Identification of *O*-acetyl-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranoses by gas chromatography and mass spectrometry

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Trifluoroacetyl derivatives of all theoretically possible isomers of *O*-acetyl-1,6-anhydro-β-D-glucopyranoses have been prepared and studied by electron-impact mass spectrometry as well as by combination with gas chromatography. On the basis of the knowledge obtained on fragmentation, from the intensities of three characteristic fragments, namely $m/z = 81$, 131, and 194, a procedure for simple identification of the compounds of this type has been elaborated.

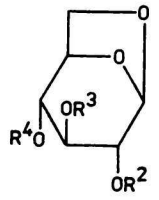
The method is suitable for following hydrolyses of acetates of 1,6-anhydrohexopyranoses by means of gas chromatography and mass spectrometry.

Синтезированы и исследованы с помощью масс-спектрометрии электронного удара и ее комбинации с газовой хроматографией трифторацетильные производные всех теоретически возможных изомеров *O*-ацетил-1,6-ангидро-β-D-глюкопиранозы. На основе результатов, полученных при фрагментации, а именно, интенсивностей трех характеристических фрагментов с m/z 81, 131 и 194, разработана процедура для легко проводимой идентификации соединений этого типа.

Предлагаемый метод пригоден для изучения гидролиза ацетатов 1,6-ангидрогексопираноз с помощью газовой хроматографии и масс-спектрометрии.

In structural analysis of natural compounds trifluoroacetyl esters [1] have found wide application as volatile derivatives. Due to their excellent properties utilizable in gas chromatography [1] as well as to the present improved methods of preparation on micro-scale [2], they have contributed to extensive use of qualitative and quantitative analysis of saccharides by the combined technique of gas chromatography and mass spectrometry [1, 3].

Additional trifluoroacetylation was utilized also in the study of partial hydrolysis of acyl 1,6-anhydro-β-D-glucopyranoses with purpose to prepare saccharides with free hydroxyl groups in various positions [4]. The aim of the present work, which is a continuation of the foregoing one, is to afford a simple method for determination of partially acetylated derivatives of 1,6-anhydro-β-D-glucopyranose, based on monitoring the chosen ions in gas chromatography—mass spectrometry. The model compounds investigated are the following



I–X

	R^2	R^3	R^4		R^2	R^3	R^4
I	TFAc	TFAc	TFAc	*VI*	Ac	TFAc	Ac
II	Ac	TFAc	TFAc	*VII*	TFAc	Ac	Ac
III	TFAc	Ac	TFAc	*VIII*	Ac	Ac	Ac
IV	TFAc	TFAc	Ac	*IX*	TFAc	TDAc	TFAc
V	Ac	Ac	TFAc	*X*	TFAc	Me	TFAc

TFAc — trifluoroacetyl, Ac — acetyl, TDAc — trideuteroacetyl, Me — methyl.

Experimental

Preparation of *O*-acetyl-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranoses *I*—*VIII* is described in [4]. 3-*O*-Trideuteroacetyl-1,6-anhydro-β-D-glucopyranose (*IX*), m.p. = = 110—111 °C, $[\alpha]$(D, H_2O) = −61.9°, was prepared similarly as 3-*O*-acetyl-1,6-anhydro-β-D-glucopyranose [5], except for using acetic ^2H-anhydride for acetylation. 3-*O*-Methyl-2,4-di-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranose (*X*), m.p. = 65—66 °C, $[\alpha]$(D, acetone) = −64.2°, was prepared according to [6].

Separation of compounds *I*—*VIII* was performed on a JGC 20K chromatograph, choosing a stainless-steel column (2 m long, 3 mm i.d.) of Supelcoport coated with 3 % OV 225 stationary phase as the most suitable one. Heating of the column was programmed to hold the temperature of 120 °C for 6 min, then increase it by 2 °C min^{-1} to 220 °C. The separator temperature was 230 °C, the injection port temperature 220 °C, and the flow rate of the carrier gas (helium) was 30 cm^3 min^{-1}. Good separation was achieved also on the SP-2340 column at the same working conditions. The values of relative retention times of the individual positional isomers were calculated relative to the fully acetylated compound *VIII* as the average of 5 measurements of mixtures of all model compounds *I*—*VIII*.

