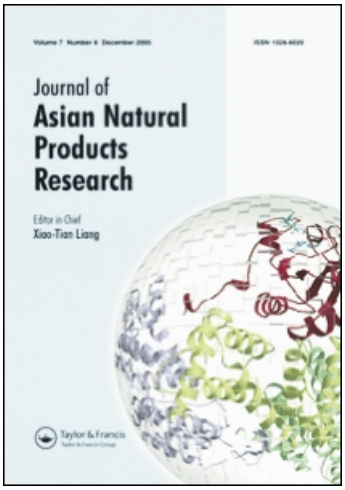

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A new steroid 24β-methylcholasta-1,8,14,22,25-penten-3-one-5α-ol (**1**) and a new alkaloid 1-(1H-indol-3-yl)-2,3-dihydroxy-5-methyl-hexane (**2**), together with four known compounds, were isolated from the EtOH extract of the South China Sea sponge *Axinella* sp. The structures of **1** and **2** were determined on the basis of extensive spectroscopic analysis, including 1D and 2D NMR spectral data.

Keywords: sponge; *Axinella* sp.; 24β-methylcholasta-1,8,14,22,25-penten-3-one-5α-ol; 1-(1H-indol-3-yl)-2,3-dihydroxy-5-methyl-hexane; steroid; alkaloid

1. Introduction

Marine sponges are known to be a rich source of secondary metabolites having unusual functionalization and structures. Previous studies on the chemical constituents of sponges in the genus *Axinella* have resulted in the isolation of bromopyrrole alkaloids [1,2], sterols [3], cyclic peptides [4], and triterpenes [5]. Now, in our chemical investigation on the EtOH extract of the South China Sea sponge *Axinella* sp., a new steroid 24β-methylcholasta-1,8,14,22,25-penten-3-one-5α-ol (**1**) and a new alkaloid 1-(1H-indol-3-yl)-2,3-dihydroxy-5-methyl-hexane (**2**), together with four known compounds, 24β-methylcholasta-7,22,25-triene-3β,5α,6β-triol (**3**) [6], *N*-methyltryptamine (**4**) [7], valcyl-leucine (**5**), and 3,5-dibromo-1-hydroxy-4,4-dimethoxy-2,5-cyclohexadiene-1-acetamide (**6**) [8], were obtained (Figure 1). This paper deals with the isolation and structural elucidation of **1** and **2**. The ^{1}H and ^{13}C NMR spectral data of **5**, and ^{13}C NMR spectral data of **6** are also reported for the first time.

2. Results and discussion

Compound **1** has the molecular formula of $C_{28}H_{38}O_2$ as deduced from HR-EI-MS and NMR spectral data, and it contains 10 degrees of unsaturation. The ^{1}H NMR spectrum revealed the presence of three tertiary methyls (δ_H 0.97, 1.10, 1.69), two secondary methyls (δ_H 1.11, 1.07), and seven olefinic protons (δ_H 6.55 (1H, d, $J = 9.6$ Hz), 6.42 (1H, d, $J = 9.6$ Hz), 5.68 (1H, br s), 5.33 (1H, dd, $J = 5.6$, 15.1 Hz), 5.28 (1H, dd, $J = 7.0$, 15.1 Hz), 4.72 (2H, br s)). The ^{13}C NMR spectrum showed the presence of 28 carbons, including five methyl, six methylene, three methine, three quaternary carbons (δ_C 39.0, 43.7, 71.5), four double bonds (δ_C 108.8 (t), 116.0 (d), 126.6 (s), 132.1 (d), 134.8 (d), 143.7 (s), 144.8 (s), 149.4 (s)), and an

