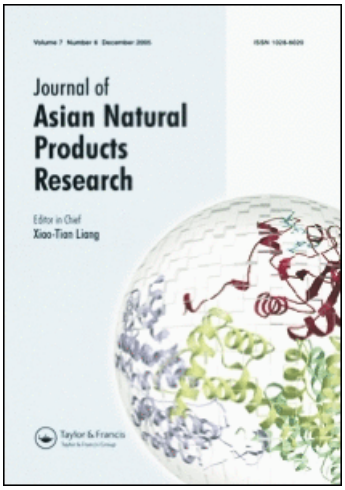

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Huang, X. -C. , Guo, Y. -W. and Song, G. -Q.(2006) 'Fortisterol, a novel steroid with an unusual seven-membered lactone ring B from the Chinese marine sponge *Biemna fortis* Topsent', Journal of Asian Natural Products Research, 8: 6, 485 — 489
To link to this Article: DOI: 10.1080/10286020410001690127
URL: http://dx.doi.org/10.1080/10286020410001690127

Journal of Asian Natural Products Research, Vol. 8, No. 6, September 2006, 485–489

Fortisterol, a novel steroid with an unusual seven-membered lactone ring B from the Chinese marine sponge *Biemna fortis* Topsent

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(Received 5 November 2003; revised 25 December 2003; in final form 30 December 2003)

Fortisterol (**1**), a novel steroid with a rare seven-membered lactone ring B, has been isolated from the marine sponge *Biemna fortis* and its structure gas been elucidated on the basis of spectroscopic data.

Keywords: *Biemna fortis* Topsent; Marine sponge; Fortisterol

1. Introduction

Marine sponges of the genus *Biemna* have a broad spectrum of metabolites, including steroids [1–5], alkaloids [6–8], ceramides [9], and glycolipids [10]. Some of them exhibit cytotoxic activities [2,6–8].

As part of our ongoing research on the biologically active substances of Chinese marine invertebrates [11,12], we collected the sponge off the Lingshui Bay, Hainan province, China. On separation of the EtOAc-soluble fraction of a methanol extract of this sponge we isolated the novel seven-membered lactone B ring steroid, fortisterol (**1**) (figure 1). This paper describes the isolation and structure elucidation of the new steroid.

2. Results and discussion

The sponge was collected off Lingshui Bay in November 2001 at a depth of 10 m in the South Chin.a Sea; it was kept frozen prior to process. Specimens (5 kg, dry wt.) were exhaustively extracted with MeOH and the methanolic extract was partitioned between EtOAc and H_2O. Repeated column chromatographic separation of the EtOAc-soluble portion on silica gel gave fortisterol (**1**).

Fortisterol (**1**) was isolated as colorless crystals, $[\alpha]_D^{20} = +1.8$ (*c* 0.2, $CHCl_3$). It showed a molecular ion peak in the EIMS at *m/z* 424 and the molecular formula $C_{28}H_{40}O_3$ was

