Carbohydrate Research, 191 (1989) 343–350
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

DETERMINATION OF THE STRUCTURES OF PENTOSE-CONTAINING CARBOHYDRATES BY THE REDUCTIVE-CLEAVAGE METHOD

HEINRICH HEIMS, HANS STEINHART*,
Institut für Biochemie und Lebensmittelchemie der Universität, Grindelallee 117, D-2000 Hamburg 13 (F.R.G.)

AND PETRA MISCHNICK
Institut für Organische Chemie der Universität, Martin-Luther-King-Platz 6, D-2000 Hamburg 13 (F.R.G.)

(Received January 3rd, 1989; accepted for publication, March 21st, 1989)

ABSTRACT

Some di- and poly-saccharides containing arabinosyl and xylosyl residues have been methylated and then subjected to reductive-cleavage with triethylsilane in the presence of either trimethylsilyl trifluoromethanesulfonate or boron trifluoride etherate and trimethylsilyl methanesulfonate. The products were converted into the partially methylated anhydroalditol acetates and analysed by g.l.c. and g.l.c.–m.s. The results demonstrate that the reductive-cleavage method is an attractive alternative to methylation analysis in determining the structure of polysaccharides containing pentosyl residues.

INTRODUCTION

In conventional methylation analysis[1–5] (methylation, acid hydrolysis, reduction, and acetylation), the resulting partially methylated alditol acetates are analysed by g.l.c. and g.l.c.–m.s.[6–8]. The acid hydrolysis is a critical step and the conditions have to be optimised for different carbohydrates in order to eliminate degradation, which is more extensive for pentoses and furanoses than for hexoses and pyranoses. The polysaccharides of the dietary fiber complex are normally rich in pentoses, so that a procedure for the determination of structure that avoids degradation has potential importance. Knowledge of the structure of dietary fiber may help to explain physiological effects in the gut (adipositas, cancer in the large intestine, *etc.*)[9].

The disadvantages of standard methylation analysis can be avoided by using the reductive-cleavage method[10–13] and we now describe its application to 3-*O*-β-D-galactopyranosyl-D-arabinofuranose (**1a**), 4-*O*-β-D-xylopyranosyl-D-xylopyranose (**5a**), arabinogalactan from larchwood (**10**), and xylan from oat spelts (**16**).

*Author for correspondence.

0008-6215/89/$ 03.50

EXPERIMENTAL

General. — 3-*O*-β-D-Galactopyranosyl-D-arabinofuranose, 4-*O*-β-D-xylopyranosyl-D-xylopyranose (xylobiose), arabinogalactan from larchwood, and xylan from oat spelts, purchased from Sigma, were used, without further purification, for methylation according to Ciucanu and Kerek[5]. Reductive-cleavage and acetylation were performed by the general procedure of Gray and his co-workers[10–14].

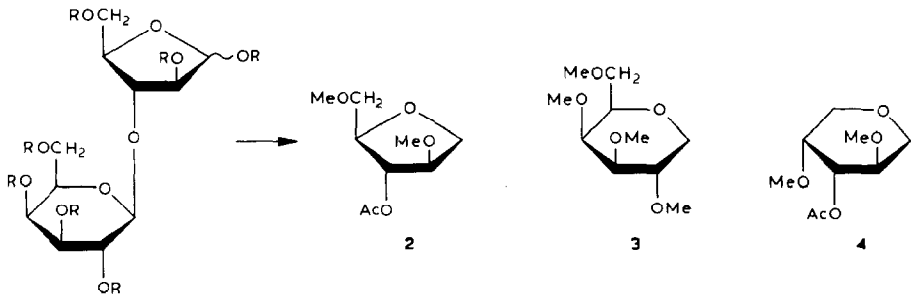
G.l.c. was carried out on a fused-silica capillary column (0.32 mm × 30 m) coated with DB-5 (J & W Scientific) by the on-column injection mode. A Carlo Erba 5160 gas chromatograph equipped with a flame-ionisation detector and a Shimadzu model C-R3A integrator were used. The retention times (*T*) are relative to that of erythritol tetra-acetate (Table I). The g.l.c. conditions were as follows: 1 min at 70°, →120° at 20°/min, 6 min at 120°, then →300° at 5°/min. 3-*O*-Acetyl-1,4-anhydro-2,5-di-*O*-methyl-D-arabinitol and 4-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-xylitol, which were co-eluted on DB-5, were separated and quantified on a fused-silica capillary column coated with DB-60 N (J & W Scientific) (data not reported).