Mass spectra were measured on a JMS D-100 spectrometer at 300 μA emission current in combination with GC at the energy of electrons 23 eV and in direct inlet at 70 eV and 12 eV. In the latter case the evaporation temperature ranged from 80 °C to 150 °C in dependence on the volatility of compounds. Fragmentation of the chosen metastable ions in the first field free region was monitored by scanning the accelerating voltage, using an MS-MT-01 detector. Elemental composition of ions with m/z = 81, 97, and 131 was measured on the same apparatus at resolution 5000.

In order to test the differences in the spectra, they were first normalized according to

the relationship $\Sigma_{81}\sqrt{Pn} = 1$ (Pn — peak height). The discrepancy factor D of two spectra compared was calculated from the sum of differences of the absolute values of the normalized peaks [7].

The mixture of all positional isomers was prepared by hydrolysis of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose (1 g) with methanolic HCl (10 cm^3, 0.4 %) at room temperature. Hydrolysis was stopped after 8 h by neutralization of the mixture with Amberlite IRA-402 (HCO_3^-).

Results and discussion

Almost all positional isomers of compounds *I*—*VIII* were well separated by gas chromatography on OV-225 and SP-2340 columns. Trifluoroacetylated 2,4- and 3,4-di-*O*-acetyl-β-D-glucopyranoses *VI* and *VII* were the only pair not separable on the packed columns. In spite of that, pure spectra of both isomers can be obtained from the mixed peaks by measuring the bottom ascending and descending sections. Relative retention times obtained on the OV-225 column, related to fully acetylated compound *VIII*, are presented in Table 1.

Table 1

Relative retention times (RRT) of *O*-acetyl-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranoses

Compound	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
Position of OAc	—	2	3	4	2,4	3,4	2,3	2,3,4
RRT	0.38	0.45	0.51	0.59	0.71	0.72	0.77	1.00

Conventional mass spectra (70 eV) of compounds *I*—*VIII* are presented in Table 2. The 23 eV mass spectra differ from those of 70 eV only negligibly in their peak intensities. By decreasing the energy of ionizing electrons to 12 eV a small increase in peak intensities of ions formed by primary and secondary fragmentations of molecular ions was achieved. In spite of that the difference in the 70 eV and 12 eV spectra is not very significant, the calculated D values vary for the individual isomers in the range of 0.1—0.3.

Reproducible peaks of molecular ions were not observed in any spectra. The bottom parts of the spectra with $m/z < 80$ contain very intense peaks of ions CH_3CO^+ with $m/z = 43$, CF_3^+ with $m/z = 69$, and $CH_3COOH^{\bullet+}$ with $m/z = 60$, which are formed from the present or released acetic acid, trifluoroacetic acid, and their anhydrides, respectively. Due to instability of their intensities in dependence on the conditions of measurement (the way of sample preparation, temperature program, energy of electrons in measurement, *etc.*), the spectra of compounds (Table 2) were normalized from $m/z = 81$ upwards. The ions in this

Table 2

Mass spectra (70 eV) of compounds *I*—*VIII*

m/z	I_r/%							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
405	2.1							
351			0.7	1.4				
337			3.5					
336	12.9		2.1					
318			2.1					
300			2.8					
299			3.5					
297					10.4	3.4	3.2	
291	3.8							
290	4.8							
284				3.8	8.8		6.0	
283		7.9	5.6	24.0	18.4	3.4	49.1	
282						1.7	3.7	
279		3.4						
265	10.7		3.5					
255					3.2			
254					4.8	1.7	2.7	
253					3.2			
252	2.1							
243								2.6
241		2.3			5.6	1.7	2.7	
240		12.5	3.5		44.8	6.8	23.8	
239					4.0		2.3	
237					2.4		1.8	
236				1.4	9.6		1.8	
231	2.1							
230						1.7		1.7
229					2.4	8.5	4.1	10.4
228					1.6	1.7		4.3
225					2.4			
223	3.8	2.3			1.6			
222	4.8		4.9		5.6		2.7	
221	1.9	5.7	13.3					
212					4.8	1.7	3.2	
211			4.2	7.2	24.8	6.8	25.2	
209			2.1					
198	3.8		3.5					
196	3.2							
195	8.1	14.8	7.7	4.1	15.2	1.7	7.3	
194	7.0	70.4	14.7	2.4	100	11.9	18.3	
193	19.3	29.5	13.3	10.0	20.0	12.7	4.1	

