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A New 5α,8α-Epidioxy Sterol from a Marine Sponge *Psammocinia* Species

Hye Jin Park[1], Xuan Luo[1], Jongki Hong[2], Dong-kyoo Kim[3], Kwang Sik Im[1], and Jee H. Jung[1]*

[1]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*
[2]*College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*
[3]*Department of Chemistry, Inje University, Kimhae 621-749, Korea*

Abstract – An investigation of the MeOH soluble fractions of a marine sponge *Psammocinia* sp. (Order: Dictyoceratida) led to the isolation of a new epidioxy sterol (**1**) and four known sterols (**2-5**). Their planar structures were defined by analyses of the spectroscopic data. The 27-nor-24-methylcholestan type side chain with an epidioxy nucleus (**1**) was unprecedented. Compounds **1-5** were isolated from a sponge *Psammocinia* sp. for the first time.

Keywords – *Psammocinia* species, epidioxy sterol, 7-keto sterol, marine sponge

Introduction

The sponge *Psammocinia* (order Dyctyoceratida, family Thorectidae) is known to produce sesterterpenes (Choi *et al.*, 2004; Liokas *et al.*, 1989), pederin family metabolites (psymberin, pederin, mycalamide A) (Cichewicz *et al.*, 2004), and a halogenated hexapeptide (cyclocinamide A) (Clark *et al.*, 1997). Our previous investigation of the polar fractions of the marine sponge *Psammocinia* sp. resulted in the isolation of ten cytotoxic furanosesterterpenes (Choi *et al.*, 2004). In a continuing study, a new epidioxy sterol (**1**) was isolated along with known ones (**2** and **3**) and two 7-keto sterols (**4** and **5**) from the MeOH soluble fraction of the same sponge. The planar structures of these compounds were elucidated with the aid of ^{1}H NMR, COSY, HSQC, HMBC, and mass spectroscopy.

Experimental

General Procedures – The ^{1}H and 2D NMR data were collected on UNITY Plus 300 (Varian) and UNITY Inova 500 (Varian, Palo Alto, California, U.S.A.) spectrometers. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). LR FAB MS was measured on a JEOL JMS-SX-102A double-focusing instrument. HR FAB MS data were obtained on a JEOL JMS SX-101A spectrometer. HPLC was performed on a Gilson (Villiers-le-Bel, France) 370 pump with Shodex 5C8-10E (250×10 mm I.D., 4 μm, 80 Å) column, Shodex C18-10E (250×10 mm I.D., 4 μm, 80 Å) column, and Alltech Econosphere C18 guard column, using a Shodex RI-71 detector (Minato-ku, Tokyo, Japan). Solvents for extraction, partition, and TLC were distilled from industrial grade solvents. TLC plates used were Kieselgel 60 F254 (Art.1.05554, Merck) and RP-18 F254s (Art. 1.05560, Merck).

Animal Material – The sponge was collected in October 2001, off the coast of Ulleung Island, Korea. The specimen was identified as *Psammocinia* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen of this horny sponge (registry No. Spo. 42) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere (Choi *et al.*, 2004).

Extraction and Isolation – The frozen sponge (4 kg, wet weight) was extracted with MeOH at room temperature. The MeOH extract of the sponge displayed significant toxicity against brine shrimp larvae (LD_{50}, 126 μg/mL). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between 90% MeOH and *n*-hexane to yield 90% MeOH soluble (21.4 g) and *n*-hexane soluble (4.9 g) fractions. The 90% MeOH fraction was subjected to a stepped-gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh) eluting with a solvent system of 66% → 100% MeOH/H_2O, to afford 20 fractions (Fr.1~Fr.20). These fractions were evaluated for activity in the brine shrimp lethality assay, and fractions

