SPECTRA OF THE METASTABLE IONS ON STEROID SAPOGENINS AND THEIR DIHYDRO DERIVATIVES

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Characteristic distinguishing features of the mass spectra of steroid sapogenins of the spirostan series and their dihydroderivatives are considered. The MD and B/E = const spectra of all the key ions have been studied. It has been established that the relative intensities of the metastable peaks obtained by the two methods have close values. The orders of these magnitudes are the same for monotypical fragmentation processes. The correlation between the parameters of the spectra is disturbed if a daughter of one has a number of alternative formation pathways. On the whole, the linked-scanning spectra are characteristic to the extent that they contain metastable peaks of analogous transitions possessing close parameters.

Investigations to establish the nature of the characteristic fragments of steroid sapogenins [1] have long ago entered the category of mass-spectrometric classics. So far as concerns the breakdown under electronic impact of their dihydro derivatives, an investigation by Albert et al. [2] has been devoted to this and has elucidated the fragmentation of the side chain at C-22 in fairly great detail in connection with the main task of the investigation – the study of the mechanism of the hydrogenation of sapogenins by $LiAlH_4 + AlCl_3$. Nevertheless, the interpretation of the mass spectra of the above-mentioned derivatives deserves a more detailed review, since with respect to their structure they are close to the aglycons of the furostanol glycosides the production of which by the hydrolysis of the latter is impossible.

In addition to the search for new characteristic methods of fragmentation of the dihydro derivatives, we set ourselves the aims of: 1) finding criteria of the monotypical nature of the fragmentation processes for a set of compounds differing by the number and positions of the OH groups in rings A and B, which leads to a substantial redistribution of the intensities of the peaks of the characteristic fragments; and 2) comparing the structures of ions coinciding with respect to the position of cleavage of the bonds in the molecules of the dihydro derivatives and of the initial sapogenins. To solve these problems we used the mass spectra of the breakdown of metastable ions, the metastable transitions being determined by the methods of metastable defocusing (MD) and linked scanning (LS), B/E = const. In the latter method we recorded all the daughter ions (fragments) corresponding to given parental ions [3]. In this work we have used the calculation of the parameters of the LS spectra for the first time.

In order to establish or confirm the structures of the fragments under consideration, we made use of high-resolution mass spectrometry.

Figure 1 gives the formulas of the compounds investigated: the genins of the spirostan series (25S)-ruscogenin (I), neotigogenin (II), yuccagenin (III), and alliogenin (IV), and the dihydro derivatives of compounds (II-IV) – dihydroneotigogenin (V), dihydroyuccagenin (VI), and dihydroalliogenin (VII) [4]. The dashed lines and arrows indicate the main fragments. The mass numbers and relative intensities of the ions in the mass spectra of (I-VII) are given in Table 1.

For the genins of the spirostan series the most characteristic ion was k with m/z 139 [1], the peak of which is the maximum in intensity, as a rule. The cleavage of the bonds of

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TABLE 1. Mass Numbers and Relative Intensities (I, %) of the Ions in the Mass Spectra of Compounds (I-VII)