Table 2 (Continued)

m/z	I_r/%							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
187					1.6	4.2	2.3	5.6
186					4.0	2.5	1.4	16.5
185	2.1	2.3			1.6			
183	1.6							
182					2.4	2.5	1.4	
181	5.9	2.3	4.9		1.6	1.7	1.8	
178	7.0		4.2					
177	80.9	9.1	46.8		4.0			
170	5.9							2.6
169	24.7	11.4	23.8	3.1	9.6		2.3	
168					2.4			
167	8.6	6.8	11.2	2.1	4.0	2.5	3.7	
166	5.9	32.9	4.9	1.4	20.0	23.7	11.5	
165	8.6	4.5	6.3	1.0	2.0			
158								6.1
157	18.3	9.1		1.2	4.0	4.2	4.6	63.5
156			2.1					
155	2.1							
154			2.8		1.6			
153	15.6	12.5	20.9	5.1	16.8	4.2	2.3	
152	2.1							
151	1.6							
150	3.2							
149		3.4		1.7	4.8			
148				1.0				
145					1.6			12.2
144					15.2	2.5	2.3	13.0
143					6.4	2.1	3.2	6.1
142					1.6	1.7		
141	4.8	6.8	6.3	1.4	15.2	11.0	2.7	12.6
140	7.5	2.3	6.3	0.7	6.4	3.4	3.7	20.0
139	8.1	3.4	11.2	2.1	12.0		8.7	8.7
137	3.8	3.4	4.9					
135	3.2							
132			7.7		2.4		2.7	
131	6.4		100	1.4	39.2	6.8	42.2	3.5
130	1.6			1.0				
129								3.5
128		3.4		1.0	4.0	2.5	1.8	2.2
127	3.8	50.0	7.0	2.4	31.2	14.4	9.2	14.8
126		4.5			68.0	4.2	2.3	9.6
125	10.7	7.9	14.7	1.7	7.2	2.5	1.8	
121	1.6							

Table 2 (Continued)

m/z	I_r/%							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
119			4.9	1.4				
117			2.8					
116							2.7	5.2
115	7.0	7.9	5.6	2.4	10.4	5.9	12.8	55.6
114	20.4	20.4	14.0	6.9	7.2	5.9	2.3	3.5
113	3.2		3.5	1.0	3.2	1.7	1.8	4.3
112	2.1		2.8	1.0	14.4	11.0	9.2	27.8
109	7.5	11.4	6.3	1.7	5.6	1.7		
104			3.5					
103			36.4		21.6	6.8	15.6	26.9
102		14.8		2.4	96.0	6.8	6.9	37.4
101	4.3	6.8	6.3	4.8	10.4	5.1	9.6	7.8
100	1.6							
99	8.6	13.6	7.7	3.4	12.8	14.4	7.3	20.9
98	3.8	10.2	7.0	2.1	40.8	22.9	10.1	75.6
97	133.8	112.5	92.3	39.8	68.0	17.8	22.0	32.6
96	2.7		4.9	1.7	5.6	1.7	2.3	
95	58.4	56.8	30.8	28.5	13.6	9.3		
94	5.4	5.7	5.6	2.7				
93	3.8							
91	4.8			1.4				
89	5.9	9.1	18.2	2.1	15.2	1.7	4.1	2.6
87	3.8	3.4	5.6	1.4			1.4	
86	34.9	44.3	26.6	13.7	15.2	6.8	17.0	5.2
85	5.4	9.1	7.7	4.1	8.8	3.4	6.0	8.3
84	3.2		4.2		3.2	2.5	1.8	
83	9.7	7.9	14.0	3.1	7.2	2.5	3.7	
82	6.4	6.8	7.7	4.8	8.8	7.6	6.4	8.7
81	100	100	79.0	100	73.6	100	100	100

region of the spectrum originate from the saccharide skeleton of the derivative investigated. Only the ions with $m/z = 97$ contain a mixture of hydroxypyronium and CF_3CO^+ ions. For this reason they were not considered the main peak in the spectrum of compounds *I* and *II*. The spectra adjusted in this way (from $m/z = 81$ upwards and without $m/z = 97$) were evaluated also from the aspect of reproducibility and similarity (factor *D*).

The spectra of compounds *I* and *VIII* are qualitatively identical with those published for 2,3,4-trifluoroacetyl-1,6-anhydro-β-D-galactose and 2,3,4-tri-*O*--acetyl-1,6-anhydro-β-D-idose [8]. Compounds *II*—*VII*, remarkable for the presence both of acetyl and trifluoroacetyl groups, retain the features of com-

pounds *I* and *VIII* in fragmentation. In the region of higher masses, for determination of molecular mass there are the peaks of $[M - HCOO^{\bullet}]^+$ ions, characteristic of 1,6-anhydro grouping of hexopyranose derivatives [8]. In fragmentation series the eliminations of acetic acid and ketene are overlapped with those of trifluoroacetic acid.

