

1.12 (6 H, s), 1.21 (3 H, s), the protons of tert-Me groups, 2.09 (3 H, s), 1.96 (3 H, s), the protons of acetate Me groups, 3.72 (1 H, triplet, C<sub>24</sub>-H), 4.59 (1 H, triplet, J < 4 Hz, C<sub>3</sub>-H), 5.16 (1 H, multiplet,  $\Sigma J \sim 20$  Hz, C<sub>11</sub>-H). Mass spectrum, m/e: 542 (M<sup>+</sup>-H<sub>2</sub>O), 545 (M<sup>+</sup>-CH<sub>3</sub>), 501 (M<sup>+</sup>-59), 500 (M<sup>+</sup>-AcOH), 143 (100%), 125, 59.

Saponification of the Diacetate (XI). A mixture of 150 mg of (XI) and 5 ml of 0.9 N methanolic KOH was heated under reflux for 2 h. After the usual working up, the residue was chromatographed on silica gel in the hexane-acetone (10:1) system. The reaction yielded 15 mg of a mixture of (II) and (III), and also 95 mg of the triterpene (V).

#### SUMMARY

1. From the unsaponifiable fraction of an ethereal extract of the leaves of *Betula lanata*, in addition to 3-epiocotillol (I) we have isolated a new triterpene of the dammarane series 29(S),24(R)-epoxydammarane-3 $\alpha$ ,11 $\alpha$ ,25-triol (V), and also derivatives of it: 11 $\alpha$ -acetoxy-20(S),24(R)-epoxydammarane-3 $\alpha$ ,25-diol (II), 2 $\alpha$ -acetoxy-20(S),24(R)-epoxydammarane-11 $\alpha$ ,25-diol (III), and 11 $\alpha$ ,25-dihydroxy-20(S),24(R)-epoxydammaran-3-one (IV).

2. It has been established that the triterpenoids are present in the leaves of *B. lanata* in the form of acetates.

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#### CHARACTERISTICS OF THE MASS SPECTRA OF ECDYSTEROIDS WITH DIFFERENT NUMBERS OF -OR GROUPS

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The mass spectra of 15 phytoecdysteroids and acetyl derivatives have been compared. With a decrease in the number of -OR groups, the contribution of cleavages of the bonds of the steroid skeleton increases. 20,22-Diols are characterized by the greatest significance of fragmentation at the C-20-C-22 bond. In all the spectra, clear indications of fragmentation of the side chain at the C-22-C-23, C-23-C-24, and C-24-C-25 bonds are observed.

The detection in the plant *Silene praemixta* M. Pop. of several phytoecdysones belonging to the subgroup of 2-deoxy- $\alpha$ -ecdysone [1, 2] has substantially supplemented the general pattern of distribution of compounds of this type in the plants of Central Asia. To ecdysteroids found previously the molecules of which contain seven or eight hydroxy groups (integristerones A and B [3, 4]) compounds with three or four hydroxy functions have been added. The necessity for an all-sided study of the ecdysteroids of the latter group follows, for example, from the work E. Ohnishi et al. [5], who have shown that 2-deoxy- $\alpha$ -ecdysone is not only one of the main metabolites of  $\alpha$ -ecdysone but also plays an independent role in the metamorphosis of insects.

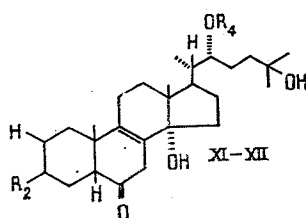
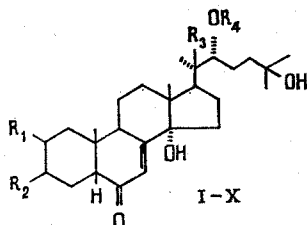
The analysis of mass-spectrometric characteristics is important for establishing the structures of the ecdysones [6]. The basic laws of fragmentation of these compounds have

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been expounded previously [6-8]. At the same time, the existence of a set of ecdysteroids with different numbers of hydroxy functions will enable us to compare their spectra in order to evaluate the relative contribution of each of the main directions of the fragmentation. Furthermore, the use of high-resolution mass spectrometry is opening up a route for the discovery of previously unknown fragmentation reactions of the steroid nuclei and of the side chains of the ecdysteroids.

We have compared the spectra of 2-deoxy- $\alpha$ -ecdysone (I) and its 22-monoacetate (II) and 3,22-diacetate (III), silenosterone (IV) [1] and its 22-monoacetate (V),  $\alpha$ -ecdysone (VI), 2-deoxyecdysterone (VII) and its 3,22-diacetate (VIII), ecdysterone (IX) and its 2,3,22-triacetate (X), and premixisterone (XI) [2] and its 3,22-diacetate (XII).



