STEROID SAPONINS AND SAPOGENINS OF *Allium*.

XIX. THE STRUCTURE OF KARATAVIGENIN C

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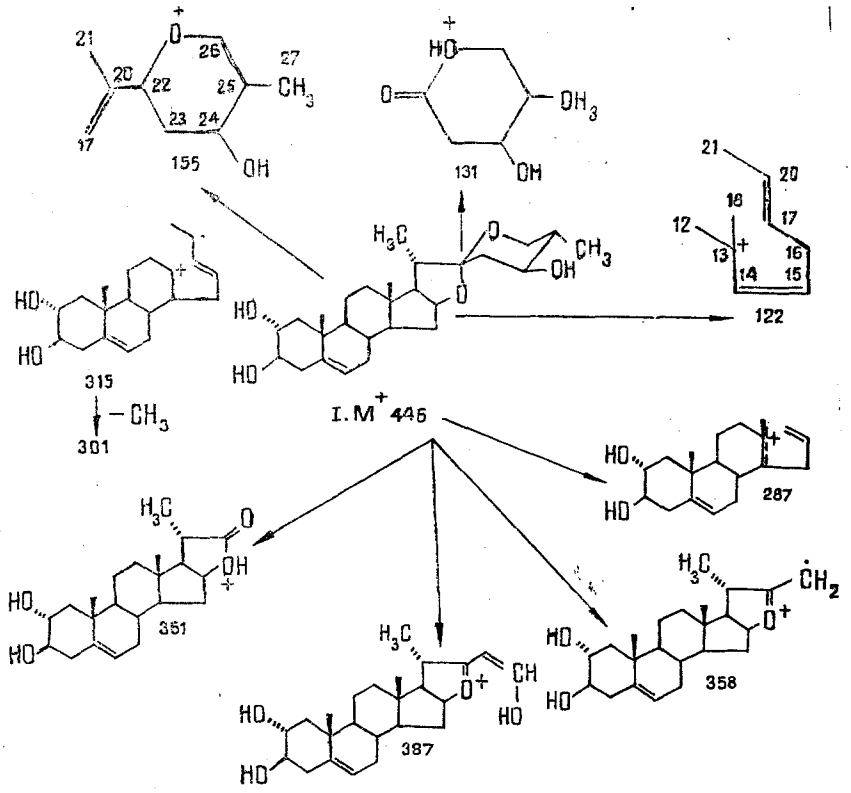
Two new steroid glycosides of the spirostan series — karataviosides E and F — have been isolated from inflorescences of *Allium karataviense* Regel. Smith degradation of these compounds has given a steroid sapogenin, karatavigenin C, which is (25S)-spirost-5-ene-2α,3β,24S-triol. Its structure was established from an analysis of spectral characteristics.

We have previously reported on karataviosides A and C [1] and B [2] — new steroid glycosides isolated from inflorescences of *Allium karataviense* Regel. (family Liliaceae). Analysis of the intermediate fractions and mother liquors collected during the accumulation of these substances showed that the mixtures obtained contained two minor glycosides. The less polar compound we have called karatavioside E and the more polar one karatavioside F. In the present paper we give a proof of the structure of the aglycone of these glycosides — karatavigenin C (I) (for karatavigenins A and B, see our previous publications [3, 4]).



The main fragments of the mass-spectrometric fragmentation of karatavigenin C (I) (m/z).

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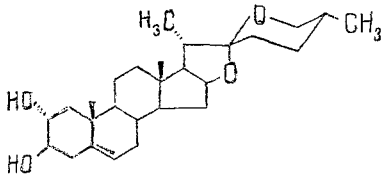
The complete acid hydrolysis of karataviosides E and F led to several products, which indicated the degradation of the aglycone under these conditions. Smith degradation [5] of glycosides E and F led to the same compound in each case — karatavigenin C (I).

