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Carbohydrate Research 343 (2008) 1390–1393
Carbohydrate RESEARCH

Note

An arabinogalactan containing 3-*O*-methyl-D-galactose residues isolated from the aerial parts of *Salvia officinalis* L.

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Received 27 April 2006; received in revised form 18 March 2008; accepted 18 March 2008
Available online 27 March 2008

Abstract—An arabinogalactan with a high content of 3-*O*-methyl-D-galactose residues has been isolated from the aerial parts of sage (*Salvia officinalis* L.). Structural studies of the polymer indicated a β-1,6-D-galactopyranose backbone in which at least one-third of D-galactopyranosyl residues carries methoxyl groups and another one-third L-arabinosyl side chains at C-3.

Keywords: Sage; Arabinogalactan; 3-*O*-Methyl-D-galactose; Madurose; NMR spectroscopy; Mass spectrometry

Sage (*Salvia officinalis* L., Lamiaceae) is a medicinal plant used throughout the world. The aerial parts of sage are used as a remedy in traditional medicine and as a flavouring for culinary purposes. Sage contains a great number of bioactive compounds possessing a variety of biological activities.[1] It has been shown that sage has a relatively high polysaccharide content exhibiting immunomodulatory[2,3] and antitussive[4] activities. However, no details are available about the fine structural features of these polymers. Consequently, the present study reports on the isolation and structural characterization of the α-L-arabino-β-3,6-D-galactan containing 3-*O*-methyl-D-galactose (madurose) residues from the aerial parts of sage.

The crude polysaccharide (PS) was obtained by water extraction of the defatted and decoloured aerial parts of the plant, followed by ethanol precipitation and freeze-drying (Table 1). Ion-exchange chromatography of PS afforded six fractions by the step-wise elution with water (PSA_1), sodium chloride solutions (PSA_2-A_5) and sodium hydroxide (PSA_6).

The non-retained fraction (PSA_1) was recovered in 7.5% yield and showed a single peak of lower molecular weight on HPLC (Table 1). Compositional analysis revealed the dominance of arabinose, galactose, glucose and mannose residues. However, paper chromatography showed, inter alia, the presence of another monosaccharide having chromatographic mobility between arabinose and xylose. Its brown colour indicates the presence of hexose. The unknown saccharide, isolated by preparative paper chromatography, was converted into its acetate derivative and identified by GC–MS as the 3-*O*-methylhexopyranose tetraacetate. The ^{13}C NMR spectrum (Fig. 1A) showed signals at δ 97.3 and 93.0 due to β and α anomers of galactose or glucose residues. Characteristic chemical shifts due to signals

Table 1. Characterization of sage polysaccharides PS, PSA_1 and PSA_1-AG

Monosaccharide composition (%)	PS	PSA_1	PSA_1-AG
Galactose	17.9	22.1	35.3
3-*O*-Methyl-galactose	3.0	10.6	19.9
Glucose	15.5	13.0	tr
Mannose	8.3	10.1	tr
Arabinose	30.4	35.0	44.8
Xylose	7.6	2.9	tr
Fucose	2.6	0.4	
Rhamnose	6.7	1.2	
Uronic acid	8.0	4.7	
Yield[a] (%)	3.8		
Protein (%)	9.4		
Molecular mass (M_w)	2–93,000	8000	

PS: crude polysaccharides, PSA_1: ion-exchange, no retained fraction of PS, AG: arabinogalactan, tr: traces.
[a] Based on air-dried plant.

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0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.03.026

at δ 82.8 (β) and 79.4 (α) indicated the substitution of unknown sugar at C-3 whilst those at δ 57.0 and 56.8 suggested the presence of methoxyl[5] groups.

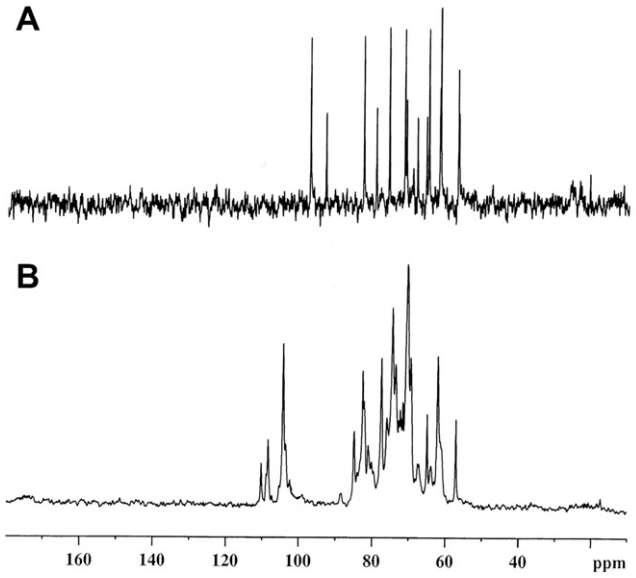

Figure 1. ^{13}C NMR spectra of the 3-*O*-methyl-galactose (A) and the arabinogalactan (B).