Ions	I		II		III		IV		V				VI	
	m/z	I, %	*m/z*	I, %	*m/z*	I, %	*m/z*	I, %	*m/z*	I, %	*m/z*	I, %	*m/z*	I, %
M+	430	2	416	17	430	15	464	16	418	6	432	3	466	5
$(M-CH_3)+$			401	3	415	2	449	1	403	2	417	2	451	2
$(M-nH_2O)+$	412	57	398	1	412	0,5	446	2	400	3	414	4	448	3
	394	7					428	0,5						
$(M-nH_2O-CH_3)+$	379	2	383	1	397	0,5	431	1	385	2	399	2	433	2
													417	1
a $-H(a_1)$	371	3	357	6	371	8	405	9	359	2	373	2	407	4
a (a_1-2H)	—	—	—	—	—	—	—	—	357	1	371	1	405	2
b (b_1-H)	358	2	344	22	358	23	392	23	344	4	358	5	392	7
c +H	361	1	347	13	361	8	395	11	—	—	—	—	—	—
d (d+H)	316	4	302	36	316	29	350	10	303	2	317	5	—	—
d $-CH_3$	301	5	287	24	301	16	335	11	—	—	—	—	—	—
d $(d_1+H)-nH_2O$	298	30	284	4	298	44	332	36	285	6	299	1	333	6
	280	6			280	10	314	24					315	5
e+H	115	7	115	17	115	25	115	17	—	—	—	—	—	—
e $(e_1)-H$	287	5	273	54	287	31	321	21	275	100	287	100	321	93
e $(e_1)-H-nH_2O$	269	3	255	6	269	7	303	9	255	5	269	6	303	23
							285	16					285	16
g $(g_1)-3H$			245	3	259	2			245	3	259	2		
h $(h_1)-2H$			232	4	246	2			232	2	246	2		
i $(i_1)-nH_2O$	174	3	176	6	174	4			176	4	174	4		
j $(j_1)-H-nH_2O$	159	6	161	9	159	6			161	12	159	8	175	3
	139	100	139	100	139	100	139	100	—	—	—	—	—	—
ℓ	—	—	—	—	—	—	—	—	331	41	345	16	379	21
ℓ $-nH_2O$	—	—	—	—	—	—	—	—	313	3	327	2	361	10
											309	2	343	6
o	—	—	—	—	—	—	—	—	121	11	144	62	121	100
p	—	—	—	—	—	—	—	—	287	4	301	7	335	3
o $-nH_2O$	—	—	—	—	—	—	—	—	269	5	283	5	317	5
													299	13
o +H	—	—	—	—	—	—	—	—	288	6	302	5	336	1
Other ions														
$(M-CH_2O)+$	400	0 5	386	2	400	2	434	2	—	—	—	—	—	—
m $-H_2O$	—	—	—	—	—	—	—	—	126	5	126	8	126	10
	342	3			342	3							332	5
					274	3							331	4

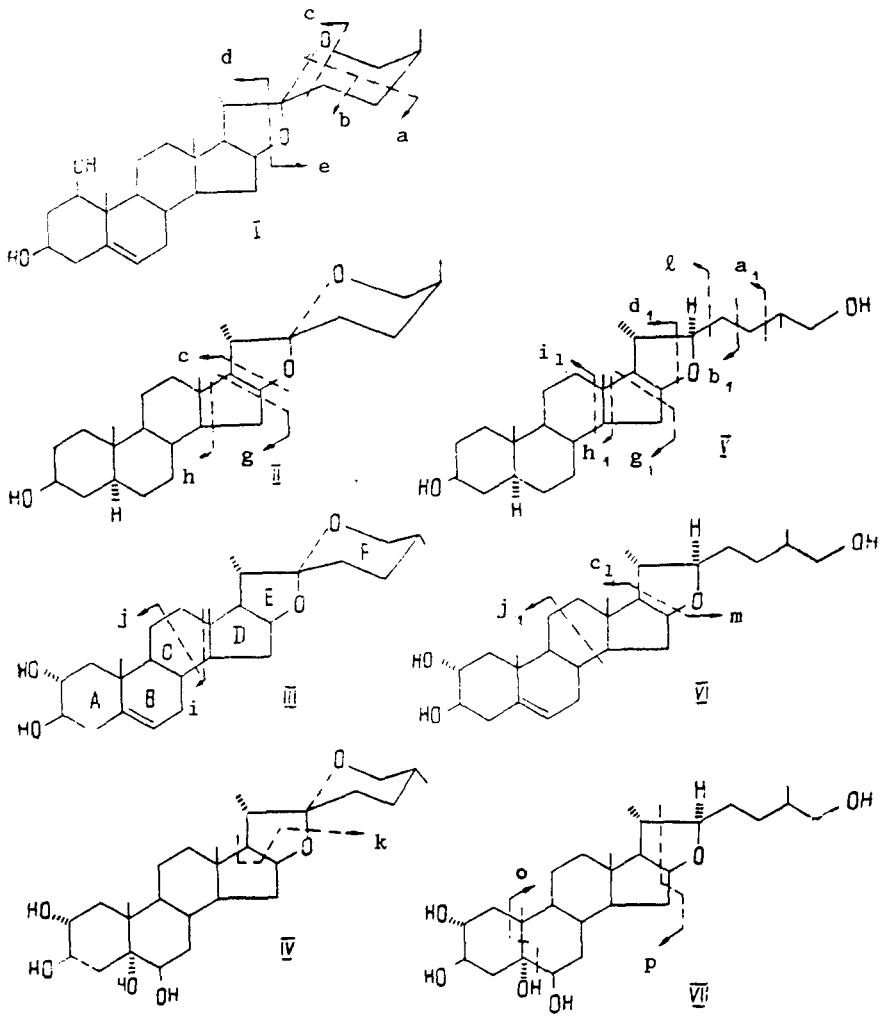

Fig. 1. Structural formulas of compound (I-VII).

