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Carbohydrate Research 321 (1999) 128–131

CARBOHYDRATE RESEARCH

Note

A new trisaccharide, α-D-glucopyranuronosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranose from *Chlorella vulgaris*

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Received 7 April 1999; accepted 30 June 1999

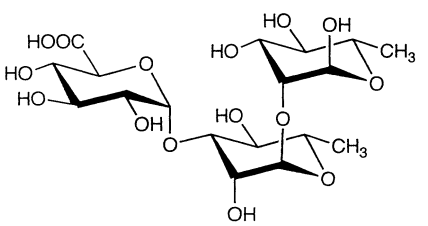
Abstract

A new acidic trisaccharide, α-D-glucopyranuronosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranose, was isolated from the hydrolyzate of an acidic polysaccharide, glucuronorhamnan, of *Chlorella vulgaris* K-22 cells.

Keywords: *Chlorella vulgaris*; Acidic polysaccharide; Glucuronorhamnan; Acidic trisaccharide; α-D-Glucopyranuronosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranose

1. Introduction

In 1972, White and Barber obtained an acidic polysaccharide [1] from the cell wall of the green alga, *Chlorella pyrenoidosa*, and a glucuronosylrhamnose was isolated from its acid hydrolyzate, but they did not describe the chemical structures of the disaccharide and the parent polysaccharide. We also isolated an acidic polysaccharide [2,3], rendered soluble from a commercial preparation of *Chlorella vulgaris* K-22 cells, that contains high contents of glucuronic acid and rhamnose. The isolation and structural elucidation of three constituents, 2-*O*-methyl-L-rhamnose, 3-*O*-methyl-L-rhamnose [2], and 3-*O*-α-D-glucopyranuronosyl-L-rhamnopyranose [3], have already been reported. A new acidic trisaccharide, glucuronosylrhamnosylrhamnose, has been isolated from a hydrolyzate of the acidic polysaccharide. The polysaccharide therefore seems to be a new type of plant polysaccharide, a glucuronorhamnan. In this paper, the isolation and structural determination of the new acidic trisaccharide in the glucuronorhamnan are reported, and the methylation analysis of the glucuronorhamnan is also described.



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0008-6215/99/$ - see front matter
PII: S0008-6215(99)00176-7

2. Results and discussion

The glucuronorhamnan was isolated from a defatted preparation of *C. vulgaris* K-22 cells using the method described in our previous paper [2]. Monosaccharide analyses indicated 14% glucuronic acid, and 86% rhamnose, arabinose, xylose, mannose, galactose, 2-*O*-methylrhamnose, and 3-*O*-methylrhamnose in the molar ratio of 25:2.3:1.7:1.0:3.9:2.1:1.2 as constituents of the glucuronorhamnan [2].

The configurations of D-glucopyranuronic acid and L-rhamnopyranose were determined by their optical rotation values. Several rhamnitol and glucitol derivatives were detected by methylation analysis after reduction with $NaBD_4$ [4–7], showing the presence of 2-*O*-, 3-*O*-, and 2,3-di-*O*-glycosylated rhamnose moieties, and a 4-*O*-glycosylated glucuronic acid moiety in the glucuronorhamnan, as shown in Table 1.

Partial acid hydrolysis of the glucuronorhamnan with 0.2 M trifluoroacetic acid, followed by column chromatography on anion-exchange resin and preparative paper chromatography, gave 3-*O*-α-D-glucopyranuronosyl-L-rhamnose [3], and a new acidic trisaccharide as a colorless solid. The positive- and negative-ion FABMS spectra of the trisaccharide showed *m/z* 487 $[M+H]^+$ and *m/z* 485 $[M-H]^-$, indicating a molecular formula $C_{18}H_{30}O_{15}$. As shown in Table 2, ^1H and ^{13}C chemical-shift assignments were made by standard 1D and 2D NMR techniques, such as DEPT, $^1H-^1H$ COSY, HMQC, and HMBC. The α anomeric configuration for the D-glucopyranuronic acid was assigned based on the coupling constant of H-1 ($J_{1,2}$ 3.9 Hz). The anomeric configuration of each L-rhamnopyranose moiety was determined to be α, by comparison of chemical-shift data for H-1 (a low-field shift in the α anomer), C-3, and C-5 (high-field shifts in the α anomer) [3,8–10]. There was no β anomeric proton signal for the reducing-end L-rhamnopyranose. The substituted sites in the two rhamnopyranose moieties were determined by their low-field shifts for C-3 (for the central rhamnose) and C-2 (for the reducing end). Thus, the structure of the acidic trisaccharide was elucidated to be α-D-glucopyranuronosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranose.

