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Note

Structures of pectic polysaccharides isolated from the Siberian apricot (*Armeniaca siberica* Lam.)

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The carbohydrates of the fruit of the wild Siberian apricot have been studied in relation to dietary fibre composition and potential uses in food and in the pharmaceutical industry[1]. Previous studies dealt with the isolation, purification, and characterisation of hemicelluloses from the delignified cell-wall material of the apricot[2,3]. We now report on the structures of two pectic polysaccharides.

TABLE I

Data on the pectin fractions from Siberian apricot

	AP	*AP-I*	*AP-II*
Uronic acid (%)[a]	64.5[b]	90.6[b]	8.3[c]
$[\alpha]_D$ (degrees)	+152	+150	−14
Protein (%)[d]	0	0	32.3
Neutral sugars (mol.%)			
Rhamnose	25.7	16.8	15.5
Fucose	4.8	22.8	2.2
Arabinose	47.6	28.5	43.5
Xylose	2.4	4.3	8.3
Glucose	3.8	8.0	3.0
Mannose	traces	4.3	3.0
Galactose	15.7	15.4	23.5
Methoxyl (%)	6.5	traces	2.3
O-Acetyl[e]: uronic acid (mol)	1:11.8	–	–
$M_n \times 10^{-3}$	33.4	22.5	21.5

[a] As galacturonic acid. [b] Determined by alkalimetry. [c] Determined by the carbazole method. [d] Calculated from 6.25 × N%. [e] Determined by ^{1}H-n.m.r. spectroscopy.

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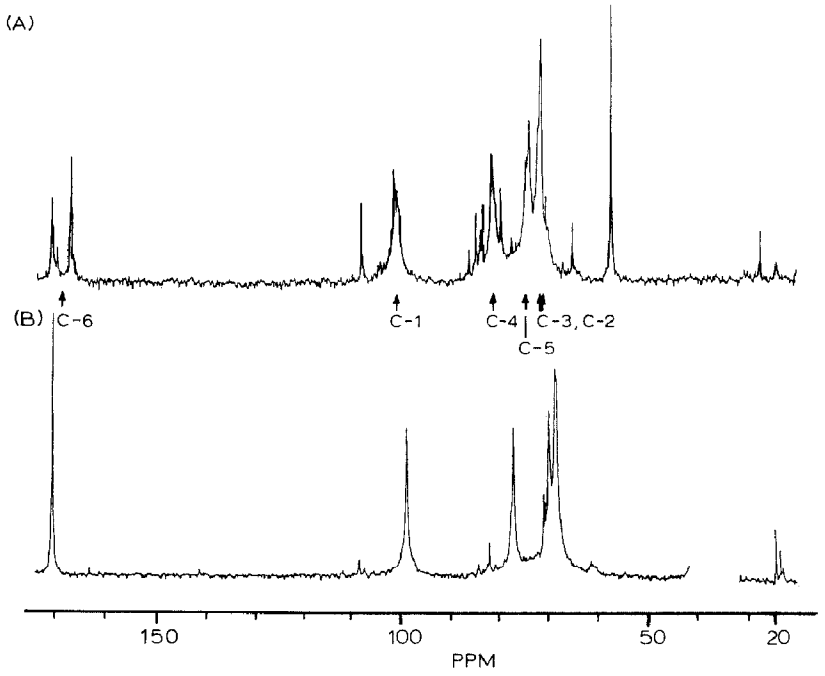


Fig. 1. ^{13}C-N.m.r. spectrum of solutions of *A*, AP in D_2O; and *B*, AP-I in $(CD_3)_2SO$. The arrows indicate the resonances of a commercial polygalacturonic acid.

Fraction 1, extracted from apricot[2] with EDTA, was precipitated with ethanol to yield pectic polysaccharide AP (3.7% of the original solids). After delignification of the fruit[2] with sodium chlorite, a minor (0.9%) pectic fraction (AP-II) was obtained by extraction with EDTA. Treatment of AP with Cetavlon (HO^- form)[4] precipitated AP-I (58% of AP). Data on AP, AP-I, and AP-II are given in Table I.

