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Note

A highly substituted glucan that coats the seeds of *Helipterum eximium*

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Helipterum eximium (Asteraceae, Asterales) is grown in South Africa and its dried flower-heads are sold as "everlastings"; the samples used were collected in the autumn at Riversdale, Cape Province. The seeds were coated with a white granular powder and both were subjected to scanning electron microscopy (Fig. 1). The covering, which consisted of a monolayer of discrete bilobal capsules (Fig. 1a–e), occurred on both fertile and infertile seeds. Fungal hyphae were present (Fig. 1d). The arrangement of capsules on the seed surface and indentations that showed their positions prior to removal were revealed (Fig. 1e). The nature of the capsules, which interfere with the antifungal treatment of seeds, is now reported.

The powder (*A*, 36% by wt. of the original seed on average), collected by brushing the dried seeds, dissolved with difficulty in water and gave a highly viscous, jelly-like solution that contained carbohydrate. Complete acid hydrolysis of *A* gave (p.c.) Ara, Xyl, Gal, and Glc in approximately equal proportions that were unchanged after a solution of the polysaccharide had been extensively dialysed against running water and freeze-dried prior to hydrolysis.

On methylation analysis, each sugar constituent of *A* gave a single partially methylated derivative. The results indicated that Ara*f* and Gal*p* occupied non-reducing terminal positions, Xyl was 2-substituted, and Glc was 2-, 4-, and 6-substituted. These data suggest that *A* contains a glucan backbone to which the other sugar units are attached as side chains.

Mild acid hydrolysis (40 mM trifluoroacetic acid, 100°, 40 min) of *A* removed most of the Ara and traces of Gal, and provided further evidence that the Ara units were furanoid. Methylation analysis of the resulting degraded polysaccharide *B* showed almost complete loss of 2-substitution of Glc and suggested that Ara*f* had been attached at this position in *A*. Identification of traces of terminal Ara and 2,4,6-linked Glc indicated that the release of Ara was not complete.

Partial hydrolysis of *A* with 0.1 M trifluoroacetic acid at 100° for 4 h yielded *C* ($\bar{M}_w$ 52 000) that contained no Ara and had partially lost Gal and Xyl, although to different extents, and the proportions of Xyl, Gal, and Glc were 6:1:10. The facile release of Gal

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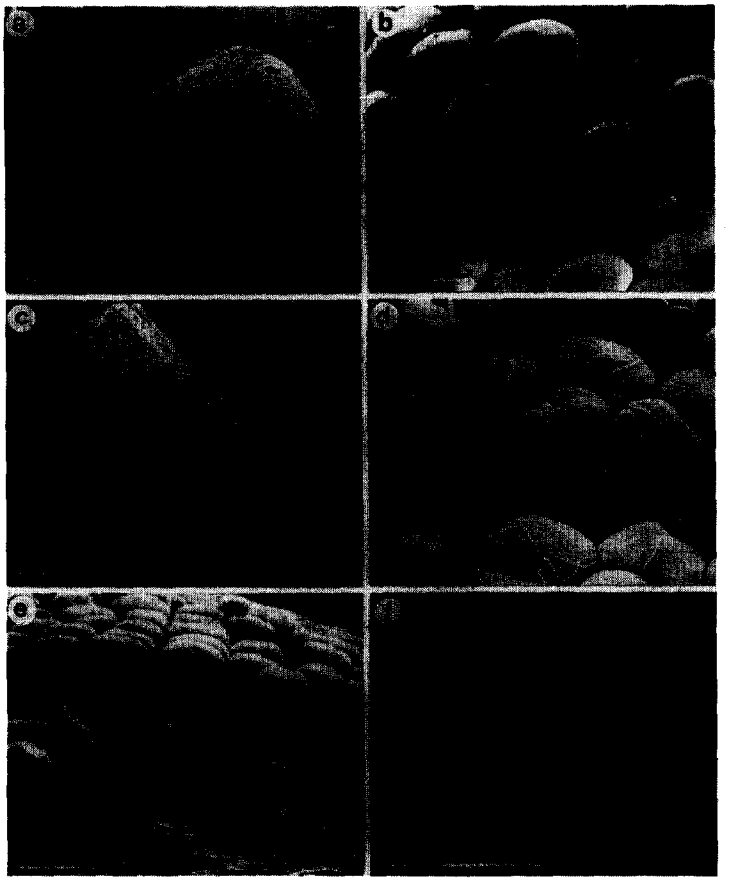


Fig. 1. (*a*) Fertile seed (scale bar = 1 mm); (*b*) top surface of the capsules on fertile seed (bar = 100 μm); (*c*) infertile seed (bar = 1 mm); (*d*) top surface of the capsules on infertile seed, with fungal hyphae visible (bar = 100 μm); (*e*) lateral view of the capsules and part of the seed surface (bar = 100 μm); and (*f*) isolated capsule with the point of detachment from the seed shown at the left extremity (bar = 100 μm).

relative to Xyl and the retention of most or all of the Glc, in conjunction with the results of methylation analysis of *A*, suggested that the Gal was located peripherally and external to the Xyl which, in turn, was linked to Glc in the backbone.