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020410001690127

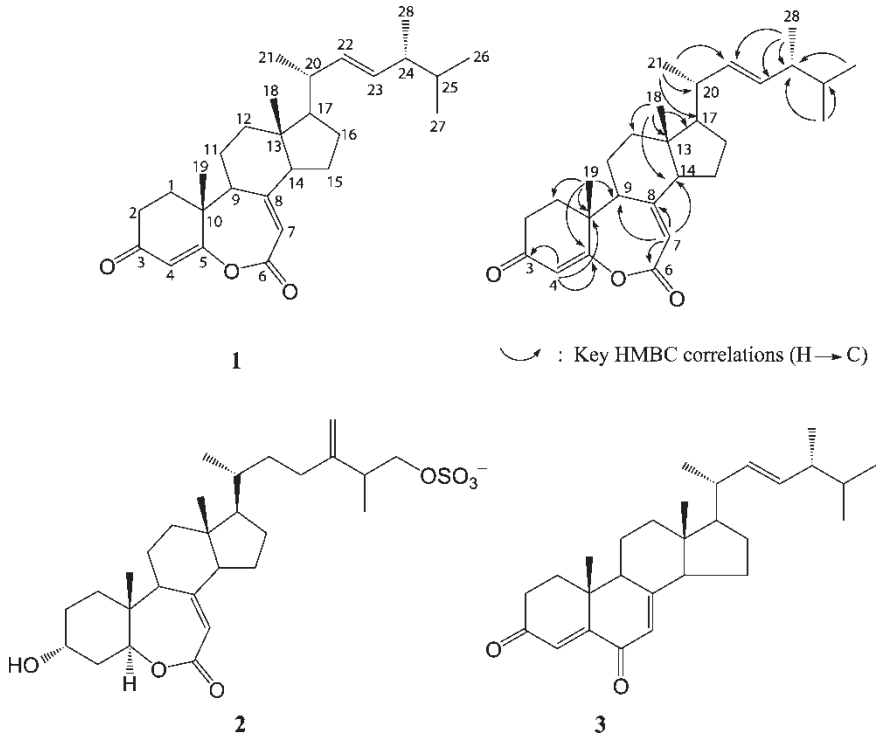

Figure 1. Structures of compounds **1**–**3** and selected HMBC correlations for **1**.

established by HREIMS (*m/z* 424.2993, $[M]^+$ $\Delta = -1.5$ mmu). The IR spectrum of **1** shows absorption bands of a carbonyl group belonging to an α,β-unsaturated ketone (1679 cm^{-1}) and a carbonyl group of an α, β-unsaturated ester (1716 cm^{-1}). The presence of these two functional groups was further confirmed by the absorption maximum at 237 nm (log ε 4.56) in the UV spectrum. The ^1H NMR spectrum of **1** clearly shows signals for two methyl singlets (δ 0.63, 18-Me; 1.23, 19-Me), four methyl doublets (δ 0.82, 6H, d, $J = 6.8$ Hz, 26, 27-Me; 0.87, 3H, d, $J = 6.8$ Hz, 28-Me; 1.01 3H, d, $J = 6.6$ Hz, 21-Me), as well as two olefinic double doublets (δ 5.25, dd, $J = 7.7, 15.2$ Hz, H-22; 5.14, dd, $J = 8.4, 15.2$ Hz, H-23), indicating that **1** has an ergostane skeleton and the same side-chain as that of ergosta-4,7,22-triene-3,6-dione (**3**) [13]. Furthermore, two additional olefinic proton signals at δ 5.71 (1H, s) and 5.74 (1H, s) for **1**, together with two tertiary carbon signals at δ 113.2 and 114.6, two quaternary carbon signals at δ 159.5, 173.9 and two carbonyl carbon signals at δ 162.4 and 198.5 in its ^{13}C NMR spectrum, indicate the presence of not only an α,β-unsaturated ester function but also an α,β-unsaturated ketone moiety. Interpretation of COSY, HMQC and HMBC cross peaks of **1** identified the structural moieties from C-1 to C-10, C-11 to C-17, and C-20 to C-27. In particular, the HMBC spectrum of **1** shows clear long-range cross peaks from the methyl signal (δ 1.23, s, 19-Me) to C-1 (33.8), C-5 (173.9), C-9 (47.2), C-10 (40.3), from the olefinic proton signal (δ 5.74, s, H-4) to C-3 (198.5), C-5, C-10, and from the other olefinic proton (δ 5.72, s, H-7) to C-6 (162.4), C-8 (δ 159.5), C-9, and C-14 (58.0), which imply an α,β-unsaturated ketone moiety in the A ring and an α,β-unsaturated ester function in the B ring. On the basis of the above findings, the structure of the novel steroid, named fortisterol, was unambiguously determined as depicted. Complete ^1H and ^{13}C NMR assignments were achieved on the basis of detailed analysis of ^1H—^1H COSY, HMQC,

Table 1. ^{1}H and ^{13}C NMR of compound **1** (in $CDCl_3$)[a] and ^{13}C NMR of **3**.