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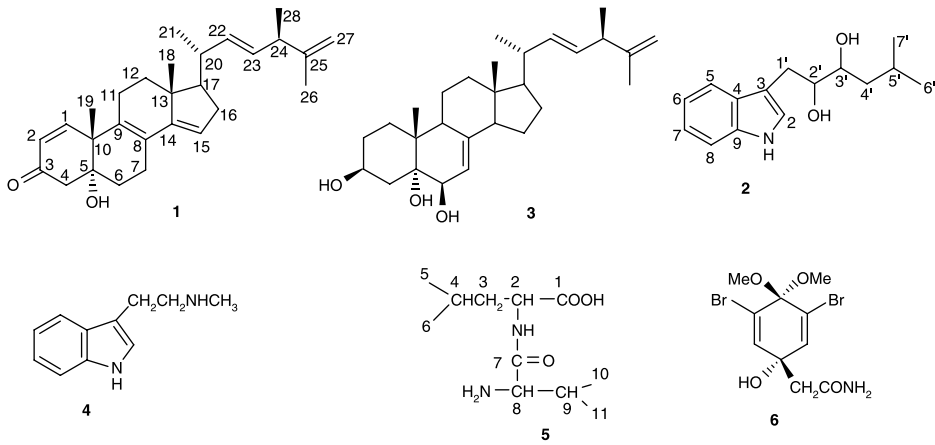

Figure 1. Structures of compounds **1**–**6**.

α,β-unsaturated carbonyl group (δ_C 158.5 (d), 128.4 (d), 201.7 (s)). The signals for the side chain corresponded well to those observed in the ^1H and ^{13}C NMR spectra of 24β-methylcholasta-7,22,25-triene-3β,5α,6β-triol (**3**) [6]. Combined with the above data, it is obvious that **1** should also be a 24β-methylcholestane with a 22,25-diene side chain.

In the HMBC spectrum (Figure 2), HMBC correlations of H-26 with Me-27, C-25, C-24, H-27 with C-26, C-24, H-28 with C-23, C-25, H-23 with Me-28, H-22 with Me-21, C-24, and H-21 with C-22 proved the presence of a 24-methyl-22,25-diene side chain. HMBC correlations of H-1 with Me-19, C-3, and H-2 with C-10, C-4 suggested the presence of the α,β-unsaturated carbonyl group. HMBC correlations of Me-19, H-1 with C-5 suggested the oxygenation of C-5 (δ_C 71.5, s). Moreover, HMBC correlations of H-19 with C-9, H-6 with C-8, H-18 with C-14, and H-15 with C-8, C-17 suggested the presence of —C(8)═C(9)—C(14)═C(15)— conjugated double bonds. In the NOESY spectrum of **1**, NOE correlations of Me-21 with H-17 suggested the α-configuration of Me-21, while no NOE correlation was observed between H-17 and Me-18, Me-21, and Me-24, which indicated the β-configuration of Me-18 and Me-28. Combined with the analysis of the NOESY spectrum and comparison of the NMR spectral data of **1** and **3**, the relative stereochemistry of asymmetry carbons in **1** was deduced to be the same as that in **3**. Thus, the structure of **1** was elucidated as 24β-methylcholasta-1,8,14,22,25-penten-3-one-5α-ol.

Compound **2** has the molecular formula of $C_{15}H_{21}NO_2$ as deduced from

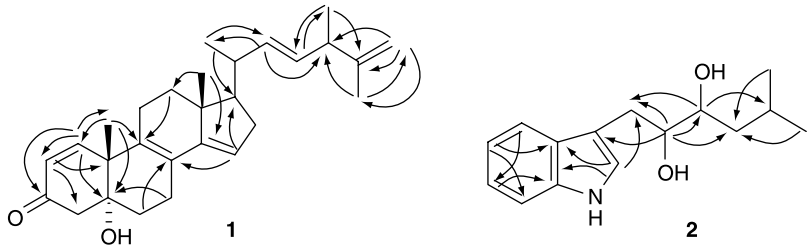

Figure 2. Key HMBC correlations of **1** and **2**.

