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Note

Glucomannoglycans (glucomannans) from the tubers of *Satyrium coriifolium* and *S. carneum*

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Satyrium coriifolium and *S. carneum*, which usually become dormant during the non-vegetative season, have subterranean tubers that serve as storage reservoirs for essential nutrients. The main polysaccharide components found in tubers of some species of Orchidales are glucomannoglycans[1,2] (referred to usually as glucomannans). Structural studies of the major polysaccharides in the tubers of *S. coriifolium* and *S. carneum*, originating in the Southern Hemisphere, were undertaken in order to relate them to those isolated from other species.

Tubers from each species were extracted successively with EtOH, water, and aqueous 24% KOH (in the presence of borohydride to minimise degradation), to yield *A–E* in relative amounts of 6, 7, 5, 10, and 72%, and 8, 7, 6, 17, and 62%, respectively. For both species, *A* comprised Glc, and *B–D* were polysaccharides, each of which on hydrolysis furnished Glc; *C* also contained a trace of Man, *D* an equivalent proportion of Man, and *E* more than 3 mol of Man.

The major components, glucomannoglycans *E* (3.38 g and 3.66 g from single tubers of *S. coriifolium* and *S. carneum*, respectively), were isolated as unusually hard, insoluble pellets of relatively uniform size. Electron micrographs of these pellets are shown in Fig. 1. Although the pellets were insoluble in water, methyl sulphoxide, and aqueous 24% KOH, those from *S. carneum* were soluble in 6M urea and 4-methylmorpholine *N*-oxide[3], whereas the pellets from *S. coriifolium* were not soluble. Each polysaccharide dissolved, albeit slowly, in aqueous 24% NaOH at ambient temperature. The resulting, highly viscous 0.2% solution yielded copper complexes[4] from which the purified polysaccharide was obtained. Each polysaccharide had a Man–Glc ratio of 3.6:1, and the $[\alpha]_D$ values were $-46°$ and

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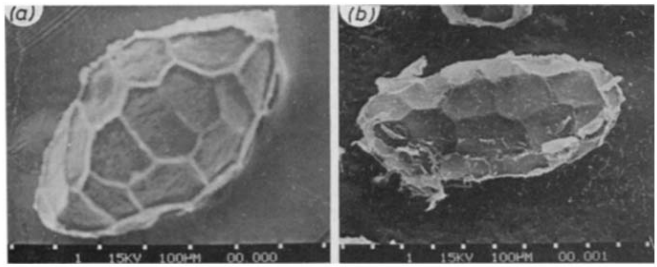

Fig. 1. Electron micrographs of glucomannoglycan pellets from (*a*) *Satyrium coriifolium* (dimensions: 0.75 × 0.40 mm) and (*b*) *Satyrium carneum* (0.29 × 0.23 mm).

−48° (*c* 0.25 and 0.35, in aqueous 24% NaOH) for *S. coriifolium* and *S. carneum*, respectively. The sugar ratios resemble that for a polysaccharide isolated from another orchid species, *Orchis morio*[1]. Neutralisation of alkaline solutions of the polysaccharides gave gelatinous precipitates, a property that is characteristic of glucomannoglycans[5,6].

Methylation analyses of the purified polysaccharides indicated the preponder-

Fig. 2. ^{1}H-N.m.r. spectrum (90 MHz) of oligosaccharides obtained from the glucomannoglycan of *Satyrium coriifolium*.

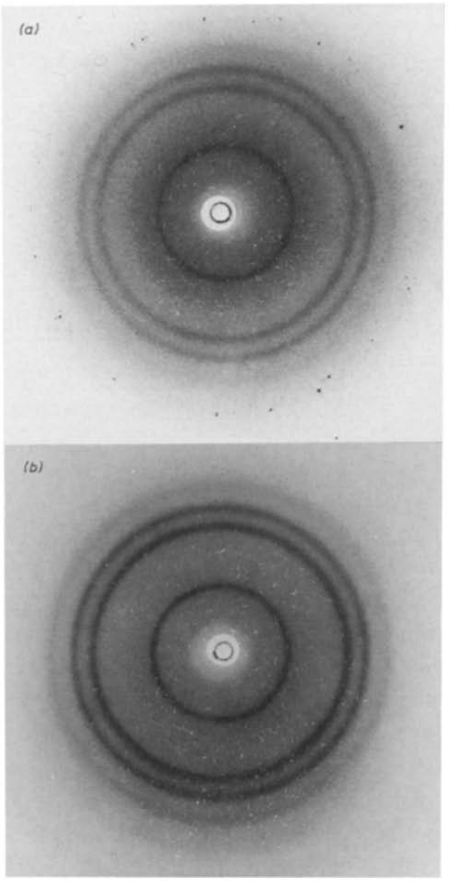

Fig. 3. X-ray diffraction photographs of glucomannoglycan pellets from (*a*) *Satyrium coriifolium*, and (*b*) *Satyrium carneum*. The discrete spots in (*a*) are from a calcite calibrant.

ance of unbranched chains of 4-linked mannosyl and glucosyl residues. Some branching has been described for other glucomannoglycans[1,6]. That there were some contiguous glucosyl residues was evident from the presence of cellobiose among the products of partial acid hydrolysis. This structural feature too has been observed for other glucomannoglycans[1,6].