AP had a relatively low molecular weight and consisted mainly of galacturonic acid and the neutral sugars common in pectic substances[5]. Fig. 1 shows the ^{13}C-n.m.r. spectra of AP and AP-I. The signals were assigned on the basis of data for related oligo- and poly-saccharides[6-11], and by comparison with those for a commercial polygalacturonic acid. The main resonances, which appeared at δ 100.5–101.5 (C-1), 69.21 (C-2), 69.60 (C-3), 80.14 (C-4), and 71.70 (C-5), were related to the (1→4)-linked α-D-Gal*p*A backbone of the pectin molecule. Of the GalA residues, 57.6% were methyl esterified (MeO: ^{13}C, δ 54.06; ^{1}H, δ 3.81) and 8.5% were acetylated at different positions (AcO: ^{13}C, δ 21.39 and 21.58; ^{1}H, δ 2.09 and 2.19). The ^{13}C signal at δ 171.8 was due to COOH of galacturonic acid[12] and that at δ 175.68 (downfield shifted[7]) to COO^-. The i.r. spectrum of AP contains bands at 1611 and 1404 cm^{-1} for the COO^- group.

The ^{13}C-n.m.r. spectrum of AP resembled that of the acetylated sugar-beet pectin[13] and showed broadening of the ^{13}C signals of the galacturonan chain due to its restricted mobility. However, the ratios of the integrated intensities of the C-1 signals for GalA, Ara, and Gal corresponded well with those found by sugar analysis (Table I).

Arabinose, the main neutral sugar component of AP, was present as terminal and 5-linked α-L-Ara*f* residues. The corresponding signals were located, in accord with reported data[14], at δ 108.48 (C-1), 82.52 (C-2), 77.82 (C-3), 85.21 (C-4), and 62.36 (C-5) for the non-reducing end group, and at δ 108.66 (C-1), 82.07 (C-2), 77.99 (C-3), 83.50 (C-4), and 68.05 (C-5) for the 5-linked residues. By comparison of the integrated intensities of the C-1 signals, an average chain-length of ~ 3.7 was found for the arabinan side chains, similar to that for pectic polysaccharides from onion[10].

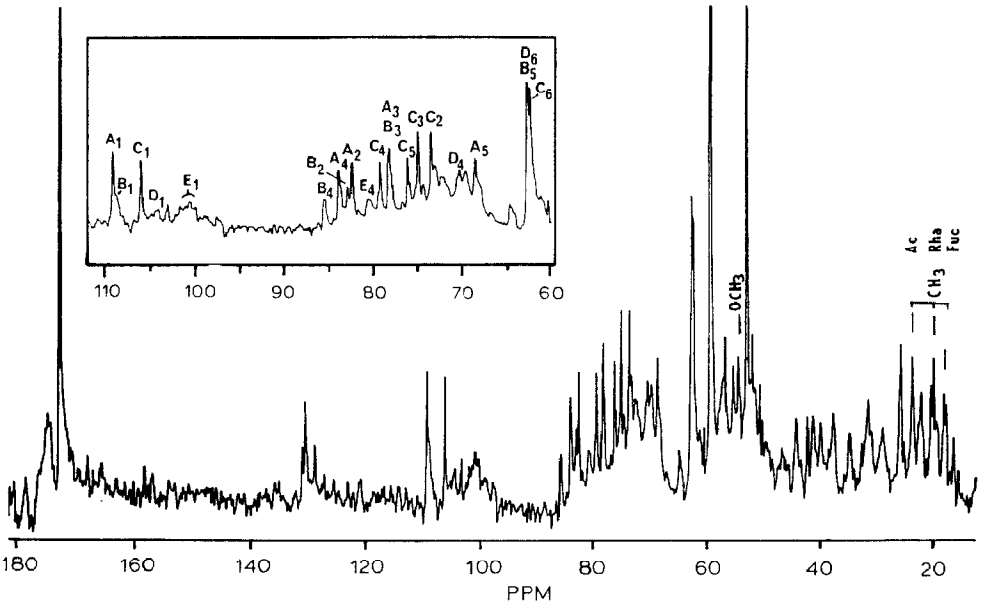
Galactose occurred as β-D-Gal*p* residues, as indicated[12] by the signals of C-1 at δ 104.2 and 104.6; no other signals for Gal were detected reliably. The doublets at δ 17.81 and 18.02 were assigned to C-6 of two differently linked Rha moieties, which are known to be present in the rhamnogalacturonan RG-I sequence[15,16] of pectin molecules, and α-D-Gal*p*A-(1→2)-L-Rha was isolated from a partial acid hydrolysate of AP-I.

