Carbohydrate Research, 38 (1974) 247–255

RAPESEED HULL PECTIN

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(Received June 4th, 1974; accepted with revisions, July 22nd, 1974)

ABSTRACT

A pectin isolated from rapeseed, hulls by extraction with aqueous ammonium oxalate, had a degree of esterification of 83% and contained residues of hexuronic (mainly D-galacturonic) acid (76%), D-galactose (2–3%), L-arabinose (8–9%), D-xylose (2%), L-rhamnose (2–3%), and L-fucose (1%). Partial acid hydrolysis of the derived pectic acid furnished 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid and the polymer-homologous tri- and tetrasaccharides, and 4-*O*-(glucopyranosyluronic acid)-L-fucose. The cleavage products from the methylated pectin were examined by g.l.c. and the partially methylated alditol acetates from the methylated carboxyl-reduced polysaccharide by g.l.c.–mass spectrometry. Parallel methylation studies on lemon-peel pectin have established a close similarity between the two pectins.

INTRODUCTION

Structural studies on pectins from a variety of sources[1] have shown that galacturonans contain, as integral constituents, small but significant proportions of some or all of the neutral sugars, D-galactose, L-arabinose, D-xylose, L-rhamnose, and L-fucose. As part of an investigation of the polysaccharide components of rapeseed hulls we report the first examination of a pectin from this source.

RESULTS AND DISCUSSION

Rapeseed hulls were extracted successively with light petroleum and acetone to remove fats and colouring matter, with ethanol–water to remove soluble sugars, with water at 60°, and then with aqueous ammonium oxalate at 80° to give a pectin relatively uncontaminated by other polysaccharides. The purified pectin, obtained by chromatography on DEAE-Sephadex A-50 (formate form), contained 76% of hexuronic acid residues, and the methoxyl content (10.3%) indicated a degree of esteri-

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fication of 83%. Saponification of the pectin under controlled conditions[2], followed by graded precipitation with sodium acetate gave a major and two minor sodium pectate fractions. Pectic acid, which was regenerated from the major fraction, contained 83% of hexuronic acid residues, gave on hydrolysis galactose, arabinose, xylose, rhamnose, and fucose in the ratio of 2.1:18:3.7:4:1, and was used for structural studies.

Partial acid hydrolysis of the pectic acid resulted in the separation of an insoluble, degraded polysaccharide, essentially a galacturonan. The soluble sugars were separated by chromatography on DEAE-Sephadex A-25 (formate form), and the neutral sugars, which were eluted with water, were characterized by formation of crystalline derivatives as D-galactose, L-arabinose, D-xylose, L-rhamnose, and L-fucose. Elution of the column with aqueous formic acid, followed, as required, by filter-sheet chromatography, furnished D-galacturonic acid and five acidic oligosaccharides (**1**–**5**). 2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose (**1**) was characterized by conversion into the crystalline methyl glycoside pentamethyl ether. 4-*O*-(α-D-Galactopyranosyluronic acid)-D-galacturonic acid (**3**) and the polymer-homologous galacturonotriose (**4**) and galacturonotetraose (**5**) each furnished crystalline calcium salts. The nature of the linkages was established by methylation of the corresponding neutral methyl glycosides obtained by the sequence of reactions involving conversion of the acidic oligosaccharides into the methyl ester methyl glycosides, trimethylsilylation, reduction with lithium aluminum hydride, and removal of protecting groups[3]. Oligosaccharide **2**, isolated only in small proportion, was shown by methylation analysis to be 4-*O*-(glucopyranosyluronic acid)-L-fucose.

1 α-D-Gal*p*A-(1→2)-L-Rha

2 Glc*p*A-(1→4)-L-Fuc

3 α-D-Gal*p*A-(1→4)-D-Gal*p*A

4 α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-D-Gal*p*A

5 α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-D-Gal*p*A

For purposes of structural comparison, methylation studies were performed in parallel on rapeseed hull and lemon-peel[2] pectic acids. Pectic acids were methylated by the Hakomori[4] procedure as described by Sandford and Conrad[5]. Björndal *et al.*[6] have commented that (1→4)-linked glycuronans may be methylated in a single operation by the Hakomori procedure, when etherification presumably occurs more rapidly than base-catalyzed β-elimination, although repetition of the procedure with fresh base caused extensive depolymerization. The cleavage products from the methylated pectin were characterized qualitatively by g.l.c. of the methyl glycosides and the neutral components were additionally characterized by g.l.c. of the partially methylated alditol acetates. Samples of rapeseed hull and lemon-peel pectic acids were reduced by the procedure of Taylor and Conrad[7], and repetition of the operation gave galactans containing less than 5% of hexuronic acid residues. Depolymerization of the methylated derivatives of the carboxyl-reduced polysaccharides gave mixtures of sugars that