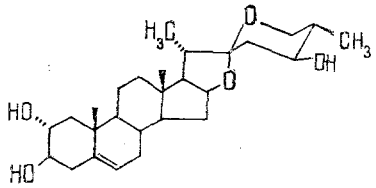
The mass spectrum of compound (I) contained, in addition to the peak of the molecular ion with m/z 446 ($C_{27}H_{42}O_5$), peaks of ions with m/z 387, 361, 358, 316, 301, 287, 155, 131, and 122 (see scheme). The composition of the molecular ion and the fragmentation of the molecule under the action of electron impact permitted the assumption that karatavigenin C (I) is a steroid trihydroxysapogenin with one double bond [6, 7]. The presence in the mass spectrum of sapogenin (I) of the peaks of ions with m/z 361, 358, 316, 301, and 287 indicates that two of the hydroxy groups and the double bond are present in the steroid moiety of the molecule. The presence of peaks of ions with m/z 387, 155, and 131 can be explained only by the assumption that one hydroxy function is present in ring F, and the peak of an ion with m/z 387 shows that this hydroxy group is attached at C-24.

In the IR spectrum of karatavigenin C (I), in addition to the band of hydroxylic absorption at 3490 cm^{-1} there are bands at 845, 895, 905, 980, and 1000 cm^{-1}. It is obvious that the absorption in the 800-1000 cm^{-1} region, which is uncharacteristic for the majority of steroid saponins, is due to the presence of a hydroxy group in ring F of compound (I) [8].

Further information on the structure of karatavigenin C (I) was obtained by a comparative analysis of the ^{13}C NMR and the PMR spectra of compound (I) and of yuccagenin (II).



II. Yuccagenin



I. Karatavigenin C

In the ^{13}C NMR spectra of compounds (I) and (II) the chemical shifts of the carbon atoms forming rings A-E are practically identical. This indicates that in the molecule of karatavigenin C (I) hydroxy groups are located at C-2 and C-3 and a double bond between C-5 and C-6. There is an appreciable difference only for the chemical shifts of the carbon atoms forming ring F (ppm relative to TMS, solvent — C_5D_5N):

Carbon atom	Karatavigenin C	Yuccagenin
C-22	111.8	109.2
C-23	40.0	31.8
C-24	70.6	29.2
C-25	40.8	30.6
C-26	65.3	66.8
C-27	13.6	17.3

On comparing the facts given, it can be seen that on passing from yuccagenin (II) to karatavigenin C (I), the greatest change is undergone by the chemical shift of the C-24 atom, and less pronounced ones by the C-23 and C-25 atoms. The shifts of the signals are due to the presence of a hydroxy group at C-24.

The chemical shifts of the protons of aglycones (I) and (II) are given in Table 1.

In the PMR spectrum of karatavigenin C (I) three one-proton multiplets are observed at 4.17, 4.03, and 3.83 ppm, the nature of the splitting of each of which was considerably simplified when a small amount of trifluoroacetic acid was added to the solution. Consequently, these signals correspond to the resonance of protons located geminally to the three secondary hydroxyls and undergo additional interaction with the protons of the OH groups. In the same region of the PMR spectrum of yuccagenin (II) which contains only two hydroxy groups, at C-2, and C-3, there are one-proton octets at 4.16 and 3.84 ppm. We are therefore justified in assigning the signals identical with them in terms of splitting in the PMR spectrum of karatavigenin C (I) to the corresponding protons at C-2 and C-3. The signal at 4.03 ppm in the PMR spectrum of compound (I) obviously belongs to the proton geminal to the secondary hydroxy group at C-24.

The octet nature of the splitting of the signals at 4.17 and 3.83 ppm in the PMR spectrum of the sapogenin (I), and also the use of the double-resonance method enabled us to establish that each of the two methine protons at C-2 and C-3 interacts vicinally with the other and with the neighboring methylene protons. Thus, when the proton resonating at 4.17 was irradiated with a strong radiofrequency field, the quartets at 1.44 ppm (^2J = 12.8, ^3J = 11.9 Hz) and 2.39 ppm (^2J = 12.8 ^3J = 4.6 Hz) were converted into doublets with the same SSCCs, ^2J = 12.8 Hz.

When a similar experiment was performed in relation to the proton corresponding to the octet at 3.83 ppm, another pair of signals was converted into doublets with ^2J = 13.8 Hz. They were located in a weaker field — a quartet at 2.67 ppm (^2J = 13.8; ^3J = 5.9 Hz) and a triplet at 2.73 ppm ($\Sigma J \approx 25$ Hz). The lines of the latter were appreciably broadened, but the broadening was eliminated when the resonance lines of the olefinic proton were saturated. In the spectrum of karatavigenin C (I), a doublet with broadened lines at 5.43 ppm having ^3J = 4.7 Hz, corresponds to the latter. It follows from this that there is an allyl spin—spin interaction between the olefinic proton and one of the methylene protons (resonating at 2.73 ppm). This circumstance confirms the presence of a double bond between C-5 and C-6 in the sapogenin (I).

