Carbohydrate Polymers **23** (1994) 13–18

Structure of chia seed polysaccharide exudate

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(Received 2 February 1993; revised version received 2 August 1993; accepted 3 August 1993)

Chia seed gum varies in molecular weight from 0·8 to 2·0 × 10^6 daltons, as determined by gel filtration. β-D-Xylose, α-D-glucose, and 4-*O*-methyl-α-D-glucuronic acid were obtained on hydrolysis in the respective ratios of 2:1:1. An aldobiouronic acid, 2-*O*-(4-*O*-methyl-α-D-glucopyranosiduronic acid)-D-xylose, was obtained by partial hydrolysis. A tentative structural unit proposed for the polysaccharide is a tetrasaccharide with 4-*O*-methyl-α-D-glucoronopyranosyl residues occurring as branches at *O*-2 of some β-D-xylopyranosyl residues in the main chain consisting of (1→4)-β-D-xylopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl units. A linear tetrasaccharide was characterized that incorporates these structural features.

NOTATION

Xylp Xylopyranosyl
Glcp Glucopyranosyl
GlcpA Glucopyranosyluronic acid

INTRODUCTION

Chia seeds from *Salvia Hispanica* L., *Salvia columbaria Benth*, *Salvia polystachya*, and other *Salvia* members of the family Labiatea have long been known and used as food by American Indians and rural Mexicans. When seeds are soaked in water, a clear mucilaginous gel is exuded that remains tightly bound to the seed. Although chia seed oil and protein, (Palma *et al.*, 1947; Earle *et al.*, 1960) have been examined, the structure of the exuded polysaccharide has not been elucidated. Mucilaginous properties of the gum have qualities that may give it application in the food industry. Its general structure has been examined and found to be high in uronic acid content, as expected for mucilages.

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Carbohydrate Polymers 0144-8617/94/$07.00

EXPERIMENTAL

General methods

Component sugars of chia seed gum were determined as their alditol acetates. Solvents were routinely evaporated under diminished pressure at 40°C. Paper chromatography was conducted by either descending or ascending methods, with Whatman No. 3MM paper and the following solvent systems: (A) ethyl acetate:acetic acid:water (9:2:2), (B) ethyl acetate:acetic acid:water (9:3:3), (C) 2-butanone: HOAc:water (3:1:1). Paper chromatograms were developed by spraying with aniline hydrogen phthalate and heating for 10 min at 105°C. Spray reagent was prepared by adding aniline (0·93 g) and phthalic acid (1·65 g) to water-saturated butanol (100 ml) (Partridge, 1949). Paper electrophoretic separation was conducted in 0·05 M borax (pH 9·5) buffer at 300 V for 2–8 h.

GLC of alditol acetates employed a 1·83 m × 2 mm i.d. glass column packed with 3% SP 2330 on 100/120 Supelcoport. The column oven was temperature-programmed from 210°C to 230°C (temperature was held at 210°C for 5 min prior to the program) at 2°C/min

with a nitrogen carrier gas flow rate of 20 ml/min in a gas chromatograph equipped with a flame ionization detector. The injector temperature was 300°C and the detector temperature was 250°C. Partially methylated alditol acetates were determined with the same column and an oven temperature programmed from 180°C to 240°C at 4°C/min.

The ^{13}C-NMR chemical shifts were recorded at 50·3 MHz with a Nicolet NT200 instrument and expressed in ppm relative to internal 1,4-dioxane (67·4 ppm). GLC–MS was performed with a quadrupole instrument using electron impact ionization (70 ev).

Chia seeds (20 g) were suspended in 6 N aqueous urea (400 ml) at 25°C for 7 h. After centrifugation, the residue was washed twice with distilled water (50 ml). The aqueous solutions were combined and then dialyzed 18 h against running deionized water. When freeze-dried, 0·9 g of polysaccharide was obtained (4·5% yield from seeds). Paper electrophoresis for 8 h showed only one component.

