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Note

A xyloglucan from olive pulp*

ANTONIO GIL-SERRANO AND PILAR TEJERO-MATEO

Departamento de Quimica Orgánica, Facultad de Quimica, Universidad de Sevilla, 41071 Sevilla (Spain)

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The hemicellulosic material from olive pulp has been studied in relation to its dietary fibre composition and fruit softening. Previous studies reported the isolation, purification, and characterisation of various hemicelluloses from green olives[1–3]. We now report on the isolation and characterisation of a xyloglucan from olive pulp *(Olea europaea)*.

The hemicellulose B of olive pulp of the "gordal" variety was treated with Fehling's solution to precipitate the hemicellulose. From the supernatant solution, a xyloglucan was isolated which, on hydrolysis, gave arabinose, xylose, galactose, and glucose in the molar ratios 2.2:2.8:1.0:4.0, had $[\alpha]_D^{20}$ + 33° (*c* 1, methyl sulfoxide), and showed an i.r. band at 897 cm^{-1} characteristic[4] of this type of polysaccharide. The molecular weight, determined by gel-permeation chromatography, was 103,000.

Acetolysis[5] of the xyloglucan followed by *O*-deacetylation yielded a mixture of sugars. Four oligosaccharides were detected by p.c., two of which, isolated by preparative p.c., were shown by methylation analysis to be cellobiose and 2-*O*-D-galactopyranosylxylose. The other two oligosaccharides were tentatively identified, by their chromatographic properties, as cellotriose and cellotetraose.

The results of methylation analysis[6,7] of the xyloglucan are shown in Table I.

The region for anomeric carbons of the ^{13}C-n.m.r. spectrum was interpreted on the basis of the literature data[8]. The signals at δ 109.4 and 108.1 were assigned to C-1 of internal and terminal α-L-arabinofuranose residues. The signal at δ 105.4 may be assigned to C-1 of terminal β-D-galactopyranosyl groups. The group of signals centered at δ 102.7 was assigned to C-1 of β-D-glucopyranose residues and reflects the high complexity of the xyloglucan. Finally, two signals at δ 99.3 and 98.9 may correspond to C-1 of internal and terminal α-D-xylopyranose

*Polysaccharides from Olives, Part X. For Part IX, see ref. 3.

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TABLE I

METHYLATION ANALYSIS DATA FOR XYLOGLUCAN FROM OLIVE PULP

Methylated sugars (as alditol acetates)	T[a]	T[b]	*Molar ratio*	*Linkage*
2,3,5-Me_3-Ara[c]	0.47	0.71	1.9	Ara*f*-(1→
2,3,4-Me_3-Xyl	0.64	0.77	0.9	Xyl*p*-(1→
3,5-Me_2-Ara	0.90	0.87	0.5	→2)-Ara*f*-(1→
2,3-Me_2-Ara	1.81	0.93	0.4	→5)-Ara*f*-(1→
3,4-Me_2-Xyl	1.42	0.95	2.1	→2)-Xyl*p*-(1→
2,3,4,6-Me_4-Gal	1.25	1.04	1.0	Gal*p*-(1→
2,3,6-Me_3-Glc	2.52	1.19	1.7	→4)-Glc*p*-(1→
2,3-Me_2-Glc	5.56	1.37	2.2	→4,6)-Glc*p*-(1→

[a]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on a column of ECNSS-M. [b]for a W.C.O.T. column of OV-1. [c]2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.*

residues, respectively.

Thus, it is concluded that the xyloglucan contains a (1→4)-β-D-glucan backbone substituted at positions 6 with 2-substituted α-D-xylopyranose, and α-L-arabinofuranose, α-D-xylopyranose, and β-D-galactopyranose as terminal residues, similar to other xyloglucans[9-13]. The presence of traces of internal arabinose may be due to contamination with an arabinan which occurs in olive pulp[14].

EXPERIMENTAL

Materials. — Stoned olives ("gordal" variety), produced according to the Sevillan style, were obtained from TEPESA.