These results, enabling a chain of interconnected protons to be isolated, show the presence in the molecule of compound (I) of the fragment: —C-1(H_2) — C-2(HOH) — (C-3)(HOH) — C-4(H_2) — C-5=C-6(H)—.

The signals in the spectrum of karatavigenin C (I) belonging to the protons of this fragment are assigned in the following way: 1.44 and 2.39 ppm (2 H at C-1); 2.67 and 2.73 ppm (2 H at C-4); 4.17 and 3.83 ppm (H at C-2 and H at C-3, respectively); and 5.43 ppm (H at C-6_a). It follows from an analysis of the multiplicities of these signals that the proton at C-2 interacts vicinally with two other protons of a neighboring methylene group, with ^3J = 11.9 and 4.6 Hz, and with H at C-3, with ^3J = 11.0 and 5.9 Hz. The vicinal spin—spin coupling constant of the interaction between the protons at C-2 and C-3 is 9.0 Hz. This set of observed values of ^3J shows that in the chair-shaped conformation of ring A of karatavigenin C (I), the protons at C-2 and C-3 interact trans-diaxially and axially-equatorially with the protons of neighboring methylene groups. They also enter into trans-diaxial interaction with one another; in other words, they are axial. The facts given unambiguously show that the secondary hydroxy groups at C-2 and C-3 of karatavigenin C (I) are equatorial and have the α- and β-orientations, respectively.

We showed above that the resonance signal at 4.03 ppm in the PMR spectrum of sapogenin (I) corresponds to a proton located geminally to the secondary hydroxy group at C-24. This is confirmed by the sextet nature of the splitting of this signal, with ^3J = 4.8, 10.6, and 10.6 Hz. It must be assumed that the proton corresponding to the sextet under discussion interacts vicinally with three neighboring hydrogen atoms. Consequently, the secondary hydroxy group under consideration can be located at C-24, which agrees with the results of ^{13}C NMR spectrum (see above). The observed values of the vicinal SSCCs indicate that the proton at C-24 interacts diaxially with the proton at C-25, and also diaxially and axially-equatorially with the protons at C-23. Consequently, the proton at C-24 is axial, and the CH_3-27 group and the hydroxy group at C-24 are equatorial. Thus, the chiral centers of C-24 and C-25 have the S-configuration.

The characteristics of the PMR spectra of karatavigenin C (I) and the conclusion based on them are fully comparable with literature information for 24S-hydroxypennogenin [9].

The equatorial orientation of the CH_3-27 group of karatavigenin C (I) is also confirmed by an analysis of the values of the chemical shifts and multiplicities of the signals of the protons at C-26. It is known that with the axial orientation of CH_3-27 these signals (in C_5D_5N) are less equivalent ($\Delta\delta$ = 0.7 ppm) than for the equatorial orientation ($\Delta\delta$ = 0.1 ppm) [10]. In the PMR spectrum of compound (I), the axial and equatorial protons at C-26 appear at 3.60 ppm (t, $\Sigma J \approx 22$ Hz), and 3.71 ppm (q, ^2J = 11.0; ^3J = 5.0 Hz), respectively, i.e., the difference amounts to 0.11 ppm. If, in addition we take into account the diaxial and equatorial-axial interaction between the 2H at C-26 and H at C-25, the equatorial orientation of CH_3-27 is not a matter of doubt.

We may note that the downfield shift by 0.40 ppm of the CH_3-27 resonance signal in the PMR spectrum of karatavigenin C (I) as compared with the corresponding signal of yuccagenin (II) (see Table 1) is caused not by a change in the orientation of this methyl group [10, 11], but by the influence of the hydroxy group at C-24.

