A New Secosterol from the Indonesian Octocoral *Pachyclavularia violacea*

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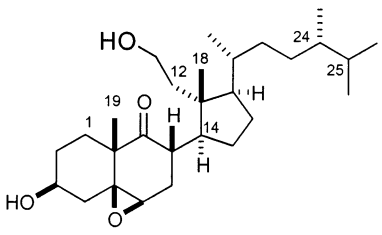
Received November 21, 2001

A new secosterol (**1**), along with pachyclavulariaenone B (**3**), was isolated from *Pachyclavularia violacea*. The structures of these compounds were established on the basis of the NMR and MS experiments.

Octocoral (phylum Cnidaria) specimens belonging to the *Pachyclavularia* genus (order Stolonifera, family Clavulariidae) are known to biosynthesize furanocembrane and briarane diterpenes.[1] The taxonomy of this genus has been the subject of some debate. K. Fabricius and P. Alderslade propose that *Pachyclavularia* is actually the same genus as *Briareum* (order Gorgonacea) on the basis of morphological reasons and similarity of chemistry.[2] *Pachyclavularia violacea* (Quoy & Gaimard, 1833) is the most common species of this genus and has been the subject of several studies.[3]

In the course of our search for novel and bioactive marine natural products from Indonesian octocorals,[4] we have isolated and identified from *P. violacea* a new secosterol (**1**) along with pachyclavulariaenone B, compound **3**, recently isolated by Sheu et al. from the same species.[3e] The isolation and structural determination of these compounds are presented in the following.



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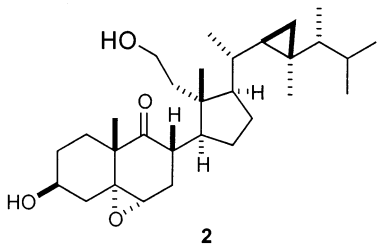

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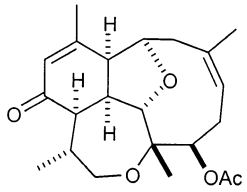

3

Specimens of soft corals were collected in the Togian Islands, near Sulawesi Island (Indonesia), and immersed in MeOH. The methanol extracts were partitioned between CH_2Cl_2/H_2O to yield a brown organic residue, which was subsequently partitioned between $MeOH/H_2O$ and solvents of increasing polarity. The CH_2Cl_2 extracts, which showed cytotoxic activity against P388, A-549, and HT-29 tumor cells (IC_{50} of 2.5 μg/mL), were submitted to repeated silica gel flash column chromatography (CH_2Cl_2 polarized with MeOH) and finally purified by reversed-phase HPLC to give compounds **1** and **3**.

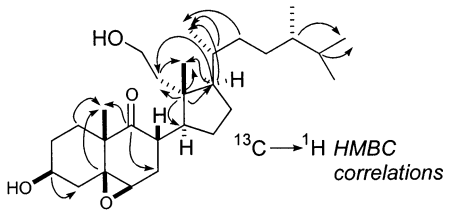

Figure 1. Selected HMBC correlations observed for **1**.

Compound **1** was isolated as an amorphous solid. The triterpenoid origin of this compound was deduced from its ^{13}C NMR and DEPT-135 spectra, which showed that the compound has 28 carbon atoms: four quaternaries, eight methines, ten methylenes, and six methyl groups. The presence of a ketone carbonyl carbon [δ_C 214.6 (s)], one oxygenated methine and one oxygenated methylene carbon [δ_C 68.0 (d) and 58.8 (t), respectively], and one epoxy group [δ_C 65.5 (s) and 58.1 (d)] required the presence of four oxygen atoms. This information, along with the pseudomolecular ions $[M + H]^+$ at *m/z* 449, $[M + Na]^+$ at *m/z* 471, and $[M + K]^+$ at *m/z* 487 observed in the (+)-APCIMS of **1**, indicated a molecular formula of $C_{28}H_{48}O_4$ and thus the existence of 5 degrees of unsaturation. The presence of a ketone group was confirmed by a sharp band at 1704 cm^{-1} in the IR spectrum, which also showed the presence of a hydroxyl group by the broad band at 3360 cm^{-1}. These data, together with the spectroscopic data from ^1H NMR and 2D NMR experiments (gHMQC, $^1H-^1H$ gCOSY, gHMBC) on **1** (see Figure 1), suggested a secosterol-type structure, and this was in part confirmed by the oxygenated methylene signal at δ_C 58.8.[5]

The 24*S** relative stereochemistry at C-24 was deduced by comparison of the NMR spectra of **1** with those of both reported epimeric 24-methylcholestanols. In the ^1H NMR spectrum of **1** the chemical shifts for methyl groups in the side chain are sensitive to the stereochemistry at C-24, and so our value of δ_H 0.77 is in agreement with the value of 0.78 reported for the 24*S* (β-H isomer) methyl group in the 24*S* methylcholestanol [vs δ_H 0.80 for the 24*R* (α-H) isomer].[6] Furthermore, the characteristic differences between 24*R* and 24*S* isomers were also recognized in the ^{13}C and ^1H NMR spectra, especially at C-25, C-26, and C-27, and our values for these carbons at δ_C 31.5, 20.4, and

