994).
2. Hänsel, R., *D. Apoth. Ztg. 132*, 2095 (1992).
3. Bezáková, L., Mišík, V., Máleková, L., Švajdlenka, E., and Košťálová, D., *Pharmazie 51*, 758 (1996).
4. Kardošová, A. and Košťálová, D., *Chem. Pap. 53*, 82 (1999).
5. Šoltés, L., Alföldi, J., and Šandula, J., *Appl. Polym. Sci. 48*, 1313 (1993).
6. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., *Anal. Chem. 28*, 350 (1956).
7. Schleucher, J., Sattler, M., and Griesinger, C., *Angew. Chem., Int. Ed. Engl. 32*, 1489 (1993).
8. Ciucanu, I. and Kerek, F., *Carbohydr. Res. 131*, 209 (1984).
9. Waeghe, T. J., Darvill, A. G., McNeil, M., and Albersheim, P., *Carbohydr. Res. 123*, 281 (1983).
10. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B., and Lönngren, J., *Chem. Commun. Univ. Stockholm 8*, 1 (1976).
11. Bock, K., Pedersen, C., and Pedersen, H., *Adv. Carbohydr. Chem. Biochem. 42*, 193 (1984).
12. Flugge, L. A., Blank, J. T., and Petillo, P. A., *J. Am. Chem. Soc. 121*, 7228 (1999).