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Steroid compounds from the marine sponge *Raspilia hirsute*

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Abstract : The methanolic extract of the marine sponge *Raspilia hirsute* collected from Keomun Island resulted in three types of sterols : a mixture of (24*S*)-Poriferasta-5, 25-diene-3β, 24-diol and (24*R*)-Stigmasta-5, 25-diene-3β, 24 -diol (1), 25,26,27-Trinorcholest-5-en-3β,24-diol (2), and Pregn-5-en-20-on-3β-ol (3). The isolation and structural determination of these sterols are reported here. Compound 1 showed moderate cytotoxicity against human Leukemia cell line K562.
Keyword: 1D and 2D NMR, marine sponge, *Raspilia hirsute,* Steroid

INTRODUCTION

All marine organisms have provided as a new source of unusual steroid metabolites. Among them, specifically marine sponges are thought to produce the most diverse and biogenetically unprecedented steroids in the entire animal kingdom.[1] The steroids isolated from sponges are sometimes highly functionalized.[2] Commonly features in these compounds include additional oxygenation of both the nucleus and the side chain, extensively alkylation and degradation of side chain part. Sulfate esters of polyoxygenated sterols in sponge has also frequenctly been found.[3-4]

In our searching of biologically active marine metabolites, some extracts from Korean marine sponges were screened for cytotoxicity against human Leukemia cell line K562. The methanolic extract of the marine sponge *Raspilia hirsute* showed some selectivity in the initial screen. We isolated three different types of steroid (compounds **1**, **2** and **3**) from the organism. The new compound **1** was identified as a mixture of (24*S*)-Poriferasta-5, 25-diene

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-3β, 24-diol and (24*R*)-Stigmasta-5, 25-diene-3β, 24 -diol (1) and compound **2** and **3**, though the known components, as 25, 26, 27-Trinorcholest-5-en-3β,24-diol and Pregn-5-en-20-on-3β-ol, respectively. In this report, we describe the isolation and structural elucidation of compound **1**.

EXPERIMENTAL

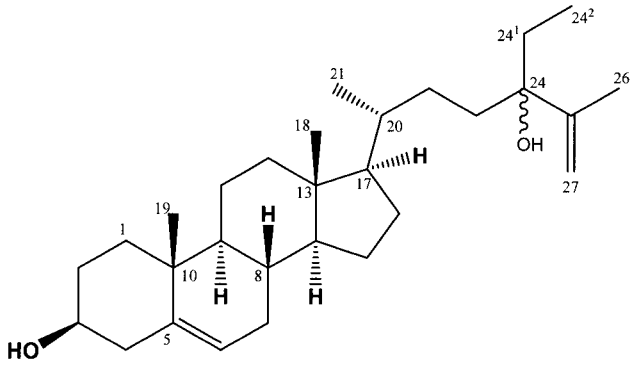
NMR experiment

The 1D and 2D NMR spectra were obtained on a Varian UNITY500 spectrometer working at 500MHz for proton and 125MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts refer to $CDCl_3$ at 7.26 and 77.0 ppm, respectively. For all experiments, the temperature was stabilized at 297K. The parameters used for 2D NMR spectra were as follows; The gradient COSY spectra were collected with a spectral width 2567 Hz in a 512(t1) × 1024 (t2) matrix applying the pulse gradient of 1ms duration with a strength 10G/m and processed with a sinebell function. The gradient HSQC spectra were measured in a 128(t1) × 1024(t2) matrix with J_{CH}=140 Hz and processed in a 256(t1) × 1024(t2) matrix by a linear prediction method for a higher resolution. The gradient HMBC experiment was optimized for the long-range coupling constant of 7Hz. The HSQC and HMBC experiments were utilized by the pulse gradients of 1ms duration and 10G/m strength to reduce the artifacts in the spectra.

