ISSN 0974 – 5211

Journal of Natural Products

Volume 8 (2015)

www.JournalofNaturalProducts.Com

Research Paper

Occurrence of a rare gorgosterol type steroid in marine sponge *Sigmadocia fibulata*

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(Received 10 Sept. 2014; Revised 22 Sept. 2014-06 Jan. 2015; Accepted 07 January 2015)

ABSTRACT

A rare gorgosterol type sterol, 22, 23-methylene (22*S*, 23*S*) cholesterol **(3)** and three other compounds **1, 2** and **4** were isolated from the marine sponge *Sigmadocia fibulata*, for the first time. Their structures were elucidated by spectroscopic (IR, 2D NMR and Mass spectral) data.

Keywords: Gorgosterol; Marine sponge; *Sigmadocia fibulata*.

INTRODUCTION

Over the last 50 years, a large number of new natural products, including sterols have been reported from marine sources (Blunt, et al., 2014). Many of them are vested with the potential for drug development and also structurally intriguing. Gorgosterol is a unique C_{30} sterol with cyclopropane ring system substituted with two methyl groups in the sterol side chain. It was first isolated from the gorgonian *Plexaura flexuosa* (Bergmann, et al., 1962; Hale, et al., 1970). Related sterols having this side chain with one or two methyl substitutions of the cyclopropane ring have subsequently been isolated from several other gorgonians. Later, they have also been reported from other marine organisms such as starfish (Sheikh, et al., 1971), mollusks, and microalgae and fungi that symbiotically live in the zooxanthellae of hexacoral (hard coral) and octocoral (soft coral) species. Surprisingly, marine sponges, which also host symbiont colonies and produce a large variety of natural products, are scarcely reported (Delseth, et al., 2004) to produce gorgosterol type sterols.

Continuing our interest on organisms occurring on Indian shores (Prakasa Rao, et al., 2010; Rambabu, et al., 1987; Sarma, et al., 1987), we report herein from the marine sponge *Sigmadocia fibulata*, a gorgosterol type sterol lacking methyl substitution of the cyclopropane ring system, namely 22, 23-methylene (22*S*, 23*S*) cholesterol **3** along with other known compounds, the sterol **1** and two non-sterol metabolites (**2** and **4**), for the first time. Previous work on this sponge reported steroid mixtures (Lo, et al., 2001), and peptides (Tan, et al., 2000). Also for the first time, we record herein the HR NMR and DEPT spectra for the gorgosterol type sterol **3**.

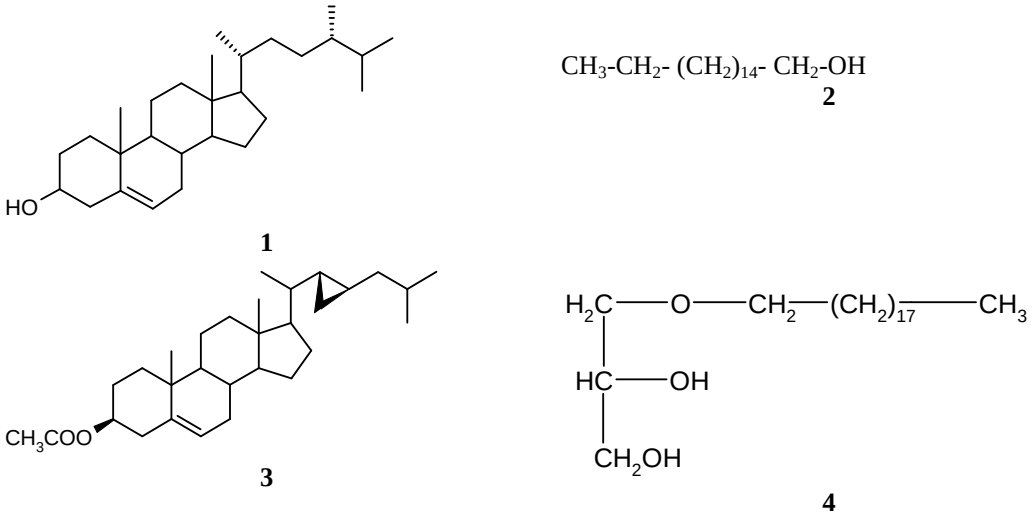
MATERIALS AND METHODS

Experimental: Melting points were measured with Kumar apparatus and are uncorrected. The IR spectra were taken on a Perkin-Elmer Paragon spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker 300 and 600MHz

spectrometers, using TMS as an internal standard. EI-MS were measured with JOEL-MS mass spectrometer. Separation and purification was performed by column chromatography on silica gel 100-200 mesh size; Merck, Mumbai, India) and TLC on silica gel plates (0.25 mm). Detection of spots was done spraying TLC plates with sulfuric acid in methanol (5%) followed by heating briefly at in an oven at 80°C.

Sponge material: *Sigmadocia fibulata* was collected during April 2003, from the Mandapam coast ($19^{o}10^{'}$ E Longitude, $9^{o}20^{'}$ N Latitude) Ramanadhapuram, Tamil Nadu State, India in the southwestern Bay of Bengal and identified by Dr. P.A. Thomas, Scientist (Retd.), Central Marine Fisheries Research Institute, Trivandrum. The collection was done manually in the intertidal rocky region, when the organism was exposed during low tide. The sponge was carefully separated and washed with fresh water; specimens were dried, sliced into small pieces, and preserved in methanol at site. The voucher specimens of the sponge are preserved with the label AU1-260 at School of Chemistry, Andhra University, Andhra Pradesh and at National Institute of Oceanography (NIO), Goa, India.