the tetrahydropyran ring F always led to the appearance of the ions (a–H), b, and (c + H), the m/z values of which permitted the determination of the additional hydroxy groups in this ring. The fragmentation of the tetrahydrofuran ring E gave the ions d, (e + H), and (f–H). Less characteristic were the processes involving the cleavage of rings C and D (the ions g, h, i, and j). The contributions of ions a-i were fairly sensitive to the set of substituents in rings A and B. Thus, the combination of an OH group at C-1 with a Δ^5 bond in the molecule of (25S)-ruscogenin (I) led to a marked decrease in the stability of the molecular ion, to an increase in the height of the peak of the $(M-H_2O)^+$ ion, to a decrease in the contributions of the ions a-f, to the disappearance of the ions g and h, and to the suppression of the contribution of the ions i and j. In the spectrum of alliogenin (IV), fragmentations of types g, h, j, and j became unimportant, and the heights of the peaks of ions d and (f–H) decreased but the stability of M^+ and the contributions of the ions (a–H), b and (c+ H) remained at the same level (Table 2).

In the spectra of the dihydro derivatives that we studied (V-VII), the stabilities of M^+ were close in magnitude and appreciably less than those of the corresponding genins (II-IV). The fragmentary ions a_1, (a_1-2H), (b_1-H), and м, which had been characterized in [2], made approximately the same contributions to the total current in the spectra of (V-VII).

The results shown in Tables 1 and 2 indicate a greater selectivity of the fragmentation of the dihydro derivatives. More than half the ion current was due to three fragments: (f_1-H) and m, which were formed on the cleavage of identical bonds of ring E, and the above-mentioned ion ℓ.

Other distinguishing features of the spectra of the dihydro derivatives are: 1) the d_1-type fragmentation of ring E takes place with the migration of hydrogen to the charged

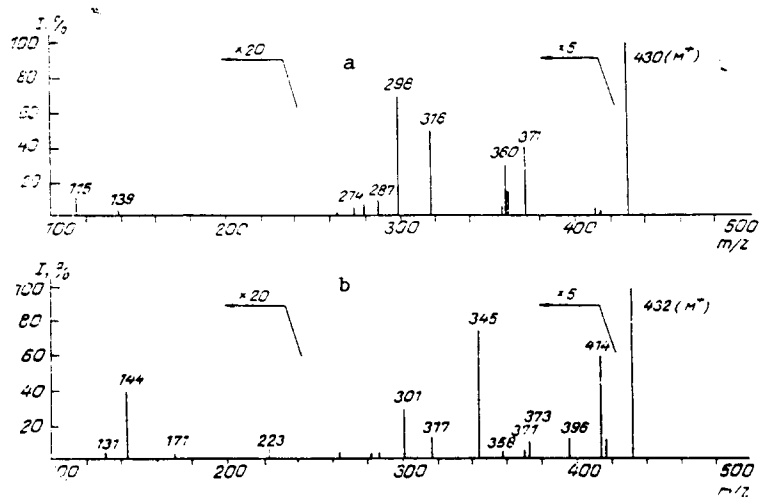

Fig. 2. Linked scanning, B/E = const, spectra of the M^+ ions of yuccagenin (a) and dihydroyuccagenin (b).

TABLE 2. Stabilities of M^+ and Contributions to the Total Ion Current (Σ_d) of the Main Fragments of the Ordinary Spectra of Compounds (I-VII)