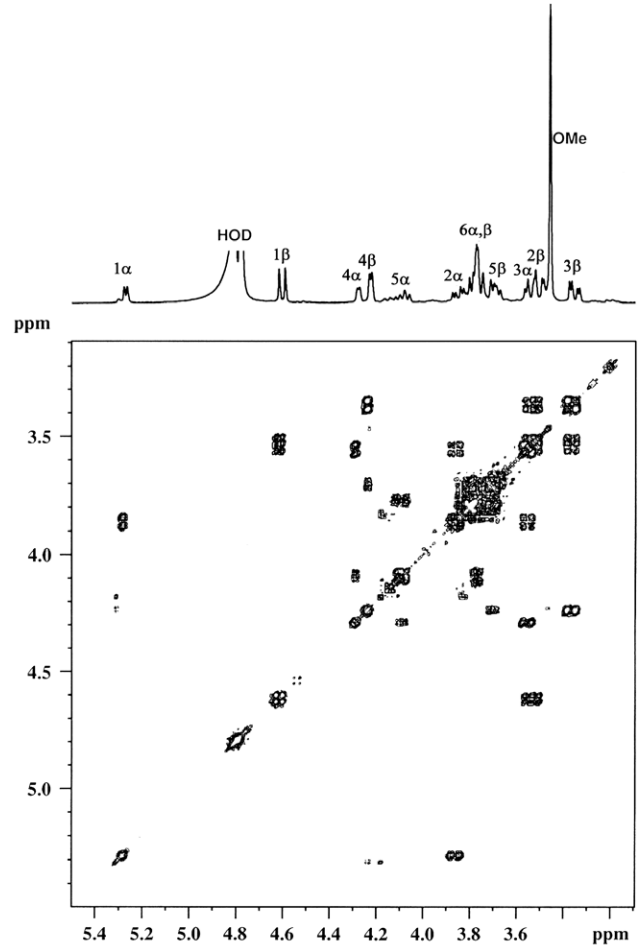

Figure 2. 2D COSY spectrum of the 3-*O*-methyl-galactose.

Detailed NMR structural studies by 2D COSY (Fig. 2), HSQC and HMBC (Fig. 3) measurements have identified galactose as the unknown sugar and the location of the methoxyl group at C-3 was found on the basis of its cross peak in the HMBC spectrum (Fig. 3, insert A). The NMR data of the 3-*O*-methyl-galactose residue are given in Table 2.

The fraction PSA_1 was further purified by Fehling reagent to give the arabinogalactan (AG) composed of galactose (~55%) and arabinose (~45%) and showed a single symmetric peak on HPLC. The high content of the 3-*O*-methyl-galactose residues in AG was determined in a similar way to PSA_1 (Table 1).

