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The Identification of Two New Sterols from Marine Organism

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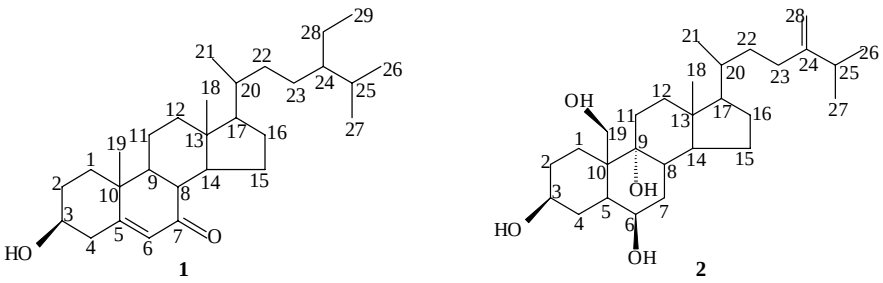
Abstract: Two new sterol have been isolated from the South China Sea marine organism. Compound **1** was isolated from the sponge *Polymastia sobustia* and compound **2** was obtained from the soft coral *Sinularia inexplicata.* Their structures were established as 3β-hydroxy-stigmast-5en-7-one and 24 - methylene cholestan -3β, 6β, 9α, 19 -tetrol by variety of spectral analysis such as IR, EIMS, 1DNMR, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY.

Keywords: Sterol, *Polymastia sobustia*, 3β-hydroxy- stigmast-5en-7-one, soft coral, *Sinularia inexplicita*, 24 - methylene cholestan -3β, 6β, 9α, 19 -tetrol.

Marine organisms produced many interesting pharmacologically and biologically activie metabolites such as antibacterium, anticancer and antimicrobial active compounds[1-2]. In recent years, a variety of sterols have been reported from marine invertebrates and sponges[3-4]. More and more chemists and biologists pay attention to the constituents of sponges and corals. Thirteen compounds have been isolated from the sponge *Polymastia sobustia* and eighty compounds have been obtained from the soft coral *Sinularia inexplicita*. In this paper we would like to report the structural elucidation of two sterols compound **1** and compound **2**. **(Figure 1)**

The ethanol extract of sponge *Polymastia sobustia* was chromatographed with silica gel and eluted using petroleum ether with increasing amounts of ethyl acetate as eluent. The fraction obtained with petroleum ether/ethyl acetate 80/20 (v/v) contained compound **1** which was an amorphous powder, m.p. 126~128^{0}C (crystallized from ethyl acetate). The molecular formula of **1** was established as $C_{29}H_{48}O_2$ by EIMS: 428 (M^+), ^{13}CNMR and element analysis (Found: C, 81.62%; H, 11.52%. $C_{29}H_{48}O_2$ Calculated C, 81.41%; H, 11.31%) and its unsaturated degree was 6.

Figure 1 The structure of compound **1** and compound **2**



1 **2**

Compound **1** gave a positive Lieberman - Burchard reaction, indicating a steroid skeleton. Peaks at *m/z* 287 $[M - C_{10}H_{21}]^+$ and 285 $[M - C_{10}H_{21} - 2H]^+$ indicated the presence of a $C_{10}H_{21}$ side chain, which contains one isopropyl group as shown in IR (1370 and 1390 cm^{-1}) and an ethyl group from δ_H 1.12 (3H) and 1.34 (2H). The side chain has been determined by fragment ions *m/z* 385 $[M- C_3H_7]^+$ and 343 $[M- C_6H_{13}]^+$. In DEPT experiment 29 resonance lines were assigned to four quaternary carbons, nine methines, ten methylenes and six methyls. The IR absorption at 3332 cm^{-1}, combined with ^{1}HNMR δ_H at 2.39 showed the presence of hydroxyl group. The ^{13}C NMR signal of **1** at δ_C 202.3, together with UV absorption maximum at 237nm (ε,22 000) and IR 1672 cm^{-1} supported the presence of a conjugated carbonyl group.

HMBC showed that C_3 was related to H_2 and H_4, C_4 related to H_6 and H_3, C_5 related to H_4 and H_6, C_7 related to H_6 and H_8, C_{10} related to H_6, H_9, H_1 and H_{19}, thus the structure of ring A and ring B was established. Based on the extensive analysis of ^{13}C NMR, ^{1}H NMR, ^{13}C - ^{1}H COSY and ^{1}H - ^{1}H COSY spectra and comparison with the known compound[5], compound **1** was elucidated as 3β-hydroxy-stigmast-5en-7-one, The spectral data were summed in **Table 1**.

The yellowish solid extracted from the soft coral *Sinularia inexplicita* was eluted stepwise with petroleum ether contained increasing amount of acetone. The fraction with 20% acetone / ether (v / v) yielded compound **2** as colorless needles, m.p. 237~238^{0}C, crystallized from acetone, $[\alpha]_D^{20}$ +25 (c, 0.053, CH_3OH). The Lieberman -Burchard reaction supported it is a steroid. The molecular formula of **2** was shown to be $C_{28}H_{48}O_4$ by EIMS: 448 (M^+) and element analysis (Found: C, 75.02%; H, 10.70%. $C_{28}H_{48}O_4$ Calculated C, 75.00%; H, 10.71%) and its unsaturated degree was 5.

