STEROID SAPONINS AND SAPOGENINS OF *Allium*

XIV. THE STRUCTURE OF KARATAVOISIDE A

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Continuing a study of the steroid saponins and sapogenins of *Allium karataviense* Rgl. (family Liliaceae) [1], we have isolated from the total extractive substances of the inflorescences of this plant four steroid glycosides which we have called, in order of increasing polarity, karataviosides A, B, C, and D. On the basis of the Sannie [2] and Ehrlich [3, 4] color reactions, glycosides A and B have been assigned to compounds of the spirostan series, and glycosides C and D to those of the furostan series. The present paper gives a proof of the structure or the main glycoside of the inflorescences of *A. karataviense* — karatavioside A (I).

The color reactions mentioned above and the presence in the IR spectrum of glycoside (I) of a series of bands characteristic for the spiroketal grouping [5, 6] enabled karatavioside A (I) to be assigned to the spirostanol compounds of the 25R series.

The methanolysis of compound (I) gave a set of monosaccharides and an aglycone (II) which was identified as yuccagenin. Analysis of the hydrolyzate by thin-layer chromatography and gas–liquid chromatography showed that the carbohydrate chain of karatavioside A (I) included D-galactose, D-glucose, and D-xylose in a ratio of 1:2:1.

Partial acid hydrolysis of the glycoside (I) yielded, in addition to yuccagenin (II), three prosapogenins (III-V). The aglycones of all three glycosides were identified as yuccagenin (II). Analysis of the hydrolyzates showed that the molecule of compound (III) contained D-galactose, (IV) contained D-galactose and D-glucose in a ratio of 1:1, and (V) contained D-galactose and D-glucose in a ratio of 1:2.

It follows from the facts given that in the molecule of karatavioside A (I) D-galactose is attached to the aglycone and D-glucose is attached to the galactose. Further information on the structure of the carbohydrate chain and the position of attachment were obtained as a result of the analysis of the products of complete methylation of glycosides (I) and (V).

The permethylate (VI) was obtained by methylating karatavioside A (I) by Hakomori's method [7]. The subsequent acid hydrolysis of compound (VI) led to the formation of the monomethyl ether of yuccagenin (VII) and a mixture of methylated sugars. The combined methylated sugars were separated, and among the individual components by thin-layer and gas–liquid chromatographies in comparison with authentic samples we identified 2,3,6-tri-O-methyl-D-galactopyranose, 4,6-di-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, and 2,3,4-tri-O-methyl-D-xylopyranose. Thus, in the molecule of karatavioside A (I), to one of the D-glucose molecules, forming a center of branching, two terminal carbohydrates are attached — D-glucose and D-xylose.

In order to determine the position of attachment of the terminal sugars to the central glucose molecule, we methylated the trioside (V) separately, and the resulting permethylate (IX) was hydrolyzed. In the permethylate, by comparison with authentic samples, we identified 2,3,6-tri-O-methyl-D-galactopyranose, 3,4,6-tri-O-methyl-D-glucopyranose, and 2,3,4,6-tetra-O-methyl-D-glucopyranose. The aglycone of the permethylate (IX) was identical with that of the permethylate (VI). The results obtained show that the terminal carbohydrates D-glucose and D-xylose in the sugar moiety of karatavioside A (I) are attached to the hydroxy groups at C-2 and C-3, respectively, of the central D-glucose molecule, which is attached to the D-galactose molecule through the hydroxy at C-4.

A calculation of molecular rotation differences [8] showed that all the four glycosidic bonds had the β configuration. Furthermore, the PMR spectrum of compound (VI) showed four

