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Analysis of the linkage positions in polydextrose by the reductive cleavage method

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The structure of the synthetic carbohydrate polymer Polydextrose® was investigated by a reliable and rapid method of methylation, combined with reductive cleavage and acetylation. Monomeric derivatives were identified by gas–liquid chromatography (GLC) retention data and electron impact (EI)– and NH_3 chemical ionization (CI)–mass spectrometry (MS) data. Ratios of permethylated, once or twice acetylated derivatives confirmed that Polydextrose® is a highly branched material. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Polydextrose® (PD) is used in several countries as a low caloric sugar and fat substitute (bulking agent). It is prepared by condensation of glucose, glucitol and citric acid (89:10:1). The resulting condensation product has no chemically defined structure, but represents a mixture of polymerization products covering a molecular weight range from 150–20 000 (Table 1).

Although a hypothetical structure has already been proposed (Torres & Thomas, 1981; Murray, 1988; Moppett, 1991), we were interested to examine previously permethylated PD by the relatively new reductive cleavage method, which is useful for elucidation of the degree of branching, position of the linkages and the type of monomeric compounds involved in a polymeric carbohydrate. Reductive cleavage of the glycosidic carbon–oxygen bonds in carbohydrates occurs by an ionic hydrogenation mechanism and leads to the corresponding anhydroalditols (Rolf & Gray, 1984). In several studies, Gray and co-workers investigated fructans (Rolf & Gray, 1984), glucans (Rolf *et al.*, 1985; Rolf & Gray, 1986) and complex plant or microbial-derived polysaccharides (Vodonik & Gray, 1988*a*,*b*; Zeller & Gray, 1992). Because of disadvantages (Hanisch, 1994) in the Hakomori method (Hakomori, 1964), we carried out methylation by the procedure of Ciucanu and Kerek (1984). The combination of both methods was first described by Heims *et al.* (1989). As anhydroalditols are not commercially available, we have treated several mono-, di-, tri-, tetra- and polysaccharides which were methylated, reductively cleaved and acetylated to yield the required reference compounds.

EXPERIMENTAL

Materials and methods

Amylopectin, 1,6-anhydroglucose, D-fructose, glucitol, D-glucose, inulin, maltitol (4-*O*-α-D-glucopyranosyl-D-glucitol) and xylitol were purchased from Fluka (Buchs, Switzerland). Amylose, maltose (4-*O*-β-D-glucopyranosyl-D-glucopyranose), raffinose (*O*-α-D-galactopyranosyl-(1,6)-α-D-glucopyranosyl-β-D-fructofuranoside), sucrose (β-D-fructofuranosyl-α-D-glucopyranoside), trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), turanose and xylose were purchased from Merck (Darmstadt, Germany). Gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucopyranose, Janssen, Beerse, Belgium), melezitose (*O*-α-D-glucopyranosyl-(1,3)-*O*-β-D-fructofuranosyl-(2,1)-α-D-glucopyranoside, Serva, Heidelberg, Germany), β-sophorose (2-*O*-β-D-glucopyranosyl-D-glucopyranose, Roth, Karlsruhe, Germany) and stachyose (*O*-α-D-galactopyranosyl-(1,6)-*O*-α-D-galactopyranosyl-(1,6)-*O*-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside, EGA, Steinheim,Germany) came from the sources indicated. Leucrose (5-*O*-α-D-glucopyranosyl-D-fructopyranose), Palatinit (1-*O*-α-D-glucopyranosyl-D-mannitol and 6-*O*-α-D-glucopyranosyl-D-glucitol) and Palatinose (isomaltulose, 6-*O*-α-D-glucopyranosyl-D-fructopyranose) were gifts from Prof. Dr. Matissek, Bundesverband der Deutschen Süßwarenindustrie e.V., Köln, Germany. 'Polydextrose® improved' (Go2280-S285) was submitted by Pfizer Inc. (Sandwich, Kent, England).