Molecular weights were estimated by gel filtration using a column (150 × 5 cm) packed with Bio-gel A 1·5 M (100–200 mesh) for separations in the range 10^4–$1{\cdot}5 \times 10^6$ daltons; fractions (5 ml) were eluted with aqueous Tris solution (0·025 M Tris/0·14 M NaCl, pH 7·4). Standards were blue dextrin, hemoglobin, bovine serum albumin, and bromothymol blue. Forty-five milligrams of polysaccharide dissolved in 15 ml of Tris solution was subjected to gel filtration. The quantities of eluted polysaccharide were determined by measuring the absorbance at 534 nm of the solution in which the polysaccharide was reacted with carbazole–sulfuric acid to produce a purple color. Three major groups of fraction were obtained: fractions 31–34 (8 mg), fractions 36–41 (12 mg) and fractions 43–59 (25 mg). Pooled fractions were dialyzed against running deionized water overnight and then freeze-dried to afford polysaccharide fractions which, on analysis as for their alditol acetates, had identical compositions.

Analysis showed an ash value of 6%. Microanalysis gave C 34·4%; H 5·27%; N 0·62%. Cations present in the native polysaccharide were determined by ashing, dissolving in dilute HCl and dilution with deionized water before atomic absorption analysis (AOAC, 1980). The major cation composition, expressed as mg per 100 g of polysaccharide, was Ca 1032, Mg 984; phosphorus as determined by standard procedures was unexpectedly low at 32·4.

Analysis of neutral sugars of the polysaccharide

Neutral components of the polysaccharide were quantitatively analyzed by GLC as their alditol acetates. Polysaccharide (10 mg) was completely hydrolyzed by a slight modification of the procedure of Albersheim *et al.* (1967) with aqueous 2 N trifluoroacetic acid (2 ml, 121°C, 3 h). Dried hydrolyzate was then reduced with $NaBH_4$ in DMSO followed by acetylation with acetic anhydride/1-methylimidazole (Blakeney *et al.*, 1983).

Uronic acids

Uronic acids were quantitatively determined by the carbazole–sulfuric acid procedure (Dische, 1947) and qualitatively by cysteine–sulfuric acid (Dische, 1948). Colorimetric quantitative determination at 534 nm was performed using D-glucuronic acid as standard and gave 22·5% uronic acid content for the polysaccharide.

Carboxyl groups in the polysaccharide were reduced by the method of Taylor and Conrad (1972), and the resulting products were analyzed as their alditol acetates by GLC and GLC–MS.

Anomeric configurations

^{13}C-NMR spectra were obtained by dissolving the polysaccharide and its carboxyl-reduced derivatives in D_2O, with *p*-dioxane as an internal standard, at ambient temperature and at 70°C. The higher temperature was used to obtain a higher solution concentration of polysaccharide. For chromic acid oxidation (Hoffman & Lindberg, 1980), completely acetylated polysaccharide (10 mg) (Tanghe *et al.*, 1963) was dissolved in 0·3 ml of acetic acid and chromium trioxide (27 mg) was added. The suspension was agitated in an ultrasonic bath at 55°C for 1·5 h. Water (2 ml) was then added and the product was extracted with five 1-ml portions of methylene chloride. After evaporation of the solvent the product was hydrolyzed with 2 N TFA, the sugars converted to alditol acetates, and then analyzed by GLC.

Methylation analysis

Uronic acids were reduced to neutral sugars after two reductions with 1-cyclohexyl-2-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate/$NaBH_4$. Native polysaccharide and its carboxyl-reduced derivative, were dissolved in 40% aqueous potassium hydroxide solution and stirred at 25°C for 1 h, followed by two additions of excess methyl iodide and reacted at 25°C for 1 h. The mixture was diluted with three volumes of water and the partially methylated products extracted thoroughly with methylene chloride. The dried, methylated polysaccharides were then dissolved in DMSO containing powdered KOH and methylated by the method of Fugedi and Nanasi (1981). The products were completely methylated as evidenced by the absence of hydroxyl absorptions in IR spectra. Partially methylated alditol acetates were prepared from hydrolyzates in the usual way and analyzed by GLC.

