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Methyl spongoate, a cytotoxic steroid from the Sanya soft coral *Spongodes* sp.

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Abstract—A new steroid with an uncommon 21-oic acid methyl ester moiety designated methyl spongoate (**1**) which exhibited potent cytotoxicity against BEL-7402 tumor cells in vitro has been isolated from the Sanya soft coral *Spongodes* sp. Its structure was determined by detailed interpretation of spectroscopic data and by comparison with related compounds.

Marine organisms have been proven to be a prolific source of unique steroids.[1] The origin of these sterols from marine invertebrates is complicated by the fact that they may be of dietary origin or produced by a symbiont and later modified biochemically in the invertebrate.[2] The diversity of these sterols is mainly displayed in the side chain. Literature survey revealed that the steroids with 21-oic acid functionality are rare and biologically interesting though their biosynthetic origin is a puzzle. So far kiheisterones A-E (exemplified by kiheisterone A **2**) are found to be the only steroids with 21-oic acid moiety, which were all isolated previously from the Maui sponge *Strongylacidon* sp.[3,4] In our search for bioactive substances from South China Sea marine organisms,[5–9] we encountered the title soft coral whose organic crude extract exhibited significant cytotoxicity against several tumor cell lines. Chemical investigation of this animal resulted in the isolation of a new cytotoxic 21-oic acid methyl ester steroid, methyl spongoate (**1**). In this paper we describe the isolation and structural elucidation of this new compound.

The soft coral *Spongodes* sp.,[10] was collected off Sanya, Hainan Province, China, in December 2001 and identified by Professor R.-L. Zhou of South China Sea Institute of Oceanology, CAS. Freshly collected soft coral tissue was frozen on site and stored at −20 °C until work-up. A voucher specimen (01-HN-99) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

The frozen soft coral (dry weight 456 g) was extracted with acetone exhaustively at room temperature. The acetone extract was concentrated in vacuo and the resulting residue was partitioned between H_2O and Et_2O. The Et_2O-soluble extract (3.2 g) was chromatographed on a silica gel column using eluents of increasing polarity from light petroleum ether to Et_2O. The fraction eluted with 10% Et_2O/petroleum ether, that mainly contained compound **1**, was further purified by Sephadex LH-20 gel column chromatography eluting with light petroleum ether/$CHCl_3$/MeOH (2:1:1) affording pure methyl spongoate (**1**, 2.5 mg, 0.0005%).

Methyl spongoate (**1**)[11] was isolated as a UV-absorbing [λ_{max} 228 nm, ε = 12,412] amorphous powder. Its molecular formula, $C_{28}H_{44}O_3$, was deduced from its HREIMS {*m*/*z* 428.3295, Δ 0.5 mmu}. ^{1}H, ^{13}C NMR and DEPT (Table 1) spectral analysis of **1** showed the presence of five methyls, ten sp^3 methylenes, seven sp^3 methines, two sp^2 methines, two sp^3 quaternary carbons, and two sp^2 quaternary carbons in the molecule. The carbon signals were correlated with proton signals by the analysis of the HMQC spectrum. The molecular formula $C_{28}H_{44}O_3$ led to seven degrees of unsaturation, two of which were due to the ketone carbonyl, one due to carbon–carbon double bond, and consequently the rest four were ascribable to four rings. In the ^{1}H NMR spectrum, two typical singlets at δ 0.72 (3H, Me-18) and 0.98

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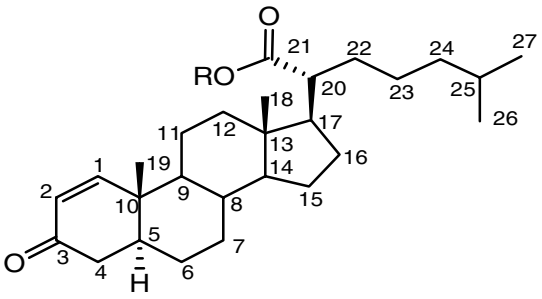
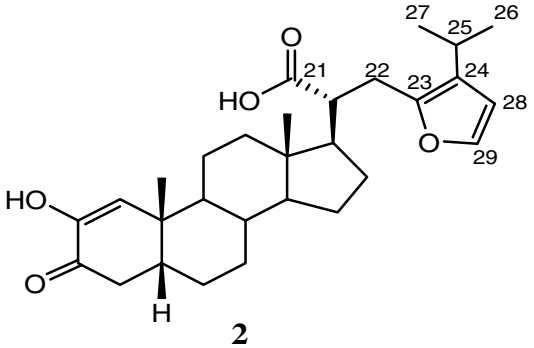


1 R = Me; **1a** R = H



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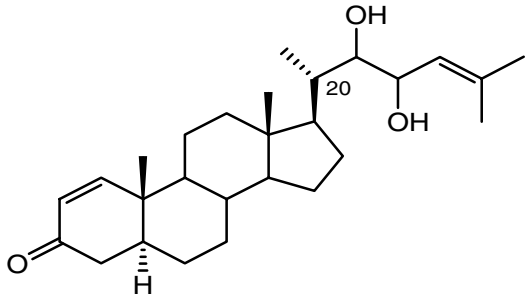