	$R_1$	$R_2$	$R_3$	$R_4$
I	H	OH	H	H
II	H	OH	H	Ac
III	H	OAc	H	Ac
IV	H	=O	H	H
V	H	=O	H	Ac
VI	OH	OH	H	H
VII	H	OH	OH	H
VIII	H	OAc	OH	Ac
IX	OH	OH	OH	H
X	OAc	OAc	OH	Ac
XI		OH		H
XII		OAc		Ac

Basic Processes in the Fragmentation of Compounds (I-XII). Table 1 gives information on the fragments by the successive splitting out from  $M^+$  of the particles R-OH and  $CH_3$  (column 2), and also on the key ions of series a, b, and c (columns 3, 5, and 7). The structures of the ions at the head of these series are given. The ions of series a and b are the well-known [6, 8] products of cleavage at the C-20-C-22 and C-17-C-20 bonds. The c ions are the closest analogs of the main key fragments in the spectra of the cardenolides [9, 10]. Their formation has been observed previously for the acetates of ponasterone and ajugasterone [8]. The nature of the particles, the transfer of which to the charged (+) or uncharged (-) fragment accompanied the cleavage of a C-C bond, is shown in columns 4, 6, and 8. The origin of the ions denoted by asterisks has been confirmed by measurements of elementary compositions. In the present paper, as 100% we have taken the maximum peaks in the  $M^+ - m/e$  200 interval.

The main property of the spectra of the 2- and 20-deoxyecdysteroids is the presence of the peak of the molecular ion, which is absent in the case of the ecdysteroid (IX) and its acetate (X).

In order to follow the change in the contribution of the processes of the fragmentation of compounds (I-XII) with a change in the number and position of the OR groups, we have drawn up Table 2, including part of the material of Table 1, in generalized form. The numerical matter of Table 2 was obtained as the sum of the relative intensities of the peaks in columns 2, 3, 5, and 7 for each compound taken individually in percentages with respect to the sum of the heights of all the peaks shown in the table.

It can be seen from Table 2 that there is no regularity whatever in the change in the contribution of the ions  $(M - nOR - CH_3)^+$ , with the exception of a tendency to a decrease in

Table I. Mass Numbers and Relative Intensities of the Main Fragments in the Mass Spectra of the Ecdysteroids (I-XII)

Compound	M <sup>+</sup> and (M - nOR - CH <sub>3</sub> ) <sup>+</sup> ions	Ions of series a (C-20-C-22 cleavage)		Ions of series b (C-17-C-20 cleavage)		Ions of series c (C-8-C-14, C-13-C-16, and C-13-C-14 cleavages)	
		m/e (I, %)	accompanying processes	m/e (I, %)	accompanying processes	m/e (I, %)	accompanying processes
I	448 (M <sup>+</sup> , 4), 430 (26)*, 415 (28), 412 (22), 397 (31), 379 (6)	332 (66)* 314 (55) 299 (32)	+H +H; -H <sub>2</sub> O +H; -H <sub>2</sub> O; -CH <sub>3</sub>	303 (5) 285 (23)* 284 (23)*	- -H <sub>2</sub> O -H <sub>2</sub> O; -H	234 (100)* 233 (63)* 215 (16)	+H -H <sub>2</sub> O
II	490 (M <sup>+</sup> , 6), 472 (12), 412 (22), 397 (41), 394 (26), 379 (16)	332 (10) 331 (11) 314 (12) 313 (11)	+H +H; -H <sub>2</sub> O -H <sub>2</sub> O	303 (10) 302 (11) 285 (47) 284 (94)	- -H -H <sub>2</sub> O -H; -H <sub>2</sub> O	234 (95) 233 (100)	+H -
III	532* (M <sup>+</sup> , 1), 514 (4), 499 (2), 496 (2), 472 (3), 454 (18), 439 (20), 435 (17), 412 (4), 394 (14), 379 (19), 361* (4)	374 (6)* 373 (6)* 314 (7) 313 (11)	+H +H; -AcOH -AcOH	345 (3) 344 (5) 327 (37) 326 (80)* 267 (42) 266 (48)	-H -H <sub>2</sub> O -H; H <sub>2</sub> O -H <sub>2</sub> O; AcOH -H; -H <sub>2</sub> O; AcOH	276 (89)* 275 (86)*	+H +H (-AcOH) -AcOH
IV	446 (M <sup>+</sup> , 1), 428* (14), 413 (12), 410 (24), 395 (30), 377 (8)	330 (21)* 312 (30) 297 (29)	+H -H; -H <sub>2</sub> O +H; -H <sub>2</sub> O; -CH <sub>3</sub>	283 (41)* 282 (49)*	-H <sub>2</sub> O -H <sub>2</sub> O; -H	233 (100)* 232 (75) 231 (50)	+2H +H +H
V	488 (M <sup>+</sup> , 1), 470 (6), 452 (3), 428 (1), 410 (16), 395 (29), 392 (21), 377 (13)	330 (2) 329 (4) 312 (8) 311 (14)*	+H +H; -H <sub>2</sub> O -H <sub>2</sub> O	301 (10) 300 (13) 283 (39) 282 (57)* 281 (20)*	- -H -H <sub>2</sub> O -H; -H <sub>2</sub> O -2H; -H <sub>2</sub> O -H <sub>2</sub> O; -H	232 (100)* 231 (92)*	+H -
VI	464 (M <sup>+</sup> , 1), 446* (26), 431 (21), 428 (68), 413 (15), 410 (10), 395* (6)	348 (22)* 330 (68) 315 (6)	+H +H; -H <sub>2</sub> O +H; -H <sub>2</sub> O; -CH <sub>3</sub>	300 (100)*	-H <sub>2</sub> O; -H	250 (20)* 249 (15) 231 (6)	+H -H <sub>2</sub> O +H
	464 (M <sup>+</sup> , 1), 446* (3), 428 (10), 413 (8), 410 (14), 395 (6)	347 (35)* 329 (100)	-H <sub>2</sub> O	303 (9) 302 (11)*	-H	234 (15) 233 (15)	+H -H <sub>2</sub> O +H