G.l.c.–m.s. of partially methylated alditol acetates was performed with a VG 70-250 S or Finnegan MAT-311-A mass spectrometer and a Carlo Erba Fractovap 2101 gas chromatograph. A 25-m fused-silica capillary column coated with OV1-CB (Macherey & Nagel) was used. For e.i.-m.s., the ionizing voltage was 70 eV (Table I). For g.l.c.–c.i.-m.s., ammonia was used as reactant gas. The integral values of all peaks in g.l.c. were corrected by the effective-carbon-response (e.c.r.) method[15], which is applicable to anhydroalditols[10].

RESULTS AND DISCUSSION

3-*O*-β-D-Galactopyranosyl-D-arabinofuranose (**1a**) was chosen as a model for pentose-containing carbohydrates. Reductive cleavage of methylated **1a** (**1b**), with $Me_3SiOSO_2CF_3$ or Me_3SiOSO_2Me and $BF_3 \cdot Et_2O$ as catalysts, followed by acetylation gave the expected 3-*O*-acetyl-1,4-anhydro-2,5-di-*O*-methyl-D-arabinitol (**2**) and 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-galactitol (**3**), but ~17% of 3-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-D-arabinitol (**4**) was also formed (Fig. 1, peaks 2–4) due to conversion of the D-arabinofuranosyl residue in **1a** into the pyranose form during methylation. Equal amounts of the hexose and pentose derivatives were found (Table I) after integration of the peaks and correction for molar response[10,15], and reductive cleavage of **1b** with both catalysts was complete within 45 min.

4-*O*-β-D-Xylopyranosyl-D-xylopyranose (xylobiose, **5a**) was chosen as a model compound for xylans. Reductive cleavage of methylated **5a** (**5b**), as for **1b**, followed by acetylation gave three components (Fig. 2, peaks 6, 8, and 9). 1,5-Anhydro-2,3,4-tri-*O*-methyl-D-xylitol (**6**) was obtained from the non-reducing unit. The isomerisation product of **6**, 1,4-anhydro-2,3,5-tri-*O*-methyl-D-xylitol (**7**), was not detected. The reducing unit of **5a** gave the expected 4-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-



1a R = H

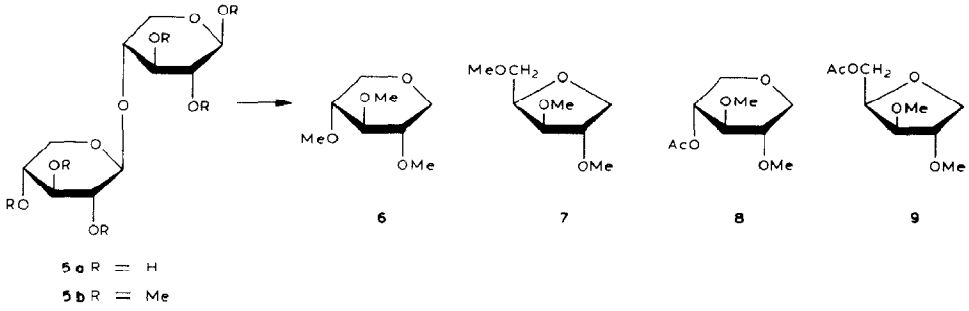
1b R = Me

TABLE I

MOLAR FRACTIONS OF PRODUCTS DERIVED BY REDUCTIVE DEPOLYMERISATION OF METHYLATED 3-*O*-β-D-GALACTOPYRANOSYL-D-ARABINOSE, 4-*O*-β-D-XYLOPYRANOSYL-D-XYLOSE, ARABINOGALACTAN, AND XYLAN

