The method was checked on five model samples of tselanid solutions. The results obtained are given in Table 3, from which it can be seen that the relative error of the determination in the analysis of tselanid solutions was less than ±3%.

The results of an analysis of industrial samples of tablets and solutions of tselanid are given in Table 4, from which it follows that the results of the chromato-photocolorimetric method correlate with those obtained by the use of high-performance liquid chromatography and the biological method [1]. On the basis of the investigations performed, procedures have been developed for the quantitative determination of small amounts of tselanid (0.12 mg) that are 4-5 times faster than the procedures included in pharmacopoeias. Reliable results can be obtained by the procedures developed, since the separation of the degradation products by thin-layer chromatography makes it possible to analyze the remaining glycoside.

SUMMARY

1. Conditions for the chromatographic separation of glycosides and the products of their degradation have been investigated and solvents have been selected for extracting glycosides from tablets and sorbents. Procedures have been developed for the quantitative chromato-colorimetric determinations of tselanid as such and in tablets and solutions. The relative error of the determination does not exceed ±4%.

2. The results of the chromato-photocolorimetric method correlate with those obtained by the biological method and by high-performance liquid chromatography. The procedure developed can be used for the analysis of glycosides during storage.

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MASS SPECTROMETRY OF PENNOGENIN GLYCOSIDES

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UDC 543.51+547.918+547.926

The electron-impact mass spectra of five unesterified pennogenin glycosides, which contain the M^+ or $(M - H_2O)^+$ peaks, have been obtained. The characteristic features of the fragmentation of these compounds have been studied. In addition to ions characterizing the successive elimination of carbohydrate units, fragments have been detected which show the breakdown of the terminal pyranose ring. Five new directions of the fragmentation of the spirostanol skeleton due to the presence of an OH group at C-17 have been found.

Pennogenin (PG) and its glycosides are discussed in a number of publications [1-10] the mass-spectral information in which relates almost entirely to features of the fragmentation of the aglycon due to the presence of an OH group at C-17. A report by Japanese authors recently appeared on the acquisition of the electron-impact (EI) mass spectra of glycosides of PG and of diosgenin and of the corresponding furostanols where the possibility was shown of

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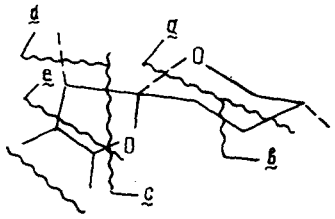
TABLE 1. Mass Numbers (m/z), Relative Intensities (%) of the Main Fragments, and Characteristics of the Fragmentation of the PG Glycosides (I-V)