VII	392 (6), 377 (5)	312 (33)* 311 (28)* 297 (14)	+H; -2H <sub>2</sub> O -2H <sub>2</sub> O 2H <sub>2</sub> O; -CH <sub>3</sub>	285 (30) 284 (35)	-H <sub>2</sub> O -H; -H <sub>2</sub> O	+H +H; -AcOH -AcOH +H
VIII	548* (M <sup>+</sup> , 0, 2), 530 (0, 5), 515 (1), 512 (0, 5), 497 (2), 488 (2), 470 (15), 455 (10), 452 (33), 437 (22)	389 (80)* 371 (45) 329 (80) 311 (100)	-H <sub>2</sub> O -AcOH -H <sub>2</sub> O; -AcOH	327 (23) 326 (18)	-H <sub>2</sub> O -H; -H <sub>2</sub> O	276 (32)* 275 (11) 216 (36) 215 (27)
IX	480 (M <sup>+</sup> , 0), 462* (6), 444 (14), 429 (11), 426 (40), 411 (4), 408 (11), 393* (4)	363 (28) 345 (100) 344 (99) 328 (55) 327 (60) 313 (6) 310 (9)	-H <sub>2</sub> O -H; -H <sub>2</sub> O +H; -2H <sub>2</sub> O +H; -2H <sub>2</sub> O; -CH <sub>3</sub> +H; -3H <sub>2</sub> O	301 (25) 300 (48) 285 (23) 267 (19)	-H <sub>2</sub> O -H; -H <sub>2</sub> O -H; -H <sub>2</sub> O; -CH <sub>3</sub> -H; -2H <sub>2</sub> O; -CH <sub>3</sub>	250 (9) 249 (8)
X	606 (M <sup>+</sup> , 0), 528 (33), 510(100), 495 (88), 492 (13), 477 (10)	447 (9) 430 (8) 429 (6) 387 (10) 327 (11) 309 (12)	+H; -H <sub>2</sub> O -H <sub>2</sub> O -2AcOH -AcOH; -H <sub>2</sub> O	404 (1) 385 (27)	+H -H <sub>2</sub> O	334 (35) 333 (19) 274 (11) 232 (30) 231 (18)
XI	448* (M <sup>+</sup> , 2), 430 (33), 415 (12), 412 (22), 397 (15), 379* (7)	332 (15)* 314 (15) 299 (10)	+H; -H <sub>2</sub> O +H; -H <sub>2</sub> O; -CH <sub>3</sub> +H	303 (13) 285 (21)* 284 (13)*	-H <sub>2</sub> O -H <sub>2</sub> O; -H	+H -H <sub>2</sub> O
XII	532 (M <sup>+</sup> , 6), 517 (3), 514 (10), 496 (3), 472 (45), 457 (28), 454 (100), 439 (10), 436 (15), 412 (27), 394 (94), 379 (28)	374 (31)* 373 (29) 314 (48) 313 (30)	+H +H; -AcOH	345 (46) 327 (20) 326 (23) 267 (25)	-H <sub>2</sub> O -H; -H <sub>2</sub> O -H <sub>2</sub> O; -AcOH	276 (21) 275 (23) 216 (66) 215 (98)

\*Ions whose composition has been determined.

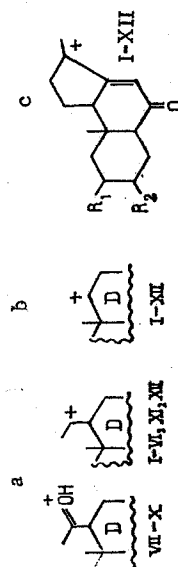


TABLE 2. Total Relative Contribution of the Main Processes in the Fragmentation of the Ecdysteroids (I-XII), %

Compound	(M-nOR-CH <sub>3</sub> ) <sup>+</sup>	a	b	c
Ecdysteroids				
I	23,3	30,4	10,2	36,1
IV	18,0	16,6	18,7	46,7
VI	38,3	25,0	26,0	10,7
VII	12,7	58,6	21,2	7,5
IX	14,6	58,0	24,7	2,7
XI	28,1	12,6	14,5	44,8
Ecdysteroid acetates				
II	23,5	8,4	31,0	38,1
III	14,9	4,0	29,6	51,5
V	20,0	6,2	31,0	42,8
VIII	15,9	55,2	9,8	19,1
X	42,8	23,7	8,2	25,3
XII	44,5	16,7	13,8	25,0

this magnitude for the 20,22-dihydroxyecdysteroids (VII) and (IX). Simultaneously, an increase in the total size of the peaks of the ions of series *a* is clearly observed for the same substances, which is completely justified for this type of compound.

