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MASS SPECTRA OF ALLIOSTEROL AND ITS DEHYDRATION PRODUCT AND OF ALLOSIDES A AND B

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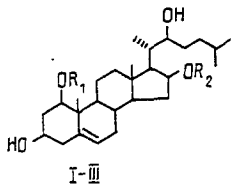
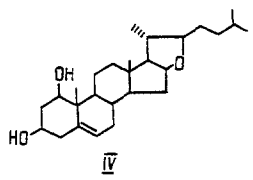
UDC 543.51+547.926

The EI and LSIMS mass spectra of alliosterol and its dehydration product and of allosides A and B have been studied. The spectra of alliosterol show features characteristic for sterols of the cholestane series. A comparison of the B/E spectra of some fragments of the ions from alliosterol and its dehydro product has shown the unimportance of the conversion of the former into the latter under mass-spectrometric conditions.

Recently, sterol glycosides (allosides A and B) the glycons of which are genetically connected with the spirostanols characteristic of these plants have been isolated from the collective fruit of the co-cultivated Allium suvorovii Rgl. and Allium stipitatum Rgl. [1]. The acid hydrolysis of allosides A (I) and B (II) formed a mixture of the corresponding aglycon – cholest-5-ene-1β,3β,16β,22-tetrol (III) and the product of its dehydration (IV), having a furostan skeleton [1]:



I-III



IV

I. R_1=H; R_2=β-D-Galp
II. R_1=β-*D*-Glcp; R_2=β-*D*-Galp
III. R_1=R_2=H

Interest in obtaining more comprehensive mass-spectrometric information than was given in [1] is due to a number of factors: the positions of the hydroxy groups, unusual for phytosterols, the probability of the (III) → (IV) transition under thermal conditions or in the dissociation of ions, and the possibility of comparing EI and LSIMS spectra and characterizing the fragmentation of compounds of the furostan series without OH groups at C-22 and C-26 (IV). The mass numbers and relative intensities of the main ions in the EI and LSIMS spectra are given in Table 1.

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TABLE 1. Mass Numbers (m/z) and Relative Intensities (%) of the Ions in the EI and LSIMS Mass Spectra of Compounds (I)-(IV)

Compound	Method of ionization	Mass numbers and relative intensities
Dehydroalli-sterol (IV)	EI	416(2), 398(100), 380(21), 365(14), 345(2) 327(2) 309(2), 298(1) 287(13) 269(7) 251(5)
	LSIMS (Gl)	417(63), 399(100), 381(44), 299(37), 287(81), 269(56)
Alliosterol (III)	EI	434(0.3), 416 (100), 398(28), 383(8), 380(6), 365(4) 329(4), 316(4), 301(8), 298(50), 287(27), 280(9), 269(9), 265(7)
	LSIMS (Gl)	435(8), 417(24), 399(100), 381(34), 363(11), 299(12) 287(56), 271(26), 269(21)
	LSIMS (Gl−NaCl)	457(85), 349(81), 381(52), 363(22), 299(22) 287(100)
Alloside A (I)	EI	596(0,1), 578(9), 560(1), 406(0,4), 478(20), 460(3) 414(8), 434(0,2), 416(38), 399(40), 398(21), 345(30), 316(18), 298(47), 287(100), 271(16), 269(23), 251(14)
	LSIMS (Gl)	597(18), 417(30), 399(100), 381(37), 363(12) 299(23), 287(63)
	LSIMS (Gl+NaCl)	619(100), 601(6), 417(3), 399(25), 381(7), 363(4) 321(5), 299(8), 287(23)
Alloside B (II)	EI	578(6), 561(3), 560(3), 533(3), 478(8), 460(5), 458(6) 449(11), 417(7), 416(100), 399(54), 398(79), 397(32) 381(46), 380(27), 316(36), 315(30), 298(89), 287(89) 271(54), 269(75), 267(46), 251(34)
	LSIMS (Gl)	759(9), 741(1), 597(2), 579(9), 561(18), 461(7), 446(14) 417(15), 399(90), 381(100), 363(18), 299(33) 287(28)
	LSIMS (Gl+NaCl)	781(100) 601(30)

In the EI spectrum of all four substances the molecular ions were unstable, and $M^{+\cdot}$ decreased in the sequence (IV) > (III) > (I) > (II). In the case of the last-mentioned compound the $M^{+\cdot}$ peak was absent and the peak with the largest mass number was that of the $(M - 180)^{+\cdot}$ ion. It is obvious that the dehydration of alliosterol (III) with the formation of (IV) may not be the only reason for the low stability of $M^{+\cdot}$. One must also bear in mind the peculiar combination of the OH groups of ring A in each of the (III) and (IV) molecules with a Δ^5 π-bond. In the spectra of spirostanols with analogous positions of the functional groups in rings A and B (ruscogenin) the stability of $M^{+\cdot}$ is 7-8 times lower than in compounds with different positions of the OH groups [2].

