Identification of methyl *O*-acetyl-*O*-trifluoroacetyl-β-D--xylopyranosides by gas chromatography and mass spectrometry

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Of all theoretically possible positional isomers of methyl *O*-acetyl-β-D--xylopyranosides, trifluoroacetyl derivatives have been prepared and studied by combination of electron impact mass spectrometry and gas chromatography. All positional isomers are separated on an OV-225 column. The positions of acetyl groups in mono-*O*-acetyl derivatives may be established unambiguously from the intensities of peaks with *m/z* 182, 225, and 278. The mass spectra of di-*O*-acetyl derivatives differ from each other only negligibly. Therefore, in characterization of these derivatives the retention times relative to fully acetylated methyl β-D-xylopyranoside (RRT) have to be considered.

Из всех теоретически возможных пространственных изомеров метил-*O*-ацетил-β-D-ксилопиранозидов были получены трифторацетилпроизводные, которые в дальнейшем исследовались с помощью масс-спектрометрии электронного удара в сочетании с газовой хроматографией. Все пространственные изомеры были разделены на колонне OV-225. Положение ацетильных групп в моно-*O*-ацетилпроизводных можно было однозначно определить на основании соотношений интенсивностей пиков с *m/z* 182, 225 и 278. Масс-спектры ди-*O*-ацетилпроизводных имеют лишь незначительные отличия; при их характеризации следует учитывать значения относительных времен удерживания (RRT), вычисляемые относительно полностью ацетилированному метил-β-D-ксилопиранозиду.

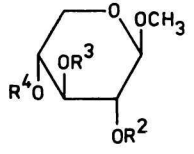
Partially acetylated saccharides are important intermediates in chemistry of saccharides, *e.g.* in synthesis of oligosaccharides [1]. They are formed also in hydrolysis of fully acetylated compounds [2] as well as in acetolysis [3] of natural polysaccharides. Some polysaccharides, *e.g.* glucuronoxylans, components of biologically important substances, are partially acetylated in natural state [4]. Therefore, structural analysis of partially acetylated saccharides is significant also from the point of view of determination of structures of polysaccharides and their conjugates.

Mass spectrometric behaviour of methyl *O*-acetyl-β-D-xylopyranosides was studied by electron impact (EI), methane and ammoniacal chemical ionization (CI) as well as by collision activated (CA) mass spectrometry [5]. While EI and

CI mass spectra do not provide complete information about the position of *O*-acetyl groups, the number and positions can be determined unambiguously from the CA spectra of $[M-\cdot OCH_3]^+$ ions. The need for a simple method with relatively cheap and available instrumentation for analysis of mixtures of partially acetylated saccharides led us to GC—MS study of fully derivatized methyl *O*-acetyl-β-D-xylopyranosides. Trifluoroacetyl esters (TFAc), having excellent gas chromatographic properties, have been chosen for preparation of volatile derivatives. By trifluoroacetylation of *O*-acetyl-1,6-anhydro-β-D-glucopyranoses, we suggested a simple method for determination of partially acetylated 1,6-anhydrohexopyranoses using gas chromatography in combination with mass spectrometry [6]. The present paper is a continuation of the aforementioned studies on *O*-acetyl derivatives of saccharides. The model compounds investigated are reviewed in Table 1.

Table 1

Compounds investigated



I–VIII

Compound	R^2	R^3	R^4	Compound	R^2	R^3	R^4
I	TFAc	TFAc	TFAc	*V*	Ac	Ac	TFAc
II	Ac	TFAc	TFAc	*VI*	Ac	TFAc	Ac
III	TFAc	Ac	TFAc	*VII*	TFAc	Ac	Ac
IV	TFAc	TFAc	Ac	*VIII*	Ac	Ac	Ac

Ac — acetyl, TFAc — trifluoroacetyl.

Experimental

Methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside *VIII* was prepared according to the literature [7]. The compounds *I*—*VII* were prepared by addition of trifluoroacetic anhydride (0.2 cm^3) to methyl *O*-acetyl-β-D-xylopyranoside (3 mg), the synthesis of which was described in [8—12].