TABLE I

G.L.C. DATA FOR SUGAR DERIVATIVES FROM METHYLATED RAPESEED HULL AND LEMON-PEEL PECTINS AND THEIR CARBOXYL-REDUCED DERIVATIVES

Sugar	*Relative retention times of*			*Sugars formed from methylated pectins*	*Proportions (%) in methylated reduced*	
	methyl glycosides on column		*alditol acetates on column* d		*rapeseed pectin*	*lemon-peel pectin*
	a (140°)	b				
2,3,4-Me_3 xylose	0.39 0.52	0.40 0.49	0.65	+	1.7	0.6
2,3,5-Me_3 arabinose	0.49 0.71	0.47 0.64	0.46	++	4.6	5.9
2,3-Me_2 arabinose	1.25 1.56 1.75	1.04 1.42	1.22[a]	+	2.0	1.9
2-Me arabinose	n.d.	n.d.	2.32	tr		
3,4-Me_2 rhamnose	0.88	0.76	0.93	+	2.1	2.6
3-Me rhamnose		2.00	1.94	tr	0.4	0.6
2,3,4,6-Me_4 galactose	1.92	1.77	1.27[a]	+	2.0	3.7
2,3,6-Me_3 galactose		2.68 3.59	2.42	+[b]	82.5	81.1
2,6-Me_2 galactose		5.72 6.23	3.66		3.7	3.6
2,3,4-Me_3 galacturonic acid		6.90		++		
2,3-Me_2 galacturonic acid		4.08 16.7		+++++		

[a]Complete separation of 2,3-di-*O*-methylarabinitol and 2,3,4,6-tetra-*O*-methylgalactitol acetates was achieved by g.l.c. on column *a*. [b]Not detected from methylated lemon-peel pectin.

were analyzed as methylated alditol acetates by g.l.c.–mass spectrometry[6]. The results are summarized in Table I.

Table II indicates the limits which the sugar composition of rapeseed hull pectin lies, based on (*a*) the hexuronic acid content and the relative proportions of neutral sugars of the pectic acid, (*b*) the proportions of sugars formed on hydrolysis of the

TABLE II

ANALYSIS OF COMPOSITION OF RAPESEED HULL PECTIC ACID AND DERIVATIVES

Sugar	*Proportions (%) of sugars*			*Estimated composition*
	Pectic acid[a]	*Carboxyl-reduced pectic acid*[b]	*Methylated carboxyl-reduced pectic acid*[c]	
Arabinose	(18)	8.5	6.6	8–9
Xylose	(3.7)	1.9	1.7	2
Rhamnose	(4)	2.4	2.5	2–3
Fucose	(1)	0.8	n.d.	1
Galactose	(2.1)	86.4	88.2	1–2
Galacturonic acid	83			83 ±2
Glucuronic acid		n.d.	n.d	1

[a]Galacturonic and glucuronic acids determined as total hexuronic acid. Figures in parenthesis are relative proportions of neutral sugars formed on hydrolysis and determined as alditol acetates. [b]Sugars determined as alditol acetates. [c]Based on the analysis of sugars as partially methylated alditol acetates.

carboxyl-reduced polysaccharide, and (*c*) the proportions of methylated sugar derivatives formed from the methylated carboxyl-reduced pectic acid. In the analysis of methylated sugars as partially methylated alditol acetates, it is possible that the proportions of tri-*O*-methylpentoses may be slightly underestimated because of the relative volatility of their derivatives, and that other components are correspondingly overestimated. It may be noted that it was not possible to determine the proportions of glucuronic acid, whose presence as a minor constituent was shown by the partial characterization of the aldobiouronic acid **2**, which has been similarly isolated from several pectins[1]. Glucose was not recognized on hydrolysis of the carboxyl-reduced polysaccharide, but in the analysis of alditol acetates, less than 1% of glucitol hexaacetate, whose retention time is high, could easily escape detection.