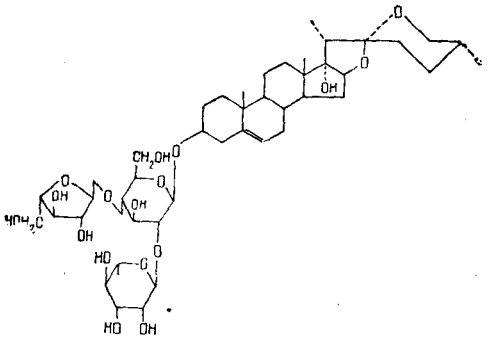
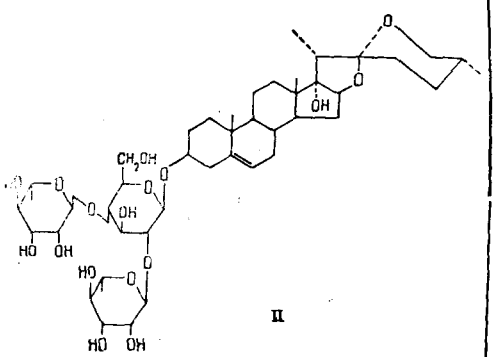
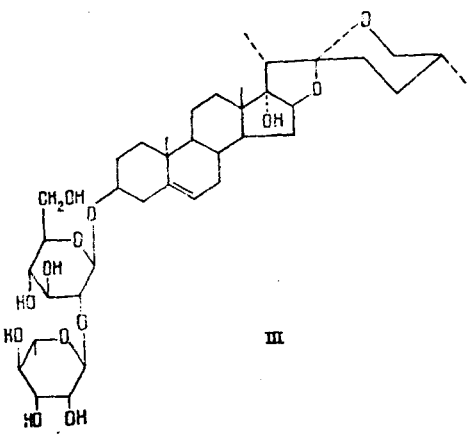
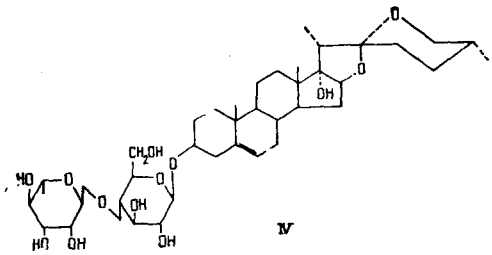
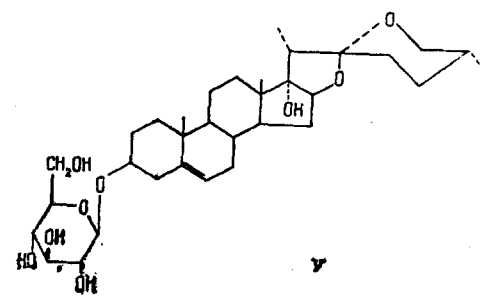
Glycoside	Fragments in the spectrum
	720 (M—H_2O—Ara_f +H; 0,06); 705 (720—CH_3; 0,05); 616 (B; 0,05); 603 (A+H; 0,08); 601 (A — H; 0,02); 592 (M —Ara_f —Rha_p + 2H; 0,16); 577 (592—CH_3; 0,5); 574 (592 — H_2O; 0,8); 559 (577—H_2O; 0,76); 502 (574 — *b*; 0,6); 467 (502—$2H_2O$+H; 0,68); 463 (592—*d*; 0,7); 430 (AglOH; 11.0; 412 (430—H_2O; 49,9); 395 (412 — H_2O+H; 21,4); 379 (395—CH_3 — H; 2,2); 377 (395—H_2O; 2,0); 371 (430 — *a*; 1,7); 358 (430—*b*; 4,5); 340 (412—*b*; 5,6); 316 (430—*c*—H; 10,4); 298 (316—H_2O; 21,4); 287 (430—*e*—H; 10,1); 281 (298—H_2O+H; 7,6); 269 (412—*e*—H; 11,0); 259 ($C_{18}H_{27}O$; 13,4); 277 ($C_{18}H_{29}O_2$; 6,2); 275 ($C_{18}H_{27}O_2$; 4.8); 257 ($C_{18}H_{25}O$; 13,4); 241 (259—H_2O; 8,3); 239 (257—H_2O; 10,1); 214 ($C_{16}H_{22}$; 30,9); 213 ($C_{16}H_{21}$; 22,0); 199 (66% $C_{15}H_{19}$+33% $C_{11}H_{19}O_3$, 21,0); 197 (66% $C_{15}H_{17}$+33% $C_{11}H_{17}O_3$, 21,0); 155 ($C_9H_{15}O_2$, 33,6); 153 ($C_9H_{13}O_2$, 73,0); 126 ($C_8H_{14}O$, 100).
 II	884 (M^+, 0,04); 866 (M — H_2O; 0,1); 762 (B; 0,1); 749 (A + H; 1,7); 738 (M—Rha_p +H; 0,1); 723 (738 — CH_3; 0,3); 720 (M—H_2O—Rha_p + H; 0,7); 705 (723—H_2O; 0,3); 648 (720—*b*; 0,2); 616 (B; 0,4); 603 (A+H; 0,6); 601 (A—H; 0,16); 592 (M—$2Rha_p$ + 2H; 0,5); 577 (592—CH_3; 1,3); 574 (592—H_2O; 1,3); 559 (574—CH_3; 1,1); 502 (574—*b*; 0,4); 533 (592—*a*; 0,4); 463 (592—*d*; 0,9); 430 (AglOH; 3,6); 412 (430 — H_2O; 37,0); 395 (412 — H_2O; 37.0); 371 (430—*a*; 1,3); 353 (412—*a*; 1,9); 340 (412—*b*; 6,3); 316 (430—*c*—H; 4,3); 298 (316—H_2O; 20,0); 287 (430—*e*;—H; 11,2); 281 (298—H_2O+H; 13,7); 277 ($C_{18}H_{29}O_2$; 63,3); 275 ($C_{18}H_{27}O_2$; 2,5); 269 (412 — *e* — H; 13,0); 259 ($C_{18}H_{27}O$; 13,7); 257 ($C_{18}H_{25}O$; 13,2); 241 (259—H_2O; 9,1); 239 (257 — H_2O; 10,7); 214 ($C_{16}H_{22}$; 29,0); 213 ($C_{16}H_{21}$; 24,7); 199 (66% $C_{15}H_{19}$+33% $C_{11}H_{19}O_3$; 20,2); 197 (66% $C_{15}H_{17}$+33% $C_{11}H_{17}O_3$; 22,0); 155 ($C_9H_{15}O_2$; 51,0); 153 ($C_9H_{13}O_2$; 88,0); 126 ($C_8H_{14}O$; 100).

