MASS SPECTRUM OF GLYCOSIDES OF ECDYSTEROIDS AND THEIR PERACETATES

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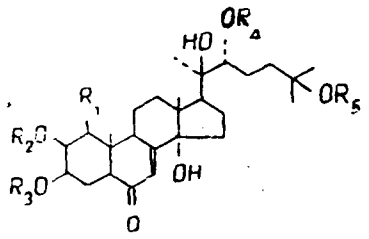
Features of the fragmentation of the carbohydrate chains of galactopyranosides of ecdysteroids (sileneosides A-D) and their peracetates under electron impact are discussed. The migration of Gal and AcGal residues from C-22 to C-20 has been detected. The secondary-ion mass spectra of the sileneosides using liquid matrices are considered.

Continuing an investigation of the LSIMS mass spectra of steroid glycosides [1], we have obtained the spectra of mon- and bisgalactosides of phytoecdysteroids [sileneosides A, B, C, and D (I-IV) [2-5]] and have compared them with the electron-impact spectra. The latter are characterized by the presence of peaks of the $(M - H_2O)^+$ ions [2-5] or by ions corresponding to the products of the splitting out of carbohydrate units [3, 4], and also by the presence of characteristic fragments formed on the cleavage of the C-20–C-22 bond [6]. In the spectrum of the less polar ponasteroside A, a weak peak of the M^+ ion has been detected [7]. The EI spectra of the sileneoside peracetates (V-X) contain the peaks either of the M^+ ions (V, VI) [2] or the $(M - H_2O)^+$ ions (VII-X) [3, 4] of the above-mentioned key fragments and the ion of a peracetylated galactose residue with m/z 331, and also ions characterizing the products of the successive splitting out of acetoxy groups in the form of AcOH molecules from all the fragments mentioned. The breakdown of the M^+ ions of compounds (I-X) is shown in scheme 1.



I. $R_1=R_2=R_3=R_5=H$; $R_4=\alpha$-Gal
II. $R_1=R_2=R_5=H$; $R_3=R_4=\alpha$-Gal
III. $R_1=OH$; $R_2=R_3=R_5=H$; $R_4=\alpha$-Gal
IV. $R_1=R_2=R_4=R_5=H$; $R_3=\alpha$-Gal
V. $R_1=R_5=H$; $R_2=R_3=Ac$; $R_4=\alpha$-AcGal
VI. $R_1=H$; $R_2=R_3=R_5=Ac$; $R_4=\alpha$-AcGal
VII. $R_1=R_5=H$; $R_2=Ac$; $R_3=R_4=\alpha$-AcGal
VIII. $R_1=H$; $R_2=R_5=Ac$; $R_3=R_4=\alpha$-AcGal
IX. $R_1=OAc$; $R_2=R_3=Ac$; $R_4=\alpha$-AcGal $R_5=H$
X. $R_1=OAc$; $R_2=R_3=R_4=Ac$; $R_5=\alpha$-AcGal

In the description of the breakdown under EI of sileneosides A-D the authors concerned [2-5] did not consider certain fragments of glycosides and their acetates to be of interest, and we shall dwell upon these before characterizing the LSIMS spectra of compounds (I-IV). These ions are formed on the fragmentation of a galactopyranose or peracetyl galactopyranose unit at C-22 and the cleavage of the C-22–C-22 bond.

The splitting out of the R_4 substituent from C-22 in the form of $Gal\dot{O}$ ($AcGal\dot{O}$) is characteristic for the spectra of sileneoside A (I) and the peracetates (V-X), while for (I) the peak of the $(M - \dot{O}R_4)^+$ ion has a fairly high intensity and in the peracetates stabilization sets in only after the additional elimination of R_5OH and H_2O.

