Koreoside A, a New Nonholostane Triterpene Glycoside from the Sea Cucumber *Cucumaria koraiensis*

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The new triterpene glycoside koreoside A (**1**) has been isolated from the sea cucumber *Cucumaria koraiensis*. Koreoside A (**1**) is the first glycoside reported from holothurians that presents a Δ^7 nonholostane aglycon without a lactone group and with a shortened side chain. Its structure has been elucidated by ^{13}C and ^{1}H NMR as well as FABMS studies.

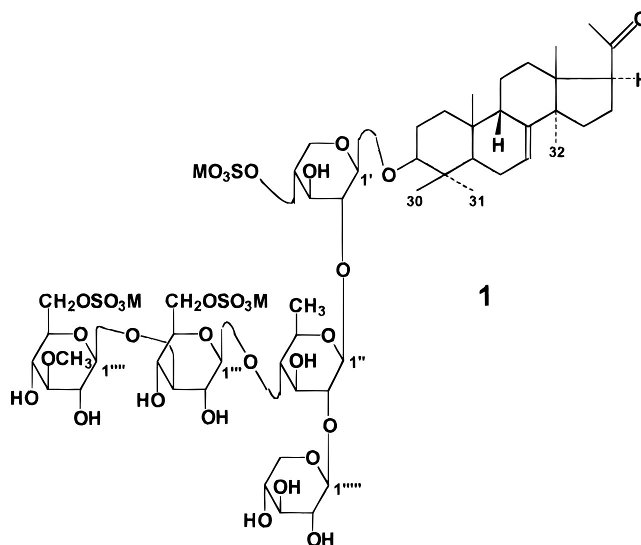
Triterpene glycosides are a known class of secondary metabolites typically found in holothurians. More than 80 of these saponins have been described to date, and most of them present a sugar chain of up to six monosaccharide units linked to C-3 of the aglycon, which is usually represented by a triterpene 18(20)-lactone with a lanostane skeleton (holostane).

As part of our research on the structure and biological role of triterpene glycosides from holothurians (sea cucumbers) belonging to the genus *Cucumaria*,[1−4] we became interested on the glycoside contain of the sea cucumber *Cucumaria koraiensis* Ostergren (Dendrochirotida, Cucumariidae). We report here the structure and isolation of koreoside A (**1**), a saponin with a nonholostane skeleton that is the major component of the polar extracts of *Cucumaria koraiensis* collected in the Pacific Ocean (Kurile Islands).

The ethanolic extracts of *C. koraiensis* (500 g wet) were sequentially submitted to column chromatography on silica gel, polychrom-1 (powdered Teflon), and again silica gel to give a fraction that was eventually purified by reversed-phase HPLC on a Silasorb C-18 column, affording 25 mg of pure koreoside A (**1**).

The molecular formula of **1** was deduced to be $C_{53}H_{83}O_{33}S_3M_3$ (M represents Na, K, or H) from the pseudomolecular ions at *m/z* 1389 ($M_{Na,Na,Na}$ − Na − H) and *m/z* 1383 ($M_{K,H,H}$ − H) in the negative FABMS and *m/z* 1429 ($M_{K,Na,Na}$ + H) in the positive FABMS. The fragment peak at *m/z* 1326 ($M_{K,Na,Na}$ − SO_3Na + H) in the positive FABMS and *m/z* 1185 ($M_{H,H,H}$ − $2SO_3$ − H) in the negative mode suggested the presence of sulfated groups in the molecule.

The ^{13}C and ^{1}H NMR data of **1** were clearly indicative of a saponin structure. The proton and carbon resonances corresponding to the sugar part of the molecule suggested the presence of five monosaccharide units in **1**, clearly indicated by signals for five anomeric carbons at δ_C 105.0, 104.5, 104.3, 103.8, and 102.4 ppm and five anomeric protons at δ_H 4.87, 4.90, 5.22, 5.25, and 5.28 ppm that resonate as doublets with coupling constants (J = 7.5 Hz), indicating a β-stereochemistry of the glycoside bonds. The ^{13}C NMR chemical shifts of the carbohydrate chain of **1** are practically identical to those of the sugar parts of known cucumariosides A_7-1 and A_7-3, isolated from *Cucumaria japonica*,[4] suggesting that the oligosaccharide part of **1** should be identical to that of those two glycosides and composed by one sulfated 3-*O*-methylglucose, one sulfated glucose, one quinovose, one xylose, and one sulfated xylose. A doublet at δ_H 1.70 ppm (3H, d, J = 6.3 Hz) and a singlet at δ_H 3.90 ppm (3H, s) in the ^{1}H NMR spectrum corroborated the presence of quinovose and 3-*O*-methylglucose. For their part, ions at *m/z* 1287 ($M_{K,K,Na}$ − 133 − Na − H) in the negative FABMS and 1311 ($M_{K,K,Na}$ − 133) in the positive FABMS assured the presence in **1** of a terminal nonsulfated xylose and the ion at *m/z* 1033 ($M_{K,K,Na}$ − 133 − 279 + H) in the positive FABMS that of a terminal sulfated 3-*O*-methylglucose.



