GLYCOSIDES OF MARINE INVERTEBRATES.

XXIII. KURILOGENIN — A NEW GENIN FROM THE GLYCOSIDES OF THE HOLOTHURIAN *Duasmodactyla kurilensis*

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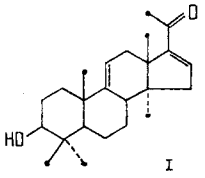
The acid hydrolysis of the total glycosides from the holothurian *Duasmodactyla kurilensis* Levin. has yielded a new aglycone which has been called kurilogenin. On the basis of the results of NMR, IR, UV, and mass spectroscopy, it has been shown that it is 3β-hydroxy-4,4,14-trimethylpregna-9(11),16-dien-20-one.

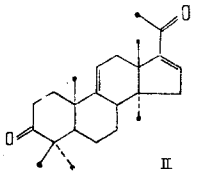
Continuing investigations of triterpene glycosides, we have isolated the total glycosidic material from extracts of the Far Eastern holothurian *Duasmodactyla kurilensis* Levin (Dendrochirota, Phyllophoridae). The acid hydrolysis of this fraction gave genin (I), differing substantially in its structure from the previously **isolated holothyrinogenins [1, 2], stychopogenins [3, 4], and cucumariogenins [4]. We propose to call it kurilogenin.**

In the mass spectrum of compound (I) the peak of the molecular ion is observed at m/z 356. The ^{13}C NMR spectrum (Table 1) showed that kurilogenin contains 24 carbon atoms, two double bonds, and keto and hydroxy groups. It follows from this that (I) (empirical formula $C_{24}H_{36}O_2$) can only be a tetracarbocyclic compound.

To establish the structure of the genins (I) we studied the high-resolution ^1H NMR spectra of this compound and of its keto derivative (II) (Table 2).

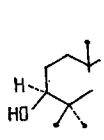


I

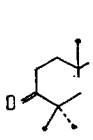


II

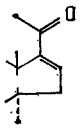
On passing from (I) to (II) the hydroxy group is oxidized with the formation of ketone group in a six-membered ring ($\nu_{C=O}$ 1700 cm^{-1}). The signals of the hydrogen atoms of the methylene groups at C-1 and C-2 in compound (II) (the fragment (IIIa)) were revealed with the aid of differential decoupling and double resonance. It can be seen from a comparison of the ^1H NMR spectra of (I) and (II) that the signals of the C-30 and C-31 protons of the methyl groups shift downfield and form a singlet, which is characteristic for the spectra of keto derivatives of the lanostane series [5]. All these facts show the presence of fragment (III) in the composition of (I).



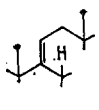
III



IIIa



IV



V

The IR, UV, and mass spectra of (I) show a similarity of its chromophoric groups to the corresponding groups in 16-dehydroprogesterone. In actual fact, (I) has λ_{max} at 242.6 nm

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TABLE 1. ^{13}C NMR Spectrum of the Genin (I) and the Corresponding Signals in the Spectra of Model Compounds (M)

Carbon atom	I	M	Carbon atom	I	M	Carbon atom	I	M
C-1	36,2 t	36,2[b]	C-9	148,6 s	148,2[b]	C-17	152,4 s	155,6[c]
C-2	27,9 t	28,3[b]	C-10	39,7 s	39,5[d]	C-18	19,3[a] q	18,3[d]
C-3	78,9 d	79,0[b]	C-11	115,6 d	115,0[b]	C-19	22,2 q	21,5[b]
C-4	39,2 s	39,2[b]	C-12	31,8 t	33,2[d]	C-20	197,0 s	195,4[c]
C-5	52,6 d	52,6[b]	C-13	47,2 t	46,3c	C-21	27,0 q	26,4[c]
C-6	21,2 t	22,3[b]	C-14	49,6 s	48,8[d]	C-30	28,3 q	27,9[b]
C-7	27,9 t	28,1[b]	C-15	42,0 t	41,0[d]	C-31	15,7 q	15,7[b]
C-8	39,5 d	41,9[b]	C-16	144,3 d	143,2[c]	C-32	19,9[a] q	18,6[b]

a) Assignment of the signals ambiguous; s, d, t, q) singlet, doublet, triplet, and quartet, respectively, on incomplete decoupling from protons; b) from the spectrum of lanost-9(11)-en-3β-ol; c) from the spectrum of 16-dehydroprogesterone; d) calculated values.