Treatment of pectin with alkali causes cleavage of the molecule[17] by β-elimination reactions, and it is assumed that parts of the neutral side chains were released in this way during the fractionation of AP with Cetavlon (HO^- form) to give AP-I. The appearance of new minor signals at δ 163.1, 141.5, and 112.0 in the ^{13}C-n.m.r. spectrum of AP-I (Fig. 1B) also reflected the presence of the resulting unsaturated residues[18]. AP-I was mainly a (1→4)-α-D-galacturonan, comprising ~ 90% of GalA, with resonances at δ 98.58 (C-1), 68.10 (C-2), 68.26 (C-3), 76.65 (C-4), 69.49 (C-5), and 170.16 (C-6) in the ^{13}C-n.m.r. spectrum [$(CD_3)_2SO$]. Some of the GalA residues were substituted, as confirmed by their resistance to periodate (0.85 mol of IO_4/176 mol of GalA). Evidently, some of the arabinan side chains were preserved in the RG-I sequence of AP-I, as seen from the C-1 signals at δ 107.3 and 108.1. The intense resonances at δ 18.78, 19.03, and 19.56 were related to C-6 of the Fuc and Rha residues, which were found in relatively high proportions in the neutral sugar fraction of AP-I. 2-*O*-Methylxylose and 2-*O*-methylfucose, which are characteristic[19] for rhamnogalacturonan RG-II, were identified in the neutral sugar portion of the AP-I hydrolysate by p.c. and by g.l.c.–m.s. of the derived alditol trifluoroacetates.

Thus, it is concluded that the apricot pectin also contains "hairy" or ramified regions as found in pectins isolated from apple juice[14], carrot[20], and grape[9].

AP-II was rich in protein (32.2%, see Experimental) and neutral sugars (Table I). The ^{13}C-n.m.r. spectrum (Fig. 2) reflects the complex character of AP-II. The polysaccharide region (Fig. 2, inset) was dominated by signals of the (1→5)-linked α-Ara*f* chains, as in AP, and by resonances of (1→4)-linked β-Gal*p* chains. The ^{13}C resonances for the latter units were as follows: δ 105.46 (C-1), 72.94 (C-2), 74.43 (C-3), 78.77 (C-4), 75.63 (C-5), and 61.88 (C-6). The signals at δ 72.75, 73.76, 69.77, and 75.40 were assigned to the terminal non-reducing residues[21]. (1→4)-β-Galactans, as short side chains and/or high molecular weight components, have been reported for pectic polysaccharides isolated from onion[10], sugar beet[13], mung bean[21], and flax[16].

The resonances for C-1 and C-4 of the (1→4)-α-galacturonan chain appeared as broad minor signals at δ 101 and 80. Resonances of the methoxyl and acetyl groups, and for C-6 of Rha and Fuc residues, could be detected only by comparison with the ^{13}C chemical shift data for AP. These resonances were overlapped by those of the protein



components and/or residual phenolic substances, as indicated[22] by the signal at δ 130. Possible linkages between protein and the neutral sugar side chains of the pectic substances from grape have been suggested[9]. AP-II may be part of a such complex, which became accessible to EDTA after the acid–chlorite treatment.