*Author for correspondence
Fax: +82-51-513-6754; E-mail: jhjung@pusan.ac.kr

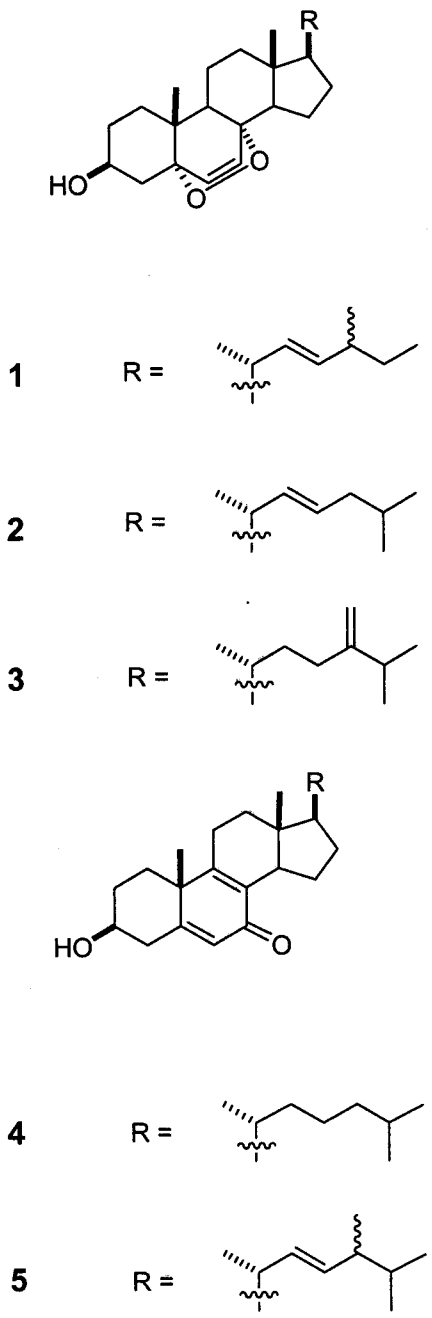

Fig. 1. Structures of compounds **1-5**.

Fr.4~Fr.16 were found active. The fraction Fr.14 was separated by reversed-phase HPLC (Shodex $5C_8$-10E, 250×10 mm I.D., 4 μm, 80 Å) eluting with 84% MeOH/H_2O, to afford 9 sub-fractions (Fr.14-1~Fr.14-9). Compounds **1** (0.86 mg) and **2** (1.8 mg) were obtained by purification of sub-fraction Fr.14-5 by reversed-phase HPLC (Shodex C_{18}-10E, 250×10 mm I.D., 4 μm, 80 Å), eluting with 95% MeOH/H_2O. Compound **3** (1.27 mg) was obtained by purification of sub-fraction Fr.14-7 by reversed-phase HPLC (Shodex C_{18}-10E, 250×10 mm I.D., 4 μm, 80 Å), eluting with 90% MeOH/H_2O. Compounds **4** (0.48 mg) and **5** (0.5 mg) were obtained by purification of sub-fraction Fr.14-4 by reversed-phase HPLC (Shodex C_{18}-10E, 250×10 mm I.D., 4 μm, 80 Å), eluting with 95% MeOH/H_2O.

Compound 1 – white powder, ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 437 $[M+Na]^+$, HRFABMS *m/z* 415.3195 $[M+H]^+$ (calcd for $C_{27}H_{43}O_3$, 415.3212), *m/z* 437.3026 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_3Na$, 437.3032).

Compound 2 – white powder, ^1H NMR (500 MHz, CD_3OD) δ 3.76 (1H, m, H-3), 6.26 (1H, d, J = 7.5 Hz, H-6), 6.52 (1H, d, J = 7.5 Hz, H-7), 0.84 (3H, s, H_3-18), 0.89 (3H, s, H_3-19), 1.00 (3H, d, J = 6.0 Hz, H_3-21), 5.30 (1H, m, H-22), 5.18 (1H, m, H-23), 0.87 (3H, d, J = 6.0 Hz, H_3-26), 0.87 (3H, d, J = 6.5 Hz, H_3-27); ^{13}C NMR (CD_3OD, Assignments based on HSQC and HMBC experiments, 500 MHz) δ 35.6 (C-1), 30.5 (C-2), 67.2 (C-3), 37.5 (C-4), 83.5 (C-5), 137.0 (C-6), 131.8 (C-7), 80.5 (C-8), 52.8 (C-9), 45.5 (C-10), 21.0 (C-11), 40.5 (C-12), 46.0 (C-13), 52.6 (C-14), 21.0 (C-15), 30.6 (C-16), 57.2 (C-17), 12.5 (C-18), 18.2 (C-19), 40.8 (C-20), 21.0 (C-21), 137.8 (C-22), 127.8 (C-23), 43.0 (C-24), 30.5 (C-25), 22.8 (C-26/27).

