Journal of Chromatography, 179 (1979) 113–122

CHROM. 12,203

GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC IDENTIFICATION OF A RANGE OF METHYL ETHERS FROM L-GLYCERO-D-MANNOHEPTOSE AND D-GLYCERO-D-MANNOHEPTOSE

JOANNA RADZIEJEWSKA-LEBRECHT, DEREK H. SHAW*, DIETMAR BOROWIAK, INGE FROMME and HUBERT MAYER

Max-Planck-Institut für Immunobiologie, Stübeweg 51, D-78 Freiburg (G.F.R.)

(Received July 13th, 1979)

SUMMARY

A procedure is described using open tubular gas chromatography followed by mass spectrometry to distinguish between the acetylated alditols of 17 methyl ethers of L-glycero-D-mannoheptose and six methyl ethers of D-glycero-D-mannoheptose. The combined technique affords unequivocal identification of this range of heptose methyl ethers.

INTRODUCTION

Methylation analysis has proven an invaluable tool in the determination of linkage positions of individual sugar residues in polysaccharides, and the techniques of methylation followed by combined gas–liquid chromatography–mass spectrometry (GLC–MS) have been recorded in detail[1]. Adequate information is available for the acetylated alditols of a large number of methylated pentoses, deoxyhexoses and hexoses[2] using a variety of column packings for the analysis; somewhat less complete listings are available for methylated amino-sugar alditol acetates[3–6].

Reports on the chromatographic and mass spectrometric characteristics of methyl ethers of L-glycero-D-mannoheptose do exist in the literature but they are fragmentary, and deal with only one or two of the many possible methyl ethers of this heptose. The fully methylated ether of D-glycero-D-mannoheptose has been similarly described, but none of the multiplicity of partially methylated ethers of this heptose has been studied. In recent years, D-glycero-D-mannoheptose has increasingly been found as a major or significant part of the heptose portion of the lipopolysaccharide molecule in such diverse species as (for example) *Veillonella*[7], *Aeromonas*[8], *Fusobacterium*[9] and *Rhodopseudomonas*[10].

This paper examines 17 methyl ethers of L-glycero-D-mannoheptose and six methyl ethers of D-glycero-D-mannoheptose from the standpoint of their GLC retention times on both packed and open tubular columns, and their mass spectra.

* On leave from: Fisheries and Oceans Canada, Microbial Chemistry Section, P.O. Box 5638, St. John's, Canada.

MATERIALS AND METHODS

Source of heptose methyl ethers

Mixtures of O-methylated heptoses were isolated from lipopolysaccharides from the following genera: *Aeromonas*, *Rhodospirillum*, *Rhodopseudomonas*, *Proteus*, *Escherichia*, *Rhizobium* and *Coxiella*. Synthetic 2,3,6-tri-O-methyl-D-glycero-L-mannoheptose and synthetic 4,6,7-tri-O-methyl-D-glycero-L-mannoheptose[11] were obtained from Dr. Patricia Szabo (Paris, France). Some of the lipopolysaccharides contained only D-glycero-D-mannoheptose or L-glycero-D-mannoheptose thus leading to unequivocal assignment of the parent heptose.

Preparation of methyl ethers of heptose

As appropriate, lipopolysaccharides, O-polysaccharides or core oligo-saccharides were methylated, hydrolysed, reduced and acetylated by the method of Lindberg[1] to give mixtures of methyl ethers of the reduced sugar components of the starting saccharides. In order to distinguish between C-1 and C-7 of the original heptose, peracetylation was generally preceded by reduction with sodium borodeuteride rather than sodium borohydride[12]. Individual methyl ethers of the heptose were not separated from the mixtures prior to GLC.

Gas–liquid chromatography–mass spectrometry

GLC of the acetylated methyl alditols was performed either on a packed column of 3% ECNSS-M on 100–120 mesh Gas-Chrom Q (150 cm × 2 mm I.D.) operated isothermally in a Varian Model 1520B gas chromatograph at 155° with a nitrogen flow-rate of 40 ml/min, or on a glass wall-coated open-tubular (WCOT) column of CP-SIL-5 (Chrompack, Berlin, G.F.R.) with a film thickness of 0.2 μm and column dimensions of 25 m × 0.25 mm I.D. The column was operated at 190° with an injector split ratio of 50:1 and helium as the carrier gas. In both cases retention times are given relative to hexaacetylmannitol.

