Pergamon 0031-9422(95)00193-X

Phytochemistry, Vol. 39, No. 6, pp. 1377–1380, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

POLYSACCHARIDES FROM SEEDS OF *STRYCHNOS* SPECIES

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(*Received in revised form 7 February 1995*)

Key Word Index—*Strychnos nux-vomica*; *S. innocua*; Strychnaceae; seeds; galactomannan; galactan; arabinogalactan; polysaccharides; structural determination.

Abstract—The chemical composition of polysaccharide fractions from *Strychnos nux-vomica* and *S. innocua* seeds and comparison with those from *S. potatorum* seeds are reported. The structural features of the galactomannans from the three *Strychnos* species are also discussed.

INTRODUCTION

Galactomannans are a very important class of polysaccharides both for their industrial applications and for polysaccharide interaction studies [1, 2]. They are mostly found in the seeds of leguminous plants [1]. We recently described the occurrence of a galactomannan, as well as a galactan, in the coagulant polysaccharide fraction of *Strychnos potatorum* seeds [3]. Furthermore, we reported the coagulant properties of the polysaccharide fractions from *S. nux-vomica* and *S. innocua* seeds.

Few reports have been published on the polysaccharide composition of *Strychnos* seeds. Andrews [4], when describing a galactan from *S. nux-vomica* suggested the presence of a galactomannan and Dea and Morrison [1] reported the occurrence of polymers containing galactose and mannose. A mannogalactan has been extracted with alkali from *S. potatorum* seeds [5], while, to our knowledge, no data have been given on *S. innocua* seeds.

The present studies were carried out on polysaccharides extracted by water from seeds of *S. nux-vomica* and *S. innocua*, and by water and by alkali from *S. potatorum*, with a view to examining chemotaxonomic relationships.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of the polysaccharide fractions of both *S. nux-vomica* and *S. innocua* showed, as main signals, a doublet at δ5.02 (3.5 Hz), a broad singlet at δ4.76 and a doublet at δ4.62 (7.8 Hz). These signals have also been observed in the NMR spectrum of the *S. potatorum* polysaccharide fraction [3] and assigned to α-galactose and β-mannose of a galactomannan, and to a β-galactose of a galactan, respectively. The corresponding ^{13}C NMR anomeric signals at δ105.2 were assigned to β-galactose of a galactan and those at δ101.0 and 99.8, to β-mannose and α-galactose, respectively, of a galactomannan [3]. Integration of ^{1}H NMR signals established galactomannan/galactan ratios of 1:0.2 and 1:2 for *S. innocua* and *S. nux-vomica*, respectively. Minor signals in the proton spectra of both *S. nux-vomica* and *S. innocua*, indicated the presence of other polysaccharides; acid hydrolysis yielded arabinose and traces of rhamnose, in addition to galactose and mannose.

From the polysaccharide fractions of both *Strychnos* species, the galactan was separated from galactomannan by precipitation of the latter with Fehling's solution (see Experimental). Structural determination of galactomannans and galactans was achieved on the basis of acid hydrolysis, methylation analysis and ^{1}H and ^{13}C spectral data. In particular, the low-field shift of the C-4 signal of galactans suggested a linear chain of 1,4-linked β-D-galactopyranosyl residues [3], in agreement with the occurrence of only 2,3,6-tri-*O*-methylgalactopyranose in the methylation of purified galactan.

In all cases, the galactomannans are made up of a main chain of 1,4-linked β-D-mannopyranosyl residues bearing terminal α-D-galactopyranosyl units linked at the O-6 position of some mannose residues. Very similar man:gal ratios were found for all three *Strychnos* species both by ^{1}H NMR integration of pertinent anomeric signals and by acid hydrolysis. This was confirmed by the finding of similar ratios (2.5–2.9) between methylated sugars (Table 1).

It is well known [1] that the ability of galactomannan to produce gels by interaction with other polysaccharides depends on the level and distribution of galactosyl substitution along the mannose backbone. The side-chain distribution has been studied by chemical [6, 7], enzymatic [8, 9] and ^{13}C NMR spectroscopic methods [10–12]. We have applied this latter technique to non-degraded polysaccharides and measured spectra at 70° and in the

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Table 1. Molar ratios of methylated monosaccharides from *Strychnos* galactomannans

Methylated monosaccharide	*S. nux-vomica*	*S. innocua*	*S. potatorum**
2,3,4,6-Tetra-*O*-methylgalactopyranose	1.0	1.0	1.0
2,3,6-Tri-*O*-methylmannopyranose	1.7	1.8	1.5
2,3-Di-*O*-methylmannopyranose	1.0	1.1	1.0

