A XYLAN FROM THE RED SEAWEED *Chaetangium fastigiatum*

A. S. CEREZO, A. LEZEROVICH, R. LABRIOLA

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Buenos Aires (Argentina)

AND D. A. REES*

Chemistry Department, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ (Great Britain)

(Received February 22nd, 1971; accepted for publication, April 5th, 1971)

ABSTRACT

The main water-soluble polysaccharide from *Chaetangium fastigiatum*, a red seaweed of the order *Nemalionales*, is a xylan which appears from methylation analysis to contain (1→4)- and (1→3)-linked β-D-xylopyranose residues in the approximate ratio 3:1. There is evidence that this polysaccharide is branched to a small degree, is polydisperse, and has a random-coil conformation in aqueous solution.

INTRODUCTION AND DISCUSSION

Most red algae apparently contain a galactan sulphate, in large amounts (up to 35% of the dry weight), as the main water-soluble polysaccharide[1,2]. These substances are quite different in structure from any polysaccharides that occur in land plants or bacteria although, in terms of both structure and conformation, there are marked resemblances to the polysaccharide sulphates of animals[3]. One species of seaweed, *Rhodymenia palmata*, has long been recognized as an exception in that it gives, as the main soluble polysaccharide, a xylan containing β-(1→3)- and β-(1→4)-linked residues[4,6]. In structure and in solubility properties, it is (*a*) unlike the typical hemicellulose xylans of land plants, which are not usually extractable with water and are based on backbone chains of β-(1→4)-linked residues only[7], and (*b*) also unlike certain xylans of green algae[8], also insoluble, which contain continuous chains of β-(1→3) linkages. It has recently been shown that xylans containing mixed β-(1→3) and β-(1→4) linkages do occur in other algae, including some of the *Rhodophyceae*, and may appear in soluble or insoluble fractions[9]. We now report the isolation and characterization of another example of the soluble type. In terms of amount (32% crude yield), it seems to be much more important than any other red-seaweed xylan so far known. It is extracted from a seaweed, *Chaetangium fastigiatum*, of the order *Nemalionales*; when we started this investigation, we knew of no representative of this order which had been examined in detail for polysaccharide content. Since that time,

*Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, England.

Turvey and Williams have briefly reported on a xylan from *Rhodochorton floridulum*[9], with which comparison is made below.

The red seaweed *Chaetangium fastigiatum* grows on the rocks, in the middle littoral floor of the shores of Patagonia and Tierra del Fuego. The sample was collected in winter near the Estación Algológica de Puerto Deseado in Southern Patagonia. It was dried in the open under strong winds and extracted with boiling water, and the crude products were precipitated from aqueous solution with isopropyl alcohol. After acid hydrolysis, xylose was the main sugar that could be detected by paper chromatography, with galactose and mannose as minor components. Sulphate ester was also present. Ultracentrifugation showed two separate peaks. An acidic polysaccharide could be removed by precipitation with cetylpyridinium chloride, leaving a neutral xylan in solution which was recovered in *ca.* 26% yield by addition of isopropyl alcohol. On acid hydrolysis, this product gave D-xylose only, which was firmly characterized as the crystalline sugar and dibenzylidene dimethyl acetal. The xylan showed a single peak in the ultracentrifuge, broad enough to suggest polydispersity. Indeed, graded precipitation by the addition of ethanol to an aqueous solution gave fractions which differed in optical rotation and in behaviour towards periodate oxidation; these properties are interpreted below.

The xylan was methylated twice with barium hydroxide and methyl iodide in a mixture of methyl sulphoxide and *N*-methyl-2-pyrrolidone, and then once with silver oxide and methyl iodide in *N*,*N*-dimethylformamide. Hydrolysis of the product, followed by cellulose-column chromatography, led to the isolation of 2-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,4-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-xylose, each of which was identified by formation of a crystalline derivative. The yields were in the approximate molar proportions, 1:62:19:1.4, respectively. If xylofuranose residues are absent, the polysaccharide therefore contains chains of (1→3)- and (1→4)-linked residues and has an average chain-length of 50–60. Although the structure seems from this evidence to be branched, the indications of molecular weight range, derived from periodate-oxidation experiments with subfractions (see below), indicate that there are only a few chains in each molecule.