The negative FABMS fragmentation of koreaside A (**1**), shown in Figure 1, shows all the expected fragments for a trisulfated branched pentaosides[4] and confirms the sequence of monosaccharides shown.

The structure of the aglycon of **1** was determined on the basis of its spectroscopic data and by their comparison with those of known terpenes. The ^{13}C and ^{1}H NMR data of the aglycon moiety of **1** indicated the presence

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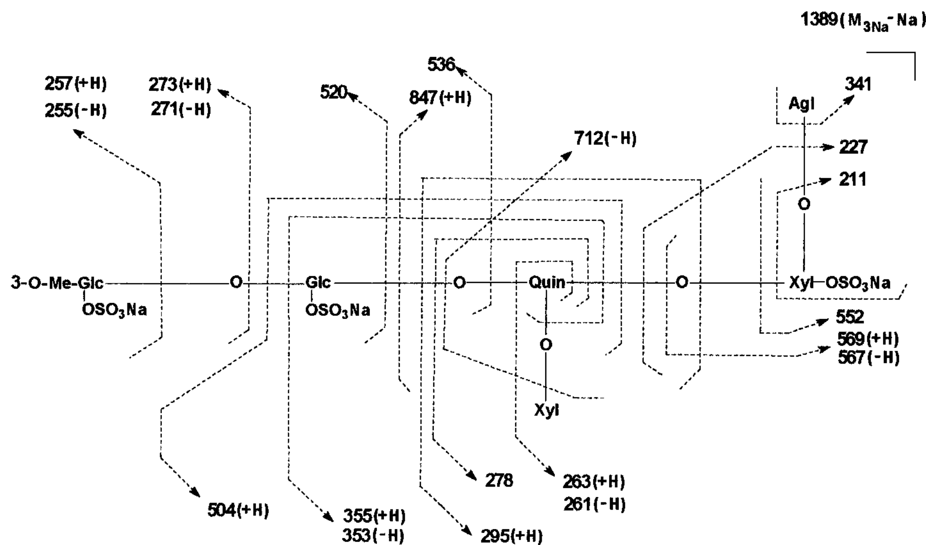

Figure 1. Negative FABMS fragmentation of koreoside A (**1**).

of a CH_3CO group [δ_C 211.5 and 30.7 ppm and δ_H 2.32 ppm (3H,s)], a trisubstituted double bond [δ_H 5.68 ppm (1H, m) and δ_C 122.5 and 147.7 ppm], and five methyl groups. Comparison of the ^{13}C NMR resonances of **1** with literature data for other saponins showed that resonances of C-1−C-8 and C-10, C-14, C-19, C-30, and C-31 are typical of lanostane triterpene glycosides with a 7(8)-double bond such as cucumarioside A_7-1[4], cucumarioside A_7-3,[4] and cucumarioside G_2.[5] In addition, the chemical shifts of C-1 through C-15, C-18, C-19, and C-30 through C-32 are coincident with those of abieslactone derivatives without the lactone ring[6] in polycyclic moiety, and the resonances of C-16, C-17, C-20, and C-21 are very close to those of the corresponding carbons in progesterone.[7] All these data suggest that the aglycon of **1** is 3β-hydroxy-22,23,24,25,26,27-hexanorlanostan-7(8)-en-20-one.