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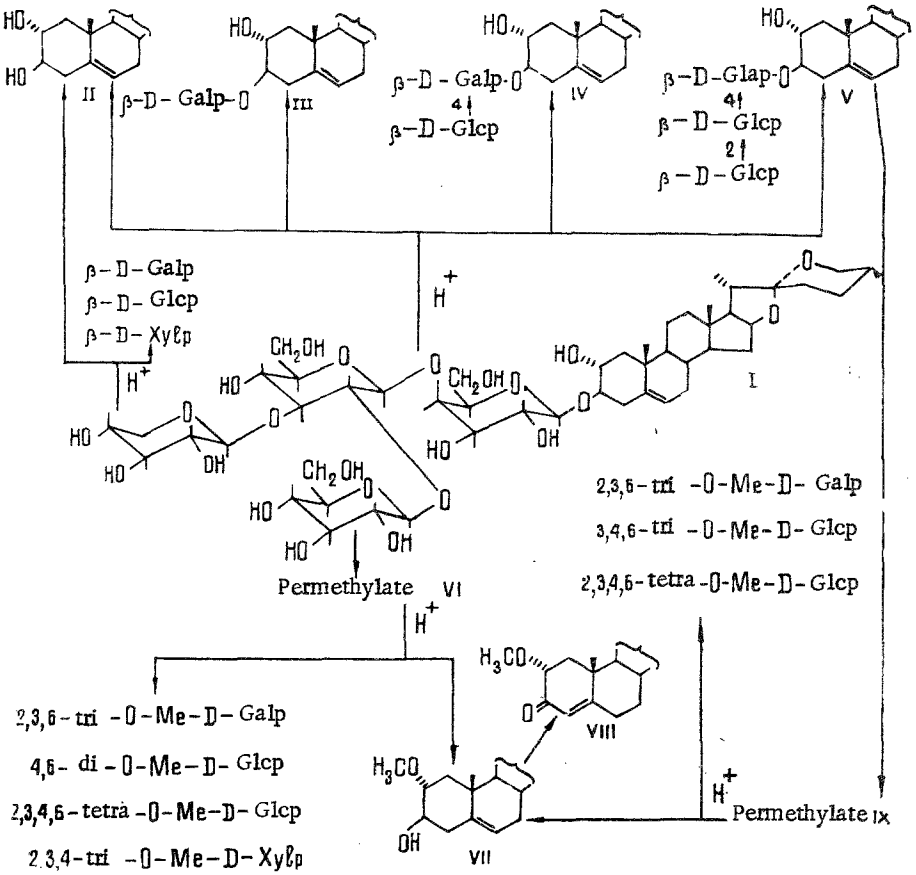

doublets of anomeric sugar protons in the 4.28-4.98 ppm region. The coupling constants of these signals (J = 7-8 Hz) also show the β configurations of the glycosidic bonds [9, 10]. It follows from the coupling constants that all the carbohydrate rings have the C1 conformation [11].

The tetrasaccharide of this structure has the name lycotetraose and is a component of many natural glycosides: the glycoalkaloids tomatine and demissine [12], and the steroid saponin F-gitonin [13], degalactotigonin [14, 15], aspidistrin [16], and purpureagitoside [17]. Glycosides including lycotetraoside — aginoside [18] and turoside A [19] — have also been found in representatives of the genus *Allium*.

The position of attachment of the lycotetraose to the yuccagenin was established on the basis of the following transformations: the monomethyl ether of yuccagenin (VII) obtained by the acid hydrolysis of the permethylate (VI) was oxidized with the Jones reagent; the reaction product, without being isolated in the pure state, was subjected to isomerization in an alkaline medium. This gave compound (VIII) with $\lambda_{max}^{C_2H_5OH}$ at 243 nm (log ε 4.07) and characteristic absorption bands in the IR spectrum at 1685 and 1625 cm^{-1}. These results showed the presence of an α,β-unsaturated keto grouping in substance (VIII). Consequently, the ketone (VIII) is 2-methoxyspirost-4-en-3-one, and compound (VII) is the 2-methyl ether of yuccagenin. Hence, it is obvious that the carbohydrate moiety in the karatavioside A (I) molecule is attached to the hydroxy group at C-3 of yuccagenin, and the structure of the glycoside corresponds to formula (I).

EXPERIMENTAL

General Remarks. Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed on fixed layers of KSK silica gel (<63 μ) containing 15% of gypsum. Column chromatography (CC) was performed on KSK silica gel (63-100 μ). The following solvent systems were used for chromatography: 1) chloroform–methanol–water (65:30:6); 2) chloroform–methanol–water (65:22:4); 3) butanol–ethanol–water (5:3:2); 4) benzene–acetone (3:1); 5) benzene–methanol (10-1); 6) benzene–methanol (15:1); 7) chloroform–methanol (20:1); 8) chloroform–methanol (8:1).