*Data from ref. [3].

presence of LiCl in order to reduce viscosity [13]. The splitting of the ^{13}C signal of the C-4 mannose units of galactomannans of the three species due [10] to the diads I, II and III is shown in Fig. 1. The low-field signal arises from groups (I), made up of two branched mannopyranosyl residues. The intermediate signal originates from diads (II), where only one of the mannose units is branched, and that at higher field from diads (III), built up of two unsubstituted mannose units [10]. The relative areas of these signals are representative of the frequencies of diads and suggested a random arrangement of D-galactosyl units for all of the three galactomannans, with a higher frequency of unsubstituted diads in *S. innocua.*

Polysaccharides containing arabinose residues (*ca* 10% of total polysaccharide fraction), remained in solution on addition of Fehling's solution. They were constituted by galactose, arabinose and rhamnose in ratios of 5.1:4.0:1.0 and 11.0:7.0:1.0, respectively, for *S. nux-vomica* and *S. innocua.* These fractions appeared as homogeneous peaks by gel-filtration chromatography. However, no further tests were performed to check on their chemical homogeneity. ^{1}H and ^{13}C NMR data of anomeric signals for *S. nux-vomica* and *S. innocua* are indicated in Table 2. An assignment of the relevant peaks has been achieved on the basis of the chemical shifts of anomeric carbons [14–16]; C, H COSY and $^{1}J_{H,C}$ values [17] were measured from a DEPT-coupled spectra.

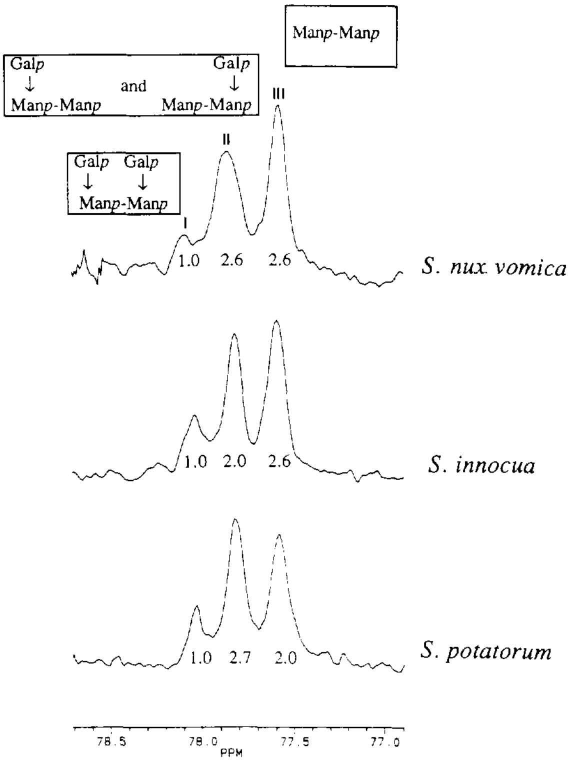

Fig. 1. C-4 Man*p* chemical shift region of ^{13}C spectra (100 MHz) of *Strychnos* galactomannans.

The large values for $^{1}J_{C,H}$ for the ^{13}C chemical shifts at 110.1, 108.6 and 101.0 ppm, allowed us to assign the first two resonances to α-Ara*f* and the third to α-Rha*p*. The signal at 105.2 ppm, was correlated with the proton doublet at δ4.62 (7.8 Hz) and can be attributed to a galactan impurity [3]. The remaining signal at 104.6 ppm is actually the centre of a very broad signal, and may be due to a chemical shift spread, but must be assigned to β-galactose on the basis of its $^{1}J_{C,H}$ value. These data allowed us to rule out the presence of arabinopyranose, because the ^{13}C anomeric signal at 102 ppm for the β-anomer [18], which is the usual configuration found in arabinogalactan [19], is missing.

In accordance with the data of Table 3, the intense low-field ^{13}C NMR signal at 83.2 ppm in the native polysaccharide spectra indicates, on the evidence of glycosylation shifts, the involvement of many O-3 positions in β-galactopyranosyl residues in glycosidic linkages. Furthermore, DEPT spectra showed low-field methylene signals at 67.5 and 70.2 ppm, indicating glycosidic linkages at oxymethylene positions. The signal at 108.6 ppm can be assigned [14] to the C-1 of terminal Ara*f* groups linked to other arabinose units on the basis of its occurrence at higher field than 110.1 ppm, which is attributed to terminal Ara*f* linked to galactose units.

