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SECONDARY-ION MASS SPECTROMETRY OF LYCOTETRAOSIDES OF THE SPIROSTAN AND FUROSTAN SERIES

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The secondary-ion mass spectra of seven steroid lycotetraosides have been obtained by the SIMS method. On the use of a glycerol matrix, all the compounds of the spirostan series, including those with an additional carbohydrate substituent a C-24, formed the $(M + H)^+$ ions, while a lycotetraoside of the furostan series formed the $(M - H_2O + H)^+$ ion. They subsequently decomposed by the alternative successive elimination of the terminal carbohydrate units of the lycotetraose. Substituents at C-25 and C-26 were ejected in the form of glucose molecules. Fragments of the aglycon and of the lycotetraoside moieties of the molecules were recorded. When NaCl was added to the same matrix, all the compounds gave preferentially the $(M + Na)^+$ and $(M + 2Na - H)^+$ ions.

The functions of the mass-spectrometric method in the study of the structures of the glycosides of the spirostan and furostan series have changed considerably during the last 15 years. Earlier, when exhaustive methylation of natural glycosides was the almost obligatory first stage of chemical investigations, the electron-impact (EI) mass spectra of these compounds gave rich information on the structures of the initial substances, thanks to the stability of the M^+ ions and the presence of a large number of characteristic fragments [1]. However, with the appearance of other methods of demonstrating structures and, above all, with the use of ^{13}C NMR spectroscopy, the modification of glycosides lost its previous urgency, but the possibilities of EI mass spectrometry of the native glycosides were limited to the production of spectra of triosides of the spirostan series containing, in addition to the weak peak of the M^+ ion, the peaks of ions corresponding to the successive elimination of the three carbohydrate units and the peaks of the key fragments of the aglycon parts of the molecule [2]. In addition to this, the wide use of new methods of "mild" ionization (fast-atom bombardment (FAB) [3], and secondary-ion mass spectrometry (SIMS) [4]) substantially broadened the possibilities of mass spectrometry in the analysis of unmodified polar and thermally labile natural compounds of various classes such as proteins and peptides [5],

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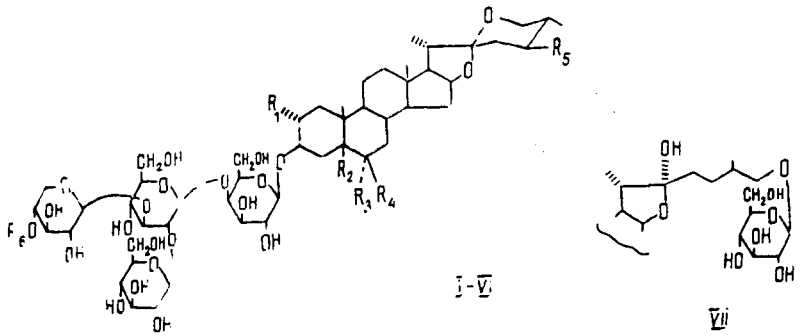
glyco- and phospholipids [6-8], prostaglandins [9], plant growth regulators [10] and others, including compounds with large numbers of carbohydrate units [11]. In the present communication, with the aim of evaluating the applicability of the method in the investigation of glycosides of steroid sapogenins, the secondary-ion spectra of six lycotetraosides of the spirostan series – derivatives of yuccagenin (karataviosides A (I) [12] and B (II) [13]), of karatavigenin C (karatavioside F (III) [14]), and of β-chlorogenin (IV), of alliogenin (V), and of alliogenone (VI) [15] and also of a lycotetraoside of the furostan series (karatavioside C (VII) [16]) have been characterized.



I-VI

VII

I. R_1=OH, $R_2+R_3=\Delta^5$, $R_4=R_5=R_6$=H

II. R_1=OH, $R_2+R_3=\Delta^5$, $R_4=R_5$=H, R_6=HOOC–CH_2–$C(CH_3)(OH)$–CH_2–C(=O)–

III. R_1=OH, $R_2+R_3=\Delta^5$, $R_4=R_6$=H, R_5=O-β-*D*-Glc

IV. $R_1=R_2=R_3=R_5=R_6$=H, R_4=OH

V. $R_1=R_2=R_4$=OH, $R_3=R_5=R_6$=H

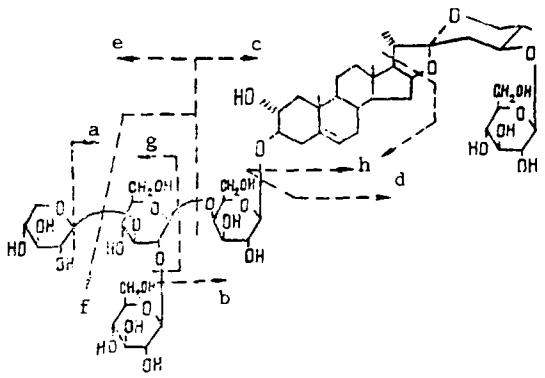
VI. $R_1=R_2$=OH, R_3+R_4=(=O), $R_5=R_6$=H

VII. R_1=OH, $R_2+R_3=\Delta^5$, $R_4=R_5=R_6$=H

Table 1 gives the main fragments from the breakdown of the above-mentioned compounds obtained by the SIMS method using a glycerol matrix. As can be seen from this Table, in the mass spectra of compound (I-VI) the peaks of the protonated molecular ions $(M + H)^+$ were considerably stronger than the peaks of the ions $(M + H - H_2O)^+$, while in the spectrum of (VII), conversely, the $(M + H)^+$ ion was not observed, which is apparently due to the presence of tertiary hydroxy group at C-22 the splitting out of which in the form of a water molecule leads to the formation of the more stable ion $(M - H_2O + H)^+$.