TABLE 1 (Continued)

Glycoside	Fragments in the spectrum
 III	720 ($M^+ - H_2O$; 0,08); 705 (720—CH_3; 0,06); 648 (720—*b*; 0.05); 647 (C + H; 0,06); 616 (B; 0,08); 603 (A+H; 0,21); 601 (A—H; 0,04); 592 (M—Rha_p + H; 0,16); 577 (592—CH_3; 0,5); 574 (720—Rha_p + H; 0,4); 559 (574—CH_3; 0.33); 533 (592—*a*; 0,02); 502 (574—*b*; 0,15); 467 (502—$2H_2O$+H; 0,04); 463 (592—*d*—H; 0,03); 430 (AglOH; 0,2); 412 (430—H_2O; 21,0); 395 (412—H_2O+H; 23,4); 379 (395—CH_3 -H; 1,5); 377 (395—H_2O; 2,1); 371 (430—*a*; 0,06); 358 (430—*b*; 0,6); 353 (412—*a*; 0,3); 340 (412—*b*; 4,1); 316 (430—*c*—H; 3,2); 298 (316 — H_2O; 13,8); 287 (430—*e*;—H, 0,5); 281 (298—H_2O + H; 8,3); 277 ($C_{18}H_{29}O_2$; 3,6); 275 ($C_{18}H_{27}O_2$; 2,7); 269 (412—*e* -H; 0,7); 259 ($C_{18}H_{27}O$; 10,5); 257 ($C_{18}H_{25}O$; 10,0); 241 (259—H_2O; 6,5); 239 (257 — H_2O; 8,2); 214 ($C_{16}H_{22}$; 18,7); 213 ($C_{16}H_{21}$; 16.4); 199 (66% $C_{15}H_{19}$+33% $C_{11}H_{19}O_3$; 15,8); 197 (66% $C_{15}H_{17}$+33% $C_{11}H_{17}O_3$, 16,4); 155 ($C_9H_{15}O_2$; 29,5); 153 ($C_9H_{13}O_2$; 64,0); 126 ($C_8H_{14}O$; 100).
 IV	720 ($M^+ - H_2O$; 0,65); 705 (720—CH_3; 0,4); 647 (C+H; 0,3); 616 (B; 0.8); 603 (A+H; 0,3); 601 (A—H; 0,6); 592 (M—Rha_p +H; 0,4); 577 (592 — CH_3; 1,3); 574 (720—Rha_p + H; 9,6); 559 (574—CH_3; 10,0); 541 (559—H_2O; 8,1); 501 (574—*b*—H; 5% O); 467 (501—$2H_2O$+2H; 1,4); 463 (592—*d*; 1,1); 430 (AglOH; 3,8); 426 (430—4H; 7,2); 412 (430 — H_2O; 47.0); 395 (AglOH—$2H_2O$ + H; 33,5); 379 (395 — CH_3—H; 5,2); 377 (395—H_2O; 5,2); 353 (412—*a*; 3,5); 316 (430—*c* —H; 3,6); 298 (316 - H_2O; 18,0); 287 (430—*e*—H; 8,7); 281 (298—H_2O +H; 10,8); 277 ($C_{18}H_{29}O_2$; 5,1); 275 ($C_{18}H_{27}O_2$; 4,9); 269 (412—*e*—H; 11,7); 259 ($C_{18}H_{27}O$; 11,8); 257 ($C_{18}H_{25}O$; 12,9); 241 (259—H_2O; 8,1); 239 (257 — H_2O; 12,2); 214 ($C_{16}H_{22}$; 20,3); 213 ($C_{16}H_{21}$; 21,0); 199 (66% $C_{15}H_{19}$+33% $C_{11}H_{19}O_3$ 24,0); 197 (66% $C_{15}H_{17}$ + 33% $C_{11}H_{17}O_3$; 21,0); 155 ($C_9H_{15}O_2$; 40,0); 153 ($C_9H_{13}O_2$; 73,7); 126 ($C_8H_{14}O$; 100,0).
 V	592 (M^+; 0,7); 577 (592— CH_3; 1,7); 574 (592—H_2O; 1,7); 559 (574—CH_3; 1,0); 556 (M—$2H_2O$; 0,6); 533 (M—*a*; 0,4); 502 (574—*b*; 0,2); 478 (M—*c*; 0,7); 467 (502—$2H_2O$+H; 1,2); 463 (M—*d*; 1,2); 454 (B+H—H_2O; 0,6); 449 (M—*e*; 0,4); 430 (AglOH; 1,6); 412 (430—H_2O; 23,0); 395 (412 — H_2O+H; 16,5); 379 (395—CH_3—H; 1,4); 377 (395—H_2O; 2;4); 340 (412—*b*; 6,3); 316 (430—*c*—H; 1,7); 298 (316—H_2O; 24,0); 281 (298—H_2O+H; 11,5); 277 ($C_{18}H_{29}O_2$; 2,6); 275 ($C_{18}H_{27}O_2$; 1,4); 269 (412 — *e*; 8,2); 259 ($C_{18}H_{27}O$; 11,0); 257 ($C_{18}H_{25}O$; 9.4); 241 (259—H_2O; 5,1) 239 (257—H_2O; 6,8); 214 ($C_{16}H_{22}$; 20,5); 213 ($C_{16}H_{21}$; 14,5); 199 (66% $C_{15}H_{19}$+33% $C_{11}H_{19}O_3$; 12,0); 197 (66% $C_{15}H_{17}$+33% $_{11}H_{17}O_3$; 9,5); 155 ($C_9H_{15}O_2$; 25,5); 13 ($C_9H_{13}O_2$; 64,0); 126 ($C_8H_{14}O$; 1 0

