Phytochemistry, Vol. 26, No. 4, pp. 1033–1035, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

ALKALI-SOLUBLE POLYSACCHARIDES FROM *CHAETANGIUM FASTIGIATUM*: STRUCTURE OF A XYLAN

MARÍA C. MATULEWICZ* and ALBERTO S. CEREZO†

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

(*Received* 9 *July* 1986)

Key Word Index—*Chaetangium fastigiatum*; Chaetangiaceae; seaweed; alkali-soluble polysaccharides; xylan.

Abstract—Extraction of the seaweed with dilute alkali after exhaustive treatment with boiling water led to the isolation of a β-D-(1 → 4)-linked xylan and a complex system of polysaccharides containing sulphate.

INTRODUCTION

D-Xylose occurs in polysaccharides of red algae in the form of either xylans or combined in heteropolysaccharides [1]. Pure xylans may be water-soluble of the 'mixed linkage' type containing both β-(1 → 3)- and β-(1 → 4)-linkages, or alkali-soluble cell-wall components either completely β-(1 → 3)-linked or completely β-(1 → 4)-linked. Xylans of the first type have been isolated from *Rhodymenia palmata* [2], *Rhodochorton floridulum* [1], *Porphyra umbilicalis* [1], *Laurencia pinnatifida* [1], *Nemalion vermiculare* [3], *Chaetangium erinaceum* [4] and *C. fastigiatum* [5]. Further extractions with alkali of *P. umbilicalis* [1, 6] and *R. palmata* [1] gave a β-(1 → 3)-linked and a β-(1 → 4)-linked xylan, respectively.

Little is known about the presence of xylose-containing heteropolysaccharides or of other polysaccharides in the cell walls of red seaweeds. We wish to report the isolation of a complex system of polysaccharides containing sulphate and of a β-D-(1 → 4)-linked xylan from the cell walls of *C. fastigiatum*.

RESULTS AND DISCUSSION

Hot-water extraction of the seaweed gave a xylan [5] with an essentially linear structure consisting of a chain of (1 → 4)- and (1 → 3)-linked β-D-xylopyranose residues. When the residue from this treatment was sequentially extracted four times with dilute alkali, fractions were obtained whose yields, carbohydrate composition, and contents of sulphate and protein are shown in Table 1.

The carbohydrate composition of the fractions was qualitatively similar but the first fraction was different from the others because it contained a small proportion of fucose and its content of xylose was much higher and that of sulphate much lower than in the other fractions.

Further subfractionation of fraction 1 with water at room temperature gave an insoluble residue ($1i_1$) in a *ca* 38% yield which contained mainly xylose and smaller proportions of mannose and galactose (Table 1). When extraction with water was repeated the contents of mannose and galactose diminished but the insoluble residue ($1i_2$) still contained a small amount of sulphate and protein. The supernatants were pooled yielding a water-soluble subfraction (1s) which contained the five sugars present in fraction 1. It is noteworthy that subfraction 1s resisted extraction with boiling water suggesting that its insolubility was due to 'interactions' with other components of the cell-wall. This is consistent with the fact that after two washings with water the xylan still contained small amounts of mannose and galactose. The structure of the xylan (subfraction $1i_2$) was worked out while the other products were reserved for further research.

The optical rotation of the xylan, $[\alpha]_D - 90.5°$ (1.0 M NaOH; *c* 0.34) was similar to that of the β-D-(1 → 4)-linked esparto grass xylan (−92°) [7]. Its ^{13}C NMR spectrum showed five main signals which were assigned to C-1 (δ102.9), C-2 (73.9), C-3 (75.4), C-4 (76.8) and C-5 (64.2) of a 4-*O*-substituted β-D-xylopyranosyl residue [8].