Acetylated 1,4- or 1,5-anhydro derivative	*E.c.r.*[a]	T[b]	*Catalyst (molar ratio)*	
			$Me_3SiOSO_2CF_3$	$Me_3SiOSO_2Me + BF_3 \cdot Et_2O$
3-O-β-D-Galactopyranosyl-D-arabinose				
2[c]	445	0.52	0.78	0.86
4	445	0.55	0.17	0.15
3	500	0.65	1.00	1.00
Xylobiose				
6	400	0.30	1.00	1.00
8	445	0.52	0.30	0.37
9	455	0.65	0.79	0.67
Arabinogalactan				
13	400	0.33	0.07	0.08
14	400	0.37	0.04	0.04
2	445	0.52	0.06	0.06
3	500	0.65	0.24	0.25
15	545	0.90	0.02	0.03
12	555	0.93	0.22	0.22
11	600	1.11	0.35	0.32
Xylan				
6	400	0.30	0.01	0.01
13	400	0.33	0.05	0.05
8 + 9	445/455	0.52/0.65	0.79	0.83
16 + 18	490/500	0.73/0.77	0.05	0.04
17 + 19	490/500	0.78/0.84	0.03	0.02
21	545	0.85	0.04	0.03
20	500	0.74	0.03	0.03

[a]Effective-carbon-response values. [b]Relative to that of erythritol tetra-acetate (20.65 min). [c]Numbers refer to peaks in Figs. 1–4 and, where appropriate, to the formulae.



D-xylitol (**8**) and its isomerisation product 5-*O*-acetyl-1,4-anhydro-2,3-di-*O*-methyl-D-xylitol (**9**), formed from **8** by ring contraction during reductive depolymerisation and obtained with both catalysts. The 4-linked xylopyranosyl residue could not isomerise during methylation. In contrast to 4-linked xylopyranosyl residues, 4-linked glucopyranosyl residues do not isomerise[13] when $Me_3SiOSO_2Me/BF_3 \cdot Et_2O$ is used as the catalyst. The molar ratio of **6** and **8** + **9** was ~1:1 (Table I) and some **6** was lost because of its volatility.

The structure **10** has been proposed[16–18] for the water-soluble arabinogalactan from larchwood.

$$
\begin{array}{cccc}
\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow \\
\begin{array}{c}6\\ \uparrow \\ 1\\ \beta\text{-D-Gal}p\\ 6\\ \uparrow \\ 1\\ \beta\text{-D-Gal}p\end{array} & \begin{array}{c}6\\ \uparrow \\ 1\\ \beta\text{-D-Gal}p\\ 6\\ \uparrow \\ 1\\ \beta\text{-D-Gal}p\end{array} & \begin{array}{c}6\\ \uparrow \\ S\end{array} & \begin{array}{c}6\\ \uparrow \\ 1\\ \text{L-Ara}f\\ 3\\ \uparrow \\ 1\\ \text{L-Ara}p\end{array}
\end{array}
$$

S = side chains of β-D-Gal*p* or L-Ara*f*

10

The arabinogalactan studied contained arabinose and galactose in the molar ratio 1:4.9, which was determined by g.l.c. of the alditol acetates prepared after acid hydrolysis (data not reported). Reductive depolymerisation of methylated **10** and acetylation of the products gave (Fig. 3, Table I), from the 3,6-linked D-galactopyranosyl residues, 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-D-galactitol (peak 11), the expected 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-galactitol (peak 3) and 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-galactitol (peak 12) from the terminal and 6-linked D-galactopyranose residues, 1,4-anhydro-2,3,5-tri-*O*-methyl-

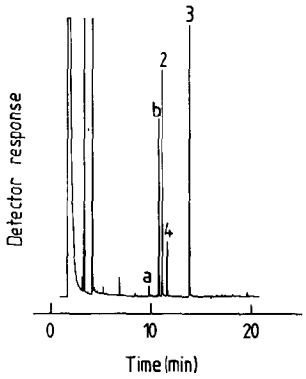


Fig. 1. G.l.c. (see Experimental) of the partially methylated anhydroalditol acetates derived by reductive depolymerisation of methylated 3-*O*-β-D-galactopyranosyl-D-arabinose with $Me_3SiOSO_2CF_3$ as catalyst. See text for the identification of peaks 2–4. Peaks a, b, and those eluted with retention time T <6 min are non-carbohydrates.