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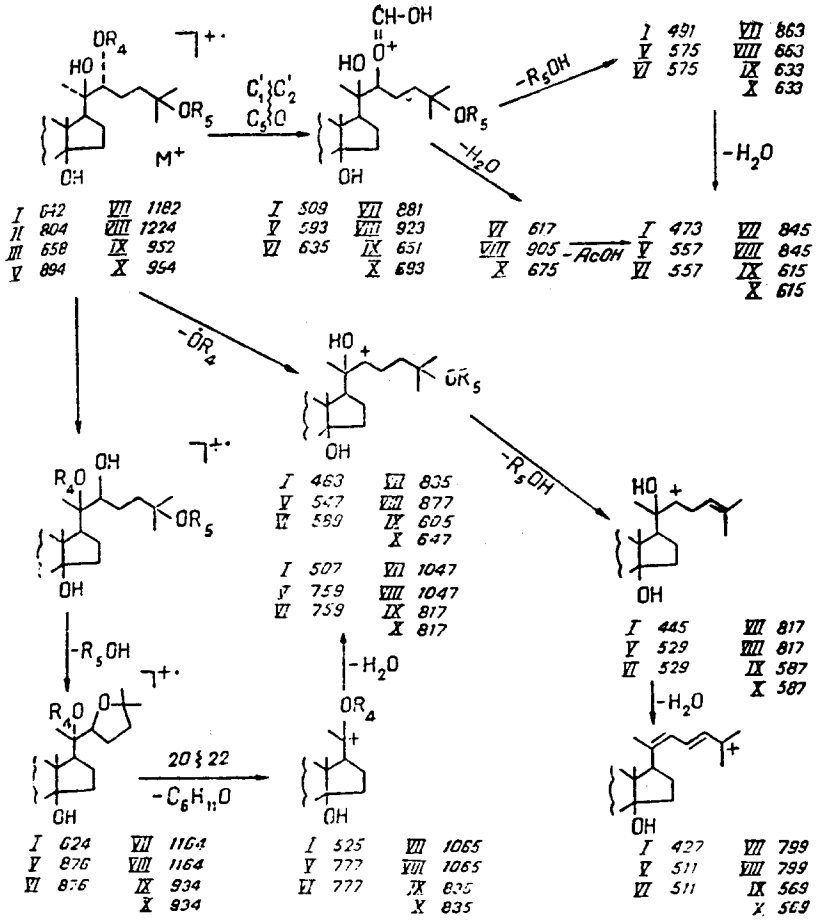


Scheme 1

In the spectra of compounds (I) and (V-X) the fragmentation of the carbohydrate chain at the C_1–C_2 and C_5–O bonds that is characteristic for glycosides [8] and their peracetates [9] was observed. The breakdown of the molecular ions by this direction did not give stable cations, and the subsequent successive ejection of H_2O and AcOH (VI, VIII, X) R_5OH and H_2O (for (I, V-X)) led to one of the strongest ion peaks in the region of medium mass numbers (see scheme 1).

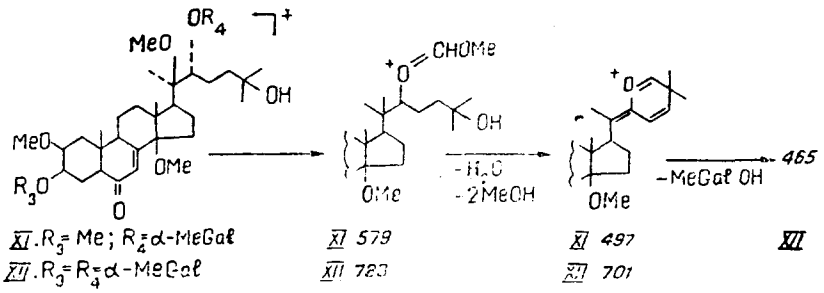
An unexpected property of the compounds under consideration with R_5 = H is the formation of $(M - 135)^+$ ions, or, for the compounds with R_5 = Ac, the $(M - 177)^+$ ions. For sileneoside A, such an ion could be the consequence of the cleavage of the same bonds of the carbohydrate unit (C_1–C_2 and C_5–O) with the opposite migration of the hydrogen atom. However, the absence of the corresponding shift in the spectra of the peracetates and, which is the main thing, the elementary compositions of the ions under consideration forced us to look for other mechanisms. The composition of the $(M - 135)^+$ ions in the spectra of (I) and (V) corresponded to the elimination of $C_6H_{15}O_3$. This fact permitted us to recognize the possibility of the migration of a galactosyl (peracetylgalactosyl) radical from the oxygen at C-22 to the oxygen at C-20 and the elimination of a R_5OH molecule from the molecular ion isomerized in this way, with the formation of an α,α-dimethyltetrahydrofuran ring [10], followed by the cleavage of the C-20–C-22 bond and the elimination of a molecule of water (see scheme 1). As a result, we had the fragments $(M - 135)^+$ (I, V, VII, IX) and $(M - 177)^+$ (VI, VIII, X). It must be emphasized here that in all the spectra, including those of sileneosides B and C (II, III) there were also indications of the cleavage of the C-20–C-22 bond without the migration of a carbohydrate unit [2, 3].

