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A New 5α, 8α-Epidioxy Sterol from the Marine Sponge *Plakortis simplex*

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Abstract : Four 5,8-epidioxy sterols were isolated from the marine sponge *Plakortis simplex*. Their structures were completely determined by an extensive NMR analysis and comparison with NMR data of similar compounds for absolute stereochemistry of the side chain. The compounds were assigned as 5,8-epidioxy-(24*S*)-ethylcholesta-6,22(*E*),25-trien-3-ol(**1**), 5,8-epidioxy-(24*S*)-methylcholesta-6,22(*E*)-dien-3-ol(**2**), 5,8-epidioxycholesta-6,22(*E*)-dien-3-ol(**3**) and 5,8-epidioxycholesta-6-en-3-ol (**4**).

Keywords : 1D and 2D NMR, Plakortis simplex, 5,8-epidioxy sterol, C-24 configuration

INTRODUCTION

Sponges have been recognized as one of the most plentiful source of diverse sterols, and a large number of novel and bioactive sterols are still being reported.[1-2] Until recently, a number of sponge sterols are mixtures, but with the advances in chromatographic techniques, it is possible to separate most of these complex mixtures and their investigation is now clearly undergoing.[3-5] During the course of our study of chemically new and biologically active secondary metabolites from marine sponges, four 5α, 8α-epidioxy sterols including a new derivative were isolated from a sponge of *Plakortis simplex* collected in Keomun island, the southeast of Korea.[6] Among sterol structures reported from sponges, 5α, 8α-epidioxy sterols were relatively limited. And often the assignment for the NMR signals of C-1~ C19 in sterol compounds was not informative due to severely overlapped signals in the up-field

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area, e.g. the resonances for C-12 and C-16 were interchanged.[7-8] In this paper we report the isolation of four sterols and structure elucidation of a new compound in details, In addition, the correct assignments of the NMR signals for rings A-D of 5α, 8α-epidioxy sterols will be provided. The structures of four isolated sterols were mainly determined based on the 1D and 2D NMR analysis and their NMR assignments completely accomplished. In particular, compound **1** is the first example of a 5α, 8α-epidioxy sterol with a 24-ethyl-22,25-diene unit in the side chain.

EXPERIMENTAL

General Experimental

Optical rotations were measured on a JASCO P-1010 polarimeter with a 5 cm cell. IR spectra were measured on a JASCO FT/IR 4100 spectrometer. All NMR spectra were recorded on a Varian VNMRS 500 spectrometer in $CDCl_3$ solution. Chemical shifts of the proton and carbon spectra were reported in reference to residual solvent peaks at 7.26 ppm 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 10 G/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 7 Hz. The HSQC and HMBC experiments were utilized by the pulse gradients of 1 ms duration and 10 G/m strength to reduce the artifacts in the spectra. HPLC was carried out on a Varian equipment (Prostar 210 pump and Prostar 355 Refractive Index detector)

Collection, Extraction and Isolation

The marine sponge *Plakortis simplex* (sample No 08K-6) was collected by hand using SCUBA at a depth of 20-30 m at Keomun island, Korea in 2008. The freeze-dried specimen (2.5Kg) was extracted with MeOH twice at room temperature. The crude extract was first

partitioned between H_2O and methylene chloride (M.C.) and then the organic layer re-partitioned between hexane and 15% aqueous MeOH solvent. The latter phase was in turn subjected to reversed-phase vacuum flash chromatography eluting with stepwise gradients of MeOH in H_2O (50%, 60%, 70%, 80%, 90%, and 100%). Among them, the 100% methanol fraction showed a moderate cytotoxic effect on brine shrimp and the ^1H NMR signals characteristic of a steroidal skeleton. First, the 100% MeOH fraction was separated by reversed-phase HPLC (YMC ODS-A column, 250mm × 10mm, Refractive Index detector) using a solvent system (H_2O / MeOH = 5 / 95) to yield four dominant chromatographic peaks, two corresponding to pure compounds **1** and **3**, and two to impure compounds **2** and **4,** whose purification is needed. Next, compound **2** and **4** were purified by silica-phase HPLC with an eluant (Hexane / ethyl acetate = 60 / 40).

