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Note

Identification and quantification of methyl ester groups in methylated sugar acids and phosphates by g.l.c.–m.s. after alkaline transesterification with sodium ethoxide*

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The analysis of sugars by g.l.c.–m.s. is most often performed on the acetylated, trimethylsilylated, and methylated alditol derivatives[1]. For the derivatisation of sugars containing such negatively charged groups as carboxyl or phosphate, methylation is the most convenient method since stable methyl esters are formed which can be transesterified with ethanolic sodium ethoxide to the corresponding ethyl esters. G.l.c.–m.s. of the methyl and ethyl ester derivatives can then give information on the number of negatively charged groups. Transesterification, which has not been applied systematically hitherto to the identification of sugar acids or phosphates, is now reported.

Carbonyl-reduction of KDO followed by methylation gave the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers of methyl 3-deoxy-2,4,5,6,7,8-hexa-*O*-methyl-octonate (**1**). G.l.c. of the mixture gave two peaks with retention times of 6.64 and 6.82 min. On c.i.(ammonia)-m.s., each peak gave rise to an ion at *m/z* 356 for $[M + 18]^+$ indicating a molecular weight of 338, and the e.i. mass spectra were identical. After transesterification, the two peaks (**2**) had higher retention times (7.94 and 8.04 min), and the molecular weight was increased by 14 mass units. In the e.i. mass spectrum of **2**, all fragments containing the alkoxycarbonyl group were shifted by 14 mass units, whereas those comprising the C-5/8 moiety (*m/z* 177 and 145) were unchanged (Table I). Thus, the two peaks represent ethyl 3-deoxy-2,4,5,6,7,8-hexa-*O*-methyl-D-*glycero*-D-*talo*/*galacto*-octonate (**2**).

The same procedure was applied to D-glucose 6-phosphate, to give compound **3**, which had a retention time of 9.93 min in g.l.c. After transesterification with sodium ethoxide, three peaks were observed with retention times of 9.93, 10.93, and 11.85 min, respectively. C.i.- and e.i.-m.s. showed that the first peak represented **3**, whereas the two other peaks were identified as the ethyl methyl and diethyl derivatives **4** and **5**, respectively. Raising the temperature during trans-

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TABLE I

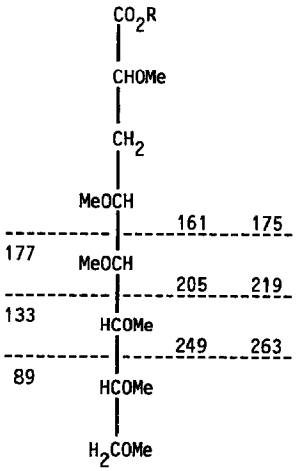
G.L.C.–M.S. OF METHYLATED KDO METHYL AND ETHYL ESTER DERIVATIVES[a]

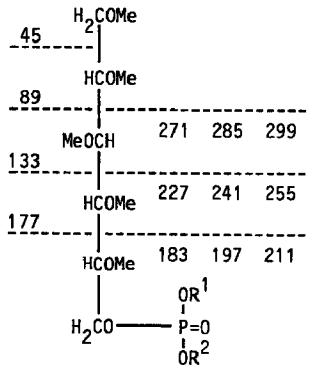
Compound	*Retention time (min)*	*Mol. wt.*[b]	*Base peak* (m/z)	*Characteristic fragment ions* (m/z) *and relative intensities*				
1	6.64/6.82[c]	338	129 (100)	145, (38)[d]	161, (15)	173, (21)	177, (8)	205 (8)
2	7.94/8.04	352	143 (100)	145, (41)	175, (26)	177, (9)	187, (24)	219 (10)

[a]For conditions, see Experimental. [b]Determined by c.i.(ammonia)-m.s. on the basis of peaks at *m/z* for $(M + 1)^+$ and $(M + 18)^+$. [c]Due to the non-stereospecific carbonyl-reduction, the compounds are mixtures of the D-*glycero*-D-*galacto* and D-*glycero*-D-*talo* isomers. [d]Intensity relative to that of the base peak.

esterification resulted in an increased yield of **5**, which was quantitative after reaction at 85° for 30 min. The e.i. mass spectra of compounds **3**–**5** are shown in Fig. 1. Fragment ions derived from the C-1/2, C-1/3, and C-1/4 moieties at *m/z* 89, 133, and 177, respectively, were present in each spectrum, whereas those containing the phosphoryl group were shifted by 14 mass units in **4**, and by 28 mass units in **5**.

