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MASS SPECTRA OF FUROSTANOL GLYCOSIDES

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UDC 543.51+547.918+547.914

The mass-spectrometric fragmentation of furostanol glycosides under electron impact is described, and a comparison is made of these spectra with the secondary-emission spectra obtained by the FABMS and LSIMS methods.

Electron-impact (EI) mass spectrometry has been used successfully in the investigation of glycosides of the spirostan series [1, 2] containing a small number of carbohydrate units. However, with an increase in this number the polarity of the unmodified glycosides also increases, which makes the EI mass spectra less informative. The efficacy of the use of this mass-spectral method in the study of furostanol glycosides becomes even lower because of their higher polarity, in comparison with that of the spiro analogues, the instability of the $M^{+\cdot}$ ions, and the less characteristic nature of the mass spectra. These factors have impelled a search for accessible highly sensitive methods of mass spectrometry with "mild" ionization capable of expanding the range of compounds of this class that can be studied and also of being used where it is impossible to obtain EI spectra without chemical modification of the compounds under investigation.

The methods of "mild" ionization that have been used successfully in recent years in the mass spectrometry of natural compounds include secondary-emission methods of ionization. Those that are used most frequently are the mass spectrometry of secondary ions from a liquid matrix (LSIMS) and fast-atom bombardment (FAB). In preceding papers on the mass spectrometry of steroid glycosides [3, 4] we have shown the applicability of the LSIMS method for the study of unmodified glycosides of the spirostan series. In the present communication we give the results of an investigation of the spectra of glycosides of the furostan series isolated from Nolina microcarpa – nolinofurosides A (I), C (II) [5], F (III) [6], G(IV), and H (V) [7] – and also the products of the desulfation of the last two glycosides – compounds (VI) and (VII) [7], obtained by the LSIMS and FAB methods. These spectra were also compared with the EI spectra of the above-mentioned compounds [with the exception of the glycosides (IV) and (V)], which have proved to be fairly informative (see scheme at top of following page).

ELECTRON-IMPACT MASS SPECTRA

The presence of sulfur groups in the steroid moieties of compounds (IV) and (V) led to their thermal decomposition in the inlet system and to the impossibility of obtaining EI

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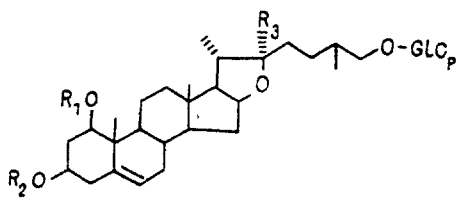


I. R_1=H; R_2=H; R_3=OH (OCH$_3$)*
II. R_1=β-*D*-Fuc$_p$; R_2=H; R_3= OH (OCH$_3$)
III. R_1=β-*D*-Fuc$_p$; R_2=α-*L*-Rha$_p$; R_3=OH (OCH$_3$)
IV. R_1=SO_3Na; R_2=H; $\Delta^{20(22)}$
V. R_1=β-*D*-Fuc$_p$; R_2=SO_3Na; $\Delta^{20(22)}$
VI. R_1=H; R_2=H; $\Delta^{20(22)}$
VII. R_1=β-*D*-Fuc$_p$; R_2=H; $\Delta^{20(22)}$

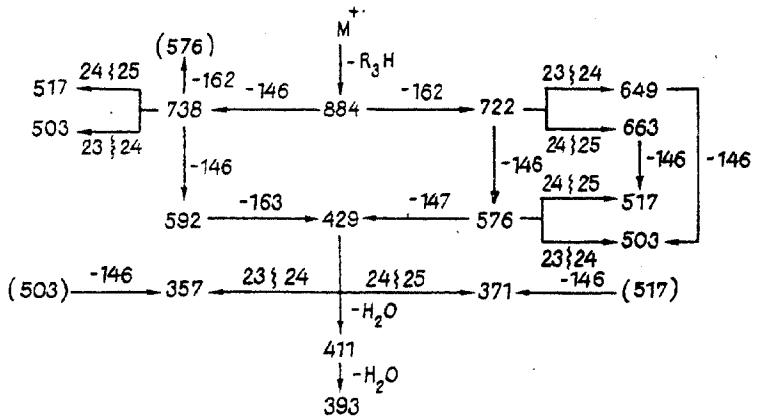
*Compounds (I-III) are mixtures of hydroxy- and methoxy-forms.

spectra. At the same time, the evaporation of samples of compounds (I-III) and (VI) and (VII) required comparatively low heating temperatures.

