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G.L.C.–M.S. OF PARTIALLY METHYLATED AND ACETYLATED DERIVATIVES OF 3-DEOXY-2-KETOALDONIC ACIDS AND 3-DEOXY-ALDITOLS*

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ABSTRACT

Partially methylated and acetylated 3-deoxyoctitols and 3-deoxyheptitols were prepared from synthetic derivatives of 3-deoxy-D-*manno*-octulosonic acid and 3-deoxy-D-*arabino*-heptulosonic acid, respectively, by derivatisation procedures used in methylation analysis (hydrolysis, carbonyl- and carboxyl-reduction, methylation, and acetylation). The compounds so obtained were characterised by g.l.c. and g.l.c.–m.s., and their retention times and fragmentation patterns are reported.

INTRODUCTION

We have reported[1] g.l.c.–m.s. data for several partially methylated and acetylated derivatives of 3-deoxyoctitols which were prepared from synthetic and natural derivatives of 3-deoxy-D-*manno*-octulosonic acid (KDO). Based on these reference compounds, a modified methylation analysis was developed which enabled the substitution pattern of KDO in bacterial lipopolysaccharide to be determined[2].

This work has been extended to another series of 3-deoxyoctitol derivatives and also to those of 3-deoxyheptitol. The latter compounds were investigated since the substitution pattern of KDO in bacterial lipopolysaccharides[3] and capsular polysaccharides[4] leads, after periodate oxidation, to the formation of 3-deoxyheptulosonic acid, yielding 3-deoxyheptitol derivatives upon methylation analysis.

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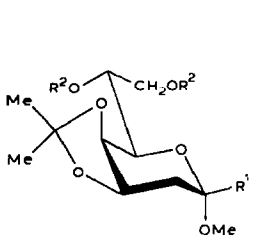
In addition, the lipopolysaccharide of *Acinetobacter calcoaceticus*[5] has been found to contain a 3-deoxy-2-ketoheptaric acid which, after reduction of both carboxyl groups, also gave 3-deoxyheptitol derivatives.

EXPERIMENTAL

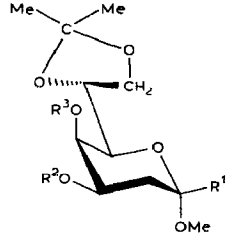
Reference compounds. — Methyl (methyl 7,8-di-*O*-acetyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-octulopyranosid)onate[6] (**1**), methyl (methyl 4-*O*-benzoyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-octulopyranosid)onate[6] (**7**), methyl 3-deoxy-4,5:7,8-di-*O*-isopropylidene-α-D-*manno*-octulopyranoside[6] (**13**), ammonium 3-deoxy-D-*manno*-octulosonate[7] (**16**), ammonium 3-deoxy-5-*O*-methyl-D-*manno*-octulosonate[8] (**23**), methyl (methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-octulopyranosid)onate[9] (**27**), ammonium 3-deoxy-D-*arabino*-heptulosonate[10] (**30**), methyl 3-deoxy-D-*arabino*-heptulopyranoside[11] (**38**), ammonium 3-deoxy-4-*O*-methyl-D-*arabino*-heptulosonate[12] (**43**) were synthesised as described in the literature.

Methylation analysis. — Details of the methods for acid hydrolysis, carboxyl- and carbonyl-reductions, methylation, acetylation, g.l.c., and g.l.c.–m.s. have been described[1]. Isopropylidene groups were cleaved by hydrolysis with 0.03M acetic acid (100°, 1 h). Carbonyl-reductions gave mixtures of the D-*glycero*-D-*talo*- and D-*glycero*-D-*galacto* isomers from D-*manno* derivatives of KDO, and of the D-*gluco* and D-*manno* isomers from derivatives of 3-deoxy-D-*arabino*-heptulosonic acid.

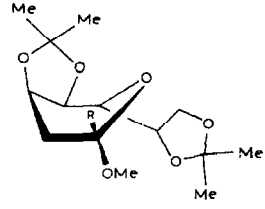
Derivatives of 3-deoxy-D-manno-*octulosonic acid (KDO).* — Compound **1** was methylated to give **2** which, after carboxyl-reduction with $NaBH_4$, yielded **3**. Aliquots of **3** were methylated, to yield **4**. After acid hydrolysis of **3** and **4**, carbonyl-reduction, and acetylation, 1,2,4,5,6-penta-*O*-acetyl-3-deoxy-7,8-di-*O*-methyloctitol (**5**) and 2,4,5,6-tetra-*O*-acetyl-3-deoxy-1,7,8-tri-*O*-methyloctitol (**6**) were obtained.