EXPERIMENTAL

General. — Descending p.c. was performed on Whatman No. 1 paper with *A*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); *B*, 1-butanol–acetic acid–water (4:1:5); *C*, ethyl acetate–pyridine–water (8:2:1); *D*, 1-butanol–pyridine–water (6:4:5); and detection with (*a*) aniline hydrogenphthalate and (*b*) benzidine–trichloroacetic acid[23]. Amino acid analysis was performed on an automatic amino acid analyser T 339 (Mikrotechna PRAHA, CSFR). The amino acid composition (mol.%) of AP-II was Asp (10.6), Thr (6.6), Ser (6.9), Glu (11.9), Pro (2.7), Gly (14.1), Ala (9.2), Val (7.0), Ile (8.0), Leu (9.7), Phe (5.0), His (2.2), Lys (1.9), and Arg (4.0). The degree of esterification was calculated from the content of methoxyl groups as the mol.% of the content of uronic acid. The content of acetyl groups was calculated from the ratio (6.8:1) of peak areas of the signals for OMe and OAc in the ^{1}H-n.m.r. spectrum. The procedures for total hydrolysis, quantitative analysis of sugars, and determination of uronic acid, optical rotation, number-average molecular weight ($\overline{M}_n$), and i.r. spectra have been described[2,3]. ^{13}C-N.m.r. spectra (75 MHz) of 3% solutions of the polysaccharide in D_2O or $(CD_3)_2SO$ were obtained with a Bruker AM-300 spectrometer at 30° in the inverse-

gated decoupling mode. Chemical shifts were measured relative to internal methanol (δ 50.15) in D_2O solutions. ^{1}H-N.m.r. spectra (300 MHz) were measured under the same conditions.

Isolation and purification of the polysaccharide. — The pectic substances were extracted from the dried apricot fruit of *Armeniaca sibirica* Lam. by a multi-step procedure[2] using EDTA. The crude fraction 1 was precipitated from aqueous solution with ethanol to give fraction AP, and AP-II was obtained in a similar manner after treatment of the residual dried fruit with sodium chlorite. A solution of AP (2 g) in water (200 mL) was treated[4] with Cetavlon (HO^- form). The precipitate, after washing with water and acidification, yielded fraction AP-I, which was recovered by ethanol precipitation and dried by solvent exchange.

Partial acid hydrolysis of AP-I. — AP-I (400 mg) was hydrolysed with 0.01M trifluoroacetic acid (400 mL) at 90° for 4 h, and the residual polymer was removed by precipitation with ethanol (3 vol.). The hydrolysate was separated into neutral and acidic sugars by ion-exchange chromatography[2]. P.c. (solvent *C*) of the neutral sugars revealed Gal, Ara, Xyl, Rha, Fuc, 2-*O*-methylxylose[23] [R_F 0.49 (solvent *B*), R_{Xyl} 1.82 (solvent *C*)], and 2-*O*-methylfucose[23] [R_F 0.56 (solvent *B*), R_{Rha} 1.36 (solvent *D*)]. The monomethylated sugars were identified[24] as the alditol trifluoroacetates by g.l.c.–m.s. of the total hydrolysate of AP-I. P.c. (solvent *A*) of the acidic fraction revealed GalA and a disaccharide (R_{GalA} 0.75) which was isolated by preparative p.c. in the same solvent, and identified by ^{13}C-n.m.r. spectroscopy[6,7,25] as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose. ^{13}C-N.m.r. data: δ 93.31 (C-1), 76.91 (C-2), 17.96 (C-6), 97.12 (C-1′), 71.93 (C-4′), and 176.23 (C-6′).

Periodate oxidation. — A solution of AP-I (0.2 g) in water (160 mL) was treated with 0.1M sodium periodate (40 mL) at 4° in the dark. The consumption of periodate was monitored spectrophotometrically[26].

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