In the spectra of compounds (I-III), the maximum mass numbers were possessed by ions with m/z 592, 738, and 884, respectively, which were formed as the result of the splitting out of the molecular ions of the substituents at C-22 in the form of R_3H. A confirmation of this was the presence of peaks of the M^+ ions of medium intensity in the spectra of the $\Delta^{20(22)}$-furostenes (VI) and (VI) (m/z 592 and 738, respectively).

The fragmentation of compounds (I-III), (VI), and (VII) on EI, taking place fairly monotypically, can be well followed by taking as example the most complex of them, nolinofuranoside F (III). The main processes in its breakdown were the successive splitting out of carbohydrate units, the cleavage of the bonds of the C-22–C-26 chain, and the fragmentation of the steroid skeleton. The splitting out of carbohydrate units under EI conditions took place by alternative routes: The peaks of ions with m/z 738 and 722 corresponded to fragments formed by the ejection from $(M - R_3H)^+$ with m/z 884 of particles having 146 m.u. (a Fuc$_p$ or Rha$_p$ residue) and 162 m.u. (a Glc$_p$ residue), respectively. Ions with m/z 592 and 576 were formed by the same scheme from the ion with m/z 738 [the B/E = const linked-scanning (LS) spectrum of the m/z 738 ion contained the peaks of daughter ions with m/z 592 and 576]. An ion with m/z 576 was also formed from the m/z 722 ion upon the loss by the latter of a fragment with 146 m.u., as was also confirmed by the LS spectrum of the m/z 722 ion. The subsequent splitting out of carbohydrate units led to the ions of the aglycon moiety of compound (III) and of the products of its degradation (ions with m/z 429, 411, and 393; scheme).

The most characteristic alternative direction of fragmentation of furostan (III), also appearing in the spectra of compounds (I), (II), (VI), and (VII), is the cleavage of the C-22 side chain at the C-23–C-24 and C-24–C-25 bonds. These processes are characteristic for the ions with m/z 738, 722, and 576 and also for the Ag10 ion with m/z 429. All the transitions shown in the scheme have been confirmed by B/E spectra.



Scheme of the main direction of fragmentation of nolinofuroside F (III).

The peaks of the pairs of ions with m/z 663 and 649 (III), and also 517 and 503, and 371 and 357 (II, III, VI, and VII) were comparable in height and had a medium intensity. As compared with other compounds, nolinofuroside A (I) was characterized by more selective breakdown. In its spectrum the peaks of the ions with m/z 371 (cleavage of the C-24–C-25

bond) and 287 (cleavage of the C-16–O and C-17–C-20 bonds [8]) had intensities far exceeding all the others. In our opinion, this was due to the predominant formation of a $(M - R_3H)^+$ ion through the splitting out of hydrogen from C-23. In the spectrum of the isomeric $\Delta^{20(22)}$-furostene (VI), the intensities of these peaks were lower (see Experimental).

Another feature of the EI spectra of compounds (I-III), (VI), and (VII) was the breakdown of ring B at the C-13–C-17 and C-14–C-15 bonds, which is characteristic for steroid compounds of various groups, including the spirostan series [9, 10]. Together with the more common fragments having m/z 212-214 with the charge on rings A-C, here charged particles were observed with m/z 179 ($C_{11}H_{15}O_2$) and 181 ($C_{11}H_{17}O_2$) formed from the elements of ring D and E and the side chain at C-22. The linked-scanning spectra of the ions m/z 430 and 394 confirmed this direction of fragmentation.

In the interval of mass numbers of from 440 to 500 a.m.u. in the spectra of (I-III), (VI), and (VII) there were the peaks of ions formed by the cleavage of bonds in the carbohydrate residue m/z 459, 458, 441 (cleavage of the C_1–C_2 and C_5–O bonds), 471 and 472 (cleavage of the C_1–O, and C_2–C_3 bonds), and 501 (cleavage of the C_1–O and C_3–C_4 bonds).

SECONDARY-EMISSION MASS SPECTRA (LSIMS AND FABMS)

The spectra of compounds (I-V) were obtained by the LSIMS method using as liquid matrices pure glycerol (Gl) and glycerol with the addition of NaCl (Gl + NaCl).

