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Note

Polysaccharides of the red seaweed *Rhodymenia pertusa* Part I. Water-soluble glucan

J. N. C. WHYTE

Fisheries Research Board of Canada, Vancouver Laboratory, 6640 N.W. Marine Drive, Vancouver 8, B.C. (Canada)

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The Pacific coast, red seaweed *Rhodymenia pertusa*, which is of the same family as the commercially available "dulse" (*R. palmata*), is being studied to appraise its potential commercial worth. In this communication, studies of a glucan isolated from *R. pertusa* are reported. Glucans, known as floridean starches, have been isolated from other algae[1].

Solvent-treated[2] *R. pertusa* was exhaustively extracted with hot water to afford a mixture of polysaccharides shown by chromatography on DEAE-Sephadex to consist of a glucan and a sulphated galactan. The isolation of water-soluble xylans from the familial *R. palmata*[3] contrasts with their absence in *R. pertusa*, and, on the basis of polysaccharide composition, these algae would be taxonomically dissimilar[4].

Removal of the sulphated galactan (by precipitation with hexadecyltrimethylammonium bromide) from the isolated polysaccharide mixture afforded a polysaccharide which on hydrolysis gave only glucose (estimated 86%). Attempts to purify this polysaccharide by the Sevag procedure or by extraction with aqueous trichloroacetic acid were unsuccessful. However, elution of the impure glucan from DEAE-Sephadex provided a pure polysaccharide containing an estimated 100.1% of D-glucose [characterised as 2,3,4,6-tetra-*O*-acetyl-*N*-(*p*-nitrophenyl)-β-D-glucopyranosylamine].

The glucan consumed 1.06 moles of sodium periodate and released 75 mmoles of formic acid per anhydrohexose unit when oxidised in buffered solution. Reduction and hydrolysis of the polyaldehyde obtained on oxidation afforded glycerol and erythritol, in the molar proportion of 1:12. Glucose was not detected in the hydrolysis products of the polyalcohol, and this substantiated the periodate-consumption figures which indicated the absence of (1→3)-linked glucose residues.

Treatment of the glucan with the Haworth[5] and Kuhn[6] reagents furnished a methylated polysaccharide (OCH_3 44.6%), a portion of which was hydrolysed, reduced, and acetylated to yield partially methylated alditol acetates subsequently analysed by gas–liquid chromatography and mass spectrometry[7]. A proportion of 2,6-di-*O*-methyl-D-glucose in the hydrolysate indicated incomplete substitution with the reagents employed and the degree of difficulty encountered in fully methylating

O-3 of the constituent glucose units. Further methylation with the Hakomori reagents[8] provided a fully methylated derivative containing 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-glucose in the molar proportions 1:11.5:1, providing evidence for a structure with twelve to thirteen (1→4)-linked-D-glucopyranose residues for each residue bearing a branch point at C-6.

The p.m.r. spectra of the methylated derivatives of the glucan, amylopectin, and amylose in benzene-d_6–chloroform-*d* (6:1) are illustrated in Fig. 1. The signals at τ 4.3 in all spectra were attributed to the anomeric protons, and this assignment was substantiated by the resonance of H-1 of the non-reducing D-glucose moiety of methyl hepta-*O*-methyl-α- and -β-maltoside at τ 4.29 and 4.34. respectively. In spectrum *C*, the doublet at τ 4.3 had a spacing of 3.6 Hz, whereas the corresponding signals in spectra *A* and *B* derived from the ramified structures were less well-resolved. Had the methylated glucan contained a substantial amount of β-D-glycosidic linkages, a signal would have been expected in the τ 5.6 region of the spectrum, since H-1 in the non-reducing D-glucose moiety of methyl hepta-*O*-methyl-α- and -β-cellobioside resonates at τ 5.60 and 5.59, respectively.

A lack of resolution in the high-field portion of these spectra can be attributed

