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THE STRUCTURES OF SOME XYLANS FROM RED ALGAE

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Abstract—Polysaccharides in which xylose is the chief constituent monosaccharide have been isolated and purified from some red algae. Using a small-scale methylation procedure, followed by methanolysis and quantitative analysis by GLC, the chief structural features of the xylans have been ascertained. The results indicate that xylose occurs in these algae in two types of structure: *A* as either a separate xylan, or combined in a heteropolysaccharide, but in which both 1,3- and 1,4-xylosidic linkages occur together in a structure that may be branched or linear; *B* as a cell-wall constituent which is essentially linear and which is either completely 1,3-linked or completely 1,4-linked.

INTRODUCTION

IT IS generally recognized that xylans from land plants consist of linear chains of β-1,4-linked D-xylopyranose units, to which may be attached side chains of arabinose units or other sugars. In contrast, xylans in the cell-walls of certain siphonaceous green algae have been shown to consist of linear chains of β-1,3-linked D-xylopyranose units,[1-3] such chains readily forming a triple helix in the cell-wall microfibrils.[4] Yet a third type of xylan is exemplified by the water-soluble xylan of the red alga, *Rhodymenia palmata*, which contains about 80 per cent of β-1,4-links and 20 per cent of β-1,3-links in an essentially unbranched chain.[5] Some heterogeneity in this xylan has been demonstrated,[6] but both linkages occur in a single polysaccharide chain.[7] The cell-walls of this alga also contain microfibrils constituted of roughly equal numbers of glucose and xylose units, but whether these sugar units are present in a xyloglucan, or as separate polysaccharides, has not been determined.[8] The xylose units in the microfibrils are believed to be 1,3-linked although no definite proof of this is given. The red alga, *Porphyra umbilicalis*, also contains a xylan, present as microfibrils in the cell-wall and extracted from the wall by alkali. This xylan gives the X-ray diffraction pattern of a β-1,3-linked xylan.[9] In contrast, it is believed that a xylan extracted by dilute alkali from the red alga, *Rhodochorton floridulum*, is 1,4-linked.[10] Xylose is a known constituent of many other algal polysaccharides but its mode of linkage has seldom been established. We wish to report some studies into methods of extracting xylan-rich fractions from certain red algae and the results of routine methylation analyses on the isolated fractions.

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RESULTS AND DISCUSSION

The method of sequential extraction of dried algae was used to give a series of polysaccharide fractions, which were then analysed for xylose contents after acid hydrolysis. The extracting solvents employed in sequence were cold dilute acid, hot water, cold dilute sodium hydroxide, and hot sodium hydroxide under nitrogen. Where the algal tissue was not broken down after the hot-water extraction, a chlorite treatment was introduced to break up the tissue. Almost every fraction examined liberated some xylose on hydrolysis, but in only a few cases was it possible to isolate a xylose-rich polysaccharide from the fraction. It is possible that much of the xylose, known to be present in these algae, is combined in various heteropolysaccharides. For this reason, only those polysaccharides that gave xylose as a predominant sugar on hydrolysis were examined by the methylation technique, and it should be borne in mind that these polysaccharides may not in fact be representative of the bulk of the xylose present in the algae.

TABLE 1. SEPARATION OF XYLANS FROM ALGAE

Alga	Extracting conditions	Sugars obtained on hydrolysis*	Xylose content† (%)
Rhodymenia palmata	0·5 M-HCl, 18°	xyl	70
	Water, 100°	xyl	87
	M-NaOH, 20°	xyl	55
	3 M-NaOH, 60°	xyl	85
Laurencia pinnatifida	2 M-NaOH, 20°	xyl, glc(t)	—
Porphyra umbilicalis	Chlorite, 70°	xyl(2+), glc(2+)	—
	3 M-NaOH, 20°	xyl(3+), glc(t)	—
Rhodochorton floridulum	Water, 100°	xyl(3+), glc(+) gal(+)	—
	Chlorite, 70°	xyl(3+), glc(+)	—

* Purified preparation hydrolysed and sugars estimated visually from chromatogram; xyl = xylose, glc = glucose, gal = galactose, (t = trace).