Table 2. Correlation between anomeric NMR signals by one-bond C,H 2D-NMR

^{13}C δ	^{1}H δ($^{3}J_{H,H}$ Hz)	$^{1}J_{C,H}$ Hz	Assignment
110.1	5.29 *s*	174	α-Ara*f*
108.6	5.11 *s*	177	α-Ara*f*
101.0	4.89 *s*	171	α-Rha*p*
104.6*	4.73†	164	β-Gal*p*
105.2	4.62 *d* (7.8)	164	β-Gal*p*
104.6*	4.54‡	164	β-Gal*p*

*Broad signal.

†*d* (6.3 Hz) for *S. innocua* and broad singlet for *S. nux-vomica.*

‡Centre of a pair of doublets partially superimposed.

Table 3. Methylation data for *Strychnos* arabinogalactans

Sugar derivative	Mode of linkage	*S. innocua*	*S. nux-vomica*
2,3,4-Me_3-Rha	Rha*p*-(1 →	0.7	1.9
2,3,5-Me_3-Ara	Ara*f*-(1 →	1.6	1.8
2,3-Me_2-Ara	→ 5)-Ara*f*-(1 →	1.4	3.2
3-Me-Ara	→ 2,5)-Ara*f*-(1 →	0.4	2.3
2,4,6-Me_3-Gal	→ 3)-Gal*p*-(1 →	1.0	1.0
2,4-Me_2-Gal	→ 3,6)-Gal*p*-(1 →	2.1	1.3
2,3,6-Me_3-Gal	→ 4)-Gal*p*-(1 →	0.2	0.1

The presence of 3,6-linked Gal*p* and 3-linked Gal*p* suggested an arabinogalactan structure of type II [19], where a backbone of 1,3-linked galactan bears branches with terminal Rha*p*, Ara*f* or Ara*f*(1 → 5) Ara*f*(1 → at the O-6 positions). In agreement with this suggestion, the ^{13}C NMR DEPT spectrum of the fraction obtained by mild acid hydrolysis of the arabinogalactan polysaccharide, showed relevant signals at 104.8 and 82.9 ppm attributable to the anomeric carbon and to the C-3 of 3-linked β-galactose unit [20], respectively.

A lower proportion of methylated galactose derivatives (Table 3) was recovered than expected from the proportions of galactose found in hydrolysates. Accordingly, from methylation of *S. nux-vomica* some unmethylated polysaccharide was recovered which had galactose and arabinose in the ratio of 4.6:1. This result is difficult to explain, but might indicate heterogeneity of the arabinogalactan fraction.

Some interesting differences are apparent in the composition of the seed polysaccharide fractions of the three *Strychnos* species. In particular, *S. innocua* shows a galactan/galactomannan ratio of 0.2 that is inverted with respect to those of 1.7 and 2.0 found for *S. potatorum* [3] and *S. nux-vomica*, respectively. In addition, the arabinogalactan was found only in trace amounts in *S. potatorum* seeds.

In a control experiment, *S. potatorum* seeds were extracted with 10% NaOH using the condition reported by Rao and Rao [5]. The polysaccharide fraction so isolated showed ^{1}H and ^{13}C NMR spectra in agreement with a mixture of galactan and galactomannan, but no evidence of terminal mannopyranose or 3,4-linked galactopyranose as previously suggested [5].

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively, using a dual probe at 70° in the FT mode. The DEPT experiment was performed using a polarization transfer pulse of 135° and a delay adjusted to an average C,H coupling of 160 Hz. ^{13}C and ^{1}H chemical shifts were measured using 1,4-dioxane (δ67.4 from TMS) and sodium 3-(trimethylsilyl)-propionate-d_4 as internal standards, respectively. ^{13}C NMR of galactomannan were performed in a 5% LiCl/D_2O soln at 70°. Optical rotations were determined in H_2O.

Samples of the polysaccharide were hydrolysed [21] with 2 M TFA at 120° for different times (1, 2 and 4 hr). Neutral sugars in hydrolysates were analysed as alditol acetates by FID GC on an SP 2330 capillary column (Supelco, 30 m × 0.25 mm i.d., flow rate 1 ml min^{-1} at 235°, N_2 as carrier gas). Molar ratios of sugars were determined using *myo*-inositol as int. standard with the appropriate response factors.