Partial hydrolysis of polysaccharide to isolate oligosaccharides and aldobiouronic acid (Whistler *et al.*, 1954)

Polysaccharide (98·8 mg) was dissolved in 50 ml of water and 1·4 ml of neat sulfuric acid was added to produce a 1 N solution. The acidic mixture was refluxed for 8 h and adjusted to pH 6·5 by addition of a saturated hot solution of barium hydroxide. Inorganic salts were removed by filtration through Celite and the clear filtrate was passed through a column of Amberlite IR-120 (H^+) resin and then through a column of IR-45 resin. The IR-45 resin was washed with water to remove neutral sugars. Uronic acids were then displaced by stirring for 2 h with an excess of 2 N sulfuric acid. After filtration, the resin was washed twice with water. The combined filtrates were adjusted to pH 6·5 by addition of $Ba(OH)_2$ solution and filtered. The filtrate was passed through an Amberlite IR-120 column and the acidic effluent concentrated to dryness to afford 34·4 mg of aldobiouronic acid. Aldobiouronic acid (250 mg) was also isolated from chia polysaccharide (500 mg) by descending preparative paper chromatography (solvent A) and had $[\alpha]_D^{25}$ + 85°C (*c* 2·0, H_2O).

Structural determination of aldobiouronic acid

Aldobiouronic acid was refluxed with 2% methanolic hydrogen chloride for 4 h and evaporated to dryness. The product was dissolved in THF and reduced with excess lithium aluminum hydride at 25°C for 1·5 h (Whistler *et al.*, 1954; Das Gupta *et al.*, 1976). Excess reducing agent was decomposed by addition of ethyl acetate and then water. On hydrolysis, monomers were monitored by TLC (solvent C) and analyzed as their alditol acetates in the usual way. Methylation of the aldobiouronic acid with KOH/DMSO/MeI (Fugedi & Nanasi, 1981) afforded fully methylated aldobiouronate. Aldobiouronic acid (200 mg) was dissolved in 2 ml of DMSO containing 0·5 g of powdered KOH and stirred at 25°C for 1 h, then 1 ml of methyl iodide was added and reacted at 25°C for 1 h. Then an additional 1 ml of methyl iodide was added and reacted for another hour. The resulting reaction mixture was diluted with water (15 ml) and then extracted four times with methylene chloride. The organic solution was dried over $MgSO_4$ and evaporated to dryness to give a syrup (52·0 mg) that had $[\alpha]_D^{25}$ + 110°C (*c* 0·5, CH_3OH).

Permethylated aldobiouronate was carboxy-reduced by the procedure of Das Gupta *et al.* (1976). A 3-fold excess of $LiAlH_4$ was added to the methyl aldobiouronate dissolved in diethyl ether. After 2 h at 25°C, excess lithium aluminum hydride was decomposed by careful addition of ethyl acetate and then diluted with water. The organic solvent was evaporated, the solution filtered, and the filtrate deionized with Amberlite IR-45 and IR-120 resins and then evaporated to a syrup. This product was methylated again as described to afford fully methylated disaccharide. After the fully methylated disaccharide was hydrolyzed, reduced, and acetylated, GLC analysis showed peaks with the same retention times as 2,3-di-*O*-methyl-1,4,5-tri-*O*-acetylxylitol (or 3,4-di-*O*-methyl-1,2,5-tri-*O*-acetylxylitol) and 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetylglucitol.

Isolation of tetrasaccharide

Polysaccharide (0·6 g) was soaked in 20 ml water, refluxed in 3% hydrochloric acid for 3·5 h and deionized as previously described. A tetrasaccharide was obtained by preparative paper chromatography (solvent B). Paper electrophoresis of the isolated oligosaccharide showed a single spot after color development. Uronic acid content was 26·1% as determined by the carbazole–sulfuric acid method. The tetrasaccharide and its methylated derivatives were hydrolyzed with 2 N trifluoroacetic acid, reduced with $NaBH_4$/DMSO, and finally acetylated with acetic anhydride to form alditol acetates. The reducing end was also determined by sequential reduction ($NaBH_4$/DMSO), hydrolysis, and acetylation. The products were analyzed by GLC and compared to authentic samples.