Information about linkage configuration and about polymer chain-conformation can be inferred from the optical rotation by use of a new quantitative approach to the optical rotations of polysaccharides[10,11]. The molecular rotation to be expected for the polysaccharide is estimated by summation of "residue contributions" and "linkage contributions". These various terms can be taken from appropriate model compounds; the (1→3)-linkage contribution, for reasons given elsewhere[12], can be taken from the glucose series in which the relevant oligomers have been thoroughly characterized. Thus the residue contribution for β-D-xylose (taken, according to convention[10], as the molecular rotation of methyl β-D-xylopyranoside[13]) is −107.4°, the linkage contribution ($[A]_D$; see Ref. 10) derived from the (1→4)-linked xylodextrins[14], adjusted for the proportion of (1→4) links actually present [(62 × −40.6°)/(62+19)], is −31.2°, the linkage contribution derived from laminaridextrins[15], adjusted for the proportion of (1→3) links [(19 × 13)/(62+19)], is 3.1°, and the

total, *i.e.* the estimated molecular rotation for the polysaccharide, is −135.5°. This corresponds to a specific optical rotation of −102.5°, in close agreement with the measured value of −99.3°. Hence, we conclude that all residues probably have the β-D configuration, and that the conformation at each glycosidic linkage corresponds to that in the model oligosaccharides, *i.e.* the polysaccharide has a random, fluctuating conformation ("random coil"), rather than any ordered secondary structure. This, in turn, suggests that the (1 → 3) linkages are interspersed throughout the structure rather than grouped contiguously, because the optical rotations of glucans having contiguous β-(1 → 3)-D linkages[16] are quite anomalous[17], probably because of conformational effects which, from evidence for the solid state[18], are also likely for the xylose series.

The properties of the xylan subfractions are shown in Table I. Their precipitation behaviour during isolation did not suggest that they were likely to be structurally distinct, rather than arbitrary, parts of a continuous range of molecular structure. The optical rotation and periodate-oxidation properties both indicate differences in the proportions of (1 → 3) and (1 → 4) linkages, although the estimates do not agree quantitatively, presumably because of experimental error. Although the amounts of formic acid and formaldehyde formed by periodate oxidation (Table I) do not have unique interpretations in terms of polysaccharide structure, the figures do confirm that the subfractions are different and give an *approximate* idea of (i) the chain length, which is probably a factor of 1.0–2.0 times the "moles of anhydroxylose per mole of formic acid" (Table I); and (ii) the degree of polymerisation, which is probably a factor of 1.0–2.0 times the "moles of anhydroxylose per mole of formaldehyde" (Table I). Thus, the results are consistent with a rather small polysaccharide molecule,

TABLE I

SOME PROPERTIES OF FRACTIONS *A*, *B*, AND *C*

Fraction	*Yield* (%)	$[\alpha]_D$ (*degrees*)	*Moles of "anhydroxylose" per mole of*			(*1→4*)-*Linkages* (%)
			periodate	*formic acid*[a]	*formaldehyde*[b]	
A	80	−112	1.18	54	78	85
B	9.1	−78	1.19	24	37	83
C	10.9	−57	1.28	97	42	77

[a]This was determined after direct oxidation of the polysaccharide, without prior reduction with sodium borohydride. [b]This was determined after oxidation of a polysaccharide sample which had previously been reduced with sodium borohydride.

having up to 4 branch points. Possibly, the subfractions differ in average degree-of-branching as well as other properties.

Turvey and Williams[9] have recently pointed out that, when xylose residues exist in red algae, both (1 → 3)- and (1 → 4)-xylosidic linkages may occur together in homoxylans or heteropolysaccharides which may be branched or linear; alternatively,

when the xylan is a skeletal component of the cell wall, the structure is essentially linear and may be completely (1→3)- or (1→4)-linked. *Chaetangium* xylan falls neatly into this scheme, as a representative of the "mixed linkage", branched, homoxylan, type. The resemblance to *Rhodymenia* xylan is quite marked, in types of linkage[6], molecular weight[6], polydispersity[6], optical rotation[4,5], and, very probably, degree-of-branching[9]. Curiously, such a low but definite degree-of-branching is also characteristic of the land-plant xylans, which are otherwise rather different in structure[19]. *Rhodochorton floridulum*, another member of the *Nemalionales*, contains a polysaccharide having (1→3)- and (1→4)-linked xylose residues which seems to contain at least some of the xylose in a heteropolysaccharide[9].

EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 paper and t.l.c. on microcrystalline cellulose (Avicel), using the following solvents: (*A*) ethyl acetate–pyridine–water (10:4:3), (*B*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (*C*) butyl alcohol–ethanol–water (4:1:5, upper layer), (*D*) butanone–water–ammonia (200:17:1), and (*E*) butanone–water azeotrope. The spray reagents used were: (*A*) aniline hydrogen phthalate in butyl alcohol saturated with water, and (*B*) *p*-anisidine hydrochloride in butyl alcohol.

Infrared spectra were recorded with a Perkin–Elmer 137B spectrometer. All evaporations were in a rotatory evaporator, under reduced pressure, at 35–40° (bath). Optical rotations are equilibrium values. Melting points are uncorrected.

Extraction of polysaccharides. — *Chaetangium fastigiatum* (600 g, which had been dried in the open under strong winds), collected near the Estación Algológica de Puerto Deseado in Southern Patagonia, was extracted with boiling water (12 l) for 3.5 h. The solution was filtered through muslin and centrifuged to give a supernatant solution (9 l) which was dialysed against running-tap water for 4 days, concentrated, and freeze-dried. The extraction was repeated until no further precipitate was obtained, and the solution was then poured into two volumes of isopropyl alcohol; yield 193.5 g (32.4% by weight), $[\alpha]_D^{20}$ −31.5° (*c* 0.2, water). Part (0.100 g) of this crude product was dissolved in 0.5M sulphuric acid and heated for 4 h at 100°. The solution was neutralized (barium carbonate), centrifuged, and concentrated to dryness. Paper chromatography showed xylose as the major component, with galactose, mannose, and a small proportion of glucose.

The sedimentation pattern in the ultracentrifuge showed that the crude extract was a mixture of at least two major components.

Fractionation of the mixture and isolation of the xylan. — The crude mixture (20.0 g) was dissolved, with mechanical stirring, in warm water (4 l). After cooling to room temperature, an aqueous solution of cetylpyridinium chloride (10% w/v, 250 ml) was slowly added, previous experiments having shown that this amount caused complete precipitation. After centrifugation, aqueous potassium iodide (10%,

50 ml) was added to the supernatant solution, and the precipitated detergent was removed by further centrifugation. The solution was concentrated to 500 ml and dialyzed against running-tap water for 2 days, isopropyl alcohol was then added to turbidity, and the solution was poured into two volumes of isopropyl alcohol. The liquors were decanted, and the precipitate was squeezed between sheets of filter paper and dried by solvent exchange with methanol and then ether, and finally in a vacuum oven at 45°; yield 5.23 g, $[\alpha]_D^{20}$ −90.5° (*c* 0.23, water). This product (0.2 g) was dissolved in water (20 ml) and reprecipitated by addition of isopropyl alcohol (40 ml); $[\alpha]_D^{18}$ −99.5° (*c* 0.21, water).

The purified polymer (0.03 g) was heated overnight in a sealed tube with 50% formic acid (2 ml). Concentration to dryness and paper chromatography showed xylose as the only detectable monosaccharide.

Identification of D-*xylose.* — After hydrolysis of purified xylan (0.6 g) with 0.5M sulphuric acid (50 ml) for 3 h at 100°, the solution was neutralized (barium carbonate), filtered, and concentrated to dryness. Evaporation of ethanol (10 ml) from the residue, followed by storage in a vacuum desiccator, gave a crystalline product, m.p. 131–138°. Recrystallization from ethanol–ether gave D-xylose, m.p. 138–141°, $[\alpha]_D^{16}$ +18.3°. The derived dibenzylidene dimethyl acetal had m.p. and mixed m.p. 201–203° [after recrystallization from chloroform–light petroleum (b.p. 40–60°)].

Fractionation of the xylan. — The supernatant solution (500 ml) obtained after removal of acidic polysaccharides was dialyzed against running-tap water for 48 h, and any remaining detergent was removed by extraction with a mixture of chloroform–butyl alcohol (1:1). Ethanol was added slowly to a concentration of 35.5% (w/w), and a small amount of flocculent precipitate was then removed by centrifugation and discarded. Further addition of ethanol to 62.0% concentration gave a precipitate which, after allowing 3 h to flocculate, was isolated by centrifugation and dried by solvent exchange with absolute ethanol and then ether, and finally *in vacuo* at room temperature; yield 0.73 g, $[\alpha]_D^{18}$ −112.0° (*c* 0.5, water). This product is hereafter called Fraction *A*. More ethanol was added to the supernatant solution, to 70% concentration, and the new precipitate was treated in the same way to give Fraction *B*; yield 83 mg, $[\alpha]_D^{18}$ −78° (*c* 0.4, water). Addition of more ethanol gave no further precipitate, and the solution was therefore concentrated to 50 ml, dialyzed for 48 h against running-tap water, and freeze-dried to a white powder, Fraction *C*; yield 0.1 g, $[\alpha]_D^{18}$ −57.0° (*c* 0.6, water). Hydrolysis of each of the fractions gave only xylose (paper chromatography).