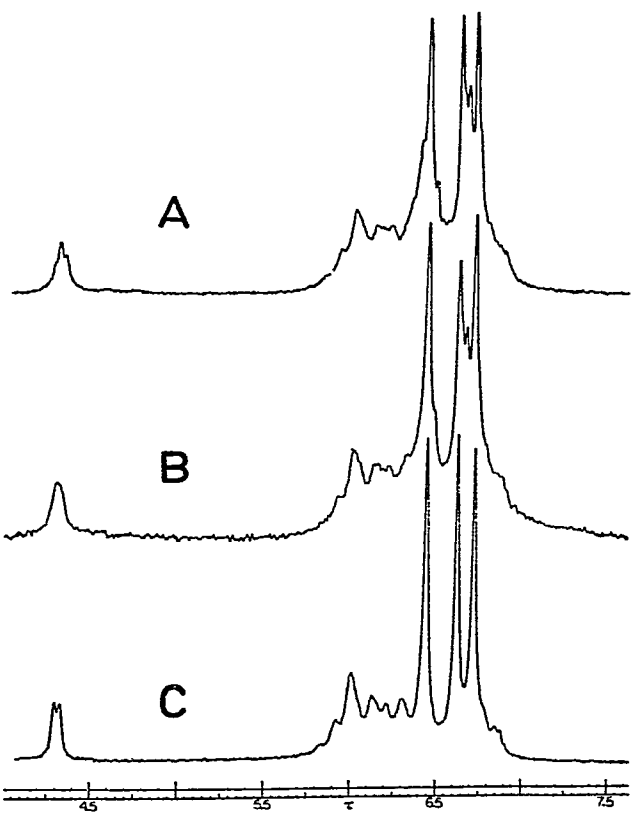

Fig. 1. P.m.r. spectra (100 MHz) in benzene-d_6–chloroform-*d* (6:1) of the methylated derivatives of *A*, Glucan from *R. pertusa*; *B*, amylopectin from waxy maize; *C*, amylose from potato.

to the viscous nature of the polymers in the solvent mixture which, nevertheless, separated the methoxyl signals; those at τ 6.44, 6.62, and 6.72 were ascribed[9] to the, methoxyl groups at C-3, C-2, and C-6, respectively. The additional signals at τ 6.49 and 6.67, in the similar spectra *A* and *B* of the glucan and amylopectin, are derived from the non-reducing, end-group residues and the residues having branch points at C-6.

A comparison of the properties of branched α-D-glucans[10] indicates that the glucan from *R. pertusa* has a structure more closely resembling glycogen and the phytoglycogen from *Zea Mays* than that of amylopectin.

EXPERIMENTAL

General methods. — Gas–liquid chromatography (g.l.c.) was carried out on a column (170 × 0.45 cm) containing 0.2% poly(ethylene glycol adipate), 0.2% poly-(ethylene glycol succinate), and 0.4% silicone XF-1150 on DMCS treated Chromosorb W (60–80 mesh) at 150° with a gas flow-rate of 60 ml of nitrogen per min. Retention values T_F and T_G are given relative to the chromatographic mobility of 6-deoxy-L-galactitol penta-acetate and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, respectively. Proton magnetic resonance (p.m.r.) spectra were obtained with a Varian HA-100 spectrometer, and the mass spectra were obtained with a Nuclide spectrometer.

Isolation of polysaccharides. — Seaweed (4750 g, fresh weight) was exhaustively extracted in a Waring Blendor with chloroform–methanol–water (20.1). The resulting material (430 g) was extracted with water (150 l) at 90° and centrifuged, and the supernatant was concentrated and poured into ethanol to give crude polysaccharide, containing galactose, glucose, and a trace of 6-*O*-methylgalactose. A sample of the polysaccharide was chromatographed on DEAE-Sephadex (A-25, 100 g), with 0.5, 1.0, 1.5, and 2.0M sodium chloride at pH 2.0, to afford a polysaccharide fraction containing glucose, which was eluted with 0.5M sodium chloride, and a second fraction composed of galactose and sulphate ions, which was eluted with 2.0M sodium chloride.

Separation of the glucan. — The polysaccharide mixture (32 g) in water (2.5 l) was treated with hexadecyltrimethylammonium bromide (40 g). The resultant precipitate was removed, and the supernatant and washings were concentrated, dialysed, and freeze-dried to afford crude glucan (1.1 g) (estimated 86% of glucose), $[\alpha]_D$ +136° (*c* 1.0, water); iodine complex λ_{max} 525 nm. Impure polysaccharide (250 mg) was added to a column of DEAE-Sephadex (A-25, 100 g, chloride form), and elution with water gave glucan (190 mg), $[\alpha]_D$ +177° (*c* 1.0, water). This operation was repeated to afford pure polysaccharide (650 mg total, estimated 100.1% of glucose); iodine complex λ_{max} 500 nm. The hydrolysate of this material (50 mg), on treatment with *p*-nitroaniline followed by acetylation, yielded 2,3,4,6-tetra-*O*-acetyl-*N*-(*p*-nitrophenyl)-β-D-glucopyranosylamine (40 mg), m.p. and mixed m.p. 182–183°, $[\alpha]_D$ −101° (*c* 1.4, chloroform).

Periodate oxidation of the glucan. — The glucan (10.8 mg) was oxidised with

30 mM sodium periodate (10 ml) at 5°, and the consumption was followed spectrophotometrically[11] to a constant value of 1.06 moles per anhydrohexose unit after 170 h. Oxidation of a sample of glucan (21 mg) with 0.3M sodium periodate (12.5 ml) in aqueous sodium chloride (3%, 12.5 ml) at 1° produced 75 mmoles of formic acid per anhydrohexose unit. The resulting polyaldehyde was reduced and hydrolysed, and the derived acetates of glycerol (T_F 0.17) and erythritol (T_F 0.61) (no glucitol) were analysed by g.l.c.; the parent compounds were estimated to be in the molar ratio 1:12.