In order to estimate the universality of the processes involving the migration of the carbohydrate unit at C-22, we considered the spectra of the 2,3;3',4'-diacetonide of sileneoside A [2] and of the 3',4';3",4"-diacetonide of sileneoside B [3], and also the spectra of the octamethyl ether of sileneoside A (XI) and the undecamethyl ether of sileneoside B (XII).

TABLE 1. Mass Numbers of Characteristic Ions in the Spectra of Sileneosides A-D (I-IV)

Compound	Matrix – glycerol							Matrix – glycerol + NaCl			
	$(M+H)^+$	$(M+H-H_2O)^+$	$(M+H-2H_2O)^+$	$(M-Gal+2H)^+$	$(M-GalO)^+$	$(M-GalO-H_2O)^+$	$(M-GalO-2H_2O)^+$	$(M+Na)^+$	$(M+Na-H_2O)^+$	$(M+2Na-H)^+$	$(M+2Na+Cl)^+$
I	643	625	607	—	463	445	427	665	647	687	723,725
II	805	787	769	643 463	625 445	607 427	589	827	809	849	885/887
III	659	641	623	—	479	461	443	681	663	703	739/741
IV	643	625	607	481	463	445	427	665	647	687	723/725

The spectrum of each of the diacetonides had the peak of the $(M - 135)^+$ ion, and the elementary composition of one of them ($C_{33}H_{47}O_9$) with m/z 587 confirmed the analogy in its origin by migration of the carbohydrate unit and the cleavage of the C-20–C-22 bond. The breakdown of the molmolecular ions of compounds (XI) and (XII) is shown in scheme 2. The mass numbers of these compounds (754 and 958) indicated that one of the OH groups of each molecule had remained unsubstituted. The presence in these spectra of strong ions of fragments with m/z 219 and 187 showed that the galactopyranose unit was completely methylated. THe decomposition of the M^+ ion at the C-20–C-22 bond gave the peaks of ions with m/z 419 and 623 (XI and XII, respectively), from which it followed that the C-1–C-20 chain contained no free hydroxy groups. Thus, the OH group at C-25 had remained unsubstituted. Neither spectrum contained the peaks of $(M - 131)^+$ or $(M - 163)^+$ ions that would be analogous to the $(M - 117)^+$ and $(M - 135)^+$ ions in the spectra of compounds (I, V, VII, and IX) and could arise as a consequence of the opposite migration of the carbohydrate unit and a methyl radical from C-22 and C-20 followed by the cleavage of the corresponding C–C bond. It may be concluded that the etherification of the OH group at C-20 acts as an obstacle to the transfer of a carbohydrate unit from C-22 to C-20.



Scheme 2

The central part of the spectrum of the octamethyl ether of sileneoside A (XI) has weak peaks of fragments with m/z 579, 561, 547, and 529, and a strong peak of an ion with m/z 497. The first of these ions is the well-known product of the cleavage of the C_1–C_2 and C_1–O bonds of a permethylated galactopyranose ring with the migration of MeO from C_3 to C_1 [11, 12]. The other ions are formed by the alternative elimination of H_2O and 2 MeOH (see scheme 2). In the spectrum of the undecamethyl ether of sileneoside B (XII) the peaks of the analogs of these ions with m/z 783, 765, 751, 733, and 701 are less pronounced but the elimination from the last-mentioned ion of a MeGalOH molecule from C-3 leads to a stable fragment with m/z 465.