The methylation results summarized in Table I and the partial hydrolysis studies on the rapeseed pectic acid, taken together with those previously reported for the lemon-peel pectic acid[3], indicate a close similarity between the two polysaccharides and between the parent pectins. The neutral sugars occupy similar structural roles to those in other pectins. L-Rhamnose residues are present in interior chains, interrupting the galacturonan chain at intervals. As acidic disaccharide **1** was the only rhamnose-containing oligosaccharide isolated from the rapeseed pectic acid it is not known

whether residues of this sugar are concentrated in certain regions of the polysaccharide chain as in lemon-peel pectic acid[3]. Other neutral sugars occur in side-chains as single L-arabinofuranose, D-xylopyranose, and D-galactopyranose residues, and, less frequently, in longer chains such as those of arabinobiose (**6**) and galactobiose (**7**). The proportions of nonterminal arabinose and galactose residues preclude the existence of a significant proportion of chains longer than two residues. It was not possible to obtain an exact balance between end groups and branch points, but the major branch points are those through *O*-3 of D-galacturonic acid residues. New methods of structural elucidation are required before these aspects of the fine structure of pectins can be established unambiguously.

6 L-Ara*f*-(1→5)-L-Ara*f*

7 D-Gal*p*-(1→4)-D-Gal*p*

EXPERIMENTAL

General. — Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ±2° and, unless otherwise stated, were for aqueous solutions. Melting points are uncorrected. Paper chromatography was performed on Whatman Nos. 1 and 3 MM papers with the following solvent systems (v/v): (A) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water; (B) 18:8:3:9 ethyl acetate–acetic acid–formic acid–water; (C) 8:2:1 ethyl acetate–pyridine–water; (D) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water; (E) 90:8:2 butanone–water–ammonia; (F) 9:2:2 ethyl acetate–acetic acid–water.

G.l.c. was performed with a Hewlett–Packard model 5750 chromatograph using columns of dichlorodimethylsilane-treated Celite coated with (*a*) 5% of neopentylglycol adipate polyester (operating temperature, 140° or 180°), (*b*) 5% of silicone gum XE-60 (140°), (*c*) 1% of silicone–polyester copolymer ECNSS-M (200°), and (*d*) 3% of ECNSS-M (180°). Retention times of methyl glycosides are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, and those of partially methylated alditol acetates relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. For g.l.c.–mass spectrometry, column *d* was used in a Perkin–Elmer 990 gas chromatograph connected via a Watson–Biemann separator to a Perkin–Elmer–Hitachi RMU-6 mass spectrometer; the latter was operated with an inlet temperature of 250°, an ionization potential of 70 eV, and an ion-source temperature of ~250°.

Evaporations were carried out under diminished pressure at bath temperatures of 40° or less. Hexuronic acid contents were determined by the modified carbazole–sulfuric acid procedure[8].

Isolation and fractionation of rapeseed hull pectin and a derived pectic acid. — Rapeseed hulls were extracted successively with boiling light petroleum (b.p. 30–60°), boiling acetone, and boiling 4:1 ethanol–water. Preliminary experiments indicated that selective extraction of pectin was achieved best with hot ammonium oxalate. Accordingly, rapeseed hulls (165 g) were extracted three times each with water (3 l) at

room temperature and at 60°, and then with 0.5% aqueous ammonium oxalate (3 l) at 80°. Crude pectin (14.6 g), $[\alpha]_D$ +208° (*c* 1.0) [Found: uronic acid residues, 68%] was isolated after regeneration from the insoluble calcium salt[8]. Crude pectin (10 g) was chromatographed on a column of *O*-(2-diethylaminoethyl)-Sephadex A-50 (80 g, formate form); elution with 0.2M sodium formate gave a minor fraction (~0.85 g) containing 25% of uronic acid residues, and elution with 0.6M sodium formate gave rapeseed hull pectin (7.8 g), $[\alpha]_D$ +224° (*c* 1.0) [Found: uronic acid residues, 76; OMe, 10.3%].