Fractionation of PD

In order to yield fractions of PD free from monomeric residues such as glucose, glucitol and anhydroglucose,

which would distort the results of GLC–MS analyses for the polymeric material, ultrafiltration was applied. We collected five fractions of PD with molecular weights between 500–10 000 (cf. Table 1) using the ultrafiltration device Amicon 8400 and the membranes YC 05, YM 2, 5, 10, purchased from Amicon, Witten, Germany. Fractionation was confirmed by anion-exchange high performance chromatography with pulsed amperometric detection according to a method which we have described elsewhere (Stumm & Baltes, 1991, 1992; Stumm, 1994).

Permethylation, reductive cleavage and acetylation

All reference carbohydrates, PD and fractions 4 and 5 of PD ($M_r > 5000$) were methylated according to the general procedure of Ciucanu and Kerek (1984). The dried, permethylated samples were further prepared by reductive cleavage and acetylation according to Jun and Gray (1987). Deviating from the original procedure, reduction with triethylsilane, trimethylsilylmethanesulfonate and boron trifluoride etherate was stopped by the addition of methanol after 1–1.5 h since longer reaction times led in some cases to decomposition of anhydroalditol derivatives already formed. All extraction steps were performed in Lidex-Mixxor extraction flasks (Elias GmbH, Heidelberg, Germany). Dried residues were dissolved in 100 μl dichloromethane containing the internal standard 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose.

Gas–liquid chromatography

GLC analyses were performed in a Carlo Erba Fractovap series 4130 chromatograph equipped with flame-ionization detector and a SA-5 fused-silica capillary column (0.25 mm×30 m) coated with 5% diphenyl-and 95% dimethylpolysiloxan (Sigma-Aldrich).

Gas–liquid chromatography– mass spectrometry

GLC–MS analyses were carried out on a Finnigan MAT 9610 gas chromatograph. The capillary column mentioned above was directly coupled with a Finnigan 4500 mass spectrometer equipped with an INCOS-2100 data-system. Column effluents were analysed by electron impact mass spectrometry (EIMS) at 70 eV in order to prove the identity of the carbohydrate derivatives and by chemical ionization with ammonia as the reagent gas (NH_3-CIMS) which was useful for determination of the molecular weight of the eluted components. Since the latter spectra are of little comparative value they are not shown here.

Table 1. Molecular weight distribution of Polydextrose®[a] and distribution obtained by ultrafiltration

Molecular weight range of Polydextrose®	%	Molecular weight range by ultrafiltration	%
162–5000	88.7	0–500	8
5000–10 000	10.0	500–1000	28
10 000–16 000	1.2	1000–5000	42
16 000–18 000	0.1	5000–10 000	20
		> 10 000	2

[a]According to Torres and Thomas (1981), Murray (1988) and Moppett (1991).

RESULTS

GLC analysis of the mixture of products derived from PD by methylation, reductive cleavage and acetylation, revealed the presence of 11 major compounds (cf. Fig. 1I–XI). These compounds were classified according to their molecular weights which could easily be determined by means of the characteristic quasimolecular ions $[M+H]^+$ and $[M+NH_4]^+$ in the CI–mass spectra.

Compounds I and II were identified by the ions at *m/z* 221 and at *m/z* 238 as non-acetylated tetra-*O*-methyl anhydroalditols (M_r 220). Since the CIMS spectra of compounds III–VII consisted mainly of ions at *m/z* 249 and *m/z* 266, they were interpreted as being mono-acetylated tri-*O*-methyl anhydroalditols (M_r 248). Accordingly, the presence of ions at *m/z* 277 and *m/z* 294 in compounds VII–XI indicated twice acetylated di-*O*-methyl anhydroalditols (M_r 276). Two additional minor peaks were assigned as permethylated glucitol (XII, M_r 266) and monoacetylpenta-*O*-methylglucitol (XIII, M_r 294). The identified and postulated structures and their origin are summarized in Fig. 2 and Table 2.