L-arabinitol (peak 13) and 1,5-anhydro-2,3,4-tri-*O*-methyl-L-arabinitol (peak 14) from the terminal arabinose residues, and 3-*O*-acetyl-1,4-anhydro-2,5-di-*O*-methyl-L-arabinitol (peak 2) from the 3-linked arabinofuranosyl residues; only a small proportion of 3-*O*-acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-galactitol (peak 15) was detected. The molar ratio of peaks 13 + 14 + **3** and peak 11 was ~1:1, suggesting that each galactose residue in the backbone was branched.

These results confirm the highly branched structure of the arabinogalactan and accord with the structure proposed[16,18] after standard methylation analysis. Reductive cleavage therefore is an effective method for establishing the structures of pentose-containing hemicelluloses from plant tissues.

The xylan from oat spelts is insoluble in water but soluble in dilute alkali. Xylans, which are the main non-cellulosic components of grasses, contain a

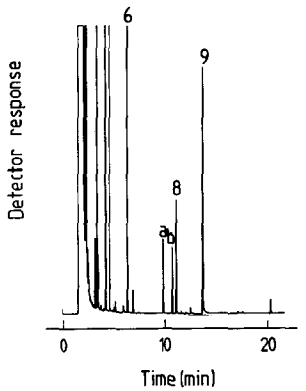


Fig. 2. G.l.c. (see Experimental) of the partially methylated anhydroalditol acetates derived by reductive depolymerisation of methylated xylobiose with $Me_3SiOSO_2CF_3$ as catalyst. See text and Fig. 1 for identification of the peaks.

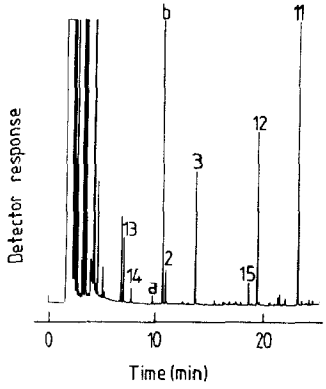
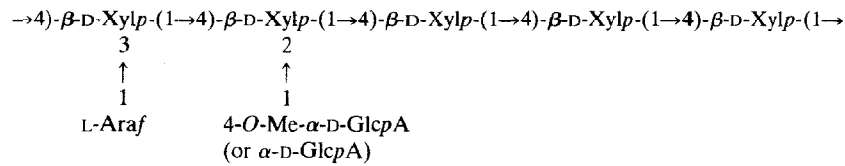


Fig. 3. G.l.c. (see Experimental) of the products derived by reductive depolymerisation of methylated arabinogalactan with $Me_3SiOSO_2CF_3$ as catalyst. See text and Fig. 1 for identification of the peaks.

backbone of (1→4)-linked β-D-xylopyranosyl residues[19–21] and the structure **16** has been proposed.