1 $R^1 = CO_2Me, R^2 = Ac$
2 $R^1 = CO_2Me, R^2 = Me$
3 $R^1 = H_2COH, R^2 = Me$
4 $R^1 = H_2COMe, R^2 = Me$



7 $R^1 = CO_2Me, R^2 = Bzl, R^3 = H$
8 $R^1 = CO_2Me, R^2 = Me, R^3 = Me$
9 $R^1 = H_2COH, R^2 = Me, R^3 = Me$
10 $R^1 = H_2COMe, R^2 = Me, R^3 = Me$

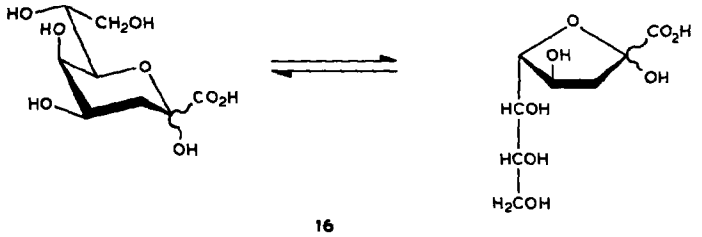


13 R = H_2COH
14 R = H_2COMe

Methylation of **7** gave **8**, carboxyl-reduction of which yielded **9**. Methylation then gave **10** which, together with **9**, was subjected in sequence to hydrolysis,

carbonyl-reduction, and acetylation, to give 2,6,7,8-tetra-*O*-acetyl-3-deoxy-1,4,5-tri-*O*-methyloctitol (**12**) and 1,2,6,7,8-penta-*O*-acetyl-3-deoxy-4,5-di-*O*-methyloctitol (**11**). Hydrolysis of **14**, obtained by methylation of **13**, gave 2,4,5,6,7,8-hexa-*O*-acetyl-3-deoxy-1-*O*-methyloctitol (**15**).

The mixture (**16**) of pyranose and furanose forms was methylated to give the corresponding derivatives **17a, 17b, 19a**, and **19b**. Compounds **18** and **20**, obtained from **17** and **19**, respectively, by carboxyl-reduction, were subjected to hydrolysis, carbonyl-reduction, and acetylation, yielding 1,2,6-tri-*O*-acetyl-3-deoxy-4,5,7,8-tetra-*O*-methyloctitol (**21**) and 1,2,5-tri-*O*-acetyl-3-deoxy-4,6,7,8-tetra-*O*-methyloctitol (**22**).



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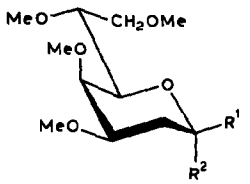
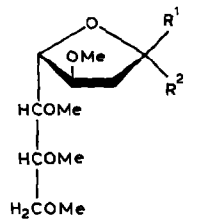


17a $R^1 = CO_2Me, R^2 = OMe$
17b $R^1 = OMe, R^2 = CO_2Me$
18a $R^1 = H_2COH, R^2 = OMe$
18b $R^1 = OMe, R^2 = H_2COH$

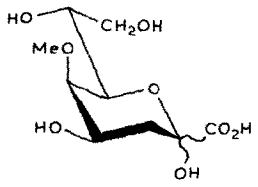


19a $R^1 = CO_2Me, R^2 = OMe$
19b $R^1 = OMe, R^2 = CO_2Me$
20a $R^1 = H_2COH, R^2 = OMe$
20b $R^1 = OMe, R^2 = CH_2OH$

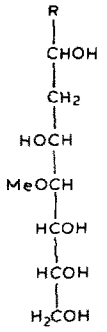
Compound **23** was carbonyl-reduced and the product was esterified with diazomethane to give **24**. Reduction of the methyl ester **25** and then acetylation gave 1,2,4,6,7,8-hexa-*O*-acetyl-3-deoxy-5-*O*-methyloctitol (**26**).

Carboxyl-reduction of **27** yielded **28** after acetylation, and **29** after methylation.

