Structure of Eximisoside A, a Novel Triterpene Glycoside from the Far-Eastern Sea Cucumber *Psolus eximius*

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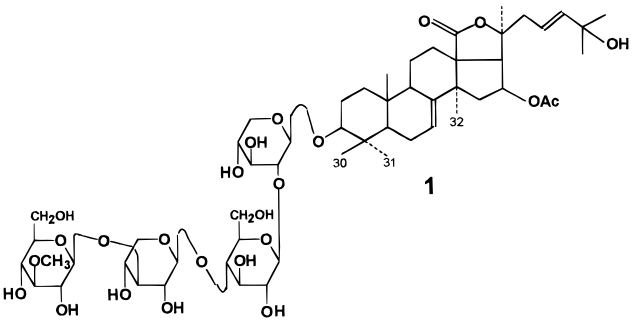
The new triterpene saponin eximisoside A (**1**) has been isolated from the Far-Eastern sea cucumber *Psolus eximius* and its structure elucidated by 1D and 2D NMR (^{13}C ,^{1}H, ^{1}H−^{1}H COSY, HMQC, and NOESY spectra), and FABMS studies. The structural features of eximisoside A (**1**) are the rare presence of a 23 double bond in the aglycon and the absence of quinovose, typically found in the oligosaccharide chain of holothurian glycosides.

As part of our ongoing investigation on triterpene glycosides of biological significance from the sea cucumbers belonging to the genus *Psolus*,[1−4] we decided to focus our attention on the glycosides of the Far-Eastern sea cucumber *Psolus eximius* Saveljeva (Psolidae, Dendrochirotida), collected in the Okhotsk Sea near Paramushir Island (Kuril Islands). In this paper we describe the isolation of eximisoside A (**1**), the main component of the polar extracts, whose structure was determined by 1D and 2D NMR (^{13}C, ^{1}H, ^{1}H−^{1}H COSY, HMQC, and NOESY spectra), FABMS studies, and by comparison with NMR spectra of related saponins.

The EtOH extracts of *P. eximius* (1.3 kg dry wt) were sequentially submitted to column chromatography on powdered Teflon (Polychrom-1, Biolar, Latvia) and Si gel, giving a fraction containing compound **1**. Pure eximisoside A (**1**), was isolated by reversed-phase HPLC on a Silasorb C-18 column.



The ^{1}H- and ^{13}C-NMR spectra suggested the presence of a triterpenoid aglycon with two olefinic bonds, one ester, and one lactone carbonyl group bonded to an oligosaccharide chain composed of four sugar units. Comparison of the spectral data with those already published for related saponins showed that the aglycon part of eximisoside A (**1**) was identical to that of cucumarioside G_4 from *Eupentacta fraudatrix*,[5] featuring the characteristic 23(24) double bond. The ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra of **1** also showed resonances for a 7(8)-double bond [δ_C 145.6 (s, C-8) and 120.6 (d, C-7); δ_H 5.70 (1H, m, H-7)] and those due to a 16β-acetoxy group [δ_C 170.0 (s) and 21.3 (q); δ_H 1.96 (3H, s). The quaternary carbon at δ_C 69.9 ppm (s, C-25) along with two methyl groups at δ_C 30.0 and 30.7 (q, C-26 and C-27) and δ_H 1.45 (6H, s) supported the presence of a hydroxyl group attached to C-25. Finally, the carbon resonances at δ_C 144.1 (d) and 120.0 (d) were indicative of an additional double bond. The chemical shift of the C-22 methylene [δ_C 41.8 (t)] and comparison with literature data[5] suggested that it is located at Δ^{23}. This conclusion was corroborated by the ^{1}H-NMR spectrum of **1**, which showed signals due to an ABXZ four-spin system composed of two diasterotopic protons (H-22 and H-22′) and two olefinic protons (H-23 and H-24). The E stereochemistry of the Δ^{23} double bond was demonstrated by the spin−spin coupling constant value (15.5 Hz). ^{1}H−^{1}H COSY, HMQC, and NOESY spectra allowed the assignment of all the proton and carbon resonances and the establishment of the relative stereochemistry of all chiral centers of the aglycon. Therefore, the aglycon of **1** is 16β-acetoxyholosta-7,23*E*-diene-3β,25-diol.

