MASS SPECTRA OF PERMETHYLATES OF SPIROSTANOL TETRAOSIDES

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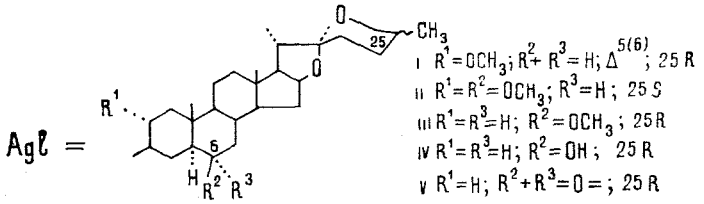
The design of the new MKh 1310 mass spectrometer with double focusing permits the recording of the spectra of sparingly volatile natural compounds with molecular weights of up to 1700 and, in particular, the permethylates and peracetates of spirostanol and furostanol saponins. The results are given of an investigation of the mass spectra of five spirostanol tetraosides in the form of their permethylates. The spectra contain the peaks of the molecular ions and of the products of their decomposition at the glycosidic bonds. The most important feature of the spectra is the fragmentation of the skeletons of the terminal saccharide residues starting directly from $M^{+\bullet}$ in directions close to the directions of decomposition of the nonreducing unit of permethylates of oligosaccharides. The origin of the ions $(M-191)^+$, $(M-175)^+$, $(M-162)^+$, $(M-147)^+$, $(M-131)^+$, $(M-118)^+$, and $(M-102)^+$ arising this way have been confirmed by measurements of their elementary compositions.

The volume of structural-analytical information arising in an analysis of the mass spectra of natural oligomers depends on a knowledge of the laws of fragmentation of these compounds, and also on the perfection of the methods of determining their molecular weights. The obstacles arising because of the low stability to electron impact, the low volatility, and the thermolability of oligomers have been in the process of being overcome in the last few years not only by the use of "mild" methods of ionization but also through an improvement in the designs of the ion sources for electron impact together with an increase in the sensitivity of mass spectrometers. These requirements are satisfied by the MKh-1310 mass spectrometer. The use of a system for direct introduction of the sample into the instrument has permitted the recording of the peaks of molecular ions of esterified and etherified spirostanol tetraosides (peracetates and permethylates) with molecular weights of up to 1700, and also of unsubstituted spirostanol biosides. The spectra obtained are distinguished by a high informative value because of the presence in them of fragments formed as a result of the cleavage of the glycosidic bonds or of the specific breakdown of the terminal pyranose rings (in the case of the permethylates). The origin of the main fragments has been confirmed by measuring their elementary compositions under high-resolution conditions.

We give the mass-spectrometric characteristics of five permethylates of spirostanol tetraosides: karatavioside A (I) [1], turoside A (II) [2], erubroside B (III) [3], and the 6-demethyl (IV) and 6-keto (V) derivatives of the permethylate of (III). These compounds were isolated and their structures were elucidated in the laboratory of glycoside chemistry of the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR. The mass numbers, relative intensities, and assignment of the main fragments in the mass spectra of (I)-(V) are given in Table 1. We shall consider only the fragments arising in the breakdown of the oligosaccharide moieties of the molecular ions (I-V). They can be divided into two main groups: ions including part of the oligosaccharide chain + the aglycone (A), and fragments formed from the sugar residue (B). Breakdown products of the aglycones (Agl) are not included in Table 1.



I $R^1=OCH_3$; $R^2+R^3=H$; $\Delta^{5(6)}$; 25R
II $R^1=R^2=OCH_3$; $R^3=H$; 25S
III $R^1=R^3=H$; $R^2=OCH_3$; 25R
IV $R^1=R^3=H$; $R^2=OH$; 25R
V $R^1=H$; $R^2+R^3=O=$; 25R

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TABLE 1. Mass Number (m/e), Relative Intensities (%), and Origins of the Main Fragments of the Mass Spectra of the Spirostanol Tetraosides (I-V)

