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New marine sterols from an algal-bearing gorgonian coral *Pinnigorgia* sp

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ABSTRACT

Four new marine sterols, (22*E*,24*R*)-ergosta-5,22-diene-3β,11α-diol (**1**), (24*S*)-ergosta-5-ene-3β,11α-diol (**2**), 5α,6α-epoxy-23-demethylgorgost-8-ene-3β,7α-diol (**3**), and 5α,6α-epoxy-23-demethylgorgost-8(14)-ene-3β,7α-diol (**4**), along with a known metabolite, 23-demethylgorgost-7-ene-3β,5α,6β-triol (**5**), were isolated from an algal-bearing gorgonian coral *Pinnigorgia* sp., collected off the waters of Taiwan. The structures of these sterols were elucidated on the basis of spectroscopic methods. Sterols **1**–**5** were tested for *in vitro* cytotoxicity in hepatic stellate cells (HSCs). Proliferation of HSCs plays a key role in the pathogenesis of liver fibrosis.

1. Introduction

Gorgonian corals have been well-recognized as marine organisms containing various steroid analogues [1] and the ergosterol- and gorgosterol-type metabolites isolated from algal-bearing gorgonian corals were suggested be originally synthesized by the symbiotic zooxanthellae and not by the host corals [2,3]. In continuation of our research into new substances from marine invertebrates collected off the waters of Taiwan, a series of novel 24-methyl sterols derivatives, including pinnigorgiols A–C [4], pinnisterols A–C [5], 11-acetoxy-24*S*-methyl-3*β*,5α,6α-trihydroxy-9,11-secocholest-7-en-9-one, and 5*β*,6*β*-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3*β*,7*β*-diol [6], along with two plant-orignated metabolites, pubinernoid and apo-9′-fucoxanthinone [7], have been isolated from an algal-bearing gorgonian coral identified as *Pinnigorgia* sp. (phylum Cinidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Gorgoniidae). Recently, chemical examination of this interesting organism resulted in the isolation of five marine sterols, including two new ergosterols, (22*E*,24*R*)-ergosta-5,22-diene-3β,11α-diol (**1**), (24*S*)-ergosta-5-ene-3β,11α-diol (**2**), two new 23-demethylgorgosterols, 5α,6α-epoxy-23-demethylgorgost-8-ene-3β,7α-diol (**3**), 5α,6α-epoxy-23-demethylgorgost-8(14)-ene-3β,7α-diol (**4**), and a known metabolite, 23-demethylgorgost-7-ene-3β,5α,6β-triol (**5**) [8] (Fig. 1). The structures of new sterols **1**–**4** were elucidated by spectroscopic methods and by comparison of their NMR features with those of related sterol

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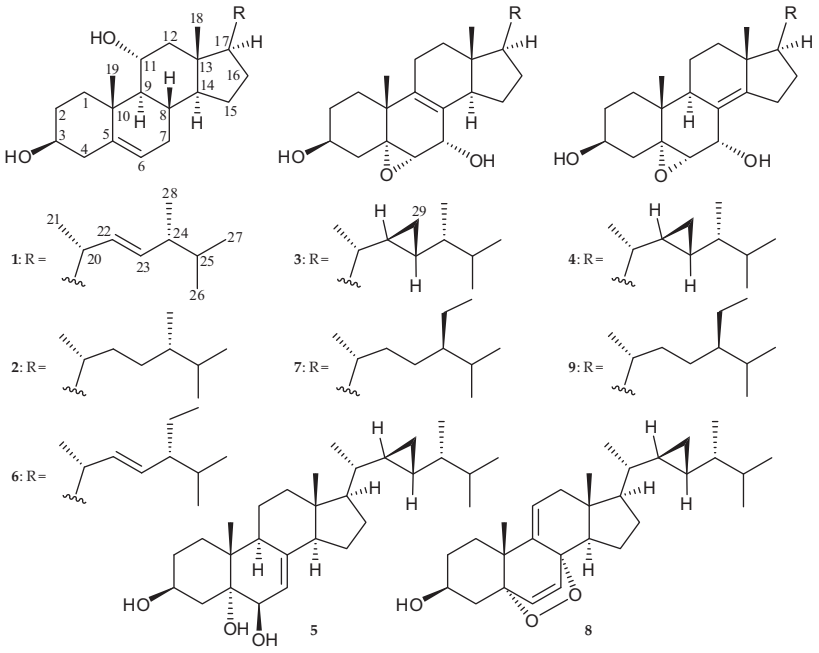

Fig. 1. The structures of (22*E*,24*R*)-ergosta-5,22-diene-3β,11α-diol (**1**), (24*S*)-ergosta-5-ene-3β,11α-diol (**2**), 5α,6α-epoxy-23-demethylgorgost-8-ene-3β,7α-diol (**3**), 5α,6α-epoxy-23-demethylgorgost-8(14)-ene-3β,7α-diol (**4**), 23-demethylgorgost-7-ene-3β,5α,6β-triol (**5**), stigmasta-5,22*E*-diene-3β,11α-diol (**6**), 5α,6α-epoxy-24*R*-ethylcholest-8-ene-3β,7α-diol (**7**), (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methyl-6,9(11)-dien-3β-ol (**8**), and 5α,6α-epoxy-24*R*-ethylcholest-8(14)-ene-3β,7α-diol (**9**).

analogues. We report herein the isolation, structure determination, and bioactivity of sterols **1–5**.