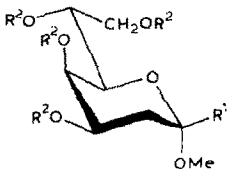
Derivatives of 3-deoxy-D-arabino-*heptulosonic acid.* — Carbonyl-reduction of **30** followed by methylation, carboxyl-reduction, and acetylation gave 1-*O*-acetyl-3-deoxy-2,4,5,6,7-penta-*O*-methylheptitol (**31**). Methylation of **30** gave the α,β-pyranosides **32** and the α,β-furanosides **34**. Methylation analysis, as described above, yielded 1,2,6-tri-*O*-acetyl-3-deoxy-4,5,7-tri-*O*-methylheptitol (**36**) and 1,2,5-tri-*O*-acetyl-3-deoxy-4,6,7-tri-*O*-methylheptitol (**37**).



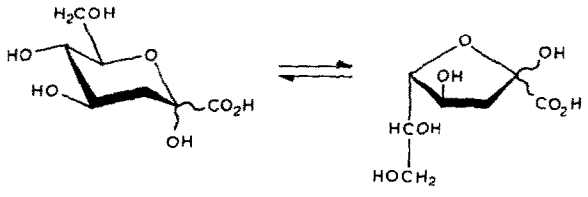
23



24 R = CO_2Me
25 R = H_2COH



27 R^1 = CO_2Me, R^2 = Ac
28 R^1 = H_2COAc, R^2 = Ac
29 R^1 = H_2COMe, R^2 = Me



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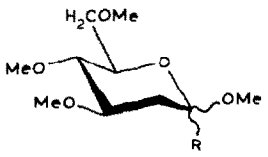
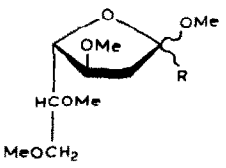
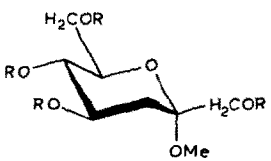


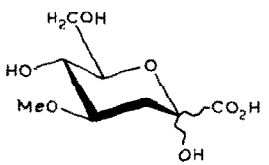
32 R = CO_2Me
33 R = H_2COH



34 R = CO_2Me
35 R = H_2COH



38 R = H
39 R = Me



43

Hydrolysis of **38** followed by reduction and acetylation or methylation gave **40** and **41**, respectively. Hydrolysis of **39**, obtained from **38** by methylation, followed by reduction and acetylation yielded 2,6-di-*O*-acetyl-3-deoxy-1,4,5,7-tetra-*O*-methylheptitol (**42**).

Carbonyl-reduction of **43** and then esterification with diazomethane gave **44**. Reduction of the methyl ester **45** and then acetylation afforded 1,2,5,6,7-penta-*O*-acetyl-3-deoxy-4-*O*-methylheptitol (**37**).

RESULTS AND DISCUSSION

Synthetic derivatives of 3-deoxy-D-*manno*-octulosonic acid (KDO) and 3-deoxy-D-*arabino*-heptulosonic acid were subjected to methylation analysis to give defined, partially methylated and acetylated derivatives of 3-deoxyoctitol and 3-deoxyheptitol, respectively. These derivatives were analysed by g.l.c. and g.l.c.–m.s. The g.l.c. retention times are listed in Table I. Each product was obtained as a mixture of D-*glycero*-D-*talo* and D-*glycero*-D-*galacto* derivatives (derived from KDO) or as the D-*gluco* and D-*manno* isomers (derived from 3-deoxy-D-*arabino*-heptulosonic acid). Most of these pairs of diastereomers were resolved by g.l.c., but **5, 6, 31, 36, 37**, and **40** each gave only a single peak. The molecular weights of the derivatives were determined by c.i. (ammonia)-m.s. on the basis of peaks for $[M + 1]^+$ and $[M + NH_4]^+$, of which the latter had the higher intensity (spectra not shown).

The characteristic fragment ions obtained in e.i.-m.s. are listed in Tables II and III. The rules proposed[1] for the fragmentation of partially methylated and acetylated 3-deoxyoctitol derivatives were obeyed. A fragment corresponding to the C-1/2 moiety was observed only in those compounds carrying a methoxyl group at position 2, and the appearance of this fragment ion was not influenced by the