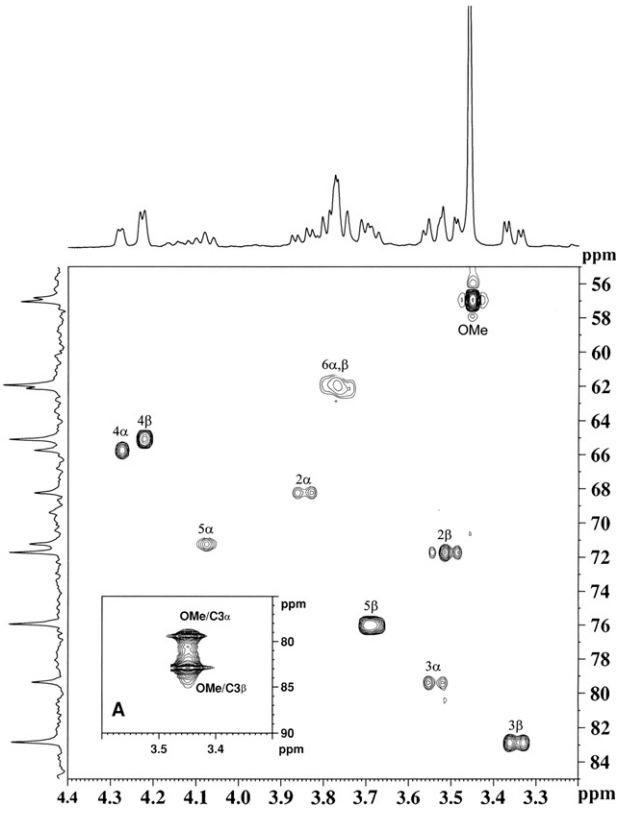

Figure 3. HSQC spectrum of the 3-*O*-methyl-galactose. The inserted part (A) represents H–OMe/C3α,β connectivities in HMBC spectrum.

Table 2. ^{1}H and ^{13}C NMR data of the 3-*O*-methyl-D-galactose

H-1 α/β	5.27/4.61 (d,[a] $J_{1,2} = 3.90/7.74$)	C-1 α/β	95.04/99.33
H-2 α/β	3.85/3.53 (dd[b])	C-2 α/β	70.26/73.73
H-3 α/β	3.55/3.36 (dd)	C-3 α/β	81.37/84.83
H-4 α/β	4.28/4.23 (dd)	C-4 α/β	67.76/67.11
H-5 α/β	4.09/3.70 (se[c])	C-5 α/β	73.23/77.95
H-6,6 α/β	3.75/3.79 (ovm[d])	C-6 α/β	64.65/63.93
OCH3 α/β	3.46 (se[c])	OCH3 α/β	58.80/59.05

[a] Doublet.
[b] Doublet of doublets.
[c] Sextet.
[d] Overlapped multiplet.

Table 3. Methylation analysis data of the arabinogalactan

Sugar derivative	mol %	Mode of linkage
2,3,5-Me_3-Ara[a]	19.3	Ara*f*-(1→
2,3,4-Me_3-Ara	5.7	Ara*p*-(1→
3,5-Me_2-Ara	1.0	→2)-Ara*f*-(1→
2,5-Me_2-Ara	8.8	→3)-Ara*f*-(1→
2,3-Me_2-Ara	11.6	→5)-Ara*f*-(1→ or →4)-Ara*p*-(1→
3-Me-Ara	1.0	→2,5)-Ara*f*-(1→
Per-*O*-Ac-Ara	2.4	→2,3,5)-Ara*f*-(1→
2,3,4,6-Me_4-Gal	1.0	Gal*p*-(1→
2,4,6-Me_3-Gal	1.2	→3)-Gal*p*-(1→
2,3,4-Me_3-Gal	30.2	→6)-Gal*p*-(1→
2,3-Me_2-Gal	tr	→4,6)-Gal*p*-(1→
2,4-Me_2-Gal	17.8	→3,6)-Gal*p*-(1→

[a] 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-arabinitol, etc.

Sugar linkage analysis of AG (Table 3) revealed the prevalence of 6- and 3,6-linked galactopyranose residues. Arabinose residues were found to occupy mostly the terminal positions in both furanose and pyranose forms and as 2-, 3-, 5- and 2,5-linked residues in the side chains.

The linkage pattern (1,3 or 1,6) of AG was determined after the elimination of arabinosyl side chains by mild acid hydrolysis. Linkage analysis of the dearabinosylated polymer afforded 2,3,4-tri-*O*-methyl-galactose as the main product and indicated the 1,6-linked backbone in AG. The ^{13}C DEPT NMR spectrum showed a downfield shifted C-6 signal at δ 70.4 and confirmed the 1,6-galactan core in the native AG.

The ^{13}C NMR spectrum of AG (Fig. 1B) showed signals at δ 110.2–108.3 and 104.3–103.4 due to C-1 resonances of α-L–arabinofuranosyl and β-D-galactopyranosyl residues, respectively, at δ 88.2 – 81.0 derived from skeletal carbons involved in linkages (C-2 and C-3 of Ara, and C-3 of Gal), at δ 78.5–61.8 due to skeletal carbons[6] and at δ 57.1 generated by the resonances of methoxyl[5] groups.