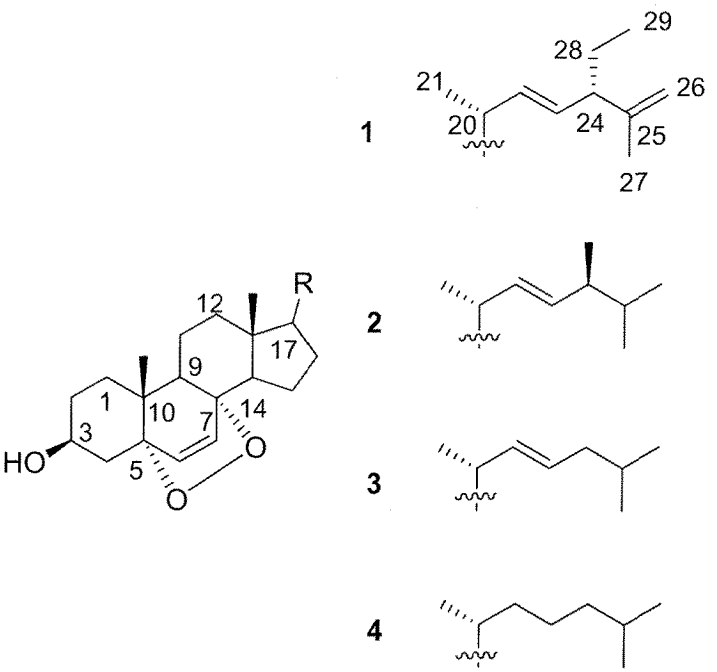

Figure 1. Four sterol compounds isolated from the sponge *P. simplex*.

RESULTS AND DISCUSSION

The methylene chloride soluble portion of *P. simplex* was subjected to a reversed-phase flash chromatography to yield seven fractions. Among them, the methanol fraction showing characteristic signals of steroids from the ^{1}H NMR spectrum was further separated to afford four sterols (**1 - 4**) as shown in the Fig. 1.

Compound **1** ($[\alpha]_D$ -20.4, *c* = 0.17) was isolated as a white, amorphous solid. The molecular formula was determined as $C_{29}H_{44}O_3$ on the basis of the pseudo molecular ion $[M + Na]^+$ at *m/z* 463.3188 in the HRESIMS, consistent with eight unsaturation degrees. The IR spectrum showed absorption at 3398 cm^{-1} arising from a hydroxyl group. The ^{1}H NMR spectrum of **1** clearly showed two up-field methyl singlets at δ 0.81 (H-18) and 0.88 (H-19), one methyl doublet at δ 1.00 (H-21), and one methine proton on an oxygenated carbon at δ 3.97 (H-3), which represented a typical steroidal skeleton. In addition, one olefinic methyl at δ 1.64 (H-26), two exomethylene protons at δ 4.69 (H-26) and 4.71 (H-26), four olefinic protons at δ 6.24 (H-6), 6.50 (H-7), 5.23 (H-22), and 5.19 (H-23) were apparent in the ^{1}H NMR spectrum [Fig. 2(a)]. In the ^{13}C spectrum [Fig. 2(b)], besides the signals corresponding to the exomethylene group [(δ 109.6, (C-26) and 148.4 (C-25)] and four olefinic carbons [δ 135.4 (C-6), 130.7 (C-7), 136.5 (C-22) and 130.6 (C-23)], two quaternary carbons [δ 82.2 (C-5) and 79.4 (C-8)] bearing an oxygen atom were also observed. The HMBC correlations from H-6 and H-7 to C-5 and C-8 suggested the presence of 5α, 8α-epidioxy functionality in the B ring of **1**. This finding was supported by the mass fragment ion $[M - O_2 + H]^+$ of *m/z* 409 from the molecular ion by loss of an oxygen molecule loss. Furthermore, NOE correlations of H-6 / H-19 and H-7 / H-18 supported the configuration of ring B. The coupling constant pattern at δ 1.91 (H-4, *J* = 13.5, 11.7 Hz) also suggested the methine proton at H-3 to be the α orientation, indicating an OH-3β at C-3. Further interpretation of 2D NMR correlations of protons and carbons determined the rings A-D system of compound **1** as 5α, 8α-epidioxycholest-6-en-3β-ol, encountered frequently in steroidal skeletons.