Periodate oxidation of fractions A, B, *and* C. — Each polysaccharide fraction (50–60 mg) was dissolved in 50 mM sodium metaperiodate (25 ml) and kept at room temperature in the dark for 5 days. At intervals, samples (0.15 ml) were removed and diluted to 100 ml for determination of periodate by the spectrophotometric method[20]. Formic acid was determined by potentiometric titration with 10 mM sodium hydroxide under nitrogen. Because some over-oxidation was observed, results were extrapolated to zero time. The values are shown in Table I.

Formaldehyde formed by periodate oxidation of the reduction products of fractions

A, B, *and* C. — Each polysaccharide fraction (20–25 mg) was dissolved in water (3 ml) and reduced with sodium borohydride (15 mg) at room temperature for 55 h. After the addition of cold acetic acid to pH 3–4, solid sodium metaperiodate (53.5 mg) was added, and the solution was diluted to 5 ml (to give a final concentration of 50 mM with respect to periodate) and kept in the dark at room temperature. Samples (2 ml) were taken after oxidation for 96 and 140 h, and lead formate (0.4 g) was added to remove iodate and periodate. After centrifugation, a part (1.5 ml) of each supernatant solution was dialyzed against the same volume of water and the determination of formaldehyde by the chromotropic acid method[21] was performed on a sample (1 ml) of the diffusate. Identical results were obtained in both determinations.

Methylation of the xylan. — The polysaccharide (3.0 g) was dissolved in a mixture of methyl sulphoxide (100 ml) and *N*-methyl-2-pyrrolidone (100 ml) by shaking overnight. The mixture was cooled to 0°, and barium hydroxide octahydrate (12.0 g) was added with methyl iodide (10 ml). After shaking for 3 h, the ice bath was removed, and shaking was continued for 16 h. After a further addition of reagents and shaking in the same way, the mixture was diluted with chloroform (500 ml) and water (500 ml). The chloroform layer was separated and retained, and the aqueous layer was extracted 4 times with chloroform (4 × 200 ml) and then rejected. The combined chloroform extracts were washed with water, dried (sodium sulphate), and concentrated to a syrup which was diluted with benzene and then with light petroleum (40–60°) until turbid. The methylated derivative was precipitated by pouring the product into an excess of light petroleum (b.p. 40–60°); yield 1.75 g, $[\alpha]_D^{21}$ −57.1° (*c* 0.3, chloroform). A further methylation by the same method gave a product which still showed some i.r. absorption in the OH stretching region of the spectrum.

The product (1.5 g) was dissolved in *N*,*N*-dimethylformamide (75 ml), and freshly prepared silver oxide (15 g) was added with methyl iodide (30 ml). The system was stirred magnetically for 4 h at 35° and, after a further addition of the same quantities of silver oxide and methyl iodide, stirring was continued for 16 h. The mixture was filtered, and the precipitate was washed with chloroform. The solution was extracted with aqueous sodium cyanide (10%, 200 ml) to give an aqueous layer which was back-extracted with chloroform (5 × 50 ml). The combined chloroform extracts were dried (sodium sulphate), concentrated to 50 ml, and then poured into light petroleum (b.p. 40–60°, 300 ml). The syrup which separated was re-dissolved in chloroform (50 ml), light petroleum was added to turbidity, and the mixture was poured, slowly with stirring, into an excess of light petroleum (b.p. 40–60°, 300 ml). The solid precipitate was removed on the centrifuge, washed with light petroleum, and dried in a vacuum desiccator at room temperature; yield 1.2 g, $[\alpha]_D^{21}$ −79.7° (*c* 0.3, chloroform) (Found: OCH_3, 36.95. Calc. for tri-*O*-methylxylan: OCH_3, 38.8%). The i.r. spectrum showed no hydroxyl band. Hydrolysis (0.5M sulphuric acid, overnight at 95°) and chromatography (solvents *C* and *D*) showed the presence of *O*-methylxyloses but the absence of xylose itself, even when the paper was heavily spotted.

