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Preliminary communication

Extraction of hemicelluloses from plant cell-walls with water after preliminary treatment with methanolic sodium methoxide*

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The most frequently-used method for the isolation of hemicelluloses from plant tissue is by alkaline extraction[1]. Preliminary fractionation of the extract involves[2] neutralization to give a precipitate (hemicellulose A), followed by the addition of ethanol to the supernatant solution to give hemicellulose B. Any carbohydrate left in solution (usually very little) is called hemicellulose C. With this method, the relative proportions of A, B, and C obtained in replicate determinations on the same sample are never very consistent. However, a method involving dialysis and treatment with an ion-exchange resin has been reported to give more consistent results[3].

O-Acetyl groups[4] and phenolic acid residues[5] are present in grass cell-walls. The phenolic acids (mainly *p*-coumaric and ferulic acids) appear to be linked to the cell-wall carbohydrates by ester bonds[6]. The acetyl groups and the phenolic acid residues have both been implicated in influencing the digestibility of grass cell-walls. Sodium methoxide solution has been used in the determination of *O*-acetyl groups in woods[7], and the same procedure has been used on grass tissue[8].

A tetraploid variety of perennial ryegrass (*Lolium perenne* – Barvestra) was harvested at a late vegetative stage and freeze-dried. Crude cell-walls, prepared as previously described[9] and ball-milled at 4°, were stirred for 18 h at room temperature with 0.25M methanolic sodium methoxide. After centrifugation and washing with methanol, a de-esterified cell-wall preparation was obtained (yield >98%). A portion of this was extracted with water (1:20) at 65° for 18 h. After centrifugation and washing twice with water, the residue (1) and the extract (2) were freeze-dried (yields, 74% and 28%, respectively). The neutral sugar residues present in 1 were arabinose (11), xylose (100), and glucose (240), whereas those present in 2 were arabinose (30), xylose (100), glucose (14), and galactose (4).

The u.v. spectrum of the extract 2 showed strong absorbance at 280 nm, with a shoulder at ~325 nm. In 0.1M NaOH, the areas of absorbance were shifted to higher wavelengths, but the relative intensities remained similar. In 0.1M HCl, the intensity in the

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

region of 280 nm was decreased, and another distinct peak appeared at ~315 nm. After 4 h, there was little change in the spectra of the neutral and the alkaline solutions, but the acidic solution became cloudy initially, and finally a precipitate formed. The spectrum of the filtered solution indicated that the intensity was diminished by ~50%, with peaks of absorbance at ~285 and ~310 nm.

When a larger sample of **2** was treated with 0.1M HCl and the insoluble material (**3**) recovered, it accounted for ~30% of the total weight of **2**, and ~35% of the total carbohydrate in **2** (based on xylose). In 0.1M NaOH, **3** absorbed strongly in the region of 280 nm, but showed very little absorption at higher wavelengths. The neutral sugar residues present after hydrolysis were arabinose (19) and xylose (100). The sugar residues in the acid-soluble material (**4**) were arabinose (53), xylose (100), glucose (4), and galactose (7).

A fractional-precipitation curve of **2** with ethanol indicated that there were three distinct components (**5**–**7**); **5** precipitated in 45% ethanol and **6** in 80% ethanol, whereas **7** was soluble even in 90% ethanol. The u.v. spectrum of **5** showed a small peak at 280 nm, and that of **6** a larger peak, while that of **7** showed strong absorption at 280 nm and also a shoulder at 310 nm. The samples **5**, **6**, and **7** contained 70%, 73%, and 11% of carbohydrate by weight (based on xylose), respectively. The neutral sugar compositions, after hydrolysis, were **5**, arabinose (12), xylose (100), and glucose (6); **6**, arabinose (46), xylose (100), glucose (8), and galactose (7); and **7**, arabinose (123), xylose (100), and glucose (53).

Fractionation of **2** by gel filtration on Sephadex G25 gave three components: **8** was eluted at the void volume of the column and contained virtually all of the carbohydrate as well as showing absorption at 280 nm. The other two fractions, **9** and **10**, contained very little carbohydrate, but absorbed strongly at 280 nm. They were incompletely separated and were adsorbed by the Sephadex gel, as their K_d values were ~1.08 and ~1.30, respectively. The mixture of **9** and **10** was partitioned between ethyl acetate and water, but the spectra of the two subfractions were identical. No free phenolic acids were found on h.p.l.c. of either the ethyl acetate-soluble or -insoluble fractions.

When the original 0.25M sodium methoxide extract was diluted with an equal volume of water, kept overnight, and then adjusted to pH 2 with HCl and extracted with ethyl acetate, *p*-coumaric and ferulic acids were identified in the fraction soluble in ethyl acetate.

It is considered that treatment of plant cell-walls with anhydrous sodium methoxide in methanol may hydrolyse certain ester bonds (possibly involving phenolic acids) which hold the cell wall together. The rupture of these bonds allows reagents that are much milder than alkali to be used to extract part of the hemicellulose complex. It appears that, with the sample examined, a fairly pure galactoarabinoxylan could be obtained by treatment with acid. Phenolic acids or lignin-like materials are still closely associated with all of the fractions, adding strength to the view that lignin is linked to cell-wall carbohydrates.

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