The structure of the side chain comprising 10 carbons was readily revealed by COSY, HSQC, and HMBC spectra. As shown in Fig. 3, the triplet protons at δ 0.83 (H-29) was attributed to a terminal methyl and its HMBC signals with C-28 and C-24 were observed.

The olefinic singlet methyl at δ 1.64 (H-27) showed the HMBC correlations to C-24, -25, and -26. Similarly, the HMBC correlations of the methyl doublet at δ 1.00 (H-21) with C-17, -20, and -22 enabled the connection between the side chain and the rings A-D system. The absolute configuration of C-24 in the side chain was defined as 24*S* by comparison of the NMR data with those of the partially identical structure reported previously.[9] Finally, the di-substituted olefin in the side chain was assigned as *E* configuration on the basis of the coupling constant (J = 15.2 Hz). Accordingly, the gross structure of **1** was established as 5α, 8α-epidioxy-(24*S*)-ethylcholesta-6,22(*E*),25-trien-3β-ol, a new derivative of 5α, 8α-epidioxy sterols. Table 1 listed the assignment of all the protons and carbons.

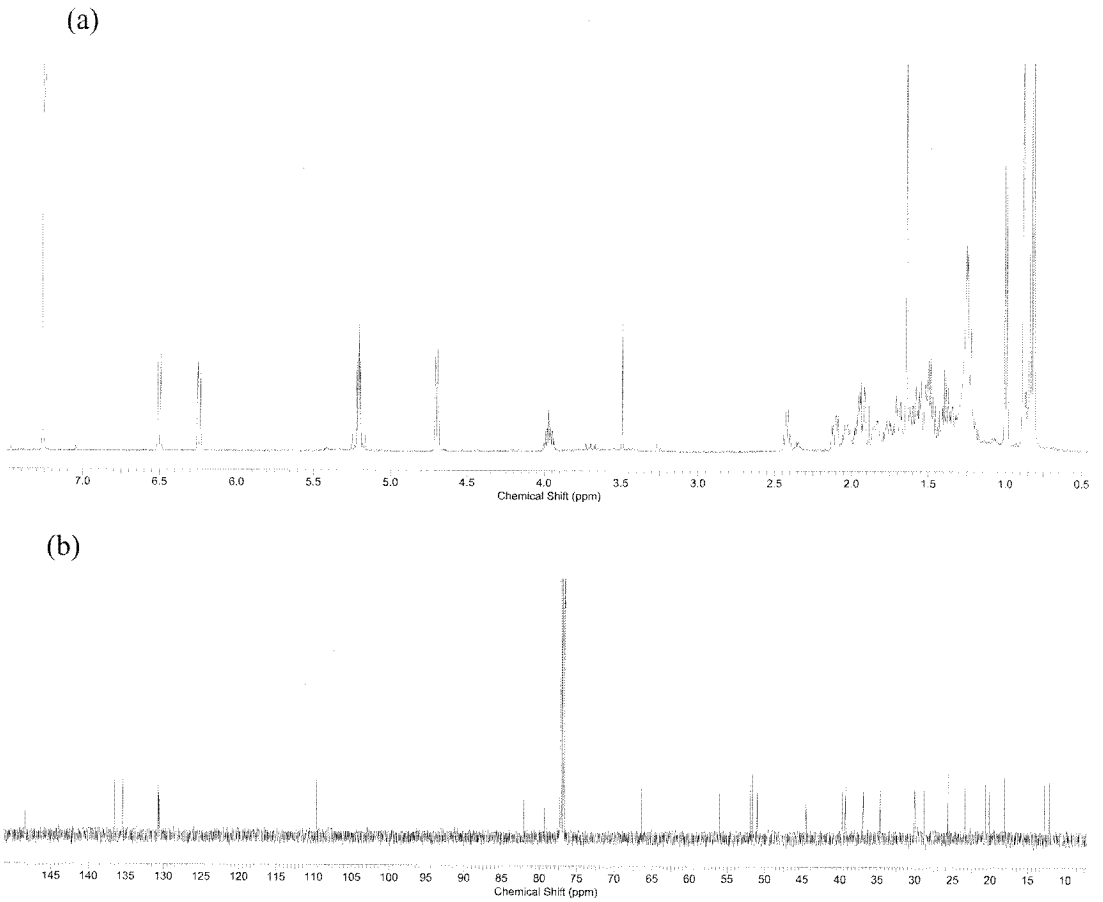

Figure 2. (a) ^{1}H and (b) ^{13}C NMR spectra for compound **1**.

Table 1. NMR spectral data for compounds **1 - 4** in $CDCl_3$

No	1		2	3	4
	δ_C	δ_H (multi, *J* Hz)	δ_C	δ_C	δ_C
1	34.7, CH_2	1.69 (dd, 13.5, 3.2); 1.94 (m)	34.7, CH_2	34.7, CH_2	34.7, CH_2
2	30.1, CH_2	1.52 (m); 1.84 (m)	30.1, CH_2	30.1, CH_2	30.1, CH_2
3	66.5, CH	3.97 (m)	66.5, CH	66.5, CH	66.5, CH