Mass spectrometry was performed on a Finnigan Model 3200E quadrupole gas chromatograph–mass spectrometer with jet separator and an ionizing voltage of 70 eV coupled to a Finnigan Model 6000 data system. The gas chromatograph was fitted with the open tubular column and operated under the same conditions as above. All mass spectra were normalized to *m/e* 43 as 100%.

RESULTS AND DISCUSSION

L-Glycero-D-mannoheptose has been recognized for many years as a ubiquitous constituent of a large number of gram-negative lipopolysaccharides[13]. In recent studies it has become obvious that the epimeric D-glycero-D-mannoheptose is more widespread than previously realised. Since its initial isolation and characterisation from *Chromobacterium violaceum*[14], it has been found either as the only heptose or, more often, in combination with L-glycero-D-mannoheptose. We have recently shown that D-glycero-D-mannoheptose can substitute for L-glycero-D-mannoheptose in some, but not all, positions in the R-core of *Rhodospirillum tenue*[15]. A similar situation may be present in *Proteus mirabilis* lipopolysaccharide[16]. In strains where only D-glycero-D-mannoheptose is present one occasionally finds that O-specific chains are present as haptens unattached to the R-type lipopolysaccharide[15].

The concomitant presence of both heptoses in R-lipopolysaccharide has posed problems of identification during methylation analysis of these core structures. We have resolved some of these problems by using open-tubular GLC–MS as an analytical tool.

Retention times (relative to hexaacetylmannitol) for the two column systems used are given in Table I. It should be noted that the relative retention times of the methyl ethers of L-glycero-D-mannoheptose are consistently higher than the values of the equivalent methyl ethers from D-glycero-D-mannoheptose, and that methyl ethers with the same degree of substitution are difficult to separate when the 7-position is methylated. This phenomenon also leads to considerable overlap between (for example) the tri-O-methylheptose ethers methylated at C-7, and the tetra-O-methyl ether which does not carry a methoxy group in this position. The use of open-tubular GLC does to a very large extent overcome this difficulty owing to the superior separation characteristics of both the technique and the liquid phase. Generally, the elution sequence is the same for both columns.

TABLE I

RETENTION TIMES OF PARTIALLY METHYLATED HEPTITOLS RELATIVE TO MANNITOL HEXAACETATE

Positions of methyl groups in parent heptose	*WCOT CP-SIL5*		*3% ECNSS-M*	
	LD-hept	*DD-hept*	*LD-hept*	*DD-hept*
2,3,4,6,7-	0.67	0.63	0.20	0.15
2,3,4,7-	0.81	—	—	—
2,3,6,7-	0.81	—	0.40	—
3,4,6,7-	0.83	0.78	0.39	0.30
2,4,6,7-	0.86	0.80	0.42	—
2,3,4,6-	0.96	0.87	0.60	0.41
2,6,7-	0.94	—	0.57	—
2,3,7-	0.97	—	0.65	—
4,6,7-	1.04	—	0.73	—
2,3,6-	1.16	—	1.08	—
2,4,6-	1.29	—	1.33	—
6,7-	1.12	1.04	0.80	—
2,7-	1.16	—	1.08	—
2,6-	1.36	—	1.59	—
3,6-	1.51	—	2.06	—
4,6-	1.67	1.55	2.19	—
3-	1.83	—	2.90	—

It will be obvious from Table I that reliance on relative retention time as a diagnostic tool for the identification of the heptose methyl ethers would be at best inconclusive in many cases. This makes it almost imperative that the definite identification be made by MS in combination with the relative retention times. The mass spectra of each of the methyl ethers shown in Table I are presented in Figs. 1–17; compounds reduced with sodium borohydride rather than sodium borodeuteride are indicated by an asterisk.

