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Note

G.l.c.–m.s. of methylated derivatives of 3-deoxy-2-ketoaldonic acid phosphates*

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3-Deoxy-2-ketoaldonic acids are naturally occurring carbohydrates, among which 3-deoxy-D-*manno*-octulosonic acid (KDO) is frequently found in bacterial lipopolysaccharides[1] and some capsular polysaccharides of *Escherichia coli*[2,3] and *Neisseria meningitidis*[4]. It has also been detected in certain plant cell walls[5]. The 8-phosphate is a biosynthetic precursor of KDO[6], the 5-phosphate is a constituent of the LPS from *Bordetella pertussis*[7] and from *Vibrio cholerae*[8], and a glycosidically linked phosphate is present in capsular polysaccharides of *E. coli*[9] where a KDO phosphate forms the link between the polysaccharide and the lipid moiety *via* a phosphodiester bond. The analytical procedures available for detecting these compounds in complex biopolymers are complicated by the polyfunctional character of KDO[10], and the presence of phosphoryl groups increases the possibility of artefacts due to elimination reactions or phosphate migration. We now report on the conversion of authentic 3-deoxy-2-ketoaldonic acid phosphates into the methylated derivatives suitable for g.l.c.–m.s.

3-Deoxy-D-*manno*-octulosonic acid 5-phosphate[11] (**1**), 3-deoxy-D-*gluco*-octulosonic acid 5-phosphate[11] (**2**), 3-deoxy-D-*manno*-octulosonic acid 8-phosphate[12] (**3**), 3-deoxy-D-*gluco*-octulosonic acid 8-phosphate[12] (**4**), methyl (methyl 3-deoxy-α-D-*manno*-octulopyranosid)onate 4-phosphate[13] (**5**), methyl (methyl 3-deoxy-α-D-*manno*-octulopyranosid)onate 5-phosphate[13] (**7**), and 3-deoxy-D-*arabino*-heptulosonic acid 4-phosphate[14] (**9**) were synthesised as described in the

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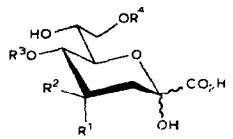
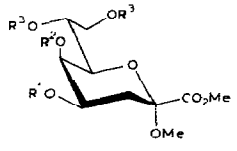
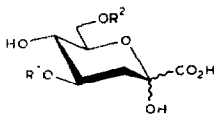
literature. 3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphate[15] (**10**) was a gift of Dr. D. B. Sprinson.



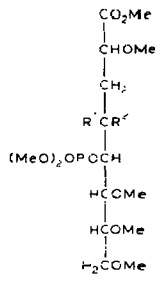
1 R^1 = H, R^2 = OH, R^3 = $PO(OH)_2$, R^4 = H
2 R^1 = OH, R^2 = H, R^3 = $PO(OH)_2$, R^4 = H
3 R^1 = H, R^2 = OH, R^3 = H, R^4 = $PO(OH)_2$
4 R^1 = OH, R^2 = H, R^3 = H, R^4 = $PO(OH)_2$



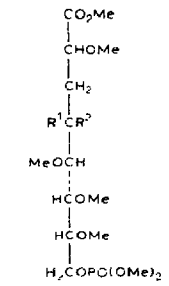
5 R^1 = $PO(OH)_2$, R^2 = H, R^3 = H
6 R^1 = $PO(OMe)_2$, R^2 = Me, R^3 = Me
7 R^1 = H, R^2 = $PO(OH)_2$, R^3 = H
8 R^1 = Me, R^2 = $PO(OMe)_2$, R^3 = Me



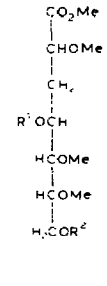
9 R^1 = $PO(OH)_2$, R^2 = H
10 R^1 = H, R^2 = $PO(CH)_2$



11 R^1 = OMe, R^2 = H
12 R^1 = H, R^2 = OMe



13 R^1 = OMe, R^2 = H
14 R^1 = H, R^2 = OMe



15 R^1 = $PO(OMe)_2$, R^2 = Me
16 R^1 = Me, R^2 = $PO(OMe)_2$

TABLE I

G.L.C. AND C.I.-M.S. DATA FOR METHYLATED DERIVATIVES OF 3-DEOXY-2-KETOALDONIC ACID PHOSPHATES

Compound	*Systematic name*	*Mol. wt.*[a]	*Retention time*[b]
12	Methyl 3-deoxy-2,4,6,7,8-penta-*O*-methyl-D-*glycero*-D-*gulo*/*ido*-octonate 5-(dimethyl phosphate)	432	2.72/2.76
11	Methyl 3-deoxy-2,4,6,7,8-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octonate 5-(dimethyl phosphate)	432	2.68/2.70
14	Methyl 3-deoxy-2,4,5,6,7-penta-*O*-methyl-D-*glycero*-D-*gulo*/*ido*-octonate 8-(dimethyl phosphate)	432	3.13/3.18
13	Methyl 3-deoxy-2,4,5,6,7-penta-*O*-methyl-D-*glycero*-D-*galacto*/*talo*-octonate 8-(dimethyl phosphate)	432	3.03/3.06
6	[Methyl (methyl 3-deoxy-5,7,8-tri-*O*-methyl-α-D-*manno*-octulopyranosid)onate] 4-(dimethyl phosphate)	416	3.12
8	[Methyl (methyl 3-deoxy-4,7,8-tri-*O*-methyl-α-D-*manno*-octulopyranosid)onate] 5-(dimethyl phosphate)	416	2.74
15	Methyl 3-deoxy-2,5,6,7-tetra-*O*-methyl-D-*gluco*/*manno*-heptonate 4-(dimethyl phosphate)	388	1.95/1.99
16	Methyl 3-deoxy-2,4,5,6-tetra-*O*-methyl-D-*gluco*/*manno*-heptonate 7-(dimethyl phosphate)	388	2.28/2.39

[a]Determined by c.i. (ammonia)-m.s. on the basis of peaks at *m/z* for $(M + 1)^+$ and $(M + 18)^+$. [b]Using a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54, a temperature programme of 140° for 3 min and then 3°/min → 220°, and H_2 as carrier gas (1.0 bar); relative to that of methyl (methyl 3-deoxy-4,5,7,8-tetra-*O*-methyl-α-D-*manno*-octulopyranosid)onate (8.39 min/1.0).

