GAS-LIQUID CHROMATOGRAPHY—MASS SPECTROMETRY OF ACETATES OF PARTIALLY METHYLATED METHYL GLYCOSIDES. VIII. TRI- AND TETRAMETHYLHEPTOSES

T. I. Vakorina and Yu. N. El'kin

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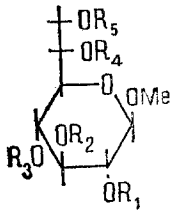
Nine tri- and five tetramethyl ethers have been obtained by the partial methylation of methyl α- and β-glucoheptopyranosides and methyl 2,3,4,6,7-penta-O-acetyl-α- and -β-glucoheptopyranosides. The retention indices of both types of methyl ethers on the liquid phase NPGS have been measured and the main directions of the fragmentation of these compounds under electron impact have been determined. The laws of the mass spectra of the position isomers that have been found will permit their use in GLC—MS analysis for determining the structure of a polysaccharide by the methylation method.

The hydroxy groups in a heptose molecule can theoretically give 31 possibilities for the formation of glycoside bonds with it in a polysaccharide chain. The determination of the positions of the glycosidic bonds from the positions of the free OH groups in the partially methylated monosaccharide residues formed as the result of the cleavage of the permethylated polysaccharide (methylation analysis) is the main method in determining its structure. The identification of the partially methylated monosaccharides is carried out with the aid of GLC and/or the GLC—MS technique on the basis of known retention indices and mass spectra. Such characteristics have been given for the acetates of mono- and dimethyl ethers of methyl heptopyranosides in the preceding paper [1]. In the present paper we consider the tri-, tetra-, and pentamethyl ethers of a methyl heptopyranoside — the most probable products of the methanolysis of polysaccharides containing heptoses. To obtain them we used both the α and the β anomers of the methyl heptopyranoside, since no 2,4,6-tri- and 2,4,6,7-tetramethyl ethers were found in the products of the methylation of the β anomer. The compound with the 3,4,7-OMe_3 pattern of methylation was not found for either of the anomers. The differences between the mass spectra of the α and β anomers of similarly methylated heptosides are far less substantial than between the position isomers of one anomer and do not affect the reliability of the determination of the positions of the methoxyls from the spectra. The retention indices of the compounds studied, measured on the liquid phase NPGS, are given in Table 1. The mass spectra of the tri- and tetramethyl ethers are given in Tables 2 and 3. The main directions of the fragmentation in the mass spectrometer of the compounds studied are shown in scheme 1 in the preceeding communication [1].

The trimethyl ethers of a methyl heptoside number ten position isomers. In the products of the partial methylation of the methyl heptoside we detected nine:



$R_1=R_4=R_5=OMe$, $R_2=R_3=OAc$ (I)
$R_2=R_4=R_5=OMe$, $R_1=R_3=OAc$ (II)
$R_3=R_4=R_5=OMe$, $R_1=R_2=OAc$ (III)
$R_1=R_2=R_4=OMe$, $R_3=R_5=OAc$ (IV)
$R_1=R_2=R_5=OMe$, $R_3=R_4=OAc$ (V)
$R_1=R_3=R_4=OMe$, $R_2=R_5=OAc$ (VI)
$R_1=R_3=R_5=OMe$, $R_2=R_4=OAc$ (VII)
$R_2=R_3=R_4=OMe$, $R_1=R_5=OAc$ (VIII)
$R_1=R_2=R_3=OMe$, $R_4=R_5=OAc$ (IX)

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TABLE 1. Retention Indices (R_T) of Tri- and Tetramethyl Ethers of Methyl β-Glucoheptopyranoside on the Phase NPGS*

R_T	Position of the OMe grps.	R_T	Position of the OMe grps.
510	3,4,6	365	3,6,7
460	2,6,7	265	3,4,6,7
425	2,3,4	210	2,3,4,6
420	4,6,7	205	2,3,7
395	2,3,6	115	2,3,6,7
370	2,4,7		

*The retention indices are given on a scale in which the R_T for the methyl 2,3,4,6,7-penta-O-methyl-β-heptoside is taken as zero and R_T for the methyl 2,3,4,6,7-penta-O-acetyl-β-heptoside is taken as 1000; glass column, 2000 × 3 mm, 150-225°C, 5°C/min, rate of flow of carrier gas 60 ml/min.

