MASS-SPECTROMETRIC STUDY OF THE SESQUITERPENE LACTONE GERMANIN-A AND ITS DERIVATIVES

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The structure of germanin-A, isolated from the leaves and flower heads of *Inula germanica* L., has been established previously [1]. In the present paper we discuss the mass-spectral behavior of germanin-A (I), [O-D]germanin-A (II), and its derivatives and their deutero analogs (III-XII).

The mass spectra of substances (I-XII) (Figs. 1-3) contain the molecular peaks with low intensities of (6-2)·10^{-4}%. The presence of an acyloxy group in position 8 of the sesquiterpene ring considerably destabilizes the molecular ions of this group of compounds.

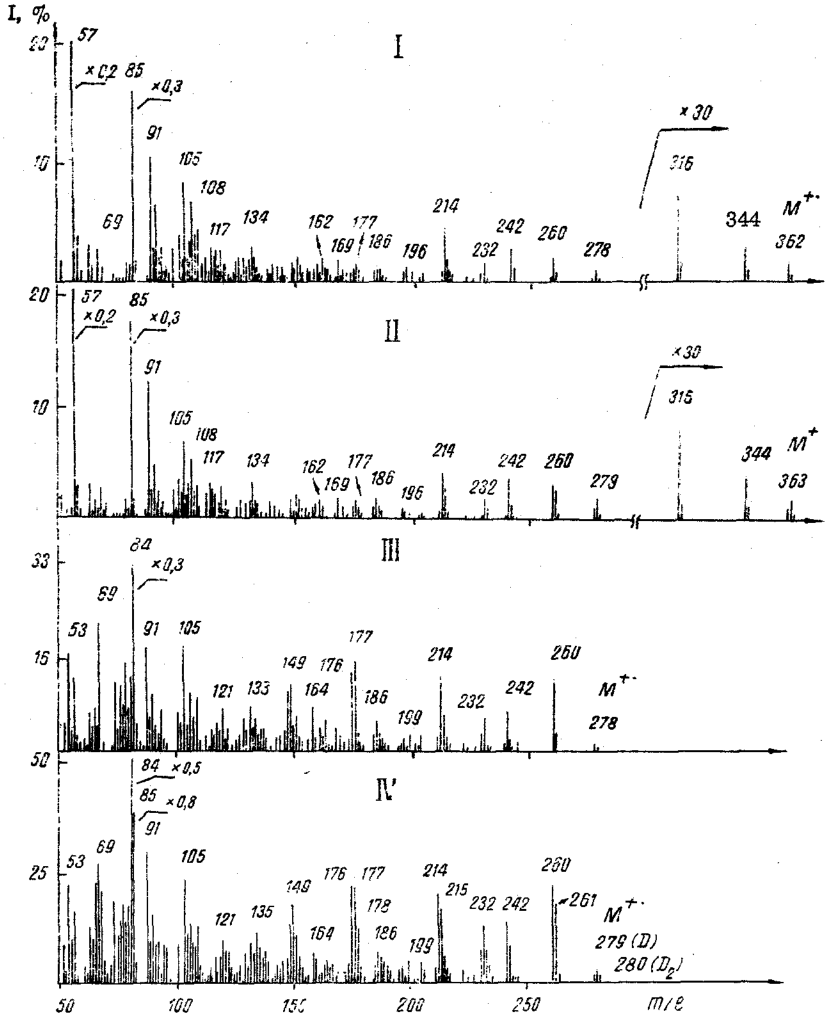

Fig. 1

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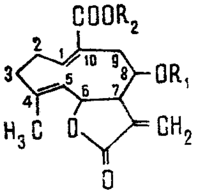
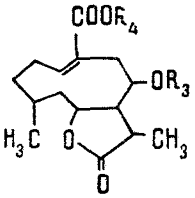