Collection, Extraction and Isolation

The sponge *Raspilia hirsuta* (sample no. 980K-2) was collected by hand using SCUBA at a depth of 20-30m at Keomun island, Korea in 1998. The freeze-dried specimen (2.5Kg) was repeatedly extracted with MeOH at room temperature. The aqueous crude extract (83.5g) was first passed through a column of Amberlite XAD-2 resin, then washed with distilled H_2O to remove salt. The adsorbed residue was eluted with MeOH, followed by chromatography on Sephadex LH-20 with CH_2Cl_2 – MeOH (1:1) as eluent. A fraction which showed the presence of sterols was subjected on a column of silica gel and a polar fraction was further separated by reversed phase HPLC(YMC ODS-A column, 250mm × 10mm, Sh-

odex RI detector) using a solvent system (H_2O : MeOH = 5 : 95). The fraction at the retention time of 23 min was finally purified on reversed phased HPLC with H_2O-MeOH (8:92) to yield compound **1** (6mg) and other fraction at 10 min gave compound **2** (1.3mg) and **3** (1.5mg) by reversed phased HPLC with H_2O-MeOH (20: 80) as eluent.



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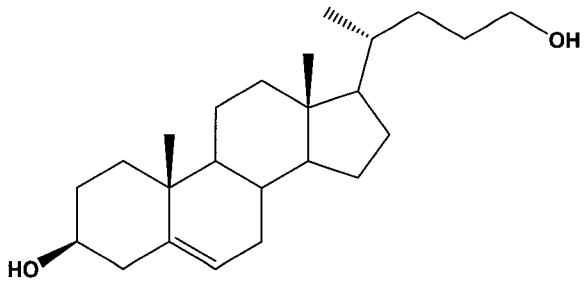

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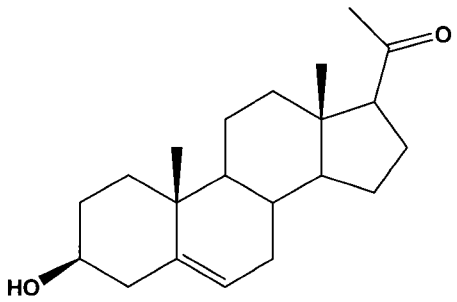

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RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder with the molecular formula of $C_{29}H_{48}O_2$ on the basis of ESI-MS and carbon spectrum. Its IR spectrum showed absorption bands at 3270 and 1650cm^{-1}, indicating the presence of hydroxyl and double bond groups, respectively. The ^{1}H NMR spectrum displayed highly overlapped signals at the range of δ 0.74-2.40, a multiplet proton at δ 3.53 and three methyl singlet protons (δ 0.68, 1.00, 1.75), which were reminiscent of a typical pattern of sterol compound. The structure of **1** was established by the extensive 2D NMR experiments. First of all, two methyl groups at δ 0.68 and 1.00 assisted to elucidate the structure by providing a number of strong HMBC correlations in the ring system of sterol compound (Fig. 1). Rings A and B were assigned using the HMBC correlations from Me-19 to four neighbor carbons C- 1, -5, -9 and -10. In a same way, rings C and D were readily assembled by the HMBC correlations from Me-18 to C-12, -13, -14 and -17. Along with the HMBC correlations, the cross peaks in the COSY and TOCSY spectra led to the structural assignment of the ring A-D in the molecule. The trisubstituted methine proton at δ 5.36 was correlated with C-5 in the HMBC spectrum and also coupled to two methylene proton at δ 1.54 and 1.98 in the COSY spectrum.

