CHROMATO-MASS SPECTROMETRIC STUDY OF 3,6-DIDEOXYHEXOSES

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The main features of the fragmentation of the acetates of methyl 3,6-dideoxyhexopyranosides and -furanosides have been established with the use of deutero and partially methylated analogs. A considerable proportion of the ion current of the pyranosides is due to the fragmentation of the monosaccharide ring: K, C, H. In the case of the furanosides, on the other hand, the main portion of the ion current is due to the ions of the furanose ring, A, E. It is proposed to use mass spectra and the gas-chromatographic properties of the 3,6-dideoxyhexoses studied for their identification in the products of the methanolysis of lipopolysaccharides.

3,6-Dideoxyhexoses (DDHs) are component parts of the lipopolysaccharides (LPSs) of Gram-negative bacteria and largely determine their O-antigenic specificity [1, 2]. Of the eight theoretically possible isomers of the 3,6-DDHs, hitherto only five have been found in the LPSs of the cell walls of microorganisms: abequose (3,6-dideoxy-D-xylo-hexose, Abe), colitose (3,6-dideoxy-L-xylo-hexose, Col), ascarylose (3,6-dideoxy-L-arabino-hexose, Asc), tyvelose (3,6-dideoxy-D-arabino-hexose, Tyv), and paratose (3,6-dideoxy-D-ribo-hexose, Par) [1, 2]. The physicochemical characteristics of these monosaccharides given in the literature are limited to the indices of their mobilities in paper chromatography [3] and electrophoresis [4] and the retention times (GLC) of the 1,5-di-O-acetyl-2,4-di-O-methyl derivatives of their alditols [5]. The latter are not at all convenient for identifying the 3,6-DDHs by chromato-mass spectrometry, since they require the preliminary methylation of the LPSs.

The establishment of the monosaccharide composition of an LPS by the GLC-MS method presupposes the assignment of the monosaccharides to a definite type (in this case, to the 3,6-dideoxyhexoses) from the mass spectrum, and the assignment to an epimeric pair (-arabino-, -xylo-, etc.) is carried out from the GLC retention indices. The absence of systematic information for the 3,6-dideoxyhexoses induced us to study their mass spectra and to measure the retention indices of these compounds in the form of the acetates of the methyl glycosides. This type of derivatives was selected in view of the fact that methanolysis is a convenient method for cleaving carbohydrate-containing biopolymers of complex composition [6].

The source of the samples consisted of the lipopolysaccharides of *Yersinia pseudotuberculosis* (types I-VI), which contain all five known 3,6-DDHs [3]. The latter were not isolated in the individual state but were studied by GLC-MS method in a mixture with the other products from the methanolysis of the LPS in the form of acetates, since the GLC peaks of the 3,6-dideoxyhexoses did not coincide with the peaks of other monosaccharides.

Analysis of the mass spectra of the acetylated methanolysis products showed that an epimer of a 3,6-DDH is characterized by four isomers (two anomers each of the pyranoside and the furanoside), each with its own value of the retention index (R_t) and partial area of the GLC peak (R_a) (Table 1). Among the five 3,6-dideoxyhexoses studied there are two pairs of enantiomers: Col/Abe and Tyv/Asc. Since enantiomers have identical R_t and R_a values and mass spectra, Table 1 includes only one representative from each pair. Thus, each pair of enantiomers, regardless of the source of isolation, has a characteristic GLC pattern (Fig. 1). The acetates of the methyl glycosides of Col from the LPSs of *Y. pseudotuberculosis* type VI and Abe from the LPSs of *Y. pseudotuberculosis* type II and of *Salmonella abortus equae* and those of Tyv from the LPSs of *Y. pseudotuberculosis* type IV and of *Salmonella typhi* and those of Asc from the LPSs of *Y. pseudotuberculosis* type V A (strain 2457) and Par from *Y. pseudotuberculosis* types I B and III had identical respective GLC patterns. The assignment of the

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TABLE 1. Retention Indices (R_t) and Ratios of the Partial Areas of the GLC Peaks (R_a, %), of Acetates of Methyl 3,6-Dideoxyhexosides

		QF-1 140°C		XE-60 140°C		NPGS 130°C	
		R_t^*	R_a	R_t	R_a	R_t	R_a
Par	p_1†	1	43	1,31	42	1,26	35
	f_1					1,37	7
	f_2	1,32	29	1,65	28	1,8	58
	p_2	1,54	28	1,88	30		
Col	p_1	1	34	1,31	24	1,32	23
	f_1			1,48	57	1,55	57
	f_2	1,19	48				
	p_2	1,51	18	2,04	19	2,1	20
Asc	p_1	0,82	64	1	67	1	61
	f_1	1	31	1,13	26	1,15	27
	f_2	1,15	tr.	1,3	tr.	1,6	4
	p_2	1,36	5	1,45	7	1,83	8

*R_t = 1, corresponds to tri-OAc-glycerol.
†p — pyranoside; f — furanoside.