RESULTS AND DISCUSSION

When chia seeds are placed in water, they quickly begin to exude a mucilage that adheres to the seed with remarkable tenacity. One method for removal of the mucilage is extraction of the seeds with 6 N urea at pH 7·4 for 7 h to obtain the polysaccharide in a dry-weight yield of 4·5%, free of protein. Gel filtration chromatography suggested the presence of three fractions with molecular weights between 8×10^5 and 2×10^6 daltons, but analysis of hydrolyzates indicated that all were of the same composition, having D-xylosyl and D-glucosyl residues in the ratio of 2:1. Paper electrophoresis also indicated that each of the three fractions was homogeneous but differed in molecular weight. The gum fractions contained 22·5% of 4-*O*-methyl-D-glucuronopyranosyl residues as evidenced by the carbazole-sulfuric acid test and by optical rotation.

Neutral sugars in hydrolyzates of the carboxyl-reduced polysaccharide were analyzed by GLC as their alditol acetates. The acetates of xylitol, glucitol, and 4-*O*-methylglucitol were present in the ratios of 8:4:3·1, respectively. With deuterium labeling before hydrolysis, mass spectrometer (MS) analysis showed deuteration in the 4-*O*-methylhexitol pentaacetate. This result indicated that 4-*O*-methyl-D-glucuronic acid is a component of the polymer. In addition, the hexitol hexaacetate was not deuterated, which indicated the absence of unmethylated D-glucuronic acid in the polysaccharide. There-

fore, components of the polysaccharide are D-xylosyl, D-glucosyl, and 4-*O*-methyl-α-D-glucopyranosyluronic acid residues in the respective ratios of about 2:1:1. Hexuronic acid determination (22·5%) with carbazole agreed closely with that from GLC analysis.

The nature of the anomeric linkage and the presence of a pyranosyl ring is indicated by ^{13}C NMR spectroscopy and by chromic acid oxidation. The interpretation of almost all the ^{13}C-NMR data is based on information derived from Bock *et al.* (1984), Gorin (1981), and others (Colson *et al.*, 1974; Mendonca-Previato *et al.*, 1979; Gast *et al.*, 1980; Kovac *et al.*, 1981; Hirsch *et al.*, 1982). The C-1 resonance of D-glucuronic acid of 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose assigned by Mendonca-Previato *et al.* (1979) and by Kovac *et al.* (1981) agrees with the assignment of the α-D-glucopyranosyluronic linkage in this work. In general, good agreement is found between ^{13}C-NMR spectra reported in the literature and observations made with the chia polysaccharide (Table 1) in regard to ring size and anomeric assignment.

Resonance at 174·9 ppm, assigned to C-6 of the glucopyranosyluronic moiety, was absent in the ^{13}C-NMR spectrum of the carboxyl-reduced polysaccharide. Usually, anomeric carbon atoms in an α-D-linkage resonate at higher field than those with a β-D-linkage. Thus, the chemical shifts at 102·8 and 102·0 ppm were assigned to C-1 of (1→4)-β-D-xylopyranosyl and the (1→4)-β-D-glucopyranosyl, respectively. Also, chemical shifts at 61·2 and 63·5 ppm were assigned to C-6 of a (1→4)-linked D-glucopyranosyl residue and C-5 of a (1→4)-linked D-xylopyranosyl residue. The assigned anomeric configurations of the residues in the polysaccharide were confirmed by oxidation with chromium trioxide.

In chromium trioxide oxidation (Hoffman & Lindberg, 1980), peracetylated β-D-aldopyranosyl residues are oxidized rapidly, whereas such α-D-anomers oxidize slowly under the same conditions. When fully acetylated chia polysaccharide was subjected to oxidation with chromium trioxide, some of the D-xylopyranosyl residues were oxidized. The ratio of unoxidized D-xylopyranosyls to D-glucopyranosyls observed by GLC was 1:2. Survival of attached D-glucosyl residues was thus evidence of their α-D-anomeric configuration. Since the majority of D-xylosyl residues are oxidized by chromic acid, the assignment to them of β-D-configurations can be generally assured.