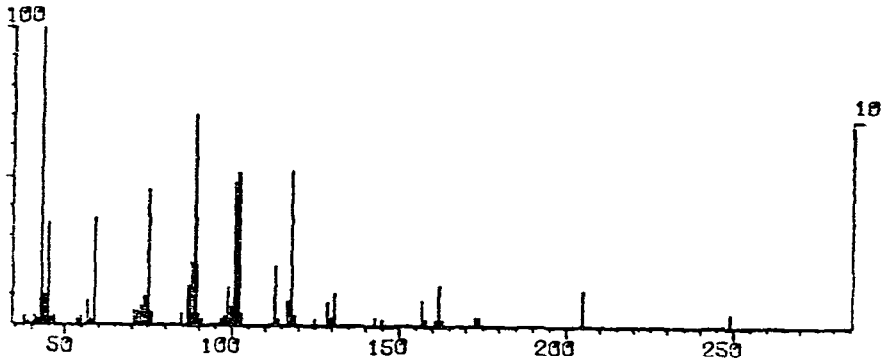


Fig. 1. Mass spectrum of alditol acetate of 2,3,4,6,7-penta-O-methylheptose.

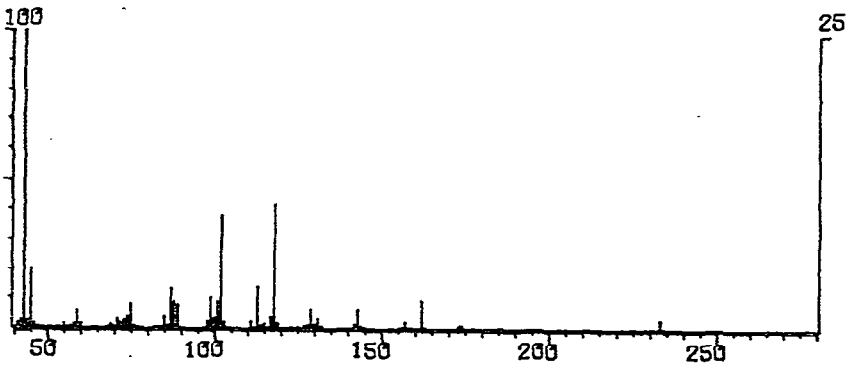


Fig. 2. Mass spectrum of alditol acetate of 2,3,4,7-tetra-O-methylheptose.

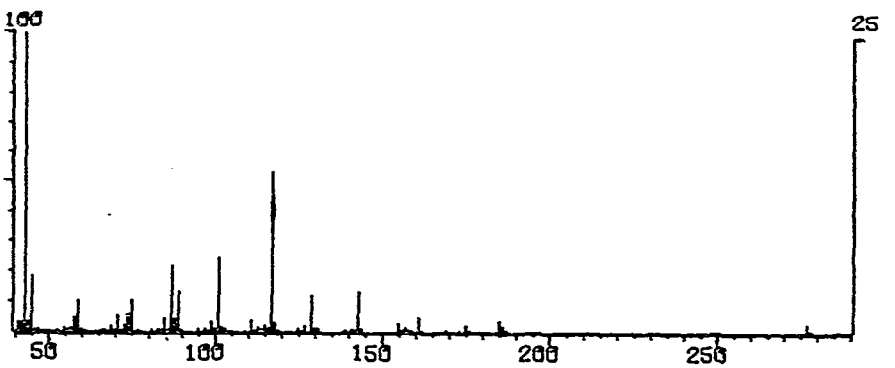


Fig. 3. Mass spectrum of alditol acetate of 2,3,6,7-tetra-O-methylheptose*.

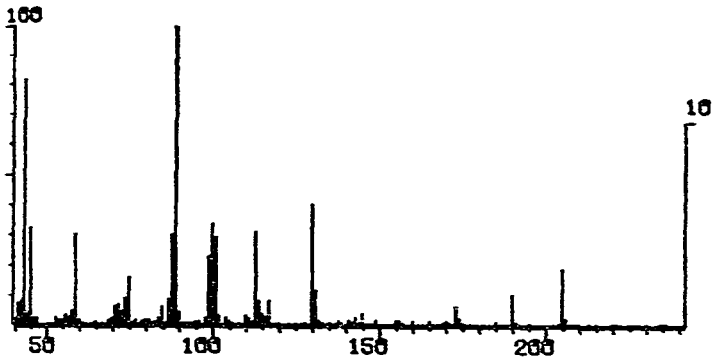


Fig. 4. Mass spectrum of alditol acetate of 3,4,6,7-tetra-O-methylheptose.