TABLE I

G.L.C. AND C.I.-M.S. DATA FOR METHYLATED AND ACETYLATED DERIVATIVES OF 3-DEOXYHEPTITOLS AND 3-DEOXYOCTITOLS

Compound	*Systematic name*[a]	*Mol. wt.*[b]	*Retention time*[c]
21	1,2,6-Tri-*O*-acetyl-3-deoxy-4,5,7,8-tetra-*O*-methyloctitol	408	1.96/1.97
22	1,2,5-Tri-*O*-acetyl-3-deoxy-4,6,7,8-tetra-*O*-methyloctitol	408	1.90/1.93
6	2,4,5,6-Tetra-*O*-acetyl-3-deoxy-1,7,8-tri-*O*-methyloctitol	436	2.02
12	2,6,7,8-Tetra-*O*-acetyl-3-deoxy-1,4,5,tri-*O*-methyloctitol	436	2.23/2.26
5	1,2,4,5,6-Penta-*O*-acetyl-3-deoxy-7,8-di-*O*-methyloctitol	464	2.44
11	1,2,6,7,8-Penta-*O*-acetyl-3-deoxy-4,5-di-*O*-methyloctitol	464	2.66/2.68
15	2,4,5,6,7,8-Hexa-*O*-acetyl-3-deoxy-1-*O*-methyloctitol	492	2.66/2.68
25	1,2,4,6,7,8-Hexa-*O*-acetyl-3-deoxy-5-*O*-methyloctitol	492	2.84/2.88
41	3-Deoxy-1,2,4,5,6,7-hexa-*O*-methylheptitol	280	0.40/0.43
31	1-*O*-Acetyl-3-deoxy-2,4,5,6,7-penta-*O*-methylheptitol	308	0.74
42	2,6-Di-*O*-acetyl-3-deoxy-1,4,5,7-tetra-*O*-methylheptitol	336	0.98/1.00
37	1,2,5-Tri-*O*-acetyl-3-deoxy-4,6,7-tri-*O*-methylheptitol	364	1.31
36	1,2,6-Tri-*O*-acetyl-3-deoxy-4,5,7-tri-*O*-methylheptitol	364	1.40
46	1,2,5,6,7-Penta-*O*-acetyl-3-deoxy-4-*O*-methylheptitol	420	2.11/2.12
40	1,2,4,5,6,7-Hexa-*O*-acetyl-3-deoxyheptitol	448	2.33

[a]All octitol derivatives were mixtures of the D-*glycero*-D-*galacto*/*talo* isomers, and the heptitol derivatives were mixtures of the D-*gluco* and D-*manno* isomers. [b]Determined by c.i. (ammonia)-m.s. on the basis of peaks at *m/z* for $(M + 1)^+$ and $(M + 18)^+$. [c]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).

substituent at position 1 (MeO or AcO). The general rule[13,14] for the fragmentation of partially methylated and acetylated alditols, *i.e.*, fission between vicinal methoxyl groups is more significant than between vicinal acetoxyl and methoxyl groups, was obeyed by 3-deoxyalditols. However, a fragment comprising the C-1/4 moiety was also observed when the substituents at positions 4 and 5 were MeO and AcO, respectively (*e.g.*, **22** and **46**).

Cyclic derivatives of 3-deoxyoctulose and 3-deoxyoctulosonic acid were also investigated. The g.l.c. retention times are listed in Table IV. Since the α- and

TABLE II

CHARACTERISTIC FRAGMENT IONS OF PARTIALLY METHYLATED AND ACETYLATED DERIVATIVES OF 3-DEOXYOCTITOLS AFTER G.L.C.–M.S. (E.I., 70 eV)

Compound	*Base peak* (m/z)	*Primary fragment ions* (m/z) *of the moieties*								*Characteristic and abundant daughter ions* (m/z)[a]
		C-1/4	*C-1/5*	*C-1/6*	*C-1/7*	*C-2/8*	*C-5/8*	*C-6/8*	*C-7/8*	
21	101	203	247	N.d.[b]	363	335	205	N.d.	89	113, 143, 173
22	143	203	275	319	363	N.d.	N.d.	133	89	101, 143
6	89	203	N.d.	N.d.	391	N.d.	N.d.	N.d.	89	113, 129, 143, 155, 169, 183, 229, 317
12	115	175	N.d.	N.d.	N.d.	N.d.	261	N.d.	N.d.	127, 231
5	183	231	N.d.	N.d.	419	391	233	N.d.	89	123, 129, 169, 201, 225, 243, 273, 345
11	143	203	N.d.	N.d.	N.d.	N.d.	261	N.d.	145	113, 127, 173
15	123	203	N.d.	N.d.	419	N.d.	289	217	N.d.	115, 143, 157, 165, 225
25	201	231	N.d.	N.d.	419	419	261	N.d.	N.d.	85, 99, 113, 127, 129, 155, 159

[a]A maximum of eight fragment ions with an intensity of >10% of that of the base peak is listed. [b]N.d. = Primary fragment ion was not detected.