O-Methylated alditol acetates derived from the native and carboxyl-reduced chia polysaccharides were characterized by their GLC retention times and their distinctive EI-mass spectra (Bjorndal *et al.*, 1967, 1970). GLC of the partially methylated alditol acetates obtained from carboxyl-reduced polysaccharides showed four peaks whose identities were confirmed by GLC–MS as 2,3-di-*O*-methylxylitol triacetate (compound **1**), 2,3-6-tri-*O*-methyl-D-glucitol triacetate (compound **2**), 3-*O*-methylxylitol tetraacetate (compound **3**), and 2,3,4-6-tetra-*O*-methyl-D-glucitol diacetate (compound **4**). GLC of the partially methylated alditol acetates from unreduced chia polysaccharide gave only three peaks, the peak corresponding to 2,3,4-6-tetra-*O*-methyl-D-glucitol diacetate, compound **4**, being absent.

The finding of compounds **3** and **4** indicates the attachment of the uronic acid to either of positions 2 or 4 of a D-xylosyl residue; compounds **1** and **2** indicate that the D-glucopyranosyl and D-xylopyranosyl residues are both 4-*O*-linked. These derivatives, together with additional evidence given below, accord with a trisaccharide backbone structure consisting of 4-*O*-linked D-xylosyl, 2,4-di-*O*-linked D-xylosyl, and 4-*O*-linked D-glucosyl residues to which a uronic acid side-group is appended at either positions 2 or 4 of a D-xylosyl residue. Hydrolysis of chia polysaccharide with mild acid gave a single aldobiouronic acid that was purified by

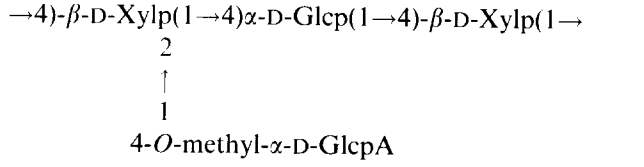
Table 1. Chemical shift assignment of chia polysaccharide, at 70°C

ppm	Assignment	Reference
174·9	C_6 α-D-glcA(1→	Kovac *et al.* (1981); Hirsch *et al.* (1982)
102·8	C_1 (1→4)-β-D-xyl	Gorin (1981); Hirsch *et al.* (1982); Gast *et al.* (1980)
102·0	C_1 (1→4)-α-D-glc	Bock *et al.* (1984); Colson *et al.* (1974)
98·7	C_1 α-D-glcA(1→	Mendonca-Previato *et al.* (1979); Kovac *et al.* (1981)
82·3	C_4 4-*O*-methylglcA, C_2(→2)xyl(1→4)	Kovac *et al.* (1981); Hirsch *et al.* (1982)
78·0	C_4 (1→4) α-D-glc	Colson *et al.* (1974)
77·1	C_4 (1→4) β-D-xyl	Hirsch *et al.* (1982); Gast *et al.* (1980)
74·6	C_3 (1→4) β-D-xyl, C_3 (1→4) α-D-glc	Gorin (1981); Colson *et al.* (1974); Hirsch *et al.* (1982); Gast *et al.* (1980)
73·5	C_2 (1→4) β-D-xyl, C_3 α-D-glcA	Gorin (1981); Colson *et al.* (1974); Hirsch *et al.* (1982); Gast *et al.* (1980)
73·1	C_2 (1→4) α-D-glc	Bock *et al.* (1984); Colson *et al.* (1974)
72·3	C_5 (1→4) α-D-glc	Colson *et al.* (1974)
71·8	C_2 α-D-glcA(1→	Kovac *et al.* (1981); Hirsch *et al.* (1982)
70·5	C_5 α-D-glcA(1→	Kovac *et al.* (1981); Hirsch *et al.* (1982)
63·5	C_5 (1→4) β-D-xyl	Gorin (1981); Hirsch *et al.* (1982); Gast *et al.* (1980)
61·2	C_6 (1→4) β-D-glc	Bock *et al.* (1984); Colson *et al.* (1974)
60·8	MeO-4-glcA	Hirsch *et al.* (1982)