Ion	I		II		III		IV		V		VI		VII	
	m/z	Σ_d	m/z	Σ_d	m/z	Σ_d	m/z	Σ_d	m/z	Σ_d	m/z	Σ_d	m/z	Σ_d
M^+	430	0,4	416	3	430	3	464	3	418	2	432	2	466	0,8
a −H(a·)	371	0,6	357	1	371	2	405	2	359	0,5	373	0,6	407	0,6
b (b_1 −H)	358	0,4	344	4	358	5	392	6	344	1	358	1	392	1
c +H	361	0,2	347	3	361	2	395	2	—	—	—	—	—	—
d (d_1+H)	316	0,7	302	7	316	6	350	2	303	0,4	317	1	—	—
e+H	115	1	115	4	115	5	115	4	—	—	—	—	—	—
f (f_1)−H	287	1	273	11	287	6	321	4	273	25	287	24	321	14
k	139	19	139	20	139	20	139	20	—	—	—	—	—	—
l	—	—	—	—	—	—	—	—	331	10	345	11	379	9
m −H_2O	—	—	—	—	—	—	—	—	313	0,6	327	0,5	361	2
	—	—	—	—	—	—	—	—	144	10	144	15	144	15

fragment, the head ion of the (d_1 + H) series of dihydroalliogenin (VII) is unstable, and in the corresponding spectrum its dehydration product with m/z 333 appears; and 2) the rearrangement fragments p and p + H (for the mechanism of their formation, see below) are present. A general feature of both groups of compounds is the formation of ions of the types f-j(f_1-j_1). In the spectrum of dihydroalliogenin (VII), in addition, an ion with the composition $C_{22}H_{36}O_2$, m/z 332 – the product of cleavage at the bonds of rings A and B (o + H−H_2O) is observed.

Before passing to a description of the main results of the investigation of the spectra of the metastable ions, we may note that the use of spectra of two types (MD and LS) was due to the mutually supplementing information that they contain. The MD method has a lower resolving capacity, which does not permit the reliable identification of transitions and the calculation of the mass numbers of the parental ions differing from one another by 1-4 m.u. The linked scanning, B/E = const, spectra do not suffer from this disadvantage but on their use it is necessary to take special measures in order to differentiate the spectra of the daughter ions formed by the material ions differing scalewise by 1-3 m.u. Examples of the spectra are given in Fig. 2. Each of them is typical for the molecular ions of related compounds. Equally monotypical are the linked-scanning spectra of the fragmentary ions of the same nature. Some differences between the spectra are mainly to different roles of the dehydration processes on the breakdown of the ions of particular compounds. In Fig. 2, the heights of the peaks of the daughter ions have been normalized relative to the height of the parental ion, taken as 100%. Thus, the parameters of the LS spectra that have been given actually coincide with the values of A (ratios of the height of the metastable and parental ions, in percentages) calculated from the MD spectra that we have used previously for various groups of natural and synthetic compounds [5-7]. Known from the literature as criteria for establishing the identity of the structures of fragmentary ions formed from the M^+ ions

TABLE 3. Values of A for the Main Metastable Transitions in the B/E = const and MD Spectra of Compounds (I-VII)

Metastable transitions	I		II		III	
	B/E	MD	B/E	MD	B/E	MD
M+→a—H(a_1)	11,5	14,3	5,6	5,9	6,0	5,7
M+→a$_1$—2H	—	—	—		—	—
M+→b (b_1—H)	1,0	1,7	0,8	0,8	0,8	0,9
M+→c+H	1,0	0,7	4,0	7,8	2 0	2,5
M+→d(d_1+H)	4,6	4,2	7,4	8,3	11 0	11,2
M+→e(e_1)—H	1,9	1,7	1,4	4,0	2,5	3,1
M+→ℓ	—	—	—	—	—	—
M+→m	—	—	—	—	—	—
M+→p	—	—	—	—	—	—
M+→p+H	—	—	—	—	—	—
a—H→f—H	15,5	12,8	5,0	6,6	8,1	8,1
a_1—2H→f_1—H	—	—	—	—	—	—
a (b_1+H)→f(f_1)—H	6,2	6,7	10,8	12,7	6,0	7,7
c+H→f—H	11,3	—	12,0	—	8,0	—
d→f—H	3,3	2,8	3,9	3,6	4,2	3,6
d(d_1)→d(d_1)—CH_3	10,0		6,0		2,7	
ℓ→d_1+H	—	—	—	—	—	—
ℓ→f_1+H	—	—	—	—	—	—
a→ℓ	—	—	—	—	—	—
f(f_1)—H→f(f_1)—H—H_2O	9,4		2,5		1,7	