Extraction and Isolation: The organism, on arrival at the laboratory was immediately freed from aqueous methanol, shade dried and the pieced dry sponge (2.5Kg) was subjected to extraction by immersion in methanol at room temperature over 24h. followed by collection of the supernatant over a number of cycles until the residue from the supernatant solution was colorless (10 times).The combined residue (45g), dark green in color, was frozen dry and preserved at (-20°C). For this report, the residue (35g) was re-extracted with ethyl acetate and the dry residue thereof, was subjected to chromatography over column of silica gel (Acme, 100-200 mesh, 200g) using increasingly polar eluents from hexane through EtOAc and finally methanol. Fractions of 250ml each were collected and monitored by silica gel TLC, the visualization of spots being done under UV light or iodine vapor or using 5% sulphuric acid in methanol spray followed by heating at (110°C) for 5 minutes, and identical fractions were combined. The Compounds **1**, **2**, **3** and **4** (Fig. 1) were obtained from fractions 42-62 (eluent: n-hexane-EtOAc, 90:1), 63-80 (85:15), 81-108 (80:20) and 109-130 (70:30) respectively and the yields of the pure compounds were (45), (20), (30) and (80mg) respectively.



1 **2** **3** **4**

Figure -1: Structures of compounds isolated.

RESULTS AND DISCUSSION

Compounds **1**, **2** and **4**, from spectral data were identified as 24-methyl cholesterol, n-heptadecan-1-ol and 3-n-octadecyloxy-1, 2-propanediol (batyl alcohol) (Prakasa

Rao, et al., 2010) respectively. Compound **3** was obtained as colorless flakes using $CHCl_3$-MeOH mixture, m. p. 162-164^oC $[\alpha]^{25}_D$ -36.0^oC (c 0.08, $CHCl_3$). The molecular formula was $C_{28}H_{46}O$ by elemental analysis and EI mass ($M^{+\cdot}$ 398). The IR spectrum showed bands at 3450 (hydroxyl), 3390cm^{-1} and UV spectrum did not show any significant absorption beyond (220nm).

The ^1H NMR spectra (Table 1) indicated the compound **3** to be a sterol with characteristic five methyl groups, two tertiary and three secondary. Unique signals were noticed in the high field region (δ<0.4) characteristic of a cyclopropyl ring (Kobayashi, et al., 1982; Armas, et al., 2000), for four protons. The ^1H NMR also showed a broad singlet characteristic of the 6-H in Δ^5 sterols. A carbinolic methine proton was observed too, and the corresponding oxycarbon in the ^{13}C NMR. 28 carbons were noticed that analyzed by DEPT spectrum, as methyl (5), methylene (10), methine (10) and quaternary (3), suggesting compound **3** to be a C_{28} steroid. Two carbons are sp^2 (Δ^5) and the rest are sp^3. In gorgosterol type of steroids reported so far[3] only two or three cyclopropyl protons are present against four in compound **3**.

Table- 1: ^1H and ^{13}C NMR spectral data of 22, 23-methylene (22S, 23S) cholesterol (3).

S. No.	**^1H NMR** (300 MHz, $CDCl_3$)	**^{13}C NMR** (75 MHz, $CDCl_3$)
1		31.4
2		31.8
3	3.52 (1H, br s)	73.9
4		40.1
5		139.6
6	5.40 (1H, br s)	122.6
7		32.9
8		42.7
9		55.6
10		48.9
11		57.9
12		42.2
13		39.7
14		39.7
15		24.2
16		27.7
17		50.7
18	0.72 (3H, s)	12.2
19	0.98 (3H, s)	16.8
20		35.2
21	0.87 (3H, d, J=5.01 Hz)	20.4
22	-0.5 (1H, dd)	31.9
23	-0.19 (1H, m)	25.7
24		24.5
25		30.5
26	0.95 (3H, d, J=5.0 Hz)	21.8
27	0.98 (3H, d, J=5.1 Hz)	15.1
28	-0.25 (1H, dd, J=6.5 Hz)	
	-0.25 (1H, dd, J=7 Hz)	
$OCOCH_3$		171.5

Methyl substitutions of the cyclopropane ring are usually present at C-22 and / or at C-23, and these are absent in compound **3** as also confirmed by correlation spectra. The ^1H-^1H COSY correlations showed interaction between the protons H-22/H-28, H-22/H-28 and H-23/H-28 confirming the absence of methyl substitution at C-22. More importantly H-23/H-28 correlation is also present. The methylene C-24 appeared at δ 24.5 (Morris, et al., 1998; Tanaka, et al., 2002). The HMQC spectrum

showed the expected correlation between the C-3 hydroxyl protons and H-6, and between the H-3, H-1 and H-4β (δ 2.42). The parent steroid is established as 23, 24-methylene (22*S*, 23*S*) cholesterol, earlier recorded in mixed unidentified species' collection comprising two sponges and a soft coral (Blanc, et al., 1980). This is first time isolation from a homogeneous collection of the sponge *S. fibulata* and provides the HR NMR and DEPT spectra of the rare gorgosterol type steroid.

CONCLUSION

Marine sponges, besides gorgonians, are a potential source for gorgosterols. Since the primary source of gorgosterols is known to be the dinoflagellates, and since the sponges are known to lack the ability to synthesize them *de novo*, a symbiotic relationship between the sponge and dinoflagellate species likely is behind the occurrence of gorgosterols in the former.

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