Next, all remaining carbons were attributed to the side-chain moiety, and most of them showed doublet signals with slightly differences in the chemical shifts ranging from δ 0.02-0.04 (Table 1), being characteristic of epimeric steroids.[5] Groups of signals at δ 145.57/145.59, 113.4 and δ 89.01/89.04 were indicative of the presence of a double bond and an oxygen-bearing carbon. From a combination of HSQCDEPT and ^{13}C spectra, the olefinic methylene protons were observed as broad singlets at δ 4.91 and 5.04. These protons were correlated with two quaternary carbons at δ 145.57/145.59 and 89.01/89.04 as well as one methyl carbon at δ 19.33/19.35. The HMBC correlations of the methyl singlet protons with three carbon groups at δ 145.57/145.59, 113.4 and 89.01/89.04 indicated that both the methyl unit and the hydroxyl-bearing carbon were connected on one olefinic carbon. And additional HMBC correlations of the methylene protons at δ 1.66 and 1.67 with the hydroxyl-bearing carbon revealed that an ethyl unit was also connected with the quaternary carbon at δ 89.01/89.04, resulting in an epimeric mixture.

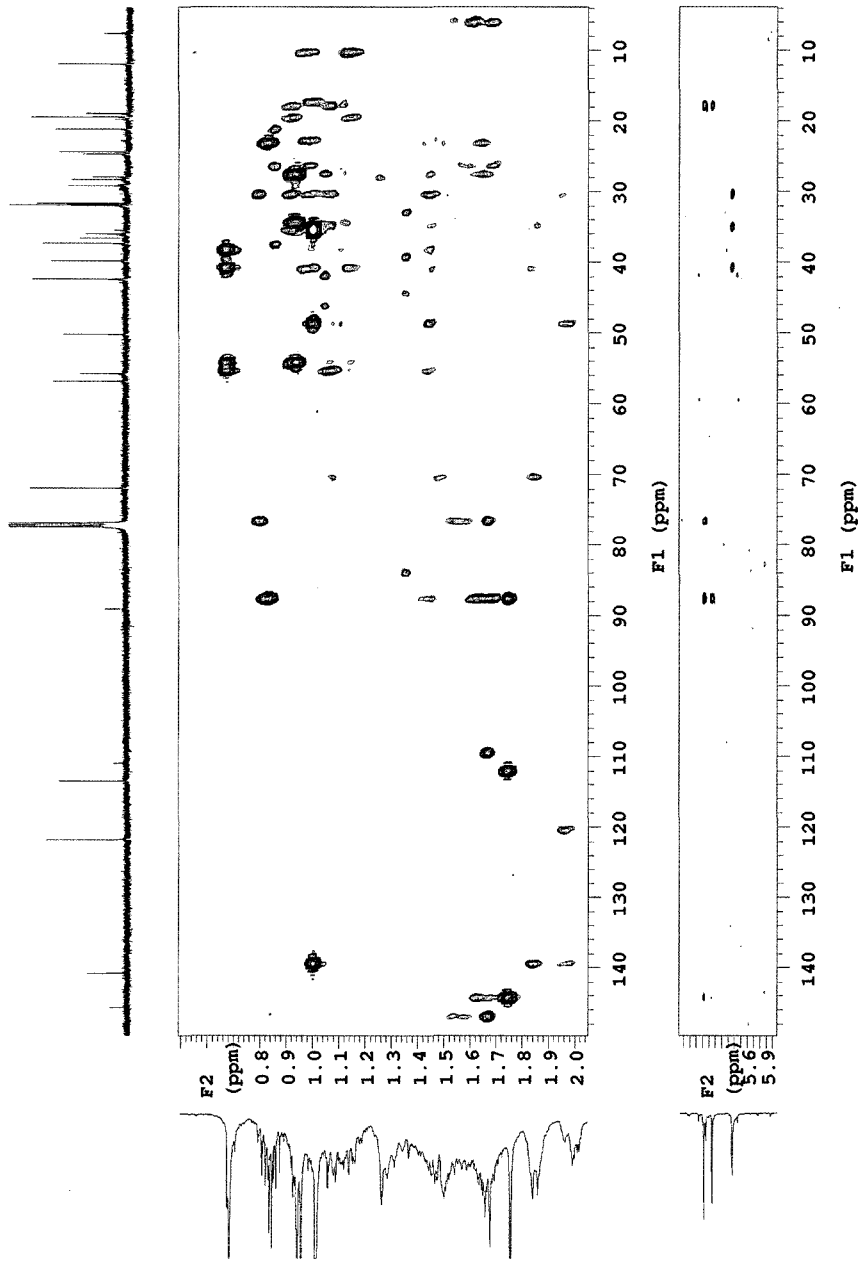

Fig. 1. HMBC spectrum for compound **1**.

