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THE POLYSACCHARIDES OF THE BROWN SEAWEED *Turbinaria murrayana*

MOHAMED M. EL-SAYED

Chemistry Department, Faculty of Marine Science, King Abdul Aziz University, Jeddah, P.O. Box 1540 (Saudi Arabia)

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ABSTRACT

Polysaccharides of the brown seaweed *Turbinaria murrayana* were isolated. Laminaran (3.2%) was isolated from the hot-water extract of the algae by using ion-exchange chromatography. Fucans (2.1%) were isolated from the hot-water extract, as well (4.7%) as from the extract of the algae with dilute acid. Acid hydrolysis of the isolated fucans revealed glucose, mannose, fucose, glucuronic acid, xylose, and galactose. Alginic acid (22.6%) was separated, and reduced to a neutral polysaccharide. The polysaccharides isolated were analyzed by methylation and Smith degradation.

INTRODUCTION

Turbinaria murrayana, an alga belonging to the Phaeophyceae, is commonly found in tropical waters. It has not been investigated chemically before, except for studies by Kalimuthu and co-workers, who examined the seasonal variation in some constituents and its relation to changes in growth[1,2].

The major components of seaweed are carbohydrate in nature, and thus the use of seaweed in food and industry is due to its carbohydrate content and, especially, to such polysaccharides as agar and algin. The characteristic polysaccharides of the brown seaweed are reserve polysaccharides, such as laminaran, and structural mucilages serving as membrane-thickening materials, such as alginic acid and fucans.

Laminaran has been isolated from many species[3–7], and has received many studies to clarify its chemical structure[3,7,8–10]. Fucans are highly sulfated polysaccharides, comprising mainly L-fucose units, presumed to occur in the mucilaginous matrix of the cell. The mucilaginous nature of these polysaccharides in marine algae is considered to give the flexibility necessary to a plant growing in a marine habitat, and yet render it sufficiently rigid to remain extended in order to make the best use of available light and nutrients. The structure of the fucans extracted from the brown seaweed with hot water or dilute acids has been studied[11–14].

Alginic acid, the major polysaccharide of the brown seaweeds, comprises 14 to 40% of the dry solids of the plant, depending on the depth at which the algae grow, and the percentage is subject to considerable, seasonal variation. The mono-

meric units were identified as D-mannuronic acid and L-guluronic acid[15-18]. The ratio of D-mannuronic acid to L-guluronic acid (M/G) was found[15,16] to vary from 0.45 to 2.35 : 1.

EXPERIMENTAL

Algal material. — *Turbinaria murrayana* was collected in October, 1979, from Jeddah beaches in Saudi Arabia, washed free from sand and foreign materials, sun-dried, and powdered mechanically.

Organic-solvent extraction. — Finely powdered weed (50 g) was extracted with 2 : 1 : 1 acetone–methanol–chloroform in a Soxhlet apparatus for 24 h, the solvent being changed twice. The residual weed (I) was air-dried.

Hot-water extraction. — Residue I (40 g) was suspended in distilled water (1 L), and the suspension was stirred on a boiling-water bath for 3 h, kept overnight in a refrigerator, and then centrifuged, to afford a supernatant liquor (II) and a residue (III). Supernatant II was concentrated to ~200 mL at 60° *in vacuo*, and dialyzed against distilled water for 24 h; the polysaccharides were precipitated by addition of ethyl alcohol (5 vol.), and the precipitate (IV) was centrifuged off, successively washed with alcohol and ether, and dried.

Fractionation of the hot-water extract[19]. — A solution of IV (200 mg) in water (40 mL) was applied to a column (50 × 2 cm) of DE-cellulose. The column was eluted with water (giving IV A) until the eluate was free from carbohydrates, and then the column was eluted with M KCl solution (giving IV B). The fractions were analyzed for carbohydrates, uronic acids, and sulfate content.

Acid extraction. — A suspension of III in distilled water (500 mL, adjusted to pH 1 with HCl) was heated for 3 h at 60°, cooled, made neutral with Na_2CO_3 solution, and centrifuged, to afford a supernatant liquor (V) and a residue (VI). Supernatant V was concentrated at 60° *in vacuo*, dialyzed against distilled water for 2 days, and centrifuged; the supernatant liquor was poured, with stirring, into ethyl alcohol (5 vol.), and the precipitate of water-soluble, acid-extractable polysaccharides (VII) was centrifuged off, successively washed with alcohol and ether, and dried.

Alkali extraction. — Residue VI was washed with distilled water until free from acid, treated with 3% Na_2CO_3 solution (500 mL), and heated at 70° with occasional shaking. After 3 h, the solution was diluted to 1500 mL with distilled water, and reheated for 3 h at 70°. The solution was kept overnight in a refrigerator, and centrifuged, affording a supernatant material (VIII) and a residue (IX). Supernatant VIII was poured, with vigorous stirring, into 25% $CaCl_2$ solution (500 mL), and the precipitated calcium alginate was centrifuged off, washed with water, resuspended in distilled water (500 mL), and treated with dilute HCl. The alginic acid liberated was centrifuged off, and washed with water until free from Ca^{2+}. The free alginic acid was suspended in distilled water (500 mL), and, with vigorous stirring, was titrated with M NaOH to pH 7. The solution of sodium alginate was dialyzed for 2 days,

centrifuged, concentrated at 60° *in vacuo*, and treated with ethyl alcohol (5 vol.), with vigorous stirring. The precipitate of sodium alginate (X) was centrifuged off, successively washed with alcohol and ether, and dried.