Methyl heptosides with a completely methylated side chain (I—III) are characterized, on fragmentation under electron impact, by the formation of the ions S_1, m/z 89, the intensity of which reaches 50% in the case of the spectrum of (I). The peak of the ion E_1^1 m/z 305, with its relatively high intensity, is also due to the methylated side chain, and in the spectra of compounds (I—III) it is represented by a series of peaks due to the loss of the substituents. But the most intense peaks in the spectra of this group of trimethyl ethers are given by the products of the breakdown of the pyranose ring of the methyl heptoside. As in the case of the 2- and 3-monomethyl ethers [1], compounds (I) and (II) are characterized by high yields of the ions H and G and the ions I and D, respectively. The value of the mass number of the D_1 ion in the spectrum of (II) — 193 — is a confirmation of the presence of a di-OMe-substituted side chain. The specific pattern of methylation of compound (III) leads to the presence in its spectrum of the strongest peak with m/z 101 of the three-carbon ion G_1^3 (the C_4—C_5—C_6 chain with substituents), and three-carbon fragments from C_2—C_3—C_4 of the monosaccharide unit are, as in the case of the 4-monomethyl ether [1], responsible for the peaks with m/z 129 and 87 in the spectrum of (III). The peak of the ion K_1 was not detected in the spectrum, and the ion K_{2p}, m/z 115, had a low yield.

Among the group of trimethyl ethers with two methoxyls in the pyranose ring and one in the side chain (IV—VII), the first two have the closest mass spectra. In actual fact, methoxy groups in positions 2 and 3 ensure a high yield of the ions H_1, m/z 88, and I_1, m/z 75, and also the less intense — but characteristic for 3-OMe substitution — peaks of the ions B_1, m/z 204, and D_1, m/z 221. The spectra of (IV) and (V) are characterized by peaks of low intensity in the region of high mass numbers. The presence of a methoxyl at C_6 is responsible for the ions of the series E, m/z 277, 245, and 185, in the spectrum of (IV) while they are absent from the spectrum of (V). The S_1 ion, which is typical for 6-OMe-substituted methyl heptosides, gives a considerable peak at m/z 117, while 7-OMe-substituted derivatives have in their spectra the peak of the S_1^1 ion at m/z 45, the intensity of which in the spectrum of (V) is twice that in the spectrum of (IV). The mass spectra of (VI) and (VIII) show that their fragmentation under electron impact differs from that of the correspondingly methylated methyl hexosides [2]. The specificity of the substitution of the side chain of the methyl heptopyranosides (VI) and (VIII) exerts an influence on their fragmentation which is shown in an increase in the yield of ions in the K direction (cleavage of the C_3—C_4 bond). In actual fact, the G_1^3 ions are responsible for the main peak at m/z 101 in the spectra of these compounds, although it is true that in the spectrum of (VI) a considerable part of this peak is due to the G_1 ion (fragment of the pyranose ring with methoxyls at C_2 and C_4). The methoxy group at C_3 in the spectrum of (VIII) is characterized by the peaks of the ions I_1, 75; B_1, 204, and D_1, 221. In the spectrum of (VII), the fragmentation in the K direction (K_1, K_1 — 32, K_1 — 59, K_{2p}, G_1^3, and G_1^3 — 42) make up the bulk of the ion current in comparison with the ions of other directions.