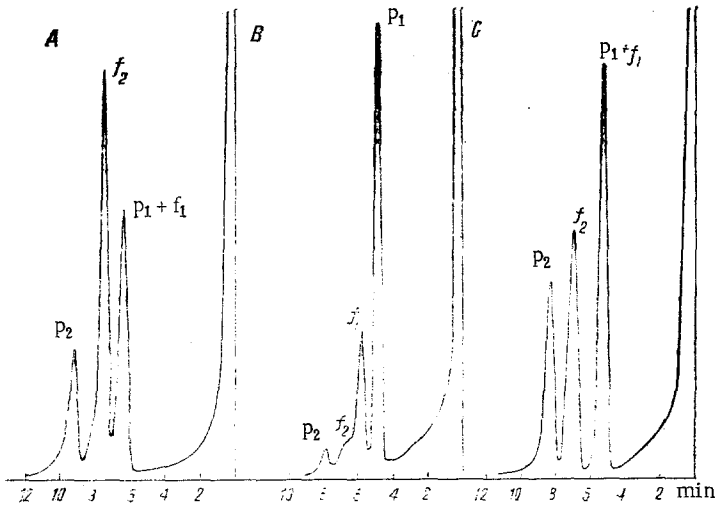


Fig. 1. GLC of the acetates of the methyl glycosides of 3,6-dideoxyhexoses of QF-1; temperature 140°C. A) Col from the LPSs of *Y. pseudotuberculosis* of type VI; B) Asc from the LPSs of *Y. pseudotuberculosis* type V A; C) Par from *Y. pseudotuberculosis* of type I B; p — pyranoside; f — furanoside.

acetates of the methyl glycosides of these sugars to the pyranosides and the furanosides was carried out from the mass spectra on the basis of systematic information for this type of monosaccharide derivative [7] and their methyl ethers [8]. However, it was impossible to identify the α and β anomers of the pyranosides and furanosides from the relative intensities of the peaks of the ions A_1 with m/z 215 according to the cis and trans configurations of the substituents at the ^1C and ^2C atoms [8]. While in the case of paratose the differences in the relative intensities of the peaks of the ions A_1, m/z 215, in the spectra of the anomers of the pyranosides are considerable, in the case of colitose and tyvelose they are already

TABLE 2. Mass Spectra of Derivatives 3,6-Dideoxyhexopyranosides

I. Methyl 2,4-di-O-acetyl-3,6-dideoxy-D-ribo-hexopyranoside

m/z	215	186	143	129	126	116	103	100	99	98	87	84	83
%	3	2	4	3	22	19	21	100	6	5	5	23	21
Ion	A_1	C_{1p}	C_2	G_1	C_{2p}; P_2	H_1	b	K_1				C^1_{3p}	C_3
m/z	74	71	61	58	57; 56	55	45	43					
%	45	30	11	23	17; 13	16	30	↑					
Ion	H_2			K_2				a					

II. Methyl 2,4-di-O-acetyl(d_6)-3,6-dideoxy-D-ribo-hexopyranoside

m/z	221	192	146	129	126	119	113	109	103	99	85	83	75	71
%	3	3	4	21	11	26	5	13	100	8	29	27	60	40
Ion	A_1	C_{1p}	C_2	C_{3p}	P_2	H_1		b	K_1		C^1_{3p}	C_3	H_2	
m/z	59	57	56	55	46									
%	24	25	14	32	↑									
Ion	K_2				a									