That the constituents were β-D was inferred from the negative $[\alpha]_D$ values of the polysaccharides. Further evidence was provided by the ^{1}H-n.m.r. spectrum (Fig. 2) of the water-soluble products (average mol. wt., 600) derived by partial hydrolysis of the parent polysaccharides. Whereas the signal at δ 4.75 (bs) could be attributed to H-1 of β-D-Man*p*, that at δ 4.5 (d, $J_{1,2}$ 8.0 Hz) was characteristic of H-1 of β-D-Glc*p*. The preponderance of D-Man residues in the polysaccharides was also evident from their occurrence as the main reducing ends in the lower oligosaccharides derived as described below; the signals at δ 5.18 and 4.88 (2 d, $J_{1,2}$ 1.5 Hz) were due to the H-1 of reducing α- and β-D-Man*p* units, respectively.

TABLE I

X-RAY INTERPLANAR SPACINGS OF GLUCOMANNOGLYCAN PELLETS FROM (*a*) *Satyrium coriifolium* AND (*b*) *S. carneum*

(a)		(b)	
Intensity[a]	*Spacing (nm)*	*Intensity*[a]	*Spacing (nm)*
S	0.815	S	0.815
S	0.448	VS	0.448
S	0.397	S	0.397
M	0.348	M	0.348
VW	0.301	VW	0.301
VW	0.269	VW	0.269
W	0.255	W	0.255

[a]Key: S, strong; M, medium; W, weak; V, very.

X-Ray diffraction of the polysaccharide pellets gave photographs (Fig. 3) which showed a series of relatively sharp diffraction rings, suggesting a high degree of regularity in the polysaccharide structures. The derived interplanar spacings, presented in Table I, are the same for both types of *Satyrium* but the relative intensities are somewhat different. Although these spacings do not correlate well with other known crystalline forms of plant polysaccharides, there was a resemblance to the diffraction spacings of mannan II, an alkali-treated mannan preparation from algal cell walls[7], which gave strong diffraction rings at 0.826 and 0.450 nm.

Since the tubers, from which the polysaccharides were isolated, were harvested during the dormant season, it appears that the polysaccharides occur as storage carbohydrate nutrients. Therefore, a tuber originating from a fully grown plant (a miniature hybrid species from the genus *Cymbidium*) of the Orchid family was investigated. The major polysaccharide material was found to be cellulose (alkali-insoluble), whereas the alkali-solubles (in both conc. aqueous KOH and NaOH) contained xylose as the main sugar component, indicating the presence of xylan and/or xyloglucan. This result is consistent with the tuber's remaining as a structural unit, serving to support the mature plant.

EXPERIMENTAL

Fractionation of the polysaccharide components. Isolation of glucomannoglycan pellets. — Tubers were harvested in February and immediately immersed and stored in aqueous 96% ethanol. A single tuber from each of the plants was ground, then stirred with cold aqueous 96% EtOH for 24 h, and the soluble components (*A*) were removed by centrifugation. The residue was stirred with water for 24 h and then centrifuged to give solubles (*B*), which were dialyzed prior to freeze-drying, and a residue. The latter was treated with aqueous 24% KOH that was

10mM with respect to $NaBH_4$, under nitrogen, yielding solubles (*C*), which were neutralised with acetic acid and dialysed. The residue was suspended in water, and then decanted to afford a dense residue (*E*) and a suspension (*D*) that was recovered by freeze-drying.

General procedures. — The analytical methods have been described[8]. The major polysaccharides (*E*) were purified by dissolution in aqueous 24% NaOH and precipitation with Fehling's solution[4], and methylated twice (Hakomori[9]) using potassium methylsulphinylmethanide in methyl sulphoxide[10] containing tetramethylurea[11]. 90-MHz ^{1}H-N.m.r. spectra (internal acetone, 2.20 p.p.m. downfield of the signal for Me_4Si) were obtained at 80° with a Bruker WH-90 spectrometer. Samples were prepared by dissolution thrice in 99.7% D_2O followed by freeze-drying. Scanning electron microscopy was performed using a Cambridge S 180 SEM model. X-Ray diffraction analyses were carried out in the laboratory of Professor E. D. T. Atkins.

Hydrolysis. — Partial acid hydrolysis of the glucomannoglycan was effected with cold 72% H_2SO_4 for 2 h, followed by neutralisation and dialysis against frequently changed distilled water. The dialysables were fractionated, after freeze-drying, into aqueous ethanol-solubles from which cellobiose was identified by g.l.c. of its methylated alditol, while the insolubles were used for n.m.r. measurements.

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