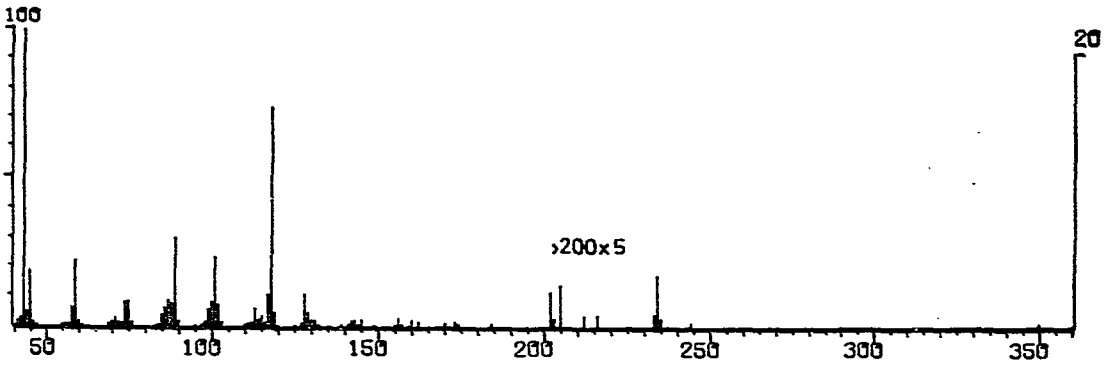


Fig. 5. Mass spectrum of alditol acetate of 2,4,6,7-tetra-O-methylheptose.

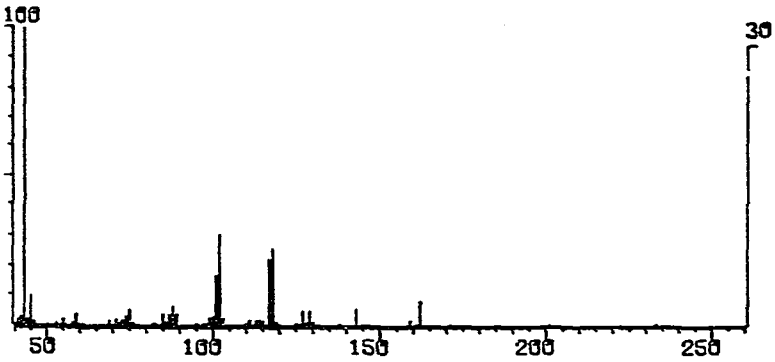


Fig. 6. Mass spectrum of alditol acetate of 2,3,4,6-tetra-O-methylheptose.

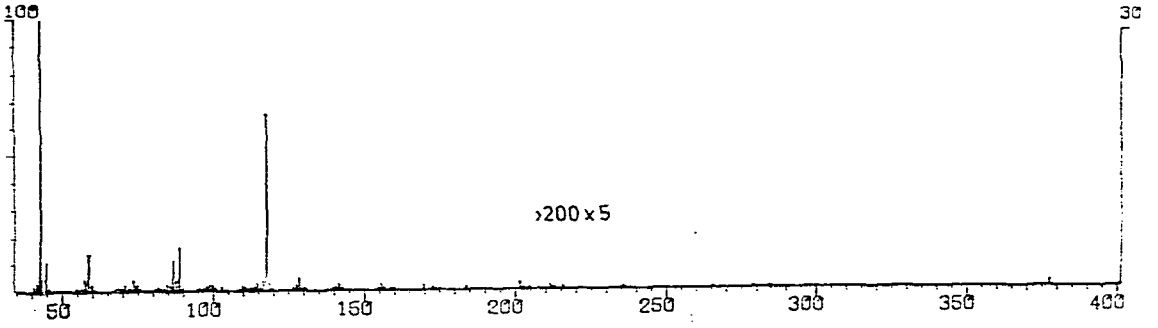


Fig. 7. Mass spectrum of alditol acetate of 2,6,7-tri-O-methylheptose.