2. Experimental

2.1. General procedures

Optical rotations were measured on a Jasco P-1010 digital polarimeter. Infrared spectra were recorded on a Jasco FT/IR-4100 spectrometer; peaks are reported in cm^{-1}. The NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer, using the residual $CHCl_3$ signal (δ_H 7.26 ppm) as an internal standard for ^1H NMR and $CDCl_3$ (δ_C 77.1 ppm) for ^{13}C NMR; coupling constants (*J*) are given in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solariX FTMS system. Column chromatography was performed on silica gel (230–400 mesh, Merck). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck); Spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Normal-phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7110 pump and a Rheodyne 7725 injection port. A normal-phase column (Supelco Ascentis Si Cat #:581515-U, 25 cm × 21.2 mm, 5 μm, Sigma-Aldrich) was used for NP-HPLC. Reversed-phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-2130 pump, a Hitachi L-2455 photodiode array detector, and a Rheodyne 7725 injection port. A reverse phase column (Luna® 5 μm C18(2) 100 Å, AXIA Packed, 25 cm × 21.2 mm) was used for RP-HPLC.

2.2. Animal material

Specimen of the gorgonian corals *Pinnigorgia* sp. was collected by hand using scuba diving off the coast of Green Island, Taiwan in August 2012 and stored in a freezer until extraction. A voucher specimen (NMMBA-TW-GC-2012-130) was deposited in the National Museum of Marine Biology and Aquarium, Taiwan. This organism was identified by comparison with previous descriptions [9]. Since collected from the same colony, the specimen that was used in this study (NMMBA-TW-GC-2012-130) and the specimen that was used in a previous study cited in Ref. [6] (NMMBA-TW-GC-2010-099) are identical.

2.3. Extraction and isolation

Sliced bodies of *Pinnigorgia* sp. (wet weight 1.98 kg; dry weight 0.86 kg) were extracted with EtOAc (1 L × 6) at room temperature. The EtOAc extract (84.9 g) was partitioned between MeOH and *n*-hexane (500 mL/500 mL × 4). The MeOH layer (12.6 g) was separated on Sephadex LH-20 and eluted using a mixture of CH_2Cl_2 and MeOH (1:1) to yield seven subfractions A–G. Fraction F was separated by silica gel and eluted using *n*-hexane/acetone (stepwise, 1:1–pure acetone) to afford eight subfractions F1–F8. Fraction F2 was purified by silica gel and eluted using *n*-hexane/acetone (stepwise, 9:1–pure acetone) to yield ten subfractions F2A–F2J. Fraction F2D was purified by NP-HPLC using a mixture of *n*-hexane/EtOAc (3:1) to yield seventeen subfractions F2D1–F2D17. Fractions F2D14 and F2D15 were purified by RP-HPLC, using a mixture of MeOH/H_2O (95:5) to afford **2** (2.7 mg) and **1** (8.9 mg), respectively. Fraction F2E was purified by NP-HPLC using a mixture of *n*-hexane/EtOAc (3:1) to yield eight subfractions F2E1–F2E8. Fraction F2E8 was repurified by NP-HPLC using a mixture of *n*-hexane/EtOAc (1:1), followed by RP-HPLC using a mixture of MeOH/H_2O (85:15) to afford **4** (1.8 mg) and **3** (1.5 mg), respectively. Fraction F4 was purified by NP-HPLC using a mixture of *n*-hexane/acetone (2:1) to yield nine subfractions F4A–F4I. Then, fraction F4I was purified by NP-HPLC using a mixture of *n*-hexane/acetone (2:1) to afford nine subfractions F4I1–F4I9. Fraction F4I6 was purified by NP-HPLC, using a mixture of *n*-hexane/acetone (2:1) to yield **5** (1.8 mg).

2.3.1. (22E,24R)-Ergosta-5,22-diene-3β,11α-diol (**1**)

White amorphous powder: mp 153–155 °C; $[\alpha]_D^{27}$ −252 (*c* 0.9, $CHCl_3$); IR (neat) ν_{max} 3391 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS, *m/z* 437 $[M + Na]^+$; HRESIMS, *m/z* 437.33888 (calcd for $C_{28}H_{46}O_2Na$, 437.33900).