† Measured, after hydrolysis, by GLC of derived alditol acetates.

Table 1 lists the algae and the extracting solvents used to give fractions from which xylose-rich polysaccharides were isolated. The purification procedures used to separate the xylans from other polysaccharides varied from the simple formation of a xylan-copper complex with Fehling's reagent for the *Rhodymenia palmata* extracts, through various modifications of copper complex formation, to fractionation on DEAE Sephadex for the extracts from *Rhodochorton floridulum*, in which the major contaminating polysaccharide was an acidic galactan. Although no xylan free from other polysaccharides was obtained from *Rhodochorton floridulum*, the presence of a separate glucan and galactan in both extracts was established by a combination of gel filtration on Sephadex G-200 and on DEAE-Sephadex A-50.

Each polysaccharide listed in Table 1 was methylated on a small scale and then methanolysed to give a mixture of methyl glycosides which were analysed by quantitative GLC. The effectiveness of the methylation procedure was checked by methylating the xylan from *Caulerpa filiformis*[3] and comparing the results of GLC analysis with those obtained from a sample of the fully methylated xylan kindly supplied by Dr. E. E. Percival. The accuracy of the analytical procedure for estimating methyl xyloses was also checked by comparing these

results with those obtained by previous workers for both the xylan from *Caulerpa filiformis* and that from esparto grass[11] (see Table 2). Finally, the identities of the methyl xyloses, provisionally identified by retention times on GLC, were confirmed by isolating and characterizing the relevant *O*-methylxyloses from a methylated xylan of *Rhodymenia palmata*.

From Table 2 it is seen that the methylation procedure is effective in giving a fully methylated xylan since the results for the two samples of *Caulerpa filiformis* xylan are almost identical and in close agreement with those reported previously.[3] Analysis of esparto xylan also gave results very similar to those reported previously.[11] The results for the xylan from the green alga, *Caulerpa racemosa*, indicate that this polysaccharide is essentially 1,3-linked but that, in contrast to the xylan from *Caulerpa filiformis*, it is highly branched. The relatively large proportions of tri-*O*-methylxylose and of 2-*O*-methylxylose indicate a considerable degree of branching through position 4 of the xylose units.

TABLE 2. MOLAR % *O*-METHYL XYLOSES FROM METHYLATED XYLANS

Source of xylan	Extracting solvent	2-*O*-Methylxylose	2,3-Di-*O*-methylxylose	2,4-Di-*O*-methylxylose	2,3,4-Tri-*O*-methylxylose
*Caulerpa filiformis**	—	2·3	0	97·0	0·7
Caulerpa filiformis†	—	2·0	0	96·0	2·1
*Caulerpa racemosa**	—	13·3	0·3	75·5	11·0
*Esparto grass**	—	3·0	94·9	0	2·1
Rhodymenia palmata	0·5 M-HCl	1·2	80·3	16·4	2·1
	H_2O	2·5	75·1	18·6	3·8
	M-NaOH	1·65	77·6	17·5	3·25
	3 M-NaOH	2·2	94·4	2·1	1·3
Porphyra umbilicalis	Chlorite	2·5	76·9	14·1	6·4
	3 M-NaOH	—	0	95	—
Laurencia pinnatifida	2 M-NaOH	4·5	74·2	12·7	8·6
Rhodochorton floridulum	H_2O	11·1	53·7	14·1	21·1
	Chlorite	11·1	38·9	10·9	39·2

* Methylated sample from Dr. E. E. Percival.
† Methylated by authors.

For *Rhodymenia palmata*, the results indicate that at least two distinct xylans are present in the alga. Sequential extraction of this alga with cold dilute acid, with hot water, and with cold dilute alkali gives three xylan fractions which differ very little in composition. Each is essentially linear and contains 1,3- –1,4-linkages in a ratio varying from 1:4 to 1:5. In contrast, extraction of the residual weed with hot 3 M-alkali (under N_2) gives a linear xylan with almost exclusively 1,4-linkages. It is probable that this xylan is derived from the skeletal material of the cell-wall, although not necessarily from the microfibrils (eucellulose).[8] A chemical examination of the microfibrils of this alga is obviously desirable since they have hitherto been assumed to contain 1,3-linked xylose units in either a xylan or in a xyloglucan.