3

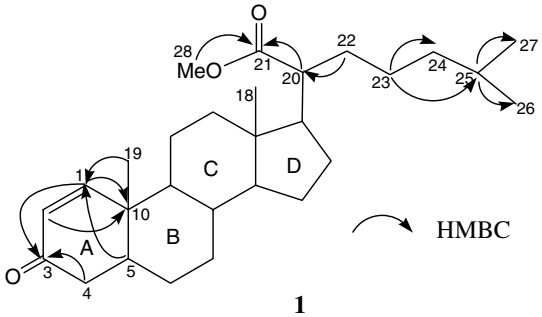
(3H, Me-19) as well as two doublets at δ 0.83 (3H, $J = 6.7$ Hz, Me-26) and 0.84 (3H, $J = 6.7$ Hz, Me-27) suggested that compound **1** is a steroid. The presence of an α,β-unsaturated carbonyl group in the molecule was straightforward from NMR signals at δ_H 5.83 (1H, d, $J = 10$ Hz)/δ_C 127.4 (d); δ_H 7.11 (1H, d, $J = 10$ Hz)/δ_C 158.5 (d), and δ_C 200.0 (s), as well as from an intense IR absorption at 1687 cm^{-1}. The ^{13}C NMR data from C-1 to C-19 were found to be in excellent agreement with those of 22, 23-dihydroxycholesta-1,24-dien-3-one (**3**),[12] suggesting that compound **1** had the same steroidal nucleus as **3**. A comparison of overall ^1H and ^{13}C NMR data (Table 1) revealed that **1** shared A, B, C, and D rings with **3** but differed at the side chain, where the presence of a methoxycarbonyl group was evident by the NMR signals at δ_H 3.65 (3H, s)/δ_C 50.9 (q) and δ_C 176.7 (s). Furthermore, the carbonyl methyl ester was located at C-21 by the fact of lack of typical NMR data of Me-21 and confirmed by $^2J_{CH}$ HMBC cross-peaks of H-20 (δ 2.25)/δ 176.7 (C-21) and H-20/C-17(δ 52.7) (Fig. 1).

Table 1. ^{13}C NMR data[a] of compounds **1**–**3** in $CDCl_3$

No.	δ (mult.)		
	1	**2**[3]	**3**[12]
1	158.4 d	129.6 d	158.5 d
2	127.4 d	144.9 s	127.4 d
3	200.1 s	196.1 s	200.0 s
4	41.0 t	37.4 t	41.0 t
5	44.3 d	41.4 d	44.3 d
6	27.1 t	26.0 t	27.6 t
7	31.2 t	25.9 t	31.2 t
8	35.7 d	35.1 d	35.7 t
9	50.0 d	47.0 d	49.9 d
10	38.9 s	38.0 s	39.0 s
11	21.1 t	22.3 t	21.3 t
12	37.3 t	37.4 t	39.8 t
13	42.3 s	42.5 s	42.5 s
14	55.7 d	55.3 d	56.3 d
15	23.6 t	23.6 t	24.0 t
16	27.6 t	27.1 t	27.9 t
17	52.7 d	52.5 d	52.6 d
18	12.3 q	12.0–	12.0 q
19	13.0 q	21.8 q	13.0 q
20	47.4 d	46.8 d	36.8 d
21	176.7 s	180.6 s	12.5 q
22	32.2 t	28.6 t	77.0 d
23	38.8 t	146.0 s	70.3 d
24	25.1 t	127.1 s	123.8 d
25	27.8 d	24.2 d	138.5 s
26	22.3 q	23.7 q	18.6 q
27	22.7 q	24.0 q	26.0 q
28	50.9 q	108.6 d	—
29	—	140.8 d	—

[a] The ^{13}C NMR data were measured at 125 MHz; assignments were deduced from analysis of 1D and 2D spectra and comparison with known compounds.

Comparison of ^{13}C NMR chemical shift values of **1** with **3** (Table 1) in combination of NOE correlations of H_3-19/H-8, H-5/H-9, H_3-18/H-8, and H-17/H-14, disclosed the expected all-*trans* stereochemistry at the ring junctures of **1**. The absolute stereochemistry of C-20 was



1

Figure 1. Key HMBC correlations of compound **1**.