Compound 3 – white powder, ^1H NMR (500 MHz, CD_3OD) δ 3.76 (1H, m, H-3), 6.26 (1H, d, J = 8.0 Hz, H-6), 6.52 (1H, d, J = 8.0 Hz, H-7), 0.83 (3H, s, H_3-18), 0.89 (3H, s, H_3-19), 0.96 (3H, d, J = 7.5 Hz, H_3-21), 1.02 (3H, d, J = 7.0 Hz, H_3-26), 1.03 (3H, d, J = 7.0 Hz, H_3-27), 4.65 (1H, s, H-24^{1}a), 4.71 (1H, s, H-24^{1}b); ^{13}C NMR (CD_3OD, Assignments based on HSQC and HMBC experiments, 500 MHz) δ 34.8 (C-1), 30.4 (C-2), 66.0 (C-3), 36.5 (C-4), 82.5 (C-5), 136.0 (C-6), 130.4 (C-7), 79.5 (C-8), 51.8 (C-9), 44.2 (C-10), 20.2 (C-11), 37.0 (C-12), 43.5 (C-13), 51.6 (C-14), 21.2 (C-15), 30.2 (C-16), 56.5 (C-17), 11.4 (C-18), 16.8 (C-19), 33.5 (C-20), 18.0 (C-21), 37.0 (C-22), 28.0 (C-23), 156.0 (C-24), 33.8 (C-25), 21.2 (C-26/27), 105.5 (C-24^1).

Compound 4 – white powder, ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 399 $[M+H]^+$, HRFABMS *m/z* 399.3235 $[M+H]^+$ (calcd for $C_{27}H_{43}O_2$, 399.3263), *m/z* 421.3094 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_2Na$, 421.3083).

Compound 5 – white powder, FABMS *m/z* 411 $[M+H]^+$, HRFABMS *m/z* 411.3223 $[M+H]^+$ (calcd for $C_{28}H_{43}O_2$, 411.3263), *m/z* 433.3058 $[M+Na]^+$ (calcd for $C_{28}H_{42}O_2Na$, 433.3083); ^1H NMR (500 MHz, CD_3OD), δ 3.53 (1H, m, H-3), 6.00 (1H, brs, H-6), 0.68 (3H, s, H_3-18), 1.38 (3H, s, H_3-19), 1.06 (3H, d, J = 6.5 Hz, H_3-21), 5.21 (2H, m, H-22, 23), 0.87 (3H, d, J = 7.0 Hz, H_3-26), 0.85 (3H, d, J = 7.0 Hz, H_3-27), 0.94 (3H, d, J = 7.0 Hz, H_3-24^1); ^{13}C NMR (CD_3OD, Assignments based on HSQC

Table 1. NMR Data of Compounds **1** and **4** in CD_3OD

Position	1		4	
	δ $^1H^a$	δ $^{13}C^b$	δ $^1H^a$	δ $^{13}C^b$
1	1.87 (m)	35.5	1.40 (m)	35.7
	1.68 (m)		1.24 (m)	
2	1.76 (m)	31.5	1.92 (m)	31.5
	1.51 (m)		1.72 (dt, 6.5, 2.5)	
3	3.76 (m)	66.6	3.53 (m)	72.3
4	1.88 (m)	37.0	2.57 (m)	42.8
	1.70 (m)		2.53 (m)	
5		82.2		166.2
6	6.26 (d, 8.5)	137.0	6.00 (brs)	126.2
7	6.53 (d, 8.5)	131.8		188.5
8		80.5		134.3
9	1.45 (m)	52.5		165.5
10		35.0		43.7
11	1.52 (m)	24.0	2.50 (m)	25.5
	1.24 (m)		1.30 (m)	
12	1.96 (m)	40.8	2.15 (m)	35.5
	1.24 (m)		1.25 (m)	
13		45.0		43.2
14	1.32 (m)	53.2	2.25 (m)	49.2
15	1.50 (m)	21.2	2.57 (m)	25.5
	1.42 (m)		1.40 (m)	
16	1.75 (m)	30.5	1.99 (m)	29.8
	1.25 (m)		1.39 (m)	
17	1.23 (m)	57.8	1.20 (m)	54.5
18	0.84 (s)	12.4	0.67 (s)	11.2
19	0.89 (s)	18.5	1.37 (s)	23.0
20	2.01 (m)	41.5	1.52 (m)	37.8
21	1.00 (d, 6.5)	22.0	0.99 (d, 6.5)	19.2
22	5.17 (m)	136.2	2.00 (m)	29.8
23	5.17 (m)	135.0	1.19 (m)	36.5
24	1.90 (m)	40.5	1.15 (m)	40.5
25	1.28 (m)	30.5	1.52 (m)	29.0
26	0.84 (t, 6.5)	12.4	0.88 (d, 6.0)	23.5
27			0.89 (d, 6.0)	23.5
24^1	0.94 (d, 7.0)	21.5		