I-IV



V-XII

I. $R_1 = -CO\underset{\overset{|}{CH_3}}{C}HCH_2CH_3$; $R_2 = -H$;

II. $R_1 = -CO\underset{\overset{|}{CH_3}}{C}HCH_2CH_3$; $R_2 = -D$;

III. $R_1 = R_2 = -H$; IV. $R_1 = R_2 = -D$;

V. $R_3 = -CO\underset{\overset{|}{CH_3}}{C}HCH_2CH_3$; $R_4 = -H$;

VI. $R_3 = -CO\underset{\overset{|}{CH_3}}{C}HCH_2CH_3$; $R_4 = -D$;

VII. $R_3 = -CO\underset{\overset{|}{CH_3}}{C}HCH_2CH_3$; $R_4 = -CH_3$;

VIII. $R_3 = R_4 = -H$; IX. $R_3 = R_4 = -D$;

X. $R_3 = -H$; $R_4 = -CH_3$; XI. $R_3 = -D$; $R_4 = -CH_3$;

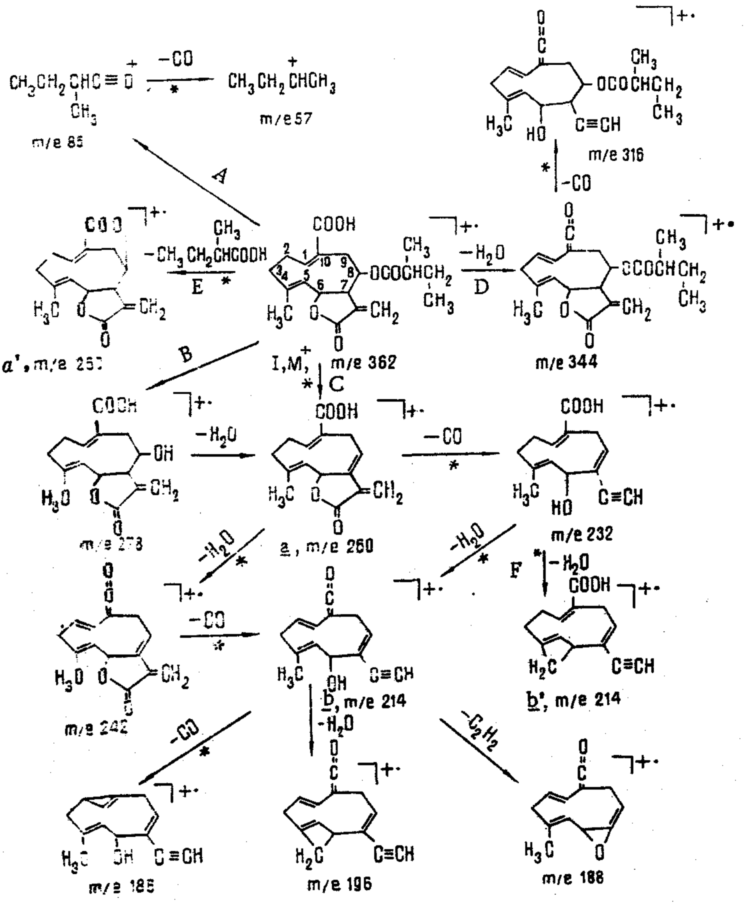
XII. $R_3 = -COCH_3$; $R_4 = -CH_3$;

The dissociative ionization of germanin-A (I) in the high-mass region leads to the fragments $(M - H_2O)^{+\cdot}$ with m/e 344 and $(M - H_2O - CO)^{+\cdot}$ with m/e 316 having very low intensities (Scheme 1, pathway D). In the mass spectrum of [O-D]germanin-A (II) the $(M - H_2O)^{+\cdot}$ ion undergoes no displacement into the region of higher masses, which confirms the splitting out of the hydroxyl of the carboxy group in the formation of the H_2O molecule. The elimination of a hydrogen atom of the methylene group from position 2 for the formation of H_2O is more likely than from position 9, since in this case, according to the McLafferty principle [2], the formation of the $(M - H_2O)^{+\cdot}$ ion would take place from the energetically more favorable six-membered transition state. The appearance of the $(M - H_2O - CO)^{+\cdot}$ fragment is apparently due to the splitting out of the CO group from the lactone ring, since in the mass spectrum of tetrahydrogermanin-A (V), where there is a methyl radical in place of the exocyclic methylene group in this ring, the value of the ratio $I_{(M-H_2O-CO)^+}/I_{(M-H_2O)^+}$ falls sharply. In the mass spectra of (I) and (V) it is equal to 5.0 and 0.25, respectively.