The most pronounced change in the phytoecdysteroids studied is that in the contributions of the ions of series *c*. The 2,20-dideoxyecdysteroids (I), (IV), and (XI) are characterized by the highest values. In the spectrum of the 20-deoxyecdysteroid (VI), this magnitude decreases sharply, and it becomes least in the 20,22-dihydroxyecdysteroids (VII), particularly (IX). The observed characteristic falls well within the framework of the idea of an increase in the role of skeletal cleavages with a rise in the stability of the molecular ions [11].

So far as concerns the acetyl derivatives, the regular change in the contribution of the ions of series *c* in their spectra does not appear so strongly, although a decrease in this magnitude can well be seen for the 22-acetoxy-20-hydroxyecdysteroids (VIII) and (X). Its low value for premixisterone (XII) can be explained by a whole series of factors based, above all, on the position of the double bond, which is different from that for the other compounds. At the same time, the size of the contribution of the *c* ions in this compound substantially exceeds the corresponding values for the ions of series *a* and *b* (Table 2).

An analysis of Table 2 also permits other conclusions. On passing from the ecdysteroids to their acetyl derivatives [again with the exception of the pair (XI) and (XII)], a tendency is observed to a decrease in the significance of fragmentation of type *a*, which is particularly striking for the 2,20-dideoxyecdysteroids (I) and (IV), and (II), (III), and (V). This phenomenon is explained by the presence of the 22-acetoxy group, which creates some hindrance to breakage at C-20-C-22. At the same time, in comparison with the corresponding nonacetylate samples, in the spectra of the acetates of the 2,20-dideoxyecdysteroids there is an increase, and in the case of the 22-acetoxy-20-hydroxy compounds a decrease, in the contribution of the ions of series *b*.

Attention is attracted also by a qualitative change in the process of formation of the ions of this series in the case of the 2-deoxyecdysteroids. The spectra of all these compounds with the exception of silenosterone (IV) contain the peaks of fragments arising as the result of simple cleavage of the C-17-C-20 bond, while the 2-hydroxyecdysteroids (VI) and (IX) are characterized by the stabilization of the fragments of series *b* only after the accompanying ejection of H and H<sub>2</sub>O [6, 8, 13] (Table 1).

We may also note an interesting feature of the 20,22-dihydroxyecdysteroid acetates (VIII) and (X): Their spectra contain weak peaks of fragments whose compositions confirm that they belong to ion series *b* - m/e 346 (VIII) and 404 (X). The appearance of these fragments is accompanied by the migration of one hydrogen atom to the charged fragment.

Nonuniversal Modes of Fragmentation of the Skeleton. The increase in the role of skeletal cleavages with a decrease in the number of OR groups is also reflected in the appearance of a series of previously undescribed types of ions in the spectra of the 2- and 20-deoxyecdysteroids. Thus, in the range of mass numbers considered, in the spectrum of 2-deoxy- $\alpha$ -ecdysterone (I) the second-strongest peak with m/e 263 has the composition C<sub>16</sub>H<sub>23</sub>O<sub>3</sub>. This shows the

TABLE 3. Elementary Compositions and Origins of Some Fragments in the Mass Spectra of the Ecdysteroids

