GLUCURONOXYLOFUCAN, A CELL-WALL COMPONENT OF *Ascophyllum nodosum*

PART II*. METHYLATION

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(Received July 27th, 1970; accepted for publication, September 3rd, 1970)

ABSTRACT

Methylation studies of the sulphated glucuronoxylofucan from *Ascophyllum nodosum* have confirmed the highly branched nature of the molecule and the presence of end-group and (1→4)-linked xylose. Evidence for the presence of end-group and (1→2)-linked fucose, for (1→2)-linked and (1→3)-linked 4-sulphated fucose residues, and for 1,2,3,4-linked/or sulphated fucose has been obtained.

INTRODUCTION

Extraction of the weed residues of the brown seaweed *Ascophyllum nodosum*, from which the laminarin, fucoidan, and alginic acid had previously been removed, led to the separation of a glucuronoxylofucan[1]. Partial hydrolysis, periodate-oxidation studies, and alkali-treatment of the polysaccharide provided evidence of the mutual linkage of glucuronic acid and fucose and of fucose and xylose. Xylose was shown to be near the periphery of the molecule, probably as end-group and (1→4)-linked units. Some, at least, of the glucuronic acid residues appeared to be (1→4)-linked, and some of the fucose residues were vulnerable to periodate. The sulphate groups proved to be stable to alkali. From this and the other evidence, it was concluded that the majority of the ester sulphate is linked to the fucose residues.

The present paper describes the results of methylation of this glucuronoxylofucan and of a partly desulphated, degraded sample of this polysaccharide.

DISCUSSION

The sulphated glucuronoxylofucan from *Ascophyllum nodosum*[1], after three Haworth methylations[2] and a further methylation in methyl sulphoxide[3], was recovered in a 55% overall yield with a methoxyl content of 13.9%. Since the product also contains ester sulphate, ash, and protein, this corresponds to complete methylation.

*For Part I, see Ref. 1.

Analysis of the glycosides in a methanolysate of the methylated material by gas–liquid chromatography (g.l.c.) gave a large number of peaks with considerable overlapping (Table I). In order to establish the identity of the methylated sugars, the material was hydrolysed, and the neutral sugars were separated from acidic material on an ion-exchange resin. The methylated uronic acid recovered from the resin was contaminated with sulphated fragments, and attempted separation was unsuccessful. The presence of 2,3,4-tri-*O*-methylxylose, 2,3,4-tri-*O*-methylfucose, and a trace of 2,3,4-tri-*O*-methylglucuronic acid in a chloroform extract of the neutral fraction was confirmed by paper chromatography, and by g.l.c. of the derived glycosides (Table I). Paper chromatography of the chloroform-insoluble material gave 3,4-di-*O*-methylfucose and 3-*O*-methylfucose, and a mixture of 2,3-di-*O*-methylxylose, 2,4-di-*O*-methylfucose (trace), and 3,4-di-*O*-methylfucose. Each substance was characterised by g.l.c. of the methyl glycosides (Table I) and alditol acetates[4] (Table II). 2-*O*-Methylfucose, which partly crystallised, was characterised in the same way and

TABLE I

RELATIVE RETENTION TIMES (*T*) OF METHYL GLYCOSIDES[a] FROM METHYLATED GLUCURONOXYLOFUCAN

	Column 1	*Column* 2	*Column* 5
2,3,4-Tri-*O*-methylfucose	0.71	0.67	0.72
2,4-Di-*O*-methylfucose	(1.7) (1.5)	0.87, (1.0)	(1.7) > (1.6)
3,4-Di-*O*-methylfucose	1.62 > 2.61	(0.94) > (1.07), (1.26)	3.3, 4.9
3-*O*-Methylfucose	3.56 > 6.48 > 5.37	1.2 > 1.5, 1.63	5.67, 7.9
2-*O*-Methylfucose	3.9, 4.4	(0.94), (1.3) (1.1)	3.6 > 6.9
2,3,4-Tri-*O*-methylxylose	0.48, 0.61	0.41, 0.50	0.5, 0.6
2,3-Di-*O*-methylxylose	(1.48), (1.67), 1.8	0.59, 0.76, (0.96)	(1.7) > (1.55)
2,3,4-Tri-*O*-methylglucuronic acid[b]		2.46	
2,3-Di-*O*-methylglucuronic acid[b]		4.5	8.8

[a]Figures in parenthesis indicate *T*-values of incompletely resolved compounds. [b]As methyl ester methyl glycoside.