The main direction of fragmentation of germanin-A (I) (Scheme 1, pathway A) is connected with the splitting out of the α-methylbutyric acid residue with the localization of the positive charge on it, which is characteristic for the decomposition of acyl derivatives of other classes of substances [3, 4]. The ion with m/e 85 formed then loses a molecule of CO, giving the fragment of maximum intensity with m/e 57.

Auxiliary directions of the dissociative ionization of (I), apart from splitting out of H_2O, are the ejection of a molecule of ketone and of the acid with the formation of ions with m/e 278 and 260, respectively (Scheme 1, pathways B and C). As can be seen from the mass spectrum of the deuterium analog (II), these ions show different degrees of displacement by one unit into the region of higher masses. The proportion of displacement of the first of them is the same as for the molecular ion M^+, while for the second it is considerably smaller ($I_{279}/I_{278} = I_{263}/I_{262} = 1.4$, and $I_{261}/I_{260} = 0.75$). This fact can be explained by the partial participation of the hydrogen of the carboxy group in the elimination of the molecule of acid for the formation of the ion $[M - CH_3CH_2CH(CH_3)COOH]^{+\cdot}$ with m/e 260 (Scheme 1, pathway E). It follows from an analysis of a Dreiding model of germanin-A (I) that the occurence of this process it most likely when orientations of the carboxyl and ester groupings are close to axial. An equatorial position of these groups prevents the steric interaction of the hydrogen atom and the CH_3 radical located on carbon atoms C_1 and C_4, respectively. Thus, in the light of the features of the NMR spectrum of (I) [1], the structure of the molecule can be represented in the form shown in Fig. 4.

The further decomposition of the fragment with m/e 260 is due to the loss of H_2O and CO molecules with the formation of ions with m/e 242 and 232, which also split out CO and H_2O, respectively, given a fragment with m/e 214. The partial displacement of the ion with m/e 214 by one unit in the direction of higher masses in the mass spectra of the deuterium derivatives (II) and (IV) shows that its formation from the ion with m/e 232 probably takes place partially through the loss of the hydroxy group at C_6 and of a hydrogen atom of the methyl group in position 4 (Scheme 1, pathway F). The ions with m/e 197 and 196 are probably formed from the ions with m/e 215 and 214 in the region of medium masses by the same mechanism



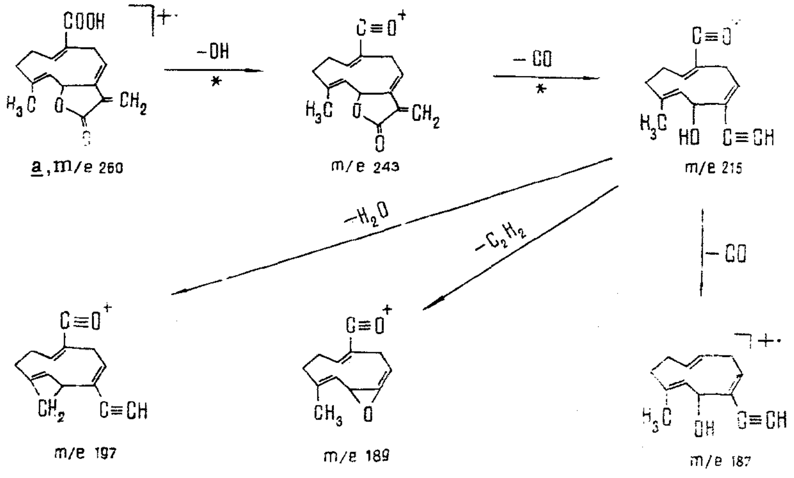
Scheme 1

(Schemes 1 and 2). The appearance of fragments with m/e 189 and 188 (Schemes 1 and 2) is probably connected with the loss of acetylene molecules by the ions with m/e 215 and 214.