TABLE 2. Mass Spectra of the Acetates of Trimethyl Ethers of Methyl Glucoheptopyranoside (relative intensity, %)

m/z	Type of ion	Positions of the OMe groups								
		2, 3, 6	2, 4, 6	3, 4, 6	2, 3, 7	2, 4, 7	2, 6, 7	3, 6, 7	4, 6, 7	2, 3, 4
43		31,1	50,0	73,5	37,5	112,6	139,0	123,1	70,9	43,7
45	S_1'	10,0	8,7	18,7	21,9	45,7	39,0	53,6	28,2	12,7
59		3,1	3,7	—	2,5	4,7	15,0	16,1	13,6	3,7
69		1,7	9,5	11,4	2,2	5,6	15,0	13,8	—	2,8
71		3,7	4,5	6,7	3,8	8,6	10,5	19,2	8,2	3,1
73		7,7	4,5	5,7	11,3	—	4,5	7,7	10,0	11,7
74	H_1—42	6,5	45,8	46,7	9,4	91,5	100,0	17,0	21,8	4,1
75	I_1	100,0	37,5	100	93,9	100	48,0	100,0	27,3	50,7
81		—	1,6	—	2,2	4,4	3,4	11,5	2,7	1,1
83		—	2,9	—	—	4,7	3,0	12,3	4,5	1,3
85		8,0	3,7	5,0	6,9	10,0	22,5	15,4	10,0	4,4
87	G_1—42, G_1^3—42	12,7	5,0	12,4	8,0	59,8	57,0	53,8	27,3	10,6
88	H_1	91,4	20,8	33,4	100,0	22,9	18,0	14,6	13,6	100,0
89	S_1	5,4	2,9	5,7	5,0	3,9	48,0	14,6	24,6	8,4
95		—	1,2	1,3	—	3,7	1,8	10,0	—	0,7
97		2,0	1,6	2,0	4,7	6,3	7,2	13,8	7,3	2,4
99		—	4,1	6,3	3,8	5,6	10,8	8,5	9,1	2,1
100		—	—	—		3,9	16,5	6,1	8,3	3,3
101	G_1, G_1^3	11,4	100,0	100,0	9,0	70,4	16,5	13,8	100,0	46,5
102		—	5,8	—	—	4,2	6,0	—	9,1	3,1
103		3,1	1,6	—	2,2	—	—	6,9	7,3	1,7
111		2,8	2,5	3,3	5,0	5,3	19,4	11,5	7,3	2,2
113	E_3	3,4	2,5	3,7	4,7	5,3	4,6	7,7	7,3	2,5
114		1,7	—	9,4	—	5,3	4,5	—	—	0,8
115	K_{2p}	2,8	3,3	3,7	—	72,1	6,1	20,7	9,1	2,7
116	H_1'	2,2	33,3	18,4	2,2	56,3	67,1	35,4	4,5	0,8
117	S_1'	11,4	41,6	36,7	2,2	6,7	7,5	9,2	—	1,4
125	E_4	2,0	2,0	2,0	3,4	4,4	11,9	5,4	7,3	3,5
127		4,5	2,5	4,0	2,2	2,8	—	9,2	6,5	—
129	G_1, G_1^3	6,5	2,9	6,3	6,9	36,9	40,3	27,7	36,4	5,8
130		1,1	—	1,0	—	2,6	4,0	—	—	—
131		1,1	0,8	1,0	—	2,6	—	4,6	—	—
139	A_4	0,8	2,9	1,7	1,3	13,0	—	7,7	9,1	2,4
141	E_3	1,7	—	2,3	1,6	—	—	9,2	7,3	1,0
142	K_1—32	1,4	—	—	—	10,4	5,9	4,6	—	1,3
143	K_{2p}, E_3'—42	3,4	6,2	4,7	2,2	4,2	36,0	8,5	11,8	1,8
144		—	2,0	1,3	—	1,6	—	—	—	—
157		2,0	4,1	1,3	2,2	1,8	11,9	4,6	7,3	1,1
159		2,0	1,2	3,3	0,8	0,5	—	3,8	2,7	0,6
170		—	—	—	0,8	1,2	2,4	—	8,2	0,5
171	C_3	0,7	—	1,4	1,9	17,6	7,9	8,5	5,5	0,7
172		—	—	—	—	1,6	1,2	—	4,5	0,4
173	E_2	0,7	2,9	4,0	0,8	1,9	1,0	2,6	2,7	0,3
174	K_1	—	—	0,5	—	1,6	—	—	—	—
185	E_3'	4,3	1,2	1,4	0,6	3,2	47,7	5,0	10,9	1,5
187		—	—	0,3	—	—	1,9	—	3,6	0,2
189		0,9	1,2	0,3	—	1,6	1,3	3,1	4,5	0,6
193	D_1	—	—	—	—	—	—	4,1	—	—
199	A_3	0,7	—	0,6	0,6	0,6	3,3	1,9	5,5	0,6
201	E_2'	0,6	—	0,5	—	—	1,0	2,5	2,7	0,1
203	E_2'—42	0,5	0,6	1,0	—	—	2,5	1,1	—	0,1
204	B_1	2,3	0,5	3,3	1,9	0,4	—	—	—	0,2
213	E_2—32	0,2	0,9	1,3	1,9	0,2	3,8	8,5	6,4	0,4
217	A_2—42, E_2'	0,2	0,7	0,4	—	0,2	0,4	—	0,9	0,2
221	D_1	5,1	—	3,0	5,3	—	—	—	—	—
230		—	0,6	—	—	1,6	1,6	—	—	—
231	C_2	—	1,1	0,4	0,2	4,0	3,1	2,3	1,8	0,1
232	B_1'	—	—	—	—	—	0,4	0,9	—	—
233	E_1	0,4	0,5	3,7	0,2	0,5	0,1	1,1	—	0,2