Compound I was identified as 1,5-anhydrotetra-*O*-methylglucitol which originates from non-reducing terminal bonded glucopyranose units or, to a minor

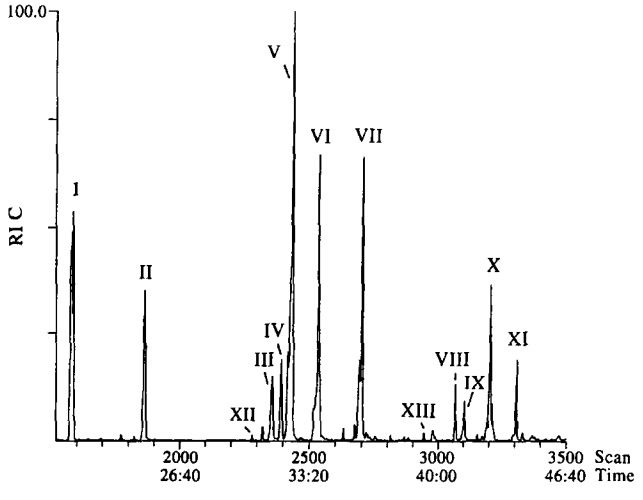


Fig. 1. GLC–MS chromatogram of reductively cleaved and derivatized Polydextrose® Chromatographic conditions: column, 30 m×0.25 mm internal diameter ×0.25 μm SA-5 capillary fused silica column; injection port temperature, 250°C; temperature programme oven, 100°C (3 min), 2°C min^{-1}, 250°C (10 min); injection mode, 1 μl split injection (10:1); carrier gas, helium.

extent, from free glucose which is contained in the original PD material. To judge the contribution of free glucose to the formation of I (and II, see below), fractions 4 and 5 of PD ($M_r > 5000$) which were almost free of unreacted adducts and compounds of lower molecular weight were investigated as well. From GLC analyses of these fractions it was evident that less than 15% of I are yielded from monomeric glucopyranose. The EI spectrum of I (Fig. 3) displayed fragment ions corresponding to (M—CH_2OH) at *m/z* 188 and (M—CH_2OMe) at *m/z* 175 and ions resulting from further losses of those ions (188—CH_2OMe) and (175—CH_2OH) at *m/z* 143. Fragment ions of lower *m/z* could arise from ring cleavage followed by different losses and rearrangements as described for permethylated monosaccharides (Heyns *et al.*, 1966; Radford & DeJongh, 1972).

Compound II could not be assigned by comparison of the retention times with those of authentic references. The peak area of this compound was about 50% of that of compound I. Actually, the EI spectrum of II (Fig. 4) differed distinctly from the well-known spectra of the expected 1,5-anhydrotetra-*O*-methylalditols, i.e. derivatives of glucose, mannose, galactose and of 2,5-anhydrohexitols, corresponding to fructose derivatives, all of which might be formed during the production process of PD.

The somewhat higher intensities of the complementary ions at *m/z* 131 and *m/z* 89 (M_r 220) gave hints that II might be a 1,4-anhydrotetra-*O*-methylalditol (cf. Scheme 1) which could arise from terminal (non-reducing) or free 1,4-glucofuranose. This proposed compound does not occur naturally in carbohydrates but might be formed during the synthesis of PD.

