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## Note

# Structural features of two pectic fractions from field-bean (*Dolichos lablab*) hulls

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The importance of pectic and other unavailable carbohydrates in the physiological effects of dietary fibre is well documented[1]. Precise chemical, structural, and biochemical knowledge of the various dietary-fibre components is yet far from understood. A study was accordingly initiated to investigate in chemical and structural terms the nature of some of the components of dietary fibre from legume pulses. Structural features of two pectic fractions from field-bean (*Dolichos lablab*) hulls are reported in this communication.

The water-insoluble residue, obtained in 87.6% yield[2] from field-bean hulls (FBH), was successively extracted with cold (room temperature) and hot (~85°) ammonium oxalate (0.5%), and two pectinic acids, PA-1 and PA-2, were recovered by alcohol precipitation. Recovery of these fractions by dialysis followed by lyophilization was not possible as these extracts gave highly thixotropic gels exhibiting non-Newtonian behaviour, and they were difficultly reconstitutable. Further extraction of the ammonium oxalate-insoluble residue with aq. ethylenedinitrilo(tetraacetic acid) gave PA-3 of considerably lower viscosity. The yield of the fractions, their chemical characteristics, and carbohydrate compositions are given in Table I. The pectic preparations varied in their degree of esterification and their content of neutral sugar. In particular, PA-3 had a complicated neutral-sugar profile, indicating it to be a composite aggregate of pentoglycan–hexoglycan–pectic-type polysaccharides. Because of its complexity and poor gelling property, studies on PA-3 were not pursued further. The presence of glucose is interesting, and it could originate from the alkali-soluble hemicellulosic polysaccharides[3]. Xyloglucans (amyloid) of unusual structures are identified[3] as components of hemicellulose A of FBH. Thus far glucose has not been reported as a constituent of pectic fractions. Rees and Wight[4], in the course of methylation analysis of mustard-seed pectic polymer, recovered a small proportion of glucose as 2,3,6-tri- and 2,3-di-*O*-methylglucose. This was cited as

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TABLE I

CHEMICAL CHARACTERISTICS[a] OF PECTINIC ACIDS FROM FBH

| | *Cold ammonium oxalate-soluble (PA-1)* | *Hot ammonium oxalate-soluble (PA-2)* | *Hot EDTA-soluble (PA-3)* |
|---|---|---|---|
| Yield (%) | 3.00 | 4.20 | 0.50 |
| $\eta$ | 4.80 | 3.60 | 2.10 |
| Protein | 3.90 | 2.65 | 5.60 |
| Total sugar | 80.10 | 84.50 | 78.00 |
| Uronic acid | 70.00 | 65.00 | 37.00 |
| Sugars detected | | | |
| Rha | 1.20 | | |
| Ara | 6.00 | 12.50 | 13.10 |
| Xyl | 2.40 | 7.00 | 14.30 |
| Gal | | | 6.60 |
| Glc | | | 6.90 |
| OMe | 2.60 | 2.20 | 1.90 |
| Esterification | 15.80 | 13.20 | 11.80 |

[a]The values denote percent composition.