The positive-ion and negative-ion mass spectra (PI-FABMS and NI-FABMS, respectively) of the ions of compounds (I-III), (VI), and (VII) were recorded by mass spectrometry with ionization by fast-atom bombardment (FABMS) (the liquid matrix being glycerol).

The LSIMS (Gl) and PI-FABMS spectra of compounds (I-III) were extremely similar to one another but differed somewhat in the intensities of the peaks of the ions (which was probably due to different conditions for recording the spectra). The absence from them of the peaks of the protonated molecular ions and the presence of the peaks of $(M + H - R_3H)^+$ ions and ions formed as the result of the successive splitting out of the carbohydrate units (II, III), of peaks of ions of the aglycon part of the molecule, and also of the peaks of ions with m/z 287, 269, and 251 make these spectra qualitatively similar to the EI spectra of the same compounds. The latter were more informative, since they contained a number of other ion peaks characteristic for steroid compounds.

The LSIMS (Gl + NaCl) and NI-FABMS spectra of compounds (I-III) contained a smaller number of characteristic peaks than in the spectra described above but, nevertheless, these gave information on the molecular masses of these compounds.

In the LSIMS (Gl + NaCl) spectra it was possible to single out two groups of peaks in the molecular-ion region, one of which related to furostanols of the hydroxy form (R_3 = OH) and the other to the methoxy form (R_3 = OCH_3). For example, in the mass spectrum of glycoside (III) the peaks of the $(M + Na)^+$ and $(M + 2Na - H)^+$ ions with m/z 925 and 947 characterized the hydroxy form while the peaks of ions with m/z 939 and 961 corresponded to the methoxy form. The spectra of compounds (I-III) also had the peaks of ions formed by the splitting out from the $(M + Na)^+$ ion of the substituent R_3 in the form of R_3H from C-22, and also the $(M + H - R_3H)^+$ ions.

The NI-FABMS spectra of the above-mentioned compounds contained the peaks of the $(M - H)^-$ ions of both forms which, in contrast to the LSIMS (Gl + NaCl) spectra were not the only analytically important peaks. Thus, in the NMI-FABMS spectrum of nolinofuranoside F (III), in addition to the peaks of the $(M - H)^-$ ions with m/z 915 (R_3 = OCH_3) and 901 (R_3 = OH) there were peaks of ions with m/z 769 and 755 $(M - H - 146)^-$ (R_3 = CH_3 and OH, respectively), 737 (769 or 755 – $R_3H)^-$, 609 $(755 - 146)^-$, 591 $(609 - H_2O)^+$, and 447 $(AglOH - H)^-$. As can be seen from the example given, the information provided by NI-FAB spectra leads to an unambiguous interpretation of EI and PI-FAB spectra.

The absence of the peaks of fragmentary ions in the LSIMS spectra obtained with the use of a glycerol matrix to which NaCl has been added may play a positive role in the investigation of natural mixtures of compounds similar to the steroid glycosides.

Particular interest is aroused by the secondary-ion LSIMS spectra of nolinofuranosides G and H [in view of the instability of compounds (IV) and (V) it was impossible to obtain their FAB spectra].

In the molecular region of the LSIMS (Gl) spectrum of compound (IV) there were the peaks of ions with m/z 711, 695, and 673. The last-mentioned ion had the composition $C_{33}H_{53}O_{12}S$, i.e., it was the molecular protonated ion the sulfo group of which did not contain an alkali-metal atom. The first two ions were also protonated molecular ions but contained a K or Na ion. This was confirmed by the following facts: the presence of two cluster ions with m/z 787 and 803, the first of which was the $(M + H + Gl)^+$ ion containing a Na atom in the sulfur group, and the second a K atom, and also by the presence in the region of low mass numbers of the peaks of ions with m/z 115 and 131 the compositions of which corresponded to the $(Gl + Na)^+$ and $(Gl + K)^+$, respectively.

An ion with m/z 575 was formed from the $(M + H)^+$ ion by the ejection of a $MHSO_4$ molecule (where M is an atom of Na or K), while the ion with m/z 597 was an analogue of the ion with m/z 575 in which a hydrogen atom had been replaced by a Na atom.