[a] Spectra were recorded at 500 MHz.
[b] Assingments were made on the basis of HMBC and HSQC data (500 MHz).

and HMBC experiments, 500 MHz) δ 34.5 (C-1), 30.5 (C-2), 73.2 (C-3), 42.5 (C-4), 165.0 (C-5), 126.2 (C-6), 188.5 (C-7), 133.5 (C-8), 164.5 (C-9), 42.4 (C-10), 24.6 (C-11), 35.6 (C-12), 43.0 (C-13), 47.4 (C-14), 25.8 (C-15), 30.5 (C-16), 53.6 (C-17), 12.1 (C-18), 23.5 (C-19), 40.5 (C-20), 22.0 (C-21), 135.5 (C-22), 131.8 (C- 23), 43.2 (C-24), 34.5 (C-25), 20.2 (C-26), 20.0 (C-27), 19.2 (C-24^1).

Results and Discussion

The MeOH extract of the sponge showed significant activity in the brine shrimp lethality assay (LD_{50}, 126 μg/mL). Guided by this assay, the MeOH extract was successively fractionated employing reversed-phase flash column chromatography and reversed-phase HPLC to afford compounds **1-5**.

Compound **1** was isolated as a white powder. The molecular formula of **1** was assigned as $C_{27}H_{42}O_3$ on the basis of combined NMR and HRFABMS spectral analyses. The HRFABMS spectrum of **1** showed the $[M + Na]^+$ ion at *m/z* 437.3026 (calcd for $C_{27}H_{42}O_3Na$, 437.3032). In the ^1H NMR spectrum of **1**, two olefinic proton signals at δ 6.26 (1H, d, J = 8.5 Hz, H-6) and 6.53 (1H, d, J = 8.5 Hz, H-7) were observed, which are characteristic of 5α,8α-epidioxy sterols. The ^1H NMR spectrum indicated an oxygenated methine proton at δ 3.76 (1H, m, H-3) and two olefinic protons at δ 5.17 (2H, m, H-22/23). The side chain of the structure was determined with the aid of ^1H NMR, COSY, and HMBC experiments. The ^1H NMR spectrum showed signals for two angular methyl groups at δ 0.84 (3H, s, H_3-18) and δ 0.89 (3H, s, H_3-19), and three methyl groups at δ 1.00 (3H, d, J = 6.5 Hz, H_3-21), δ 0.84 (3H, t, J = 6.5 Hz, H_3-26), and δ 0.94 (3H, d, J = 7.0 Hz, H_3-24^1). The H_3-18 singlet and H_3-26 triplet were overlapped at δ 0.84. The correlations between H-20 and H-22, and H-23 and H-24 were observed in the COSY spectrum. In the HMBC spectrum, key correlations from H-21 to C-17/20/22, from H-24^1 to C-23/24/25, and from H-26 to C-24/25 were observed. The above NMR data suggested that compound **1** has a 27-nor-24-methylcholesta-6,22-dien-3β-ol skeleton. The stereochemistry at C-24 remains to be assigned. This side chain was reported with other sterol nuclei from the sponge *Esperiopsis edwardii* and marine dinoflagellate *Gymnodinium simplex* (Seldes *et al.*, 1988; Goad and Withers., 1982), but the one with a 5α,8α-epidioxy sterol nucleus was unprecedented.