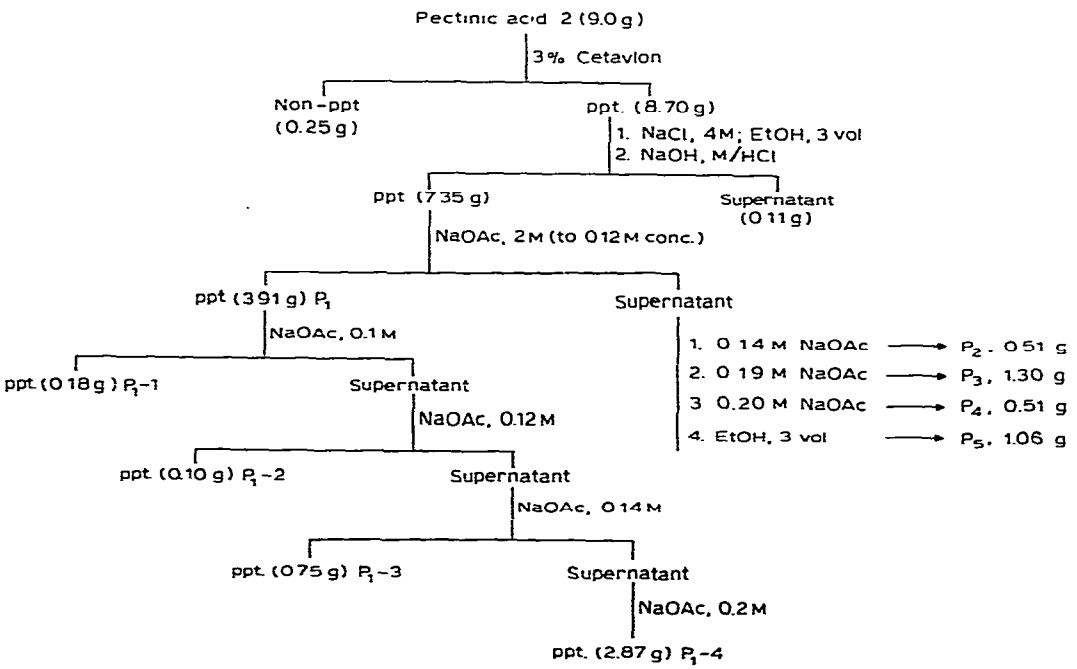


Fig. 1. Scheme of fractionation of pectinic acid 2 (PA-2).

evidence of a covalent linkage between the amyloid and pectic materials[5,6]. The presence of associated glucose in pectic fractions is also reported in oxalate-soluble rape-seed polysaccharides[7]. Such covalent bonding between pectic polymer and xyloglucan is also recognised within the primary cell-walls of cultured sycamore cells[8].

In order to assess the extent of heterogeneity of PA-1 and PA-2, the preparations were fractionated, as summarised in Fig. 1. Pectic acid 1, obtained by saponification of the Cetavlon-precipitable PA-1, was homogeneous in comparison with that obtained from PA-2. The latter was highly heterogeneous and on further fractionation with sodium acetate[9,10] afforded a series of polysaccharide fractions of decreasing uronic acid and increasing neutral-sugar content. Lemon-peel[9] pectic acids, when fractionated in this way, provided merely a subfractionation of polydisperse systems in which each fraction contained a continuous spectrum of closely-related molecular species with substantial overlaps in content of galacturonic acid.

TABLE II

METHYLATION ANALYSES OF PECTIC ACID FRACTIONS

| *Peak no.* | $R_T$ | *Sugar methyl ether* | *Molar ratio* | *Mode of linkage* |
|---|---|---|---|---|
| *Pectic acid 1* | | | | |
| 1 | 0.31 | 1,2,3,5,6-Penta-*O*-methyl-D-galactose | Trace | →4)-D-Gal*p*-OH |
| 2 | 0.45 | 2,3,5-Tri-*O*-methyl-L-arabinose | 0.8 | L-Ara*f*-(1→ |
| 3 | 0.89 | 3,4-Di-*O*-methyl-L-rhamnose | 2.0 | →2)-L-Rha*p*-(1→ |
| 4 | 1.05 | 2,3-Di-*O*-methyl-L-arabinose | 2.5 | →5)-L-Ara*f*-(1→ |
| 5 | 1.19 | 2,3,4,6-Tetra-*O*-methyl-D-galactose | 0.7 | D-Gal*p*-(1→ |
| 6 | 2.20 | 2,3,6-Tri-*O*-methyl-D-galactose | 21.6 | →4)-D-Gal*p*-(1→ |
| 7 | 3.10 | 2,6-Di-*O*-methyl-D-galactose | 1.7 | →3,4)-D-Gal*p*-(1→ |
| *Fraction $P_1$-4* | | | | |
| 1 | 0.45 | 2,3,5-Tri-*O*-methyl-L-arabinose | 0.8 | L-Ara*f*-(1→ |
| 2 | 1.05 | 2,3-Di-*O*-methyl-L-arabinose | 3.0 | →5)-L-Ara*f*-(1→ |
| 3 | 1.18 | 2,3,4,6-Tetra-*O*-methyl-D-galactose | 0.3 | D-Gal*p*-(1→ |
| 4 | 2.22 | 2,3,6-Tri-*O*-methyl-D-galactose | 24.7 | →4)-D-Gal*p*-(1→ |
| 5 | 3.15 | 2,6-Di-*O*-methyl-D-galactose | 0.4 | →3,4)-D-Gal*p*-(1→ |