Characterization, and quantitative determination, of component sugars. — The polysaccharides were hydrolyzed with 0.5M sulfuric acid for 12 h at 100°. The acid was neutralized with $BaCO_3$, and the $BaSO_4$ was removed by filtration. The filtrate containing the monosaccharides was evaporated, and the residue was converted into poly-*O*-acetylhexitols by reduction with $NaBH_4$, followed by acetylation with Ac_2O–pyridine; these were identified, and their approximate mol-percentages calculated, from g.l.c. peak areas[20].

Partial hydrolysis was conducted by heating the polysaccharide (20 mg) with sulfuric acid (2 mL) of various concentrations (250, 125, 50, and 25mM) for various time-intervals (1, 2, and 5 h) at 100°. The hydrolyzates were treated as before, and the sugar components were chromatographed on Whatman No. 3 filter paper, using[21] 7:1:2 1-PrOH–EtOAc–H_2O, and detection with aniline phthalate reagent.

Methylation of the isolated polysaccharides. — The polysaccharides (50 mg each) were twice methylated by the Hakomori method[22]. The methylated products were hydrolyzed[23], the sugars were converted into their *O*-acetyl-*O*-methylalditols, and these were estimated by g.l.c.

Periodate oxidation. — The polysaccharides (300 mg each) were treated with 15mM $NaIO_4$ (100 mL) in the dark for 5 days at 2°, and then for 2 days at room temperature. At various times, aliquots were taken for spectrophotometric determination (223 nm) of the periodate reduced, and for potentiometric determination of the formic acid produced[24]. Following the addition of ethylene glycol, dialysis, reduction with $NaBH_4$, and re-dialysis, the polyalcohol was obtained by freeze-drying.

Smith degradation[25] of the polyol (100 mg) was conducted with 0.5M trifluoroacetic acid (25 mL) for 48 h at 23°, and the solution was evaporated to dryness. The residue was separated on Whatman No. 1 filter paper with pyridine–ethyl acetate–water (2:5:7, upper phase), and the chromatograms were sprayed with benzidine–sodium metaperiodate.

Reduction of alginic acid[25]. — Alginic acid (5 g) was permitted to swell in *N*,*N*-dimethylformamide (40 mL) for 1 h at 45°, followed by swelling in pyridine (50 mL) during 0.5 h, with stirring, and the mixture was allowed to cool to 30°. With continuous stirring, propanoic anhydride (50 mL) was added during 4 h, and the stirring was continued for a further 4 h. The solution was kept overnight at room temperature, and was then poured, with stirring, into ice-cold M HCl (1 L). The precipitate was removed by centrifugation, and washed successively with dilute HCl and water. The partially esterified product was re-esterified by dispersion in pyridine (75 mL) and treatment with propanoic anhydride as already described. The mixture was kept for 1 week at room temperature, and poured into an excess of light petroleum (b.p. 60–80°). The precipitate was separated by centrifugation, washed, and dried. The dipropanoate (3 g) in diglyme (diethyleneglycol dimethyl ether) (100 mL) was treated with $NaBH_4$ (10 g) in diglyme (50 mL), followed by

boron trifluoride etherate (50 g) in diglyme (125 mL), during 2–2.5 h, with continuous shaking. After 2 days at room temperature, the solution was concentrated, treated with M NaOH to pH 10, kept for 2 h at 60°, the suspension filtered, and the filtrate dialyzed, and traces of borate were removed by several additions and evaporations of methanol.

The solution was concentrated, and the neutral polysaccharide was precipitated by pouring the concentrate into 3:1 methanol–ether. The precipitate was separated by centrifugation, washed, and dried.

RESULTS AND DISCUSSION

The algal material was first extracted with organic solvents in order to eliminate the lipids and any coloring matter that might affect the quality of the polysaccharides to be extracted.

After this extraction, the dry algae were sequentially extracted by different solvents as a preliminary, fractionation step. The yields and properties of each fraction are listed in Table I.

The relatively high content of carbohydrate indicated low degraded products. The high contents of ash in fractions VII and X were due to the presence of acidic groups (sulfate and uronic acid, respectively).

On acid hydrolysis, fraction IVA gave glucose (90%), and fraction VIB gave fucose, glucose, galactose, glucuronic acid, xylose, and mannose, in the approximate mol-percentages of 33.6, 19.7, 15.2, 12.1, 12.1, and 7.3.