TABLE 1. Chemical Shifts of the Protons of Karatavigenin C (I) and Yuccagenin (II) (C_5D_5N, δ, ppm, 0 — TMS)

Proton	Compound	
	I	II
H-2	4,17; oc	4,16; oc
	^3J=4,6; 9,0; 11,9 Hz	^3J=4,4; 9,0; 11,5 Hz
H-3	3,83; oc	3,84; oc
	^3J=5,9; 9,0; 11,0 Hz	^3J=6,1; 9,0; 11,0 Hz
H-6	5,43; br.d ^3J=4,7 Hz	5,43; br.d ^3J=4,8 Hz
H-16	4,56; q $\Sigma^3 J$=22,4 Hz	4,55; q Σ ^3J=22,0 Hz
H-24	4,03 sx	
	^3J=4,8; 10,6; 10,6 Hz	
2H-26	3,60; t Σ J=22 Hz	3,59; t Σ J=21 Hz
	3,71; q J=11,0; 5,0 Hz	3,59; q J=10,6; 4,0 Hz
3H-18	0,84; s	0,85; s
3H-19	1,09; s	1,10; s
3H-21	1,17; d ^3J=7,0 Hz	1,14; d ^3J=6,8 Hz
3H-27	1,10; d ^3J=6,2 Hz	0,70; d ^3J=5,2 Hz

Arbitrary symbols: s – singlet; d – doublet; br.d – broadened doublet; t – triplet; q – quartet; sx – sextet; oc – octet.

Thus, karatavigenin C (I) is (25S)-spirost-5-ene-2α,3β,24S-triol, and its structure corresponds to formula (I).

EXPERIMENTAL

General Observations. Silufol was used for thin-layer chromatography (TLC). Silica gels KSK and L (63-100 μ) were used for column chromatography (CC). Chromatography was performed in the following solvent systems: 1a) chloroform–methanol–water (65:35:8); 1b) (65:30:6); 1c) (65:22:4); and 2) chloroform–methanol (20:1). IR spectra were taken on a UR-20 instrument in KBr, mass spectra on a MKh-1303 instrument, PMR spectra on a XL-200 (Varian) spectrometer, and ^{13}C NMR spectra on a CFT-20 (Varian) instrument. The solvent was C_5D_5N.

Isolation of Karataviosides E and F. The mixed fractions and the mother liquors collected in the accumulation of karataviosides A and C [1, 2] obtained from 12 kg of the air-dry inflorescences of *Allium karataviense* were subjected to repeated rechromatography in solvent systems 1a, b, and c. After recrystallization of the corresponding fractions from methanol, 0.6 g of karatavioside E and 2.1 g of karatavioside F were obtained. The yields amounted to 0.005% and 0.017%, calculated on the weight of the air-dry raw material. Both glycosides were revealed on TLC by the Sannié reagent [12] in the form of blue-violet spots.

Smith Degradation of Karatavioside E. Glycoside E (200 mg) was suspended in 100 ml of 50% aqueous methanol, 400 mg of $NaIO_4$ was added, the mixture was stirred at room temperature for 16 h. The unchanged oxidizing agent was destroyed with ethylene glycol, and then the methanol was distilled off, after which 50 ml of water was added and extraction was carried out with butanol (3 × 20 ml). The butanolic extract was washed with water and concentrated, 50 ml of 50% aqueous methanol and 500 mg of $NaBH_4$ were added, and the mixture was left at room temperature for 24 h. Then dilute H_2SO_4 was added to pH ~2 and the reaction mixture was left at room temperature for 48 h, after which the reaction products were diluted with water, the methanol was evaporated off, and extraction was carried out with butanol (3 × 20 ml). The butanolic extracts were washed with water and evaporated to dryness. The residue was subjected to column chromatography in solvent system 2. The fractions containing the individual aglycone were recrystallized from methanol. This gave 55 mg of karatavigenin C, $C_{27}H_{42}O_5$, mp 309-311°C, $[\alpha]_D^{20}$ —95.9 ± 2° (c 1.46; pyridine); ν_{max}^{KBr} (cm^{-1}): 845, 895, 905, 980, 1000, 3490 (OH). Mass spectrum, m/z (%): M^+ 446(4), 431(0.8), 428(1.7), 413(2.8), 387-(14), 358(35), 316(71), 30(100), 287(80), 155(89), 131(62), 122(27).

Smith Degradation of Karatavioside F. Glycoside F (600 mg) was dissolved in 100 ml of water and the operations described above for karatavioside E were performed. This gave 130 mg of karatavigenin C, identical with the aglycone obtained in the preceding experiment.

SUMMARY

A new steroid saponogenin — karatavigenin C — had been isolated from the inflorescences of *Allium karataviense* Regel. It has the structure of (25S)-spirost-5-ene-2α,3β,24S-triol.

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