Examination by zone electrophoresis[11] on Millipore Phoroslides and precoated Polygram (cellulose) sheets in different buffer-systems; by ultracentrifugation; and by molecular-sieve chromatography, revealed the pectic acid from PA-1 and three sub-fractions ($P_1$-4, $P_2$, and $P_3$) from PA-2 to be homogeneous. Sugar analysis showed significant amounts of D-galacturonic acid (~85%) in pectic acid 1 and $P_1$-4. Mild acid hydrolysis of the former released all of the arabinose and xylose in small but equal amounts, indicating their presence in the side-chains. Complete hydrolysis of the insoluble residue showed major amounts of galacturonic acid and small proportions of rhamnose (~6%). The release of rhamnose under these conditions is indicative of its involvement in the backbone glycosidic linkages. On the other hand, $P_1$-4, having an $[\alpha]_D$ value of +232° (*c* 0.5 in 0.1M sodium hydroxide), contained only arabinose as the neutral sugar. Gel filtration on a precalibrated column of Biogel P-60 indicated $\overline{M}_n$ 35,000 for both fractions.

Because of difficulties in the direct methylation of uronic acid-rich materials, pectic acid 1 was carboxyl-reduced by the carbodiimide method[12]. The twice-reduced product had a uronic acid content of <5% and was subjected in sequence to Hakomori methylation[13], depolymerization, reduction, and *O*-acetylation. The derived alditol acetates were examined by g.l.c. and combined g.l.c.–m.s.[14]. The results are summarised in Table II. Identification of 3,4-di-*O*-methyl-L-rhamnose (~6.3%) is structurally significant and suggests that most of the L-rhamnose is present as (1→2)-linked residues in the backbone, as had been predicted. The high proportion of 2,3,6-tri-*O*-methyl-D-galactose suggests that most of the D-galactose residues, and in turn the D-galacturonic acid residues, are (1→4)-linked.

Periodate oxidation followed by Smith degradation of the reduced pectic acid 1 gave threitol and glycerol as the main products, together with small proportions of galactose. The periodate consumption of 0.95 mol per sugar residue confirmed the preponderance of (1→4) linkages in the macromolecule. The release of formic acid was negligible.

The foregoing results for pectic acid 1 were consistent with a (1→4)-linked D-galacturonan core punctuated with occasional blocks of 2-*O*-linked L-rhamnose residues. Branching in the galacturonan framework is through non-reducing single or multiple side-stubs of L-arabinofuranose and D-xylose, as in tragacanthic acid and pollen galacturonan[15].

In contrast, methylation analysis (methylation, reduction with lithium aluminium hydride[16], remethylation, depolymerization, and derivatization; Table II) of $P_1$-4 demonstrated a (1→4)-linked D-galacturonan core with occasional branches through O-3 involving single or short arabinose side-chains. The periodate consumption of 0.97 mol per sugar residue and negligible release of formic acid was also in close agreement with the methylation results. Threitol and glycerol were the main Smith-degraded products.

The primary structures of the two pectic polysaccharides of field-bean hulls just mentioned are similar to those of rape-seed[7] and mustard-seed[4] pectins, but differ considerably from those of soybean[17] and citrus[17] pectins, which contain, in

addition, side-chains of (1→4)-linked β-D-galactosyl residues. These structures are also generally consistent with the one proposed for the sycamore-cell pectic polymer[6]. By analogy with other pectic polymers, the glycosidic links in FBH pectic fractions are assumed to be α-D. The mucilaginous, fibrillar galacturonan found in, on, and between plant cell-walls has been shown to act as a filler substance and cementing agent between cells, as a carrier of lignin precursors, as a promoter of symbiosis, and also may serve to localize enzyme complexes and anchor helpful foreign cells[18]. It is an appealing idea that, henceforth, cellulose alone cannot be regarded as the sole, fibrillar component of plant cell-walls.