III. 1,2,4-Tri-O-acetyl-3,6-dideoxy-D-ribo-hexopyranoside

m/z	231	215	189	172	171	157	154	145	144	143		129	126	
%	15	5	25	4	4	1,5	4	4	3	14		8	4	
Ion	L_1	A_1	L^1_2	P^1_2	L_2		P_2	c	H_1	C_2		L^1_3	C_{2p}	
m/z	113	112	103	102	101	100	97	85	84	83	68	57	56 55	43
%	12	26	16	9	6	13	12	10	30	100	13	23	12 20	↑
Ion		P^1_3	b	H_2		K_1			C^1_{3p}	C_3				a

IV. 1,2,4-Tri-O-acetyl(d_9)-3,6-dideoxy-D-ribo-hexopyranoside

m/z	237	221	193	176	174	60	157	154	150	146	132	130	129	114
%	24	5	6	5	7	23	4.7	3	3,6	22	2	5	15	7
Ion	L_1	A_1	L^1_2	P^1_2	L_2		P_2	c	H_1	C_2		L^1_3	C_{2p}	
m/z	113	112	110	109	106	103	102	98	85	84	83	68 57	56 55	46
%	30	10	8	10	12	16	11	6	22	88	100	18 26	13 27	↑
Ion	P^1_3			b	H_2	K_1			C^1_{3p}		C_3			a

V. Methyl 4-O-acetyl- 2-O-methyl-3,6-dideoxy-D-ribo-hexopyranoside

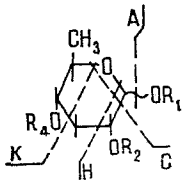
m/z	187	127	111	109	103	101	100	99	98	97	95	88	85	83
%	7	6	9	7	34	38	42	9	34	13	11	100	19	27
Ion	A_1	A_2			b	G	K_1		C_{2p}			H_1		
m/z	81	75	73	71	58	57	56	55	45					
%	11	42	21	38	53	44	13	23	35					
Ion		J_1			K^1_2									

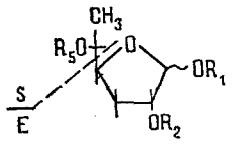
smaller, and the anomers of the furanosides are not distinguished at all. Consequently, the anomers are denoted by the indices 1 and 2 in the order in which they issued from the GLC column. No differences were detected between the spectra of the epimers, either. A discussion of the spectra of the 3,6-DDHs will be given with the paratose derivatives (compounds I-IX, scheme 1) as examples.

The assignment of the main peaks in the mass spectra of the methyl 3,6-dideoxyhexopyranoside acetate (I) and the methyl 3,6-dideoxyhexofuranoside acetate (VI) was made by comparison with the spectra of the OAc-d_3 analogs (II) and (VII), those of the full acetates of the 3,6-DDHs (III, IV, VIII), and those of the methyl ethers (V, IX) (Tables 2 and 3).

To denote the ions, on the whole, we used the nomenclature proposed previously [8], with some supplementation. The ions formed by the loss of substituents from the monosaccharide ring (excluding the ^1C substituent) have been denoted by the symbol P, and the ions

Scheme 1





I. $R_1=CH_3$, $R_2=R_4=CH_3CO$
II. $R_1=CH_3$, $R_2=R_4=CD_3CO$
III. $R_1=R_2=R_4=CH_3CO$
IV. $R_1=R_2=R_4=CD_3CO$
V. $R_1=R_2=CH_3$, $R_3=CH_3CO$
VI. $R_1=CH_3$, $R_2=R_5=CH_3CO$
VII. $R_1=CH_3$, $R_2=R_5=CD_3CO$
VIII. $R_1=R_2=R_5=CH_3CO$
IX. $R_1=R_5=CH_3$, $R_2=CH_3CO$

resulting from the loss of acetyl from the ^{1}C–OAc group by the symbol L. The spectra of the acetates of the methyl glycosides contain the peaks of the ions of series C formed by the loss of neutral fragments from the molecular ions $M^{+\cdot}$ and having even mass numbers [7]. These ions are provided with the additional subscript index p. Since the decomposition of the ions at the acetoxy group takes place with the loss of acetic acid or ketene, to introduce the corresponding ions formed of series A, E, P, and C we have introduced the superscript index 1, reflecting the splitting out of CH_2CO.