ion exchange and preparative paper chromatography. Its high optical rotation is indicative of an α-D-linkage. Literature $[\alpha]_D^{25}$ values of 2-*O*-(4-*O*-methyl-α-D-glucuropyranosyluronic acid)-D-xylose cover the range +70 to +110° (Marchessault & Timell, 1963), and for the β-D anomer it is +5·7° (Bowering & Timell, 1960).

Methanolysis of the aldobiouronic acid with 3% methanolic hydrochloric acid followed by reduction of the ester to give methyl glycosides was confirmed by GLC and GLC–MS; the alditol acetates obtained after hydrolysis and per-*O*-acetylation were identified as D-xylitol pentaacetate and 4-*O*-methyl-D-glucitol pentaacetate in the ratio of 1:1, which revealed that the aldobiouronic acid consisted of D-xylose and 4-*O*-methyl-D-glucopyranosyluronic acid residues. For further identification, the reduced, methylated aldobiouronic acid was hydrolyzed and the products reduced with $NaBD_4$/DMSO. The deuterated, partially methylated alditols were then acetylated in the usual way. Identification of a labelled xylitol by MS as 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylxylitol-1-*d* revealed that the uronic acid is linked to *O*-2.

An apparent tetrasaccharide was isolated from partially hydrolyzed polysaccharide. Uronic acid content, as determined by the carbazole method, was approximately 25%. The reducing end was determined by reduction of the oligosaccharide to an oligoalditol, followed by hydrolysis, acetylation, and GLC–MS analysis. The three peaks obtained were tetra-*O*-acetyl-D-xylose, penta-*O*-acetyl-D-glucose, and xylitol pentaacetate. Hence, a D-xylopyranosyl is at the reducing end of the oligosaccharide. The neutral sugars were D-xylose and D-glucose in 2:1 ratio as was also found in the original polysaccharide. Sequential methylation, reduction ($LiAlH_4$) and hydrolysis of the oligosaccharide, followed by reduction and acetylation of the methylated sugars gave 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl-D-glucitol (compound **5**), 2,3-di-*O*-methyl-1,4,5-tri-*O*-acetylxylitol (compound **1**), and 2,3,6-tri-*O*-methyl-1,4,5-tri-*O*-acetyl-D-glucitol (compound **2**) in the respective molar ratios of 1:2:1. Similar analysis of the unreduced, methylated tetrasaccharide gave two peaks that corresponded to compounds **1** and **2**, the peak of compound **5** being absent. Overall, the results indicate a linear tetrasaccharide having the sequence of residues depicted in a structure for chia gum that also takes into account the aldobiouronic acid: two D-xylopyranosyl residues, and one each of D-glucopyranosyl and side branches of 4-*O*-methyl-D-glucopyranosyluronic acid. Thus, the proposed repeating unit structure for chia seed gum may be as shown:



ACKNOWLEDGEMENT

The authors gratefully acknowledge support of this work by a grant from the National Science Foundation (Grant No. DMB-8211086), Washington, DC 20550, USA.

REFERENCES

Albersheim, P., Nevins, D.J., English, P.D. & Karr, A. (1967). A method for the analysis of sugars in plant cell wall polysaccharides by gas–liquid chromatography. *Carbohydr. Res.*, **5**, 340–5.

AOAC (1980). *Official Methods of Analysis*, 1st edn. Association of Official Analytical Chemists, Washington DC, USA.

Bjorndal, H., Lindberg, B. & Svensson, S. (1967). Mass spectrometry of partially methylated alditol acetates. *Carbohydr. Res.*, **5**, 433–40.

Bjorndal, H., Lindberg, B., Pilotti, A. & Svensson, S. (1970). Mass spectra of partially methylated alditol acetates, Part II. *Carbohydr. Res.*, **15**, 339–49.