A solution of each sugar (2 mg) in water (1 mL) was treated with AG 50W-X8 (H^+) resin (Bio-Rad), and **1–4**, **9**, and **10** were reduced with sodium borohydride (20 mg) and then, after conventional removal of the boric acid, methylated by a modified[16] Hakomori[17] procedure to yield **11–16**, respectively. Compounds **5** and **7** gave **6** and **8**, respectively. The methylated derivatives were investigated by g.l.c. and g.l.c.–m.s., the details of which have been described[17]. The g.l.c. retention times are listed in Table I. Each of the pairs of D-*glycero*-D-*gulo*/*ido* and D-*glycero*-D-*galacto*/*talo* diastereoisomers derived by carbonyl-reduction of the KDO phosphates and the D-*gluco* analogues could be resolved by g.l.c. Compounds (**13, 14,** and **16**) with a phosphoryl substituent at a primary hydroxyl group were eluted later than those (**11, 12,** and **15**) carrying the substituent at a secondary hydroxyl group.

Molecular weights were determined by c.i. (ammonia)-m.s. on the basis of peaks for $(M + 1)^+$ and $(M + NH_4)^+$, of which the latter were always more intense. The intensities of characteristic fragment ions obtained in e.i.-m.s. are listed in Table II. Compounds with an *O*-phosphoryl group on a primary carbon atom (C-8 in **13** and **14**, and C-7 in **16**) gave fragment ions at *m/z* 129, 161, 173, and 205, representing the C-1/4 and C-1/5 moieties and their corresponding sub-

TABLE II

CHARACTERISTIC FRAGMENT IONS OF METHYLATED DERIVATIVES OF 3-DEOXY-2-KETOALDONIC ACID PHOSPHATES AFTER G.L.C.–M.S. (E.I., 70 eV)

Compound	m/z *(% of base peak)*
11	45 (13.8), 71 (14.3), 75 (45.4), 89 (6.1), 101 (69.7), 109 (11), 127 (19.8), 129 (100), 139 (4.7), 161 (17.8), 183 (18.5), 195 (22), 343 (3.1), 387 (0.6)
13	45 (8.3), 71 (15.4), 75 (31.5), 89 (3.6), 101 (100), 109 (14.9), 127 (9.6), 129 (61.4), 139 (2.2), 141 (13.2), 161 (9.7), 173 (9.1), 183 (18.9), 196 (2.2), 205 (2.6), 227 (3.9), 239 (7.9), 271 (1.2)
6	45 (28.1), 69 (10.1), 71 (16.4), 75 (51.2), 89 (32.3), 101 (100), 109 (44.4), 127 (76.7), 129 (47), 141 (50.8), 167 (25.6), 172 (67.9), 182 (51.4), 199 (12.7), 213 (7.1), 231 (50.4), 320 (4)
8	45 (91.6), 69 (66.4), 71 (10.1), 75 (65.7), 89 (10.9), 101 (16.5), 109 (68.6), 127 (100), 129 (7.3), 141 (47.4), 167 (33), 172 (3.9), 182 (37.8), 195 (52.7), 199 (55.6), 213 (83.2), 231 (1.1), 279 (26.7), 325 (21.2), 371 (22.8)
15	45 (40.9), 71 (97.7), 75 (51.1), 89 (45.5), 101 (83), 109 (47.7), 127 (100), 142 (64.8), 153 (27.3), 171 (29.5), 185 (48.9), 239 (11.4), 299 (34.1), 343 (2.3)
16	45 (11.2), 71 (51.4), 75 (41.1), 89 (13), 101 (86.5), 109 (34.6), 127 (31.9), 129 (100), 139 (20), 161 (8.6), 173 (7), 183 (19.5), 196 (20.5), 205 (0.8), 227 (2.7)

fragments after loss of methanol. In compound **13**, fragments of the C-1/6 moiety (*m/z* 217 and 249) were also observed. The KDO 5-phosphate **11** gave a strong fragment ion at *m/z* 161 and 129 (base peak), resulting from fission of the C-4–C-5 bond followed by loss of methanol. However, in the 4-phosphate **15**, this fission did not occur, which accords with the rule, proposed[16] for the fragmentation of methylated derivatives of 3-deoxyoctonates and 3-deoxyoctitols, that fission of this bond is significant only when there is a methoxyl group at position 4. In both groups of compounds, higher fragments containing the methylated phosphoryl group were observed: for example, the ions at *m/z* 299 and 343 (C-1/5 and C-1/6 moieties) for **16**, and *m/z* 343 and 387 (C-1/6 and C-1/7 moieties) for **11**.

The general fragmentation rules[18,19] for e.i.-m.s. of methylated sugar glycosides were also obeyed by the cyclic derivatives **6** and **8**, but the intensity of individual fragments was influenced by the position of the phosphoryl group (Table II).

The methylated derivatives described herein are useful reference compounds for the methylation analysis of complex carbohydrates containing 3-deoxy-2-keto-aldonic acid phosphates.

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