Samples of polysaccharides were methylated by a modified Hakomori procedure [22, 23]. Methylated products were recovered by Sep-Pak filtration [24] and hydrolysed with acid. Methylated products in hydrolysates were reduced with $NaBD_4$, acetylated and analysed by GC-MS on an SP–2330 capillary column (Supelco, 30 m × 0.25 mm i.d., flow rate 0.8 ml min^{-1}, He as carrier gas), with the temp. prog.: 80° for 2 min, 80° to 170° at 30° min^{-1}, 170° to 240° at 4° min^{-1}, 240° for 15 min [25]. GC of methylated alditol acetates was carried out on a column identical to that used for GC-MS, with a flow rate of 1 ml min^{-1}, using effective carbon response factors [26] and normalizing peak areas with respect to that of *myo*-inositol hexaacetate used as int. standard. Unmethylated arabinogalactan fractions were sepd from methylated ones by elution with H_2O from Sep-Pak, dialysed, freeze-dried and analysed for monosaccharide composition after acid hydrolysis. Apparent M_rs were estimated by gel-filtration, using dextran standards (Fluka).

Extraction and isolation of polysaccharide fractions. Seeds of *S. potatorum*, *S. innocua* and *S. nux-vomica* originated from India and were identified by Prof. G. Aliotta (Botanical Garden, Naples, Italy). Seed powder (1 g) was stirred in distilled H_2O (50 ml) for 3 hr at room temp. After centrifugation, the supernatant soln was stirred with an equal vol. of a 1:1 mixt. of 10 mM, pH 7.5 TRIS-satd phenol and $CHCl_3$. The aq. layer was removed, washed in $CHCl_3$ and mixed with EtOH (2.5 vols). The polysaccharide fraction was precipitated overnight at −20°, washed in EtOH and dried in a vacuum desiccator.

Fractionation. Crude polysaccharide frs (100 mg) were dissolved in H_2O and Fehling's solution [27] added until precipitation of the galactomannan-copper complex was just complete. The ppt. was collected by centrifugation, washed with H_2O and decomposed by maceration for 1 min at 0° with EtOH containing 5% (v/v) of conc. HCl.

The residue was washed with EtOH until the washings gave a negative test for chloride. Increase of the galactomannan/galactan ratio was checked by ^1H NMR. The above procedure was repeated several times, until a galactomannan, containing *ca* 5% of galactan, from both *S. innocua* (60 mg) and *S. nux-vomica* (20 mg) was obtained. Physical data of galactomannas: from *S. innocua* $[\alpha]_D = +40^\circ$ (*c* 0.05) M_r 150 000; from *S. nux-vomica* $[\alpha]_D = +27^\circ$ (*c* 0.1), M_r 65 000.

Supernatant liquors of Fehling's soln treatments were collected, neutralized with 2 M HCl and dialysed against tap H_2O for 24 hr. The dialysate was freeze-dried and chromatographed on Bio-Gel A-0.5m (BIO-RAD), using 50 mM (pH 5.2) NaOAc as eluant. The chromatographic profile, revealed by the phenol test, showed two peaks. The higher M_r fr. proved to be the arabinose-containing fr., the other was galactan. Physical data of arabinogalactans and galactans: from *S. innocua* $[\alpha]_D = -4.6^\circ$ (*c* 0.11) and $[\alpha]_D = +59^\circ$ (*c* 0.11), M_r 80 000, respectively, from *S. nux-vomica* $[\alpha]_D = -10.4^\circ$ (*c* 0.12), $M_r = 800 000$ and $[\alpha]_D = +90^\circ$ (*c* 0.07) $M_r = 15 000$, respectively.

Partial acid hydrolysis of arabinogalactan fraction. A sample of arabinogalactan fraction from *S. nux-vomica* (10 mg) was treated with 0.05 M TFA (0.5 ml) at 90° for 2 hr [14] and the soln dried by repetitive codistillation with *iso*PrOH. The sample obtained was dialysed (cut-off = 1000) and freeze-dried.

Alkaline extraction of S. potatorum. Seed powder (1 g) was extracted [5] with 10% NaOH (2 × 10 ml). The combined extracts were filtered, neutralized with 2 M HCl and poured into 3 vols of Me_2CO. The precipitated polysaccharide was centrifuged, washed with Me_2CO and then with EtOH. The crude polysaccharide was suspended in H_2O, dialysed and lyophilized. ^1H and ^{13}C NMR showed the same signals of galactan and galactomannan as those displayed by the material obtained by H_2O extraction.

Acknowledgements—NMR spectra were recorded at the Centro Interdipartimentale di Metodologie Chimico-Fisiche, University of Naples Federico II. This work was supported by M.U.R.S.T. and CNR, Rome.

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