For its part, the sugar part of **1** gave proton and carbon resonances suggesting the presence of four monosacharide units (four anomeric carbons at δ_C 105.9, 105.7, 105.4, and 104.9 ppm and four anomeric protons at δ_H 5.17, 5.14, 4.85, 4.70 ppm). The β stereochemistries at the anomeric carbons were deduced from the coupling constant values (J = 7.2−7.9 Hz). The DEPT and ^{13}C-NMR data of the carbohydrate chain of **1** are coincident with those of the sugar parts of thelenotoside B from *Thelenota ananas*[6] and the progenin obtained by enzymatic hydrolysis of stichoposide D isolated from *Stichopus variegatus*.[7] Altogether, this information indicates that the oligosaccharide part of **1** is identical to that of these two glycosides and composed of two xyloses, one glucose, and one 3-*O*-methyl glucose. Furthermore, the FABMS (positive ion mode) of **1** showed pseudomolecular ions at m/z 1153 [M + Na] and 1169 [M + K], in concordance with a molecular formula $C_{55}H_{86}O_{24}$. The sequence of monosaccharide residues in the carbohydrate chain of **1** was confirmed by

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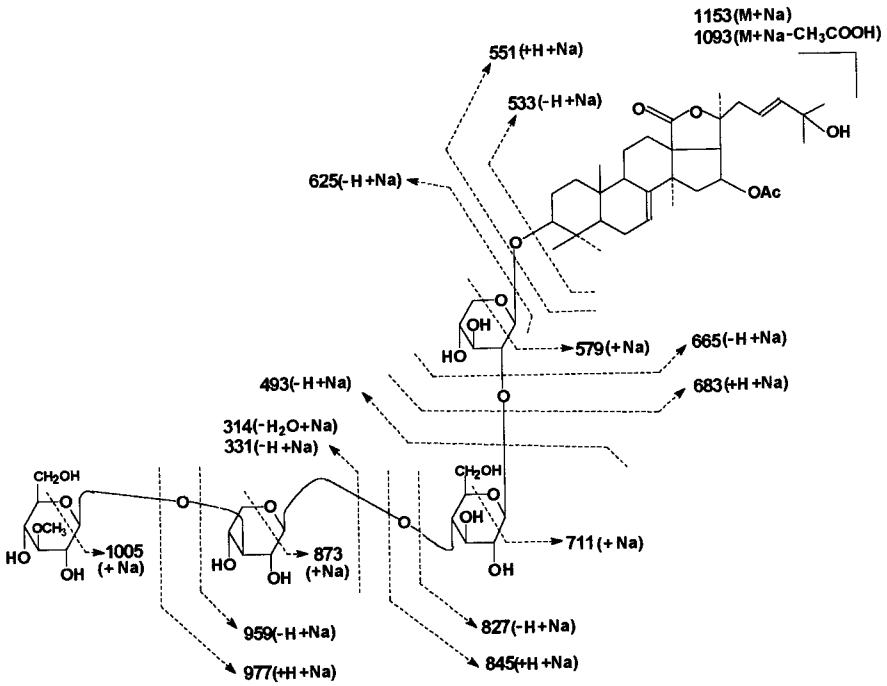

Figure 1. Fragmentation of glycoside **1** in the positive FABMS.

fragments at *m/z* 977 [M + Na − 3-O-MeGlc + H], 845 [M +Na − 3-O-MeGlc-O-Xyl + H], 683 [M + Na − 3-O-MeGlc-O-Xyl-O-Glc + H], and 551 [M + Na − 3-O-MeGlc-O-Xyl-O-Glc-O-Xyl + H] (Figure 1).

On the basis of all the above data, the structure of eximisoside A (**1**) was established as 3-*O*-[3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranosyl]-16*β*-acetoxyholosta-7,23*E*-diene-3*β*,25-diol.

More than 80 triterpene glycosides have been reported from sea cucumbers, and the great majority have a quinovose as the second unit in the carbohydrate chain.[8,9] Glycosides with glucose or xylose instead of quinovose are very rare, and this structural characteristic is associated with their smaller membranotropic (toxic) action.[10] The only such substances previously reported are stichoposides D and E and thelenotoside B and their dehydroanalogues from sea cucumbers belonging to the family Stichopodidae[6,7] and psolusoside B from the sea cucumber *Psolus fabricii*.[4] Eximisoside A (**1**) is thus one of a small number of compounds of this class of holothurians that are devoid of quinovose. For its part, the 23(24)double bond is also very rare in the aglycon of marine glycosides and to date cucumarioside G_4 from *Eupentacta fraudatrix*[5] is the single precedent to eximisoside A.