judging the nature of the aglycon and distinguishing spirostanol glycosides from furostanol glycosides [11].

Nevertheless, our experiments with PG glycosides have shown that the electron-impact mass spectra may contain a considerably larger amount of information thanks to the presence in them of the M^+ or $(M - H_2O)^+$ ions, and also those of the products of the successive elimination of carbohydrate units. This enables us to deduce the number and nature of the sugar residues in the molecule of a glycoside and to study the laws of its fragmentation.

With this aim, we have obtained the EI mass spectra of five PG glycosides, namely: α-L-Rha_p-(1 → 2)-[α-L-Ara_f(1 → 4)]-β-D-Glc_p-PG (I) [4], α-L-Rha_p-(1 → 2)-[α-L-Rha_p-(1 → 4)]-β-D-Glc_p-PG (II) [4], α-L-Rha_p-(1 → 2)-β-D-Glc_p-PG (III) [6], α-L-Rha_p-(1 → 4)-β-D-Glc_p-PG (IV) [6], β-D-Glc_p-PG (V) [5].

The formulas of the compounds with indications of the main fragmentation pathways of the molecular ions and the mass numbers and relative intensities of the fragments are given in Table 1.

As can be seen from Table 1, the peaks of the molecular ions are present only in the spectra of (II) and (V), and in the spectra of (III) and (IV) the fragments with the highest mass numbers correspond to the $(M - H_2O)^+$ ions (m/z 720). The molecular ion of compound (I) with an arabinofuranose ring is less stable and here the peak of the ion with the highest mass is that of the $(M - Ara_f - H_2O)^+$ ion.

The sequence of carbohydrate units is shown by the process of successive formation of ions of glycosides with smaller numbers of sugar residues, down to the formation of the ions of the aglycon. Both the M^+ ions and the $(M - H_2O)^+$ ions act as the precursors of this series of fragments, but the peaks of the ions of the second series are weaker. In agreement with this, the spectrum of (I) shows a $(M - Ara_f + H)^+$ peak corresponding to M^+ of (III), and the spectra of (III) and of (IV) have the peaks of the $(M - Rha_p + H)^+$ peaks coinciding with M^+ in the spectrum of (V).

The spectra of the compounds that we studied show indications of the breakdown of the terminal carbohydrate units at C–C and C–O bonds. In Table 1, these fragmentations are denoted by the letters A, B, and C, just as in a paper [12] on the mass spectra of cardenolide monosides, where these fragments were detected for the first time.

We did not have available a sufficient number of facts and a large enough set of samples to characterize the differences between the breakdown of the isomeric glycosides (III) and (IV). However, attention was directed to the fact that the attachment of rhamnose to the C-4 atom of the glucose residue led to the formation of the ions A + H and A − H with m/z 603 and 601 of approximately the same order (IV), while the presence of rhamnose in the C-2 position of the glucose residue increased the intensity only of the A + H ion (III).