The xylan was insoluble in dimethyl sulphoxide and permethylation was achieved through a two-step procedure according to Haworth and Hakomori [9]. Hydrolysis of the permethylated derivative gave a mixture of partially methylated xyloses (molar %), namely: 2,3-di-*O*-methylxylose (91.3), 2,3,4-tri-*O*-methylxylose (4.2), 2,4-di-*O*-methylxylose (2.4) and 2-*O*-methylxylose (2.1). Traces of 2,3,6-tri-*O*-methylmannose and 2,4,6-tri-*O*-methylgalactose were also detected. This composition confirmed the linear structure with almost exclusively (1 → 4)-linkages but suggested the presence of small amounts of (1 → 3)-linkages and branching, as judged by the yields of 2,4-di-*O*-methylxylose and 2-*O*-methylxylose, respectively. Small proportions of (1 → 3)-linkages and branching were also found in the xylan from *R. palmata* [1].

The degree of polymerization calculated from the non-reducing end-chain xylose content was 50 after discounting the branching, and similar (60) to that obtained from the reducing end unit content [10]. It is noteworthy that the structure of this polysaccharide closely resembles the xylan previously extracted from esparto grass [7].

In summary, a β-D-(1 → 4)-linked xylan has been identified among the components of the cell-walls of *C.*

*Research Member of the National Research Council of Argentina (CONICET).

†To whom correspondence should be addressed.

Table 1. Yields and composition of the fractions extracted with alkali from the red seaweed *C. fastigiatum*

Fraction	Yield* (%)	Carbohydrate composition (mol %) Xyl	Man	Gal	Glc	Fuc	Sulphate* (%)	Protein (%)
1	0.46	77.2	9.5	9.1	2.1	2.0	6.7	35.4
2	0.34	20.5	12.0	19.3	48.1	—	29.3	68.2
3	0.24	19.8	25.4	39.8	14.9	—	20.8	36.3
4	0.13	22.2	19.0	45.3	13.6	—	30.6	13.1
ls		10.9	37.5	37.6	3.4	10.6	26.4	47.5
li_1†		94.9	2.7	2.4	—	—	n.d.§	n.d.
li_2‡		98.2	0.2	1.6	—	—	2.0	5.5

*After subtraction of the protein.
†Obtained after one extraction of fraction 1 with water at room temp.
‡Obtained after two extractions of fraction 1 with water at room temp.
§n.d., not determined.

fastigiatum; the cell-walls also comprise a complex system of polysaccharides containing sulphate.

EXPERIMENTAL

Materials. Chaetangium fastigiatum was collected near Puerto Deseado in Southern Patagonia (Argentina) and was dried in the open under strong winds.

Extractions. The alga (60 g) was exhaustively extracted with boiling water [5] and the residue extracted with 8% NaOH (1 l.) containing $NaBH_4$ (0.1 g) and previously bubbled with N_2, under mechanical stirring for 4 hr at room temp. The insoluble residue was removed by centrifugation and the supernatant dialysed, concd to half-vol., and poured into 3 vol. (1.5 l.) of EtOH. The resultant ppt was removed by centrifugation and dried by solvent exchange (EtOH and Et_2O) and finally *in vacuo*. The extraction procedure was repeated × 3. Yields: fraction 1, 0.424 g; fraction 2, 0.644 g; fraction 3, 0.225 g; fraction 4, 0.090 g.

Fraction 1 (0.107 g) was suspended in H_2O (25 ml) and stirred overnight at room temp.; the ppt was removed by centrifugation and dried as described before ($1i_1$, 41 mg). This procedure was repeated once more on the insoluble residue ($1i_2$, 38 mg). The supernatants were pooled, concd and freeze-dried (1s, 54 mg).

General methods. Sulphate (expressed as $NaSO_3$) was analysed by the method of Wagner [11], after removing sodium cations. Nitrogen was determined by the method of Dumas-Pregl [12] and the protein content was calculated by multiplying the nitrogen content by 6.25.

The ^{13}C NMR spectrum was measured at 25.2 MHz with the sample (16 mg) dissolved in 1.0 M NaOH–D_2O (2:1, 0.4 ml) and with complete proton decoupling; chemical shifts were referred to external TMS.