Metastable transitions	IV		V		VI		VII	
	B/E	MD	B/E	MD	B/E	MD	B/E	MD
M+→a—H(a_1)	7,8	11,2	0,2	0,5	0,2	0,2	0,5	0,4
M+→a_1—2H	—	—	0,7	1,3	0,7	1,0	4,0	3,1
M+→b(b_1—H)	0,9	2,1	0,4	0,7	0,8	1,2	0,8	0,8
M+→c+H	2,3	3,6	—	—	—	—	—	—
M+→d(d_1+H)	5,7	5,5	1,2	1,0	2,4	1,4	0 5	—
M+→f(f_1)—H	2,4	3,0	0,3	0,4	0,5	0,3	1,0	1,1
M+→ℓ	—	—	14,1	12,8	14,5	13,0	22,5	23,3
M+→n	—	—	1,4	—	1,7	—	2,5	—
M+→p	—	—	9,8	1,2	5,2	1,2	12,0	1,6
M+→p+H	—	—	1,2	10,4	1,3	10,4	1,5	14,8
a—H→f—H	5,3	5,7	—	—	—	—	—	—
a_1—2H→f_1—H	—	—	12,0	17,5	8,1	15 0	4,0	7,8
b(b_1+H)→f(f_1)—H	3,3	3,6	5,0	5,4	3,9	3,7	3,3	3,3
c+H→f—H	7,5	—	—	—	—	—	—	—
d→f—H	3,9	8,9	—	—	—	—	—	—
d(d_1)→d(r_1)—CH_3	4,1	—	19,4	18,7	12,1	15,9	16,1*	
ℓ→d_1+H	—	—	0,4	0,5	1,4	1,4	0,8**	0,7
ℓ→f_1+H	—	—	16,2	13,7	16,0	13 5	20,0	14,5
a_1→ℓ	—	—	2,2	2,7	2,5	3,1	2,0	—
f(f_1)—H→f(f_1)—H—H_2O	1,4	—	2,2	—	4,5	—	3,5	—

*Passage from the ion (m_1—H_2O).
†Passage to the ion (m_1 + H—H_2O).

of small molecules [8], in our investigations they served to substantiate the monotypical nature of the fragmentation reactions of complex compounds.

The results of the measurements of A are given in Table 3. The most general conclusion from these results consists in the establishment of the closeness, with rare exceptions, of the values of A calculated by the two methods for one and the same metastable transition. This conclusion confirms the reliability of these parameters as quantitative characteristics of the fragmentations taking place in the first field-free space of the mass spectrometer.

The second conclusion consists in the characteristic nature of the values of A for monotypical transitions in the spectra of compounds belonging to each of the subgroups. As a rule, these are magnitudes of the same order. The most indicative in this respect were the transitions $M^+ \rightarrow m$ of the genins and the transitions $M^+ \rightarrow m$ and $j \rightarrow (j_1$—H) of the dihydro derivatives.

Let us further consider the results obtained for concrete fragmentation processes.

The ions (a-H) of compounds (II-IV) and the ions (a_1—2H) of the corresponding dihydro derivatives (V-VII) had identical mass numbers. However, in the first case they arose by the cleavage of two bonds of the tetrahydropyran ring and the migration of one hydrogen atom, and in the second case as the result of the cleavage of the C-24—C-25 bond with the migration

of 2H to the split-out fragment. The value of A for the transition $M^+ \rightarrow (a_1-2H)$ was on an average, almost an order of magnitude lower than the analogous magnitudes of the $M^+ \rightarrow (a-H)$ transition for (II-IV), although both processes are rearrangement processes, even if differing in their mechanism. If we compare the parameters of the process $M^+ \rightarrow a_1$ with those of the rearrangement process $M^+ \rightarrow (a_1-2H)$ in identical dihydro derivatives, it is completely explicable that the latter were increased severalfold in comparison with the former (Table 3).