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10.1021/np010592e CCC: $22.00
Published on Web 08/10/2002

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts (ppm), gHMBC, and NOESY Correlations for Secosterol **1** in $CDCl_3$

C	δ_C mult	δ_H mult (*J* in Hz)	HMBC (C→H)	NOESY
1	28.5 t	α: 1.86 dt (14.3, 14.3, 3.8)	H-19	H-3
		β: 1.76 m		
2	30.5 t	α: 2.00 br d (13.0)	H-4*β*	
		β: 1.50 m		
3	68.0 d	3.86 m	H-4*β*	H-1α, H-4α
4	38.5 t	*β*: 2.10 dd (11.5, 13.2)		
		α: 1.40 m		H-3, H-6
5	65.5 s		H-19	
6	58.1 d	3.10 br s	H-8	H-4α
7	26.3 t	α: 2.46 ddd (15.8, 10.2, 1.8)	H-8	H-19
		β: 2.25 ddd (15.8, 4.4, 2.9)		
8	38.8 d	2.68 m		H-12, H-18, H-19
9	214.6 s		H-7α, H-19	
10	46.5 s		H-1, H-19	
11	58.8 t	3.76 m		H-14
		3.65 m		H-14, H-21
12	41.1 t	1.76 m	H-11	H-21
		1.47 m	H-18	
13	45.6 s		H-12	
			H-14, H-17, H-18	
14	45.2 d	2.55 dt (9.6, 9.6, 2.5)		H-11
15	22.5 t	*β*: 1.54 m		
		α: 1.25 m		
16	25.8 t	*β*: 1.76 m		
		α: 1.33 m		
17	49.5 d	1.54 m	H-12, H-18, H-21	H-21
18	18.1 q	0.67 s		H-8, H-20
19	19.7 q	1.30 s		H-8
20	34.4 d	1.33 m	H-21	H-18
21	19.6 q	0.94 d (6.6)		H-11, H-12, H-17
22	33.0 t	1.40 m	H-21	
		0.94 m		
23	31.4 t	1.43 m		
		0.94 m		
24	39.1 d	1.20 m	H-26, H-27, H-28	
25	31.5 d	1.54 m	H-26, H-27	
26	20.4 q	0.85 d (6.8)		
27	17.6 q	0.78 d (6.8)		
28	15.4 q	0.77 d (6.6)		

17.6 are in excellent agreement with the values of δ_C 31.5, 20.4, and 17.6 found for 24*S*(*β*-H)-methylcholestanol [vs 32.4, 20.2, and 18.3 for the 24*R*(α-H) isomer].[6,7]

The relative stereochemistries in both the A and B rings were determined by using coupling constants, NOESY, and by comparison with known secosterols. The equatorial orientation of the hydroxyl group at C-3, assigned on the *β* face, was easily deduced by the presence of an axial−axial coupling constant found for the vicinal H-4*β* (dd, $J_{H3-H4\beta}$ 11.5 Hz and $J_{H4\beta-H4\alpha}$ 13.2 Hz). The relative configuration of the epoxy group at C-5/C-6 on the *β* face was deduced by comparison of the NMR spectral data of **1** to those of 3*β*-hydroxy-5α,6α-epoxy-9-oxo-9,11-secogorgostan-11-ol (**2**), recently isolated from *Pseudopterogorgia americana*.[8] Thus, the difference of the ^{13}C NMR data found for C-5, C-6, and C-7 (δ_C 65.5, 58.1, and 26.3, respectively, in **1**, in contrast to δ_C 60.9, 60.0, 32.0, respectively, reported for **2**) and the different coupling constant and chemical shift found for H-6 (δ_H 3.10 brs in compound **1** versus δ_H 3.22, d J = 4.5 Hz in **2**) indicated that the epoxy group must have the opposite configuration in these compounds. The relative stereochemistry in the D ring and at C-20 was deduced by comparison with known secosterols bearing a similar substitution partner. Indeed, the ^{13}C NMR chemical shift values from C-13 to C-17 and at C-18, C-20, and C-21 were almost identical to those reported for those positions in secosterol **2**,[8] eurypongiol B2,[9] and herbasterol,[10] which suggested that these centers have the same orientation as shown in the figures. On the other hand, NOE correlations between H-14 (δ_H 2.55) and both protons at C-11 and between H-8 (δ_H 2.68) and the methyl protons H_3-18 (δ_H 0.67) and H_3-19 (δ_H 1.30) confirmed the α orientation for H-14 and the *β* disposition for H-8 and H_3-18 (see Figure 2). With all these data the structure of compound **1** was proposed as (24*S**)-3*β*,11-dihydroxy-5*β*,6*β*-epoxy-24-methyl-9,11-secocholestan-9-one.