The sapogenins and their derivatives and gycosides were detected with the Sannie reagent [3], and the sugars and their derivatives with o-toluidine salicylate, the sugars being chromatographed on plates impregnated with a 0.3 M aqueous solution of NaH_2PO_4.

The methanolysis of the glycosides was carried out in anhydrous methanol containing 5% of HCl at 100°C for 12 h; an equal volume of water was added to the cooled reaction mixture and the aglycone was separated off by filtration. Part of the filtrate after evaporation of the methanol was heated at 100°C for 4 h and, after neutralization with ÉDÉ 10P anion-exchange resin, it was analyzed by the TLC method. The other part was neutralized with Ag_2CO_3, filtered, evaporated to dryness, silylated, and analyzed by the GLC method.

Gas–liquid chromatography was performed on a "Tsvet-4" chromatograph. Monosaccharides were chromatographed in the form of the trimethylsilyl ethers of the methyl glycosides [21, 22] on a column (3 m × 4 mm) containing Chromaton N-AW bearing 5% of SE-30 silicone phase (phase 1). The temperature of the thermostat was 190°C, and here and below the carrier gas was helium at a rate of flow of 45 ml/min. The methyl glycosides of the methylated sugars were obtained by boiling the methyl ethers in 5% HCl in anhydrous methanol. The reaction time was 4 h. The compounds obtained were chromatographed on a column (1 m × 4 mm) of Celite containing 20% of poly(butanediyl succinate) (phase 2) at a thermostat temperature of 160°C and a rate of flow of gas of 50 ml/min, and also on a column (1 m × 4 mm) of Chromaton N-AW bearing 10% of a polyphenyl ether (phase 3) at a thermostat temperature of 180°C and with a rate of flow of gas of 50 ml/min. The retention time (T_{rel}) for the methylated methyl glycosides was calculated with respect to the retention time of methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside [18, 23]. The methylated sugars obtained in a previous investigation [18] were used as the authentic samples.

The mass spectra were taken on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source at an ionizing voltage of 40 V and a temperature of 110-160°C. The molecular weights were determined mass-spectrometrically. The IR spectra were obtained on a UR-20 instrument in KBr or paraffin oil, and the PMR spectra on a JNM-4H-100 instrument (100 MHz) with HMDS as internal standard (δ scale).

Isolation of Karatavioside A (I). The sun-dried inflorescences of *Allium karataviense* collected on May 20, 1971, in Southern Kazakhstan and on the slopes of Mount Kakhpak (a spur of the Karzhan-Tau range) (28 kg) were exhaustively extracted with methanol. The yield of total extractive substances was 10%. Of this crude resinous total, 500 g was suspended in 2 liters of water and the insoluble material was filtered off. The filtrate was exhaustively extracted with butanol, the butanolic extracts were evaporated to dryness, the dry residue was dissolved in methanol, and the saponins were precipitated with acetatone. This gave 50 g of a nonhygroscopic cream-colored powder including, according to TLC, karataviosides B, C, and D with a very small amount of glycoside A (I) as impurity (total 1).

The residue insoluble in water was dissolved in a mixture of chloroform and methanol (1:1). After elimination of the chloroform a cream-colored precipitate (43 g) deposited which, according to TLC, consisted of karataviosides A and B (total 2). Total 2 was separated by CC (eluent — system 1). Fractions were obtained which contained karatavioside A (I) alone (30 g) and fractions including a mixture of glycosides A and B (10 g). The yield of compound (I) on the air-dry raw material was 0.6%.

Karatavioside A (I), $C_{50}H_{80}O_{23}$, when recrystallized from methanol, had mp 283-285°C (decomp.), $[\alpha]_D^{20}$ −72.9 ± 2° [c 0.87; chloroform–methanol (10:1)], ν_{max}^{KBr}: 3250-3550 (OH), 870, 905 > 930 cm^{-1} (spiroketal chain of the 25R series).