16

Reductive depolymerisation of the methylated xylan followed by acetylation gave (Fig. 4, Table I) 1,5-anhydro-2,3,4-tri-*O*-methyl-D-xylitol (peak 6) from the terminal xylopyranose residues, but the major products (combined yield, ~80%) were 4-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methyl-D-xylitol (peak 8) and its isomerisation product 5-*O*-acetyl-1,4-anhydro-2,3-di-*O*-methyl-D-xylitol (peak 9), derived from 4-linked xylopyranosyl residues. The ratio of peaks 8 and 9 depended on the reaction conditions (time, presence of water in the reaction mixture). Isomerisation of non-reducing 4-linked xylopyranosyl residues was caused by both catalysts. The branched units of the xylopyranosyl backbone gave 3,4-di-*O*-acetyl-1,5-anhydro-2-*O*-methyl-D-xylitol (peak 16) and 2,4-di-*O*-acetyl-1,5-anhydro-3-*O*-methyl-D-xylitol (peak 17) together with the respective isomerisation products 3,5-di-*O*-acetyl-1,4-anhydro-2-*O*-methyl-D-xylitol (peak 18) and 2,5-di-*O*-acetyl-1,4-anhydro-3-*O*-methyl-D-xylitol (peak 19). The terminal L-arabinose residues gave 1,4-anhydro-2,3,5-tri-*O*-methyl-L-arabinitol (peak 11), and the D-glucuronic acid residues gave methyl 1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucuronate (peak 20). The m.s. data were identical with those reported by Gray *et al.*[22]. 4-*O*-Acetyl-1,5-anhydro-2,3,6-tri-*O*-methyl-D-glucitol (peak 21) was also obtained, suggesting contamination by D-glucans.

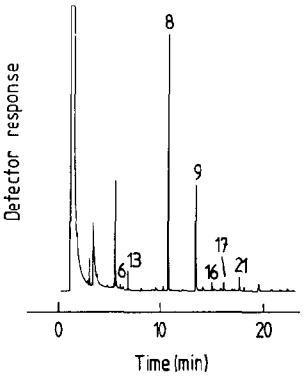


Fig. 4. G.l.c. (see Experimental) of the partially methylated anhydroalditol acetates derived by reductive depolymerisation of methylated xylan with Me_3SiOSO_2Me and $BF_3 \cdot Et_2O$ as catalysts. See text for identification of the peaks.

The results indicated the xylan to be a lightly branched arabinoglucuronoxylan. The amounts of terminal arabinose and peaks 16 + 18 corresponded with the amounts of glucuronic acid and peaks 17 + 19.

Mass spectra. — Partially methylated anhydropentitols are not commercially available and, in order to obtain reference standards, arabinose, xylose, and the disaccharides **1a** and **5a** were subjected to methylation, reductive cleavage, and

TABLE II

FRAGMENT IONS OF METHYLATED AND ACETYLATED ANHYDROPENTITOL DERIVATIVES OBTAINED BY E.I.-M.S. AT 70 eV

Acetylated 1,4- or 1,5-anhydro derivative	*Mol. wt.*[a]	m/z *(% of base peak)*
2,5-Me_2-Ara*f* (2)[b]	204	43(100), 58(16), 69(13), 71(5), 85(60), 87(8), 99(4), 113(10), 127(14), 159(28)
2,4-Me_2-Ara*p* (4)	204	43(100), 58(65), 71(20), 74(81), 75(34), 99(9), 101(15), 114(86)
2,3,4-Me_3-Xyl*p* (6)	176	58(100), 71(20), 73(21), 75(47), 88(35), 114(15), 117(7), 176(3)
2,3,5-Me_3-Xyl*f* (7)	176	58(31), 71(100), 73(30), 75(11), 99(26), 101(67), 131(37)
2,3-Me_2-Xyl*p* (8)	204	43(100), 58(66), 71(32), 74(40), 75(10), 88(10), 101(8), 112(10), 114(8), 131(3), 144(4), 145(4), 162(1)
2,3-Me_2-Xyl*f* (9)	204	43(100), 58(62), 71(83), 74(5), 75(6), 88(4), 101(17), 112(6), 114(17), 131(9), 144(10)
2,3,5-Me_3-Ara*f* (13)	176	58(35), 71(100), 73(39), 75(12), 99(29), 101(78), 102(15), 112(14), 131(48)
2,3,4-Me_3-Ara*p* (14)	176	58(100), 71(19), 73(23), 75(53), 88(42), 114(14),176(18)

[a]Determined by c.i. (ammonia)-m.s. [b]The numbers in parentheses (except 7) refer to peaks in the Figs. The text includes formulae for **2, 4**, and **6–9**.