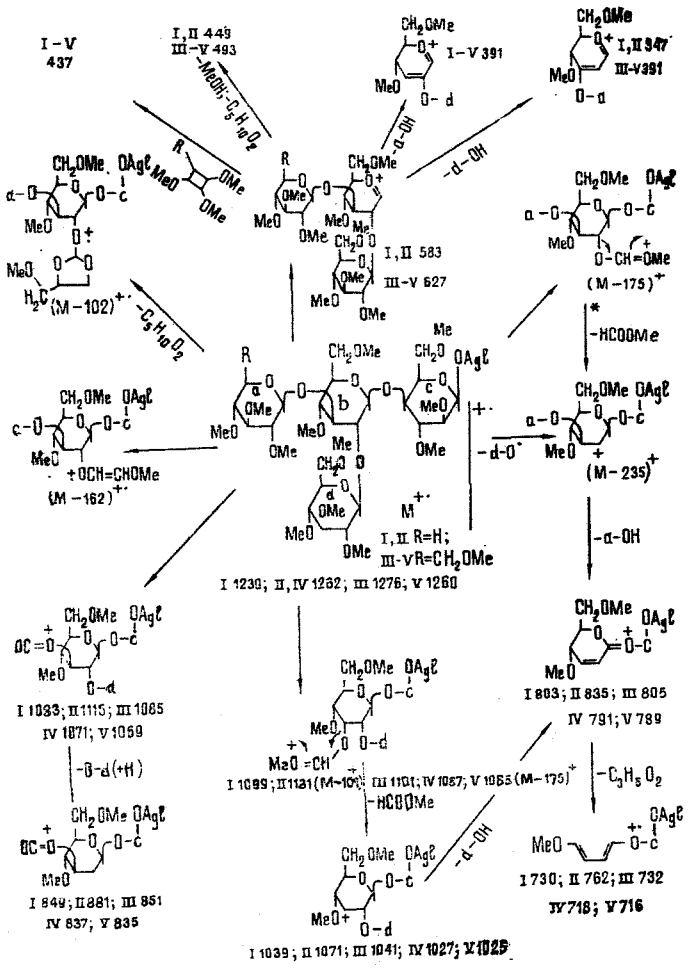
No.	I		II		III		IV		V		Origins of the fragments
	m/e	%	m/e	%	m/e	%	m/e	%	m/e	%	
1	1230	0,2	1262	0,2	1276	0,7	1262	0,2	1260	0,7	M^+
2	1199	0,1	1231	0,1	1245	0,1	1231	0,1	1229	0,1	$(M-OMe)^+$
3	1198	0,1	1230	0,1	1244	0,1	1230	0,1	1228	0,1	$(M-MeOH)^+$
4	1166	0,2	1198	0,1	1212	0,2	1198	0,1	1196	0,1	$(M-2MeOH)^+$
5	1153	0,1	1185	0,1	1199	0,1	1185	0,1	1183	0,1	$(M-MeOH-\dot{C}H_2OMe)^+$
6	1128	0,2	1160	0,2	1174	0,2	1160	0,1	1158	0,2	$*(M-C_5H_{10}O_2)^+$
7	1121	0,3	1153	0,3	1167	0,1	1153	0,1	1151	0,1	$(M-2MeOH-\dot{C}H_2OMe)^+$
8	1112	0,1	1144	0,1	—	—	—	—	—	—	$(M-C_5H_{10}O_3)^+$
9	1099	0,1	1131	0,1	1101	1,5	1087	0,7	1085	0,7	$*(M-OCHCHCH=CHOMe)^+$ (R, OMe)
10	1083	0,1	1115	0,1	1085	0,1	1071	0,1	1069	0,2	$*(M-RCH_2CH-CH-\dot{C}H)^+$ (OMe, OMe, OMe)
11	1068	0,1	1100	0,1	1114	0,2	1100	0,1	1098	0,2	$*(M-162)^+$
12	1055	0,7	1087	0,6	1101	1,5	1087	0,7	1085	0,7	$*(M-175)^+$
13	1039	0,3	1071	0,3	1041	1,3	1027	0,9	1025	0,6	$*(M-a\text{-}\dot{O})^+$
14	1025	0,1	1057	0,1	1071	0,1	—	—	1055	0,1	†(12$-CH_2O)^+$
15	1011	0,1	1043	0,2	1057	0,1	1043	0,1	1041	0,1	$(M-d)^+$
16	1009	0,1	1041	0,2	1055	0,1	1041	0,1	1039	0,2	$(12-Me_2O)^+$
17	995	0,4	1027	2,0	1041	1,3	1027	0,9	1025	0,6	$*(M-235)^+$
18	979	0,1	1011	0,1	1025	0,1	1011	0,1	—	—	$(15-MeOH)^+$
19	963	0,2	995	0,8	1009	0,5	995	0,3	993	0,3	$(17-MeOH)^+$
20	—	—	—	—	869	0,2	855	0,2	—	—	?