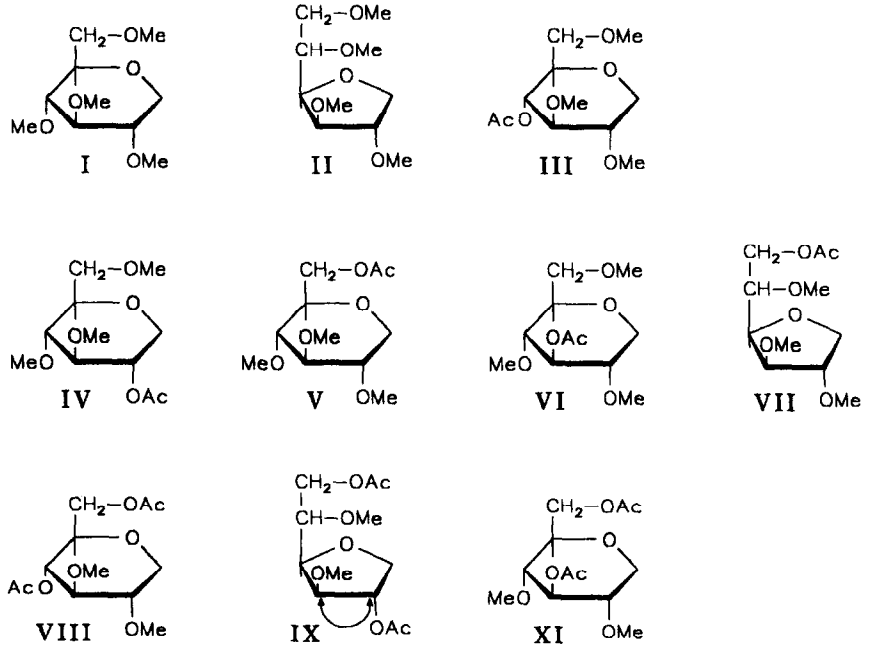


Fig. 2. Structures of the monomeric components of Polydextrose®, II, IX and X are postulated compounds.

Table 2. Identified and postulated (*) compounds and their positions in Polydextrose®

No.	Derivatives resulting from PD	Structure and linkage in PD
		Terminal (non-reducing) or free
I	1,5-Anhydro-2,3,4,6-tetra-*O*-methylglucitol	Glucopyranose
II*	1,4-Anhydro-2,3,5,6-tetra-*O*-methylhexitol	(Gluco-)furanose
		In the chain or terminal (reducing)
III	4-*O*-Acetyl-1,5-anhydro-2,3,6-tri-*O*-methylglucitol	1,4-Linked glucopyranose
IV	2-*O*-Acetyl-1,5-anhydro-3,4,6-tri-*O*-methylglucitol	1,2-Linked glucopyranose
V	6-*O*-Acetyl-1,5-anhydro-2,3,4-tri-*O*-methylglucitol	1,6-Linked glucopyranose
VI	3-*O*-Acetyl-1,5-anhydro-2,4,6-tri-*O*-methylglucitol	1,3-Linked glucopyranose
VII	6-*O*-Acetyl-1,4-anhydro-2,3,5-tri-*O*-methylglucitol	1,6-Linked (gluco)-furanose
		Branches
VIII	4,6-Di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methylglucitol	1,4,6-Linked glucopyranose
IX*	(2 or 3),6-Di-*O*-acetyl-1,4-anhydrodi-*O*-methylglucitol	1,(2 or 3),6-Linked (gluco)-furanose
X**	Unknown di-*O*-acetyl-di-*O*-methylanhydrohexitol	Compound not identified
XI	3,6-Di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methylglucitol	1,3,6-Linked glucopyranose

Compounds III–VI were identified as mono-*O*-acetyl-1,5-anhydrotri-*O*-methylglucitols (M_r 248) with the acetyl group in the 4- (compound III), 2- (IV), 6-(V) and 3- (VI) positions, respectively. These derivatives originate either from chain-glucopyranosyl residues linked in the respective manner or from monomeric anhydroglucose, as observed in the case of 1,6-anhydroglucose which gives compound V. EI spectra of compounds III–VI are reproduced in Figs 5–8.

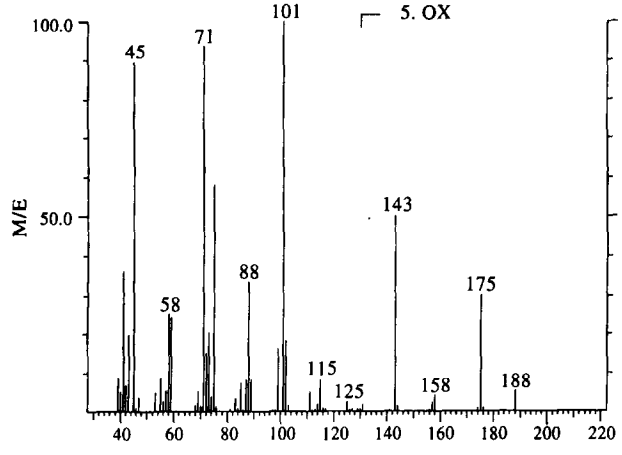


Fig. 3. EI–MS spectrum of I. For chromatographic conditions, see Fig. 1.

