Chemistry of Natural Compounds, Vol. 41, No. 3, 2005

STEROLS AND OTHER METABOLITES FROM THE FAR-EAST SPONGE *Dysidea* SP.

I. I. Kapustina, T. N. Makar′eva, V. A. Denisenko, P. S. Dmitrenok, and V. A. Stonik

UDC 547.92:639.29

Of all animals, sponges contain the widest variety of sterols [1, 2]. However, the sterol composition of many sponge species has not been studied.

We investigated the chemical composition of the alcohol extract of the Far-East sponge *Dysidea* sp. that was collected using the trawler "Sigsby" in August 2003 in the Sea of Okhotsk (Shantar Islands, Akademiya Bay, 54°17.9N, 137°55.5E) at a depth of 36 m.

Ground animals were extracted with ethanol. The extract was concentrated in vacuo. The solid was partitioned between alcohol (90%) and hexane. The hexane extract was separated over a silica-gel column (KSK, 100-150 mesh) using the gradient solvent system hexane→hexane:ethylacetate (100:0→97:3) to afford the sterol fraction (0.057% yield of the wet animal mass). The structures of the free sterols were identified as the acetylated derivatives by comparing capillary GC (HP-5MS) retention times and using GC-MS as previously described [3-5].

We identified 11 free sterols (Table 1). They belonged mainly to the Δ^5-series. The main components were 24-methylcholesta-5,24(28)-dien-3β-ol, cholesterol, and cholesta-5,24-dien-3β-ol. The content of nor-derivatives was rather high for cold-water animals.

The alcohol solution containing polar substances was concentrated and separated over a silica-gel column (KSK, 100-150 mesh) using the gradient solvent system $CHCl_3$:ethanol (100:0→0:100). Each of the three most polar fractions was purified by flash chromatography (SiO_2) using the gradient system $CHCl_3$:ethanol (90:10→50:50) and then was chromatographed over LH-20 ($CHCl_3$:ethanol, 75:25) and by HPLC (Hypersil ODS, 5 μm, 40 × 250 mm, 0.5 mL/min) in ethanol (30%) to afford uridine (**1**) and glycine (**2**). Compounds **3** and **4** were not separated under these conditions.

The structures of these compounds were identified by spectral methods (NMR and mass spectrometry including 1D COSY). The mass spectra of **1** and **2** contained peaks corresponding to the molecular ions. The ^{13}C and ^{1}H NMR spectra corresponded with those in the literature [6-8].

Uridine (1), $C_9H_{12}O_6N_2$, MW (EIMS, *m/z*): 226 $[M - 18]^+$, 133, 112.

^{13}C NMR spectrum (125 MHz, C_5D_5N, δ, ppm): 61.6 (C-5′), 71.0 (C-3′), 75.9 (C-2′), 86.1 (C-4′), 90.3 (C-1′), 102.3 (C-5), 141.0 (C-6), 152.1 (C-2), 164.3 (C-4).

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 4.22 (1H, dd, J_1 = 2.4, J_2 = 12.0, H-5′a), 4.33 (1H, dd, J_1 = 2.6, J_2 = 12.1, H-5′b), 4.68 (1H, m, H-4′), 4.93 (2H, m, H-2′,3′), 5.81 (1H, d, J = 8.0, H-5), 6.85 (1H, d, J = 3.6, H-1′), 8.57 (H, d, J = 8.0, H-6).

Glycine (2), $C_2H_5O_2N$, MW (EIMS, *m/z*): 75 $[M]^+$, 30.

^{13}C NMR spectrum (125 MHz, D_2O, δ, ppm): 41.7 (C-2), 172.5 (COOH).

PMR spectrum (300 MHz, D_2O, δ, ppm): 3.48 (2H, s, H-2).

Amino-acid analysis (Biochrom-30, Li^+, Ultropac, 8 μm, 200 × 4.6 mm) showed that **3** was alanine; **4**, proline. These data were confirmed by studying the NMR spectra [8, 9].

The composition of the sponge *Dysidea* sp. has not been previously studied.

Pacific Institute of Bioorganic Chemistry, Far-East Division, Russian Academy of Sciences, 690022, Vladivostok, pr. 100-Letiya Vladivostoku, 159, fax 7-(4232) 31 40 50, e-mail: piboc@stl.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 286-287, May-June, 2005. Original article submitted January 24, 2005.

0009-3130/05/4103-0355

TABLE 1. Composition of Free Sterols in the Marine Sponge *Dysidea* sp.

Sterols	Composition, %
24-Norcholesta-5,22*E*-dien-3β-ol	5.16
24-Nor-5α-cholest-22*E*-en-3β-ol	0.59
Cholesta-5,22*E*-dien-3β-ol	0.65
27-Nor-24ξ-methyl-5α-cholesta-7,22*E*-dien-3β-ol	6.51
Cholest-5-en-3β-ol	26.80
5α-Cholestan-3β-ol	0.48
Cholesta-5,24-dien-3β-ol	22.85
24-Methylcholesta-5,24(28)-dien-3β-ol	32.60
24-methyl-5α-cholest-24(28)-en-3β-ol	0.65
24ξ-Ethylcholest-5-en-3-ol	1.28
24ξ-Ethylcholesta-5,24(28)*Z*-dien-3β-ol	1.15

ACKNOWLEDGMENT

We express our gratitude to V. B. Krasokhin for identifying the animal and to Drs. L. P. Ponomarenko, O. P. Moiseenko, and L. Ya. Tishchenko for carrying out the GC, GC-MS, and amino-acid analyses.

The work was supported financially by the Program of the RAS Presidium "Molecular and Cellular Biology" and a grant of the RF President NSh-725.2003.4.

REFERENCES

1. B. J. Baker and R. G. Kerr, in: *Marine Natural Products - Diversity and Biosynthesis*, P. J. Scheuer, ed., Springer-Verlag, Berlin (1993), p. 32.
2. E. A. Santalova, T. N. Makarieva, I. A. Gorshkova, A. S. Dmitrenok, V. B. Krasokhin, and V. A. Stonik, *Biochem. Syst. Ecol.*, **32**, 153 (2004).
3. I. I. Kapustina, L. P. Ponomarenko, O. P. Moiseenko, and V. A. Stonik, *Khim. Prir. Soedin.*, 441 (2001).
4. L. P. Ponomarenko, T. N. Makarieva, V. A. Stonik, A. S. Dmitrenok, and P. S. Dmitrenok, *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, **111**, 575 (1995).
5. L. P. Ponomarenko, I. V. Stonik, N. A. Aizdaicher, T. Yu. Orlova, G. I. Popovskaya, G. V. Pomazkina, and V. A. Stonik, *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, **138**, 65 (2004).
6. J. B. Stothers, *Carbon-13 NMR Spectroscopy*, Academic Press, New York and London (1972).
7. G. Gosselin, M. C. Bergogne, and J. L. Imbach, *J. Heterocycl. Chem.*, **30**, 1229 (1993).
8. P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D. E. Williams, et al., *J. Am. Chem. Soc.*, **121**, 5899 (1999).
9. K. Fujii, K. Sivonen, T. Kashiwagi, K. Hirayama, and K. Harada, *J. Org. Chem.*, **64**, 5777 (1999).