The procedure described is useful for the investigation of unknown samples because it allows the determination of the number of methyl ester groups in a methylated sample and was used in the identification[2] of a new sugar acid, 3-deoxy-





1 R = Me

2 R = Et

3 R^1 = Me, R^2 = Me

4 R^1 = Me, R^2 = Et

5 R^1 = Et, R^2 = Et

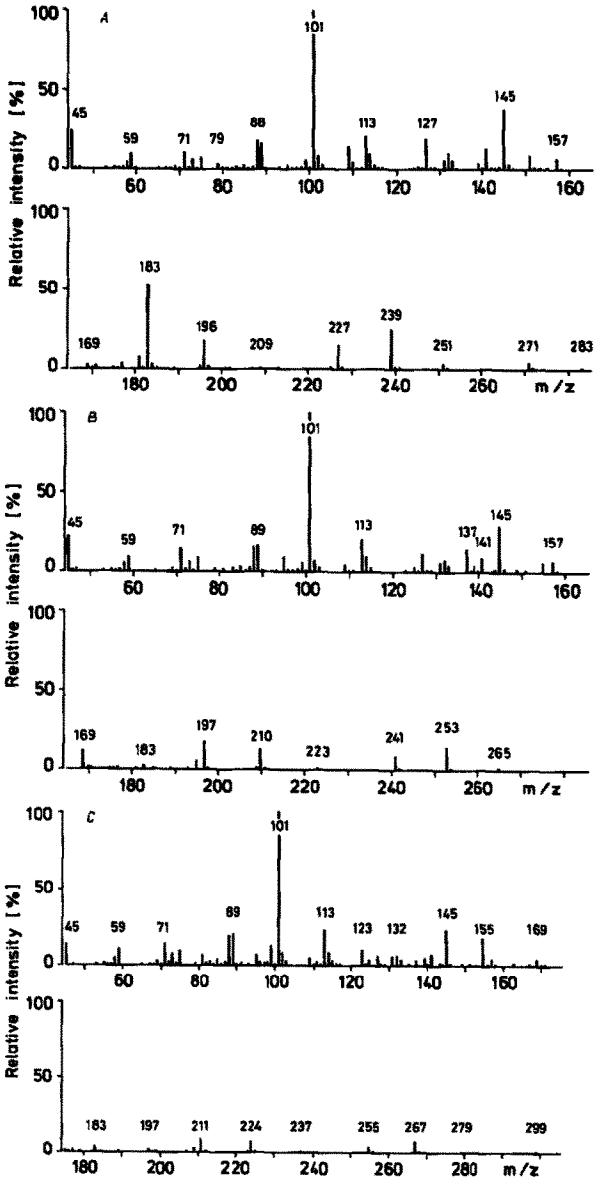


Fig. 1. E.i. mass spectra (70 eV) of *A* dimethyl (**3**), *B* ethyl methyl (**4**), and *C* diethyl (**5**) derivatives of 1,2,3,4,5-penta-*O*-methyl-D-glucitol 6-phosphate.

2-heptulosaric acid, isolated from the lipopolysaccharide of *Acinetobacter calcoaceticus*. When the method was applied to the methyl esters of long chain (C_{10}–C_{17}) hydroxylated and non-hydroxylated fatty acids, quantitative transesterification was achieved at 37° for 30 min (data not shown). The technique is simple and rapid and proved to be useful in separating accidentally co-migrating contaminants from a compound to be analysed.

EXPERIMENTAL

KDO and D-glucose 6-phosphate were reduced and methylated according to a modified[3] Hakomori procedure, and details of the g.l.c.–m.s. procedure have been described elsewhere[4]. Sodium ethoxide was prepared by adding sodium (1.5 g) to freshly distilled ethanol (100 mL) at room temperature. After 2 h, the sodium ethoxide solution was diluted to 0.5M with ethanol. Each sample to be transesterified was dried in a screw-capped, Teflon-lined tube, ethanolic sodium ethoxide (0.5 mL) was added, the mixture was kept at 37° for 30 min and then cooled (ice-bath), chloroform (2 mL) and water (8 mL) were added, and the solution was acidified to ~pH 4–5 with acetic acid. The chloroform layer was washed with water (5 × 8 mL), dried (Na_2SO_4), and concentrated to dryness, and the residue was subjected to g.l.c.–m.s. in parallel with the original samples, G.l.c. was performed on a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54 and a temperature programme of 150° for 5 min and then 5°/min→250°.

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REFERENCES

1 J. LÖNNGREN AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.
2 H. BRADE AND E. T. RIETSCHEL, *Eur. J. Biochem.*, 153 (1985) 249–254.
3 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
4 A. TACKEN, H. BRADE, F. M. UNGER, AND D. CHARON, *Carbohydr. Res.*, 149 (1986) 263–277.