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Further study on *Penares* sp. from Vietnamese waters: Minor lanostane and *nor*-lanostane triterpenes

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ABSTRACT

Eight new oxidized lanostane and *nor*-lanostane derivatives (**1**–**8**) along with the previously known penasterol (**9**) and 24-ethylcholesta-4,24(28)-dien-3-one (**10**) were isolated from a sponge *Penares* sp. collected from the Vietnamese waters. Structures of these minor compounds were elucidated by the detailed NMR spectroscopic and mass-spectrometric analyses and by comparison with earlier reported spectroscopic data. A hypothetic scheme of metabolism of the lanostane derivatives in sponges belonging to *Penares* and *Erylus* genera was proposed and discussed.

1. Introduction

Marine organisms are a prolific source of novel natural products, including steroids and their biosynthetic precursors such as tri-, di- and monomethylsterols with unique structures and biological activities of interest. Actually, as it is well known, marine sponges often produce unusual sterols and related metabolites, especially oxidized polar derivatives [1]. The steroidal and related compounds isolated from sponges form, as a rule, very complex mixtures of highly functionalized metabolites, many of which have no terrestrial counterpart and are bioactive [2].

From the sponges belonging to the genus *Penares*, diverse indole and azetidine alkaloids [3] along with sphingolipids, [4] proline containing macrolides, [5] and triterpenes [6–8] were earlier isolated.

Triterpenoids, their demethylated derivatives and thereof derived steroids from *Penares* spp. represent a particular group of natural products, some of which are of physiological relevance as antileukemic agents [6] and inhibitors of histamine release from rat peritoneal mast cells [7]. Structurally, the peculiarity of these compounds consists in the presence of a rare in nature carboxy group, attached to C-14 or 30,9-lactone fragment in lanostane nucleus. In continuation of our studies on the natural products of the *Penares* sp. from Vietnamese waters [8,9] and attempts to obtain the more detailed picture of biosynthetic transformations of lanostane triterpenoids in this animal, herein we describe the isolation and structure elucidation of new minor polar constituents from its ethanol extract.

2. Experimental

2.1. General methods

Optical rotations were measured using a Perkin-Elmer 343 polarimeter. Melting points were determined using a hot stage melting point apparatus model Leica GALEN III equipped with microscope. ^{1}H NMR (700.13 MHz, 500.13 MHz) and ^{13}C NMR (176.04 MHz, 125.75 MHz) spectra were recorded on Avance III 700 and DRX-500 Bruker spectrometers, using TMS as an internal standard. HRESI mass spectra and ESIMS/MS spectra were recorded on an Agilent 6510 Q-TOF LC/MS mass spectrometer; samples were dissolved in MeOH (c 0.01 mg/mL). HPLC separations were carried out on an Agilent 1100 Series chromatograph equipped with a differential refractometer. YMC-Pack ODS-A (250 × 10 mm), YMC-Pack ODS-A (5 μ, 250 × 4.6 mm) and ULTRASPHERA Si (5 μ, 250 × 10 mm) columns were used. Low pressure column liquid chromatography was performed using Sephadex LH-20 (Sigma Chemical Co.) and Si gel KSK (50–160 μm, Sorbpolimer, Krasnodar, Russia). Sorbfil Si gel plates (5–17 μm, Sorbpolimer, Krasnodar,

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Russia) were used for thin-layer chromatography. Yields are based on dry weight of the sponge.

2.2. Animal material

The specimens of *Penares* sp. were collected in Vietnamese waters in January 2005, as previously described [9]. A voucher specimen (PIBOC O30-271) has been deposited at the collection of marine invertebrates of the G.B. Elyakov Pacific Institute of Bioorganic Chemistry (Vladivostok, Russian Federation).

2.3. Extraction and isolation

The sponge specimen (wet weight 400 g) was finely chopped and extracted with EtOH. The ethanol soluble materials (2.8 g) were fractionated using LH-20 column chromatography ($CHCl_3$–EtOH, 1:1) into four fractions. A half of the second fraction (1.3 g of yellow oil; 2.6 g total) was further separated by silica gel column chromatography (12 × 3 cm, step-wise gradient $CHCl_3$–EtOH) to yield four subfractions (A–D).

From the subfraction A, a number of triterpenoids were isolated and described previously [8]. In the present study, we also isolated 24-ethylcholesta-4,24(28)-dien-3-one (**10**) $[\alpha]_D^{27}$ + 48.2 (c 0.28, $CHCl_3$) lit $[\alpha]_D^{26}$ + 52.0 (c 0.24, $CHCl_3$) [10] (analytical reversed phase HPLC, 100% EtOH) (2.8 mg; 0.002%) as a minor compound in addition to ergosta-4,24(28)-diene-3-one [11,12] (36.1 mg; 0.032%).