The fact that the $(M - H_2O)^{+\cdot}$ ion is the most stable in the spectrum of (III) and (IV) shows the predominant elimination of an H_2O molecule from the hydroxyls of ring A in the first stage. A confirmation of the cyclization of some of the molecules or of the M^+ ion of (III) to the corresponding particles with the structure of (IV) may be the presence in the spectrum of (III) of ions characteristic for the dehydro derivative (IV). However, the only distinguishing features of the latter are the peaks of the $(M - C_5H_{11})^+$ and $(M - H_2O - C_5H_{11})^+$ ions with m/z 345 and 327, which have a low intensity. But ions with the same mass number may arise from the $(M - H_2O)^{+\cdot}$ and $(M - 2H_2O)^{+\cdot}$ ions of alliosterol (III) on the simple cleavage of the C-22–C-23 bond [3]. Nevertheless, an ion with m/z 345 is absent from this spectrum, and the peak of the ion with m/z 327 is of low intensity.

On the other hand, an obvious characteristic of the fragmentation of alliosterol (III) in the form with a cyclized side chain at C-17 is the presence in its spectrum of the peaks of ions with m/z 316 and, particularly, 298 formed in the cleavage of the C-20–C-22 bond with the migration of a hydrogen atom to the charged fragment, which is characteristic, for example, for 20-deoxyphytoecdysteroids [3]. Ions with the corresponding mass arising in the cleavage of the bonds of ring E of dihydrospirostans (d_1 + H) [2] are of low intensity.

There are ions with m/z 287 and 269 in both spectra, but, in spite of their identical compositions, they have different origins and, probably, different structures. In the case of alliosterol, the first of the ions arises by the cleavage of the C-17–C-20 bond with the elimination of a molecule of water, and in the case of the dehydro derivative (IV) as a consequence of the breakdown of ring E at the C-16–O and C-17–C-20 bonds (the e_1 − H) ions [2].

The spectrum of compound (IV) with a furostan skeleton possesses two features distinguishing it from the spectrum of dehydrospirostans with an analogous skeleton [2]. In them,

TABLE 2. Mass Numbers and Relative Intensities (%) of the Daughter Ions in the B/E Spectra of the Metastable Ions with m/z 398 (EI), 399 (LSIMS*), and 287 (EI and LSIMS*) of Alliosterol and Dehydroalliosterol

Compound	Method of ionization	m/z of the parental ions	Daughter ions
Alliosterol (III)	EI	398	383 (33), 380 (100), 365 (30), 362 (10), 287 (4). 279(9), 269 (11), 251 (11).
	LSIMS	399	381 (100), 363 (19), 307 (11), 299 (6), 287 (5), 273 (5), 271 (6), 269 (2), 261 (2).
	EI	287	269 (100), 251 (25), 241 (11).
	LSIMS	287	269 (100), 251 (27), 241 (10).
Dehydroalli-sterol (IV)	EI	398	383 (15), 380 (100), 365 (40), 345 (6), 309 (5), 287 (3), 269 (3), 261 (1) 251 (4), 176 (5), 175 (6)
	LSIMS	399	381 (100), 363 (7), 299 (9), 287 (6), 273 (4), 271 (8), 269 (5), 261 (2).
	EI	287	269 (100), 251 (23), 241 (9), 199 (5), 197 (6).
	LSIMS	287	269 (100), 251 (22), 241 (15), 197 (15), 195 (32)

*The LSIMS spectra were obtained with the use of a glycerol matrix.

the peaks of the products of breakdown at the C-22–C-23 bond are of low intensity and analogues of the m ions with m/z 128 (C-16–O and C-17–C-20 cleavages; charge localized on the elements of ring E [2] are absent. Both types of ions are the strongest in the spectra of the dihydrospirostans. It is obvious that this is due to the presence of OH groups at C-26 in the molecules. In relation to the ions of the first type (cleavage of the C-22–C-23 bond), Albert's version of the rearrangement character of this process taking place with the participation of the above-mentioned OH group, based on a study of the spectra of 22-D derivatives of dehydrospirostans [4] is confirmed.