Fig. 8. Mass spectrum of alditol acetate of 2,3,7-tri-O-methylheptose*.

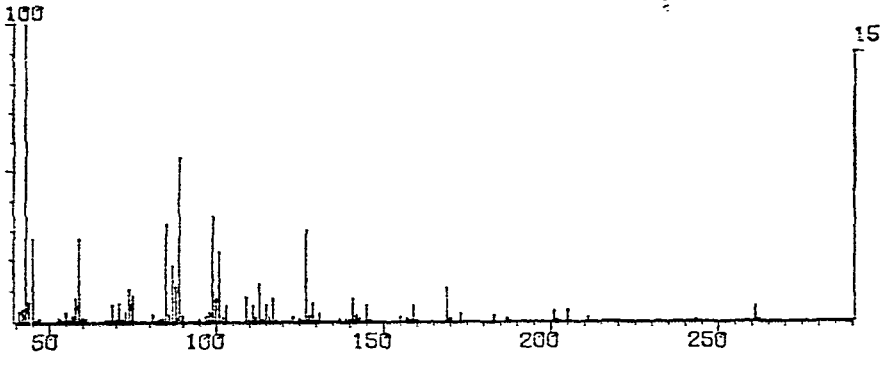


Fig. 9. Mass spectrum of alditol acetate of 4,6,7-tri-O-methylheptose*.

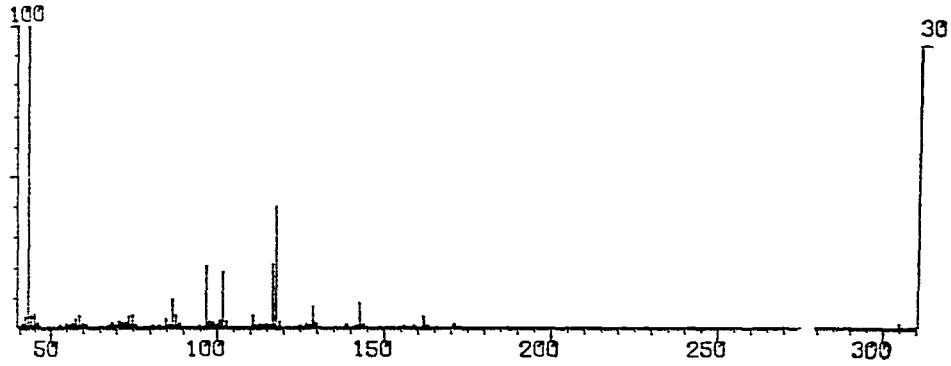


Fig. 10. Mass spectrum of alditol acetate of 2,3,6-tri-O-methylheptose.

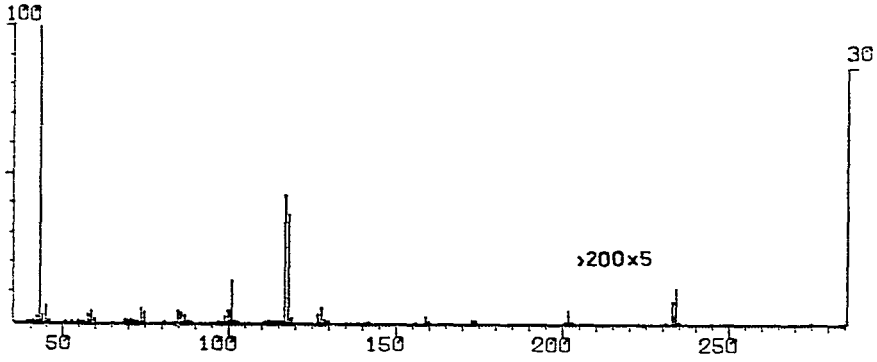
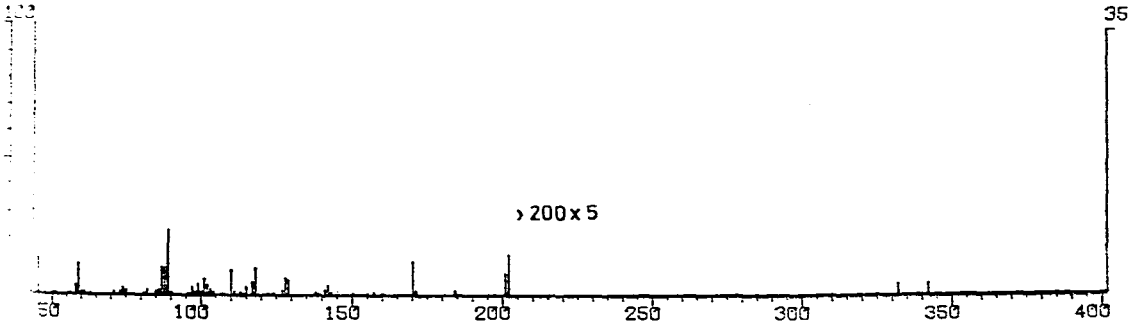