The ^1H NMR spectrum of compound **2** showed characteristic proton signals of 5α,8α-epidioxy sterol, and it was identified as 5α,8α-epidioxycholesta-6,22-dien-3β-ol. This compound was previously reported from marine sponges *Homaxinella* sp., *Luffariella* cf. *variabilis*, *Pleraphysilla papyracea*, and *Axinella cannabina* (Gunatilaka *et al.*, 1981, Gauvin *et al.*, 2000; Mansoor *et al.*, 2005).

Compound **3** was identified as 5α,8α-epidioxy-24-methylcholesta-6,24(24^1)-dien-3β-ol. This compound was previously reported from marine organisms such as tunicate *Ascidia nigra*, sponge *Thalysias juniperina*, opisthobranch mollusk *Adalaria* sp., and sea pen *Virgularia* sp. (Stonard *et al.*, 1980; Gunatilaka *et al.*, 1981; Gauvin *et al.*, 2000).

Compound **4** was isolated as a white powder. The molecular formula of **4** was assigned as $C_{27}H_{42}O_2$ on the basis of combined NMR and HRFABMS spectral analyses. The HRFABMS spectrum of **4** showed the $[M + H]^+$ ion at *m/z* 399.3235 (calcd for $C_{27}H_{43}O_2$, 399.3263)

and the $[M+Na]^+$ ion at *m/z* 421.3094 (calcd for $C_{27}H_{42}O_2Na$, 421.3083). The upfield methyl region in the ^1H NMR spectrum indicated the presence of characteristic steroidal methyl signals, except the downfield-shifted H_3-19 methyl singlet. The ^1H NMR spectrum of **4** showed signals for two methyl singlets at δ 0.67 (3H, s, H_3-18), δ 1.37 (3H, s, H_3-19), three doublet methyls at δ 0.99 (3H, d, J = 6.5 Hz, H_3-21), δ 0.88 (3H, d, J = 6.0 Hz , H_3-26), δ 0.89 (3H, d, J = 6.0 Hz, H_3-27), and one oxygenated methine at δ 3.53 (1H, m, H-3). The ^{13}C NMR data showed the presence of one oxymethine function (δ 72.3) and four olefinic carbons (δ 126.2, 134.3, 165.5, and 166.2). The ^1H NMR signal of the olefinic proton at δ 6.00 (1H, brs, H-6) and HMBC correlations to the signals at δ 166.2, 134.3, and 165.5 (C-5, C-8, and C-9, respectively) suggested the presence of an α,β-unsaturated system in the B-ring. In the HMBC spectrum, key correlations from H-4 to C-3/5, from H_3-19 to C-5/9 were observed. The side chain was confirmed by COSY and HMBC experiments. On the basis of these data, compound **4** was defined as a 3β-hydroxycholesta-5,8-dien-7-one. This compound has been previously reported from industrial source (Suzuki *et al.*, 1963). To the best of our knowledge, this is the first report of its NMR data and occurrence in nature.

The molecular formula of compound **5** was assigned as $C_{28}H_{42}O_2$ on the basis of combined NMR and HRFABMS spectral analyses. The HRFABMS spectrum of **5** showed the $[M+H]^+$ ion at *m/z* 411.3223 (calcd for $C_{28}H_{43}O_2$, 411.3263) and the $[M+Na]^+$ ion at *m/z* 433.3058 (calcd for $C_{28}H_{42}O_2Na$, 433.3083). The NMR data of **5** were almost same with those of **4**, with the only difference in the side chain. The ^1H NMR spectrum of **5** showed two olefinic proton signals at δ 5.21 (2H, m, H-22/23). The side chain was confirmed by COSY and HMBC experiments. The correlations from H_3-21 to C-17/20/22, from H_3-24[1] to C-23/24/25, and from H_3-26/27 to C- 24/25 were observed in the HMBC spectrum. Therefore, compound **5** was identified as a 3β-hydroxy-24-methylcholesta-5,8,22-trien-7-one. This compound was previously reported from the mediterranean sponge *Clathrina clathrus* and the fruit bodies of *Grifola frondosa* (Aiello *et al.*, 1988; Ishizuka *et al.*, 1997).

Compounds **1-5** are reported for the first time from a marine sponge *Psammocinia* species.

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