General methods. — Descending p.c. was performed on Whatman No. 3MM paper, using 1-butanol–pyridine–water (6:4:3). Optical rotations were measured with a Perkin–Elmer 141 polarimeter and i.r. spectra with a Perkin–Elmer 299 spectrophotometer. ^{13}C-N.m.r. spectra (100.57 MHz) were recorded at 90° with a Bruker AM 400-WB instrument on solutions in $(CD_3)_2SO$ (internal Me_4Si). G.l.c. was performed with a Hewlett–Packard 5710A chromatograph fitted with a flame-ionisation detector, using a stainless steel column (2 m x 0.3 cm) packed with 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180° for alditol acetates and 170° for partially methylated alditol acetates. G.l.c.–m.s. was performed with a Hewlett–Packard 5995B system fitted with an OV-1 W.C.O.T. column (12 m x 0.2 mm), using a temperature programme 120° →220° at 8°/min. The ionisation potential was 70 eV.

Routine polysaccharide hydrolysis (10 mg) was performed with aqueous 72% sulphuric acid (1 mL) for 1 h at 30° followed by dilution (28 mL of water/mL of acid) and heating (3 h at 100°). The hydrolysates were neutralised with barium carbonate and deionised, and the products were reduced with sodium borohydride

and acetylated.

Isolation and purification of the xyloglucan. — The olive pulp (1.4 kg) was triturated in a mixer, stabilised with hot ethanol (4.5 L) for 12 h, and extracted with chloroform–methanol (1:1). The residue (70 g) was stirred with methanolic 0.25M sodium methoxide[15] (700 mL) for 24 h at room temperature. The insoluble material was collected, washed until neutral with methanol, and then extracted twice with water (1.5 L) at room temperature. The remaining solid was delignified with sodium chlorite and acetic acid[16]. The resulting holocellulose was stirred with aqueous 10% sodium hydroxide[17] (1 L) for 24 h at room temperature under nitrogen. The extract was neutralised with aqueous 50% acetic acid, then dialysed against water for 3 days, and concentrated. Acidification with acetic acid gave no precipitate. The acidified solution was diluted with ethanol (4 vol.) to give the hemicellulose B (2.6 g).

A solution of hemicellulose B (2 g) in aqueous 5% potassium hydroxide (200 mL) was treated[18] with Fehling's solution to precipitate a hemicellulose which contained arabinose, xylose, mannose, galactose, and glucose in the molar ratios 0.9:2.9:1.9:1.3:5.2. The supernatant solution was neutralised, dialysed, concentrated, and diluted with ethanol. The precipitate was treated with ethanol containing 5% (v/v) of conc. hydrochloric acid and washed with ethanol. This procedure was repeated twice to yield the xyloglucan (370 mg).

Investigation of the xyloglucan. — *(a) Molecular weight.* A solution of the xyloglucan (0.5 mL, 0.5%) in 0.5M sodium chloride was applied to a column (55 x 1.6 cm) of Sephacryl S-300 and eluted with 0.5M sodium chloride. The column was calibrated with dextrans of known molecular weight.

(b) Methylation analysis. The xyloglucan (20 mg) in methyl sulphoxide (2 mL) was methylated twice by the Hakomori method[6], using ~2M methylsulphinylmethanide (1.5 mL) and methyl iodide (1 mL). The methylated xyloglucan was hydrolysed and the products were reduced with NaB^2H_4. The partially methylated alditols were acetylated with acetic anhydride–pyridine (1:1) overnight. A similar procedure was applied to the oligosaccharides.

(c) Acetolysis[5]. The xyloglucan (75 mg) was stirred with acetic acid–acetic anhydride–sulphuric acid (1.5:1.5:0.15) for 4 days at room temperature. The acetylated oligosaccharides were deacetylated with methanolic M sodium methoxide and examined by p.c. Several oligosaccharides were detected (R_{XYL} 0.06, 0.24, 0.62, and 0.79); the last two were isolated by preparative p.c.

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