In the mass spectrum of the product of the hydrolysis of germanin-A (III), the intensities of the ions with m/e 177, 176 and also those of the fragments with m/e 84, 83 have risen considerably. Their appearance can be explained by the cleavage of the $C_2—C_3$ and $C_9—C_{10}$ bonds on the further decomposition of the fragment with m/e 260 (Scheme 3). The displacement of the ions with m/e 177 and 84 in the mass spectrum of the deuterium analog (IV) by one unit into the region of higher masses, and also the elementary compositions of the ions with m/e 84 and 83 (the empirical formulas of the last two ions are, respectively, $C_4H_4O_2$, and $C_4H_3O_2$ and their accurate masses are 84.0142, 83.0138) confirm the pathway for their formation shown in Scheme 3.

The absence of an intense peak of the ion with m/e 84 in the mass spectrum of (I) can be explained by the assumption that a considerable part of the internal energy of the M^+ ion is borne by the polyatomic neutral particle $CH_3CH_2CH(CH_3)COOH$ in the formation of the fragment with m/e 260 in the mass spectrum of (I), in contrast to the iron with m/e 260 in the mass spectrum of (III), which is formed by the splitting out of an H_2O molecule. As a result, fragment with m/e 260 in the mass spectrum of (I) possesses a considerably smaller reserve of energy, which proves inadequate for the intensive cleavage of the two C—C bonds taking place in the formation of the ion with m/e 84.

The low intensity of this ion in the mass spectra of (V) and (VIII) can be explained by the fact that the maternal ion with m/e 264 lacks a double bond in position 4. Consequently, the probability of the cleavage of the $C_3—C_2$ bond and, together with this, of the formation of an ion with m/e 84 falls sharply (there is no allyl cleavage). In the mass spectrum of (V), the fragment with m/e 264 appears, as in the mass spectrum of (I), as the result of the elimination of a polyatomic neutral particle, which also decreases the probability of the appearance of the ion with m/e 84.



