# Chemistry of Natural Compounds and Bioorganic Chemistry

## Asterosaponin $P_2$ from the Far-Eastern starfish *Patiria (Asterina) pectinifera*

*A. A. Kicha,* N. V. Ivanchina, A. I. Kalinovsky, P. S. Dmitrenok, and V. A. Stonik*

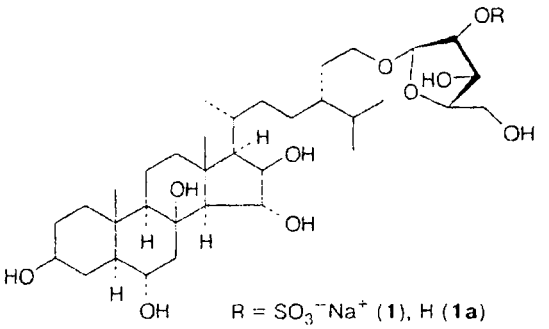
*Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences, 159 prosp. 100-letiya Vladivostoka, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050. E-mail: piboc@stl.ru*

A new polyhydroxylated steroidal glycoside, asterosaponin $P_2$, was isolated from the Far-Eastern starfish *Patiria (Asterina) pectinifera*. The glycoside was identified as the (24*R*)-29-*O*-[2-*O*-sulfo-α-L-arabinofuranosyl]-24-ethyl-5α-cholestane-3β,6α,8β,15α,16β,29-hexol Na salt.

**Key words:** starfish, *Patiria pectinifera*, glycoside, polyhydroxy steroid.

Unlike many other sea animals, starfishes contain highly oxygenated steroids encountered in free, sulfated, and glycosylated states.[1] We studied repeatedly a difficultly separable mixture of highly hydroxylated steroid metabolites from Far-Eastern starfish *Patiria pectinifera* (=*Asterina pectinifera* Muller and Trochel)[2–5] and isolated a new sulfated stigmastane glycoside from a water—ethanol extract of liver. We named the glycoside asterosaponin $P_2$ (**1**) because previously we isolated a glycoside called asterosaponin $P_1$ from the same starfish.[2] Compound **1** was obtained and purified by column chromatography on Polychrom-1, Sephadex LH-20, silica gel, and Florisil and by high-performance liquid chromatography (HPLC) on the reverse phase Nucleosil $C_{18}$.

The structure of compound **1** was established by $^1$H NMR spectroscopy. The $^1$H NMR chemical shifts and the relevant spin—spin coupling constants of the protons of the steroid moiety of glycoside **1** virtually coincide with those found for miniatoside A isolated from the starfish *Patiria miniata* and having a stigmastane type aglycone with six hydroxy groups in positions 3β,6α,8β,15α,16β,29.[6] The signals of the protons of the monosaccharide residue in the $^1$H NMR spectrum of compound **1** were compared with the signals of the 5-*O*-methyl-2-*O*-sulfo-α-arabinofuranose residue in the spectrum of miniatoside A[6] and




R = $SO_3^-Na^+$ (**1**), H (**1a**)

with the signals of the α-arabinofuranose residue in the spectrum of desulfated glycoside from the starfish *Oreaster reticulatus*.[7] The signals of the HC(2′), HC(4′), HC(5′), and HC′(5′) protons can clearly be seen in the $^1$H NMR spectrum of compound **1**. The chemical shifts and the spin—spin coupling constants suggest that the monosaccharide residue in glycoside **1** is represented by 2-*O*-sulfo-α-arabinofuranose. The signal of HC(1′) is substantially overlapped by the signal of $H_2O$. The position of this signal was determined more precisely (δ 5.13) by heating the sample to 50 °C, which induced an upfield shift of the signal of $H_2O$. A homodecoupling experiment made it possible to determine the chemical shift and the spin—spin

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coupling constant of HC(3′). Upon irradiation of the HC(2′) proton, the doublet of doublets due to HC(3′) collapsed into a doublet. The data obtained indicate that the monosaccharide residue in glycoside **1** is 2-*O*-sulfo-α-arabinofuranose. The MALDI-TOF mass spectrum of **1** exhibits the [M-cation]$^-$ peak at *m/z* 707, which confirms the presence of a sulfate group in compound **1**. According to atomic-adsorption analysis, compound **1** contains an $Na^+$ ion. Thus, asterosaponin $P_2$ (**1**) was identified as the sodium salt of 29-*O*-[2-*O*-sulfo-α-L-arabinofuranosyl]-24-ethyl-5α-cholestane-3β,6α,8β,15α,16β,29-hexol.

Apparently, asterosaponin $P_2$ is a native form of stigmastane-type desulfated glycoside **1a**, which was isolated in our previous study from the products of mild solvolytic cleavage of a mixture of steroid compounds from the same starfish.[4] Indeed, solvolysis of glycoside **1** resulted in compound **1a**, which was identified by direct comparison with the authentic sample (TLC, $[\alpha]_D$, $^1$H NMR). The arabinofuranose residue in compound **1** was assigned to the L-series because this had been established previously for compound **1a**.