The analysis of the hydrolysis products confirmed that the hot-water extract (IV) was constituted of two distinct polysaccharides. The fractionation of this extract was achieved by using ion-exchange chromatography, as the laminaran (fraction IVA) was completely eluted from the column by using distilled water. The presence of the SO_4^{2-} groups in the fucans (fraction IVB) makes this fraction more resistant to elution, and so an eluant having a high ionic strength (M KCl) is needed. The

TABLE I

YIELDS AND PROPERTIES OF THE DIFFERENT MATERIALS EXTRACTED

Fraction		*Yield* (%)	*Ash* (%)	N_2 (%)	*Carbohydrates*[a] (%)	*Uronic acid*[b]	SO_4[c] (%)
Hot-water extract (IV)	Water Fraction (IV A)	3.2	0.04	0.05	91	nil	nil
	M KCl Fraction (IV B)	2.1	1.7	0.06	85	12.1	9.4
Acid extract (VII)		4.7	3.1	0.9	78	15.3	19.5
Alkali extract (X)		22.6	13.6	0.08	67	76	nil

[a]Estimated by the PhOH–H_2SO_4 method[26]. [b]Determined by the *m*-hydroxydiphenyl method[27]. [c]Determined by the 4-amino-4′-chlorobiphenyl reagent[28].

fucans dissolved in the hot-water fraction may be degraded compounds or substances of low molecular weight.

On acid hydrolysis, fraction VII also gave fucose, galactose, glucuronic acid, xylose, glucose, and mannose, but, in contrast to fraction IVB, in the approximate mol-percentages of 45.5, 15.7, 15.3, 8.4, 7.8, and 7.4.

Complete hydrolysis of the reduced alginic acid with acid revealed the presence of mannose and gulose in the approximate mol-percentages of 59.7 and 40.3, respectively, indicating an M/G ratio of 1.48:1. Unlike the water-insoluble, acid-resistant, β-(1→4)-linked D-mannans, the reduced alginic acid was water-soluble, and rather labile, even to dilute acids. These properties may possibly be an indication that at least part of the alginic acid is not composed of separate chains of mannuronic acid and of guluronic acid, respectively, but that both acids are present in a single sequence.

Partial hydrolysis of the reduced alginic acid with sulfuric acid of different concentrations showed that the best yield was given by 25mM acid for 2 h at 100°. Residual polymeric material was precipitated with ethanol, and this appeared to consist almost entirely of mannosyl residues. The material recovered from the alcoholic, supernatant liquor was separated by p.c., as described. Two main oligosaccharides were separated. On complete hydrolysis of the separated oligosaccharides, the resulting monosaccharides were converted into their alditol acetates; g.l.c. thereof revealed mannose and gulose for the main oligosaccharide, and mannose (only) for the lesser oligosaccharide. The separation of oligosaccharides consisting of mannose and gulose supported the concept that at least part of the alginic acid is not composed of separate chains of mannuronic and guluronic acids, respectively, but that both acids are present in a single sequence.

Laminaran (IVA) was methylated according to Hakomori, followed by hydrolysis, and the methylated sugars were converted into their alditol acetates. G.l.c. analysis of the *O*-methylalditol acetates showed derivatives of 2,4,6-tri-*O*-methyl glucose (80), 2,3,4,6-tetra-*O*-methylglucose (12), and 2,4-di-*O*-methylglucose (8 mol%). The presence of 2,4,6-tri-*O*-methylglucose indicated (1→3)-linked glucose. The presence of 2,4-di-*O*-methylglucose indicated a branched molecule, with C-6 as the point of branching. The molar ratios of the tri:tetra:di-*O*-methyl sugar were 20:3:2, indicating two points of branching, three nonreducing ends, and repeating units consisting of 25 sugar residues.

Reduced alginic acid was methylated, and the methylation product was hydrolyzed. The methylated sugars were converted into their alditol acetates, and these were analyzed by g.l.c. The two main peaks were those of 2,3,6-tri-*O*-methylmannose (52.1) and 2,3,6-tri-*O*-methylgulose (46.6 mol%), indicating an M/G ratio of ~1.12:1. There were some components consisting of partially methylated sugars. The presence of 2,3,6-tri-*O*-methyl sugar indicated that alginic acid consists of (1→4)-linked D-mannuronic and (1→4)-linked L-guluronic acid residues. The absence of any di-*O*-methyl sugars indicated that the molecule is an unbranched chain.

Laminaran was oxidized with periodate; after 60 h, 0.4 mol of periodate was

consumed, and 0.15 mol of formic acid was released per sugar residue. The oxidized laminaran was reduced with $NaBH_4$, and the resulting polyalcohol was hydrolyzed with sulfuric acid; chromatography of the hydrolyzate showed that nearly all of the sugar residues were periodate-resistant, indicating that the sole mode of linkage possible is the (1→3) linkage. The formic acid liberated per mol of polysaccharide indicated three branches per mol, and repeating units consisting of ~33 sugar residues.

Reduced alginic acid consumed 0.6 mol of periodate after oxidation for 100 h, and 4 mmol of formic acid was released per sugar residue. The formic acid released per sugar residue indicated repeating units of ~750 sugar residues. After reduction of the polyaldehyde product to the polyalcohol, followed by hydrolysis with sulfuric acid, the hydrolyzate was examined by p.c.; this showed spots corresponding to mannose, gulose, erythritol, and threitol. The mol-percentage of erythritol to threitol was ~1.22:1, which indicated the same ratio for M/G.

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