Note

The fine structure of *Chaetangium fastigiatum* xylan: studies of the sequence and configuration of the (1→3)-linkages*

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The water-soluble xylan extracted from the red seaweed *Chaetangium fastigiatum* has an essentially linear structure, consisting of a chain of (1→4)- and (1→3)-linked D-xylopyranose residues in proportions of ~80–85% and 20–15%, respectively[1]. The β-D configuration of the linkages has been inferred[1] from the optical rotation by use of Rees' quantitative approach to the optical rotation of polysaccharides[2,3]. The agreement between the experimental value of the specific rotation of the polysaccharide and that calculated on the basis of model oligosaccharides having fluctuating conformations suggested that the xylan has a "random coil" conformation, and also that the (1→3)-linkages are interspersed in the structure rather than grouped contiguously[1]. Evidence to support these proposals has now been obtained by Smith degradation[4] of the xylan.

When the *Chaetangium fastigiatum* xylan was sequentially oxidised with periodate, reduced with sodium borohydride, and subjected to mild hydrolysis with acid, a mixture of non-reducing products and xylose was obtained. Paper chromatography indicated the presence of 2-*O*-β-D-xylopyranosylglycerol as major component together with traces of other components, possibly of higher molecular weight. Similar treatment of the water-soluble xylan obtained from *Rhodymenia palmata* also gave a mixture of products, including xylose, glycerol, and xylosyl-glycerol[5].

The mixture was resolved, using preparative, paper chromatography, into two fractions. One fraction contained 2-*O*-β-D-xylopyranosylglycerol which, after further purification by the same procedure, was identified by its optical rotation and by preparation of the penta-(*p*-nitrobenzoate)[6].

The other fraction contained at least two products, and these gave xylose and glycerol (paper chromatography) on complete hydrolysis with acid. The low R_{Xyl} values (0.12 and 0.20, solvent *B*) for these products suggest that the number of residues [(1→3)-linked] is higher than three [*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-glycerol has R_{Xyl} 0.35 in the same solvent[7]], thus indicating the presence of a small number of zones in the xylan chain which have several adjacent (1→3)-linked "anhydroxylose" units.

*Dedicated to Professor M. Stacey, C.B.E., F.R.S., in honour of his 65th birthday.

In summary, Smith-degradation of the water-soluble xylan extracted from the red seaweed *Chaetangium fastigiatum* supports the previous suggestion[1] that the (1→3)-linkages are interspersed in the chain. It indicates also that some (1→3)-linked units are grouped in zones containing several residues.

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 paper; Whatman No. 3MM paper was used for the preparative separation of compounds. The solvents used were: (*A*) butanone–water azeotrope and (*B*) ethyl acetate–pyridine–water (10:4:3). The spots were developed with (*a*) aniline phthalate in 1-butanol saturated with water, and (*b*) the periodate–benzidine reagent. All evaporations were carried out in a rotatory evaporator, under diminished pressure, at 35–40° (bath temperature). The optical rotations are at equilibrium. Melting points are uncorrected.

Periodate oxidation of the xylan and reduction of the oxopolysaccharide. — A solution of the purified xylan (1.5 g) in 0.15M sodium periodate (100 ml) was kept in the dark at room temperature. Aliquots (0.15 ml) were taken at intervals and diluted to 1.000 ml, and the consumption of periodate was determined by the spectrophotometric method. Because over-oxidation was observed, the results were extrapolated to zero time. The consumption was 0.80 mole of periodate per mole of "anhydroxylose".

The solution containing the oxopolysaccharide was dialysed against distilled water until periodate and iodate salts were removed. Sodium borohydride (2 × 0.1 g) was added and the mixture was left at room temperature for 24 h. After dialysis, the solution was freeze-dried to yield the polyalcohol (0.6 g).

Mild, acid hydrolysis. — A solution of the polyalcohol (0.6 g) in 50mM sulphuric acid (50 ml) was kept at room temperature for 12 h. The solution was then neutralized (barium carbonate) and centrifuged, and the supernatant was concentrated to dryness. Paper chromatography of the residue (solvents *A* and *B*; reagent *a*) indicated the presence of xylose. When reagent *b* was used, a major spot having R_{Xyl} 0.76 (solvent *A*) and 1.0 (solvent *B*) was obtained, together with xylose, glycerol, and minor amounts of another two products (at least) having R_{Xyl} 0.12 and 0.20 (solvent *B*).

The mixture (0.3 g) was resolved by preparative, paper chromatography (solvent *B*), and two fractions were collected. Fraction 1 (60 mg) contained the product with R_{Xyl} 1.0 (solvent *B*) which, after purification by the same procedure (but using solvent *A*), had $[\alpha]_D^{22}$ −32.5° (*c* 1.0, water). Its properties suggested that it was 2-*O*-β-D-xylopyranosylglycerol; lit.[6] $[\alpha]_D$ −33.0° (water), R_{Xyl} 0.76 (solvent *A*) and 1.0 (ref. 7; solvent *B*). Treatment with *p*-nitrobenzoyl chloride in pyridine gave 2-*O*-β-D-xylopyranosylglycerol penta-(*p*-nitrobenzoate), m.p. 100–102°, $[\alpha]_D^{22}$ −37.5° (*c* 0.8, ethyl acetate); lit.[6] m.p. 101–102°, $[\alpha]_D$ −37.1° (ethyl acetate).

Fraction 2 (10 mg) contained products with R_{Xyl} 0.12 and 0.20 (solvent *B*). Hydrolysis with 0.5M sulphuric acid (2 ml, 7 h, 95°), followed by neutralisation (barium carbonate), gave (paper chromatography, solvent *A*) xylose and glycerol.

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REFERENCES

1 A. S. CEREZO, A. LEZEROVICH, R. LABRIOLA, AND D. A. REES, *Carbohyd. Res.*, 19 (1971) 289.
2 D. A. REES, *J. Chem. Soc.*, (1970) 877.
3 D. A. REES, W. E. SCOTT, AND F. B. WILLIAMSON, *Nature (London)*, 277 (1970) 390.
4 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1966) 361.
5 D. J. MANNERS AND J. P. MITCHELL, *Biochem. J.*, 89 (1963) 92P.
6 G. G. S. DUTTON AND A. M. UNRAU, *Can. J. Chem.*, 40 (1962) 348.
7 G. O. ASPINALL AND K. M. ROSS, *J. Chem. Soc.*, (1963) 1681.