According to the data published for 29-hydroxysterols, the doublets corresponding to the protons of the $H_3C(26)$ and $H_3C(27)$ methyl groups overlap in the case of (24*S*)-configuration, whereas in the case of (24*R*)-configuration, the difference between the signals due to the $H_3C(26)$ and $H_3C(27)$ protons is ~0.03 ppm.[8] The difference between the chemical shifts of the $H_3C(26)$ and $H_3C(27)$ protons in the spectra of glycoside **1** is 0.02 ppm; therefore, we assumed that the C(24) asymmetric center in asterosaponin $P_2$ has the *R*-configuraion.

## Experimental

$^1$H NMR spectra were recorded on a Bruker WM-250 spectrometer using $SiMe_4$ as the internal standard. The optical rotation was measured on a Perkin—Elmer 141 polarimeter. The MALDI-TOF mass spectra were run on a Biflex III mass spectrometer (Bruker, Germany, $N_2$ laser, 337 nm). The sample was dissolved in MeOH (1 mg mL$^{-1}$) and a 1-μL aliquot was analyzed using 2,5-dihydroxybenzoic acid as the matrix. HPLC was performed on a Du Pont Model 8800 chromatograph (with a refractometer as the detector) using a column with Nucleosil $C_{18}$ (5 μ, 250×4.6 mm) and a Chromatopac C-R2A(X) integrator (Shimadzu, Japan).

The sorbents used in column chromatography were Polychrom-1 (Biolar, Latvia), Sephadex LH-20 (Sigma Chemical Co.), silica gel L (40/100 μm, Chemapol, Czech Republic), and Florisil (100—200 mesh, Koch-Light Laboratories Ltd., UK). Thin layer chromatography (TLC) was performed on glass plates (4.5×6.0 cm) with a fixed layer of Sorbphil silica gel (5—17 μ, Russia).

The starfishes were gathered in July 1998 in Posjet Bay of the Sea of Japan at a depth of 1—1.5 m and identified by Yu. M. Yakovlev (Institute of Marine Biology of the Far-Eastern Branch of the RAS, Vladivostok).

**Isolation of glycoside 1.** Starfish liver (190 g) was homogenized and extracted twice with 70% ethanol (3 mL g$^{-1}$) at room temperature and the extract was centrifuged. To remove lipids, the supernatant was extracted with benzene (1 mL per 3 mL of the supernatant). The aqueous-ethanolic layer was concentrated *in vacuo*, the residue was dissolved in 0.5 L of water, and the solution was passed through a 7×10 cm column with Polychrom-1. The column was washed with water until the eluate was free from $Cl^-$ ions and with 50% ethanol, and the ethanolic eluate was concentrated. The resulting total fraction of steroid compounds (1.5 g) was chromatographed successively on a 4×100 cm column with Sephadex LH-20 in the ethanol—$H_2O$ system (2 : 1), a 4×18 cm column with silica gel in the chloroform—ethanol system (3 : 1 → 1 : 1), and a 2×15 cm column with Florisil in the chloroform—ethanol system (2 : 1). This gave a fraction containing compound **1** (TLC, butanol—ethanol—water, 4 : 1 : 2, $R_f$ 0.58). Then the fraction was purified by HPLC on a column with Nucleosil $C_{18}$; the product was eluted with 65% aqueous methanol to give 3 mg of compound **1**, $C_{34}H_{59}NaO_{13}S$, $[\alpha]_D$ + 12° (*c* 0.1, MeOH).

$^1$H NMR ($CD_3OD$), δ: (agiycone) 0.84 (d, 3 H, Me(27), $J$ = 7 Hz); 0.86 (d, 3 H, Me(26), $J$ = 7 Hz); 0.92 (d, 3 H, Me(21), $J$ = 7 Hz); 1.01 (s, 3 H, Me(19)); 1.10 (s, 3 H, Me(18)); 2.40 (dd, 1 H, $H_2C(7)$, $J$ = 3.5 Hz and 12.5 Hz); 3.46 (m, 1 H, HC(3)); 3.61 (m, 1 H, HC(6)); 3.74 (m, 1 H, HC(29)); 4.02 (dd, 1 H, HC(16), $J$ = 8 Hz and 2.5 Hz); 4.06 (dd, 1 H, HC(15), $J$ = 11 Hz and 2.5 Hz); (monosaccharide residue) 3.62 (dd, 1 H, HC(5′), $J$ = 12.5 Hz and 6 Hz); 3.74 (dd, 1 H, H′C(5′), $J$ = 12.5 Hz and 3.5 Hz); 3.94 (td, 1 H, H′C(4′), $J$ = 6.5 Hz and 3.5 Hz); 4.03 (dd, 1 H, H′C(3′), $J$ = 6.5 Hz and 2.5 Hz); 4.56 (d, 1 H, HC(2′), $J$ = 3 Hz); 5.13 (s, 1 H, HC(1′)).

MS (MALDI-TOF), *m/z* ($I_{rel}$ (%)): 707 [M − Na]$^-$ (100%).

**Desulfation of glycoside 1.** Compound **1** (1.5 mg) was heated for 2 h at 100 °C with 2 mL of a dioxane—pyridine mixture (1 : 1). The solvent was evaporated *in vacuo* and the dry residue was chromatographed on a column with silica gel (1.5×3 cm) in the chloroform—ethanol system (6 : 1) to give 1 mg of compound **1a**, which was identified by direct comparison (TLC, $[\alpha]_D$, $^1$H NMR) with a sample isolated in our previous study from the same starfish.[4]

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