## EXPERIMENTAL

*General methods.* — Polysaccharides were hydrolysed with either 0.5M sulphuric acid (5 h, 95°) or by prior solubilization with 72% sulphuric acid (at 4°) followed by 0.5M acid. Total sugar was estimated by the phenol–sulphuric acid[19] and uronic acid by the modified carbazole[20] method. All rotary evaporations were performed at a bath temperature of below 40°. P.c. was conducted on Whatman No. 1 or 3 MM papers in (*a*) 10:1:2 1-butanol–ethanol–water; (*b*) 4:1:5 1-butanol–ethyl acetate–water; and (*c*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water. Sugars were located by aniline phthalate[21] or Tollens'[22] spray reagents. G.l.c. of the alditol acetates was performed on 3% ECNSS-M or a 3% OV-225 column, and g.l.c.–m.s. was effected with a Varian 2700 g.l.c. instrument coupled to a Varian 311 mass spectrometer. Spectra were processed with a Spectrosystem SS 100 data unit. Electrophoresis[11] was performed on Millipore Phoroslide membranes and Polygram cellulose t.l.c. strips in ammonium carbonate–sodium chloride (pH 9.3), ethylenedinitrilo(tetraacetic acid) (pH 7.4), or acetate (pH 4.8) buffers. Gel filtration was conducted on a pre-calibrated column (1.5 × 80 cm) of Biogel P-60 eluted with 0.5M ethylenedinitrilo-(tetraacetic acid) buffer (pH 7.4). The flow rate was between 10–15 mL/h and 3-mL fractions were collected.

*Extraction of pectinic acids.* — Powder (60 mesh) of defatted and depigmented FBH was extracted with aq. ethanol. The alcohol-insoluble residue was successively extracted with cold (~26°) and hot (~85°) water[2]. The hot water-insoluble residue (215 g) was then extracted with (*a*) cold (~26°), (*b*) hot (~85°), 0.5% ammonium oxalate (pH 6.8), and (*c*) hot 0.5% ethylenedinitrilo(tetraacetic acid). Polysaccharide materials (PA-1, PA-2, and PA-3) were precipitated with ethanol, washed, and dried by solvent exchange.

*Fractionation methods.* — Addition of 3% aq. Cetavlon[23] to aq. pectinic acid solution (1%) gave precipitable and non-precipitable fractions. Saponification by treatment with dilute sodium hydroxide and then hydrochloric acid (under nitrogen) furnished pectic acids. These were further fractionated[9,10] by graded precipitation with 0.2M sodium acetate, to give a series of polysaccharide fractions of different sugar proportions.

*Carboxyl reduction and methylation analyses.* — Pectic acid 1 (100 mg) was

carboxyl-reduced by the method of Taylor and Conrad[12]. The twice-reduced product (~80 mg) had a uronic acid content of <5%. It was then successively methylated (~5 mg) by the method of Hakomori[13], hydrolysed, and the neutral *O*-methyl sugars reduced and *O*-acetylated. The products were analysed by g.l.c. and g.l.c.–m.s.[14]. Fraction $P_1$-4 (10 mg) was directly methylated, reduced by lithium aluminium hydride[16], remethylated, hydrolysed, and derivatized. The permethylated alditol acetates were identified by (*a*) $R_T$ with reference to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, and (*b*) the presence of characteristic mass-fragments in the mass spectrum.

*Periodate-oxidation and Smith-degradation studies.* — Reduced polysaccharide (50 mg) in water was mixed with 0.1M sodium metaperiodate and kept at 4° in the dark. Aliquots (5 mL) were removed at intervals, and the consumption of $IO_4^-$ and liberation of formic acid were determined[24,25]. The oxidised polysaccharide was reduced with sodium borohydride (100 mg) overnight, the excess of borohydride decomposed, and the product hydrolysed (0.25M sulphuric acid for 5 h at 100°).

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