A series of ions (m/z 549, 533, and 511) was formed as the result of the splitting out of a glucose residue in each of the ions with m/z 711, 695, and 693, while the peaks of ions with m/z 431, 413, and 395 were also found in the spectra of compounds (I-III).

The spectrum of nolinofuroside G (V) (Gl + NaCl) differed from the spectrum described above by the more intensive nature of the peaks of ions with m/z 717 $(M + Na)^+$ and 597 $(M + Na - NaHSO_4)^+$, and also by the appearance of the peaks of ions with m/z 749 and 629 corresponding to a compound with the structure of nolinofuroside G but with the substituent $R_3 = OCH_3$ at C-22. In the PMR spectrum the corresponding signals were absent, and it is possible to explain the appearance of the two last-mentioned peaks only by the cleavage of the double bond and the formation of a methoxy derivative under the action of the methanol used to prepare the sample for recording. The fragmentation of nolinofuroside H took place in the same way (see Experimental).

The spectra of the nolinofurosides (VI) and (VII) obtained by the fast-atom bombardment (FAB) method in the regime of recording positive ions resembled those of compounds (I) and (II) since compounds (VI) and (VII) lack substituents at C-22 and belong by nature to the $\Delta^{20(22)}$-furostenes. However, in them the ions with the largest mass numbers were the protonated molecular ions, while in compounds (I) and (II) ions with the same m/z values have the composition $(M - R_3H)^+$.

The NI-FABMS spectra of the $\Delta^{20(22)}$-furostenes (VI) and (VII) contained the peaks of the deprotonated molecular ions $(M - H)^-$ with m/z 591 and 737, and also a series of peaks of ions characteristic of the aglycon moiety of the molecule. In the spectrum of glycoside (VI) an ion with m/z 183 was recorded; this was the cluster ion $(2M - H)^-$.

EXPERIMENTAL

Electron-impact spectra were obtained on MKh 1310 instrument; SVP 5 system for the direct introduction of the sample; ionizing voltage 70V; collector current 50 μA; temperature of the evaporator ampul and of the ionizing chamber 150-200°C. For the measurements of elementary composition and the conditions for obtaining B/E spectra, see [8].

The LSIMS spectra were recorded on a M 80-A mass spectrometer (Hitachi, Japan) with a M-003 computer data-processing system. The conditions for their recording are given in [3].

The spectra of positive and negative ions obtained by the FAB method were taken on a Finnigan MAT-8430 mass spectrometer with reverse-geometry double focusing fitted with an Ion Tech. ion source and a Spectrosystem MAT-300 data-processing system. Ionization was brought about by a beam of accelerated Xe atoms with an energy of 8 kV, and the accelerating voltage was 3 kV. The specimens were dispersed in glycerol and deposited on a steel target for the direct introduction of the sample. The spectra from 15 to 1600 m.u. were recorded at the rate of 3 sec/dec. The resolving power of the instrument was ~1000.

Mass spectra:

Nolinofuroside A. EI spectrum: 592($(M - R_3H)^+$, 16); 574(2); 501(2); 472(1); 471(2); 459(2); 458(2); 441($C_{28}H_{41}O_4$, 6); 430(25); 429(41); 413(12); 412(21); 411(13); 399(6); 396(34); 385($C_{25}H_{37}O_3$, 25); 378(10); 371(92); 357(7); 353(8); 340(8); 298(10); 287(100); 269(39); 251(25); 241(8); 229(9); 214(10); 213(13); 199(9); 197(8); 181($C_{11}H_{17}O_2$, 25); 179($C_{11}H_{15}O_2$, 14); 139(30), 121(23); 119(18). LSIMS spectrum (Gl + NaCl): 647(31); 633(100); 669(5); 655(16); 629(8); 615(25); 593(7). LSIMS spectrum (Gl): 593(90); 575(4); 459(3); 441(2); 431(21); 413(10); 371(10); 357(2); 287(100); 269(72); 251(48). FABMS-PI spectrum:

593(100); 575(8); 459(3); 431(26); 413(14); 371(8); 357(2); 287(56); 269(46); 251(28). FABMS-NI spectrum: 623(40); 609(100); 591(9); 447(12).

