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Preliminary communication

The cation-exchanger of *Sphagnum* mosses: an unusual form of holocellulose*

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It is well-established that the *Sphagnum* mosses synthesise a cation-exchanger in its hydrogen form, which is largely responsible for the acidity of peat-bog water[1−7]. Although several workers have reported on the general carbohydrate composition of the fresh mosses and of peat[8,9], little is known about the cation-exchanger, except that it contains residues of galacturonic acid[8,10,11]. This has led to the assumption that the material is pectic acid[12].

Our interest in this matter was aroused by reports[8,10] that the cation-exchanger survived delignification with chlorite, and that only a small part of it could be extracted from the holocellulose, even with concentrated alkali[10]. Ordinary pectic acid would not behave in this way, and so we have investigated the matter further. We now report preliminary findings for Norwegian samples of *Sphagnum quinquefarium* (Braithw.) Warnst. (synonym: *S. acutifolium* Lindb.) and *S. girgensohnii* Russ. (synonym: *S. strictum* Lindb.), and for a Canadian sample of *S. teres* (Schimp.) Ångstr. kindly provided by Drs. Craigie and Maass[10]. The results were similar for all three species.

After preliminary extraction of the dried and milled moss with acetone and methanol, successive extraction with hot (90°) distilled water and 0.05M ammonium oxalate removed only 0.3% of its weight as an acidic gum (*A*), containing 0.45% of nitrogen, residues of galacturonic acid, galactose, xylose, arabinose, and fucose, and possibly traces of rhamnose, identified by paper chromatography. All of these sugars have been previously identified by other workers[8−11].

Subsequent delignification with chlorite[13] led to the isolation of further traces of *A* from the chlorite liquor, and a holocellulose having a very low density and the property of swelling to many times its dry volume in water. The holocellulose represented ~ 50% of the weight of the moss, and had an ion-exchange capacity (determined by direct titration) of 1.36 mequiv./g. It accounted essentially entirely for the ion-exchange capacity of the original moss (0.66 mequiv./g).

Extraction of the holocellulose with 20% (w/v) aqueous potassium hydroxide at 20° removed 4% of its weight as water-soluble, polysaccharidic material. This product

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*Dedicated to Dr. Elizabeth Percival.

was fractionated by free-boundary electrophoresis at pH 7, and by fractional precipitation with ethanol in the presence of calcium ions, into about equal parts of a component that was essentially identical with *A* (except that it was nitrogen-free), and an apparently typical hemicellulose of the 4-*O*-methylglucuronoarabinoxylan type. Thus, conventional hemicellulose accounted for not more than 1% of the dry weight of the moss.

The residual "α-cellulose" still had an ion-exchange capacity of 1.16 mequiv./g, and contained the same sugar residues as *A*, in addition to ~ 45% of D-glucose residues, as measured by the D-glucose oxidase method[14] after complete hydrolysis with acid. Compared to a conventional α-cellulose (prepared from maize cobs), it was very resistant to cellulase, and it was unaffected by pectinase under conditions that rapidly converted apple pectin into dialysable mono- and oligo-saccharides (*cf.* Theander[8]).

Since methyl pectate is extensively degraded (by β-elimination) in mild alkali[15], it seemed likely that the insoluble, cellulose-bound form of *A* was the native state of the cation-exchanger, and that the small proportion removed by 20% potassium hydroxide was due to chemical degradation. Similarly, the traces of *A* removed under the mildly acidic conditions of extraction with ammonium oxalate and chlorite delignification were probably due to intramolecularly catalysed autohydrolysis[16], which takes place readily at pH ~ 3 in pectic and alignic acids[17-19]. These considerations prompted the following experiments, which clearly demonstrated the unique character of the *Sphagnum* holocellulose.

(*a*) The free-acid form of the holocellulose was converted into a propylene glycol ester by treatment with propylene oxide, prior to extraction with 20% potassium hydroxide as before. Forty percent of the weight was then removed by a single extraction, and was largely recovered as a non-dialysable polysaccharide similar in composition to *A*. Re-esterification of the insoluble residue and a second extraction with alkali increased the yield of soluble polysaccharide to ~ 55%.

(*b*) The free-acid form of the holocellulose was heated at 90° in distilled water, at an initial pH of ~ 6. At daily intervals, the insoluble material was filtered off and heated again in distilled water, while the acidic filtrates (pH ~ 3) were combined, concentrated, and freeze-dried. After 8 days, ~ 60% of the weight of the holocellulose had been converted into soluble material; the residue appeared to be a microcrystalline form of cellulose. Approximately 90% of the soluble autohydrolysate was non-dialysable, polysaccharidic material.

Further experiments have been carried out on the last-named material. It contained residues of D-glucose (5%), galacturonic acid (40%, by Dische carbazole assay[20]), xylose, galactose, and arabinose. Tests for esterified sulphate or phosphate were negative. The material was electrophoretically homogeneous at pH 7, but had no electrophoretic mobility at pH 2, which confirmed that all the acidic groups were carboxylic. Additionally, its equivalent weight (435) was fully accounted for by its content of galacturonic acid. Prolonged exposure to concentrated pectinase removed only minor amounts of monomeric xylose and arabinose. The calcium and barium salts of the polymer were soluble in water.

In conclusion, the cation-exchanger of *Sphagnum* mosses is a cellulose-bound mucilage or gum which is remarkably similar, at least in composition, to the cellulose-bound mucilages of quinces[21,22], and the seeds of white mustard[23], cress[24], and flax[24]. After cleavage from the cellulose, the soluble gum is also rather similar in composition and properties to tragacanthic acid[25].

Although the *Sphagna* are notorious for their high ion-exchange capacities[1-7], fairly high values have been reported for whole plants of other mosses[2,26]. We have therefore delignified samples of five other mosses that grow in Norway; in the following list, the figures in parenthesis represent the yield of chlorite holocellulose, based upon the dry weight of the moss, followed by the ion-exchange capacity in mequiv./g: *Hylocomium splendens* (34%, 0.90), *Polytrichum commune* (28%, 0.34), *Ptilium crista-castrensis* (54%, 1.02), *Rhacomitrium lanuginosum* (33%, 0.33), and *Rhytidiadelphus loreus* (49%, 1.16). Further investigation of these materials is in progress.

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