The ions b of the genins and the ions (b_1-H) of the corresponding dihydro derivatives were also identical in composition. The condition for the coincidence of their structures is migration of H from C-22 in the dihydro derivatives to the fragments split out at the C-22–C-24 bond. The closeness of the values of A for the transitions $M^+ \rightarrow$ ᵇ(b_1-H) in compounds (I-VII) is not sufficient grounds for judging a similarity of structures, since the two types of M^+ ions form the daughter ions b and (b_1-H) by different pathways. In such a case, as criteria of the identity of structures it is possible to use the spectra of the daughter ions b and (b_1-H). The main transition in these spectra was the $b(b_1 - H) \rightarrow f(f_1 - H)$ transition, and, as can be seen from Table 3, its parameters were always of the same order of magnitude although they differed from one another by factors of 2-3. However, the complete qualitative agreement of the LS spectra of the above-mentioned ions was not always observed: While the spectra of the daughter ions b and (b_1-H) in the pairs of compounds (II and V) and (IV and VII) with m/z 344 and 392 practically coincided, the LS spectra of the ions with m/z 358 of yuccagenin (III) and dihydroyuccagenin (VI) differed considerably. The spectrum of (III) contained the metastable peak of the transition $b \rightarrow (f-H-H_2O)$, while in the spectrum of (VI) no analogous transition was recorded but in return, the peak next to height to that of the transition $b_1 - H \rightarrow f_1 - H$ was that of the transition of ($_1$–H) to an ion with m/z 274 having the composition $C_{18}H_{26}O_2$. The same transition in the LS spectrum of yuccagenin (III) was weakly expressed. Nevertheless, in the ordinary spectra of these compounds the opposite pattern was observed: The height of the peak of the ion with m/z 274 in yuccagenin was twice as great as for its dihydro derivatives. Judging from the linked-scanning spectra of the other fragementary ions, no fragment with this mass was formed from them. However, this transition was also observed in the spectrum of M^+ for yuccagenin, in contrast to the analogous LS spectrum of dihydroyuccagenin. In the breakdown $b(b_1-H) \rightarrow 274^+$ for (III) and (VI), the C-22–C-23 chain, the oxygen atom of ring E, and one of the carbon atoms of ring D – C-16 or C-17 – was eliminated. The quantitative differences between the spectra of the metastable ions of (III) and (VI) probably show that not all the (b_1-H) ions of the latter were formed by the migration of a hydrogen atom from C-22; there are also other mechanisms capable, in their turn, of changing the pathways of the formation of the ion with m/z 274.

Although there are no grounds for doubting the monotypical nature of the processes involved in the formation of the ions (c + H) for compounds (I-IV), the magnitudes of A for the transition $M^+ \rightarrow$ (c + H) differed substantially from one another, particularly in the case of the MD spectra of ruscogenin (0.7) and of neotigogenin (7.8). Here we applied a stricter criterion of the monotypical nature of the fragmentation reactions which is based on the closeness of the values of A/Σ_d, where Σ_d is the contribution of the daughter ion to the total ion current [3, 5]. For the MD spectra of the ions c + H, these ratios varied between 1.5 and 3.1, i.e., they were fairly close. So far as concerns the subsequent breakdown of the (d + H) ions, which was used to evaluate the degree of common nature of their structures, it may be mentioned that the LS spectra possessed common qualitative characteristics and, above all, showed a charcteristic transition in the f–H ion with values of A of the same order (Table 3). However, the same spectra contained the peaks of processes involving the ejection of nH_2O differing in relative intensity that were specific for each of the ions.

Let us now pass to the characteristics of the main products of fragmentation and the bonds of ring E. The parameters of the spectra of the transitions $M^+ \rightarrow$ d of the genins and $M^+ \rightarrow$ (d + H) of the corresponding dihydro derivatives must not be compared, since the first of the daughter ions is an odd-electron ion and the second an even-electron ion. The values of A for these transitions within each of the subgroups of compounds were of the same order, but for the genins (I-IV) there was no symbatic relationship between A and Σ_d. The same pattern was observed in the case of the transitions $M^+ \rightarrow (f(f_1)-H)$. Furthermore, in spite of the probable common nature of the structures of the daughter ions in a given series, in compounds (II and V), (III and VI), and (IV and VII) the parameters of the peaks here dif-

fered substantially, and the values of A/Σ_d were even more different; for example, 0.36 for (II) and 0.016 for (V) (results of the MD spectra). If we judge the structures of the ions $(f(f_1)-H)$ from their characteristic fragmentation, then in the LS spectra only the peaks corresponding to specific dehydration processes appeared. Nevertheless, the orders of magnitude of A for the processes involving the elimination of one molecule of water [the transition $f(f)-H \rightarrow f(f_1)-H-H_2O$] for compounds (II-VII) were the same.