4	36.9, CH_2	1.91 (dd, 13.5, 11.7); 2.11 (dd, 13.5,	36.93, CH_2	36.9, CH_2	36.89 CH_2
5	82.2, C	5.1)	82.1, C	82.1, C	82.1, C
6	135.4, CH		135.4, CH	135.4, CH	135.4, CH
7	130.7, CH	6.24 (d, 8.6)	130.7, CH	130.7, CH	130.8, CH
8	79.4, C	6.50 (d, 8.6)	79.4, C	79.4, C	79.4, C
9	51.1, CH		51.1, CH	51.1, CH	51.0, CH
10	36.94, C	1.49 (d, 7.3)	36.96, C	36.94, C	36.91, C
11	23.4, CH_2		23.4, CH_2	23.4, CH_2	23.4, CH_2
12	39.3, CH_2	1.23 (m); 1.51 (m)	39.3, CH_2	39.3, CH_2	39.39, CH_2
13	44.6, C	1.22 (m); 1.95 (m)	44.5, C	44.5, C	44.7, C
14	51.7, CH		51.7, CH	51.7, CH	51.5, CH
15	20.66, CH_2	1.56 (dd, 12.5, 7.3)	20.7, CH_2	20.6, CH_2	20.6, CH_2
16	28.8, CH_2	1.42 (m); 1.61 (m)	28.7, CH_2	28.7, CH_2	28.2, CH_2
17	56.0, CH	1.35 (m); 1.76 (m)	56.1, CH	56.1, CH	56.4, CH
18	12.8, CH_3	1.24 (m)	12.8, CH_3	12.8, CH_3	12.6, CH_3
19	18.2, CH_3	0.81 (s)	18.2, CH_3	18.2, CH_3	18.2, CH_3
20	39.7, CH	0.88 (s)	39.8, CH	39.7, CH	35.2, CH
21	20.71, CH_3	2.03 (m)	20.9, CH_3	20.8, CH_3	18.6, CH_3
22	136.5, CH	1.00 (d, 6.6)	135.39, CH	137.4, CH	35.9, CH_2
23	130.6, CH	5.23 (dd, 15.2, 7.3)	122.4, CH	126.8, CH	23.8, CH_2
24	51.9, CH	5.19 (dd, 15.2, 7.1)	43.1, CH	41.9, CH_2	39.4, CH_2
25	148.4, C	2.42 (q, 7.1)	33.2, CH	28.5, CH	27.9, CH
26	109.6, CH_2		20.2, CH_3	22.3, CH_3	22.8, CH_3
27	20.2, CH_3	4.69 (br s); 4.71 (br s)	19.6, CH_3	22.2, CH_3	22.5, CH_3
28	25.7, CH_2	1.64 (s)	18.0, CH_3		
29	12.2, CH_3	1.40 (m); 1.46 (m)			
		0.83 (t, 7.3)			