Separation of compounds *I*—*VIII* was performed on a JGC 20 K gas chromatograph with a stainless-steel column (2 m long, 3 mm i.d.) of 3 % OV-225 on Supelcoport. Column heating was isothermal for 8 min at 120 °C, then programmed so that the

temperature increased to 220 °C by 3 °C min^{-1} Injection port temperature 200 °C, separator temperature 230 °C, flow rate of the carrier gas (helium) 30 $cm^3 min^{-1}$ The RRT values of the individual positional isomers are the average of three measurements of mixtures of all model compounds *I—VIII*. The maximum error in measurement of RRT was 2 %. The mass spectra were recorded on a JMS-D 100 spectrometer at emission of ionizing electrons 300 μA and energy 23 eV when connected with GC, or at 70 eV when using direct-inlet system. In this case the evaporation temperature of compounds ranged from 80 °C to 130 °C in dependence on their volatility. The ionization chamber temperature was 230 °C.

To test the differences in the spectra, first normalization was carried out according to the relationship $\Sigma_{81} \sqrt{Pn} = 1$. (Pn — peak height.) Discrepancy factor *D* of two spectra compared was calculated from the sum of differences of absolute values of normalized peaks [13].

Results and discussion

All positional isomers *I—VIII* were separated on a column packed with OV-225. The relative retention times, related to fully acetylated compound *VIII*, are presented in Table 2. The conventional 70 eV spectra of compounds *I—VIII*

Table 2

Relative retention times (RRT) of methyl *O*-acetyl-*O*-trifluoroacetyl-β-D-xylopyranosides *I—VIII*

Compound	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Position of OAc	—	2	3	4	2,3	2,4	3,4	2,3,4
RRT	0.18	0.44	0.42	0.40	0.71	0.70	0.65	1.00

are presented in Table 3. The 23 eV spectra differ from the 70 eV spectra only quantitatively in some peak intensities. The bottom parts of the spectra with $m/z < 80$ contain very intensive peaks of ions CH_3CO^+ with m/z 43 and CF_3^+ with m/z 69. Due to instability of their intensities, depending on conditions of measurements, the spectra of compounds *I—VIII* were normalized from m/z 81. Also the ions with m/z 114, 103, 97, and 95 of high intensities originate from acetic and/or trifluoroacetic acids. They represent the ions $CF_3COOH^{+\cdot}$ $CH_3C\overset{+}{O}\overset{H}{O}COCH_3$, CF_3CO^+, and $[CF_3COOH - \cdot F]^+$ As these ions do not contain fragments of the saccharide skeleton, the peaks with m/z 114, 103, 97, and 95 were not considered the main peak of the spectra even in the case of highest intensity.

Mass spectrometric EI fragmentation of methyl 2,3,4-tri-*O*-acetyl-β-D--xylopyranoside *VIII* was studied in detail first by *De Jongh* and *Biemann* [14]

Table 3

Mass spectra (70 eV) of methyl *O*-acetyl-*O*-trifluoroacetyl-β-D-xylopyranosides *I*—*VIII*

m/z	I_r/%							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
421	2							
367		2		7				
343							1	
339	5							
338		6		9				
313					1	3	3	
309	2							
308			1					
307	3		2					
285		2			1			
284						9	6	
279	100	3	15	2				
278	98	13	100	6				
265	50		2					
259								1
255		1						
253		2			4		3	
252	9							
242		2		2				
225	16	40	6	100	27	20	50	
224		10	2	2	100	100	100	
211	3	4	2	2	4	12	43	
209	6						2	
199								1
196	14						3	
194	4			2		8	18	
193	61	2	14	8		5	22	
188								1
183	12	10	2	2	3	23	10	
182		100	7	30	44	52	80	
181	8	7	1	8	1	5		
171	4	3	2	2	6	77	22	15
170	30		6	11	27		10	50
169		1	2	2	3		2	
167	12	1	3	2			6	
165	72	1	10	2				
157	41	8	4	3	3	12	1	34
155	4			2				
153	32	22	3	9	4	35	1	
145	4							7
144					9			3
141	2	2	1		3		2	

Table 3 (Continued)

m/z	I_r/%							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
140	38	6	9		5			
139	8	1	2	2		13	3	6
137			7					
135	6							
129	44	9	5	2	11	35	10	14
128			1	2	24	47	14	100
127	6		1		4		4	8
125	15	8		3		5		
117	15		2				2	
116		1			1	2	7	
115	9	3	1	2	4	26	9	43
114		10	4	11	16	54	26	
112	18	4	3		3		1	
111	21	14	4	6	8	26	7	9
109	31	3	4					
103		68	10	7	96	79	31	29
102					53		3	27
101	4							
100						5	2	8
99	17	11	4	6	13	23	11	13
97	84	87	26	19	71	130	75	17
95	15	15	10	11	23	100	47	
89	20		3	2				
87		4	3	4	7	35	41	22
86	16	23	4	9	16	56	45	34
85	11	6	9	6	30	21	25	28
83	8	4	1	2		2	2	
82							2	1
81	24	6	3	3	3	2	3	