Blakeney, A.B., Harris, P.J., Henry, R.J. & Stone, B.A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.*, **113**, 291–9.

Bock, K., Pedersen, C. & Pedersen, H. (1984). Carbon-13 nuclear magnetic resonance data for oligosaccharides. *Adv. Carbohydr. Chem. Biochem.*, **42**, 193–225.

Bowering, W.D.S. & Timell, T.E. (1960). Synthesis and characterization of 2-*O*-(β-D-glucopyranosyluronic acid)-D-xylopyranose. *J. Am. Chem. Soc.*, **82**, 2827–30.

Colson, P., Jennings, H.J. & Smith, I.C.P. (1974). Composition, sequence, and conformation of polymers and oligomers of glucose as revealed by carbon-13 nuclear magnetic resonance. *J. Am. Chem. Soc.*, **96**, 8081–6.

Das Gupta, P.C., Sen, S.K. & Day, A. (1976). Glucomannan from the fibre of sunn hemp. *Carbohydr. Res.*, **48**, 73–80.

Dische, Z. (1947). A new specific color reaction of hexuronic acids. *J. Biol. Chem.*, **167**, 189–98.

Dische, Z. (1948). A new specific color reaction of galacturonic acid. *Arch. Biochem.*, **16**, 409–14.

Earle, F.R., McGuire, T.A., Mallan, J., Bagby, M.O., Wolff, I.A. & Jones, Q. (1960). Search for new industrial oils. II. Oils with high iodine values. *J. Am. Oil Chem. Soc.*, **37**, 48–50.

Fugedi, P. & Nanasi, P. (1981). A convenient method for the alkylation of carbohydrates. *J. Carbohydr., Nucleosides, Nucleotides*, **8**, 547–55.

Gast, J.C., Atalla, R.H. & McKelvey, R.D. (1980). The ^{13}C-N.M.R. spectra of the xylo- and cello-oligosaccharides. *Carbohydr. Res.*, **84**, 137–46.

Gorin, P.A.J. (1981). Carbon-13 nuclear magnetic resonance spectroscopy of polysaccharides. *Adv. Carbohydr. Chem. Biochem.*, **38**, 13–104.

Hirsch, J., Kovac, P. & Petrakova, E. (1982). An approach to the systematic synthesis of (1→4)-β-D-xylooligosaccharides. *Carbohydr. Res.*, **106**, 203–16.

Hoffman, J. & Lindberg, B. (1980). Oxidation of acetylated carbohydrates with chromium trioxide in acetic acid. *Methods in Carbohydr. Chem.*, **8**, 117–22.

Kovac, P., Petrakova, E. & Kocis, P. (1981). Chemical synthesis of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. *Carbohydr. Res.*, **93**, 144–7.

Marchessault, R.H. & Timell, T.E. (1963). Four polymer-homologous series of oligosaccharides from a 4-*O*-methyl-

glycuronoxylan. *J. Polym. Sci., Part C: Polym. Symp.*, **2,** 49–61.

Mendonca-Previato, L., Gorin, P.A.J. & Previato, J.O. (1979). Investigation on polysaccharide components of cell of *Herpetomous samuelpessoai* grown on various media. *Biochemistry*, **18,** 149–54.

Palma, F., Donda, M. & Lloyd, W.R. (1947). Fixed oils of Mexico. I. Oil of Chia-*Salvia hispanica*. *J. Am. Oil Chem. Soc.*, **24,** 27–8.

Partridge, S.M. (1949). Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. *Nature*, **164**, 443.

Tanghe, L.J., Genung, L.B. & Mench, J.W. (1963). Acetylation of cellulose. *Methods in Carbohydr. Chem.*, **3,** 193–212.

Taylor, R.L. & Conrad, H.E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry*, **11,** 1383–8.

Whistler, R.L., Conrad, H.E. & Hough, L. (1954). 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose from hemicellulose-β of corn cob. *J. Am. Chem. Soc.*, **76,** 1668–70.