Nolinofuroside C. EI spectrum: 738($(M - R_3H)^+$, 13); 720(4); 592(22); 576(50); 574(12); 558(20); 517(24); 503(11); 471(7); 453(6); 430(39); 429(78); 413(47); 412(70); 411(86); 399(8); 396(28); 395(33); 394(30); 393(29); 385(10); 371(28); 357(18); 353(17); 340(10); 298(11); 287(47); 269(67); 251(33); 229(37); 213(32); 199(15); 197(15); 181(100); 179(45); 139(33); 121(45); 119(32). LSIMS spectrum (Gl + NaCl): 815(12); 793(100); 779(32); 761(29); 739(22). LSIMS spectrum (Gl): 739(92); 721(8); 593(12); 577(28); 575(28); 559(6); 557(4); 517(5); 459(4); 441(6); 431(8); 413(28); 395(13); 287(31); 269(100); 251(53). FABMS-PI spectrum: 739(100); 721(5); 593(5); 575(15); 577(17); 559(6); 557(2); 459(3); 431(9); 413(20); 395(12); 377(7); 287(25); 269(94); 251(44). FABMS-NI spectrum: 769(36); 755(100); 737(20); 609(16); 593(10); 447(8).

Nolinofuroside F. EI spectrum: 884(3); 738(5); 722(5); 720(1); 704($C_{45}H_{68}O_6$, 5); 663(2); 649(3); 592(16); 576(24); 574(7); 558(13); 556(3); 540(2); 517(5); 503(9); 471(6); 453(5); 430(51); 429(68); 413(50); 412(77); 411(73); 399(5); 396(17); 395(25); 394(30); 393(22); 371(9); 357(17); 340(10); 298(21); 287(20); 269(28); 251(21); 229(29); 213(31); 199(14); 197(15); 181(100); 179(39); 139(86); 121(64); 119(43). LSIMS spectrum (Gl + NaCl): 961(16); 947(8); 939(100); 925(37); 907(32); 885(9). LSIMS spectrum (Gl): 885(57); 739(10); 723(10); 721(10); 593(7); 575(18); 559(8); 557(6); 517(6); 431(10); 413(32); 411(18); 395(17); 287(10); 269(100); 251(80). FABMS-PI spectrum: 885(24); 739(100); 723(5); 721(5); 593(6); 577(22); 575(18); 559(6); 557(4); 457(5); 431(13); 413(37); 395(20); 287(32); 269(88); 251(56). FABMS-NI spectrum: 915(50); 901(16); 769(8); 755(92); 737(100); 609(7); 591(12); 447(3).

Nolinofuroside G. LSIMS (Gl + NaCl) spectrum: 749(52); 717(59); 629(40); 597(100). LSIMS (Gl): 803(19); 787(31); 711(60); 695(100); 673(52); 597(42); 575(20); 511(20); 431(15); 413(30); 395(14).

Nolinofuroside H. LSIMS (Gl + NaCl) spectrum: 895(57); 863(67); 775(47); 743(100); 629(23); 597(50). LSIMS spectrum (Gl): 857(47); 841(50); 819(40); 775(25); 761(50); 743(100); 597(60); 431(25); 413(85); 395(75).

Compound (VI). EI spectrum: 592(36); 574(2); 471(3); 453(2); 430(36); 429(74); 413(9); 412(17); 411(27); 399(3); 394(8); 393(11); 371(14); 357(10); 340(3); 287(23); 269(16); 251(13); 229(23); 213(33); 181(100); 179(72); 139(17); 121(46). FABMS-PI spectrum: 593(100); 575(4); 431(60); 413(17); 395(7); 371(3); 357(4); 287(28); 269(21); 251(13). FABMS-NI spectrum: 1183(46); 683(20); 591(100); 429(4).

Compound (VII). EI spectrum: 738(10); 720(1); 592(9); 576(13); 574(5); 558(4); 517(3); 503(3); 471(3); 457(3); 453(4); 430(21); 429(41); 413(24); 412(30); 411(48); 399(3); 395(14); 394(14); 393(19); 385(3); 371(5); 357(8); 353(4); 340(4); 312(10); 287(15); 269(24); 251(16); 229(32); 213(25); 199(12); 197(13); 181(100); 179(45); 139(24); 121(42). FABMS-PI spectrum: 739(96); 721(2); 593(17); 577(65); 575(42); 559(13); 431(40); 413(72); 395(37); 287(43); 269(100); 251(62). FABMS-NI spectrum: 737(100); 591(10); 575(5).

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