Subfraction B, eluted with 100% $CHCl_3$, was chromatographed using a semi-preparative reversed phase HPLC column (95% EtOH) to give a series of previously published major components of sponge extract [8] and the sum (57.3 mg) of minor compounds. The latter was further separated using a semi-preparative reversed phase HPLC column (85% EtOH) to give subsubfractions B1 and B2. The subsubfraction B1 was purified by preparative HPLC (75% CH_3CN) to yield compounds **1** (0.5 mg; 0.0005%) and **2** (0.8 mg; 0.0007%). The subsubfraction B2 was chromatographed on a preparative HPLC column (75% CH_3CN) to obtain two fractions (B2a and B2b) and epimers **3** (1.4 mg; 0.0013%) and **4** (1.1 mg; 0.0010%) as individual compounds. Further purification of B2a on an analytical normal phase HPLC column (*n*-hexane–EtOAc, 3:1) led to the isolation of triterpenoids **5** (0.7 mg; 0.0006%) and **7** (0.1 mg; 0.0001%). The fraction B2b contained compound **6** (0.3 mg; 0.0003%), which was purified by analytical normal phase HPLC (*n*-hexane–EtOAc, 4:1).

The subfraction C, eluted with $CHCl_3$–EtOH (20:1), was rechromatographed using a semi-preparative (90% EtOH) and analytical (85% EtOH) reversed phase HPLC columns to yield penasterol (**9**), mp 193–202 °C, lit [6] 197–201 °C (3.0 mg; 0.0027%) and compound **8** (0.3 mg; 0.0003%), the latter that was further purified using analytical normal phase HPLC column ($CHCl_3$–EtOH, 40:1) and analytical reversed phase HPLC column (75% CH_3CN).

2.3.1. 29-Nor-24(R),25-dihydroxypenasterone (*1*)

White amorphous powder; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 3; ms/ms (−) ESI *m/z*: 473 $[M-H]^-$, 429 $[M-COOH]^-$, 427, 413, 411, 383, 369, 341; HRESI *m/z* 473.3272 $[M-H]^-$ (calcd. for $C_{29}H_{45}O_5$, 473.3272).

Table 1
^1H NMR data of compounds **1**–**4** (δ in ppm, *J* in Hz, $CDCl_3$).

Position	1	2	3	4
1	2.10, m	α: 2.08, m	α: 2.03, m	α: 2.03, m
	1.62, m	β: 1.88, m	β: 1.70, m	β: 1.70, m
2	α: 2.38, m	α: 2.34, dt (14.5; 3.8)	α: 2.60, ddd (15.8; 11.1; 7.3)	α: 2.59, m
	β: 2.48, ddd (15.0; 5.1; 2.4)	β: 2.43, td (14.5; 5.8)	β: 2.43, ddd (15.8; 6.9; 3.7)	β: 2.44, ddd (15.9; 6.8; 3.6)
3				
4	2.31, m	2.16, m		
5	1.45, m	1.76, m	1.65, m	1.64, m
6	1.45, m	α: 2.11, m	1.63, m	1.62, m
	1.80, m	β: 1.63, dd (15.6; 13.1)	1.65, m	1.67, m
7	2.05, m	3.01, d (6.7)	2.05, m	2.05, m
	2.17, m		2.17, m	2.17, m
8				
9				
10				
11	2.25, m	α: 2.02, m	2.17, m	2.17, m
	2.19, m	β: 2.09, m	2.17, m	2.17, m
12	1.80, m	α: 1.60, m	1.78, m	1.78, m
	2.12, m	β: 2.02, m	2.12, m	2.14, m
13				
14				
15	1.61, m	α: 1.55, m	a: 1.61, m	1.62, m
	2.06, m	β: 1.28, m	b: 2.07, m	2.08, m
16	1.46, m	a: 1.39, m	1.47, m	1.44, m
	2.18, m	b: 2.18, m	2.17, m	2.18, m
17	1.62, m	1.69, m	1.61, m	1.62, m
18	0.83, s	1.04, s	0.81, s	0.81, s
19	1.24, s	1.20, s	1.16, s	1.16, s
20	1.47, m	1.56, m	1.47, m	1.47, m
21	0.94, d (6.5)	0.91, d (6.6)	0.94, d (6.6)	0.94, d (6.6)
22	1.05, m	1.88, m	1.02, m	1.28, m
	1.77, m	2.16, m	1.77, m	1.50, m
23	1.13, m	5.65, ddd (15.7; 8.8; 5.4)	1.14, m	1.38, m
	1.57, m		1.57, m	1.38, m
24	3.30, dd (10.0, 2.0)	5.56, d (15.9)	3.28, dd (10.0; 1.8)	3.33, dd (6.8, 5.6)
25				
26	1.16, s	1.31, s	1.16, s	1.16, s
27	1.21, s	1.35, s	1.21, s	1.21, s
28	1.05, d (6.5)	1.03, d (5.9)	1.08, s	1.08, s
29			1.09, s	1.10, s
−OOH		7.61, br d		