21	849	0,3	881	0,3	851	0,3	837	0,1	835	0,4	$*[(10-O\text{-}d)+H]^+$
22	786	0,1	786	0,3	830	0,1	830	0,1	830	0,2	$*[M-(AglO+H)]^+$
23	819	0,1	851	0,2	821	0,4	807	0,1	805	0,2	$(15-a\text{-}OH)^+$
24	803	0,8	835	1,0	805	1,4	791	0,8	789	1,5	$*(17-a\text{-}OH)^+$
25	773	0,3	805	0,4	775	0,8	761	0,5	759	0,5	$(24-CH_2O)^+$
26	771	0,3	803	0,4	773	0,8	759	0,7	757	1,0	$(24-MeOH)^+$
27	730	0,6	762	0,7	732	2,3	718	1,0	716	1,3	$*(24-C_3H_5O_2)^+$
28	677	0,3	709	0,3	679	0,2	665	0,3	663	0,5	$AglO-c-O-CH=\overset{+}{O}H$
29	675	0,3	707	0,3	677	0,3	663	0,3	661	0,4	$AglO-c-\overset{+}{O}=CO$
30	649	0,5	681	0,7	651	0,5	637	2,5	635	0,5	$[(AglO-c-OH)+H]^+$
31	543	6,0	583	6,0	627	8,5	627	3,0	627	2,2	$*(M-AglO\text{-}c\text{-}O)^+$
32	551	1,0	551	0,9	595	0,5	595	1,0	595	0,3	$(31-MeOH)^+$
33	519	2,1	519	3,0	563	6,0	563	3,0	563	1,5	$(31-2MeOH)^+$
34	473	1,5	473	1,3	517	2,5	517	1,3	517	0,4	$(33-Me_2O)^+$
35	449	1,9	449	2,3	493	6,0	493	3,0	493	1,4	$*(32-C_5H_{10}O_2)^+$
36	437	1,0	437	1,5	437	3,5	437	4,0	437	1,0	$*(31-RC_4H_4(OMe)_3)^+$
37	427	100	459	94	429	62	415	26	413	57	$(Agl-\dot{O}H)^+$
38	391	93	391	100	391	100	391	72	391	32	$*(31-a\text{-}OH)^+$
39	347	2,3	347	3,5	—	—	—	—	—	—	$*(31-d\text{-}OH)^+$
40	219	9,0	219	19	219	95	219	33	219	15	a^+ (III—V); d^+(I—V)
41	187	53	187	61	187	89	187	100	187	100	$(40-MeOH)^+$
42	175	14	175	31	—	—	—	—	—	—	a^+(I, II)
43	155	18	155	30	155	62	155	44	155	27	$(41-MeOH)^+$