The greatest interest is presented by the ions formed as the result of the successive splitting out of the individual carbohydrate units, since they may give a considerable amount of information on the structure of the carbohydrate moiety of the molecule. Thus, in the spectra of compounds (I) and (IV) there are the peaks of ions the mass numbers of which differ from $(M + H)^+$ by 132 and 162 m.u., which indicates the competitive splitting out of the terminal xylose and glucose residues. For compound (II), the carbohydrate moiety of which contains a β-hydroxy-β-methylglutaric acid residue at the C-4' atom of the pentose, the splitting out of the carboxy acyl group of this acid with the migration of hydrogen to the charged fragment is characteristic, this leading to the formation of the $(M + H)^+$ ion of compound (I) with m/z 1049, which then, just like the protonated molecular ions of compound (I) and (IV) loses 132 and 162 m.u. in alternative sequences. Compound (III) gives a protonated molecular ion with m/z 1227 which splits out a molecule of glucose from the C-24 atom, leading to the strong peak of an ion with m/z 1047. Then the above-mentioned elimination of fragments with 132 and 162 m.u. takes place.

In the case of compound (VII), the splitting out of the xylose and glucose residues of the lycotetraose substituent takes place from the $(M - H_2O + H)^+$ ion with m/z 1211. The splitting out from this ion of the glucose molecule attached to the C-26 atom of the side chain leads to the formation of a weak peak of an ion with m/z 1031. Thus, glycosides of the spirostan series with an additional carbohydrate substituent in ring F can be distinguished from glycosides of the furostan series by analyzing the sequence of elimination of the sugar residues and the relative intensities of the corresponding peaks.



Scheme of the directions of fragmentation of compounds (I-VII) illustrated for the case of karatavioside F (III).

The successive ejection of both terminal carbohydrate units [with an additional substitutent at C-4' (II)] leads to the formation of ions with m/z 755 (I) and (II) and 753 (III) (after the splitting out of GlcOH at C-26), 757 (IV), 789 (V), 787 (VI), and 917 (VII) [from $(M - H_2O + H)^+$]. The splitting out of three carbohydrate units leads to the formation of the peaks of ions of type (Scheme) in all compounds (Table 1). The spectra of

TABLE 1. Mass Numbers and Relative Intensities (%) of the Main Fragments of the SIMS Spectra (Glycerol Matrix) of Compounds (I-VII)

Direction of breakdown	I	II	III
$(M+H)^+$	1049 (7)	1193 (3)	1227 (2)
		1049 (1,3)	1047 (4)
$(M+H-H_2O)^+$	1031 (0,4)	1031 (0,7)	1029 (0,5)
a		917 (0,6)	915 (1)
$a-H_2O$	899 (0,5)	899 (0,6)	897 (0,7)
b	887 (1)	887 (0,5)	885 (1)
$b-H_2O$		869 (0,8)	867 (0,6)
(a+b)	755 (0,5)	755 (1,5)	753 (1)
c	593 (19)	593 (16)	771 (4)
d	431 (100)	431 (100)	447 (19)
$d-H_2O$	413 (61)	413 (76)	429 (100)
	395 (28)	395 (26)	411 (53)
			393 (15)
e	457 (5)	601 (3)	457 (11)
f	325 (21)	325 (14)	325 (40)
g	295 (35)	295 (18)	295 (38)
h	287 (28)	287 (33)	287 (38)
$h-H_2O$	269 (42)	269 (57)	269 (66)
	251 (21)	251 (37)	251 (32)

Direction of breakdown	IV	V	VI	VII
$(M+H)^+$	1051 (3)	1083 (6)	1081 (2)	—
$(M+H-H_2O)^+$	1033 (0,3)	1065 (0,6)	1063 (0,7)	1211 (5)
a	919 (0,4)	951 (0,2)		1079 (0,6)
$a-H_2O$	901 (0,2)			1061 (0,4)
b	889 (0,5)		919 (0,3)	1049 (1)
$b-H_2O$	871 (0,2)	903 (0,6)	901 (0,3)	1031 (0,6)
(a+b)	757 (1)	789 (0,3)	787 (0,3)	917 (0,6)
c	595 (25)	627 (16)	625 (8)	593 (36)
				575 (12)
d	433 (100)	465 (100)	463 (61)	431 (23)
$d-H_2O$	415 (40)	447 (59)	445 (36)	413 (18)
	397 (23)	429 (69)	427 (48)	395 (10)
		411 (31)	409 (18)	
e		457 (16)	457 (15)	
f	325 (23)	325 (38)	325 (38)	325 (20)
g	295 (31)	295 (53)	295 (48)	295 (22)
h				287 (71)
$h-H_2O$				269 (100)
				251 (62)