Compound	Mass number, m/e	Elementary composition	Origin of the fragment
I	420	C <sub>20</sub> H <sub>14</sub> O <sub>4</sub>	(M-CO) <sup>+</sup>
	361	C <sub>22</sub> H <sub>33</sub> O <sub>4</sub>	(M-C <sub>5</sub> H <sub>11</sub> O) <sup>+</sup> ; C-22-C-23
	343	C <sub>22</sub> H <sub>31</sub> O <sub>3</sub>	361-H <sub>2</sub> O
	263	C <sub>16</sub> H <sub>23</sub> O <sub>3</sub>	C-13-C-17; C-14-C-15; +H
III	193	C <sub>11</sub> H <sub>17</sub> O <sub>2</sub>	C-11-C-12; C-8-C-14; +H
	504	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	(M-CO) <sup>+</sup>
	426	C <sub>28</sub> H <sub>42</sub> O <sub>3</sub>	(M-CO-AcOH-H <sub>2</sub> O) <sup>+</sup>
	403	C <sub>24</sub> H <sub>35</sub> O <sub>5</sub>	(M-C <sub>5</sub> H <sub>11</sub> O-CH <sub>2</sub> CO) <sup>+</sup>
IV	366	C <sub>26</sub> H <sub>38</sub> O	426-AcOH
	400	C <sub>26</sub> H <sub>40</sub> O <sub>3</sub>	(M-H <sub>2</sub> O-CO) <sup>+</sup>
	341	C <sub>22</sub> H <sub>29</sub> O <sub>3</sub>	(M-C <sub>5</sub> H <sub>11</sub> O-H <sub>2</sub> O) <sup>+</sup>
VI	261	C <sub>16</sub> H <sub>21</sub> O <sub>3</sub>	Analogous to m/e 263 (I)
	418	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	(M-H <sub>2</sub> O-CO) <sup>+</sup>
VII	388	C <sub>24</sub> H <sub>36</sub> O <sub>4</sub>	(M-H <sub>2</sub> O-(CH <sub>3</sub> ) <sub>2</sub> CO) <sup>+</sup>
	377	C <sub>22</sub> H <sub>33</sub> O <sub>5</sub>	(M-C <sub>5</sub> H <sub>11</sub> O) <sup>+</sup>
	372	C <sub>23</sub> H <sub>32</sub> O <sub>4</sub>	(M-H <sub>2</sub> O-(CH <sub>3</sub> ) <sub>3</sub> C-OH) <sup>+</sup>
	354	C <sub>23</sub> H <sub>30</sub> O <sub>3</sub>	372-H <sub>2</sub> O
	279	C <sub>16</sub> H <sub>23</sub> O <sub>4</sub>	Analogous to m/e 263 (I)
	342	C <sub>22</sub> H <sub>30</sub> O <sub>3</sub>	(M-2H <sub>2</sub> O-C <sub>5</sub> H <sub>10</sub> O) <sup>+</sup>
VIII	336	C <sub>23</sub> H <sub>28</sub> O <sub>2</sub>	(M-3H <sub>2</sub> O-(CH <sub>3</sub> ) <sub>3</sub> C-OH) <sup>+</sup>
	520	*C <sub>30</sub> H <sub>48</sub> O <sub>7</sub>	(M-CO) <sup>+</sup>
IX	460	C <sub>28</sub> H <sub>44</sub> O <sub>5</sub>	(M-CO-AcOH) <sup>+</sup>
	401	C <sub>24</sub> H <sub>33</sub> O <sub>5</sub>	(M-AcOH-C <sub>5</sub> H <sub>11</sub> O) <sup>+</sup>
	383	C <sub>24</sub> H <sub>31</sub> O <sub>4</sub>	401-H <sub>2</sub> O
	378	C <sub>25</sub> H <sub>30</sub> O <sub>3</sub>	(M-3H <sub>2</sub> O-AcOH-C <sub>4</sub> H <sub>8</sub> ) <sup>+</sup>
	341	C <sub>22</sub> H <sub>29</sub> O <sub>3</sub>	401-AcOH
	358	C <sub>22</sub> H <sub>30</sub> O <sub>4</sub>	(M-2H <sub>2</sub> O-C <sub>5</sub> H <sub>10</sub> O) <sup>+</sup>
XI	357	C <sub>22</sub> H <sub>29</sub> O <sub>4</sub>	(M-2H <sub>2</sub> O-C <sub>5</sub> H <sub>11</sub> O) <sup>+</sup>
	352	C <sub>23</sub> H <sub>28</sub> O <sub>3</sub>	(M-3H <sub>2</sub> O-(CH <sub>3</sub> ) <sub>3</sub> C-OH) <sup>+</sup>
	361	C <sub>22</sub> H <sub>33</sub> O <sub>4</sub>	(M-C <sub>5</sub> H <sub>11</sub> O) <sup>+</sup>
XV	343	C <sub>22</sub> H <sub>31</sub> O <sub>3</sub>	361-H <sub>2</sub> O
	277	C <sub>18</sub> H <sub>29</sub> O <sub>2</sub>	?
	263	C <sub>16</sub> H <sub>23</sub> O <sub>3</sub>	Analogous to m/e 263 (I)
	507	C <sub>30</sub> H <sub>35</sub> O <sub>7</sub>	(M-2H <sub>2</sub> O-2AcOH-C <sub>3</sub> H <sub>7</sub> ) <sup>+</sup> ; C-24-C-25
	495	C <sub>29</sub> H <sub>35</sub> O <sub>7</sub>	(M-2H <sub>2</sub> O-2AcOH-C <sub>4</sub> H <sub>7</sub> ) <sup>+</sup> ; C-23-C-24

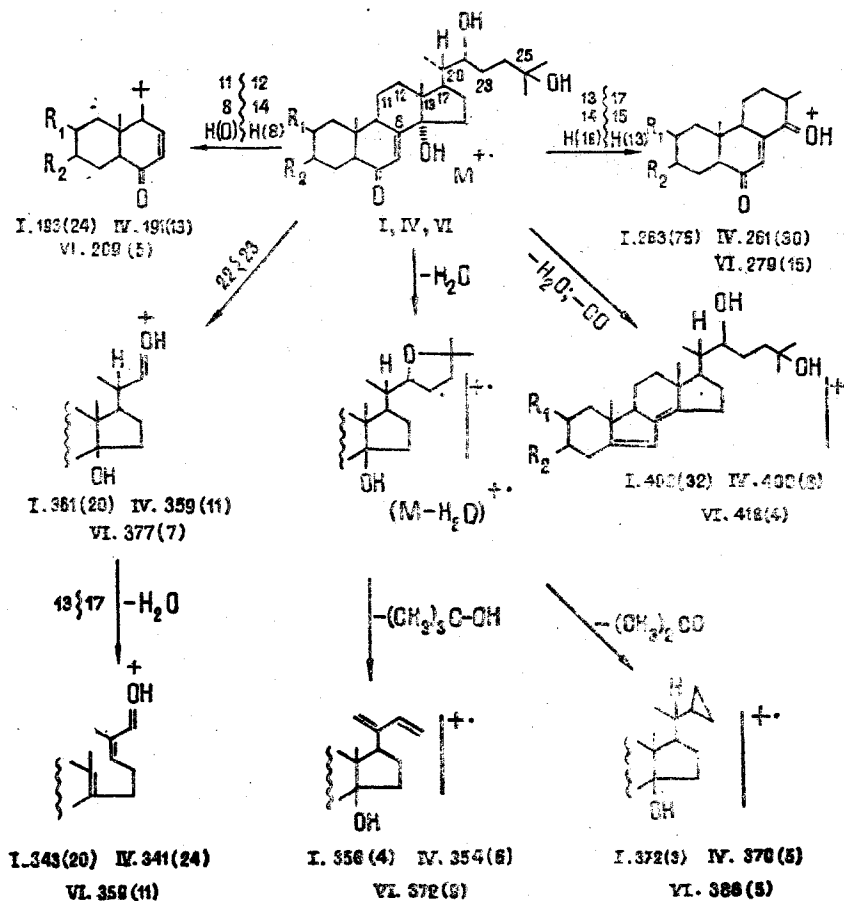
breakage of ring D at the C-13-C-17 and C-14-C-15 bonds, which is confirmed by the appearance of analogous fragments with m/e 261 and 279 in the spectra of silenosterone (IV) and  $\alpha$ -ecdysone (VI) (Scheme 1 and Table 3). However, the fragmentation products of corresponding composition in the spectra of the acetates of these ecdysteroids have a low intensity.