These series of glucuronosyl-rhamnose oligosaccharides are new ones that differ from such known galacturonosyl-rhamnose series as 2-*O*-α-D-galactopyranuronosyl-L-rhamnopyranose, which are widespread as constituents of plant polysaccharides, particularly of plant mucilages and gums (galacturonorhamnans) [11,12]. These results show that the acidic

Table 1
Substitution patterns of Rha*p* and Glc*p*A residues by methylation analysis of the glucuronorhamnan

Methylated residue	Retention time [a]	Molar ratio	Mode of linkage
2,3,4-Rha [b]	0.74	1.0	Rha*p*-(1→
3,4-Rha [b]	0.91	2.5	→2)-Rha*p*-(1→
2,4-Rha [b]	0.93	4.5	→3)-Rha*p*-(1→
4-Rha [b]	1.11	2.5	→2,3)-Rha*p*-(1→
2,3,6-GlcA [c]	1.31	3.1	→4)-Glc*p*A-(1→

[a] Relative to that of 1,5-di-*O*-acetyl-(1-deuterio)-2,3,4,6-tetra-*O*-methylglucitol.
[b] Peracetylated (1-deuterio)-tri, di, or mono-*O*-methylrhamnitol.
[c] Peracetylated (1,6,6-trideuterio)-2,3,6-tri-*O*-methylglucitol.

Table 2
^1H and ^{13}C NMR chemical shifts of α-D-Glc*p*A-(1–3)-α-L-Rha*p*-(1–2)-α-L-Rha*p* [a]

	Position	δ_C	δ_H	*J* (Hz)
α-D-Glc*p*A-	1	96.7	5.15 d	3.9
	2	72.2	3.64 dd	3.9, 10
	3	73.8	3.82 m	
	4	72.8	3.57 dd	9.5, 10
	5	72.5	4.38 d	10
	6	176.0		
-(1→3)-α-L-Rha*p*-	1	102.8	5.02 d	2
	2	68.0	4.28 dd	2, 3
	3	76.9	3.88 m	
	4	71.4	3.57 dd	9.5, 10
	5	70.3	3.82 m	
	6	17.8	1.30 d	6.4
-(1→2)-α-L-Rha*p* (Reducing end)	1	93.7	5.23 d	<1
	2	80.6	3.92 m	
	3	70.9	3.89 m	
	4	73.5	3.46 dd	9.5, 10
	5	69.6	3.84 m	
	6	17.9	1.27 d	6.4

[a] Spectra (δ, ppm) were measured in D_2O at 50 °C with a Jeol JNM-A500 spectrometer.

polysaccharide of *C. vulgaris* is a new type of plant polysaccharide, a glucuronorhamnan, or strictly, a glucuronorhamnoglycan, because a small proportion of methylated monosaccharides corresponding to 2,4-di-*O*-methylxylose and 2,4,6-tri-*O*-methylgalactose constituents, was also detected (data not shown). Interestingly, the backbone chain, consisting of 4-*O*-substituted α-D-glucuronic acid, 2-*O*-, 3-*O*- and 2,3-di-*O*-substituted α-L-rhamnose, is similar to that formed by substituting for galacturonic acid in galacturonorhamnoglycan [11,12]. Aqueous solutions of the glucuronorhamnan were slightly turbid and lacked viscosity, and therefore the polysaccaride seems to play a different role from a galacturonorhamnoglycan, and to be characteristic of green algae, because *C. pyrenoidosa* also appears to have this type of polysaccharide [1].