Fig. 11. Mass spectrum of alditol acetate of 2,4,6-tri-O-methylheptose.



g. 12. Mass spectrum of alditol acetate of 6,7-di-O-methylheptose.

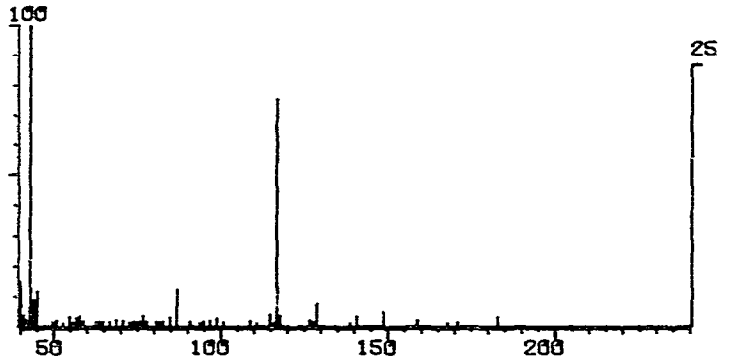


Fig. 13. Mass spectrum of alditol acetate of 2,7-di-O-methylheptose*.

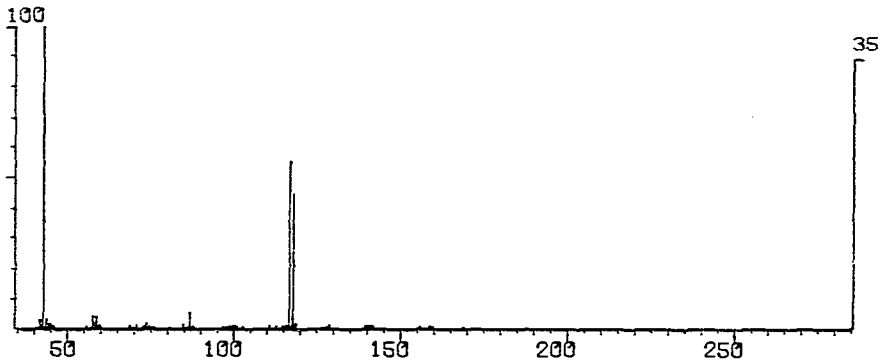


Fig. 14. Mass spectrum of alditol acetate of 2,6-di-O-methylheptose.

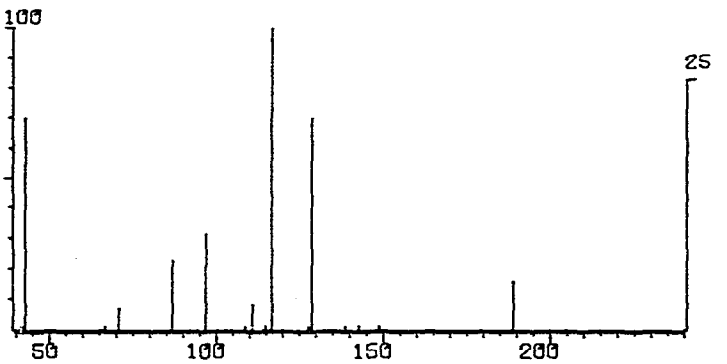


Fig. 15. Mass spectrum of alditol acetate of 3,6-di-O-methylheptose*.