TABLE 2. Mass Numbers and Relative Intensities (%) of the Main Fragments of the SIMS Spectra (with Glycerol + NaCl as matrix) of Compounds (I-VII)

Direction of breakdown	I	II	III	IV	V	VI	VII
$(M+2Na-H)^+$	1093(12,5)	1237 (37)	1271 (12)	1059 (17)	1127 (15)	1125 (13)	1273 (9)
$(M+Na)^+$	1071 (100)	1215 (100)	1249 (100)	1073 (100)	1105 (100)	1103 (100)	1251 (100)
$(M-H_2O+Na)^+$	1053 (12)	— 1071 (17)*	1231 (10)	1055 (12)	1087 (18)	1085 (25)	1233 (24)

*$(M + Na - 144)^+$

compounds (III) and (VII) contain the peaks of ions including hexose residues in the C-3 and C-24 positions (III, m/z 771) and in the C-3 and C-26 positions (VII, m/z 755). The further elimination of glucose from the C-24 (III) and C-26 (XXVII) positions leads to the formation of ions with m/z 591 and 575, respectively, while the splitting out of a galactose residue (d, scheme) from the same ions (m/z 711 (III) and m/z 755 (VIII)) leads to the formation of ions with m/z 609 and 593, respectively.

One of the most characteristic directions of fragmentation is the splitting out of the whole carbohydrate moiety of the molecule (d, scheme), leading to the formation of the protonated ion $(AglOH + H)^+$. These are the key ions in the analysis of steroid glycosides by the EI method and also by the SIMS method. The ions formed by the further loss of water molecules corresponding to the number of hydroxyls in the steroid moiety also bear important information on the structure of the aglycon moiety of the molecule.

Other fragmentation processes in the aglycon moiety take part through an insignificant degree in the SIMS spectra. Some of the strongest ions in the spectra of compounds (I-III) and (VII) are the peaks of ions with m/z 287, 269, and 251, formed by the cleavage of the C-17–C-20 and C-16–O bond with the successive loss of two water molecules (h, scheme).

Information on the carbohydrate moiety of the molecule is also supplemented by the peaks of ions formed by the cleavage of the bond between the glucose and galactose units (e, scheme). Thus, the spectrum of each of compounds (I), (III), (V), and (VI) contains the peak of an ion with m/z 457. In the spectrum of compound (II), this direction of fragmentation leads to the formation of the peak of an ion with m/z 601. The peaks of ions with m/z 325 and 295 that are present in the spectra of all the compounds are formed by the breakdown of the ion with m/z 457 in directions f and g, respectively.

In spite of the fact that the spectra of the lycotetraosides of alliogenin (V) and alliogenone (VI), unlike that of karatavioside C (VII), contain the peaks of ion $(M + H)^+$, the breakdown of the latter in directions and b is expressed more feebly than in compounds (I-IV). Thus, in the case of glycoside (V), the intensity of the peak of ion A is smaller than for the other compounds, while it is impossible to judge the presence of ion b because its mass number coincides with that of one of the peaks of the glycerol matrix (m/z 921). The spectrum of alliogenone lycotetraoside (VI) lacks the peak of ion while the peak of ion b is weak. At the same time, in both spectra there are weak peaks of the ions b $- H_2O$ with m/z 903 (V) and 901 (VI). The products of the successive splitting out of both terminal carbohydrate units – ions with m/z 789 (V) and 787 (VI) – are likewise weak.

The spectra of substances (I)-(VII) taken in a glycerol matrix with the addition of NaCl contain mainly the peaks of the $(M + Na)^+$, $(M + 2Na - H)^+$, and $(M + Na - H_2O)^+$, the fragmentation of which is expressed more weakly (Table 2) and does not include general features, as for the $(M + H)^+$ ions (Table 1).

Thus, as has been observed previously in the investigation of fusicoccin terpenoids [10], the use of different liquid matrices favors the acquisition of reliable information on the molecular masses of glycosides and of valuable information on the structure of the carbohydrate chains and the aglycon moieties of the molecules.

EXPERIMENTAL

The SIMS mass spectra were taken on a M 80-A mass spectrometer (Hitachi, Japan) fitted with an ion source working in the EI and SIMS regimes of mass spectrometry with a M-003 computer data-processing system. As the bombarding beam of primary ions was used Xe^+ with an energy of 8 keV, the temperature of the ionization chamber was 30°C, the accelerating voltage 3 kV, and the range of mass numbers from 100 to 1500 m.u.

The samples for analysis were dissolved in methanol, and 1 μl of a methanolic solution, containing 1-10 μg of the substance to be analyzed, was added to 1 μl of the liquid matrix placed on a silver target holder. Glycerol or glycerol with trace amounts of an aqueous solution of NaCl was used as matrix.

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