Hydrolyses of polysaccharides were carried out in sealed tubes with 2 M TFA for 16 hr at 95° and the sugar mixtures were derivatized for analysis by GC and GC/EIMS.

GC was performed on glass columns (a) 3% ECNSS-M on Gas-Chrom Q (100–120 mesh) (1.8 m × 2 mm) and (b) 3% OV-17 (1.8 m × 2 mm). Chromatography on column (a) was carried out at 190° for the alditol acetates [13] and at 170° for the partially methylated alditol acetates [14] and partially methylated aldononitrile acetates [15]; the N_2 flow rate was 25 ml/min, the injector temp. 210° and the FID temp. 210°. For the determination of the degree of polymerization [10] column (b) was used at 170° with a N_2 flow rate of 27 ml/min, an injector temp. of 250° and a FID temp. of 250°. Computerized GC/EIMS of the partially methylated alditol acetates [14] and partially methylated aldononitrile acetates [16] was performed at 70 eV; chromatography was carried out on a glass column (1.2 m × 3 mm) of 3% ECNSS-M on Gas-Chrom Q (100–120 mesh) at 120° for 1 min and then 4°/min to 190°. The He flow rate was 25 ml/min.

Methylation analysis. The xylan was initially methylated by the method of Haworth and further fully methylated according to Hakomori [9] as described by Lindberg [14]. The Hakomori procedure was repeated until constant composition.

The permethylated polysaccharide (2.0 mg) was hydrolysed with 2 M TFA (0.5 ml). Half of the resulting mixture was reduced with $NaBD_4$ and the product acetylated; the other aliquot was converted into the corresponding aldononitrile acetates. The mixtures of alditol acetates and aldononitrile acetates were analysed by GC and GC/EIMS and identified by RR_t (known standards) and mass spectra.

Acknowledgements—The authors are indebted to Lic. Guillermo A. Kopp and to UMYMFOR (FCEyN-CONICET) for technical assistance. This work was supported by grants from CONICET, UNESCO (Rostlac 036/84), the University of Buenos Aires and the International Foundation for Science (Sweden).

REFERENCES

1. Turvey, J. R. and Williams, E. L. (1970) *Phytochemistry* **9**, 2383.
2. Percival, E. G. V. and Chanda, S. K. (1950) *Nature* **166**, 787.
3. Usov, A. I., Adamyants, K. S., Yarotskii, S. V., Anoshina, A. A. and Kochetkov, N. K. (1974) *Zh. Obshch. Khim.* **44**, 416.
4. Nunn, J. R., Parolis, H. and Russell, I. (1973) *Carbohydr. Res.* **26**, 169.
5. Cerezo, A. S., Lezerovich, A., Labriola, R. and Rees, D. A. (1971) *Carbohydr. Res.* **19**, 289.
6. Frei, E. and Preston, R. D. (1964) *Proc. R. Soc. B* **160**, 314.
7. Chanda, S. K., Hirst, E. L., Jones, J. K. N. and Percival, E. G. V. (1950) *J. Chem. Soc.* 1289.
8. Kovač, P., Hirsch, J., Shashkov, A. S., Usov, A. I. and Yarotskii, S. V. (1980) *Carbohydr. Res.* **85**, 177.
9. Hakomori, S. (1964) *J. Biochem. (Tokyo)* **55**, 205.

10. Morrison, I. M. (1975) *J. Chromatogr.* **108**, 361.
11. Wagner, H. (1957) *Mikrochim. Acta* 19.
12. Dumas-Pregl (1958) in *Quantitative organische Mikroanalyse* (Pregl, F. and Roth, H., eds.) p. 91. Springer, Vienna.
13. Sloneker, J. H. (1972) *Methods Carbohydr. Chem.* **6**, 20.
14. Lindberg, B. (1972) *Methods Enzymol.* **28**, 178.
15. Tanner, G. R. and Morrison, I. M. (1984) *J. Chromatogr.* **299**, 252.
16. El'kin, Yu. N., Rozynov, B. V. and Dzizenko, A. K. (1972) *Khim. Prir. Soedin.* **8**, 642.