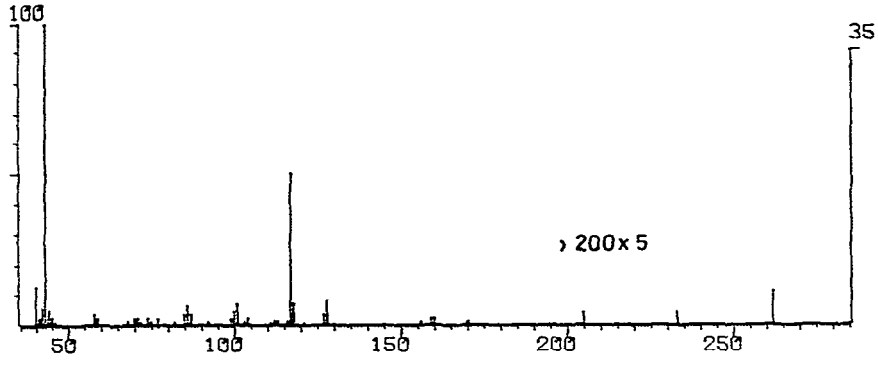


Fig. 16. Mass spectrum of alditol acetate of 4,6-di-O-methylheptose.

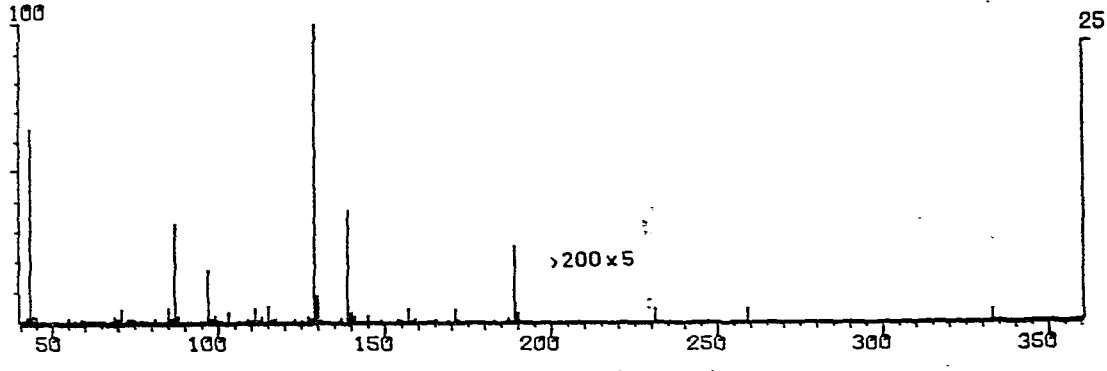


Fig. 17. Mass spectrum of alditol acetate of 3-O-methylheptose*.