On analyzing these facts, we came to the conclusion that for daughter ions having a whole series of precursors and arising not only from the molecular ions but also as the result of successive transitions [for example, $M^+ \rightarrow d \rightarrow f-H$ or $M^+ \rightarrow a_1 \rightarrow M \rightarrow f-H$], the values of $A/\Sigma_d(f_1-H)$ cannot be criteria of a common structure. However, these considerations relate primarily to the direct transition $M^+ \rightarrow (f(f_1)-H)$. In relation to other, one-stage, processes for the formation of these ions – from the ions a—H, a_1, a_1—2H, b, b_1—H, c + H, and c – not only identical orders of magnitude of A in monotypical transitions but also, with few exceptions, their proportionality to the values of Σ_d were observed (Tables 2 and 3).

Each of the two processes for the formation of ions ℓ that were recorded – from M^+ and from d_1 – was characterized by close values of A for all three compounds. In [2], by the introduction of a deutero label in the C-22 position of tigogenin and its derivative, it was shown that a considerable part of the ℓ ion (40%) was formed with the loss of the deuterium atom, i.e., as the result of a rearrangement. Of course, it is impossible to evaluate the contributions of this process to the breakdown of the dihydro derivatives (V-VII) from the figures of Table 3 and all that can be done is to note that they are probably of the same order.

It is quite impossible to judge the complexity of the processes occurring with the loss of large neutral particles on the basis of calculations of the values of A or A/Σ_d. Thus, the close values of A for the transition $M^+ \rightarrow m$ corresponded to the ions m formed with the cleavage of two bonds of ring E, but the ratios A/Σ_d were extremely small in comparison with the analogous magnitudes of the rearrangement processes $M^+ \rightarrow (a_1-2H)$ or $M^+ \rightarrow (b_1-H)$, where relatively small fragments were split out. This ratio was still smaller for the transition $M^+ \rightarrow 139$ (k) for the genins (Fig. 2a), although the process shown is even more complicated. Unfortunately, neither remote transition can be monitored by MD spectra. The reasons for the discrimination of remote transitions by height are unclear, and although in a monograph by Chapman [9] it has been shown that linked scannings permit the recording of high-intensity ions over a wide range of masses, analysis of literature information has demonstrated that at $m_1/m_2 \geq 3$ the heights of the metastable peaks decrease [10]. At the same time, to check the common nature of the structure of the ions k (I-IV) and m (V-VII) we obtained the B/E = const spectra and convinced ourselves of their qualitative agreement and of the closeness of the values of A for concrete transitions (Table 4).

The formation of the ions p and (p + H) was the only example of the nonagreement of the results of the two types of spectra used – MD and B/E = const. For the transition $M^+ \rightarrow p$ the value of A in the LS spectra was 4-8 times higher than in the MD spectra, while for the transition $M^+ \rightarrow p + H$ the opposite pattern was observed (Table 3). The only explanation of this fact is the existence of two processes for the formation of C_{20} fragments of the same composition – the ions p and the ions (d_1-CH_3). In spite of the uncharacteristic nature of the d_1 ions for the spectra of the dihydro compounds (V-VII), the transition $d_1 \rightarrow (d_1-CH_3)$ was recorded by both types of spectra of the metastable ions [for dihydroalliogenin (VII) the LS spectrum of the ion (d_1-H_2O) with m/z 332 was measured]. As can be seen from Table 3, these transitions were charcterized by the closeness of the values of A. This circumstance may totally place under doubt the reality of the process involved in the formation of the p [but not the (p + H)] ions. But it is then necessary to explain the reason for the small height of the peak of the one-stage transition $M^+ \rightarrow d_1$ and the high intensity of the peak of the two-stage transition $M^+ \rightarrow (d_1-CH_3)$ in the LS spectra of the dihydro compounds (V-VII). This contradicts the essence of linked scanning spectra which primarily reflect one-stage processes of the formation of daughter ions. The question requires further investigation.