Information on the stereochemistry of **1** was obtained by use of selective NOE irradiation. Thus, irradiation at δ_H 1.16 ppm (CH_3-14) enhanced the signals at δ_H 2.89 ppm (H-17α) and 5.68 ppm (H-7), while irradiation at δ_H 0.88 ppm (CH_3-13) gave NOE on H-9β (2.25 ppm) and CH_3-20 (2.32 ppm). For its part, irradiation at δ_H 1.02 ppm (CH_3-10) gave the NOE on H-9β (δ_H 2.25 ppm) and CH_3-4β (δ_H 1.13 ppm). Finally, irradiation at δ_H 3.26 ppm (H-3α) led to NOE effect on the signals at δ_H 1.28 ppm (CH_3-4α) and 4.87 ppm (H-1′), demonstrating the linkage of the xylose unit to C-3 of the aglycon.

On the basis of all the above data, the structure of koreoside A (**1**) was established as 3β-hydroxy-22,23,-24,25,26,27-hexanor-3-*O*-[[6′′′′-*O*-sulfonyl-3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6′′′-*O*-sulfonyl-β-D-glucopyranosyl-(1→4)]-[β-D-xylopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→2)-4′-*O*-sulfonyl-β-D-xylopyranosyl]-lanostan-7(8)-en-20-one.

Most of the glycosides isolated so far from sea cucumbers belong to the holostane series: they are lanostane derivatives containing an 18(20)-lactone cycle,[8,9] and only a few nonholostane glycosides have been reported in the literature. Kurilosides A and C from the sea cucumber *Duasmodactyla kurilensis*[10] and the desulfated penaustrosides A and B from *Pentacta australis*[11] are examples of nonholostane saponins characterized by the presence of a 9(11)-double bond and the lack of lactone ring. Other two nonholostane glycosides, psolusoside B from *Psolus fabricii*[12] and cucumarioside G_2 from *Eupentacta fraudatrix*,[5] contain both the 18(16)-lactone function and the 7(8)-double bond. For their part, three of those nonholostane saponins, namely kurilosides A and C and cucumarioside G_2, present a shortened side chain.

Koreoside A (**1**) is the first nonholostane holothuroid glycoside reported in the chemical literature that lacks the lactone cycle and containing a 7(8)-double bond. It is also the third example of a hexanorlanostane glycoside from sea cucumbers.

From a biogenetic point of view, the 4,4,14-trimethylpregnane skeleton of the aglycon of **1** suggests its biosynthesis to be similar to that of pregnane hormones,[13] although in the case of **1** the triterpene precursor must shorten its side chain during the biosynthetic process.

Experimental Section

General Experimental Procedures. All melting points were determined with a Kofler-Thermogenerate apparatus. Specific rotation was measured on a Perkin-Elmer 141 polarimeter. NMR spectra were obtained on a Bruker WM-250 spectrometer at 250 MHz (^1H) and 62.7 MHz (^{13}C) in C_5D_5N:D_2O (4:1) with TMS as an internal reference ($\delta = 0$). The positive and negative FABMS were taken on a micromass apparatus, Model Autospec-M, on a glycerol−thioglycerol + NaCl matrix. HPLC was performed with a Dupont 8800 chromatograph equipped with a RIDK-102 differential refractometer (Laboratorni Pristroje, Prague).

Animal Material. The sea cucumber *C. koraiensis* (family Cucumariidae, order Dendrochirotida) was collected during a scientific cruise of the research fishery vessel "Tikhookeansky" in December 1987. The collection was carried out with a fishery bottom trawl at 290 m depth in the Krusenschtern strait (Kurile Islands). The sea cucumber was identified by Dr. V. S. Levin at the Institute of Marine Biology of the Far East Division of Russian Academy of Sciences, Vladivostok (present address: Kamchatka Institute of Fishery and Oceanography, Petropavlovsk-Kamchatsky, Russia), and stored frozen at −20 °C. A voucher specimen (st. 433, sample 1235) is on deposit in the collection of Zoological

Institute of the Russian Academy of Sciences, Saint-Petersburg, Russia.