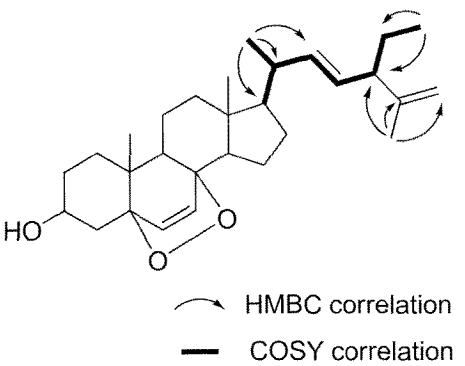

Figure 3. HMBC and COSY correlations in the side chain of **1**.

In the same way, the structures of compounds **2** - **4** were elucidated by a combination of 1D and extensive 2D NMR experiments. Each structure of three compounds was concluded to possess different side chain on the same ring skeleton as that of compound **1**. The structure of compound **2** was identified as 5α, 8α-epidioxy-24-methylcholesta-6,22(*E*)-dien-3β-ol, reported already somewhere.[3,10] The configuration of C-24 could be determined by comparison with carbon chemical shifts of 24*R* or 24*S* compounds. The carbon chemical shifts of compound **2** were in good agreement with those of 24*S* compound, except for the interchange in the assignment of C-11 and C-15. Our assignment was clearly confirmed by the coupling of δ 1.23 (H-11) and 1.51 (H-11) with δ 1.95 (H-12) as well as δ 1.42 (H-15) and 1.61 (H-15) with δ 1.75 (H-16) in the TOCSY spectrum shown in Fig. 4.

Compared with the known structures, compounds **3** and **4** were recognized as 5α, 8α-epidioxycholesta-6,22(*E*)-dien-3β-ol and 5α, 8α-epidioxycholesta-6-en-3β-ol, respectively.[11-12]

All the compounds isolated showed weak cytotoxicity on the brine shrimp lethality test.

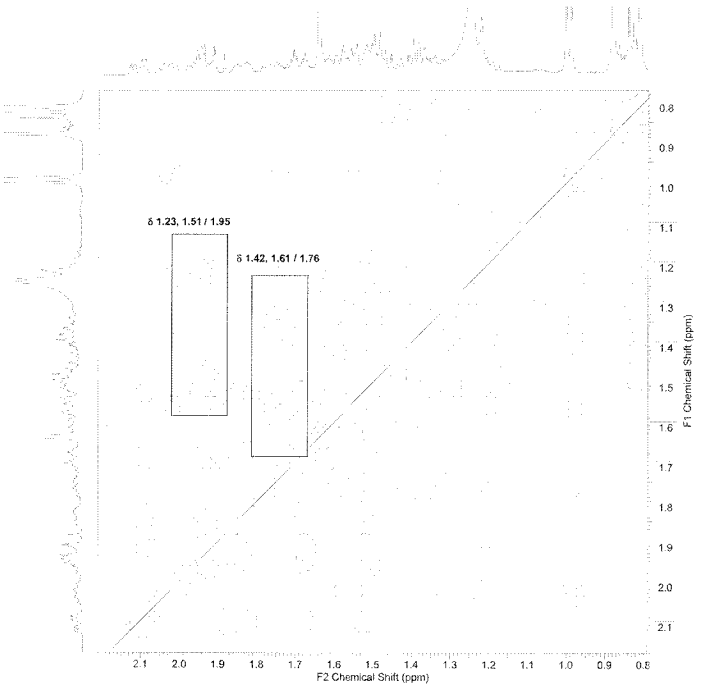

Figure 4. Expanded TOCSY spectrum of compound **2**.

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