In the literature there are the following examples of the use of "mild" methods of ionization for the analysis of glycoecdysteroids. In the spectra of the 3β-D-glucosides of 5α- and 5β-hydroxyecdysterones obtained by the plasma desorption method with californium-252 the peaks of the ions $(M + Na)^+$, $(M + H + Na)^+$, $(M + 2Na)^+$, and $(M + H + 2Na)^+$ have been detected [13]. In the desorption chemical ionization spectra of the 3β-D- and 25β-D-glucopyranosides of 20,22-dideoxyecdysterone and their peracetates there are the peaks of the $(M + H)^+$ or $(M + H + H_2O)^+$ ions, and also of the $(M - \dot{G}lcO)^+$ ions [14].

The LSIMS spectra of sileneosides A-D (I-IV) were obtained not only with the use of a pure glycerol matrix but also in glycerol with addition of NaCl. The mass numbers and natures of the ions observed in these spectra are given in Table 1. The spectra obtained with the use of the glycerol matrix were qualitatively monotypical, with the exception of certain features due to structural differences. They contained the peaks of the $(M + H)^+$ ions and the products of the successive splitting out of two molecules of water, and the peaks of the $(M - \dot{G}alO)^+$ ions and of the products of their dehydration. The spectfa of sileneosides B and D (II and IV), the molecules of which contain a carbohydrate unit at C-3 were characterized additionally by the presence of $(M - Gal + 2H)^+$ ions. In the spectrum of sileneoside B the splitting out of (Gal + 2H), ($\dot{G}alO$) and ($\dot{G}alO + H_2O$) was accompanied by the elimination of the second carbohydrate unit in the form of a GalOH molecule (Table 1).

The LSIMS spectrum of the sileneosides in a glycerol matrix wtih the addition of NaCl are distinguished by the presence of the peaks of the ions $(M + Na)^+$, $(M + Na - H)^+$, and also of the products of the degradation of the first of them. In all the spectra there are

doublets with a height ratio corresponding to the isotopes ^{35}Cl and ^{37}Cl and, from their mass numbers, ions with the composition $(M + Na + NaCl)^+$.

EXPERIMENTAL

Electron-impact mass spectra were taken on a MKh 1310 mass spectrometer with a SVP-5 system for the direct introduction of the sample at an accelerating voltage of 5 kV, an ionizing voltage of 50 V, and a collector current of 40 μA, the temperature of the ionization chamber being 170-200°C and the temperature of the heater ampul 180°C. The ordinary spectra were recorded at a resolution of the instrument of 1000. The elementary compositions of the ions were determined at a resolution of 10,000, with perfluorokerosine as the reference substance. For the conditions of recording the LSIMS spectra, see [1].

Methylation of Sileneoside A by Hakomori's Method. A solution of 160 mg of sileneoside A in 20 ml of dimethyl sulfoxide was treated with 125 mg of sodium hydride, and the mixture was shaken at room temperature for 1 h. Then 3 ml of methyl iodide was added to the reaction mixture and it was left for a day at room temperature. The mixture was then poured into a 2% solution of sodium thiosulfate and was extracted with chloroform (3 × 100 ml). The chloroform fractions were combined, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. This method of methylation was repeated three times.

The substances obtained, in their entirety, were chromatographed on a column of silica gel. When the column was eluted with the benzene–acetone (4:1) system, the methyl derivative of sileneoside A was obtained.

Mass spectrum, m/z %: 754 (M^+, 0.3), 736(0.3), 72(0.1), 529(2), 497(17), 483(3), 465(4), 451(5), 433(3), 419(12), 401(13), 387(14), 369(25), 355(12), 337(15), 219(21), 187(100).

Sileneoside B was methylated by the method described above.

Mass spectrum, m/z %: 958(M^+, 1), 926(1), 857(1), 825(1), 733(8), 705(12), 623(13), 605(31), 465(68), 451(31), 437(30), 419(50), 387(62), 369(37), 355(62), 337(63), 219(62), 187(100).

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