TABLE II

RELATIVE RETENTION TIMES (*T*) OF ALDITOL ACETATES[a] FROM METHYLATED GLUCURONOXYLOFUCAN

	Column 4	*Column* 6
2,3,4-Tri-*O*-methylfucitol	0.7	0.62
2,4-Di-*O*-methylfucitol	0.79	1.0
3,4-Di-*O*-methylfucitol	(1.1)	1.2
3-*O*-Methylfucitol	1.15	1.8
2-*O*-Methylfucitol	(1.0)	1.5
2,3,4-Tri-*O*-methylxylitol	0.58	0.54
2,3-Di-*O*-methylxylitol	0.87	1.36
3,4,5-Tri-*O*-methylgulonic acid	0.53	0.4
4,5-Di-*O*-methylgulonic acid	1.75, 1.5, (1.0)	1.3, 3.1
Penta-*O*-acetylfucitol	1.2	2.0

[a]Figures in parenthesis indicate *T*-values of incompletely resolved compounds.

also by conversion into the 3,4-*O*-isopropylidene derivative[5]. Methyl α-L-fucopyranoside was also separated. No standard 4-*O*-methylfucose was available, and it is possible that this sugar might be present in the hydrolysis products of the methylated materials, since several unidentified peaks were always present on the gas–liquid chromatograms of these mixtures.

The 3-*O*-methylfucose and fucose appeared to be present in largest quantity. Complete methylation of highly sulphated polysaccharides is very difficult, and it is possible that some of the fucose and monomethylfucoses result from undermethylation. In view, however, of the reasonably high methoxyl content of the methylated material, they must be involved to some extent in the structure of the macromolecule.

Since the sulphate groups are not labile to alkali, the 2-*O*-methyl- and 3-*O*-methyl-fucoses cannot have arisen from (1→4)-linked units sulphated, respectively, at C-3 and C-2. These methylated sugars can only have arisen, respectively, from (1→3)- and (1→2)-linked units, both branched or sulphated at C-4. The 3,4-di-*O*-methylfucose indicates the presence of (1→2)-linked fucose units. Such units would be vulnerable to periodate, thus confirming the earlier results.

For comparative purposes, a second portion of the glucuronoxylofucan was methylated with the methylsulphinyl carbanion and methyl iodide in methyl sulphoxide[6], as modified to the microscale by Björndal and Lindberg[7]. Examination of the derived methyl glycosides and alditol acetates[4] by g.l.c. confirmed the presence of the methylated sugars and fucose, and also gave peaks characteristic of methyl (methyl 2,3-di-*O*-methylglucopyranosid)uronate (Table I, column 2) and 4,5-di-*O*-methylgulonic acid 2,3,6-triacetate (from 2,3-di-*O*-methylglucuronic acid) (Table II).

A sample of the polysaccharide which had been partly desulphated with methanolic hydrogen chloride[1] was also methylated by the methods of Haworth and of Purdie until the methoxyl content was constant at 20.5%. This is consistent with full methylation for a polysaccharide containing 7% of ester sulphate and 10% of uronic acid, and isolated as the silver salt. G.l.c. examination of the glycosides derived from this methylated material revealed the same methylated sugars as for the parent polysaccharide, together with methyl (methyl 2,3,4-tri-*O*-methyl- and 2,3-di-*O*-methylglucopyranosid)uronates, and a higher proportion of methyl 2,4-di-*O*-methylfucoside and 3,4-di-*O*-methylfucoside. The proportion of mono-*O*-methylfucoses and of fucose in this partly desulphated, methylated polysaccharide was considerably less than in the methylated, initial polysaccharide. The increase in 2,4-di-*O*-methyl sugar can only have been derived from (1→3)-linked units initially sulphated at C-4, and an increase in 3,4-di-*O*-methylfucose results from (1→2)-linked units sulphated at C-3 and/or C-4. These conclusions confirm the deductions made from the presence of the monomethylfucoses found in the products of methylation of the initial polysaccharide.

The proportion of methylated xyloses was smaller in the product from the Haworth methylations, indicating a preferential loss of xylose during this methylation procedure as compared with that from the Björndal and Lindberg methylation.

The methylation results confirm earlier studies that the polysaccharide is highly branched, and that the xylose is present as end-group and (1→4)-linked residues. It has also provided evidence that the fucose is present as end-group and (1→2)-linked residues, (1→2)-linked and (1→3)-linked residues sulphated at C-4, and 1,2,3,4-linked and/or sulphated residues. Unfortunately, these studies have failed to provide conclusive evidence of the uronic acid linkages; only tentative evidence for the presence of end-group and (1→4)-linked glucuronic acid could be obtained.

EXPERIMENTAL

Details of analytical methods are given in a previous publication[1]. In addition, a column (*5*) of acid-washed, silane-treated Celite (80–100 mesh) coated with 10% w/w polyethylene glycol adipate was used at an operating temperature of 175° for the methylated glycosides. For the methylated alditol acetates, column *4* and a column (*6*) of acid-washed, silane-treated Gas Chrom Q (100–120 mesh) coated with 3% w/w ECNSS-M were used at an operating temperature of 162°. Retention times (*T*) are expressed relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside for the methyl glycosides and 2,3,4,6-tetra-*O*-methyl-D-glucitol 1,5-diacetate for the alditol acetates.

