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Note

A ^{1}H- and ^{13}C-n.m.r. study of an agar polysaccharide from *Bryothamnion triquetrum*

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Hot-water-soluble agar-type polysaccharides have been extracted from a variety of red algae (Rhodophyceae), mainly from species of Gelidium, Gracilaria, and Pterocladia[1]. Agarophytes synthesise cell-wall galactans containing distinctive disaccharide repeating-units of 3-linked β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactopyranose. The galactosyl residues in agar are unsubstituted or substituted with either methyl, carboxyethylidene, or sulfate groups[2].

Recently, the ^{13}C-n.m.r. spectra of red-seaweed galactans have been interpreted[3–7] mainly on the basis of comparison with monosaccharide reference compounds.

Bryothamnion triquetrum (Ceramiales) is very common in Cuban waters. Hot-water extraction of air-dried *B. triquetrum* yielded a viscous solution which gelled on cooling. Two successive extractions gave a total polysaccharide yield of 35%. All further work was conducted on the polysaccharide first-extract, which contained 4% of sulfate, 0.6% of total protein, and 3.3% of ash.

Acid hydrolysis of the polysaccharide gave (p.c.) galactose, 6-*O*-methylgalactose, and 5-hydroxymethyl-2-furaldehyde. The last component was detected by ^{1}H- and ^{13}C-n.m.r. spectroscopy, and it is presumed to be a decomposition product of the polysaccharide.

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TABLE I

^{13}C CHEMICAL SHIFTS (P.P.M.) OF AGAR-LIKE POLYSACCHARIDES

Disaccharide repeating-unit	*Residue*[d]	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OMe*
Neoagarobiose[a]	G	102.4	70.1	82.2	68.4	75.3	61.4	
	A	98.4	69.8	80.1	77.4	75.6	69.3	
6-*O*-Me-neoagarobiose[a]	G	102.4	70.1	82.2	68.6	73.6	71.8	59.1
	A	98.4	69.8	80.1	77.4	75.6	69.3	
2′-*O*-Me-neoagarobiose[b]	G	102.6	70.2	82.7	68.7	75.6	61.4	
	A	98.7	78.8	78.4	77.6	75.3	69.8	59.1
Bryothamnion triquetrum polysaccharide[c]	G	102.5	70.2	82.2	69.0	73.6	71.8	59.1
	A	98.3	70.0	80.2	77.4	75.7	69.4	
	G′	102.5	70.2	82.2	69.0	75.7	61.8	
	A′	98.8	79.0	78.7	77.4	75.7	69.4	59.1

[a]From ref. 6; in D_2O, at 80°. [b]From ref. 4; in D_2O, at 80°. [c]In D_2O, 80°. [d]G,A and G′,A′ refer to the main and minor β-D-galactopyranosyl and 3,6-anhydro-α-L-galactopyranosyl residues.

Quantification of the constituent sugars that were not acid-labile showed a molar ratio of galactose to 6-*O*-methylgalactose of 1:3.43. The low content of sulfate and the presence of 3,6-anhydrogalactose residues in the polysaccharide were confirmed by i.r. bands at 1240 (S=O stretching) and 930 cm^{-1} (*cf.* ref. 12).

Table I contains the ^{13}C-n.m.r. signals of the polysaccharide sample and some reference substances. The polysaccharide gave 13 strong signals, with the main resonances for anomeric carbons at 102.5 and 98.3 p.p.m. corresponding to 3-linked β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactopyranose in the neoagarobiose repeating-unit, respectively[6].

The low intensity of the signal at 61.8 p.p.m. indicated almost all of the β-D-galactopyranose residues to be substituted at O-6. The presence of an intense peak at 59.1 p.p.m. corresponds to an *O*-methyl group. The multiplicities of the lines were determined by an APT experiment[14] and the signal at 71.8 p.p.m. was assigned thus to C-6 of the 3-linked 6-*O*-methyl-β-D-galactopyranose.

The assignment of the other signals is based on comparison with the spectra of appropriate polysaccharides[4,6,7,13] and monosaccharides[8–10]. As contradictions have been found in the assignments of C-3 and C-5 of methyl 3,6-anhydro-α-D-galactopyranoside[8–10], its 2D heteronuclear-correlation spectrum was recorded (Fig. 1, see Experimental for the ^{13}C- and ^{1}H-n.m.r. data). The ^{1}H parameters and the assignments accord with previous data[11]. The ^{13}C assignment is identical to that given by Shashkov *et al.*[8] and Balza *et al.*[9], and opposite to that of Bock and Pedersen[10].

Besides 6-*O*-methylation, another explanation of the weak intensity of the signal at 61.8 p.p.m. may be the presence of a 6-sulfate group in the 3-linked β-D-