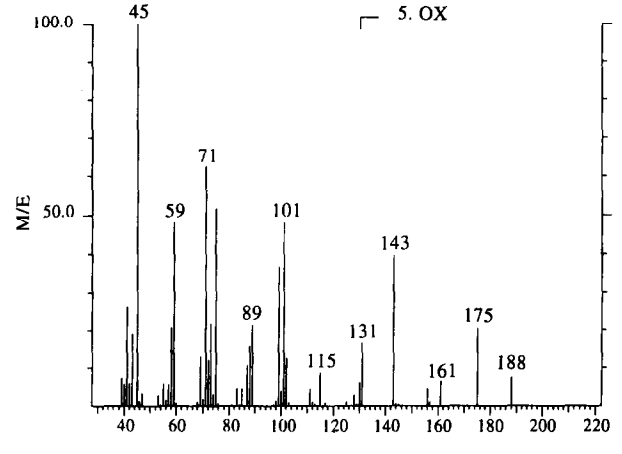
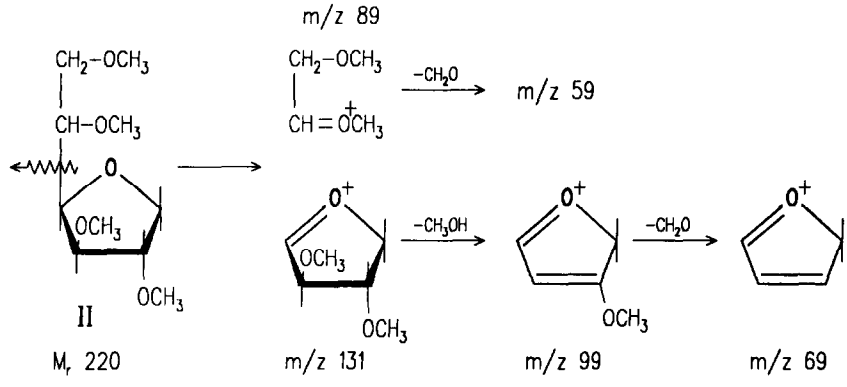


Fig. 4. EI–MS spectrum of II. For chromatographic conditions, see Fig. 1.

The structure of the fifth monoacetylated compound (VII) is postulated. The EI–MS spectrum (Fig. 9) contained a prominent ion at *m/z* 117 which did not occur with noticeable intensity in the spectra of known mono-*O*-acetyl-1,5-or-2,5-anhydrotri-*O*-methylalditols. With respect to this rarely observed ion and the corresponding ion at *m/z* 131 we proposed 6-*O*-acetyl-1,4-anhydro-2,3,5-tri-*O*-methylalditol with the ions mentioned resulting from the cleavage of the bond between the glucofuranose ring and the exocyclic substituent (cf. Scheme 2). The analogous compound with an acetoxy group in the 5-position provided a completely different spectrum (van Langenhove & Reinhold, 1985). Considering peak areas within these once acetylated tri-*O*-methylethers, the dominating compound was the 6-linked glucopyranose derivative (V, 30%) followed by the postulated 6-linked glucofuranose derivative (VII, 24%). Compounds IV and VI exhibited peak area proportions of 20% each, and the 4-linked glucopyranose derivative III had a peak area of 7%.

Among the four di-*O*-acetylanhydrodi-*O*-methylalditols (VIII–XI, M_r 276) which represent branches in the carbohydrate chain, only two compounds were identified, namely VIII as 4,6-di-*O*-acetyl-1,5-anhydro-2,3-di-*O*-methylglucitol (EI spectrum, see Fig. 10) and XI as 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methylglucitol (EI spectrum, see Fig. 13). Compound IX, like VII, exhibited the abusive intense ion at *m/z* 117 but not at *m/z* 131 (Fig. 11). Since a fragment ion at *m/z* 159 was present, we postulated another glucofuranose derivative with one acetoxy moiety at the ring and one at the exocylic substituent, i.e. (2 or 3),6-di-*O*-acetyl-1,4-anhydro-(3 or 2),5-di-*O*-methylalditol. Compound X was assigned by CI–MS as another di-*O*-acetyldi-*O*-methylanhydroalditol. Its molecular structure cannot be elucidated by EI–MS alone. The EI spectrum is shown in Fig. 12. The peak areas of these four diacetylated compounds decreased in the order X > XI > VIII > IX.



Scheme 1. Possible fragment ions of II with intact ring structures.