Methylation. — The polysaccharide [A, 1.6 g; carbohydrate content, 54; uronic acid (decarboxylation), 15.8; sulphate, 18; N, 1.45%] and methanolysed polysaccharide[1] [*B*, 435 mg; carbohydrate content, 65; uronic acid (decarboxylation), 10.7; sulphate, 7%] were each dissolved in water (10 ml) and separately methylated three times with methyl sulphate and sodium hydroxide[2]. After each methylation, the resulting mixture was neutralised with acetic acid and dialysed until free from sulphate ion. The solution from the dialysis sac was concentrated and freeze-dried. Both polysaccharides gave biscuit-coloured solids [0.9 g (OMe, 12.63; N, 1.99%) from *A*, and 225 mg from *B*].

The methylated polysaccharide *A* (0.5 g) was dissolved in methyl sulphoxide (30 ml), and powdered sodium hydride (0.15 g) was added in small portions with gentle stirring during 1 h. To the resulting yellow solution, methyl iodide (2 ml) was added[3] dropwise during 1.5 h, and stirring was continued overnight. Nitrogen was bubbled into the mixture throughout the procedure. Sodium hydride (0.25 g) and methyl iodide (2 ml) were added dropwise during the next 2 h, and the stirring was continued overnight. The mixture was diluted with water, dialysed, concentrated, and freeze-dried to a cream solid (460 mg) (Found: OMe, 13.9; N, 1.73; SO_4^{2-}, 28%).

The methylated polysaccharide *B* (116 mg) in water was stirred with Amberlite IR-120 (H^+) resin for several hours. The resin was removed, silver carbonate (150 mg) was added to the solution, and stirring was continued overnight. The mixture was freeze-dried to a grey solid (153 mg) which was subjected to two Purdie methylations with silver oxide and methyl iodide. The recovered polysaccharide had OMe, 20.5%. Further Purdie methylation failed to raise the methoxyl content.

A fresh portion of the initial polysaccharide (*C*, 5 mg) was methylated as described by Björndal and Lindberg[7].

Methanolysis of the methylated sugars. — A portion of the methylated polysaccharide *A* and the methylated polysaccharides *B* and *C* were hydrolysed separately with formic acid[1], and the derived methylated sugars were converted into their methyl glycosides[3] and analysed by g.l.c. A second portion of *A* and the mixture of glycosides from *B* and *C* were separately hydrolysed, reduced, and acetylated[4], and analysed by g.l.c. (Table II). Authentic samples of each methylated sugar and alditol acetate were run for comparison. Paper chromatography (solvents *1* and *2*) of the formic acid hydrolysate of *A* confirmed the presence of the methylated sugars and revealed the presence of fucose. The latter was also detected, as fucitol penta-acetate, by g.l.c.

The remainder of the methylated material from *A* was hydrolysed[1], and the resulting mixture was passed down a column of Biodeminrolit (carbonate form) resin, and the column was washed with deionised water and then with methanol until the washings were free from carbohydrate[1]. The combined aqueous and methanolic washings were concentrated to a syrup, and the latter was extracted with chloroform. Concentration of the chloroform extracts gave a syrup which was analysed by paper chromatography and by g.l.c. of the derived methyl glycosides.

The residual syrup not extracted into the chloroform was separated on Whatman No. 17 paper[9] with solvent *1*. Considerable contamination from the paper occurred, which necessitated repeated purification with charcoal.

Fraction 1, a syrup (R_G 0.77; 0.86), was divided into two portions. One portion was converted into the methyl glycosides and the second portion into the alditol acetates[4]. Analysis of both products by g.l.c. gave peaks having the same retention times as the peaks given by authentic 2,3-di-*O*-methylxylose and 3,4-di-*O*-methylfucose as their methyl glycosides (Table I) and their acetylated alditols, respectively, (Table II).

Fraction 2, a syrup (R_G 0.77), was examined as for fraction 1 and gave, as the methyl glycosides and as the alditol acetates, g.l.c. peaks, having the retention times of the same derivatives of 3,4-di-*O*-methylfucose.

Fraction 3, a syrup (R_G 0.61), which partly crystallised, m.p. 145–147° (*cf.* 2-*O*-methyl-L-fucose[10], 150–152°). Analysis by g.l.c., as the methyl glycosides and alditol acetate, confirmed the identity of this fraction. The methyl fucoside was converted into the 3,4-*O*-isopropylidene derivative[5] which had m.p. and mixed m.p. 124–128°.

Fraction 4, a syrup (R_G 0.52), was identical with 3-*O*-methyl fucose run on the same chromatogram. G.l.c. examination of the derived glycosides and alditol acetate confirmed this identity (Tables I and II).

Fraction 5, a syrup, had the chromatographic mobility of fucose. After conversion into the methyl glycoside, it crystallised as methyl α-L-fucopyranoside, m.p. and mixed m.p. 154°.

Elution of the resin column with 0.5M sulphuric acid, followed by neutralisation

of the eluate with barium carbonate, filtration, and concentration, gave a syrup which, on analysis by paper chromatography, (solvent *1*) gave a long streak. Removal of barium ions with Amberlite IR-120 (H^+) resin resulted in a very acid solution which was freeze-dried to a viscous mass. All attempts to esterify and reduce this material were unsuccessful.

ACKNOWLEDGMENTS

The author thanks Professor E. J. Bourne for his interest in this work, and the Wellcome Trust for the provision of a gas chromatograph.

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