

## Preliminary communication

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### Isolation of three water-soluble glucan components from *Pelvetia canaliculata*\*

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Following the recent preparative-scale fractionation of laminarin into its component mannitol-terminated chains (M-chains) and glucose-terminated chains (G-chains), using a column of DEAE-cellulose(or Sephadex)—molybdate<sup>1</sup>, the present work describes the use of column chromatography for investigations into the water-soluble polysaccharides of *Pelvetia canaliculata*. This seaweed is of interest because it contains, in addition to a large proportion of free mannitol, a similar amount of free volemitol. The presence of volemitol (D-glycero-D-talo-heptitol) and its mono- and di-D-glucosides in extracts of *Pelvetia canaliculata* was reported by Lindberg<sup>2</sup>. Although it is therefore possible for this seaweed to contain a  $\beta$ -D-glucan component with a volemitol-terminated chain, there are no reports in the literature of the nature of the reducing terminal groups in the reserve polysaccharide of this seaweed.

Initial studies in the present work were hampered by the viscous nature of the aqueous extracts. This problem was overcome by extracting an alcohol-dried, finely ground preparation with filtered sea-water. The product from this extraction, in addition to being less viscous, contained a larger proportion of glucose than a distilled-water extract. The polysaccharide material extracted with sea water was subjected to column chromatography on Sephadex G100 and on DEAE-cellulose—Tris; direct application of the crude extract onto a DEAE-cellulose—molybdate system proved unsuccessful. Using Sephadex G100, it was shown that the major portion of the glucan was present in the zone of lower molecular weight, although a complete separation from the xylose, fucose, galactose, and uronic acid-containing components could not be achieved. Laminarin isolated from *Laminaria hyperborea* (*L. cloustoni*) also has a low molecular weight<sup>3</sup>. With a column of DEAE-cellulose—Tris (pH 7.5), the glucan could be separated from the other components, but only in small quantities. Heavier loading of the column led to overlap of the polysaccharide peaks. When the small amounts of glucan from this column were subjected to further chromatography on a DEAE—molybdate column, two glucan fractions were obtained in the ratio of 1:3.

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\*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.

The problem of producing reasonably pure glucan in quantities large enough for further studies by DEAE—molybdate chromatography was solved by adding barium chloride (4 g/100 ml) to the sea-water extract. The precipitate so formed was removed and the supernatant solution was fractionated with ethanol. The fraction of lower molecular weight (requiring six volumes of ethanol for precipitation) was largely glucan and contained only very small quantities of other polysaccharides. In the other fractions, which required only one or two volumes of ethanol for precipitation, the non-glucan polymers were preponderant. Chromatography of this partly purified glucan on DEAE-cellulose—molybdate yielded three components (*A*, *B*, and *C*) which were eluted with water, 0.25M sodium chloride, and M sodium chloride, respectively. Rechromatography of the separated components *A*—*C* on DEAE—molybdate columns indicated that the original fractionation was not an artefact of the column procedure. An examination of *A*, *B*, and *C* by total acid hydrolysis, partial acid hydrolysis, and enzymic degradation with a fungal  $\alpha$ -D-glucanase<sup>4</sup> showed that each fraction consisted mainly of (1→3)-linked  $\beta$ -D-glucosyl residues. Component *A* was a pure glycan, whereas *B* and *C* contained traces of other sugar residues. The pattern of the products on enzymic hydrolysis was the same for each component; large proportions of D-glucose were produced, together with small proportions of gentiobiose and traces of other glucose-containing oligosaccharides. The present work therefore illustrates a further extension of the use of the DEAE-cellulose—molybdate column, in showing the presence of a three-component glucan of low molecular weight in *Pelvetia canaliculata*.

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