Experimental Section

General Experimental Procedures. Melting points were determined with a Kofler Thermogenerate apparatus. Specific rotations were measured on a Perkin-Elmer 141 polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker WM-250 spectrometer at 250 and 62.7 MHz in C_5D_5N with TMS as an internal reference, or Bruker AMX 500 (500.12 and 125 MHz) spectrometers. The FABMS (positive ion mode) were performed on a Fisons VG-Quatto spectrometer with Cs atoms at 7−9 keV and a matrix of glycerol−thioglycerol + NaCl. HPLC was performed with an Yanako L-2000L chromatograph equipped with differential refractometer.

Animal Material. Specimens of sea cucumber *P. eximius* were collected at 100 m depth with a Sigsby trawl during the seventh scientific cruise of r/v *Akademik Oparin* (August 1988) in the Okhotsk Sea near Paramushir Island (Kuril Islands). The sea cucumbers were identified by Dr. A. V. Smirnov (Zoological Institute of Russian Academy of Sciences, Saint-Petersburg, Russia), and immediately extracted by EtOH. Voucher specimens are kept in the collection of Zoological Institute of the Russian Academy of Sciences.

Extraction and Isolation. In all, 2200 specimens of *P. eximius* (1.3 kg dry wt) were extracted twice with hot EtOH. The combined extracts were evaporated, and the residue, dissolved in EtOH, was passed through a Polychrom-1 (powdered Teflon; Biolar, Latvia) column filled with EtOH and washed with H_2O, to retain the organic material. Inorganic salts and highly polar impurities were eluted with H_2O, and the glycoside-containing fraction with 50% Me_2CO. This fraction was chromatographed on a Si gel column, using $CHCl_3$−MeOH−H_2O (650:300:50) and $CHCl_3$−MeOH−H_2O (75:25:1) as eluents. The resulting 110 mg of impure glycoside were submitted to HPLC on a Silasorb C-18 (4.8250 mm, 1.2 mL/min) column. Elution with 37.5% Me_2CO afforded 25 mg of compound **1**.

Eximisoside A (**1**): mp 218 −221 °C, $[\alpha]^{20}_D$ −25° (*c* 0.1, pyridine); ^{13}C NMR (ppm, C_5D_5N, 62.7 MHz); aglycon carbons 36.3 (t, C-1), 27.3 (t, C-2), 89.3 (d, C-3), 39.7 (s, C-4), 48.4 (d, C-5), 23.5 (t, C-6), 120.6 (d, C-7), 145.6 (s, C-8), 47.3 (d, C-9), 35.8 (s, C-10), 22.7 (t, C-11), 31.6 (t, C-12), 59.5 (s, C-13), 47.6 (s, C-14), 43.8 (t, C-15), 74.9 (d, C-16), 54.9 (d, C-17), 179.2 (s, C-18), 24.1 (q, C-19), 84.5 (s, C-20), 28.5 (q, C-21), 41.8 (t, C-22), 144.1 (d, C-23), 120.4 (d, C-24), 69.9 (s, C-25), 30.0 (q, C-26), 30.7 (q, C-27), 17.6 (q, C-30), 29.0 (q, C-31), 32.3 (q, C-32), 170.0 (s, CH_3CO), 21.3 (q, CH_3CO); oligosaccharide chain carbons 105.7 (d, C-1Xyl_1), 83.6 (d, C-2Xyl_1), 77.9 (d, C-3Xyl_1), 70.9 (d, C-4Xyl_1), 66.6 (t, C-5Xyl_1), 104.9[#] (d, C-1Qui), 76.5 (d, C-2Qui), 75.8 (d, C-3Qui), 80.9 (d, C-4Qui), 76.5 (d, C-5Qui), 61.7 (t, C-6Qui), 105.4[#]