Saponification of the pectin (15 g), as described previously[2], gave pectic acid (13.8 g), $[\alpha]_D$ +246° (*c* 1.0) [Found: uronic acid residues, 82%], and, from the supernatant liquid, acid-soluble polysaccharide (380 mg) [Found: uronic acid residues, 43%] that on hydrolysis gave galactose and arabinose as the main neutral sugars.

Pectic acid (13.8 g) was fractionated by precipitation with sodium acetate, as described previously[2], to give pectic acid A (2.76 g; precipitated with 0.12M sodium acetate), $[\alpha]_D$ +257° (*c* 1.0; as sodium salt) [Found: uronic acid residues, 86%], pectic acid B (8.56 g; precipitated with 0.14M sodium acetate, $[\alpha]_D$ +248° (*c* 1.0; as sodium salt) [Found: uronic acid residues, 83%], and pectic acid C (1.24 g; precipitated with ethanol), $[\alpha]_D$ +192° (*c* 1.0; as sodium salt) [Found: uronic acid residues, 69%]. The major fraction, pectic acid B, was taken as representative of the polydisperse system[2] and was used for structural investigations. The insolubility of this pectic acid in appropriate buffers precluded its examination by electrophoresis (we are grateful to Dr. C. T. Bishop for attempting to carry out this experiment). A sample of the pectic acid was hydrolyzed, and analysis of the products by g.l.c. of the derived alditol acetates showed the presence of rhamnose, fucose, arabinose, xylose, and galactose as neutral constituents in the molar ratio of 4:1:18:3.7:2.1.

Partial, acid hydrolysis of pectic acid. — Pectic acid (10 g) in 0.5M sulfuric acid (500 ml) was heated for 2 h on a boiling-water bath. Degraded polysaccharide was separated by centrifugation and was hydrolyzed for a further 2 h. Insoluble degraded polysaccharide (4.2 g) was removed by centrifugation and was shown to give galacturonic acid and a trace of rhamnose on extended hydrolysis. The combined aqueous hydrolyzates were neutralized with barium hydroxide and barium carbonate, and the filtrate was treated with Amberlite resin IR-120 (H^+) to remove barium ions and concentrated to a syrup (5.3 g). The syrup was adsorbed onto a column of *O*-(2-diethylaminoethyl)-Sephadex A-25 (40 g, formate form). Elution of the column with water gave a mixture of neutral sugars (2.2 g), and further elution with 0.05M, a gradient of 0.05–0.4M, 0.5M, and M formic acid gave a series of fractions from which chromatographically homogeneous samples of D-galacturonic acid and five acidic oligosaccharides were obtained after further separation by paper chromatography in solvents A and B.

Characterization of neutral sugars. — The neutral sugars from the water eluate were separated by paper chromatography in solvents C and F to give: L-rhamnose (20 mg), $[\alpha]_D$ +7° (equil.) (*c* 1.0), characterized by conversion into the phenylhydrazone, m.p. and mixed m.p. 160°, $[\alpha]_D$ +29° (*c* 1.0, 4:1 ethanol–water); L-fucose (5 mg),

characterized as the *p*-tolylsulfonylhydrazone, m.p. and mixed m.p. 158°, $[\alpha]_D$ −12° (*c* 0.5, pyridine); D-xylose (62 mg), m.p. and mixed m.p. 152°, $[\alpha]_D$ +18° (equil.) (*c* 1.0), characterized by conversion into the di-*O*-benzylidene dimethyl acetal, m.p. and mixed m.p. 210–212°, $[\alpha]_D$ −8° (*c* 1.0, chloroform); L-arabinose (650 mg), m.p. and mixed m.p. 158°, $[\alpha]_D$ +107° (equil.) (*c* 1.0), characterized by conversion into the *p*-tolylsulfonylhydrazone, m.p. and mixed m.p. 156–157° (*c* 1.5, pyridine); and D-galactose (14 mg), $[\alpha]_D$ +80° (equil.) (*c* 1.0), characterized by conversion into β-D-galactopyranose pentaacetate, m.p. and mixed m.p. 141–142°, $[\alpha]_D$ +25° (*c* 0.8, chloroform).

Characterization of acidic sugars. — D-Galacturonic acid was isolated as the monohydrate (2.1 g), m.p. and mixed m.p. 106–108°, $[\alpha]_D$ +51° (equil.) (*c* 1.0), and was further characterized by conversion into galactaric acid, m.p. and mixed m.p. 213–214°.