The ion with m/e 193 and the composition C<sub>11</sub>H<sub>17</sub>O<sub>2</sub> (I) and its analogs in the spectra of (VI) and (VI) may be a consequence of the process of fragmentation illustrated in Scheme 1. Both the types of cleavage of skeletal bonds considered are also characteristic of the spectra of cardenolides [9, 10, 12].

In the spectrum of premixisterone (XI), the strongest peak [2] is that of the ion with m/e 277 (C<sub>18</sub>H<sub>29</sub>O<sub>2</sub>). Although the nature of this fragment has not yet been established, its appearance is also due to the cleavage of skeletal bonds.

One more feature of the spectra of the 2- and 20-deoxyecdysteroids and their acetates consists in a tendency to the splitting out of a molecule of CO (Scheme 1) taking place as an alternative to the elimination of R-OH and CH<sub>3</sub> particles from M<sup>+</sup>. The elimination of carbon monoxide from the ions of series  $\alpha$ , b, and c is expressed less clearly in these spectra. The source of the particle eliminated is apparently the 6-keto group conjugated with the  $\Delta^{7(8)}$  bond. When there is no double bond in the molecule [compounds (XI) and (XII)], the (M-nROH-CO)<sup>+</sup> ions are not observed.

It is also necessary to direct attention to the fact that not only a decrease in the number of OR groups but also features of their mutual positions lead to unusual modes of frag-

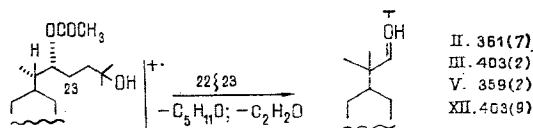


Scheme 1. Subsidiary directions of the fragmentation of the 20-deoxyecdysteroids.

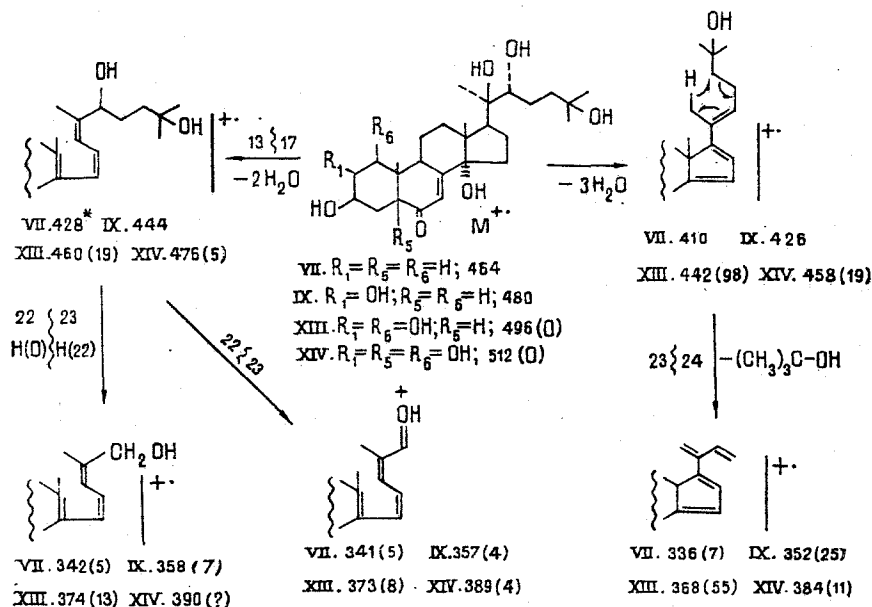
mentation of the skeleton of an ecdysteroid, as has been observed for integristerone B [4] the molecule of which contains  $\beta$ -OH groups at C-1, C-2, C-3, and C-5.