Table 1. NMR spectral data for compound **1** in $CDCl_3$

carbon	^1H (mult, J Hz)	^{13}C
1	1.08 (1H, m) ; 1.85 (1H, m)	37.2, CH_2
2	1.51 (1H, m) ; 1.84 (1H, m)	31.6, CH_2
3	3.53 (1H, tt, 11.1, 4.9)	71.8, CH
4	2.23 (1H, dd, 13.2, 11.1)	42.28, CH_2
	2.28 (1H, ddd, 13.2, 4.9, 1.9)	
5		140.7, C
6	5.36 (1H, m)	121.7, CH
7	1.54 (1H, m) ; 1.98 (1H, m)	31.9, CH_2
8	1.45 (1H, m)	31.9, CH
9	0.92 (1H, m)	50.1, CH
10		36.5, C
11	1.49 (1H, m) ; 1.46 (1H, m)	21.1, CH_2
12	1.15 (1H, m); 2.00 (1H, dt, 12.9, 3.4)	39.7, CH_2
13		42.32, C
14	0.98 (1H, m)	56.7, CH
15	1.08 (1H, m) ; 1.58 (1H, m)	24.3, CH_2
16	1.27 (1H, m) ; 1.85 (1H, m)	28.2, CH_2
17	1.12 (1H, m)	55.7, CH
18	0.68 (3H, s)	11.8, CH_3
19	1.00 (3H, s)	19.4, CH_3
20	1.40 (1H, m)	35.9, CH
21	0.94 (1H, d, 6.4)	18.89 / 18.85, CH_3
22	1.31 (2H, m)	29.1, CH_2
23	1.64 (1H, m) ; 1.44 (1H, m)	27.87 / 27.85, CH_2
24		89.01 / 89.04, C
25		145.57 / 145. 59, C
26	1.75 (3H, s)	19.33 / 19.35, CH_3
27	4.91 (1H, br s) ; 5.04 (1H, br s)	113.4, CH_2
24^1	1.66 (2H, q, 7.8) / 1.67 (2H, q, 7.3)	24.6(broad), CH_2
24^2	0.84 (3H, t, 7.8) / 0.83 (3H, t, 7.3)	7.54 / 7.57, CH_3

The relative stereochemistry in the A-D ring was assigned from the ROESY spectrum and the interpretation of coupling constant data. The A/B *trans* ring junction was identified by the presence of NOE correlations between Me-19 and H-1β, -4β and -8. The C/D *trans* ring junction was also revealed by the NOEs between Me-18 and H-8, -11β. On the other hand, the large coupling value (J = 11.1 Hz) for H-3 showed that the hydroxyl group was placed on the β-position in the ring system. Therefore, compound **1** was concluded to be mixture of (24*S*)-poriferasta-5, 25-diene-3β, 24-diol and (24*R*)-stigmasta-5, 25-diene-3β, 24 -diol which proved to be new compound. Although this compound was not responsible for the initial strong cytotoxicity, **1** showed moderate cytotoxicity against human leukemia cell line K562.

Compound **2** and **3** were also analyzed by extensive 2D NMR experiments. Their structures were known as 25, 26, 27-Trinorcholest-5-en-3β,24-diol and Pregn-5-en-20-on-3β-ol, respectively. The values of 13C chemical shifts were confirmed by comparison of those of reported in the literatures.[6-7]

Acknowledgement

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