The methanolysis of karatavioside A (I) (200 mg) and the working up of the reaction mixture were performed as described in the "General remarks" section. After recrystallization from methanol, 35 mg of yuccagenin (II), $C_{27}H_{42}O_4$, was obtained with mp 242-244°C, $[\alpha]_D^{20}$ −118 ± 2° [c 1.39; chloroform–methanol (10:1)]; ν_{max}^{KBr}: 3420 (OH), 870, 905 > 930 cm^{-1} (spiroketal chain of the 25R series); M^+ 430. According to the literature: mp 243°C, $[\alpha]_D$ −120° [24]. D-Galactose, D-glucose, and D-xylose were detected by TLC in system 3 and, according to GLC (phase 1), their ratio was 0.96:2.00:1.10.

Partial Hydrolysis of Karatavioside A (I). A solution of 1.2 g of glycoside (I) in 800 ml of 2% aqueous methanolic (1:1) hydrochloric acid was heated in a boiling water bath at 100°C for 2 h. After elimination of the methanol, the reaction mixture was exhaustively extracted with butanol. The butanolic extracts were washed with water and evaporated to dryness. The residue obtained (900 mg) was separated by CC (eluent — system 2). The eluates

with identical compositions were combined. Fractions containing the following substances were collected: yuccagenin (II), 100 mg; monoside (III), 30 mg; bioside (IV), 50 mg; trioside (V), 250 mg; and the initial glycoside (I), 200 mg. The weight of the other fractions, containing mixtures of the substances mentioned, was 200 mg.

3-O-β-D-Galactopyranosylyuccagenin (III). After recrystallization of the monoside fraction from methanol, 18 mg of the glycoside (III) was obtained; $C_{33}H_{52}O_9$, mp 268-272°C (decomp.), $[\alpha]_D^{20}$ −84.2 ± 2° [c 0.74; chloroform–methanol (10:1)]. Compound (III) (10 mg) was subjected to methanolysis as described in the "general remarks" section. By means of TLC in system 5, the aglycone was identified as yuccagenin. The presence of D-galactose was found by TLC (system 3) and GLC (phase 1).

3-O-[O-β-D-Glucopyranosyl-(1→4)-β-D-galactopyranosyl]yuccagenin (IV). Recrystallization of the bioside fraction from methanol gave 30 mg of compound (IV) $C_{39}H_{62}O_{14}$, mp 267-271°C (decomp.), $[\alpha]_D^{20}$ −73.9 ± 2° [c 0.67; chloroform–methanol (10:1)]. The methanolysis of 10 mg of the bioside (IV) and working up of the reaction mixture were carried out as described above. The aglycone was identified as yuccagenin by TLC in system 5. The presence of D-galactose and D-glucose was established by TLC (system 3) and GLC (phase 1). The ratio of the sugars according to the GLC results was 0.93:1.00.

3-O-[O-β-D-Glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-galactopyranosyl]yuccagenin (V) was obtained with a yield of 180 mg by recrystallizing the trioside fraction from methanol. The prosapogenin (V), $C_{45}H_{72}O_{19}$, had mp 242-246°C (decomp.), $[\alpha]_D^{20}$ −70.3 ± 2° [c 0.84; chloroform–methanol (10:1)]. After methanolysis of 10 mg of the glycoside (V) and the working up of the reaction mixture (under the conditions described above), the aglycone was identified by TLC in system 5 as yuccagenin. The presence of D-galactose and D-glucose was shown by the TLC method in system 3. The ratio of the carbohydrates according to GLC (phase 1) was 0.48:1.00.

Permethylate of Karatavioside A (VI). A solution of 1.2 g of the glycoside (I) in 100 ml of dimethyl sulfoxide was treated with 1.05 g of sodium hydride and the mixture was stirred at room temperature for 1 h. Then 15 ml of methyl iodide was added over 30 min, after which stirring was continued for another 3 h. The reaction mixture was poured into 0.5 liter of a 1% solution of sodium thiosulfate and was exhaustively extracted with chloroform. The combined chloroform extracts were washed with water and dried over anhydrous sodium sulfate. The CC method (eluent system 4) yielded 1.1 g of the amorphous permethylated (VI), $C_{63}H_{106}O_{23}$, $[\alpha]_D^{20}$ −73.8 ± 2° (c 1.2; methanol); ν_{max}^{Nujol}, cm^{-1}: 870, 905 > 930 (spiroketal chain of the 25R series).