TABLE 3. Mass Spectra of Derivatives of 3,6-dideoxyhexofuranosides

VI. Methyl 2,5-di-O-acetyl-3,6-dideoxy-D-ribo-hexofuranoside

m/z	215	159	126	117	113	112	103	99	97	95	87	84	83
%	5	25	6	3	5	2	8	100	2	3	5	12	9
Ion	A_1	E_1	C_{2p}, P_2	E_2^1	A_3^1		b	E_2		A_3		C_{3p}^1	C_3
m/z	74	71	69	57	56	55	45	43					
%	2	10	3	4	5	12	40	↑					
Ion								a					

VII. Methyl 2,5-di-O-acetyl(d_6)-3,6-dideoxy-D-ribo-hexofuranoside

m/z	221	162	129	126	119	118	114	113	109	99	87	85	83
%	3	23	3	2	1	1	2	3	3	100	4	9	7
Ion	A_1	E_1	C_{2p}	P_2		E_2^1	A_2^1		b	E_2		C_{3p}	C_3
m/z	71	69	57	56	55	46							
%	8	3	4	4	9	↑							
Ion						a							

VIII. 1,2,5-Tri-O-acetyl-3,6-dideoxy-D-ribo-hexofuranoside

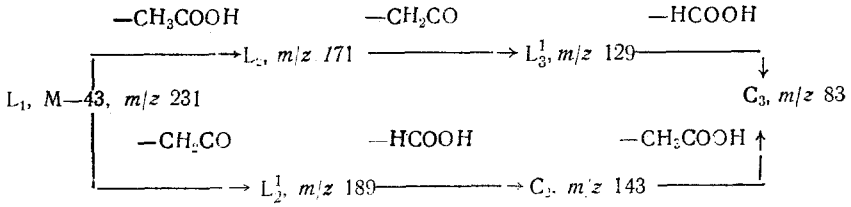
m/z	231	215	187	172	171	170	157	155	154	145	129	127	117
%	6	45	80	6	4	5	13	6	9	33	20	100	5
Ion	L_1	A_1	E_1	P_2^1	L_2			A_2	P_3	E_2^1	L_3	E_2	
m/z	115	113	112	103	97	95	87	85 83	74	71	69 68	58	57
%	8	36	34	18	16	13	24	73 72	15	13	14 26	8	16
Ion		A_3	P_4^1					E_3^1 C_3					
m/z	56	55	45	43									
%	8	13	25	↑									
Ion				a									

IX. Methyl 2-O-acetyl-5-O-methyl-3,6-dideoxy-D-ribo-hexofuranoside

m/z	187	159	127	117	103	99	97	95	87	85	83	75	74
%	5	27	3	2	7	100	4	4	5	7	6	6	2
Ion	A_1	E_1	A_2	E_2^1	b	E_2							
m/z	71	69	59	57	56	55	45	43					
%	12	7	31	9	4	6	20	↑					
Ion			S_1					a					

The decomposition of the methyl 2,4-di-O-acetyl-3,6-dideoxyhexopyranoside (I) takes place mainly in three directions: K, H, and C (scheme 1), thereby recalling the decomposition of methyl 2,4,6-tri-O-methyl-3-deoxy-α-D-glucopyranoside [8]. The ions of this series make up half the ion current of (I) (the acetyloxonium ions a, m/z 43; b, m/z 103; and c, m/z 145 were not taken into account in the calculation). The spectrum of (I) shows the formation of two types of ions of series C one of which, C_2 with m/z 143, is connected with the loss of a radical [M − CH_3OCOH − $\cdot CH_3CO$]. In the spectrum of deutero analog (II), this peak is shifted to m/z 146. Another type of C ions forms a whole series: C_{1p}, m/z 186 (192); C_{2p}, m/z 126 (129); C^1_{3p}, m/z 84 (85). Noteworthy in the decomposition of (I) is the formation of the ion P_2 [M − CH_3COOH − CH_3COOH] which makes up about 40% of the peak with m/z 126, as follows from the mass spectrum of the deutero analog (II). In the case of the 6-deoxy homolog, however, the proportion of the P_2 ion in the m/z 184 peak is less than 10%, and a larger proportion is due to the C_{2p} ion.

The most characteristic difference of the mass spectra of the full acetates of the 3,6-dideoxyhexoses from the spectra of the acetates of their methyl glycosides is the presence in the former of intense peaks of ions of series P and L. The splitting off of acetyl from the ^{1}C—OAc group is apparently characteristic for acetates of deoxysugars. Thus, if we compare the spectra of glucose pentaacetate, 2-deoxyglucose tetraacetate, and 3,6-dideoxyglucose triacetate (III) (Table 2), it is possible to detect an increase in the intensity of the M − 43 peak in the order in which these monosaccharides have been mentioned. Furthermore, in the spectrum of the last-mentioned compound there is a ground of peaks due to the ions of series L. The intensities of the peaks of the ions C_2, m/z 143, and C_3, m/z 83, in the spectrum of (III) are also higher than in the spectrum (I). This can be explained by the assumption that these ions arise from the ions of series L with the loss of a molecule of formic acid (scheme 2).