In conclusion, a highly branched α-L-arabino-β-3,6-D-galactan consisting of 1,6-linked galactopyranose backbone carrying α-L-arabinosyl side chains and methoxyl groups at C-3 has been isolated from the aerial parts of *S. officinalis* L. The occurrence of arabinogalactans with the β-1,6-galactan core branched at C-3 by α-L-arabinosyl side chains has been described in some plant sources.[6–8] However, the highly methylated α-L-arabino-β-3,6-D-galactan was not reported even if the 3-*O*-methyl-D-galactose was identified in some fungi,[9] in red alga,[10,11] in green alga,[12] in leaves of lycophytes[13] and in some higher plants.[14,15]

1. Experimental

1.1. Plant material and general methods

The medicinal plant *S. officinalis* L. was purchased from Slovakofarma, Medicinal plants, Malacky, Slovakia. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C. The quantitative determination of the neutral sugars was carried out in the form of their trifluoroacetates[16] by gas chromatography (GLC). The uronic acid content was determined with the 3-hydroxybiphenyl reagent.[17] Gas chromatography–mass spectrometry of partially methylated alditol acetates[18] was effected on a FINNIGAN MAT SSQ 710 spectrometer equipped with a SP 2330 column. The absolute configuration of monosaccharides was established by the method of Gerwig et al.[19] HPLC measurement was performed with a LC-10AD pump (Shimadzu, Japan) equipped with a refractive index detector.

1.2. Extraction and fractionation

Crude mixture of polysaccharides (PS) was extracted as described.[2] Material (PS; 1 g) was dissolved in distilled water (50 mL) and applied to a column (8 × 20 cm) of DEAE-Sephacel and eluted successively with water (PSA_1), 0.1 M (PSA_2), 0.25 M (PSA_3), 0.5 M (PSA_4) and 1 M (PSA_5) NaCl solutions, and finally with 1 M NaOH (PSA_6). Fractions of 10 mL were collected and analyzed for the carbohydrate content by the phenol–sulphuric acid assay.[20] Pooled fractions were dialyzed and freeze-dried.

Treatment of a solution of PSA_1 in distilled water with the Fehling reagent[21] forms a suspension, which on centrifugation gives the glucomannan–copper complex (GM) as the pellet and the arabinogalactan (AG) as the supernatant. Polysaccharides were recovered from the pellet and the supernatant as described.[21]

1.3. Hydrolysis and chromatography

The polysaccharide sample PSA_1 (40 mg) was hydrolyzed with 2 M TFA (5 mL) at 120 °C for 1 h. TFA was evaporated, and the hydrolyzed mixture was subjected to preparative paper chromatography by the descending method on Whatman 3MM in the solvent system S, 8:2:1 ethyl acetate–pyridine–water. A part of the paper was stained with anilinium hydrogenphthalate, and monosaccharides eluted from the rest part with water were freeze-dried.

The portion of polysaccharide PSA_1 (100 mg) was partially hydrolyzed with 1 M TFA (10 mL) for 30 min at 100 °C. TFA was evaporated and the hydrolyzed mixture was separated on a column (200 × 2.5 cm) of Bio-Gel P-2 by water elution. Fractions of 4 mL were collected and analyzed for the carbohydrate content.[20] The elution profile showed six fractions. The dominant components were the polymeric residue (1F) and monosaccharides (6F), whilst the other fractions (2-5F) were negligible.

1.4. Methylation analysis

The dry sample of polysaccharide (~5 mg) was solubilized in dry Me_2SO (1 mL) and methylated by the Hakomori method.[22] The methylated polysaccharide was purified using the Sep-Pak C_{18} cartridge (Waters Assoc.), hydrolyzed with 90% formic acid at 100 °C for 1 h and with 2 M trifluoroacetic acid at 120 °C for 1 h, reduced with sodium borodeuteride, acetylated and analyzed[18] by GLC–MS.

1.5. NMR spectroscopy

The sample was dissolved in 0.5 mL D_2O (99.99 atom %) in 5-mm tubes. ^1H and ^{13}C NMR spectra of carbohydrates were recorded at 25 °C, on a Bruker DPX AVANCE 300 spectrometer operating at 300 MHz for ^1H and 75.46 MHz for ^{13}C. The acetone was used as the internal standard (δ 2.225 ppm for ^1H and 31.07 ppm for ^{13}C). The following pulse programmes were used: 2D COSY, HSQC and HMBC.

Acknowledgements

The author is grateful to J. Alföldi for NMR measurements, E. Marková for technical assistance and to A. Graydon. This work was supported by the Slovak Grant Agency VEGA No. 2/0155/08 and VEGA Grant No. 2/613126.

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