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AN ALKALI-SOLUBLE POLYSACCHARIDE FROM THE OAK LICHEN *Cetraria islandica* (L.) Ach.

MIRJANA HRANISAVLJEVIĆ-JAKOVLJEVIĆ, JELENA MILJKOVIĆ-STOJANOVIĆ, RADMILA DIMITRIJEVIĆ, AND VUKIĆ M. MIĆOVIĆ

Department of Chemistry, Faculty of Science, University of Belgrade and Institute of Chemistry, Technology and Metallurgy, Belgrade (Yugoslavia)

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ABSTRACT

An alkali-soluble polysaccharide, $[\alpha]_D^{20}$ +43° (M sodium hydroxide), containing D-glucose and D-glucuronic acid has been isolated from the oak lichen *Cetraria islandica* (L.) Ach. On the basis of methylation, periodate oxidation, and partial hydrolysis studies, the polysaccharide has been shown to contain (1→3)-linked glucopyranose and glucuronic acid residues and (1→4)- and/or (1→6)-linked glucopyranose residues as the main structural features of the basic chain. A preponderance of β linkages, indicated by the low optical rotation and the i.r. spectrum, was corroborated by the formation of laminaribiose, cellobiose, and gentiobiose on partial hydrolysis.

INTRODUCTION

Water-soluble polysaccharides from the oak lichen *Cetraria islandica* (L.) Ach. have been extensively investigated[1], but data on the alkali-soluble polysaccharides are scarce and indicate only the general structural features[2]. We now report on a new alkali-soluble polysaccharide isolated from *Cetraria islandica* (L.) Ach.

RESULTS AND DISCUSSION

All non-carbohydrate and carbohydrate components soluble in organic solvents and water, respectively, were removed from the lichen material prior to alkali extraction. Alkali-soluble polysaccharide material was fractionated with barium hydroxide[3] and Fehling's solution[4]. Fractional precipitation of the copper complex from aqueous solution, by the addition of ethanol, gave a polysaccharide, $[\alpha]_D^{20}$ +43 ±1° (M sodium hydroxide), which afforded only D-glucose and D-glucuronic acid on acid hydrolysis. The former was characterised as the crystalline *N*-*p*-nitrophenyl-D-glucopyranosylamine[5], and the latter by conversion into D-glucose *via* reduction and hydrolysis of the methyl ester methyl glycoside. The polysaccharide had i.r. absorptions at 1620 cm^{-1} for carboxyl, and at 920, 890, and 785 cm^{-1} characteristic[6] of axial H-1, *i.e.*,

TABLE I

HYDROLYSIS PRODUCTS[a] FROM THE METHYLATED ACIDIC POLYSACCHARIDE FROM THE OAK LICHEN *Cetraria islandica* (L.) Ach.

Methylated D-*glucose*	*Linkage indicated*	*Approximate molar proportions*		*G.l.c.* (T)[d]
		A[b]	B[c]	
2,3,4,6-Tetra-	Glc*p*-(1→	2	2	1.00
2,4,6-Tri-	→3)-Glc*p*-(1→	7	8	1.96
2,3-Di-	→4→6)-Glc*p*-(1→	2	2	5.37
2,4-Di-*O*-methyl-D-glucuronic acid	→3)-Glc*p*A-(1→	1	—	

[a]*A*, native polysaccharide; *B*, carboxyl-reduced polysaccharide. [b]Quantitative p.c. [c]G.l.c. of partially methylated alditol acetates. [d]Relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

the β configuration. The $[\alpha]_D$ value of the polysaccharide also indicated the presence of β linkages.

Hypoiodite oxidation of the polysaccharide indicated the degree of polymerisation to be 100.

On hydrolysis, the fully methylated polysaccharide, $[\alpha]_D^{20}$ +3 ±0.5° (chloroform), yielded the four *O*-methyl sugars listed in Table I.

The isolation of 2,3,4,6-tetra- and 2,3-di-*O*-methyl-D-glucose in equimolecular proportions indicated that the polysaccharide was essentially fully methylated and highly branched, with an average repeating-unit of 10 sugar residues. Characterisation of non-terminal residues as 2,4,6-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-glucuronic acid, and 2,3-di-*O*-methyl-D-glucose revealed that the main chain comprised 80% of (1→3)- and 20% of (1→4)- and/or (1→6)-glucosidic bonds. The isolation of 2,3-di-*O*-methyl-D-glucose proved that branching occurs through positions O-4 and/or O-6.