DISCUSSION

Methylation and reductive cleavage of Polydextrose® is a reliable and easy method to obtain information about the nature of monomeric residues, positions of the linkages, degree of branching and average chain length, repectively. Examination of peak area data from multiple sample analyses revealed differences in absolute peak areas and, furthermore, relative peak areas varied especially for compounds I and II. This is obviously due to the inhomogeneous sample material, since PD is a mixture of polymerization products, and to losses of analyte during the three step sample preparation. Therefore, we did not evaluate peak areas of each compound, but summarized and averaged corrected relative peak areas according to three groups: the non-acetylated compounds I and II, the once acetylated (III–VII) and the twice acetylated compounds (VIII–XI). Ratios of these three groups were calculated for Polydextrose® and for fractions 4 and 5 and are shown in Table 3.

These ratios clarify that PD and its higher molecular weight fractions are highly branched polymeric carbohydrates. A schematic model constructed on the basis of these data can be characterized by a main chain, branched at about every third glucose residue on average, with the branches consisting of two chain residues and one terminal residue. Since several similar models are conceivable a special model is not depicted here. Our results disagree so far with the published hypothetical structure (Torres & Thomas, 1981; Murray, 1988; Moppett, 1991), as we postulate a higher number of furanoic residues.

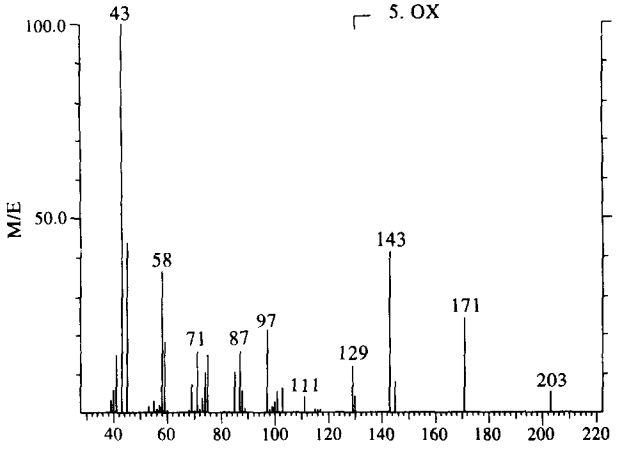


Fig. 5. EI–MS spectrum of III. For chromatographic conditions, see Fig. 1.

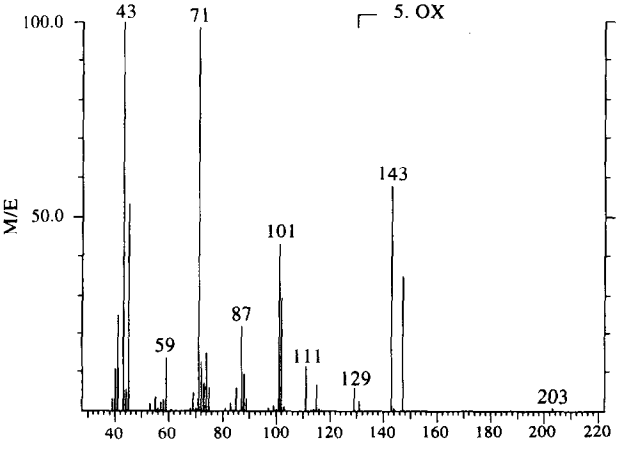


Fig. 6. EI–MS spectrum of IV. For chromatographic conditions, see Fig. 1.

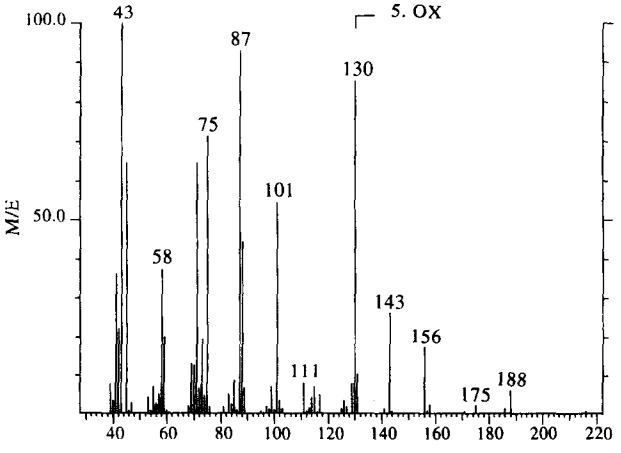


Fig. 7. EI–MS spectrum of V. For chromatographic conditions, see Fig. 1.