*Ions the origin of which is shown in the Scheme and has been confirmed by measurements of elementary composition.
†The first figure within a pair of parentheses is the serial number of an ion from Table 1.

Ions of Group A. In a study of the mass spectra of permethylates of oligosaccharides [4-9] it was found that in addition to the cleavage of the glycosidic bonds and the ejection of the substituents in the form of $CH_3\dot{O}$, CH_3OH, and $\dot{C}H_2OCH_3$, rearrangement processes take place that are accompanied by the elimination of parts of the skeleton of a monosaccharide unit.

The previous paper most closely related to the subject investigated in our work is one by Komori et al. [10], in which the mass spectra of a large series of permethylates and peracetates of spirostanol and furostanol glycosides are considered. Here, tetraoside permethylates are represented only by a diosgenin glycoside, and this has a different type of branching of the oligosaccharide chain. The spectrum of this compound shows the elimination from $M^{+\cdot}$ of a neutral particle in the form of a 2,3,4-tri-O-methylrhamnose molecule. In the spectra of (I-V), however, one of the terminal pyranose rings is eliminated in the form of the radical α—$\dot{O}$ or d—$\dot{O}$, and in the subsequent stage of decomposition the molecule of a tri- or tetramethylpyranoside respectively (α—OH or d—OH, Scheme 1) is split off.

The most important and fully reproducible feature in the spectra of (I-V) is the presence in the region of high mass numbers of fragments formed as the result of the cleavage of C—C and C—O bonds of the terminal pyranose rings. The highest intensity among the ions of this type is shown by the peaks of the rearrangement ions $(M - 175)^+$, the mechanism of the origin of which was first described by O. S. Chizhov, L. A. Polyakova, and N. K. Kochetkov [4] for the case of the spectra of disaccharide methyl ethers. Subsequently, these peaks were found, for example, in the spectra of permethylates of biosides and triosides of oleanolic acid [11]. As a result of transitions, confirmed by metastable peaks, with the isolation of a methyl formate molecule the $(M - 175)^+$ ions are converted into $(M - 235)^+$ ions.



Scheme 1

An analysis of our results based also on an estimate of the relative intensities of the $(M - 175)^+$ peaks in the spectra of compounds with different types of glycosidic bond [8, 11] indicates that the $(M - 175)^+$ and $(M - 235)^+$ ions are formed largely at the expense of the glucopyranose unit d attached by a 1 → 2 bond. In actual fact the peaks of the ions with m/e 1099, 1039 (I) and m/e 1131, 1071 (II) (Scheme 1), which are formed similarly by the decomposition of the xylopyranose ring *a* (1 → 3 bond) are considerably weaker than the peaks of the $(M - 175)^+$ and $(M - 235)^+$ ions.

The spectra of compounds (I-V) show weak peaks of ions with even mass numbers $(M - 162)^+$. According to measurements of elementary composition, they each contain one methine group more than the $(M - 175)^+$ ions and are apparently formed by the cleavage of the C_1—O and C_2–C_3 bonds of the glucopyranose ring d (Scheme 1). An ion with the same origin has been recorded in the spectrum of one of the biosides of oleanolic acid [11]. The spectra of compounds (I) and (II) contain the peaks of the $(M - 118)^+$ ions probably arising by a similar pathway at the expense of the xylopyranose link *a*.

The loss of a fragment with the composition $C_5H_{10}O_2$ leads to the formation of the ion $(M - 102)^+$. In the cited literature on the spectra of oligosaccharide permethylates [4-9], noneven-electron fragments of this type are not discussed. At the same time, various mechanisms have been proposed to explain the processes of formation of the $(M - 101)^+$ ions by a pathway involving the splitting out of the C_2—C_4 chain of pyranose rings [4, 9]. It may be assumed that the $(M - 102)^+$ ions also arise through the splitting out of the C_2—C_4 chains of the terminal units *a* or d, but with migration of one additional hydrogen into the neutral fragment and the stabilization of the decomposing unit in the form of a dioxolane ring (Scheme 1). However, the possibility is not excluded either, that the $(M - 102)^+$ ions are formed by the elimination from unit d of the C_4—C_5 chain, also having the composition $C_5H_{10}O_2$.