(d, C-1Xyl$_2$), 73.6 (d, C-2Xyl$_2$), 87.9 (d, C-3Xyl$_2$), 69.2 (d, C-4Xyl$_2$), 66.6 (t, C-5Xyl$_2$), 105.4[#] (d, C-1MeGlc), 75.1 (d, C-2MeGlc), 87.5 (d, C-3MeGlc), 70.9 (d, C-4MeGlc), 78.3 (d, C-5MeGlc), 62.5 (t, C-6MeGlc), 60.6 (q, O*C*H$_3$ of MeGlc); ^{1}H NMR (ppm, C$_5$D$_5$N, 500 MHz) aglycon protons 5.89 (1H, d, J = 15.5 Hz, H-24), 5.84 (1H, q, J = 8.6 Hz, H-16), 5.74 (1H, ddd, J = 15.5, 7.0, 5.7 Hz, H-23), 5.70 (1H, m, H-7), 3.24 (1H, br d, J = 17.3 Hz, H-9), 3.20 (1H, dd, J = 14.0, 5.7 Hz, H-22), 3.19 (1H, dd, J = 14.1, 4.7 Hz, H-3), 2.73 (1H, d, J = 8.6 Hz, H-17), 2.57 (1H, dd, J = 14.0, 7.0 Hz, H-22′), 2.51 (1H, dd, J = 12.6, 7.7 Hz, H-15), 1.96 (3H, s, CH$_3$CO), 1.56 (1H, dd, J = 12.6, 10.6 Hz, H-15′), 1.53 (3H, H$_3$-21), 1.45 (6H, H$_3$-26 and H$_3$-27), 1.13 (3H, H$_3$-31), 1.05 (3H, H$_3$-32), 1.04 (3H, H$_3$-19), 1.02 (3H, H$_3$-30), 0.85 (1H, b rd, J = 8.8 Hz, H-5); oligosaccharide chain protons 5.17* (1H, d, J = 7.6 Hz, H-1MeGlc), 5.14* (1H, d, J = 7.9 Hz, H-1Xyl$_2$), 4.85* (1H, d, J = 7.7 Hz, H-1Qui), 4.70 (1H, d, J = 7.2 Hz, H-1Xyl$_1$), 3.55 (1H, t, J = 11.0 Hz, H5-Xyl$_1$), 3.79 (3H, s, OCH$_3$-MeGlc); FABMS (positive ion mode) m/z (rel int) = 1169 ([M + K]$^+$, 2), 1153 ([M + Na]$^+$, 11), 977 ([M − 176 + Na]$^+$, 3), 959 ([M − 176 − H$_2$O + Na]$^+$, 2), 845 ([M − 176 − 132 + Na]$^+$, 4), 827 ([M − 176 − 132 − H$_2$O + Na]$^+$, 3), 683 ([M − 176 − 132 − 162 + Na]$^+$, 4), 665 ([M − 176 − 132 − 162 − H$_2$O + Na]$^+$, 3), 549 ([M − 176 − 132 − 162 − 132 − 2H + Na]$^+$, 5), 533 ([M − 176 − 132 − 162 − 132 − H$_2$O + Na]$^+$, 6), 165 (100).

Note: Xyl: β-D-xylopyranose, Qui: β-D-quinovopyranose, MeGlc: β-D-3-*O*-methylglucopyranose. #, * these signals may be interchanged.

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References and Notes

(1) Kalinin, V. I.; Stepanov, V. R.; Stonik, V. A. *Khim. Prirod. Soedin.* **1983**, 789−789.
(2) Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A. *Khim. Prirod. Soedin.* **1985**, 212−217.
(3) Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A. *Khim. Prirod. Soedin.* **1987**, 674−678.
(4) Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A. Dmitrenok, P. S. Elkin, Y. N. *Khim. Prirod. Soedin.* **1989**, 361−368.
(5) Kalinin, V. I.; S. A. Avilov, Kalinovsky, A. I.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. W. *Khim. Prirod. Soedin.* **1992**, 691−694.
(6) Stonik, V. A.; Maltsev, I. I.; Elyakov, G. B. *Khim. Prirod. Soedin.* **1982**, 624−627.
(7) Stonik, V. A.; Maltsev, I. I.; Kalinovsky, A. I.; Elyakov, G. B. *Khim. Prirod. Soedin.* **1982**, 200−204.
(8) Stonik, V. A.; Elyakov, G. B. Secondary Metabolites from Echinoderms as Chemotaxonomic Markers. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer: Berlin, 1988; Vol. 2, pp 43−86.
(9) Habermehl, G. G.; Krebs, H. C. Toxins of Echinoderms. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, 1990; Vol. 7, pp 265−316.
(10) Kalinin, V. I.; Anisimov, M. M.; Prokofieva, N. G.; Avilov, S. A.; Afiyatullov, Sh. Sh.; Stonik, V. A. Biological Activities and Biological Role of Triterpene Glycosides from Holothuroids (Echinodermata). In *Echinoderm Studies* , Jangoux, M., Lawrence, J. M., Eds.; Rotterdam: Brookfield, 1996; Vol. 5, pp 139−181.

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