Four quaternary carbons, eight methines, twelve methylenes and four methyls were shown by DEPT experiment. Peaks at *m/z* 323 $[M - C_9H_{17}]^+$ and 321 $[M - C_9H_{17} - 2H]^+$

Table 1 ^{13}C NMR and ^{1}H NMR spectra of data of compound **1** (TMS, $CDCl_3$, 600MHz, δ, ppm)

C	δ	C	δ	H	δ_1 J (Hz)	^{1}H-^{1}H COSY
1	38.73	16	36.35	H-2	1.94 (m)	H-2
2	31.17	17	54.74	H-3	3.68 (m)	H-1, H-3
3	70.17	18	11.98	H-4	2.41 (d d, J=4.1, 12.1)	H-2, H-4
4	41.84	19	17.35		2.38(d d, J=2.5, 12.1)	H-2, H-4
5	165.04	20	36.21	H-6	5.69 (s)	H-3
6	126.15	21	18.93	H-8	2.25 (d d, J=10.98, 12.3)	H-9, H-14
7	202.27	22	36.37	H-18	0.69 (s)	
8	45.21	23	28.17	H-19	1.21 (s)	
9	49.79	24	39.53	H-21	0.92 (d, J=6.5)	H-20
10	38.3	25	28.01	H-26	0.87 (d, J= 2.7)	H-25
11	21.25	26	22.56	H-27	0.87 (d, J= 2.7)	H-25
12	71.00	27	22.83	H-28	1.34 (m)	H-24, H-29
13	43.14	28	23.04	H-29	1.12 (t, J= 4.14)	H-28
14	54.84	29	18.65			
15	23.86					

indicated the presence of a C_9H_{17} side chain contain one double bond. IR 1637and 878 cm^{-1}, corresponding to ^1H NMR, ^{13}C NMR data δ_H 4.63 (1H, d, J = 1Hz), 4.69 (1H, d, J = 1Hz), δ_C 156.0 (s), 106.1 (t), revealed the existence of a terminal double bond to be placed at C-24 in the side chain[6]. In the EIMS of compound **2**, the significant fragment ions at 430 $[M - H_2O]^+$, 412 $[M - 2H_2O]^+$, 394$[M - 3H_2O]^+$ and 376$[M - 4H_2O]^+$, corresponding to successive losses of 18 units, suggested the presence of four hydroxyl groups. In the ^{1}HNMR spectrum of **2**, δ_H 3.85 (1H,m), 3.31 (1H, m),3.52 (1H, d, J = 12 Hz) and 3.99 (1H, d, J = 12Hz),combined ^{13}CNMR data with δ_C 66.1(d), 73.9 (d), 74.7 (s) and 62.2 (t), revealed the presence of one primary, two secondary and one tertiary hydroxyl groups. No absorption was compatible with the presence of C_{19} methyl. Several ion peaks at *m/z* 418 $[M- CH_2O]^+$, 400 $[M- CH_2O - H_2O]^+$, 382 $[M- CH_2O - 2H_2O]^+$, 364 $[M- CH_2O -3H_2O]^+$seemed to arise from the lose of a CH_2O unit, this suggested that compound **2** was 19- hydroxyl sterol [7]. ^{1}HNMR signal at 4.21 (1H,m), 3.74 (1H,m), 4.54 (1H,m), 5.07 (1H br) revealed the presence of four hydroxyl. Two groups of key peaks *m/z* 321, 303, 285, 267 and 281, 263 and 245 indicated that three hydroxyl groups must be located in ring A, B and C of the steroid nucleus. The structure of steroid nucleus was supported by HMBC which showed that C_3 was related to H_2 and H_4, C_6 related to H_5 and H_7, C_9 related to H_8 and H_{11}, C_{10} related to H_1, H_5 and H_{19}. In NOESY spectra, we observed the NOESY of H_8 to H_{19}, and H_{18}, $H_{5\alpha}$ to H_6 and H_3. The structure of compound **2** was established as 24 - methylene cholestan -3β, 6β, 9α, 19 -tetrol.

Spectral data of compound **2:** IR (ν_{max}KBr, cm^{-1}) 3640~3140, 1035, 1637 ; ^{1}HNMR (DMSO-d_6, 600MHz, δ ppm) 0.66 (3H,s,18-CH_3), 0.99 (3H, d, J=3.6Hz, 26-CH_3), 0.97 (3H, d, J= 3.6Hz, 27-CH_3), 1.01 (3H, d, J=6.4Hz, 21-CH_3), 3.85 (1H, m,3α-H), 3.31 (1H, m, 6α-H), 3.52 (1H, d, J=12Hz, 19-Ha), 3.99 (1H, d, J=12Hz, 19-Hb), 4.63 (1H, d, J=1Hz, 28-Ha),4.69 (1H, d, J=1Hz, 28-Hb) ; ^{13}CNMR (DMSO-d_6, 600MHz, δ ppm) 27.1 (C-1, t), 31.3 (C-2, t), 66.1 (C-3, d), 41.1 (C-4, t), 45.2 (C-5, d), 73.9 (C-6, d), 34.1 (C-7, d), 31.1 (C-8, d), 74.7 (C-9, s), 42.8 (C-10, s), 21.9 (C-11, t), 40.6 (C-12, t), 42.7 (C-13, s), 56.7 (C-14, d), 24.0 (C-15, t), 28.1(C-16, t),55.8 (C-17, d), 12.4 (C-18, q), 62.2 (C-19, t), 35.4 (C-20, d), 18.7 (C-21, q), 34.4 (C-22, t), 30.7 (C-23, t), 156.0 (C-24, s), 33.2 (C-25, d), 21.9 (C-26, q), 22.0 (C-27, q), 106.1 (C-28, t).

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