TABLE 2. ^{1}H NMR Spectra of Compounds (I) and (II)

Positions of the protons	I $CDCl_3$	I (C_6D_6)	II ($CDCl_3$)	Spin–spin coupling constants (Hz)
H-1			2,13 m	I, $CDCl_3$
H-1			1,82 m	$J_{15_\alpha,\ 15_\beta}=17,1$ $J_{15_\alpha,\ 16}=3,4$
H-2			2,74 m	$J_{15_\beta,\ 16}=2,2$
H-2			2,41	$(\Sigma J)_3 = (J_{aa} + J_{a\phi})$
H-3	3,22 dd	3,01 m		
H-8	2,40 m	2,25 m	2,46 m	I, C_6D_6
H-11	5,32 m	5,30 dt	5,39 m	$J_{12_\beta,\ 12_\alpha}=17,6$ $J_{12_\beta,\ 11}=6,0$
H-12	2,33 m	2,71 m	2,35 m	$J_{12_\alpha,\ 11}=1,9$ $J_{12_\beta,\ 8}=2,0$
H-12	2,33 m	2,51 m	2,35 m	$J_{12_\alpha,\ 8}=3,6$ $J_{12_\alpha,\ 18}\sim 0,8$
H-15	2,30 dd	1,97 dd	2,30 m	$J_{11,8}\sim 2,0$ $J_{15_\alpha,\ 15_\beta}=17,0$
H-15	2,15 dd	1,80 dd	2,15 m	$J_{15_\alpha,\ 16}=3,4$ $J_{15_\beta,\ 16}=2,0$
H-16	6,68 dd	6,07 dd	6,70 dd	$J_{15_\beta,\ 32}\sim 0,8$
CH_3-18	0,832[a] s	0,992 s	0,885 s	II, $CDCl_3$
CH_3-19	1,065 d	1,028 s	1,256 s	$J_{15_\alpha,\ 15_\beta}=17,0$ $J_{2_\beta,\ 1_\beta}=6,2$
CH_3-21	2,270 s	1,980 s	2,270 s	$J_{15_\alpha,\ 16}=3,2$ $J_{2_\beta,\ 1_\alpha}=13,4$
CH_3-30	0,995 s	0,980 s	1,082 s	$J_{15_\beta,\ 16}=1,9$ $J_{2_\alpha,\ 1_\beta}=3,2$
CH_3-31	0,850[a] s	0,856 s	1,082 s	$J_{15_\beta,\ 32}\sim 0,7$ $J_{2_\alpha,\ 1_\alpha}=5,3$
CH_3-32	0,820 s	0,816 s	0,824 d	$J_{2_\beta,\ 2_\alpha}=15,4$ $J_{1_\beta,\ 1_\alpha}=13,2$
				$J_{1_\alpha,\ 19}\sim 1,0$

a) Assignment of the signals ambiguous; s, m, singlet, multiplet, respectively; dd) doublet of doublets; dt) doublet of triplets.

(ε 7200) and a band of stretching vibrations at 1661 cm^{-1} ($\nu_{C=O}$). The singals in the ^{13}C NMR spectrum of (I) observed at 27.0 (C-21), 197.0 (C-20), 152.4 (C-17), and 144.3 ppm (C-16) are close to the signals of C-21, C-20, C-17, and C-16 in the spectrum of 16-dehydroprogesterone [6]. The values of the chemical shifts of H-16 (6.68 ppm) and CH_3-21 (2.27 ppm) are close to the values of 6.72 and 2.25 ppm, respectively, in the spectrum of allopregn-16-en-20-one [7]. The H_α-15 and H_β-15 signals (see Table 2) appear in the form of doublet of doublets. Consequently, (I) contains the fragment (IV).

The position and spin–spin coupling constants of the signals of the protons of the allyl methylene group (at C-12) and of the methine group (at C-8) were determined by differential decoupling, which showed the presence of fragment (V) in compound (I).

On the basis of these facts, it may be assumed that (I) is 3β-hydroxy-4,4,14-trimethylpregna-9(11),16-dien-20-one.

A comparison of the ^{13}C NMR spectra of kurilogenin and of lanost-9(11)-en-3β-ol [8] showed that the signals of the C-1–C-9, C-11, C-19, and C-30–C-32 atoms in both compounds are close or coincide. The values of the chemical shifts of C-12, C-14, C-15, and C-18 atoms for structure (I) were estimated with allowance for the contributions of the C-32 group, the 16-17 double bond, and the acyl group. The calculations were based on the spectra of lanost-9(11)-en-3β-ol and of 16-dehydroprogesterone together with information on the spectra of androst-9(11)-enol [9] and of progesterone [6]. The results so obtained agree well with the observed values (see Table 1).

Thus, kurilogenin, unlike all the genins of holothurian glycosides known previously, has no lactone ring and contains a shortened side chain.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were obtained on Bruker HX-90E and Bruker WM-250 spectrometers. The signals in the NMR spectra are given in the δ scale with TMS as standard. Specific rotations were measured on a Perkin-Elmer 141 polarimeter. IR spectra were recorded on a Specord 75-IR instrument in chloroform, and UV spectra on a Specord UV-VIS instrument in ethanol, and mass spectra on a LKB 9000S mass spectrometer with direct introduction. The ionization energy was 70 eV.