The LSIMS spectra of the compounds under consideration were obtained with the use of glycerol (Gl.) and glycerol with the addition of NaCl as liquid matrices. The stability of the $(M + H)^+$ ion of alliosterol (Gl.) only slightly exceeded that of $M^{+\cdot}$ in the EI spectrum. The most stable ion was $(M + H - 2H_2O)^+$ with m/z 399. The peak of an ion with m/z 287 was one of the most intense, and the ion with m/z 298 present in the EI spectrum appeared in protonated form – m/z 299. The addition of NaCl to the glycerol matrix led to the formation of a stable cation $(M + Na)^+$ with m/z 457. However, the contribution of the ion with m/z 399 decreased only slightly and the peak of the ion with m/z 287 became the maximum peak (Table 1).

The stability of $(M + H)^+$ in the LSIMS spectrum of compound (IV) was substantially greater than that of $M^{+\cdot}$ in the EI spectrum; the peak of the $(M + H - H_2O)^+$ ion remained the maximum and the peak of the ion with m/z 287 had also increased considerably in the LSIMS spectrum.

Thus, the general spectra of alliosterol and its dehydro derivatives recorded in the EI and LSIMS regimes do not permit us to judge the contribution of the (III) → (IV) transition since the spectra of (IV) do not possess appreciable specific features. Another approach to the solution of this problem consists in an analysis of the spectra of metastable ions. For this purpose it is necessary to select fragments of the two substances with the same mass numbers the structures of which coincide as the result of cyclization with the participation of the OH groups at C-16 and C-22. As such we selected the ions with m/z 398 (EI), 399 (LSIMS), and 287 (EI and LSIMS). We used the B/E = const. method of linked scanning, which gives information on all the daughter ions of a given metastable parental ion and shows similarities and differences in the parental ions [5]. The relative intensities of the peaks in the B/E spectra of the above-mentioned metastable ions are given in Table 2.

A comparison of the B/E spectra of the metastable ions with the same mass numbers for compounds (III) and (IV) showed substantial qualitative and quantitative differences between them. Thus, the spectrum of the metastable ion with m/z 398 from alliosterol (III) contained ions with m/z 362 and 279, which were absent from the spectrum of the analogous ion of compound (III). Conversely, daughter ions with m/z 345, 309, 176, and 175 were observed in the latter. The B/E spectra of the ion with m/z 399 in LSIMS differed to a smaller degree:

the intensity of the peak of the ion with m/z 363 had increased and the peak of an ion with m/z 307 had appeared in the case of alliosterol (III).

Characteristic for the B/E spectrum of the ion with m/z 287 was a qualitative similarity on recording in the EI and LSIMS regimes, while for alliosterol quantitative ratios of the intensities of the peaks of the daughter ions were also observed. In the B/E spectra of the ion with m/z 287 of dehydroalliosterol (IV) ions were observed with m/z 199 and 197 (EI) and 197 and 195 (LSIMS). These differences between the B/E spectra of the ions with m/z 287 of compounds (III) and (IV) gave grounds for considering that the cyclization of (III) under thermal conditions or on dissociation is unimportant.

In the EI spectrum of alloside A (I) the peak of the $(M-H_2O)^{+\cdot}$ stood out in a similar way to the spectra of (III) and (IV). The $M^{+\cdot}$ and $(M-H_2O)^{+\cdot}$ ions, losing a $C_6H_{12}O$ particle (C-20–C-22 cleavage) formed ions with m/z 496 and 478, respectively.

The elimination of the carbohydrate chain took place by the splitting out of a galactose molecule from $M^{+\cdot}$ (ions with m/z 416) or by the elimination of GalO from the $M-H_2O^{+\cdot}$ ion (ion with m/z 399). Together with these processes, the ejection of a fragment with 162 a.m.u. (Gal – H) from the $M^{+\cdot}$ and $(M-H_2O)^{+\cdot}$ ions that is characteristic for the majority of glycosides took place, was confirmed by the MD spectra of the corresponding daughter ions.

The fragmentation of the galactopyranose ring was characterized by the peak of an ion with m/z 444, formed by the cleavage of the C^1–C^2 and C^5–O bonds with the additional loss of a molecule of water [6]. The alternative elimination of a molecule of water, galactose, and a $C_6H_{12}O$ fragment led to a stable charged fragment with m/z 298. In contrast to breakdown at the C-20–C-22 bond, the ions characterizing the cleavage of C-17–C-20 bond were stabilized only after the elimination of a galactose molecule [m/z 287 (596 – 180 – $C_8H_{17}O$)].