Scheme 2

Thus, the nature of the ^{1}C substituent has a decisive influence on the decomposition under electron impact of the acetylated pyranose nucleus of a 3,6-DDH. Similarly, the replacement of the ^{2}C—OAc group in (I) by a methoxy group leads to a situation in which the proportion of the two- and three-carbon ions H and G become dominating in the spectrum of compound (V). In addition to this, an appreciable peak of the rearrangement ion J_1, m/z 75, $[CH_3OCHOCH_3]^+$, appears.

The main direction of the decomposition of the methyl 3,6-dideoxyhexofuranoside acetate (VI) is the cleavage of the ^{4}C—^{5}C-bond with the formation of ions of series E (scheme 1). Among the fragments of the furanose ring only the ions of series C, having a low intensity, have been identified. In the spectrum of methyl 2-O-acetyl-5-O-methyl-3,6-dideoxyhexofuranoside (IX), also, no appreciable fragments of the monosaccharide nucleus have been detected and the main peaks are due to ions E_1 and S_1. In the products of the decomposition of the 3,6-dideoxyhexofuranoside triacetate (VIII), likewise, the bulk is due to the ions of series E.

On the whole, the chromatographic (Table 1 and Fig. 1) and mass spectrometric (Tables 2 and 3) information obtained permits the unambiguous identification of the epimer of a methyl 3,6-dideoxyhexoside in the products of the methanolysis of an LPS by the GLC-MS method.

EXPERIMENTAL

As the source of 3,6-dideoxyhexoses we used the LPSs of *Yersinia pseudotuberculosis* types I—VI, since they contain all five known monosaccharides of this type, Par in types I and III, Abe in type II, Tyv in type IV, Asc in type V A [3], and Col in type VI [5].

Preparation of the Acetate of the Methyl 3,6-Dideoxyhexosides. The LPSs (10 mg) were heated with a mixture of 72% perchloric acid and methanol (1:10, v/v, 1 ml) at 100°C for 3 h. After cooling, the solution was neutralized with Dowex-1 (HCO_3^-) and was evaporated in vacuum

to dryness, and the residue was acetylated with a mixture of acetic anhydride and pyridine (1:1, v/v, 1 ml).

Compounds (V) and (IX) were detected in the determination of the position of the O-acetyl groups in the LPS of *Y. pseudotuberculosis*, type I B, by the method of Hellerqvist et al. [10].

Preparation of the 3,6-Dideoxyhexose Acetates. The LPSs (10 mg) were hydrolyzed in 1 N sulfuric acid (1 ml) at 100°C for 4 h. The acid was neutralized with Dowex-1 (HCO_3^-), the solution was concentrated to dryness, and the residue was acetylated in a mixture of acetic anhydride and pyridine.

The mass spectra of the compounds studied (I-IX) were recorded on an LKB-9000 S instrument in a GLC-MS system at 70 V. GLC analysis was carried out on a Pye-Unicam 104 gas chromatograph with a flame-ionization detector using standard glass columns (150 cm × 4 mm) filled with Chromaton N-AW-DMCS (100-120 mesh) with 3% of a liquid phase (QF-1, NPGS, or XE-60).

SUMMARY

1. The mass spectra of acetates of the 3,6-dideoxyhexoses paratose, abequose, colitose, ascarylose, and tyvelose, and their methyl glycosides have been studied.

2. The GLC retention indices of the anomers of acetates of the methyl 3,6-dideoxyhexopyranosides and -furanosides of the monosaccharides mentioned above have been measured.

3. It has been established that the main direction of the fragmentation of methyl 2,4-di-O-acetyl-3,6-dideoxyhexopyranosides is the formation of the $^4C-^5C$ and $^2C-^1C$ fragments of the pyranose ring.

4. Taken together, the GLC and MS properties of the 3,6-dideoxyhexose derivatives studied permit them to be identified unambiguously in the products of the methanolysis of lipopolysaccharides, with an accuracy extending as far as the particular epimer.

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