Subsidiary Directions of the Fragmentation of the Side Chain of C-17. The possibility of the cleavage of the C-22-C-23 bond as a consequence of 1,2-elimination of R-OH from C-25 has been mentioned in a study of the spectra of ecdysterone and its acetates [13]. Here, attention is directed to fragments with  $m/e$  69, including the chain from C-23 to C-27. Their intensity rose with R = Ac. The ions formed by the cleavage of the same bond but containing the chain from C-1 to C-22 were not revealed. Ohnishi et al. [5] have reported that the ions with  $m/e$  361 in the spectrum of 2-deoxy- $\alpha$ -ecdysone arise as the result of the ejection from  $M^+$  of the fragment  $C_5H_{11}O$  (87 amu). Other authors [8], referring to unpublished results have stated that C-22-C-23 cleavage is characteristic only of certain ecdysteroids.

A certain complication in the detection of this type of fragmentation consists in the fact that the ions  $(M - 87)^+$  may also arise as the result of the elimination of  $(4 H_2O + CH_3)$  which is extremely characteristic for any polyhydroxysteroids. However, by using measures of the elementary compositions of the ions in the spectral region under consideration, we have succeeded in showing that the cleavage of the C-22-C-23 bond is a fairly common phenomenon in the fragmentation of the ecdysteroids. The  $(M - 87)^+$  ions in the spectra of (I), (IV), (VI), and (XI) are due entirely to the elimination of the  $C_5H_{11}O$  fragment (Scheme 1 and Table 3). The  $(M - 105)^+$  ions of these spectra are formed by the same route but with the loss of an additional molecule of water. The acetyl derivatives of the compounds under consideration (II) (III), (V), and (XII) show clear indications of fragmentation at the C-22-C-23 bond, but with the simultaneous elimination of a ketene molecule at the expense of the 22-acetoxy group:



In the spectra of the ecdysteroids with a 20,22-diol grouping, the products of fragmentation at the C-22-C-23 bond are stabilized after the additional elimination of two molecules of water (Scheme 2). Under these conditions, together with ions having an odd mass there are fragments with an even mass formed by the migration of the mobile hydrogen atom from the C-22 OH group to the charged fragment. For greater persuasiveness, we have included in the scheme information from the spectra of integristerones A (XIII) and B (XIV). However, it must be noted that in one case, namely in the case of the ion with  $m/e$  390 from integristerone B, there is no information on a fragmentation process at the C-22-C-23 bond, since the fragment of maximum height, with the same mass number but a different elementary composition, is formed in the fragmentation of this substance by a different mechanism [4].



Scheme 2. Subsidiary directions of fragmentation of the side chains of 20,22-dihydroxyecdysteroids.

On the basis of the diacetate of 2-deoxyecdysterone (VIII), the triacetate of ecdysterone (X), and the pentaacetate of integristerone A (XV), it can be seen (Scheme 3) that C-22-C-23 cleavage follows the elimination of a molecule of acetic acid at the expense of the substituents at C-20 and C-22.

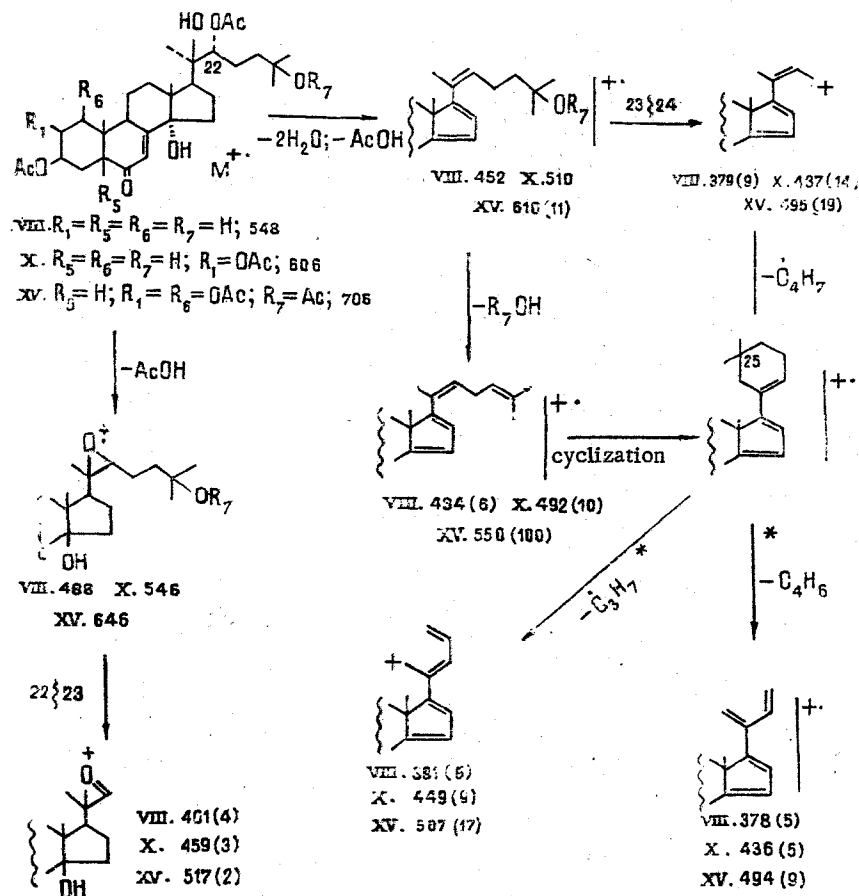
In all the spectra studied, we have been able to establish the splitting out of the C-24-C-27 chain taking place by various mechanisms (C-23-C-24 cleavage). It has been reported in the literature that this type of fragmentation is found predominantly in the 28C- and 29C-ecdysones having branching at C-24 [6, 8]. In the case of the 20-deoxyecdysteroids (I, IV, and VI), the peaks of ions with an even mass and a low intensity arising by the elimination of one molecule of water and a molecule of tert-butanol have been detected (Scheme 1).