The polysaccharide consumed 0.48 mol. of periodate with simultaneous liberation of 0.13 mol. of formic acid per "anhydrohexose" unit. This finding agrees with the structural requirements deduced from the methylation data, namely, that the repeating unit consists of two terminal non-reducing residues, eight (1→3)-linked units, and two branching residues joined through O-1, O-4, and O-6. Additional evidence for the presence of (1→3)- and (1→4)-linkages was provided by reduction of the oxopolysaccharide with borohydride and subsequent hydrolysis, which gave glucose, glucuronic acid, erythritol, and glycerol in the molar ratios 7:1:2:2.

Partial, acid hydrolysis (0.2M sulphuric acid, 1 h) of the polysaccharide gave five products, four of which were identified by paper chromatography as glucose, laminaribiose, cellobiose, and gentiobiose. The formation of gentiobiose is of interest and probably of structural significance.

Thus, it is concluded that the alkali-soluble polysaccharide has a branched structure composed of a main chain of (1→3)-linked D-glucopyranose and D-glucuronic acid residues, with (1→4)- and/or (1→6)-linked D-glucopyranose residues at the branch points.

EXPERIMENTAL

General methods. — Solvents were evaporated under reduced pressure at 30°. P.c. was performed on Whatman No. 1 and 3MM paper with *A*, ethyl acetate–pyridine–water (2.5:1:2.5); *B*, 1-butanol–ethanol–water (4:1:5); *C*, butanone saturated with water; *D*, 1-butanol–ethanol–water (3:1:1); and *E*, 1-butanol–pyridine–water (6:4:3). T.l.c. was performed on Silica Gel H with *F*, benzene–acetone (1:1), and *G*, 1-butanol–acetic acid–ethyl ether–water (9:6:3:1). Detection was effected, as appropriate, with silver nitrate–sodium hydroxide[7], aniline hydrogenphthalate[8], or triphenyltetrazolium chloride[9], or by charring with sulphuric acid. Glucose was determined by the phenol–sulphuric acid method[10], and glycerol and erythritol by the chromotropic acid method[11]. Melting points are uncorrected. Optical rotations were recorded with a Perkin–Elmer 141 MC polarimeter, and i.r. spectra with a Perkin–Elmer Model 421 spectrophotometer. G.l.c. was performed at 170° on a Varian Model 1200 gas chromatograph fitted with a glass column (200 × 0.15 cm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh).

Isolation and purification of the alkali-soluble polysaccharide. — Finely powdered oak lichen (250 g) was extracted with ether (2 litres) and then methanol (4 litres), air-dried, and exhaustively extracted with distilled water (to a negative anthrone test) at 50°. The residual material was treated with 3% aqueous sodium carbonate (2 litres), and then with 4% aqueous sodium hydroxide (3 litres) under nitrogen. The mixture of alkali-soluble polysaccharides precipitated by the addition of ethanol (4 litres) and glacial acetic acid (3 litres) was a light-brown powder (yield, 4%).

A solution of the crude polysaccharide (10 g) in 4% aqueous sodium hydroxide (500 ml) was diluted with the same volume of water, and saturated, aqueous barium hydroxide was gradually added with vigorous stirring. The precipitate was collected by centrifugation, and the supernatant solution was acidified with acetic acid (to pH 5) and then dialysed against running tap-water (2 days) and distilled water (1 day). The dialysate was concentrated to 500 ml and mixed with an equal volume of Fehling's solution. The trace of precipitate was removed and the soluble copper complexes were fractionaliy precipitated by the gradual addition of ethanol. The polysaccharide in the largest fraction (7%), which precipitated at 25% ethanol, was recovered from the copper complex by treatment with 5% methanolic hydrochloric acid and was dried by solvent exchange. The polysaccharide was purified by several precipitations, to give material with $[\alpha]_D^{20}$ +43 ±1° (*c* 0.32, M sodium hydroxide) (Found: Ash, 0.15; N, 0%). On acid hydrolysis, the polysaccharide gave only glucose and glucuronic acid (p.c., solvents *A* and *D*). The hydrolysate was fractionated by p.c. (solvent *E*). One fraction (88.7%)[10] was identified as D-glucose by its conversion into *N*-*p*-nitrophenyl-D-glucopyranosylamine[5], m.p. 183°. The second fraction (11%) was treated with methanolic hydrogen chloride, and the resulting methyl ester methyl glucoside was reduced with lithium aluminium hydride in tetrahydrofuran and then hydrolysed to give D-glucose, which was identified as described above.