The spectra of compounds (III-V) have the peaks of the $(M - 191)^+$ ions the elementary compositions of which indicate the loss by the molecular ion of a $C_9H_{19}O_4$ fragment. The $(M - 191)^+$ ions of the permethylates (I and II) were formed by the splitting out of the sugar residue *a*—O having the composition $C_8H_{15}O_5$. At the same time, the spectra of (I and II) contain the peaks of the $(M - 147)^+$ ions. On combining these facts, it may be concluded that the ions of the type under consideration are formed only through the decomposition of unit *a* (m/e 1083 (I) or m/e 1085 (III) (Scheme 1). Weak peaks of fragments of this type have recently been described in the spectra of disaccharide permethylates [8]. In the spectra of (I-V), products of the subsequent decomposition of the ions $(M - 147)^+$ and $(M - 191)^+$ by the loss of unit d are also observed (Scheme 1).

Decomposition of the nonterminal pyranose rings appears in the spectra of (I-V) after the splitting out of units *a* and d: as the result of the elimination of a $C_3H_5O_2$ fragment, ions are formed with even mass numbers (for example, an ion with m/e 730 in the spectrum of (I), Scheme 1).

Of ions including the aglycone and part of the oligosaccharide chain we must also mention ions arising by the cleavage of the b—O bond with the migration of two hydrogens to the charged fragment (for example, m/e 649 (I), Table 1).

Ions of Group B. The ions of this type with the highest mass numbers (m/e 786 (I, II), m/e 830 (III-V)) are formed by the cleavage of the c—O bond with the migration of one hydrogen to the neutral fragment. The intensities of the peaks of these ions are low. The most characteristic ions are those arising by the simple cleavage of the c—O bond (m/e 583 (I, II), m/e 627 (III-V)), showing the branched nature of the oligosaccharide chain. In the spectra of (I-V) a series of directions of the successive fragmentation of these ions can be traced (Scheme 1). More intense are the peaks of ions with m/e 391 formed by the splitting off of the molecules *a*—OH (I-V) or d—OH (III-V). The splitting off of d—OH from the ions with m/e 583 in the case of compounds (I and II) leads to fragments with m/e 347 in low abundance. The preferential nature of the splitting out of the unit attached to C_3 of a nonterminal pyranose ring as compared with the splitting out of units attached to C_2 or C_4 in this type of ions has been reported by other authors [10, 11].

The peaks of the fragments formed through the splitting out from the ions with m/e 583 and 627 of parts of rings *a* and d possess relatively low intensities. However, thanks to the presence of the corresponding peaks in all the spectra and a confirmation of their origin with the aid of measurements of elementary composition, they acquire some analytical significance. Under consideration are the ions with m/e 437 the method of formation of which resembles the

process of origin of the ions $(M - 147)^+$ and $(M - 191)^+$. Ions with m/e 449 (I, II) and 493 (III-V) reflect the contribution of ring d to the process of fragmentation of the ions with m/e 583 and 627. Their origin may be compared with that of the ions $(M - 102)^+$ with the difference that the formation of the ions with m/e 449 and 493 involves the additional splitting out of a methanol molecule.

We must also mention the presence in the spectra of fragments with m/e 219 and 175, and also the products of their subsequent decomposition taking place with the elimination of the substituting groups.

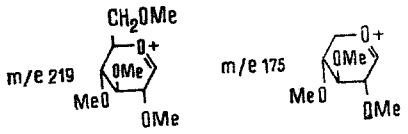


Table 1 also includes fragments whose origin does not require special explanations in the text. The methods of their formation are shown in the last column.

Experimental conditions: the synoptic spectra and the elementary compositions of the ions were obtained on a MKh-1310 mass spectrometer with double focusing using a system for the direct introduction of the sample. The ionizing voltage was 50 V, the collector current 120 μA, the temperature of the evaporator 200-270°C, and that of the ionization chamber 230-250°C. The relative error of the mass determinations does not exceed $1 \cdot 10^{-5}$.

SUMMARY

Features of the mass spectra, obtained on a MKh-1310 instrument, of spirostanol tetraoside permethylates consist in the presence, together with the molecular ions and the products of their fragmentation at glycosidic bonds, of a repeating series of fragments formed by the cleavage of the bonds of the skeleton of the terminal pyranose units.

The nature of the fragments has been confirmed by measurements of elementary composition.

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