Methylation studies. — The glucan (250 mg) was methylated successively with methyl sulphate–sodium hydroxide and *N,N*-dimethylformamide–methyl iodide–silver oxide to give methylated polysaccharide (300 mg; OCH_3, 44.6%). A portion of the methylated polysaccharide (40 mg) was hydrolysed with 90% formic acid for 1 h at 100°, followed by 0.25M sulphuric acid for 16 h at 100°. The acid was neutralised, and the sugars were converted into alditol acetates and analysed by combined g.l.c.–mass spectrometry. Fractions with retention values T_G 1.0, 2.6, 4.1, and 5.9 were shown to be derived from 2,3,4,6-tetra-, 2,3,6-tri-, 2,6-di-, and 2,3-di-*O*-methyl-D-glucose. Controlled hydrolysis of tetra- and tri-*O*-methyl-D-glucose indicated that the 2,6-di-*O*-methyl derivative resulted from incomplete substitution of the polymer and not from demethylation. Treatment of the undermethylated glucan (200 mg) with methylsulphinyl carbanion and methyl iodide afforded a derivative (207 mg; OCH_3, 45.6%), $[\alpha]_D$ +200° (*c* 2.1, chloroform), containing 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-glucose in the molar proportions 1:11.5:1.

Fully methylated, waxy-maize amylopectin {$[\alpha]_D$ +218° (*c* 2.0, chloroform); OCH_3, 45.5%}, prepared in a similar manner, contained 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methyl-D-glucose in the molar proportions 1:21:1. Similarly methylated, potato amylose {$[\alpha]_D$ +212° (*c* 2.2, chloroform); OCH_3, 45.5%} on hydrolysis gave 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-glucose in the molar ratio 1:421.

P.m.r. studies. — The spectrum of methyl tetra-*O*-methyl-α-D-glucopyranoside in the solvent mixture benzene-d_6–chloroform-*d* (6:1) was in accord with Gagnaire[9], and that of 2,3,4,6-tetra-*O*-methyl-α-D-glucose in the same solvent exhibited 4 methoxyl signals at τ 6.76 (C-6), 6.67 (C-2), 6.54 (C-4), and 6.40 (C-3). Maltose was methylated, and the product was separated by preparative t.l.c. to give methyl hepta-*O*-methyl-α-D-maltoside which provided a spectrum in the solvent mixture exhibiting signals at τ 5.33 (H-1, $J_{1,2}$ 3.6 Hz) and 4.29 (H-1′, $J_{1',2'}$ 3.6 Hz); the corresponding β-glycoside had signals at τ 5.92 (H-1, $J_{1,2}$ 7.6 Hz), 4.34 (H-1′, $J_{1',2'}$ 3.6 Hz). Methylation of cellobiose yielded a mixture of methyl hepta-*O*-methyl-α- and β-cellobioside (1:2 by integration) whose spectrum exhibited anomeric signals for the α-glycoside at τ 5.31 (H-1, $J_{1,2}$ 3.6 Hz) and 5.60 (H-1′, $J_{1',2'}$ 7.3 Hz), with the corresponding β-glycoside signals at τ 5.88 (H-1, $J_{1,2}$ 7.5 Hz) and 5.59 (H-1′, $J_{1',2'}$ 7.3 Hz).

Intrinsic viscosity measurements. — The viscosities of glycogen (oyster) and the glucan in water, and amylopectin in 0.5M sodium hydroxide, were measured by using an Ostwald viscometer at 25°. Values of $[\eta]$ obtained for the glucan, glycogen, and amylopectin were 8, 6, and 150, respectively.

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REFERENCES

1 H. OZAKI, M. MAEDA, AND K. NISIZAWA, *J. Biochem* (Tokyo), 61 (1967) 497, and references therein cited.
2 J. N. C. WHYTE AND B. A. SOUTHCOTT, *Phytochemistry*, 9 (1970) 1159.
3 H. BIORNDAL, K. E. ERIKSSON, PER J. GAREGG, B. LINDBERG, AND B. SWAN, *Acta Chem. Scand.*, 19 (1965) 2309.
4 L. STOLOFF AND P. SILVA, *Econ. Botany*, 11 (1957) 327.
5 W. N. HAWORTH, *J. Chem. Soc.*, (1915) 8.
6 R. KUHN, H. TRISCHMANN, AND I. LÖW, *Angew. Chem.*, 67 (1955) 32.
7 H. BIORNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohyd. Res.*, 5 (1967) 433.
8 S. HAKOMORI, *J. Biochem.* (Tokyo), 55 (1964) 205.
9 D. GAGNAIRE AND L. ODIER, *Carbohyd. Res.*, 11 (1969) 33.
10 C. T. GREENWOOD AND J. THOMSON, *J. Chem. Soc.*, (1961) 1534.
11 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.* (London), (1957) 1216.