The chlorite extract of *Porphyra umbilicalis* and the dilute alkali extract of *Laurencia pinnatifida* show a remarkable similarity to one another and also to the earlier extracts from *Rhodymenia palmata*. Each contains 1,3- –1,4-links in a ratio of about 1:5 or 6 and only small amounts of branching as judged by the yield of 2-*O*-methylxylose. A higher yield of 2,3,4-tri-*O*-methylxylose might indicate a chain length less than that for the *Rhodymenia* xylans. For the two xylan fractions from *Rhodochorton floridulum*, the results again show a

[11] S. K. CHANDA, E. L. HIRST, J. K. N. JONES and E. G. V. PERCIVAL, *J. Chem. Soc.* 1289 (1950).

ratio of 1,3- –1,4-links in the region of 1:4 but also indicate a considerable degree of branching. Furthermore, the high yields of tri-*O*-methylxylose compared to 2-*O*-methylxylose strongly suggest that the xylose chains are short side branches on another polysaccharide. Since it was found to be impossible to isolate a xylan completely free from other sugars, it is tentatively suggested that the fractions from *Rhodochorton* may be hetero-polysaccharides in which the xylose occurs as short, branched chains on another polysaccharide backbone (probably a glucan). The recognition of 2,4,6-tri-*O*-methylglucose as another product of methylation of these fractions suggests that a 1,3-linked glucan is present in the fraction.

The fraction obtained from *P. umbilicalis* by extraction with alkali is almost certainly a pure xylan, contaminated with traces of a glucan. A xylan has previously been obtained by such methods from this alga.[9, 12] In agreement with the conclusions of previous workers,[9] this xylan, which is probably microfibrillar in origin, is shown to be 1,3-linked.

It appears, therefore, from the narrow range of algae examined, that xylose occurs in polysaccharides of marine algae in at least two distinct types of structure; first in the form of either a separate xylan or combined in a heteropolysaccharide, neither of which is a skeletal material, and in which both 1,3- and 1,4-linkages occur; secondly as a linear polysaccharide in the cell-wall, in which it is either completely 1,3-linked, or completely 1,4-linked.

EXPERIMENTAL

General

The red algae were all collected in late summer around the coast of Anglesey, Wales. They were sorted by hand and, with the exception of *Rhodochorton floridulum*, were air-dried and milled before use. The *Caulerpa* xylans and esparto xylan were kindly given by Dr. E. E. Percival. Paper chromatography on Whatman 54 or 3MM paper employed: *A*, *n*-BuOH–pyridine–benzene–H_2O (5:3:1:3, v/v/v/v, upper layer) or *B*, *n*-BuOH–EtOH–H_2O (4:1:5, v/v/v, upper layer). *p*-Anisidine hydrochloride spray was used to detect sugar zones.[13] GLC was performed on a Pye 104 dual column chromatograph with flame ionization detectors. Columns (5 ft) contained *C*, 10% Apiezon M on silver-coated, acid-washed, silylated Chromosorb W (100–120 mesh) at 220°,[14] and *D*, 10% butan-1,4-diol succinate polyester on acid-washed, silylated Chromosorb W at 175°.[15] Column *C* was used for the analysis of alditol acetates and *D* to separate and estimate methyl glycosides of *O*-methylsugars.