This direction of fragmentation is probably favored by the cyclic form of the  $(M - H_2O)^+$  ion, the presence of which we have shown previously [4].

In the spectra of all the phytoecdysteroids with a 2-,22-diol chain there is the peak of an ion of variable intensity with an even mass number [for example,  $m/e$  336 (VII) or 384 (XIV)]. Measurement of the compositions of these ions has shown that they can be formed as the result of the elimination of three molecules of water and a molecule of  $(CH_3)_3C-OH$  (Scheme 2). The occurrence of this process is favored by the possibility of the realization of a six-membered transition state.

In contrast to the 20,22-diols, the acetates (VIII), (X), and (XV) give spectra showing a doublet of peaks of ions corresponding to cleavage at the C-23-C-24 bond. In addition to the simple cleavage of this bond, which can be realized after the elimination of  $2H_2O + AcOH$  (Scheme 3), cyclization of the cholestene chain is possible as the result of various processes of elimination of the substituent at C-25 which are enhanced, as can be seen from the intensities of the corresponding peaks, when  $R_7 = Ac$  [compound (XV)]. Fragmentation with the elimination of  $C_4H_7$ ,  $C_4H_6$ , and  $C_3H_7$  is characteristic for the 4,4-dimethylcyclohexene ring arising in this way [14]. The first two acts may be considered as processes of fragmentation





Scheme 3. Subsidiary directions of fragmentation of the side chain of the acetates of 20,22-dihydroxyecdysteroids; \*) the relative intensities are given in Table 2.

at C-23-C-24, and the last at C-24-C-25. The transformation of the ion with  $m/e$  550 into ions with  $m/e$  507 and 494 in the spectrum of the pentaacetate (XV) is confirmed by metastable peaks. The splitting out of a three-carbon-atom side chain is not observed in the spectra of the 20,22-diols (VII, IX, XIII, and XIV), but in the case of the 20-deoxyecdysteroids (I, IV, and VI) this type of fragmentation appears in the form of low-intensity peaks of the fragments  $(M - H_2O - Me_2CO)^+$  (Scheme 1).

**Experimental Procedure.** The synoptic mass spectra were taken on a MKh-1303 instrument (direct introduction of the sample, temperature of the inlet system 150-210°C, ionizing voltage 40V). The elementary compositions of the ions were measured in a MKh-1310 mass spectrometer using a sample inlet system (SVP 5) at a temperature of the heater of 50-150°C and of the ionizing chamber of 100-170°C, with an ionizing voltage of 70 V. The reference substance was perfluorokerosine. The relative error in the determination of masses does not exceed  $1 \cdot 10^{-5}$ .

#### SUMMARY

A change in the number of OR groups in the molecules of the ecdysteroids leads to a redistribution of the contributions of the three main directions of fragmentation of these compounds under electron impact.

In the spectra of the 20-deoxyecdysteroids, fragmentation of ring D at the C-13-C-17 and C-14-C-15 bonds is observed.

The subsidiary directions of fragmentation of the side chains of the compounds investigated have been analyzed.

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#### ISOLATION OF ECDYSTERONE FROM THE ROOTS OF *Rhaponticum carthamoides*

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UDC 615.322

The solubility of ecdysterone in individual solvents and mixtures has been studied. A method has been developed for isolating ecdysterone from the roots of *Rhaponticum carthamoides*. Extraction from the raw material was carried out with methanol. The catty oils and tanning and resinous substances were eliminated from the concentrated and water-diluted extract by treatment with chloroform. The combined ecdysteroids were extracted from the purified aqueous solution with chloroform-isopropanol (1:1). They were freed from pigments by chromatography on alumina (Brockmann activity grade II) with elution by methanol-chloroform (1:2). The product was re-crystallized from methanol-ethyl acetate (1:9), giving 0.05% (on the weight of the raw material) of ecdysterone.

The perennial herbaceous plant *Rhaponticum carthamoides* (Willd.) Iljin. (maral root, safflower leuzea, safflower rhubarb, safflower centaury) growing in the Asiatic part of the USSR and also in the Mongolian People's Republic [1] has been used from ancient times in folk medicine but its introduction into cultivation as a medicinal and fodder plant began only recently [2-4]. At the present time, galenical preparations with a tonic and stimulating action are being made from the roots and rhizomes of *Rh. carthamoides* [5]. An extract of the roots is one of the components of the beverage "Sayany."

The main biologically active substance of the roots of *Rh. carthamoides* - ecdysterone - was discovered in the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR by N. K. Abubakirov et al. [6]. The reserves of the wild and cultivated *Rh. carthamoides* make it a promising raw material for the production of ecdysterone. We have investigated the processes of isolating ecdysterone from the roots and rhizomes of this plant, which we obtained from the factory for the primary treatment of medicinal raw material. To select the optimum solvent for the various stages of the isolation and purification of ecdysterone we investigated its solubility in several solvents and mixtures of them:

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