Scheme 2

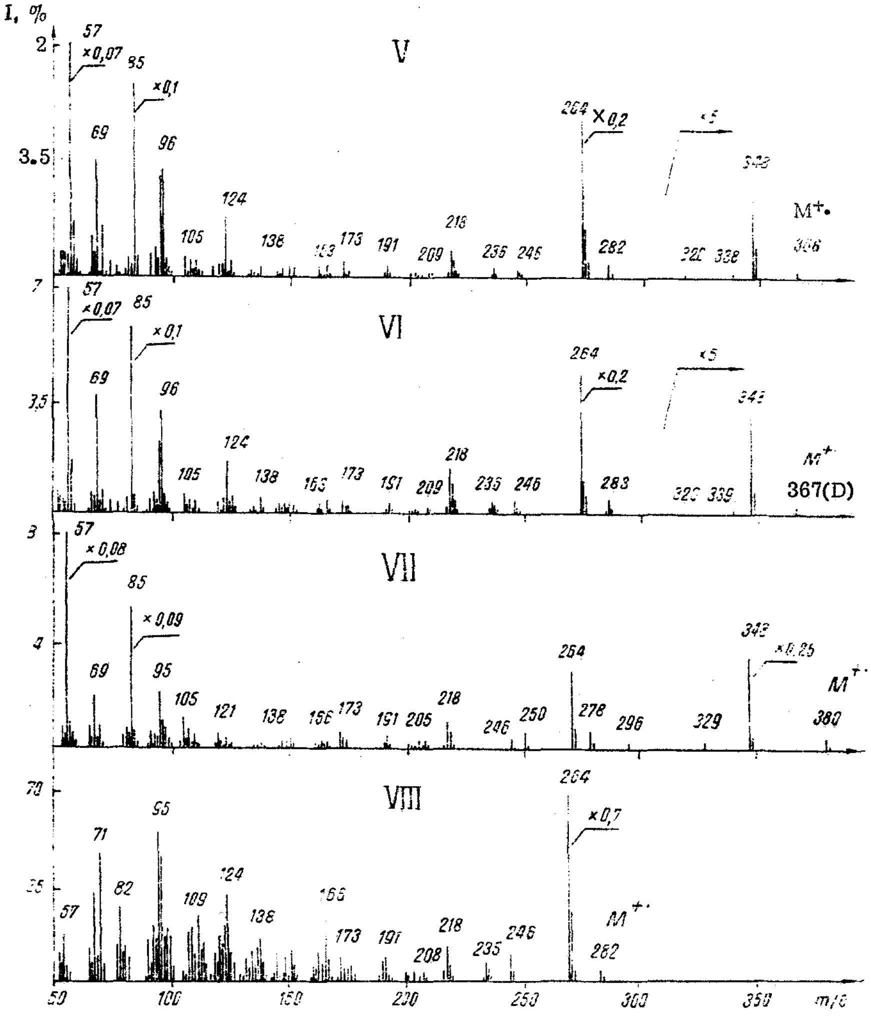
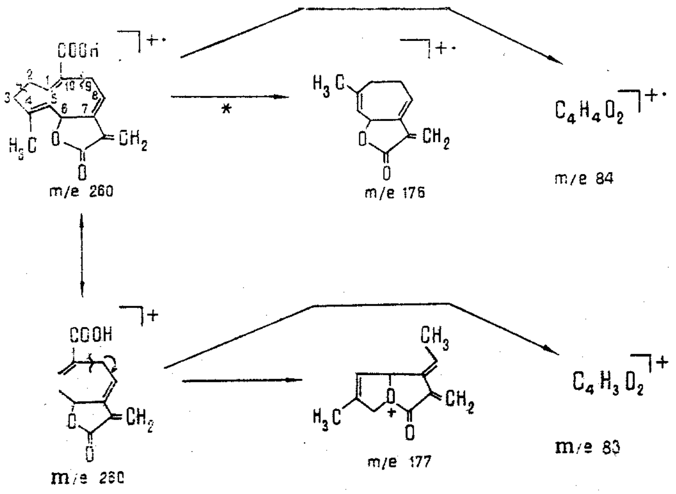


Fig. 2

The elementary compositions of the ions (see Table 1) in the region of high and medium masses in the mass spectrum of (I) also agree with the directions of their formation shown in Schemes 1 and 2.

In the decomposition of tetrahydrogermanin-A (V), the ions in the region of high and medium masses are displaced by 4 amu in comparison with the ions corresponding to them in the