The peak of an ion with m/z 345 ($C_{22}H_{33}O_3$) had a fairly high intensity in this spectrum. Judging from its composition, this ion arose as the result of the cleavage of the C-22–C-23 bond in the ion with m/z 416. However, the metastable defocusing spectrum of the ion with m/z 345 showed as precursors not only the ion with m/z 416 but also ions with m/z 496, 401, and 363. While the parental ion with m/z 401 lost the C-23–C-27 hydrocarbon chain in two stages (CH_3 + C_4H_8), the remaining precursor ions formed ions with m/z 345 by a fundamentally different method. After the loss by the $M^{+\cdot}$ ion of the $C_6H_{12}O$ fragment, elimination of part of the carbohydrate unit (cleavage of the C^1–C^2 and C^3–O bonds) and the elimination of an H_2O molecule took place.

In the LSIMS spectrum of alloside A (Gl.), the ion $(M+H)^+$ with m/z 597 had a medium intensity (Table 1). The ion having the mass number closest to this, m/z 417, was formed by the ejection of a galactose molecule. The dehydration of this ion led to charged fragments with m/z 399 (100%) and 381. The spectrum also contained ions formed as the result of the cleavage of the side chain at the C-17–C-20 and C-20–C-22 bonds (m/z 287 and 299, respectively). In spite of the fact that only the second of the ions formed contained an extra atom of hydrogen in comparison with the ion in the EI spectrum, the two ions were formed by the same rule, which is characteristic for LSIMS spectra – by the successive elimination of neutral molecules [$(M+H)^+$ – GalOH – $C_8H_{18}O$ and $(M+H)^+$ – GalOH – H_2O – $C_6H_{12}O$].

In the LSIMS (Gl. + NaCl) spectrum of alloside A, the peak of the $(M+Na)^+$ ion with m/z 619 was the maximum. Although the peaks of all the fragmentary ions corresponding to the spectrum obtained with the use of a glycerol matrix were also observed in the spectrum, their intensities were low (Table 1).

The breakdown of $M^{+\cdot}$ from alloside B (IV) on EI led to the splitting out of one hexose molecule with the formation of an ion with m/z 578, which then broke down in the same way as the analogous ion in the spectrum of alloside A (Table 1). The ion with m/z 449 had an origin analogous to that of the ion with m/z 287, but contained a carbohydrate substituent, and, in place of the ion with m/z 345, an ion with m/z 344 ($C_{22}H_{32}O_3$) also formed on the cleavage of the C-22–C-23 bond.

The LSIMS spectrum of alloside B (Gl.) was characterized by the $(M+H)^+$ ion with m/z 759, which decomposed with the subsequent elimination of the two hexoses (m/z 579 and 399). Each of these fragmentary ions was stabilized after the loss of a molecule of water. The peaks of the ions with m/z 287 and 299 were weaker than in the spectrum of alloside A.

EXPERIMENTAL

All the spectra were obtained on an MKh 1310 mass spectrometer using an IE24 source and LSIMS. Conditions for recording the EI spectra: SVP5 system for direct introduction, temperature of the ionization chamber and the evaporator bulb 200-180°C, ionizing voltage 70 V, collector current 60 μA, accelerating voltage 5 kV. For measuring the accurate masses of the ions the standard substance was perfluorokerosene, R ~ 10,000. Conditions for recording the LSIMS spectra: as the bombarding beam of primary ions we used Cs^+ with an energy of 7 keV, and accelerating voltage of 5 kV, and a temperature of the ionization chamber of 30-40°C. For the conditions used in obtaining the B/E spectra and the metastable defocussing spectra, see [2].

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TRITERPENE GLYCOSIDES OF Pulsatilla dahurica STRUCTURES OF GLYCOSIDES A, B, C, AND D

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The isolation of four triterpene glycosides from the roots of the dahurian anemone Pulsatilla dahurica (Fisch. ex DC) Spreng, is described together with their identification, on the basis of chemical transformations, spectral characteristics, and literature analogies, as hederagenin 3-O-α-L-arabinoside, hederagenin 3-O-[O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside], hederagenin 3-O-α-L-arabinopyranoside 28-O-[O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside], and hederagenin 3-O-[O-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranoside] 28-O-[O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside].

Continuing a chemical study of the Far Eastern species of anemone [1], we have isolated a fraction of substances of glycosidic nature from the roots of the dahuriam anemone (Pulsatilla dahurica) (Fisch. ex DC Spreng), family Ranunculaceae. It was found to contain six compounds, which have been designated in order of increasing polarity as substances A, B, C, D, E, and F [2, 3]. In the present paper we discuss the establishment of the chemical structures of structures A (I), B (II), C (III), and D (IV).

The acid hydrolysis of compounds (I-IV) gave a common aglycon for them, which, by a comparison of physical constants, chromatographic mobilities, and ^{13}C NMR spectra with an authentic specimen, was identified as hederagenin (Table 1).

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