The animals were collected in July, 1981 at a depth of 70-100 m in the Pacific Ocean littoral (Kurile islands).

Isolation of the Glycosidic Fraction. An ethanolic extract of the holothurians (dry weight of the animals 213 g) was evaporated, and the residue was dissolved in water and was chromatographed on a column of Polikhrom 1 [10] and then on silica gel in the chloroform–ethanol–water (300:250:8) system. This gave 170 mg of combined glycosides.

The acid hydrolysis of 150 mg of the combined glycosides was carried out with 2 N HCl at 90°C for 2 h. The resulting precipitate of aglycones was extracted with chloroform. The combined monosaccharides obtained were analyzed by GLC-MS in the form of the peracetates of the corresponding aldononitriles. D-Quinovose, D-xylose, D-glucose, and 3-O-methylglucose were identified.

The Genin (I). The combined aglycones (20 mg) obtained on hydrolysis were chromatographed on silica gel in the benzene–ethyl acetate (8.5:1.5) system. This gave 11 mg of genin (I) with mp 152-154°C (aqueous methanol), $[\alpha]_D^{20}$ +79.3° (c 2.25 chloroform). UV spectrum: λ_{max} 242.6 nm (ε 7200). Mass spectrum, m/z: 356 (M^+), 341 ($M^+ - 15$), 323 ($M^+ - 15$-18), 313 ($M^+ - 43$). IR spectrum: 1661, 3623 cm^{-1}.

The Ketone (II). A solution of 5 mg of the genin (I) in 3 ml of acetone was treated with an excess of the Jones reagent [5]. The reaction mixture was stirred vigorously at room temperature for 4 min. The excess of oxidizing agent was reduced by the addition of propan-2-ol. The reaction mixture was diluted with water fourfold and was extracted with ether (3 × 2 ml). The ketone obtained was chromatographed in the hexane–ethyl acetate (4:1) system. This gave 3.6 mg of the individual compound (II): an amorphous substance with $[\alpha]_D^{22}$ +49.3° (c 1.6; chloroform). IR spectrum: 1700, 1663 cm^{-1}. Mass spectrum, m/z: 354 (M^+), 339 ($M^+ - 15$), 311 ($M^+ - 43$).

SUMMARY

Acid hydrolysis of the combined glycosides from the holothurian *Duasmodactyla kurilensis* Levin has yielded a new aglycone the structure of which has been determined as 3β-hydroxy-4,4,14-trimethylpregna-9(11),16-dien-20-one.

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TRITERPENE GLYCOSIDES OF *Salsola micranthera*.

I. STRUCTURES OF SALSOLOSIDES C AND D

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New triterpene glycosides — salsolosides C and D — have been isolated from the epigeal part of *Salsola micranthera* Botsch. On the basis of chemical transformations and physicochemical measurements, salsoloside C has been assigned the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuropyranoside], and salsoloside D has the structure of hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuropyranoside].

Several new glycosides of the triterpene series have been isolated from the plant *Climacoptera transoxana* (Iljin) Botsch, family Chenopodiaceae [1]. We have also investigated for its glycoside content the epigeal part of the annual plant *Salsola micranthera* Botsch. from the same family. The raw material was collected in southeastern Turkmenia, in the environs of the village of Tashlyk.

When the purified combined glycosides from its methanolic extract were subjected to thin-layer chromatography (TLC), seven compounds were detected which have been named salsolosides A, B, C, D, E, F, and G. In the present paper we give information on the determination of the structures of salsolosides C (I) and D (II).

The acid hydrolysis of glycosides (I) and (II) separately led to oleanolic acid (III) and hederagenin (IV), respectively.

Analysis of the carbohydrate fractions of the hydrolysates by TLC and PC showed that the sets of monosaccharides in the two glycosides were identical, consisting of D-glucuronic acid, D-glucose, and D-xylose. It was found with the aid of gas–liquid chromatography (GLC) [2] that the monosaccharides were present in a ratio of 1:1:1.

The alkaline hydrolysis of salsolosides C (1) and D (II) led to the formation of the progenins (V) and (VI). D-Glucuronic acid and D-xylose were found in an acid hydrolysate of glycoside (V) and (VI). Consequently, the D-glucose forms the acyloside moiety of the compounds (I) and (II) under consideration.

Stepwise acid hydrolysis of salsolosides C (I) and D (II) led to the glucuronosides (VII) [3] and (VIII) [4] of known structure. Consequently, the D-glucuronic acid residue is attached directly to the genins at C-3.

To determine the position of attachment of the D-xylose residue, glycosides C (I) and D (II) separately were methylated by Hakomori's method [5]. This gave the permethylates (IX) (M^+ 1066) and (X) (M^+ 1096).

The mass spectra of the permethylates (IX) and (X) showed the characteristic peaks of ions corresponding to fragments of the carbohydrate moieties and the genins. The peaks of

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