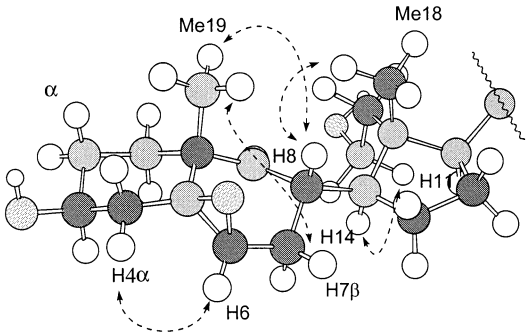

Figure 2. Selected NOESY correlations observed for **1**.

The NMR spectral data of compound **3** matched those reported by Sheu et al. for (−)-pachyclavulariaenone B, recently isolated from a Taiwanesse specimen of *P. violacea*.[3e]

Compounds **1** and **3** showed an $IC_{50} > 1$ *μ*g/mL against mouse (P-388) and human (A-549, HT-29, MEL-28) tumor cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured in CH_2Cl_2 using a JASCO DIP-1000 polarimeter with a sodium lamp operating at 598 nm. NMR spectra were recorded at 500/125 MHz ($^1H/^{13}C$), AMX-Bruker; 200/50 MHz ($^1H/^{13}C$), Bruker AC-200 NMR spectrometer using $CDCl_3$ as solvent and internal standard. Carbon multiplicities were determined using DEPT-135. Atom connectivities were determined using gHMQC, gHMBC, and gCOSY data. NOESY experiments were carried out using a mixing time of 0.8 s. ESIMS and APCIMS were obtained using a Thermoquest Navigator spectrometer. (+)-LRFABMS were measured on a VG-Quattro spectrometer, while (+)-HRFABMS were measured on an Autospec from Micromass Instruments using thioglycerol with 1% of NaI as matrix. Semipreparative HPLC was performed using μ-Bondapak C_{18} column (250 × 10 mm) from Waters with RI detection.

Biological Material. Specimens of *Pachyclavularia violacea* were collected in October 1996 in the Togian Islands near Sulawesi Island (Indonesia) (coordinates: 0°58′113 N, 126°09′417 E) at a depth range 27−33 m. Voucher samples are deposited at the Departamento de Química Fundamental, Universidade de A Coruña, under reference UDC 96039. A picture can be obtained from the authors.

Extraction and Isolation. Specimens of the octocoral (2 kg) were homogenized in MeOH (3 × 2.5 L), and the solvent was evaporated under reduced pressure. The crude extract was partitioned between CH_2Cl_2 and H_2O (1:1). The fraction soluble in CH_2Cl_2 was evaporated under pressure and partitioned between 10% aqueous MeOH (400 mL) and hexane (2 × 400 mL). Water was added to the polar fraction until the mixture became 50% aqueous MeOH and then was extracted with CH_2Cl_2 (3 × 400 mL). The fraction soluble in H_2O was extracted with *n*-BuOH saturated with water (3 × 400 mL). After evaporation, the combined organic layers yielded 4.6 g (hexane), 6.4 g (CH_2Cl_2), and 1.3 g (*n*-BuOH). The viscous oil (6.4 g) obtained from the CH_2Cl_2 fraction was repeatedly submitted to flash column chromatography (silica gel 230−240 mesh, eluting with CH_2Cl_2/MeOH mixtures of increasing polarity) to give two fractions. One of them was purified by reversed-phase HPLC eluting with MeOH/H_2O (8:2) to obtain 6.4 mg of compound **1**. The second one was separated by reversed-phase HPLC eluting with MeOH/H_2O/TFA (7:3:0.1) to give compound **3** (6.0 mg).

(24*S)-3*β*,11-Dihydroxy-5*β*,6*β*-epoxy-24-methyl-9,11-secocholestan-9-one (1):** amorphous colorless solid; $[\alpha]^{25}_D$ −23.1° (CH_2Cl_2, *c* 0.26); IR ν 3360, 1704 cm^{-1}; (+)-APCIMS *m*/*z* 487 $[M + K]^+$, 471 $[M + Na]^+$, 449 $[M + H]^+$, 431 $[M - H_2O + H]^+$, 413 $[M - 2H_2O + H]^+$; ^1H and ^{13}C NMR, see Table 1.

Pachyclavulariaenone B (3): amorphous colorless solid; IR ν 1726 cm^{-1}; (+) LRFABMS *m*/*z* 375 $[M + H]^+$, 315 $[M - AcOH + H]^+$; (+)-APCIMS *m*/*z* 315 $[M - AcOH + H]^+$; (+)-HRFABMS *m*/*z* 315.1965 $[M - AcOH + H]^+$ (calcd for $C_{20}H_{27}O_3$, Δ 0.5 mmu).

Acknowledgment. Taxonomic information was generously provided by L. von Ofwegen from the Nationaal Natuurhistorisch Museum, Leiden, The Netherlands. We appreciate the assistance of P. Crews (University of Santa Cruz, CA) in the collection of octocorals. We are grateful to BIOMAR S.A. for the pharmacological assays. This work was financially supported by grants from the Xunta de Galicia (PGIDT99-PXI10304A and PGIDT00MAR10301PN) and from CICYT (MAR99-0287). N.G. thanks the Xunta de Galicia for a grant.

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NP010592E