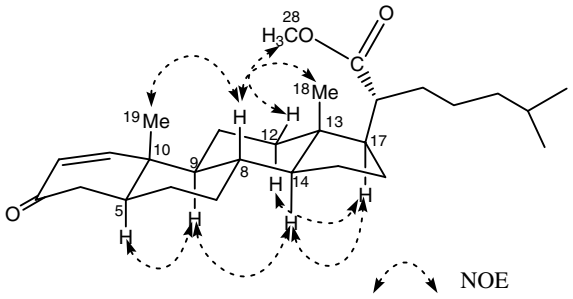


Figure 2. Key NOE correlations of compound **1**.

tentatively assigned as *R*, the same as that of model compound **2**, through comparison of ^{13}C NMR data of **1** with those of **2**, showing almost identical chemical shift values for C-17, C-20, and C-21. Therefore, the structure of compound **1** was established as 1-en-3-one-cholest-20(*R*)-oic acid methyl ester (Fig. 2).

As we mentioned above, an intriguing feature of **1** is the oxygenation pattern of the side chain. Oxidation of C-21 to an alcohol is not uncommon in marine sterols, but we are unaware of any soft coral sterols oxidized to a C-21 carboxylic acid. This is the first report of steroid with C-21 methoxycarbonyl group from soft coral though similar compounds were previously found from a marine sponge.[3,4] The origin of compound **1** is a matter needing discussion. To determine if **1** was a natural product or it was probably derived from its C-21 carboxylic acid form (**1a**) during the isolation process, we re-checked the crude extract of the soft coral by both comparison of the R_f values of metabolites in the crude extract with that of pure sample **1** on co-plate TLC and the retention time on HPLC. We did detect the compound **1** present in the Et_2O-soluble portion but failed to observe its demethyl form (**1a**) from either the Et_2O-soluble portion or the more polar fraction (*n*-BuOH extract) of the acetone extract of the specimen. These evidences excluded the possibility that **1** was an artifact obtained during the work-up. In addition, it may be worthy to point out that the crude **1**, which was eluted from the Si gel column by using petroleum ether–Et_2O (1:9), was subjected to Sephadex LH-20 column chromatography just for the purpose of removing color impurities (e.g., pigments) present in the crude **1**.

Methyl spongoate (**1**) exhibited potent cytotoxicity against BEL-7402 cells in vitro with an IC_{50} of 0.14 μg/mL and mild cytotoxicity toward several cell lines. IC_{50} values of 5 μg/mL were determined in assays against A-549 lung carcinoma and HT-29 colon adenocarcinoma human tumor cell lines, and 3.8 μg/mL against the P388 murine lymphocytic leukemia cell line. Unfortunately, limited amounts of **1** prevented further structural derivartization/modification as well as SAR studies. Further study should be conducted to perform the total or hemi-synthesis of **1** in order to carry out a more-in-depth in vivo anti-tumor study, as well as to understand the real biological role of **1** played in the life cycle of the animal.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.095.

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10. Specimens of *Spongodes* sp. were collected in December, 2001 by SCUBA techniques at a depth of −15 m off Sanya, Hainan Province, China, in the South China Sea. The sample is bushy, prickly colonies on few, short branches, polyps grouped in tight round bunches. The color of the animal is scarlet red in life and yellowish brown in alcohol.
11. Spectral data of methyl spongoate (**1**): white powder, $[\alpha]_D^{25} + 63°$ (*c* 0.34, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 2933, 1729, 1687, 1153, 775; LREIMS, *m/z*: 428 (M^+), 386, 307, 271, 225; HREIMS *m/z*: 428.3295 (calcd for $C_{28}H_{44}O_3$, Δ 0.5 mmu); UV λ_{max} (MeOH): 228.5 (ε 12,412); The center peak of ^1H value of $CDCl_3$ was taken as standard at δ 7.26. ^1H NMR chemical shifts of **1** ($CDCl_3$, 500 MHz): 7.11 (d, *J* = 10.3 Hz, H-1); 5.84 (d, *J* = 8.3 Hz, H-2); 2.20 (dd, *J* = 17.8, 4.0 Hz, H-4α); 2.36 (dd, *J* = 17.8, 14.3 Hz, H-4β); 1.91 (m, H-5); 1.85 (m, H-6α); 1.28 (m, H-6β); 1.47 (m, H_2-7); 1.45 (m, H-8); 0.96 (m, H-9); 1.70 (m, H-11α); 1.38 (m, H-11β); 1.08 (m, H-12α); 1.50 (m, H-12β); 1.08 (m, H-14); 1.61 (m, H-15α); 1.08 (m, H-15β); 1.40 (m, H_2-16); 1.62 (m, H-17); 0.72 (s, H_3-18); 0.98 (s, H_3-19); 2.25 (ddd, *J* = 4.8, 9.3, 11.2 Hz, H-20); 0.98 (m, H_a-22); 1.70 (m, H_b-22); 1.13 (m, H_2-23); 1.12 (m, H_2-24); 1.46 (m, H-25); 0.84 (d, *J* = 6.7 Hz, H_3-26); 0.83 (d, *J* = 6.7 Hz, H_3-27); 3.65 (s, H_3-28); ^{13}C NMR chemical shifts of **1** ($CDCl_3$, 125 MHz): see Table 1.
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