Oligosaccharide **1** (41 mg, eluted with 0.05M formic acid), $R_{Galacturonic\ acid}$ 0.76 in solvent A, $[\alpha]_D$ +78° (*c* 1.0), gave galacturonic acid and rhamnose on hydrolysis, and colorimetric determinations by the carbazole[9] and cysteine[10] methods indicated their presence in the molar ratio of 1:1. G.l.c. of the methanolysis products from the methylated derivative[11] on columns *a* and *b* showed the presence of methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 3,4-di-*O*-methylrhamnose. 2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose was characterized by conversion into the methyl glycoside pentamethyl ether dihydrate, m.p. and mixed m.p. 69°, $[\alpha]_D$ +92° (*c* 0.5, chloroform)[8].

Oligosaccharide **2** (26 mg, eluted with 0.05M formic acid), $R_{Galacturonic\ acid}$ 0.60 and 1.0 in solvents A and D, $[\alpha]_D$ −67° (*c* 0.5), gave glucuronic acid and fucose on hydrolysis. The methyl ester methyl glycosides were reduced with sodium borohydride and the product was successively hydrolyzed, reduced with sodium borohydride, and acetylated. G.l.c. of the mixture on column *c* showed the presence of glucitol and fucitol acetates in equimolar proportions. G.l.c. of the methanolysis products from the methylated derivative[11] showed the presence of methyl glycosides of 2,3,4-tri-*O*-methylglucuronic acid and 2,3-di-*O*-methylfucose. Confirmation of the substitution pattern in the latter sugar was obtained by converting the sugar into the partially methylated alditol acetate, whose mass spectrum was identical to that of 2,3-di-*O*-methyl-L-fucitol acetate.

Oligosaccharide **3** (530 mg, eluted with 0.4M formic acid), $R_{Galacturonic\ acid}$ 0.25 and 0.50 in solvents A and B, gave galacturonic acid only on hydrolysis and furnished a crystalline calcium salt, $[\alpha]_D$ +116° (*c* 0.5, 0.5M hydrochloric acid)[3]. The sugar (100 mg) was converted into the methyl ester methyl glycosides as described previously[3], and thence into the trimethylsilyl ethers, which were reduced with lithium aluminum hydride. The resulting methyl galactobiosides (92 mg) were methylated successively with methyl sulfate and sodium hydroxide, and methyl iodide and silver oxide in *N*,*N*-dimethylformamide. Methanolysis of a portion of the methylated disaccharide was shown by g.l.c. on columns *a* and *b* to give equimolar proportions of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose. The major

portion (60 mg) of the methylated disaccharide was hydrolyzed in M hydrochloric acid (3 ml) for 8 h on a boiling-water bath and the cooled solution was neutralized with silver carbonate. The mixture was filtered, and the filtrate concentrated to a syrup, which was separated by paper chromatography in solvent E to give (*i*) 2,3,6-tri-*O*-methyl-D-galactose (22 mg), $[\alpha]_D$ +79° (*c* 1.0, methanol), which was characterized by conversion into 2,3,6-tri-*O*-methyl-D-galactono-1,4-lactone, m.p. and mixed m.p. 97–99°, and (*ii*) 2,3,4,6-tetra-*O*-methyl-D-galactose (25 mg), $[\alpha]_D$ +80° (*c* 1.0, methanol), which was characterized by conversion into the aniline derivative, m.p. and mixed m.p. 196–197°.

Oligosaccharide **4** (165 mg, eluted with 0.5M and M formic acid), $R_{Galacturonic\ acid}$ 0.05 and 0.25 in solvents A and B, gave galacturonic acid only on hydrolysis and furnished a calcium salt, $[\alpha]_D$ +135° (*c* 0.63, 0.5M hydrochloric acid)[3]. The sugar (5 mg) was converted, as described for the disaccharide, into the methylated derivative of the neutral carboxyl-reduced trisaccharide by successive ester glycoside formation, trimethylsilylation, reduction, removal of trimethylsilyl ethers, and methylation. Methanolysis of the methylated derivative was shown by g.l.c. on columns *a* and *b* to give methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose in the molar ratio of 1:2.