PMR spectrum ($CDCl_3$, δ, ppm): 0.72 (6H at C-27 and C-18; broadened singlet); 0.95 (3 H at C-21, d, J = 6 Hz); 1.01 (3 H at C-19, s); 3.20-3.70 (signals of the protons of 13 methoxy groups); 4.28, 4.69, 4.85, 4.98 (4 H, anomeric protons of the carbohydrate moiety, d, J = 7-8 Hz; H at C-16 (the signal from this proton is superposed on the resonance line of the anomeric proton at 4.28 ppm); 5.29 (H at C-6, broadened singlet).

Hydrolysis of the Permethylate (VI). The hydrolysis of 1.1 g of compound (VI) dissolved in 100 ml of 50% aqueous methanol containing 9% of sulfuric acid was performed at 100°C for 8 h. Then 50 ml of water was added, the methanol was distilled off, and the precipitated aglycone (VII) was filtered off (340 mg). The filtrate was heated at 100°C for another 4 h. The solution was cooled and was neutralized with ÉDÉ 10P anion-exchange resin, and the water was distilled off. The mixture of methylated sugars (600 mg) was subjected to CC (eluent–system 7). Fractions enriched with the individual components were collected a) 95 mg; b) 80 mg; c) 110 mg; d) 115 mg. The weight of the other fractions amounted to 130 mg. The fractions obtained were reseparated by PTLC in systems 7 and 8. The methylated sugars were identified by the TLC and GLC methods.

2,3,4,6-Tetra-O-methyl-D-glucopyranose. From fraction a in system 7 a substance was obtained the R_f value of which on TLC in the same system did not differ from that of an authentic sample. On GLC, the methyl tetra-O-methylglucoside gave two peaks the intensities and T_{rel} values of which (1.00, 1.44 on phase 2, and 1.00, 1.35 on phase 3) were identical with the corresponding indices for an authentic sample [23].

2,3,4,-Tri-O-methyl-D-xylopyranose. From fraction b in system 7 was obtained a compound which had the same R_f value on TLC in system 7 as an authentic sample. The ratio of the areas and the T_{rel} values of the GLC peaks of the methyl glycoside of the substance described (0.45 and 0.58 on phase 2, 0.42 and 0.47 on phase 3) were identical with the corresponding parameters of an authentic sample [23].

2,3,6-Tri-O-methyl-D-galactopyranose. From fraction c in system 8 a methylated sugar was obtained. The R_f values of the compound isolated and of an authentic sample (TLC, system 8) did not differ from one another. The intensities and T_{rel} values of the GLC peaks of the methyl tri-O-methylgalactoside (3.24, 3.95, 4.36, and 4.75 on phase 2, and 1.59, 2.11, 2.23, and 2.53 on phase 3) coincided with the figures given for an authentic sample [23].

4,6-Di-O-methyl-D-glucopyranose. The carbohydrate was isolated from fraction d in system 8. The R_f value of the compound obtained on TLC in the same system coincided with that of an authentic sample. The T_{rel} value of the methyl di-O-methylglucoside on GLC (2.50 — main peak; 2.70 — shoulder; phase 3) coincided with the T_{rel} value of an authentic sample.

2-Methyl Ether of (25R)-Spirost-5-ene-2α,3β-diol (VII). The recrystallization from methanol on the aglycone obtained after the hydrolysis of the permethylate (VI) yielded 257 mg of compound (VII), $C_{28}H_{44}O_4$, mp 228-230°C, $[\alpha]_D^{20}$ −149.2 ± 2° (c 0.94; chloroform). ν_{max}^{KBr}: 3420 (OH), 870, 905 > 930 cm^{-1} (spiroketal chain of the 25R series). M^+ 444.