No	*1* ^{1}H	*1* ^{13}C	*3 [13]* ^{13}C
1a	2.21 (m)	33.8	35.4
1b	1.96 (m)		
2a	2.50 (m)	33.1	34.4
2b	1.47 (m)		
3	–	198.5	200.1
4	5.74 (m)	114.6	124.4
5	–	173.9	168.4
6	–	162.4	187.7
7	5.71 (m)	113.2	126.4
8	–	159.5	158.7
9	2.51 (m)	47.2	47.3
10	–	40.3	39.1
11a	1.55 (m)	22.6	21.9
11b	1.52 (m)		
12a	2.12 (m)	39.1	38.6
12b	1.45 (m)		
13	–	47.0	44.8
14	2.13 (m)	58.0	56.5
15a	1.84 (m)	25.3	22.6
15b	1.82 (m)		
16a	1.73 (m)	27.6	27.8
16b	1.35 (m)		
17	1.37 (m)	56.3	56.3
18	0.63 (s)	12.4	12.9
19	1.23 (s)	19.9	19.6
20	2.04 (m)	40.2	40.4
21	1.01 (s)	21.0	21.2
22	5.25 (dd, $J = 7.7, 15.2$ Hz)	134.7	135.3
23	5.14 (dd, $J = 8.4, 15.2$ Hz)	132.8	133.2
24	1.84 (m)	42.8	43.0
25	2.44 (m)	33.0	33.2
26	0.82 (d,$J = 6.8$ Hz)	19.9	20.0
27	0.82 (d,$J = 6.8$ Hz)	19.6	19.7
28	0.87 (d,$J = 6.8$ Hz)	17.5	17.6

[a] Bruker DRX-400 spectrometer; δs (ppm) referenced to TMS as an internal standard; assignments deduced from the analysis of mono- and heteronuclear spectra ($J = 9$ Hz).

HMBC spectra and are given in table 1 while figure 1 shows the selected key HMBC correlations of **1**.

Although various unconventional steroids with unusual side-chains and nuclei have been isolated from marine invertebrates [14], few naturally occurring steroids from marine organisms contain a seven-membered lactone B ring. To our knowledge, fortisterol (**1**) represents the second example of this kind of compound, following the discovery of astersterol A (**2**) [15]. Notably, this is the first isolation of seven-membered 5-oxalactone B ring steroid from a sponge source. Biogenetically, **1** could be derived from **2** by the oxidation of C-3 hydroxyl and subsequent dehydrogenation between C-4 and C-5. Pharmacological studies on compound **1** are currently in progress.

3. Experimental

3.1 General experimental procedures

The IR spectrum was recorded on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker DRX-400 spectrometer (400 MHz for ^{1}H

and 100 MHz for ^{13}C). Chemical shifts are reported in ppm relative to an internal TMS standard. ^{1}H and ^{13}C NMR assignments were supported by $^{1}H—^{1}H$ COSY, HMQC and HMBC experiments. EIMS and HR-ESIMS spectra were obtained on a Finnigan-MAT-95 mass instrument. Optical rotation was measured on a Perkin-Elmer 241MC Polarimeter in $CHCl_3$. Commercial Merck silica gel plates were used for TLC. Chromatograms were sprayed with a saturated solution of vanillin in 15% H_2SO_4 and heated at 80°C for 5 min to detect the spots.

3.2 Collection of the biological material

The sponge *Biemna fortis*, identified by Professor J.-H. Li of Institute of Oceanology, Chinese Academy of Sciences, was collected by scuba diving at Lingshui Bay, Hainan Province, China, in November 2001, and was kept frozen until used. A voucher sample (02SS-9) is available for inspection at the Herbarium of Institute of Materia Medica, SIBS-CAS.

3.3 Extraction and isolation

The frozen body of *Biemna fortis* (5 kg dry wt.) was ground and extracted (4 ×) with MeOH at room temperature. The MeOH extract was then concentrated *in vacuo* and the resulting residue partitioned between H_2O and EtOAc. The EtOAc extract was chromatographed on a silica gel column using eluents of increasing polarity from light petroleum to EtOAc. Fractions eluted with 20% light petroleum–EtOAc were further purified by repeated silica-gel column chromatography and then by Sephadex LH-20 with $CHCl_3$ as eluent to afford **1** (8.3 mg).

3.3.1 Fortisterol (1). Colorless crystals, $[\alpha]_D^{20} = +1.8$ (*c* 0.2, $CHCl_3$). IR ν_{max}(KBr) (cm^{-1}): 2958, 2869, 1716, 1679, 1625, 1459, 1236, 1188, 1137. UV: λ_{max} 237 nm (log ε 4.56). EIMS, *m/z* 424; HREIMS *m/z* 424.2993 (calcd. for $C_{28}H_{40}O_3$ 424.3008). ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR see table 1.

Acknowledgements

This research is financially supported by the "National Science Foundation for Outstanding Chinese Youth" (No. 30125044), National 863 project (2001AA620403) and the "National Natural Science Foundation" (No. 30170106).

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