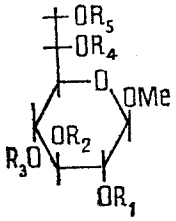
(continued)

Table 2 (continued)

m/z	Type of ion	Positions of the OMe groups								
		2, 3, 6	2, 4, 6	3, 4, 6	2, 3, 7	2, 4, 7	2, 6, 7	3, 6, 7	4, 6, 7	2, 3, 4
245	E_2', E_1—32	0,4	1,1	0,5	—	0,2	4,8	0,8	0,5	0,1
249	D_1	—	—	—	—	—	—	—	—	2,5
259	A_2	0,4	0,5	0,6	0,1	0,3	—	0,4	0,5	0,1
261	E_1	—	—	—	—	—	0,7	1,9	7,3	0,4
273	E_1'—32	—	—	--	0,1	0,1	0,7	1,7	1,7	0,2
277	E_1'	0,5	—	0,4	—	—	—	—	—	—
291	M—59	0,05	0,3	0,3	0,07	0,2	0,4	—	0,5	0,06
305	E_1'	—	—	0,2	—	0,1	5,2	14,6	1,3	0,09
319	A_1	0,06	0,4	0,4	0,3	0,1	0,15	0,23	0,5	0,2

The decomposition of compound (IX) under electron impact gives a spectrum resembling that of the analogously methylated hexoside [2]. The strongest peaks in the spectrum of (IX) are due to the ions I_1, H_1, and D_1, the peak of the ion H_1, m/z 88, being the main one. A side chain with two acetoxyls is reflected by the value of the mass number of D_1, m/z 249.

The acetylated tetramethyl ethers of a methyl heptoside number five isomers differing from one another only by the position of one acetoxyl:



$R_1=R_2=R_3=R_4=OMe$, $R_5=OAc$ (X)
$R_1=R_2=R_3=R_5=OMe$, $R_4=OAc$ (XI)
$R_1=R_2=R_4=R_5=OMe$, $R_3=OAc$ (XII)
$R_1=R_3=R_4=R_5=OMe$, $R_2=OAc$ (XIII)
$R_2=R_3=R_4=R_5=OMe$, $R_1=OAc$ (XIV)