Oligosaccharide **5** (85 mg, eluted with 0.5M and M formic acid), $R_{Galacturonic\ acid}$ 0.11 in solvent B, d.p.[12] 2, gave galacturonic acid only on hydrolysis and furnished a calcium salt, $[\alpha]_D$ +139° (*c* 0.62, 0.5M hydrochloric acid)[3]. The methylated, carboxyl-reduced neutral tetrasaccharide was prepared as described previously and examination of the methanolysis products by g.l.c. on columns *a* and *b* showed the presence of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose in the molar ratio of 1:3.

Methylation of pectic acids. — Rapeseed hull pectic acid (750 mg, acid form) was methylated with methyl iodide and sodium hydride in methyl sulfoxide to give methylated pectic acid (560 mg), $[\alpha]_D$ +201° (*c* 1.0, chloroform) [Found: OMe, 40.6% (theoretical maximum, 42.0%)]. Lemon-peel pectic acid (1 g) was similarly converted into the methylated derivative (730 mg), $[\alpha]_D$ +215° (*c* 1.0, chloroform) [Found: OMe, 41.1%]. Samples of the methylated polysaccharides were methanolyzed and the resulting methyl glycosides were examined by g.l.c. on columns *a* and *b*. The results are summarized in Table I. Samples of the methyl glycoside mixtures were hydrolyzed, reduced with sodium borohydride, and acetylated. The neutral alditol acetates were examined by g.l.c. on column *d* (see Table I) and their identities were confirmed by g.l.c.–mass spectrometry.

Carboxyl-reduced pectic acids and their methylated derivatives. — 1-Cyclohexyl-3-[2-(4-methylmorpholino)ethyl]carbodiimide *p*-toluenesulfonate (10 g) was added to rapeseed pectic acid (1 g) in water (300 ml) and the reaction mixture was maintained at pH 4.75 for 2 h by the addition of 0.1M hydrochloric acid. Aqueous 2M sodium borohydride (100 ml) was added slowly during 4 h to the reaction mixture, the pH was maintained at 7.0 by the addition of 4M hydrochloric acid, and a drop of 1-octanol was added occasionally to prevent foaming. The reaction mixture was dialyzed against

distilled water, the solution was concentrated (to 300 ml), and the whole reaction sequence was repeated. Carboxyl-reduced rapeseed hull pectic acid (0.82 g) was isolated after dialysis and freeze-drying [Found: uronic acid residues (carbazole method), 3%]. A sample of the polysaccharide was hydrolyzed, and g.l.c. analysis of the derived alditol acetates showed the presence of arabinose, rhamnose, fucose, xylose, and galactose in the molar ratio of 8.5:2.4:0.8:1.9:86.4. Reduction of lemon-peel pectic acid (1 g) similarly afforded carboxyl-reduced pectic acid (0.8 g) [Found: uronic acid residues, 4.2%], hydrolysis of which gave arabinose, rhamnose, fucose, xylose, and galactose in the molar ratio of 9.5:3.1:0.6:1.0:85.8.

Carboxyl-reduced, rapeseed hull pectic acid (0.5 g) was methylated successively with methyl sulfate and sodium hydroxide, and with methyl iodide and sodium hydride in methyl sulfoxide, to give methylated carboxyl-reduced pectic acid (0.45 g), $[\alpha]_D$ +179° (*c* 1.0, chloroform) [Found: OMe, 43.3% (theoretical maximum, 44.6%)]. Similar treatment of carboxyl-reduced lemon-peel pectic acid (0.6 g) furnished the methylated polysaccharide (0.48 g), $[\alpha]_D$ +172° (*c* 1.0, chloroform) [Found: OMe, 43.6%]. Samples of the methylated polysaccharides were methanolyzed and the resulting methyl glycosides were examined by g.l.c. on columns *a* and *b*. The mixtures of methyl glycosides were hydrolyzed, reduced with sodium borohydride, and the resulting alditols were acetylated with acetic anhydride and pyridine. The mixtures of alditol acetates were analyzed quantitatively by g.l.c. on columns *d* and *a* (see Table I), and the identities of individual components were confirmed by g.l.c.–mass spectrometry.

ACKNOWLEDGMENTS

This work forms part of the Rapeseed Utilization Assistance Programme and the authors thank the Rapeseed Association of Canada for financial assistance.

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