Analytical

The sugar content of a polysaccharide was determined, after hydrolysis, by quantitative GLC of the derived alditol acetates.[16] For the determination of *O*-methylxyloses derived from a methylated xylan, quantitative GLC on column *D* was employed. Calibration curves were constructed for 2-*O*-methyl-, 2,3-di-*O*-methyl-, 2,4-di-*O*-methyl- and 2,3,4-tri-*O*-methyl-D-xylose as follows. The methylxylose (1–10 mg) and an internal standard, 2,3,4,6-tetra-*O*-methyl-D-glucose (5 mg) were mixed and heated under reflux with 3% methanolic HCl (5 ml) for 12 hr. The mixture was neutralized (Ag_2CO_3), filtered and concentrated. After suitable dilution in $CHCl_3$, samples (1–3 μl) were pipetted on the chromatograph. The height of the peak, due to one of the glycosides of the *O*-methylxylose, was compared with that of the methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside and the ratio plotted against the weight of *O*-methylxylose originally taken. The choice of glycoside peak used for calibration purposes was governed by the need to use only peaks which where well separated from all other peaks. The methylxylose and the retention time[15] of the glycoside peak used for calibration were as follows: 2,3,4-tri-*O*-methylxylose (T = 0·58); 2,3-di-*O*-methylxylose (T = 1·74); 2,4-di-*O*-methylxylose (T = 2·0); 2-*O*-methylxylose (T = 4·04). After averaging triplicate injections, straight line calibrations were obtained for all the methylxyloses. For the analysis of methylated xylans, the internal standard (3 mg) was added to the methylated xylan (10 mg) and the mixture was methanolysed as above. Samples (1–5 μl) of the resulting mixtures in $CHCl_3$ were injected onto the column and the proportions of each methylxylose determined by reference to the calibration curves.

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[14] S. W. GUNNER, J. K. N. JONES and M. B. PERRY, *Can. J. Chem.* **39**, 1892 (1961).
[15] G. O. ASPINALL, *J. Chem. Soc.* 1676 (1963).
[16] D. M. BOWKER and J. R. TURVEY, *J. Chem. Soc.* (C) 983 (1968).

Methylation of a Polysaccharide[17]

The dried polysaccharide (100 mg) in dry DMSO (5 ml) was cooled, and BaO (0·5 g) and MeI (2 ml) were added. The mixture was shaken until a dark colour developed. Further additions of BaO (0·5 g) and MeI 1·5 ml) were then made five times with intervals of shaking between each. The semi-solid mixture was extracted in a Soxhlet with $CHCl_3$ for 24 hr. Evaporation (20°) of the extract gave a product, a small portion of which was examined, after hydrolysis, by paper chromatography (solvent *B*). Complete methylation was assumed when no free xylose was detected in the hydrolysate and a further sequence of methylation produced no change in the pattern of methylated sugars.

On a larger scale, the water-soluble xylan (5 g) of *Rhodymenia palmata* was methylated to give a product (1·2 g) with OCH_3 39·3%. This product was heated at 100° (sealed tube) with 90% formic acid (60 ml) for 1 hr and then the formic acid was removed by co-distillation with water at 30°. The product was hydrolysed with M-H_2SO_4 (120 ml) at 100° for 2 hr, neutralized with Biodeminrolit resin ($CO_3^=$ form) and evaporated to a syrup, which was resolved into four components (*a*)–(*d*) by paper chromatography (solvent *B*). The separated components were identified (by comparison with authentic specimens) by paper chromatography, by GLC of their methyl glycosides and by preparation of derivatives, as, (*a*) 2,3,4-tri-*O*-methyl-D-xylose (51 mg), the derived *p*-nitrobenzoate having m.p. and mixed m.p. 135°; (*b*) 2,3-di-*O*-methyl-D-xylose (161 mg), the *N*-phenylglycosylamine (anilide) derivative had m.p. and mixed m.p. 136–139°; (*c*) 2,4-di-*O*-methyl-D-xylose (98 mg), the anilide had m.p. and mixed m.p. 171–173°; (*d*) 2-*O*-methyl-D-xylose (130 mg), the derived triacetate had m.p. and mixed m.p. 96–98°.

Extraction of Xylans

The algae were sequentially extracted as indicated below and the polysaccharides were recovered from the neutralized extracting solution by precipitation with acetone (2 vol.). Partial purification was achieved by redissolving the polysaccharide in the extracting solvent, dialysing for 3 days against running water and either precipitating with ethanol (2 vol.), washing with ethanol and ether, and drying, or by freeze-drying. Only those polysaccharide fractions which, on hydrolysis (3 hr with M H_2SO_4 at 100°), gave xylose as a major sugar unit were further purified.