Komori et al. [2] have shown the presence in the spectra of spirostanol glycosides of fragments formed by the splitting out from M^+ of various elements of rings E and F. In the spectra that we studied, we also detected such fragments: an ion with m/z 553 in the case of compounds (II), (III), and (V) was formed as the result of the loss of 59 amu in the form of the particle C_3H_7O (denoted in the table by *a*) from M^+ (V), from $(M - Rha_p + H)^+$ (III), and from $(M - 2\ Rha_p + 2H)^+$ (II); in the case of compound (II), an ion with m/z 648 was formed as the result of the loss by the $(M - Rha_p + H - H_2O)^+$ ion of 72 amu (C_4H_8O, b); the peaks with m/z 478 and 449 in the spectrum of compound (V) were formed by the loss of 113 amu ($C_6H_9O_2$, c) and 142 amu ($C_8H_{14}O_2$, d) from M^+; and an ion with m/z 463 present in the spectra of all five compounds was formed by the ejection of 129 amu ($C_7H_{13}O_2$, d) from the ion with m/z 592.

The peaks of the ions of these series containing no sugar residues were the strongest. In the spectra of all the compounds there were ions with m/z 371 (Agl OH − *a*), 358 (Agl OH − b), 316 (Agl OH − c − H), 287 (Agl OH − e), 353 (Agl OH − H_2O − *a*), 340 (Agl OH − H_2O − b), 298 (Agl OH − H_2O − c) and 269 (Agl OH − H_2O − e) (in the case of compound (V), the peaks with m/z 371, 358, and 353 were absent).

So far as concerns the ions produced by the breakdown of the steroid nucleus, the peaks of which increase in intensity when there is an OH group present at C-17, we directed our attention to the presence in all the spectra of ions with m/z 259 and 257 and m/z 214 and 213. The ion with m/z 259 $(C_{18}H_{27}O)^+$ arises as the result of the elimination of H_2O from an ion with m/z 277 which was formed by the cleavage of the C-22–O, C-16–C-17, and C-13–C-17 bonds, i.e., essentially in a similar manner to the formation of the ion with m/z 153 from PG [11],

but with the charge on the steroid moiety. The ion with m/z 257 $(C_{18}H_{25}O)^+$ has as its precursor an ion with m/z 275, which is the complementary fragment to the ion with m/z 155 in the spectrum of PG (275 + 155 = 430). The appearance of an ion with m/z 214 as the result of the dehydration of the ion with m/z 232 was noted in the spectrum of PG in a paper by Budzikiewicz et al. [10] as the result of the cleavage of ring D at the C-13–C-17 and C-14–C-15 bonds. We measured the composition of the ions with m/z 214 $(C_{16}H_{22})^+$ and in this way confirmed that this process was the most probable one. On the cleavage of the same bonds but with the migration of one hydrogen to the neutral fragment, an ion with m/z 213 $(C_{16}H_{21})^+$ is formed.

The elimination of a methyl group from the ion with m/z 214 leads to the formation of an ion with m/z 199. An accurate measurement of the mass of this fragment showed that it had a doublet nature; one of the components corresponded to a $C_{15}H_{19}^+$ ion and the second component to a $C_{11}H_{19}O_3^+$ ion (the ratio between them being 2:1). The $C_{11}H_{19}O_3^+$ ion was probably formed in the above-mentioned cleavage of ring D, but with the localization of the charge on rings E and F and with the migration of a hydrogen atom to the charged fragment; the opposite migration of the hydrogen atom leads to the formation of the $C_{11}H_{17}O_3^+$ ion having an intensity of the same order as that of the $C_{11}H_{19}O_3^+$ ion.

Both in the spectrum of pennogenin itself and in the spectra of its glycosides (I)-(V), the intensities of the peaks with m/z 155, 153, and 126 are greater than those of the other ions, the peak with m/z 126 being the maximum peak.

Experimental Procedure. MKh 1310 mass spectrometer; SVP-5 system for direct introduction of the sample; temperature of the heater bulb of the ionizing chamber 150-250°C; collector current 40 μA; ionizing voltage 50 V. The transitions between the ions were confirmed with the aid of the metastable defocusing method. The stability of admission was checked by the constancy of the monitor current, and the distribution of the intensities of peaks by not less than two scans of the spectra. The masses of the ions were determined with an accuracy of $5 \cdot 10^{-6}$, the reference substance being perfluorokerosine.

SUMMARY

The possibility has been shown of obtaining electron-impact spectra of unesterified PG glycosides containing the M^+ or $(M - H_2O)^+$ peaks. Features of the fragmentation of these compounds have been studied. In addition to ions characterizing the successive elimination of the carbohydrate units, fragments have been detected which show the breakdown of the terminal pyranose ring. A number of new directions of the fragmentation of the spirostanol skeleton due to the presence of an OH group at C-17 have been described.

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