Scheme 3

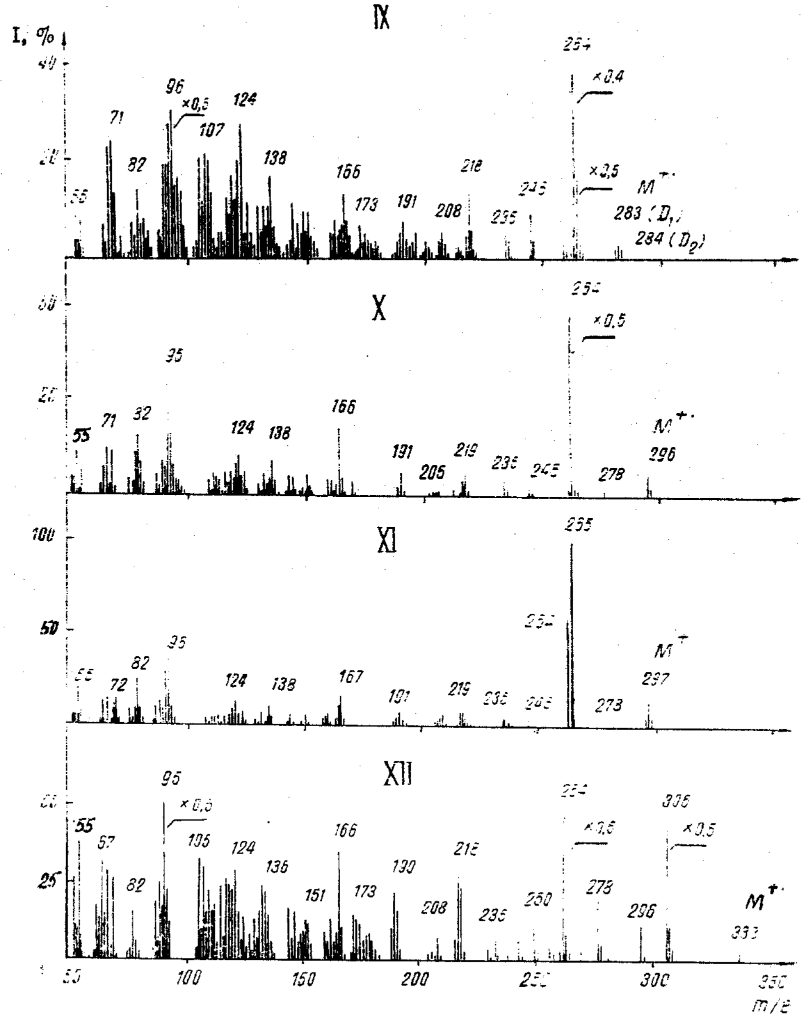


Fig. 3

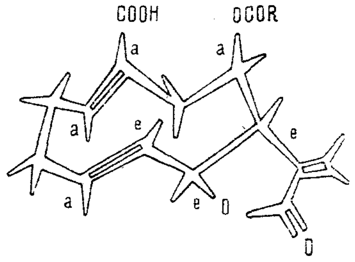


Fig. 4

mass spectrum of germanin-A (I). In contrast to the latter, the dissociative ionization of (V) is accompanied by the formation of a more intense fragment $(M - C_4H_9COOH)^{+\cdot}$ with m/e 264. A characteristic feature of its appearance is the complete absence of a shift into the region of higher masses in the mass spectrum of [O-D]tetrahydrogermanin-A (VI). This fact indicates a migration of the hydrogen of the carboxy group to the carbonyl oxygen atom of the acyl residue with a probability of 100% when the latter is split out to form a RCOOH molecule. Consequently, as compared with substance (I), the carboxy and ester groupings in the molecule of (V) are closer to one another, which is due to the absence of a double bond at the C_{10} atom in compound (V). Thus, the dissociative ionization of (V) confirms the similar spatial orientations of the carboxy and ester groupings in the germanin-A molecule (I). The fragmentation of tetrahydrogermanin-A (V) is also accompanied by the appearance of a stronger $(M - H_2O)^{+\cdot}$ ion than in the mass spectrum of (I), and for the first of them the ratio $I_{(M - H_2O)^+}/I_{M^+}$ is 20, and for the second 2. At the same time, the ejection of a ketone molecule in the case of dissociative ionization of (V) leads to an ion with m/e 282, the intensity of which is three times smaller than the corresponding magnitude for the analogous ion with m/e 278 in the mass spectrum of (I).

As can be seen from the mass spectrum of its deuterium analog (IX), the molecular ion of the product of the hydrolysis of tetrahydrogermanin-A (VIII) loses the hydrogen atom of the carboxy group to form an H_2O molecule, since on the decomposition of (IX) the $(M - H_2O)^{+\cdot}$ fragment with m/e 264 undergoes no displacement in the direction of higher masses. In the case of the dissociative ionization of the methyl ether of tetrahydrogermanin-A (VII) and of the

TABLE 1. High-Resolution Mass Spectrum of Germanin-A (I) in the Region of High and Medium Masses*