using analogues containing COC^2H_3 groups, and then by ourselves [5] after detailed study of metastable transitions. These proceed in four fragmentation series *A*, *C*, *H*, and *F* [5] (the *Kochetkov* nomenclature [15]). Fragmentation of the trifluoroacetyl analogue *I* obeys, in principle, the suggested fragmentation scheme. However, it is simplified, as with trifluoroacetyl esters the fragmentation analogous to elimination of ketene does not proceed. The representative ions of the *A* series are $[M - \cdot OMe]^+$, $[M - \cdot OMe - TFAcOH]^+$, and $[M - \cdot OMe - 2TFAcOH]^+$ at *m/z* 421, 307, and 193. The ions F_1 are at *m/z* 265, H_1 at *m/z* 252. The ions of the *C* series are represented by the fragments $[M - \cdot OTFAc]^+$ at *m/z* 339 and $[M - \cdot OTFAc - HCOOMe]^+$ at *m/z* 279. These, after elimination of the TFAcOH molecule, afford the ions with *m/z* 225

and 165 and after elimination of the CO molecule the ions with m/z 137. The peak with m/z 278 represents the $[M - HCOOMe - TFAcOH]^+$ ions.

The compounds *II*—*VII*, containing both Ac and TFAc groups, retain the features of both derivatives in fragmentation. In fragmentation series the eliminations of acetic acid and ketene are overlapped by those of the trifluoroacetic acid molecule.

In fragmentation of methyl mono-*O*-acetyl-di-*O*-trifluoroacetyl-β-D-xylopyranosides the ions of the *A* series at m/z 367, 253, 211, and 193 are distinguished. The series *C* dominates and is reflected in $[M - HCOOMe]^{+\cdot}$ ions at m/z 338, $[M - HCOOMe - AcOH]^+$ at m/z 278, $[M - HCOOMe - \cdot OTFAc]^+$ at m/z 225, and $[M - HCOOMe - TFAcOH - CH_2CO]^{+\cdot}$ at m/z 182. The ions F_1 occur at m/z 211 and 157. The intensity ratios of the ions of *C* series at m/z 182, 225, and 278 can be utilized for unambiguous determination of positions of the acetyl group in methyl mono-*O*-acetylpentapyranosides after additional trifluoroacetylation (Table 4).

Table 4

Characteristic ratios of intensities of methyl mono-*O*-acetyl-di-*O*-trifluoroacetyl-β-D-xylopyranosides

Compound	Positions of OAc group	Peak intensity/%		
		m/z 182	m/z 225	m/z 278
II	2	100	40	13
III	3	7	6	100
IV	4	30	100	6

Table 5

Values of discrepancy factors *D* of pairs of spectra of compounds *V*—*VII*

Pair of spectra of compounds	Positions of OAc groups	Discrepancy factor *D*
V *V*	2,3 2,3	0.20
VI *VI*	2,4 2,4	0.31
VII *VII*	3,4 3,4	0.12
V *VI*	2,3 3,4	0.36
V *VII*	2,3 2,4	0.32
VI *VII*	2,4 3,4	0.36

In fragmentation of methyl di-*O*-acetyl-mono-*O*-trifluoroacetyl-β-D-xylopyranosides *V*—*VII* the formation of ions of the *C* series dominates: $[M - HCOOMe]^{+\cdot}$ at m/z 284, $[M - HCOOMe - AcOH]^+$ at m/z 224, $[M - HCOOMe - AcOH - CH_2CO]^{+\cdot}$ at m/z 182, $[M - HCOOMe - TFAcOH]^{+\cdot}$

at *m/z* 170, and $[M - HCOOMe - TFAcOH - CH_2CO]^{+\cdot}$ at *m/z* 128. Mass spectra of all three positional isomers *V—VII* are qualitatively the same. In order to evaluate the effect of positional isomerism on quantitative differences in the spectra, the discrepancy factors of pairs of isomers were calculated and compared to those from repeated measurements of each isomer (Table 5).

The positional isomerism of methyl di-*O*-acetyl-*O*-trifluoroacetyl-β-D-xylopyranosides *V—VII* does not contribute to differences in the spectra more than the conditions of measurements. Due to this fact, we do not take the quantitative differences in the spectra of the isomers *V—VII* for the only sufficient criterion for determination of positional isomerism of this type of compounds. In GC—MS analysis the positions of OAc groups are confirmed by the RRT values, calculated relative to fully acetylated compound *VIII* (Table 2).

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