Methylation analysis of *C* showed that, whereas there was no terminal Xyl in *A*, ~85% of the Xyl in *C* was terminal and that the remaining 2-linked Xyl was in the same proportion as terminal Gal. The change in $[\alpha]_D$ ($-103°$ for *A*, $-6°$ for *B*, and $+114°$ for *C*) implied that, if the Ara and Gal were L and D as expected for substituted xyloglucans, they must have been α and β, respectively.

Treatment of *C* with cellulase from *Trichoderma reesei*[1] released Glc and oligosaccharides which had mobilities in p.c. (various solvent systems) similar to those, including 6-*O*-xylosylcellobiose and 6,6′-bis-*O*-xylosylcellobiose, derived[2] from sisal xyloglucan using the same enzyme preparation. The susceptibility to cellulase indicated

that *C* contained a (1→4)-β-D-glucan moiety. The resistance of *A* and *B* suggested that the cellulase required several contiguous, unsubstituted 4-linked β-D-Glc residues for activity.

The results of methylation analysis were corroborated by the finding that all the sugar constituents except Glc were oxidized by $NaIO_4$. The water-insoluble Smith-degradation product *D* was shown by methylation analysis to consist essentially of 4-linked Glc. Treatment of *D* with the cellulase preparation yielded mainly Glc, consistent with a (1→4)-β-D-glucan structure.

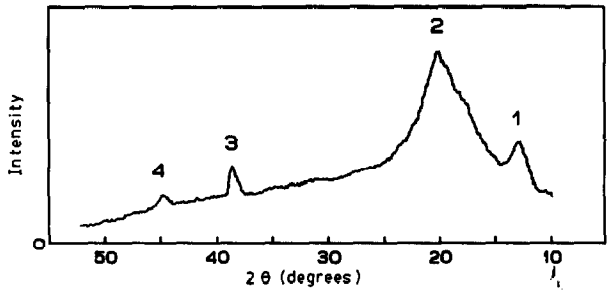


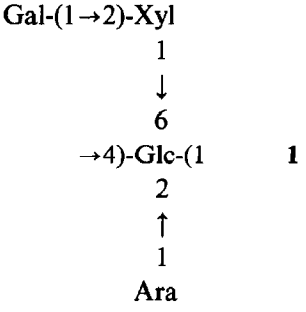
Fig. 2. X-Ray diffraction pattern for *A*. Peak numbers correspond to those in Table I.

TABLE I

X-Ray diffraction data for the polysaccharide capsule from *Helipterum eximium* seed

Peak number	*Interplanar spacing* d (Å)	*Relative intensity* I (%)
1	6.70	9
2	4.31	100
3	2.34	3
4	2.03	< 1

The X-ray diffraction pattern (Fig. 2, Table I) of *A* resembled closely that of a cellulose polymorph[3] and indicated that substitution of the cellulose molecule in *A* did not significantly perturb the structure. It is proposed that the repeating unit of *A* is **1**. Substitution of a glucan to this extent has, to our knowledge, not been reported hitherto.



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Partial acid hydrolysis may be a possible, if destructive, procedure that could be used to assist removal of the encrusting polysaccharide from seed surfaces; enzymic hydrolysis is yet to be explored in depth.

EXPERIMENTAL

General procedures. — Analytical methods have been described[4]. Methylation was carried out by an adaptation of the process described by Isogai *et al.*[5]. In a typical experiment, a suspension or solution of dry polysaccharide (50 mg) in methyl sulfoxide (4.4 mL) was heated for 0.5 h at 60°, then cooled to room temperature, and treated with a solution (0.2 mL) of SO_2 in Me_2SO (0.3 g. mL^{-1}) followed by diethylamine (0.1 mL). The mixture was stirred until a clear solution was obtained. Powdered NaOH (600 mg) was added and stirring was continued for at least 1 h. The mixture was frozen (ice–water) and, after addition of methyl iodide (2.4 mL), stirred at room temperature for 1 h. The temperature was allowed to rise gradually to 60° while stirring was continued for 1 h, and the methylated product was then isolated by dialysis, freeze-drying, and extraction of the residue with chloroform.

Degradation with cellulase. — A solution of the polysaccharide (10 mg) in 0.1M sodium acetate buffer (1 mL, pH 4.8) was incubated with the cellulase preparation (3 mg; activity, 390 IU. g^{-1}) from *T. reesei* (Rut C-30) for 3 days at 37°, in the presence of toluene. Ethanol was added to 70%, the precipitate was removed by centrifugation, and the supernatant solution was concentrated to dryness under reduced pressure. A solution of the residue in water (1 mL) was freed from sodium ions with Amberlite IR-120 (H^+) resin, then freeze-dried, and the product was examined by p.c.

Scanning electron microscopy. — Air-dried *Helipterum* seeds were mounted on Cambridge series 100 stubs, and sputter-coated with 20-nm gold/palladium. The samples were viewed in a Cambridge S180 SEM operated at 10 kV. Micrographs were recorded using low-dose techniques in order to minimise beam damage to the sample.

X-Ray powder diffraction. — A Philips PW 1050/70 automatic diffractometer, fitted with a monochromator and scintillation counter with pulse height selection, was used. Peak positions and intensities were recorded on a strip chart whilst scanning through a 2 θ range of 10–60°.

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