The discrepancy factor, calculated with compounds *I*—*VII* relative to 2,3,4--tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (*VIII*) (*D* for *I*—*VII*: 1.37, 1.19, 1.29, 1.17, 1.02, 0.78, resp. 0.96.), indicates considerable differences in the spectra of the individual isomers. The *D*-factor may be utilized in comparison of spectra by the data system.

For determination of the positional isomerism of acetyl and trifluoroacetyl groups, respectively, by simple interpretation of the spectra or mass fragmentography, the ions with $m/z = 81$, 131, and 194 were chosen. The criterion was the mutual ratio of relative intensities of these ions, graphically illustrated in Fig. 1.

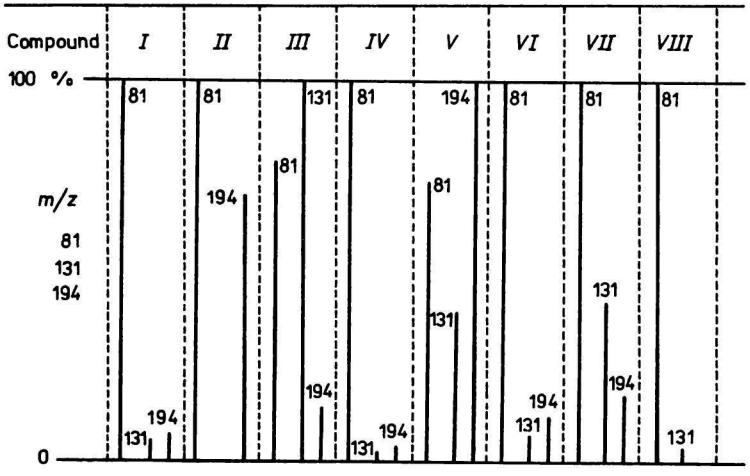
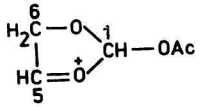


Fig. 1. Graphical illustration of relative intensities of significant ions.

The fragmentation processes leading to pyronium ions with $m/z = 81$ and elemental composition C_5H_5O are mentioned in [8]. The ions $[C_5H_7O_4]^+$ with $m/z = 131$ in the spectrum of the peracetylated compound *VIII* are of low intensity. Introduction of TFAc groups into the molecules of *O*-acetyl-1,6-anhydro-β-D-glucopyranoses *II*—*VII* increased considerably the occurrence of these ions. The most intensive formation of the $[C_5H_7O_4]^+$ ions was observed in the case of 3-*O*-acetyl-2,4-di-*O*-trifluoroacetyl derivatives. The TFAcO groups, attached to the positions C-2 and/or C-4, by their electron-accepting effects

weaken the C-1—C-2 and C-4—C-5 bonds so that they are split. Simultaneously, the •OAc radical is rearranged from C-3 to C-1 to give a stable ion with $m/z = 131$ of the following structure



The structure of the ions with $m/z = 131$ was proved by high-resolution mass spectrometry as well as by measuring the spectra of 3-*O*-trideuteroacetyl-2,4--di-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranose (*IX*) and 3-*O*-methyl-2,4--di-*O*-trifluoroacetyl-1,6-anhydro-β-D-glucopyranose (*X*). The ions discussed above occur in the case of *IX* at $m/z = 134$, and in the case of *X* represent the base peak in the spectrum with $m/z = 103$. The characteristic ions with $m/z = 194$ originate from the ions of $m/z = 240$ (after elimination of the HCOOH molecule), as it was proved by measurement of the metastable transition. The latter represent in the case of di-*O*-acetyl derivatives *V*—*VII* the ion radicals $[M - Ac_2O]^{\bullet+}$ In the case of monoacetyl derivatives *II*—*IV* the ions with $m/z = 240$ are formed after elimination of the mixed trifluoroacetic acetic anhydride from the molecular ions.

The ratio of relative intensities of the ions with $m/z = 81$, 131, and 194 (Fig. 1) was, together with relative retention times, tested for characterization of the mixture formed on hydrolysis of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose. In the mixture all positional isomers of *O*-acetyl-1,6-anhydro-β-D--glucopyranose were identified unambiguously.

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