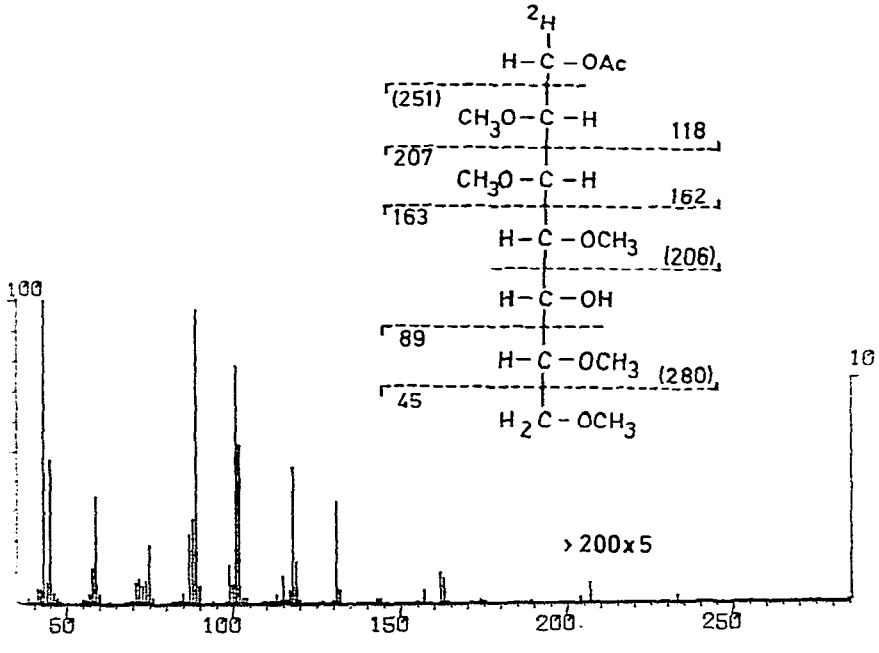


Fig. 18. Mass spectrum of 1-acetyl-5-hydroxy-2,3,4,6,7-penta-O-methyl-D-glycero-D-manno-heptitol.

We have noted that the 5-position on heptitols is sometimes not completely acetylated during the acetylation step with pyridine–acetic anhydride after reduction of the methylated heptoses with borohydride. The reason for this underacetylation is unclear, but during chromatography on ECNSS-M the underacetylated product is not usually resolved from the corresponding fully acetylated one. On the CP-SIL-5 column, however, it is differentiable and runs faster than the correct derivative. Such underacetylation can generally be diagnosed by the presence of the mass fragment *m/e* 163 in the mass spectra of the peaks as shown in Fig. 18.

ACKNOWLEDGEMENTS

The authors acknowledge gifts of 2,3,6- and 4,6,7-tri-O-methyl-D-glycero-L-mannoheptose from Dr. P. Szabo (Paris, France) of 3,6-di-O-methyl- and 3-O-methyl-L-glycero-D-mannoheptose from Dr. R. Russa (Lublin, Poland)and of the methyl ethers of lipopolysaccharides from *C.burneti* and *E.coli* from Dr. U. Feige (Münster, G.F.R.)

REFERENCES

1 B. Lindberg, *Methods Enzymol.*, 28 (1972) 178.
2 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg and J. Lönngren, *Chem. Commun., Univ. Stockholm*, 8 (1976) 75.
3 K. Stellner, H. Saito and S.-I. Hakamori, *Arch. Biochem. Biophys.*, 155 (1973) 464.
4 T. Tai, K. Yamashita and A. Kobata, *J. Biochem. (Japan)*, 78 (1975) 679.
5 S. Hase and E. Th. Rietschel, *Eur. J. Biochem.*, 63 (1976) 93.
6 J. Finne and H. Rauvala, *Carbohydr. Res.*, 58 (1977) 57.
7 T. Hofstad, *Acta Pathol. Microbiol. Scand., B*, 86 (1968) 47.
8 D. H. Shaw and H. J. Hodder, *Can. J. Microbiol.*, 24 (1978) 864.
9 G. Fredriksen and T. Hofstad, *Acta Pathol. Microbiol. Scand., B*, 86 (1978) 41.
10 J. Weckesser, H. Mayer, G. Drews and I. Fromme, *J. Bacteriol.*, 123 (1975) 449.
11 P. Szabo, *Carbohydr. Res.*, 59 (1977) 580.
12 B. Fournet, Y. Leroy, J. Montreuil and H. Mayer, *J. Chromatogr.*, 92 (1974) 185.
13 O. Lüderitz, K. Jann and R. Wheat, *Comp. Biochem.*, 26A (1968) 105.
14 D. A. L. Davies, *Nature*, 180 (1957) 1129.
15 J. Weckesser, G. Drews and H. Mayer, *Annu. Rev. Microbiol.*, 33 (1979) 215.
16 K. Kotelko, W. Gromska, M. Papierz, Z. Sidorzyk, O. Krajewska and K. Szer, *J. Hyg. Epidemiol. Microbiol. Immunol. (Praha)*, 21 (1977) 271.