TABLE II

PERIODATE OXIDATION OF THE ACIDIC POLYSACCHARIDE FROM THE OAK LICHEN *Cetraria islandica* (L.) Ach.

Time (h)	24	46	70	96	120
Periodate[a]	0.45	0.48	0.52	0.53	0.53
Formic acid[a]	0.10	0.12	0.13	0.13	0.13

[a]In mol. per mol. of hexose residue.

The number-average degree of polymerization was found by hypoiodite oxidation[12] to be 99 ±1.

Periodate oxidation. — A solution of the polysaccharide (0.2 g) in water (100 ml) was treated with 0.1M sodium periodate (100 ml) at room temperature in the dark. Oxidation was monitored by the arsenite method, and formic acid production by iodometric titration[13]. The results are recorded in Table II.

After the oxidation was complete, excess of periodate was reduced with ethylene glycol, and the solution was dialysed against tap and distilled water, concentrated to small volume, and treated with sodium borohydride (3 × 200 mg). After 26 h, excess of borohydride was decomposed with acetic acid, and the solution was dialysed and concentrated to dryness. The residue was hydrolysed with M sulphuric acid (10 h, 100°) and the hydrolysate was neutralised with barium carbonate. P.c. (solvent *E*) then revealed glycerol (R_F 0.80), erythritol (R_F 0.54), glucose (R_F 0.29), and glucuronic acid (R_F 0.07), the relative proportions of which were assessed after separation by p.c. or t.l.c. (solvent *G*) by spectrophotometry[10,11].

Methylation analysis. — (*a*) The polysaccharide, when methylated both by the Hakomori[14] or Kuhn[15] procedures, gave a fully methylated product, $[\alpha]_D^{20}$ +3° (*c* 4, chloroform), having no i.r. absorption for hydroxyl.

The methylated polysaccharide was hydrolysed[16] using 90% formic acid and 0.25M sulphuric acid. P.c. (solvents *B* and *C*) and t.l.c. (solvent *F*) of the hydrolysate revealed the following four components which were isolated by p.c. and t.l.c.

2,3,4,6-Tetra-*O*-methyl-D-glucose, R_F 0.78 (p.c., solvent *C*), m.p. 92° alone and in admixture with authentic 2,3,4,6-tetra-*O*-methyl-D-glucose[17] (from hexane), $[\alpha]_D^{20}$ +82 ±1° (*c* 0.3, ether). 2,4,6-Tri-*O*-methyl-D-glucose had the same mobility in p.c. (solvents *B* and *C*) and t.l.c. (solvent *F*) as the authentic compound, and m.p. 121–122° (from ether), $[\alpha]_D^{21}$ +71° (*c* 0.2, water)[18]. The derived anilide had m.p. 160°, $[\alpha]_D^{20}$ −110° (*c* 0.25, methanol), in agreement with the literature data.

2,3-Di-*O*-methyl-D-glucose had the same chromatographic mobility as the authentic compound and gave a negative reaction with the triphenyltetrazolium reagent, indicating HO-2 to be substituted. The derived anilide had m.p. 132–134°, in accord with the literature data[19].

2,4-Di-*O*-methyl-D-glucuronic acid had a low chromatographic mobility, and application in sequence of glycosidation–esterification, reduction, and hydrolysis gave 2,4-di-*O*-methyl-D-glucose[20], m.p. 124–127° (from methanol–benzene), $[\alpha]_D^{20}$ +75° (*c* 1.2, water).

(*b*) A solution of the methylated polysaccharide (50 mg) in tetrahydrofuran (5 ml) was added to a solution of lithium aluminium hydride (0.15 g) in ether (25 ml). The mixture was heated under reflux for 18 h and the reduced material (which had no i.r. absorption for carboxyl) was recovered by chloroform extraction. Remethylation (Hakomori[14]) of the carboxyl-reduced product, and then hydrolysis, reduction with sodium borohydride, and acetylation gave a mixture of partially methylated alditol acetates which was subjected to g.l.c.[21]. The results are presented in Table I (under B^c).

Partial, acid hydrolysis. — The polysaccharide (0.05 g) was stirred for 1 h with 0.2M sulphuric acid (2 ml) at 100°. The mixture was neutralised and concentrated, and p.c. (solvent *F*) of the residue revealed five components (R_{Glc} 1.00, 0.83, 0.67, 0.63, and 0.52); four components were tentatively identified as glucose, laminaribiose, cellobiose, and gentiobiose.

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