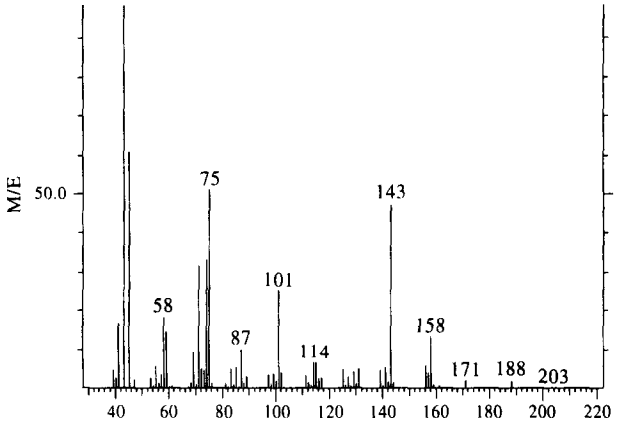


Fig. 8. EI–MS spectrum of VI. For chromatographic conditions, see Fig. 1.

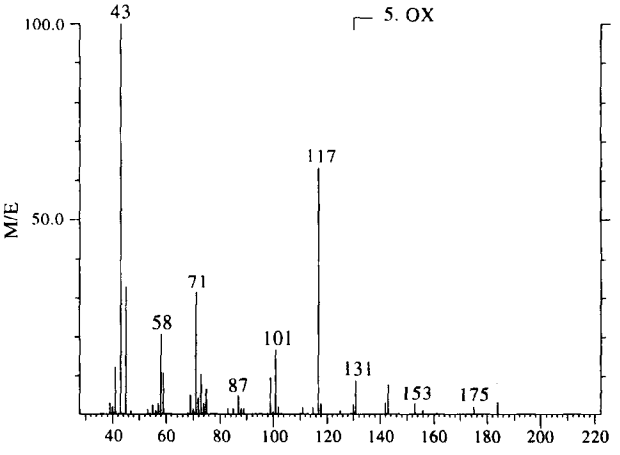
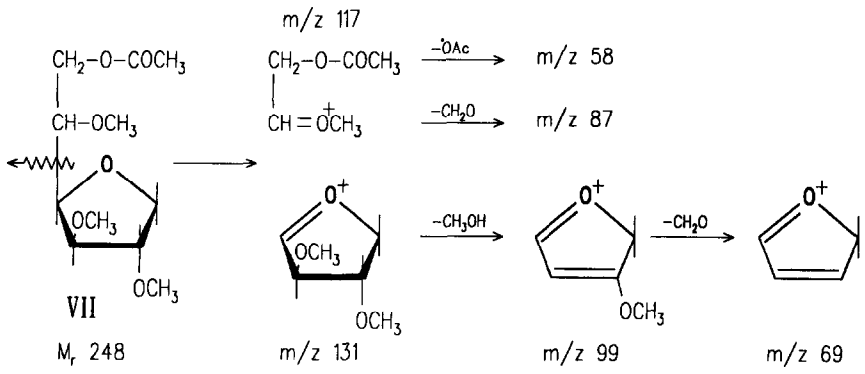


Fig. 9. EI–MS spectrum of VII. For chromatographic conditions, see Fig. 1.



Scheme 2. Possible fragment ions of VII.

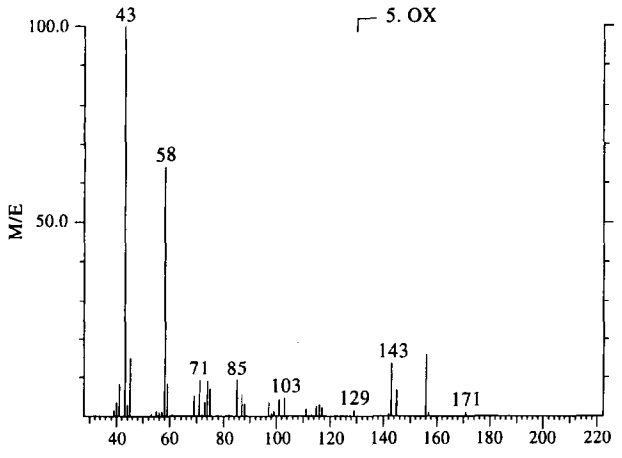


Fig. 10. EI–MS spectrum of VIII. For chromatographic conditions, see Fig. 1.