The behavior of this group of methyl esters under electron impact is more similar to the fragmentation of the completely methylated methyl glycosides, for which the overwhelming bulk of the ion current is due to the one-, two-, and three-carbon ions of series I, H, and G. Thus, the product of the fragmentation of methyl 2,3,4,6,7-penta-O-methyl-β-heptopyranoside (XV) is characterized by the six strongest ions: H_1, m/z 88-100%; I_1, m/z 75-80%; G_1, m/z 101-70%; S_1', m/z 45-25%; S_1, m/z 89-18% and D_1, m/z 193-18%. A comparison of its spectrum with the spectrum of a completely methylated methyl hexopyranoside shows a rise in the proportion of the ions I_1, D_1, and G_1 in the case of the heptoside. The high intensity of the peak at m/z 101 in the spectrum of (XV) is due to contributions of the G_2^3 ion, the proportion of which in the G ions for the heptoside is higher than for the hexoside, while the increase in the yield of the rearranged ions I_1 and D_1 is due to the size of the side chain. This rule is also followed in the spectra of the tetramethyl ethers of the methyl heptosides. The mass spectra of the heptosides with methylated pyranose rings, (X) and (XI), show that the proportion of the G_1^3 ions in the m/z 101 peak in the case of (X) amounts to about 50%. But the intensity of this peak in the spectrum of (XI) is only half that in the spectra of (X) and (XV). The intensities of the peaks of the ions I_1 and D_1 are approximately equal in the spectra of all three compounds. The methyl glycosides (X) and (XI) differ by the position of the methoxyl in the side chain. Consequently, in the spectrum of the 2,3,4,6-tetra-OMe-substituted methyl heptoside there is a considerable peak of the S_1 ion at m/z 117, and in the spectrum of (XI) the methoxyl at the C_7 ion is responsible for the increased intensity of the peak with m/z 45, thanks to a contribution of the ions S_1'. In the spectra of compounds (X), (XI), and (XV) there is the peak of the ion B_1, m/z 176, which is a confirmation of the 2,3,4-tri-OMe-substitution of the pyranose ring.

The completely methylated side chains of compounds (XII—XIV) are responsible for the appearance in the products of their fragmentation of the ions S_1, m/z 89, S_1', m/z 45, and a series of ions E. The pattern of methylation of the pyranose rings of these three methyl heptosides leads to considerable differences in the yields of the ions H_1, G, and I. In the spectrum of (XII), the 2,3-di-OMe-grouping is responsible for the predominating yield of the ions I_1, m/z 75, and H_1, m/z 88. But in the spectrum of (XIII) there is one strong peak

TABLE 3. Mass Spectra of the Acetates of Tetramethyl Ethers of Methyl Glucoheptopyranoside (relative intensities, %)

m/z	Type of ion	Positions of the OMe groups				
		2, 3, 4, 6	2, 3, 4, 7	2, 3, 6, 7	2, 4, 6, 7	3, 4, 6, 7
43		23,4	8,9	18,5	29,5	39,1
45	S_1'	11,7	17,7	29,0	25,7	27,9
59		2,8	3,0	6,6	11,4	12,1
69		1,8	0,9	1,6	—	4 5
71		3,3	2,8	6,6	4,0	7,8
73		10,7	6,7	11,1	4,5	9,3
74	H_1—42	2,7	2,3	5,0	31,4	26,0
75	I_1	73,5	68,9	92,4	45,7	81,8
83		1,3	0,9	1,0	2,9	2,6
85		2,5	5,2	8,2	4,8	8,4
87		1,7	13,3	14,8	4,3	12,1
88	H_1	100,0	100,0	100,0	18,1	46,5
89	S_1	8,3	8,0	13,2	20,1	18,6
95		0,4	0,6	0,8	1,7	1,5
97		0,7	0,9	2,1	1,7	3,0
99		2,0	2,2	4,0	2,8	4,6
101	G_1, G_1^3	66,8	35,5	13,2	100,0	100,0
102		3,7	—	0,8	1,7	6,7
103		1,0	—	3,7	—	2 6
111	A_4	1,0	1,3	3,2	3,2	4,1
113		0,8	1,4	—	—	3,3
114		1,2	2,1	2,6	4,8	3,0
115	K_{2p}	0,8	0,8	3,2	15,2	11,2
116	H_1	—	,1	1,1	13,3	7,8
117	S_1	11,7	1,5	1,0	3,0	10,0
119		0,8	—	1,1	1,2	2,3
125		1,2	0,5	1,7	1,1	2,0
127		1,3	1,0	4,0	2,4	4,1
129		1,0	10,0	6,3	2,7	7,8
131		2,5	2,8	2,6	—	2,0
143	C_3, K_{2p}	1,0	1,2	4,0	1,8	4,1
145		1,2	1,1	1,2	2,4	4,3
147		0,5	2,8	1,1	—	0,6
157	$(A_2$—32)—42	1,3	0,7	4,0	5,7	3,7
159		—	1,1	0,6	1,1	3,9
161		0,7	1,3	0,3	0,7	2,6
171	A_3	0,2	1,3	1,1	5,2	3,7
173	E_2	0,9	0,5	0,7	1,4	1,9
176	B_1	0,5	0,4	—	—	—
185	E_2—32	0,7	0,5	9,2	0,9	1,9
189		—	—	1,5	—	—
193	D_1	0 4	—	7,9	—	2,8
199		0,5	0,2	0,7	0,7	1,3
203	C_2	—	—	0,8	—	1,3
204	B_1	0,2	0,2	2,4	—	2,8
205	E_1	0,2	0,2	0,3	—	—
213		—	—	0,2	0,7	1,5
217	E_2'	0,3	0,1	0,4	1,8	—
221	D_1	8,7	7,8	—	—	0,9
231	A_2	0,2	—	0,2	—	—
233	E_1	—	1,8	0,2	3,3	3 7
245	E_1'—32	—	—	0,7	0,2	0,5
263	M—59	—	—	—	0,11	—
277	E_1'	0,09	—	1,3	0,06	0,5
291	A_1	0 07	0,13	0,05	0,6	0,1

