551. *Polysaccharides Synthesised by* Monodus subterraneus. *Part II.*[1] *The Cell-wall Glucan*

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Earlier work [1] on the non-motile unicellular alga, *M. subterraneus*, established that the cell-wall polysaccharide synthesised by this organism is an essentially β-1,4-linked glucan. The solubility and other physical properties of this material, however, were different from those of a true cellulose and gas chromatography of the methanolysed methylated polymer indicated the possible presence of a small proportion of 1,3-linkages. It was impossible from these studies to decide whether the material was a single polysaccharide of the lichenin type or a low-molecular-weight cellulose contaminated with a small proportion of laminarin. That both types of linkage are present in a single polysaccharide has now been proved by the application of the Smith degradation,[2] that is, periodate oxidation, borohydride reduction, and mild acid hydrolysis. This led to the separation of a small

[1] Part I, A. Beattie and E. Percival, *Proc. Roy. Soc. Edinburgh*, 1962, *B*, **68**, 171.
[2] G. G. S. Dutton and A. M. Unrau, *Canad. J. Chem.*, 1962, **40**, 348.

proportion of glucosyl-erythritol. The presence, after this treatment, of an oligosaccharide containing uncleaved glucose (derived from a 1,3-linked unit), and erythritol (derived from a 1,4-linked glucose), confirms the presence of both types of linkage in a single molecule of the glucan. Although the glucosyl-erythritol could result from branch points at C-2 or C-3 on the main chain of β-1,4-linked units, the presence of 2,4,6-tri-*O*-methylglucose in the hydrolysate of the methylated polysaccharide [1] proves that at least some 1,3-linked units are an integral part of the macromolecule. *M. subterraneus* therefore synthesises a lichenin-type polysaccharide although the proportion of 1,3-linkages appears to be considerably smaller than that found in lichenin (30%).[3] The absence of fragments containing more than a single glucose unit indicates that the 1,3-linkages occur singly along the mainly 1,4-linked chains.

An improved method of extraction and purification of the glucan has been developed. Attempted purification by elution from a column of DEAE-cellulose was unsuccessful; no purification was achieved and the polysaccharide was strongly bound on the cellulose, indicating an essentially linear polymer,[4] in agreement with the earlier findings.

Experimental.—Chromatography was carried out as before.[1]

Extraction and purification. *M. subterraneous* (*ca.* 6 g.), grown under bacteria-free conditions as for the earlier investigations, was frozen in liquid nitrogen and the cells completely disintegrated. The resulting powder was exhaustively extracted with n-butanol (6 × 100 ml.). The decolourised material was then extracted with cold water (3 × 200 ml.) twice for 6 hr. and once for 12 hr. The residue was then sequentially extracted with hot water, chlorite solution, and 4% sodium hydroxide solution as in the earlier work.[1] After neutralisation of the alkaline extract with glacial acetic acid and removal of the non-carbohydrate precipitate, the crude glucan (770 mg.; 40% carbohydrate [5]) was precipitated as a white powder with ethanol (10 vol.). This was dissolved in water (19 ml.), and an aqueous solution of phenol (90%; 19 ml.) added, the mixture vigorously shaken for 0·5 hr., and stored overnight in the refrigerator to allow separation into two phases. The resulting emulsion at the interface of the two phases was dispersed by warming with butyric acid (*ca.* 1 ml.). The aqueous layer containing the polysaccharide was separated, contaminating phenol was removed by extraction with ether, and the glucan (163 mg., 80% carbohydrate,[5] 10% moisture) isolated by freeze-drying.

Smith degradation. (a) *Periodate oxidation.* Glucan (135 mg.) in sodium metaperiodate (20 ml.; 0·14M) was set aside at room temperature in the dark, and the reduction of periodate measured [6] on aliquots (1 ml.) at suitable intervals.

Time (hr.)	6	12	24	48
Moles of IO_4^- reduced per C_6 anhydro-unit	0·60	0·85	0·99	0·99

After 48 hr. excess periodate in the residual solution was destroyed with sulphur dioxide, and the oxopolysaccharide precipitated with ethanol (10 vol.).

(b) *Reduction.* The oxopolysaccharide in 0·05M-boric acid (10 ml.) was treated with sodium borohydride (200 mg.) in water (5 ml.) and the reduction allowed to proceed at room temperature (24 hr.). Excess sodium borohydride was destroyed by the addition of glacial acetic acid to neutrality.

(c) *Mild hydrolysis.* Sulphuric acid was added to the above solution until the acid concentration was normal, and the mixture was allowed to stand at room temperature for 2 days. Addition of ethanol (10 vol.) to the resulting solution failed to precipitate any polysaccharide. After neutralisation, deionisation (IR-120 H^+ resin), and removal of the borate by repeated evaporation with methanol, the derived solution was concentrated to a syrup. Chromatographic examination (solvent C and D, sprays 1 and 2) revealed glycollaldehyde, erythritol, and a spot $R_{glucose}$ 0·89 (solvent C, spray 2), M_G 0·2 (borate, pH 10, 750v). The residual syrup was separated on Whatman No. 1 paper (solvent C) and the material $R_{glucose}$ 0·89 separated. Hydrolysis of this (N-H_2SO_4 at 100°), and chromatographic analysis of the hydrolysate showed

[3] K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, 1947, **30**, 751.
[4] M. A. Jermyn, *Austral. J. Biol. Sci.*, 1962, **15**, 769, 787.
[5] M. Dubois, J. K. Hamilton, K. A. Gilles, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.
[6] G. O. Aspinall and R. J. Ferrier, *Chem. and Ind.*, 1957, 1216.

spots with mobilities of glucose and erythritol. Treatment of this hydrolysate with the specific enzyme glucose oxidase, and paper chromatography and ionophoresis of the derived material, revealed spots with the mobilities of gluconic acid and erythritol (solvent C, sprays 2 and 4). No residual glucose could be detected.

The authors are grateful to Professor E. J. Bourne for providing facilities in the Department, and to the D.S.I.R. for financial support, and for a Maintenance Grant to C. W. F.

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ENGLEFIELD GREEN, SURREY. [*Received, December 1st*, 1964.]