We also directed our attention to one characteristic detail of the B/E = const spectra of the M^+ ions of genins (I-IV): The intensities of the peaks of the transitions $M^+ \rightarrow (M-70)^+$ substantially exceeded those of the transitions in the ions $(M-69)^+$ (c + H) and $(M-72)^+$ (c) (Fig. 2a), although in the ordinary spectra the peaks of the $(M-70)^+$ ions had inconsider-

TABLE 4. Values of A for Metastable Transitions in the B/E = const Spectra of the Ions k (I-IV) and j (V-VII)

Compound	k→121	k→97	k→95	k→83	k→69
I	0,4	0,2	0,1	0,1	1,2
II	0,3	0,2	0,1	0,1	1,3
III	0,3	0,2	0,1	0,1	1,2
IV	0,3	0,2	0,1	0,1	1,2

Compound	m→126	m→117	m→115	m→111	m→105	m→97
I	3,6	0,1	0,2	0,1	0,1	0,5
VI	5,3	0,1	0,2	0,1	0,1	0,6
VII	5,4	0,1	0,2	0,1	0,1	0,7

able heights. Analysis of the elementary composition of the $(M-70)^+$ ion of yuccagenin (III) showed that it consisted of the particles $(M-C_5H_{10})^+$ (c) and $(M-C_4H_6O)^+$ (b + 2H) in a ratio of 3:1. Consequently, the intensity of the metastable peak of the transition $M^+ \rightarrow (M-70)^+$ was due to the rearrangement component (b + 2H).

EXPERIMENTAL

All the measurements were performed on a MKh 1310 mass spectrometer with a SVP-5 device for direct introduction, an ionizing voltage of 60 V, a collector current of 40 μA, and temperatures of the ionization chamber of 160-190°C and of the evaporating bulb of 130-160°C. The conditions for measuring the elementary compositions, for obtaining the MD spectra, and for calculating the values of A from them have been described in [5]. The linked scanning, B/E = const, mass spectra were obtained with the aid of an additional device containing an operational amplifier for feeding the energy analyzer with the smooth regulation of the amplification factor. The amplifier was fed with a voltage proportional to B, i.e., to the signal of the Hall probe. With the magnetic field tuned to the peak of the parental ion, this coefficient was selected in such a way that the output voltage of the amplifier E corresponded to the nominal value E_0. Then automatic exponential scanning of the strength of the magnetic field (B) was carried out in which the proportionality of B and E was ensured with an error of not more than 0.1 percent. The rate of scanning was 30 sec per mass decade.

The mass numbers of the daughter ions m_2 were then determined from the formula

$$m_2 = \sqrt{m_1 \cdot m_2'},$$

where m_1 is the mass number of the selected parental ion, and m_2' is the "apparent" mass number of the daughter ions determined from the readings of the scale of the mass-number indicator.

The samples of compounds (I-VII) were provided by Yu. S. Vollerner.

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METABOLITES FROM SPONGES AS INHIBITORS OF β-1,3-GLUCANASE

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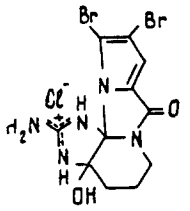
UDC 547.964

The action of a number of aromatic and nitrogen heterocyclic metabolites from sponges on β-1,3-glucanase Lo from the bivalve mollusk Chlamys albidus has been investigated. The greatest inhibiting action on the activity of α-1,3-glucanase Lo was shown by puupehenone derivatives.

The increasing interest in enzyme inhibitors is connected with their practical use in medicine for the diagnosis and treatment of a number of diseases [1, 2]. Specific inhibitors are used for studying the mechanism of the action of enzymes [3].

We have investigated the action of a number of aromatic and nitrogen heterocyclic metabolites from sponges on β-1,3-glucanase Lo from the bivalve mollusk Chlamys albidus living in the Sea of Japan.

Inhibiting action was determined from the capacity of solutions of the metabolites for interfering with the interaction of Lo with laminarin. The dependence of the inhibiting action on the concentration of the substance was determined. Table 1 gives the amounts of substance in a sample causing 50% inhibition of $2 \cdot 10^{-2}$ activity units of the enzyme.



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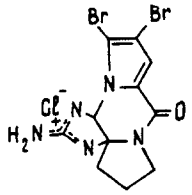

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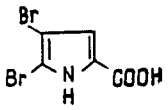

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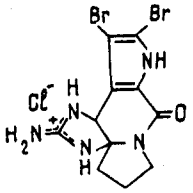

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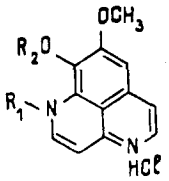

5. $R_1 = H$, $R_2 = CH_3$

6. $R_1 = CH_3$, $R_2 = H$

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