Mass		Error	Composition of the ion
measured	calculated		
362,1733	362,1728	- 0.5	$C_{20}H_{26}O_6$
344,1601	344,1622	—2,1	$C_{20}H_{24}O_5$
316,1689	316,1673	+1,6	$C_{19}H_{24}O_4$
278,1122	278,1153	—3,1	$C_{15}H_{18}O_5$
260,1040	260,1047	—0,7	$C_{15}H_{16}O_4$
242,0937	242.0942	—0,5	$C_{15}H_{14}O_3$
232,0129	232.0114	+1.5	$C_{14}H_{16}O_3$
215,1040	215,1072	—3,2	$C_{14}H_{15}O_2$
214,1205	214,1293	- 1,8	$C_{14}H_{14}O_2$
197,0964	197,0966	—0,2	$C_{14}H_{13}O$
196,0983	196,0988	—0,5	$C_{14}H_{12}O$
189,0918	189,0916	+0,2	$C_{12}H_{13}O_2$
188,0856	188.0837	+1,9	$C_{12}H_{12}O_2$
187,1121	187,1122	—0,1	$C_{13}H_{15}O$
186,1043	186,1044	—0.1	$C_{13}H_{14}O$
177,0907	177,0915	—0,8	$C_{11}H_{13}O_2$
176,0897	176,0837	+1	$C_{11}H_{12}O_2$

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*The table gives the main ions of which the formation is shown in Schemes 1-3.

acetyl derivative (XII), the intensity of the peak of the $(M - ROH)^{+\cdot}$ with m/e 348 and 306, respectively [where R = $-CH_3$ (VII, XII) or $-H$ (V)], increases considerably in comparison with tetrahydrogermanin-A (V). At the same time, the intensity of the $(M - R_1COOH)^{+\cdot}$ fragment with m/e 278 [$R_1 = C_4H_9$ (VII) or CH_3 (XII)] falls sharply, which confirms the predominant elimination of the hydrogen of the carboxy group in the formation of the molecule of acid during the fragmentation of (V).

The mass spectrum of the methyl ether of the product of the hydrolysis of tetrahydrogermanin-A (X), in contrast to the hydrolysis product (VIII) itself, contains a $(M - H_2O)^{+\cdot}$ ion having m/e 278 and a very low intensity. This fact confirms the participation of the hydrogen of the carboxy group in the splitting out of an H_2O molecule in the decomposition of the molecular ion of (VIII). The degree of displacement of the $(M - CH_3OH)^{+\cdot}$ fragment with m/e 264 in the direction of higher masses in the mass spectrum of the deuterium analog (XI) shows that approximately 18% of the hydrogen is eliminated in the composition of the CH_3OH molecule from the hydroxy group in position 8.

Thus, the dissociative ionization of germanin-A and its derivatives is determined by the presence of the lactone ring and of functional groups in positions 8 and 10 of the sesquiterpene ring, which are responsible for the occurrence of rearrangement processes connected with the appearance of $(M - ROH)^{+\cdot}$ ions, where R = H, CH_3, Ac.

EXPERIMENTAL

The mass spectra of compounds (I-XII) were obtained on a standard MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source, at an ionizing voltage U = 70 V and inlet temperatures of 100°C (III, IV, X, XI), 140°C (VIII, IX), 170°C (V, VI, VII), and 175 (III, IV, X, and XI). The high-resolution mass spectrum of germanin-A (I) was obtained on a JMS-01 SG-2 instrument with an automatic data-processing system. The derivatives were synthesized by a method described previously [5].

SUMMARY

The mass spectra of the sesquiterpene lactone germanin-A and 11 of its derivatives and deuterium analogs have been studied. A scheme has been put forward for the decomposition of germanin-A under the action of electron impact. It has been established that the elimination of a molecule of acid from position 8 in the dissociative ionization of germanin-A and of tetrahydrogermanin-A involves the hydrogen atom of the carboxy group present in position 10 of the sesquiterpene ring. On this basis, a suggested conformation of the germanin-A molecule has been put forward.

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