2α-Methoxy-(25R)-spirost-4-en-3-one (VIII). With the bubbling of nitrogen through solution and stirring, 0.2 ml of the Jones reagent was added to a solution of 100 mg of compound (VII) in 100 ml of acetone cooled to 10°C. The reaction took 5 min, after which the reaction mixture was poured into water, the precipitate was filtered off and was dissolved in boiling methanol, and after the addition of one drop of 10% aqueous KOH solution the resulting alkaline solution was heated at 100°C for 5 min. Then the alkali was neutralized with acetic acid and the oxidation product was isolated by CC (eluent — system 6). After recrystallization from methanol, 30 mg of compound (VII) was obtained; $C_{28}H_{42}O_4$, mp 210-212°C, $[\alpha]_D^{20}$ +1.93 ± 2° (c 1.04; chloroform). ν_{max}^{KBr}, cm^{-1}: 1685, 1625 (>C=C—C=O), 870, 905 > 930 (spiroketal chain of the 25R series). $\lambda_{max}^{C_2H_5OH}$: 243 nm (log ε 4.07). M^+ 442.

The Permethylate (IX) of the Yuccagenin Trioside and the Products of Its Hydrolysis. The glycoside (V) (55 mg) was methylated, and the reaction mixture was treated as described for the permethylate (VI) of karatavioside A (I). Separation by CC (eluent — system 4) gave 23 mg of the amorphous permethylate (IX) showing no IR absorption in the region of hydroxy groups. The hydrolysis of compound (IX) and the working up of the reaction mixture were performed as described for the permethylate (VI). The aglycone obtained was identified by the GLC method as the 2-methyl ether of yuccagenin (VII).

The combined methylated sugars were separated by PTLC in system 7. The two components of the material were identified by their R_f values with markers by TLC, and also by the GLC of the methyl glycosides from the areas of the peaks and the retention times, with the 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3,6-tri-O-methyl-D-galactopyranose described above. In addition, a methylated carbohydrate was isolated which gave a positive reaction with the Bonner reagent [25]. Its R_f value on direct comparison with an authentic sample (TLC, system 8), and also the ratio of the areas of T_{rel} on the GLC of the methyl glycoside of this compound (3.14 and 3.77 on phase 2; 1.69 and 2.33 on phase 3), enabled it to be identified as 3,4,6-tri-O-methyl-D-glucopyranose [23].

SUMMARY

From a methanolic extract of the inflorescences of *Allium karataviense* Rgl. we have isolated a new steroid glycoside of the spirostan series — karatavioside A, which is (25R)-spirost-5-ene-2α,3β-diol 3-O-{[O-β-D-xylopyranosyl-(1→3)-][O-β-D-glucopyranosyl-(1→2)-]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

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THE STABILITY OF STROPHANTHIDIN ACETATE IN SOLUTION

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Strophanthidin acetate is obtained by acetylating strophanthidin [1, 2]. In its chemical structure, strophanthidin acetate differs from other cardiac glycosides by the presence of an acetyl group in addition to a carbohydrate residue, which changes its capacity for hydrolysis. Some drugs, such as acetyldigoxin [3] are readily hydrolyzed in alkaline and even neutral solutions. Thus, one of the main factors determining the stability of glycosides in solution is the optimum pH value [4].

We have developed a stable medicinal form of strophanthidin acetate in the shape of an injection solution. To determine the optimum conditions for the preparation of stable solutions we studied the stability of strophanthidin acetate in buffer solutions (citrate–phosphate and acetic acid–acetate), which were added to an ethanolic glycerol solution of strophanthidin acetate in a ratio of 10:15:75.

The behavior of strophanthidin acetate in solutions with different pH values was studied after their sterilization and storage under elevated temperature (60°C) and room-temperature (15-25°C) conditions.

In solutions with pH 7.0 and above, strophanthidin acetate is hydrolyzed immediately after their sterilization, and in addition to the spot of strophanthidin acetate (R_f 0.62) the spot of strophanthidin (R_f 0.21) appears on a chromatogram.

In a solution with a pH below 4.0, hydrolysis begins after 11 days (at 60°C), and in a solution with pH 4.0-5.0 after 44 days (at 60°C). We also studied the degree of decomposition of the glycoside as a function of the pH of the solution after sterilization and storage for 10 months at room temperature.

As the figures in Table 1 show, a prepared ampul solution of strophanthidin acetate without stabilizers decomposes more rapidly, mainly with the formation of the aglycone strophanthidin.

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