Rhodymenia palmata. The milled alga (100 g) was homogenized with 0·2 M-HCl at 18° for 10 min, and the extract gave a polysaccharide (0·4 g). The washed residue was stirred twice at 100° with H_2O (1 l.) at pH 5 for 24 hr to give a polysaccharide fraction (2·0 g). To the residue in water (500 ml) and HOAc (1 ml) at 70°, sodium chlorite (6 g) was added at hourly intervals for 5 hr. The chlorite extract gave no polysaccharide. The residue was extracted for 3 days at 18° with M-NaOH (250 ml) and then with 3 M-NaOH (100 ml), containing $NaBH_4$ (3 g) at 65° for 5 hr under N_2, to give two further fractions (1·3 g and 1·9 g, respectively). Each fraction was shaken overnight in 0·2 M-NaOH, insoluble material was discarded and Fehling's reagent added to 10% concentration. After 5 hr, the copper complexes were removed, washed with water and decomposed by shaking in 0·2 M-HCl, the polysaccharide being recovered by precipitation with acetone (2 vol.). The polysaccharides were redissolved, dialysed and reprecipitated.

Laurencia pinnatifida. The dried alga (400 g) was extracted exhaustively with hot water until no further polysaccharide was extracted. The residual alga was extracted twice with 2 M-NaOH at 18° for 24 hr and the extracted polysaccharide fraction recovered (2 g). The fraction (1·9 g) was dissolved in 0·2 M-NaOH (100 ml) and Fehling's reagent (100 ml) was added followed, with stirring, by acetone (70 ml). After 16 hr, the precipitated complex was recovered, washed with 25% aq. acetone, and redissolved in 0·2 M-HCl (100 ml). Insoluble residues were discarded and the polysaccharide was recovered as described above (0·4 g).

Porphyra umbilicalis. The dried alga (500 g) was extracted exhaustively with hot water, and the residue was subjected to a chlorite treatment as described above. Polysaccharide (0·5 g) was recovered from the chlorite extracts. This fraction (0·4 g) was dissolved in hot water (35 ml), cooled and 1% $CuSO_4$ solution (100 ml) was added. After 6 hr, the precipitate was recovered, dissolved in 0·5 M-HCl and the polysaccharide (0·2 g) recovered as before. The residue remaining after chlorite treatment was extracted with 3 M-NaOH (200 ml) containing $NaBH_4$ (3 g) at 18° for 5 hr, and a polysaccharide fraction (4·2 g) was recovered from the extract. This fraction contained much silica. It was redissolved in M-NaOH and reprecipitated with ethanol, and the precipitate was extracted in a Soxhlet for 24 hr with water. The insoluble residue (100 mg) was retained for methylation.

Rhodochorton floridulum. The fresh alga was contaminated with much sand from which it could not be separated completely. The alga (1 kg, wet wt.) was extracted with cold 0·2 M-HCl and with hot water as for *Rhodymenia palmata*. The water extract gave fraction *A* (0·5 g) which was retained. The residue was subjected to a chlorite treatment, as described above, and fraction *B* (2·3 g) was recovered from the extract. Each fraction was contaminated with an acidic galactan which was removed by absorption on DEAE-Sephadex A-50 gel. Fraction *A* (250 mg) was dissolved in warm water (15 ml) and cooled. The solution was then percolated down a column (60 × 6 cm) of the gel (chloride form), the column being eluted with water. Polysaccharide

[17] H. C. SRIVASTAVA, S. N. HARSHE and P. P. SINGH, *Tetrahedron Letters* 1869 (1963).

(30 mg) was recovered from the eluate. Similar elution of fraction *B* (500 mg) through the gel gave a polysaccharide (30 mg).

Each polysaccharide was methylated, methanolysed and analysed by GLC as described previously.

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