with m/z 101 of the G_1^3 and G_3 ions. The formation of these ions with a considerable yield presupposes the presence of 4,6- and 2,4-di-OMe groupings in the methyl heptoside molecule. In the ion current of compound (XIV), the proportion of ions with m/z 101 is smaller than in the case of (XIII). In the spectrum of (XIV), the G_1 ion makes no contribution to this peak and, relative to it, the intensities of the peaks with m/z 75 (I_1), and m/z 88 (H_1), rise.

Thus, the mass spectra of the methyl heptopyranoside derivatives that have been studied are characteristic and unambiguously reflect the pattern of methylation.

EXPERIMENTAL

The trimethyl ethers (I, II, IV, V, VII, IX, and VI) were detected in the products of the partial methylation of the monomethyl ethers of methyl β- and α-heptopyranosides. The

conditions for their production and also of GLC separation and of GLC—MS analysis have been given in our previous paper [1]. The derivatives (XI, XII, XIII, and XV) were detected in the products of the methylation of methyl β- and α-heptopyranosides by Kuhn's method [3]. The same method was used to obtain compounds (III), (VIII), and (XIV) from methyl 2,3,4,6,7-penta-O-acetyl-β-heptopyranoside.

Methylation by Kuhn's Method. The methyl heptoside (20 mg) was dissolved in dry N,N-dimethylformamide, methyl iodide (0.18 ml) and barium oxide (70 mg) were added, and the mixture was stirred for 2.5 h. Then it was diluted with chloroform, filtered, and washed with water (3 × 3 ml), the wash-waters were extracted with chloroform (3 × 3 ml), and the chloroform extracts were combined, dried over sodium sulfate, and evaporated.

On the methylation of the pentaacetate of methyl β-heptoside, silver oxide was used instead of barium oxide and the mixture was stirred for from 24 to 36 h, the remaining treatment being the same as in the methylation of the methyl heptoside.

SUMMARY

1. Nine of the ten possible trimethyl ethers, all five of the tetramethyl ethers, and the completely methylated monosaccharide have been detected in the products of the partial methylation of methyl α- and β-glucoheptosides.

2. The retention indices on the liquid phase NPGS of the acetates of the tri- and tetramethyl ethers of methyl β-glucoheptoside have been measured.

3. The mass spectra of both types of methyl ethers are given and discussed. Assignments have been made of the main peaks in the spectra to known types of ions.

4. It has been established that the mass spectra of the position isomers in both types of methyl ethers unambiguously characterize the pattern of substitution of the monosaccharide skeleton of a methyl heptopyranoside.

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