acetylation. Table II shows the main fragment ions obtained by g.l.c.–e.i. m.s. The methylated 1,4-anhydropentitols (**7** and **13**) can be distinguished from the 1,5-anhydropentitols (**6** and **14**) by the primary cleavage of the exocyclic residue (→ *m/z* 131) and the secondary fragment ion at *m/z* 99, due to the loss of methanol. In the 1,5-anhydro derivatives, methanol is eliminated first from position 3 followed by loss of formaldehyde via retro-Diels–Alder cleavage. The same fragmentation pathway is exemplified for the 3-*O*-acetyl-1,4- and -1,5-anhydropentitol derivatives with fragment ions at *m/z* 159 and *m/z* 99 (**2**) and at *m/z* 144 (**4**), respectively. The 4-*O*-acetyl-1,5-anhydropentitol (**8**) and the 5-*O*-acetyl-1,4-anhydropentitol (**9**) each lose AcOH (→ *m/z* 144) then methanol (→ *m/z* 112). The fragment ion at *m/z* 131 from the loss of the exocyclic residue ($\cdot CH_2OAc$) of **9**, is also formed from **8**, probably due to the loss of a methoxy radical from *m/z* 162. However, the last fragment ion (*m/z* 162) occurs only in the mass spectra of **8**, resulting from the elimination of ketene.

Thus, the reductive cleavage method is very suitable for the elucidation of linkage positions in pentose-containing carbohydrates. Degradation products such as furfural, hydroxymethylfurfural, and C-fragments of the sugar units formed during acid hydrolysis were not detected after reductive cleavage.

REFERENCES

1 W. N. HAWORTH AND H. MACHEMER, *J. Chem. Soc.*, (1932) 2270–2277.
2 R. KUHN, H. TRISCHMANN, AND I. LÖW, *Angew. Chem.*, 67 (1955) 32.
3 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
4 J. FINNE, T. KRUSIUS, AND H. RAUVALA, *Carbohydr. Res.*, 80 (1980) 336–339.
5 I. CIUCANU AND F. KEREK, *Carbohydr. Res.*, 131 (1984) 209–217.
6 E. T. OAKLEY, D. F. MAGIN, G. H. BOKELMAN, AND W. S. RYAN, *J. Carbohydr. Chem.*, 4 (1985) 53–65.
7 P. J. HARRIS, R. J. HENRY, A. B. BLAKENEY, AND B. A. STONE, *Carbohydr. Res.*, 127 (1984) 59–73.
8 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–76.
9 T. F. SCHWEIZER AND P. WÜRSCH, *Nestle Research News*, (1984/85), 43–52.
10 D. ROLF AND G. R. GRAY, *J. Am. Chem. Soc.*, 104 (1982) 3539–3541.
11 J. U. BOWIE, P. V. TRESCONY, AND G. R. GRAY, *Carbohydr. Res.*, 125 (1984) 301–307.
12 D. ROLF, J. A. BENNEK, AND G. R. GRAY, *Carbohydr. Res.*, 137 (1985) 183–196.
13 J.-G. JUN AND G. R. GRAY, *Carbohydr. Res.*, 163 (1987) 247–261.
14 J. U. BOWIE, P. V. TRESCONY, AND G. R. GRAY, *Carbohydr. Res.*, 131 (1984) 17–28.
15 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.
16 G. A. ADAMS, *Can. J. Chem.*, 38 (1960) 280–293.
17 T. E. TIMELL, *Adv. Carbohydr. Chem.*, 20 (1965) 410–483.
18 C. SIMIONESCU, I. BYON SANG, AND A. CERNATESCU-ASANDEI, *Cellul. Chem. Technol.*, 10 (1976) 535–545.
19 G. O. ASPINALL AND R. C. CARPENTER, *Carbohydr. Polym.*, 4 (1984) 271–282.
20 K. C. B. WILKIE, *Adv. Carbohydr. Chem. Biochem.*, 36 (1979) 215–264.
21 R. R. SELVENDRAN, *J. Cell Sci. Suppl.*, 2 (1985) 51–88.
22 S. A. VODONIK AND G. R. GRAY, *Carbohydr. Res.*, 172 (1988) 255–266.