Extraction and Isolation. The sea cucumbers (0.5 kg) were defrosted, cut into pieces, and immediately extracted with refluxing ethanol. The combined extracts were concentrated, and the residue was chromatographed on a Si column. Elution with the mixture $CHCl_3$−EtOH−H_2O (100:50:4) up to elution of sterol sulfates and further elution with $CHCl_3$−EtOH−H_2O (100:150:30) produced a fraction containing the glycosides. Desalting was carried out passing this fraction through a Polychrom-1 column (powdered Teflon; Biolar, Latvia), previously filled with EtOH and washed with H_2O, eluting first the inorganic salts and polar impurities with H_2O and then the glycoside fraction with 50% ethanol. This fraction was further chromatographed on a silica gel column eluting with $CHCl_3$−EtOH−H_2O (100:100:17) and then submitted to reversed phase HPLC on a Silasorb C-18 column (10 × 150 mm, 4 mL/min) eluting with 30% methanol to give 25 mg of compound **1**. Koreoside A (**1**): mp 250 °C dec; $[\alpha]^{20}_D$ −48° (c = 0.1, pyridine); ^{1}H NMR [C_5D_5N:D_2O (4:1)] 3.26 (H-3α, 1H, dd, J = 4.2, 11.5 Hz), 5.68 (H-7, 1H, m), 2.25 (H-9β, 1H, m), 2.89 (H-17α, 1H, t, J = 7.5 Hz), 1.02 (CH_3-10, 3H, s), 2.32 (CH_3-20, 3H, s), 1.28 (CH_3-4α, 3H, s), 1.13 (CH_3-4β, 3H, s), 0.88 (CH_3-13, 3H, s), 1.16 (CH_3-14, 3H, s), 1.70 (CH_3 of Qui, 3H, d, J = 6.3 Hz), 3.90 (OCH_3, 3H, s), 4.87 (H-1 of Xyl(1), 1H, d, J = 7.5 Hz), 4.90[a] (H-1 of Qui, 1H, d, J = 7.5 Hz), 5.22[a] (H-1 of Glc, 1H, d, J = 7.5 Hz), 5.25[a] (H-1 of 3-O-Me-Glc, 1H, d, J = 7.5 Hz), 5.28[a] (H-1 of Xyl(2), 1H, d, J = 7.5 Hz), signals marked "a" may be interchanged; ^{13}C NMR [C_5D_5N:D_2O (4:1)] 35.6[b] (t, C-10), 27.0 (t, C-2), 89.1 (d, C-3), 39.4 (s, C-4), 48.5 (d, C-5), 23.4 (t, C-6), 122.5 (d, C-7), 147.7 (s, C-8), 49.3 (d, C-9), 35.7[b] (s, C-10), 22.4[a] (t, C-11), 33.6 (t, C-12), 45.1 (s, C-13), 53.2 (s, C-14), 33.6 (t, C-15), 22.5[a] (t, C-16), 62.1 (d, C-17), 24.9[c] (q, C-18), 24.7 (q, C-19), 211.5 (s, C-20), 30.7 (q, C-21), 17.5 (q, C-30), 29.0 (q, C-31), 30.5 (q, C-32), 104.5 [d, C-1 of Xyl(1)], 82.0 [d, C-2 of Xyl(1)], 74.9 [d, C-3 of Xyl(1)], 75.3[d] [d, C-4 of Xyl(1)], 63.9 [t, C-5 of Xyl(1)], 102.4 (d, C-1 of Qui), 82.5 (d, C-2 of Qui), 74.9 (d, C-3 of Qui), 86.5 (d, C-4 of Qui), 70.9 (d, C-5 of Qui), 17.9 (q, C-6 of Qui), 103.8 (d, C-1 of Clc) 73.5 (d, C-2 of Glc), 86.7 (d, C-3 of Glc), 69.3 (d, C-4 of Glc), 75.5[d] (d, C-5 of Glc), 67.6 (t, C-6 of Glc), 104.3 (d, C-1 of 3-O-Me-Glc), 74.3 (d, C-2 of 3-O-Me-Glc), 86.5 (d, C-3 of 3-O-Me-Glc), 70.1 (d, C-4 of 3-O-Me-Glc), 76.5 (d, C-5 of 3-O-Me-Glc), 67.4 (t, C-6 of 3-O-Me-Glc), 60.3 (q, OMe), 105.0 [d, C-1 of Xyl(2)], 74.9 [d, C-2 of Xyl(2)], 76.5 [d, C-3 of Xyl(2)], 69.8 [d, C-4 of Xyl(2)], 66.4 [t, C-5 of Xyl(2)], the signals with similar superscripts may be interchanged; FABMS (positive ion mode, (glycerol/thioglycerol (1:1) + NaCl as matrix), m/z (rel int) 1429 (53), 1326 (52), 1311 (19), 1209 (63), 1033 (84); FABMS (negative ion mode, (glycerol/thioglycerol (1:1) + NaCl as matrix) (see Figure 1), m/z (rel int) 1389 (7), 1383 (22), 1287 (17), 1185 (18).

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