TABLE III

CHARACTERISTIC FRAGMENT IONS OF PARTIALLY METHYLATED AND ACETYLATED DERIVATIVES OF 3-DEOXYHEPTITOLS AFTER G.L.C.–M.S. (E.I., 70 eV)

Compound	*Base peak* (m/z)	*Primary fragment ions* (m/z) *of the moieties*							*Characteristic and abundant daughter ions* (m/z)[a]
		C-1/2	*C-1/4*	*C-1/5*	*C-1/6*	*C-2/7*	*C-5/7*	*C-6/7*	
41	89	89	147	191	235	235	N.d.[b]	89	101, 115, 127, 171, 203
31	117	117	175	219	N.d.	N.d.	N.d.	89	69, 101, 143, 203
42	115	N.d.	175	219	N.d.	N.d.	161	N.d.	115, 129
37	157	145	N.d.	N.d.	N.d.	N.d.	N.d.	89	85, 111, 129, 157, 201, 231
36	143	N.d.	203	N.d.	319	291	161	N.d.	69, 101, 129, 143
46	143	N.d.	203	N.d.	347	347	N.d.	N.d.	69, 143
40	69	N.d.	231	303	375	375	217	145	69, 85, 115, 129, 153, 201

[a]A maximum of six fragment ions with an intensity of >10% of that of the base peak is listed. [b]N.d. = Primary fragment ion was not detected.

TABLE IV

G.L.C. AND C.I.–M.S. DATA FOR METHYLATED AND ACETYLATED CYCLIC DERIVATIVES OF KDO

Compound	*Systematic name*	*Mol. wt.*[a]	*Retention time*[b]
29	Methyl 3-deoxy-1,4,5,7,8-penta-*O*-methyl-α-D-*manno*-octulopyranoside	308	0.78
17a	Methyl (methyl 3-deoxy-4,5,7,8-tetra-*O*-methyl-α-D-*manno*-octulopyranosid)onate	322	1.00
17b	Methyl (methyl 3-deoxy-4,5,7,8-tetra-*O*-methyl-β-D-*manno*-octulopyranosid)onate	322	0.92
19a	Methyl (methyl 3-deoxy-4,6,7,8-tetra-*O*-methyl-α-D-*manno*-octulofuranosid)onate	322	0.90[c]
19b	Methyl (methyl 3-deoxy-4,6,7,8-tetra-*O*-methyl-β-D-*manno*-octulofuranosid)onate	322	0.88[c]
27	Methyl (methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-octulopyranosid)onate	434	2.32
28	Methyl 3-deoxy-1,4,5,7,8-penta-*O*-acetyl-α-D-*manno*-octulopyranoside	448	2.42
3	Methyl 3-deoxy-4,5-*O*-isopropylidene-1,7,8-tri-*O*-methyl-α-D-*manno*-octulopyranoside	320	0.76
10	Methyl 3-deoxy-7,8-*O*-isopropylidene-1,4,5-tri-*O*-methyl-α-D-*manno*-octulopyranoside	320	0.80
14	Methyl 3-deoxy-4,5:7,8-di-*O*-isopropylidene-1-*O*-methyl-α-D-*manno*-octulopyranoside	332	0.94
2	Methyl (methyl 3-deoxy-4,5-*O*-isopropylidene-7,8-di-*O*-methyl-α-D-*manno*-octulopyranosid)onate	334	0.94
8	Methyl (methyl 3-deoxy-7,8-*O*-isopropylidene-4,5-di-*O*-methyl-α-D-*manno*-octulopyranosid)onate	334	1.04
1	Methyl (methyl 7,8-di-*O*-acetyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-octulopyranosid)onate	390	1.83

[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). [c]Assignment exchangeable.

β-furanosides and α- and β-pyranosides of KDO and 3-deoxy-D-*arabino*-heptulosonic acid are in equilibrium, methylation of **16** and **30** gave derivatives which could be separated by g.l.c. Although the mass spectra of cyclic sugar derivatives are usually of limited value, some conclusions could be made therefrom. The methylated furanosides yielded a fragment of high intensity at *m/z* 157 representing the furanoyl cation which results from fission of the C-5–C-6 bond followed by loss of methanol. This fragment was not formed from the methylated pyranosides (data not shown).

The data reported here together with those published[1] provide a basis for interpreting the results of methylation analysis of complex carbohydrates containing 3-deoxy-2-ketoaldonic acids and enabled the substitution patterns of KDO in bacterial lipopolysaccharides to be determined[2-15].

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