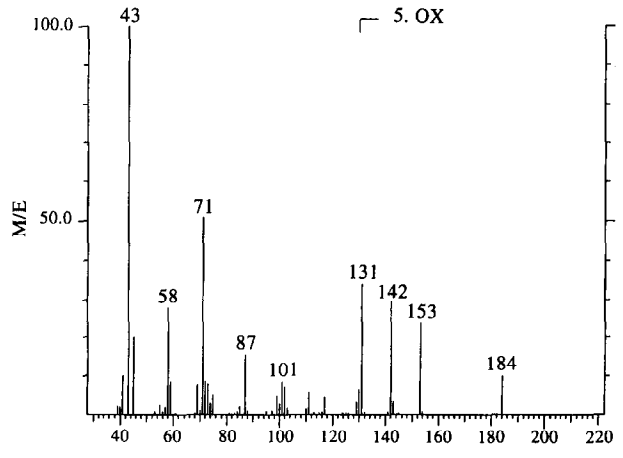


Fig. 12. EI–MS spectrum of X. For chromatographic conditions, see Fig. 1.

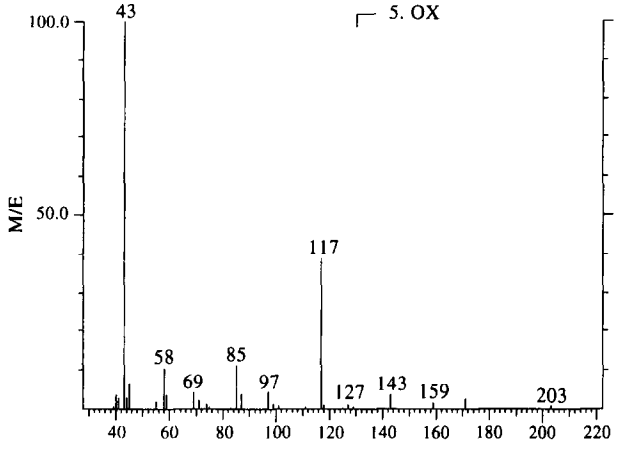


Fig. 11. EI–MS spectrum of IX. For chromatographic conditions, see Fig. 1.

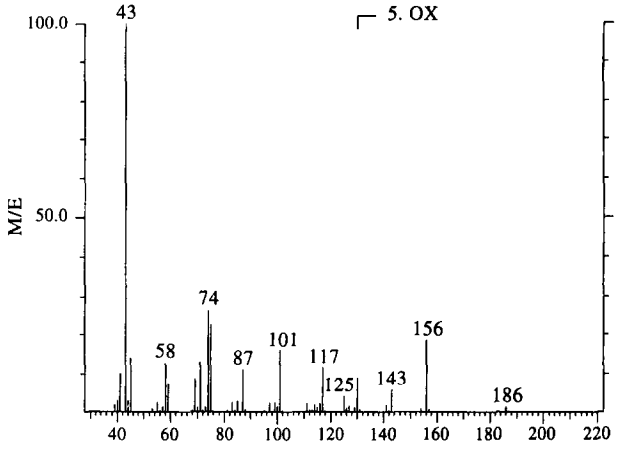


Fig. 13. EI–MS spectrum of XI. For chromatographic conditions, see Fig. 1.

Table 3. Ratios of acetylated anhydroalditol derivatives from Polydextrose®

Sample	Non-acetylated	Once acetylated	Twice acetylated
		O-Methylanhydroalditols	
Polydextrose® improved commercial product	1,3	2,7	1
Fraction 4 (M_r = 5000–10 000)	1,6	3,6	1
Fraction 5 (M_r > 10 000)	1,7	3,5	1

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