Hydrolysis of the methylated xylan. — A solution of the methylated xylan (1.0 g) in formic acid (50% v/v; 80 ml) was heated for 18 h at 95°. Formic acid was removed

by evaporation, and methanol (50 ml) was twice evaporated from the residue. The product was dried in a vacuum desiccator over potassium hydroxide pellets and then dissolved in methanol to give a solution which was filtered and concentrated to a light-red syrup. Paper chromatography (solvents *C* and *D*) showed 2-*O*-methylxylose, 2,4-di-*O*-methylxylose, 2,3-di-*O*-methylxylose, and 2,3,4-tri-*O*-methylxylose. This mixture was separated by cellulose-column chromatography (solvent *E*) with automatic collection of the eluate in fractions of 10 ml. After paper chromatography (solvent *D*) of the contents of alternate tubes, it was possible to combine them to give the following larger fractions.

*Fraction 1. Identification of 2,3,4-tri-*O*-methyl-*D*-xylose.* — The pure component (15 mg) from tubes 1–8 was chromatographically identical to 2,3,4-tri-*O*-methyl-D-xylose (R_F 0.79, solvent *D*) and had $[\alpha]_D^{22}$ +18.6° (*c* 0.2, water); lit.[22] $[\alpha]_D$ +18.0° (water). The derived 2,3,4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. 99–100°, $[\alpha]_D^{23}$ +49.3° (*c* 0.2, ethanol); lit.[22] m.p. 102°, $[\alpha]_D$ +47.0° (ethanol).

*Fraction 2. Identification of 2,3-di-*O*-methyl-*D*-xylose.* — Tubes 10–32 contained one substance only (R_F 0.53, solvent *D*). Evaporation gave a syrupy material (0.616 g), $[\alpha]_D^{22}$ +24.3° (*c* 0.3, water); lit.[5] $[\alpha]_D$ +23.0° (water). The derived 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. 143–144°, $[\alpha]_D^{22}$ +183.2° (*c* 0.5, ethyl acetate); lit.[5] m.p. 146°, $[\alpha]_D$ +185° (ethyl acetate).

Fraction 3. — Evaporation of the contents of tubes 33–37 gave a syrup (0.10 g), $[\alpha]_D^{23}$ +21.3° (*c* 0.8, water), composed of two substances with R_F 0.53 and 0.42 (solvent *D*) which were chromatographically identical to 2,3-di-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-D-xylose (see fraction 4), respectively. Visual examination of the chromatograms suggested a 1:1 ratio of these sugars.

*Fraction 4. Identification of 2,4-di-*O*-methyl-*D*-xylose.* — This component (0.20 g), from tubes 38–48, showed R_F 0.43 (solvent *D*) and $[\alpha]_D^{20}$ +23.5° (*c* 0.4. water), corresponding to 2,4-di-*O*-methyl-D-xylose; lit.[23] $[\alpha]_D$ +22.0° (water). 2,4-Di-*O*-methyl-*N*-phenyl-D-xylosylamine was obtained by treatment of the syrup with aniline in the usual way, and had m.p. 168°, $[\alpha]_D^{20}$ −82.3° (*c* 0.4, *p*-dioxane); lit.[23] m.p. 170°, $[\alpha]_D$ −82.0° (*p*-dioxane).

*Fraction 5. Identification of 2-*O*-methyl-*D*-xylose.* — Evaporation of the contents of tubes 74–147 gave a small amount of a syrupy material (8–10 mg), which showed R_F 0.18 (solvent *D*) and $[\alpha]_D^{16}$ +33.2° (*c* 0.2 water); lit.[24] $[\alpha]_D$ +36.0° (water). The derived 2-*O*-methyl-*N*-phenyl-D-xylosylamine had $[\alpha]_D^{18}$ +231° (*c* 0.1, ethyl acetate); lit.[25] $[\alpha]_D$ +237° (ethyl acetate).

After these fractions had been collected, the column was washed with a further quantity of the same solvent mixture (500 ml) and then with water (500 ml), but paper chromatography of the concentrated, combined eluates showed neither xylose nor any of its other monomethyl ethers.

ACKNOWLEDGMENT

One of the authors (A. L.) is indebted to the Consejo Nacional de Investigaciones Científicas y Técnicas for a research fellowship which was held in Edinburgh

(1964–1966), to initiate this work. The work was also supported by a grant from the Consejo Nacional de Investigaciones Cientificas y Técnicas (Argentina). We thank Professor Sir Edmund Hirst C.B.E., F.R.S., for his interest.

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