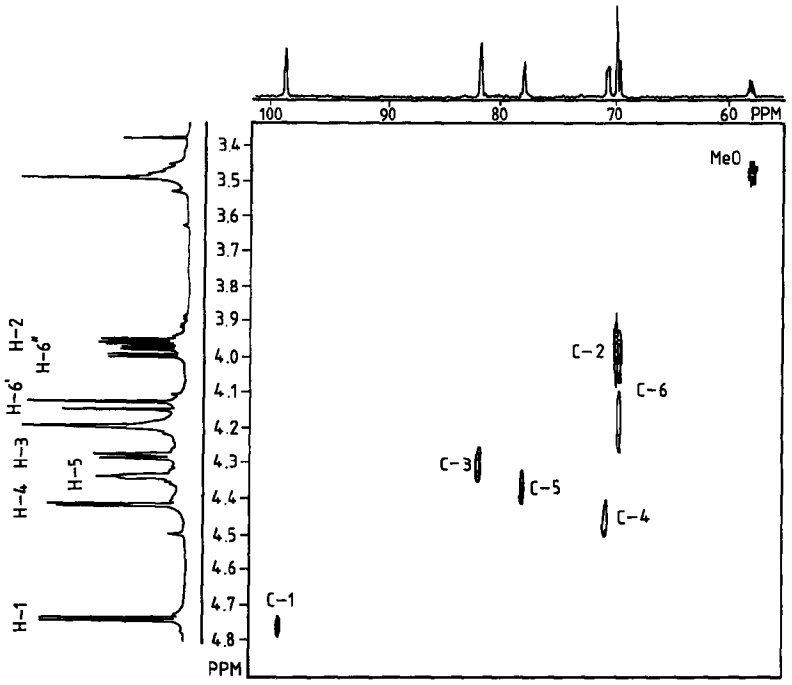

Fig. 1. ^{1}H–^{13}C 2D heterocorrelation spectrum of methyl 3,6-anhydro-α-D-galactopyranoside at 500 MHz; 80° in D_2O.

galactopyranosyl residue (signal at 67.9 p.p.m.)[6]. Of the minor ^{13}C signals given by the polysaccharide, the third signal in the region for anomeric carbons at 98.8 p.p.m. as well as those at 79.0 and 78.7 p.p.m. indicate the presence of a different *O*-methylated neoagarobiose repeating-unit containing 4-linked 3,6-anhydro-2-*O*-methyl-α-L-galactopyranose. This conclusion is supported also by the ^{1}H-n.m.r. spectrum of the polysaccharide (2 s at 3.50 and 3.48 p.p.m.).

Because of the types of substitution found (*i.e.*, 6- and 2-*O*-methyl groups), the present n.m.r. study does not indicate whether these substituents occur in the same disaccharide repeating-unit or not. However, the structure of the main component could be determined with confidence on the basis of the ^{13}C- and ^{1}H-n.m.r. data.

EXPERIMENTAL

Isolation of the polysaccharide. — The alga *B. triquetrum,* which was collected in February, 1985, on the north coast of Havana, was carefully hand-sorted, then air dried, milled, extracted exhaustively with ethanol, and dried. The seaweed (100 g, dry powder) was extracted with boiling water (3 L) for 4 h. Insoluble material was removed from the hot solution by using a cloth filter, and the filtrate was kept

for 16 h at −10°. The insoluble material was re-extracted as described above. The frozen extracts were thawed at room temperature and filtered, the solids were dried with ethanol and redissolved in boiling water, and the solutions were freeze-dried to give the polysaccharide (35%). The material in the first extract was redissolved in boiling water and the freeze–thaw operation was repeated five times. Lyophilisation of the purified gel gave the sample used for the n.m.r. studies.

Ash and sulfur contents were determined by the Microanalytical Laboratory of CENIC and total protein by the method of Lowry[15].

Descending p.c. was performed with 6:4:3 1-butanol–pyridine–water and detection with aniline hydrogen phthalate[16].

G.l.c. was performed with a Pye Series 204 chromatograph equipped with a flame-ionisation detector, a glass column (0.3 × 200 cm) packed with 3% of OV-225 on Chromosorb WHP (80–100 mesh), nitrogen as the carrier gas at 30 mL/min, and the temperature programme 180→200° at 2°/min.

Hydrolysis of polysaccharide fractions. — Hydrolysis was effected with (*a*) M sulfuric acid for 16 h at 100°, and (*b*) aqueous 90% formic acid for 1 h at 100°, followed by 0.1M sulfuric acid for 16 h at 100°. Method (*a*) was employed for the detection of 5-hydroxymethyl-2-furaldehyde.

Methyl 3,6-anhydro-α-D-galactopyranoside, prepared by the method of Ohle and Thiel[17], had m.p. 137–138°, $[\alpha]_D$ +80° (*c* 0.7, water); lit.[17] m.p. 139°, $[\alpha]_D$ +83.9° (*c* 0.82, water). N.m.r. data: ^{1}H, δ 3.99 ($J_{2,3}$ 5.3 Hz, H-2), 4.01 ($J_{5,6'}$ 3.1 $J_{6,6'}$ −10.7 Hz, H-6'), 4.17 ($J_{5,6}$ < 1.0 Hz, H-6), 4.31 ($J_{3,4}$ < 1.0 Hz, H-3), 4.37 ($J_{4,5}$ 2.0 Hz, H-5), 4.46 (H-4), 4.79 ($J_{1,2}$ 2.7 Hz, H-1); ^{13}C, δ 69.5 (C-6), 69.8 (C-2), 71.0 (C-4), 77.8 (C-5), 81.5 (C-3), 98.6 p.p.m. (C-1).

N.m.r. spectroscopy. — Spectra were obtained for solutions in D_2O at 80°. ^{13}C-N.m.r. spectra were recorded with Varian XL-100-FT-15 and JEOL FX-90-Q spectrometers. Chemical shifts were measured relative to that of internal Me_2SO (39.7 p.p.m.). ^{1}H-N.m.r. spectra were recorded with a Bruker WM-250 spectrometer. The 2D experiment was performed on Bruker AM-500 equipment.

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