3. Experimental

General methods.—Melting points were measured with a Yanagimoto micro-melting point apparatus and were not corrected. Optical rotations were taken on a Perkin–Elmer 241 polarimeter. Mass spectra were determined with a Jeol JMS-SX102 mass spectrometer (FAB mode). ^{1}H NMR spectra were measured on a Jeol JNM-EX400 or JNM-A500 spectrometer using sodium 4,4-dimethyl-4-silapentanoate ($\delta = 0$) as an internal standard, and ^{13}C NMR spectra were recorded with 1,4-dioxane ($\delta = 67.4$), in D_2O at 50 °C. Ascending paper chromatography was carried out using Toyo No. 51A filter paper (Toyo Roshi Kaisha, Tokyo), eluted with 6:4:3 1-butanol–pyridine–water and detection with $AgNO_3$ reagent.

Preparation of acidic polysaccharide.—According to the method described in a previous paper [2], the purified acidic polysaccharide (0.45 g) was prepared from the dry cells of *C. vulgaris* K-22 (500 g, Chlorella Industry Co., Tokyo).

Isolation of the major components.—The acidic polysaccharide (100 mg) was heated with 0.5 M H_2SO_4 (100 mL) for 14 h at 100 °C. Preparative paper chromatography of the hydrolyzate gave glucuronic acid (R_f 0.09, 1.7 mg) and rhamnose (R_f 0.81, 15 mg). Optical rotation values: L-rhamnose, $[\alpha]_D^{20} + 13°$ (*c* 0.75, water) (lit. $[\alpha]_D + 8.9°$ [13]) and D-glucuronic acid, $[\alpha]_D^{20} + 31°$ (*c* 0.17, water) (lit. $[\alpha]_D + 36.3°$ [14]).

Methylation analysis.—An aq solution (2 mL) of the acidic polysaccharide (20 mg) was treated twice with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (20 mg) at pH 4.5–5.0 for 6 h, and then with $NaBD_4$ (10 mg) at pH 7–8 overnight [4]. The product was dialyzed against running water, and then lyophilized to give the 6,6-dideuterio-alditol derivative of the polysaccharide (5 mg). The product was methylated twice by Hakomori's method [5] and then treated using the method of Lindberg [6]. The partially methylated alditol acetates were analyzed by GC–MS on a Jeol JMS-AX505H mass spectrometer, using a Shimadzu CBP1 capillary column (50 m × 0.2 mm, 200 °C, carrier gas N_2) [7].

Isolation of the acidic trisaccharide.—The acidic polysaccharide (50 mg) was hydrolyzed with 0.2 M trifluoroacetic acid (10 mL) in a screw-capped tube for 2 h at 100 °C. The hydrolyzate from five tubes was collected and then evaporated to dryness. The residue was purified by column chromatography (15 × 270 mm) on Dowex 1-X8 resin (formate form, Dow Chemical Co., MI), eluting with a linear gradient of HCO_2H (0–2.0 M, 400 mL). Two fractions eluted with 0.60–0.69 and 0.70–0.86 M HCO_2H were collected, and concentrated to yield 17 and 39 mg of solids, respectively. Further purification of the former solid by preparative paper chromatography gave the pure acidic trisaccharide (R_f 0.33, 3.3 mg) as a colorless solid: mp 166–176 °C (dec.); $[\alpha]_D^{24} + 46°$ (*c* 0.3, water); FABMS (pos.): *m/z* 509 $[M + Na]^+$, 487 $[M + H]^+$; FABMS (neg.): *m/z* 485 $[M-H]^-$; ^{1}H and ^{13}C data, see Table 2. From the latter, 3-*O*-α-D-glucopyranuronosyl-L-rhamnose [3] (R_f 0.32, 11 mg) was obtained.

Acknowledgements

We thank Messrs Hisashi Horiuchi, Seiichi Tomita, Tetsuya Baba, and Yasuhiko

Kurosawa for assisting with the experimental work, and Mr Isao Maruyama of Chlorella Co. Ltd. for the *Chlorella* cell preparations. Thanks are also due to Dr Hiroshi Naganawa for the measurements of MS and NMR spectra.

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