HR-EI-MS and NMR spectral data. Its ^{1}H NMR spectrum displayed two methyl groups at δ_H 1.02 and 0.97 (each 3H, d, $J = 6.6$ Hz), two oxymethine protons at δ_H 3.91 (2H, overlap), an olefinic proton at δ_H 7.11 (1H, br s), signals of a four-spin proton system at δ_H 7.63 (1H, d, $J = 7.9$ Hz), 7.39 (1H, d, $J = 7.9$ Hz), 7.23 (1H, t, $J = 7.9$ Hz), and 7.15 (1H, t, $J = 7.9$ Hz), and a downfield proton singlet at δ_H 8.09 (br s, NH). The ^{13}C NMR spectrum showed the presence of two methyls at δ_C 23.8, 21.8, two methylenes at δ_C 40.8, 26.9, one upfield methine at δ_C 24.7, two oxymethines at δ_C 74.1, 71.8, five downfield methines at δ_C 122.9, 122.4, 119.6, 118.8, 111.3, and two downfield quaternary carbons at δ_C 111.9, 127.4. These data suggested that **2** might have a mono-substituted indole nucleus. Comparison of the ^{1}H and^{13}C NMR spectral data of **1** with those of mono-methyl indoles, such as *N*-methyltryptamine (**4**) [7] and 2-hydroxy-1-(1H-indol-3-yl)-5-methyl-3-hexanone (**7**) [9], established the location of the mono-substituted chain on C-3, and the only difference between **2** and **7** was that the carbonyl group in compound **7** was replaced by a hydroxyl group in compound **2**. This was proved by the HMBC correlations of H-2 with C-4, C-9, C-1′, H-2′ with C-3, C-4′, C-3′, and H-3′ with C-5′, C-1′. Thus, the structure of **2** was elucidated as shown in Figure 1, and was named 1-(1H-indol-3-yl)-2,3-dihydroxy-5-methyl-hexane.

Based on the 1D and 2D NMR spectroscopic analysis (including HSQC and HMBC), all of the ^{1}H and ^{13}C NMR spectral data of **5** and **6** were assigned.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a Horiba SEAP-300 spectropolarimeter. UV spectra were measured using a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer with TMS as the internal standard. MS spectral data were obtained on an LCQDECA XP HPLC/MSn spectrometer for ESI-MS. Silica gel (200–300 mesh) for column chromatography (CC) and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, China.

3.2 Animal material

The South China Sea sponge *Axinella* sp. was collected in Sanya, Hainan Province, China, in October 2005. A voucher specimen (No. hm0501) is deposited in the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

3.3 Extraction and isolation

The fresh specimen (4.0 kg, wet weight) was extracted with EtOH three times at room temperature, and the solution was evaporated *in vacuo*. The residue was suspended in H_2O and extracted with EtOAc three times, and the EtOAc layer was concentrated *in vacuo* to afford 60 g of the residue. The EtOAc extract was subjected to CC on silica gel using petroleum ether–EtOAc (from 10:0 to 0:10) as the eluent. By combining the fractions with TLC (GF_{254}) monitoring, 10 fractions were obtained. Fraction 2 was repeatedly subjected to CC on silica gel using $CHCl_3$–$(CH_3)CO$ (from 10:1 to 9:1) as the eluent to yield **1** (6 mg). Fraction 3 was repeatedly subjected to CC on silica gel using $CHCl_3$–$(CH_3)CO$ (from 9:1 to 8:2) as the eluent to yield **5** (6 mg). Fraction 5 was chromatographed over Sephadex LH-20 eluted with $CHCl_3$–MeOH (1:1), then repeatedly subjected to CC on silica gel, and eluted with $CHCl_3$–MeOH (from 9:1 to 8:2) to yield **2** (8 mg), **3** (9 mg), and **6** (18 mg). Fraction 6 was chromatographed

over Sephadex LH-20 eluted with $CHCl_3$–MeOH (1:1) and then subjected to CC on silica gel, using $CHCl_3$–MeOH (from 8:2 to 7:3) as the eluent to yield **4** (7 mg).

*3.3.1 24β-Methylcholasta-1,8,14,22,25-penten-3-one-5α-ol (**1**)*

White powder; $[\alpha]_D^{20} + 14.6$ ($c = 0.14$, $CHCl_3$); UV (MeOH) λ_{max}: 204, 224, 248 nm; IR (KBr) ν_{max}: 3444, 2902, 1724, 1674 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H: 6.55 (1H, d, $J = 9.6$ Hz, H-1), 6.42 (1H, d, $J = 9.6$ Hz, H-2), 5.68 (1H, br s, H-15), 5.28 (1H, dd, $J = 7.0$, 15.1 Hz, H-22), 5.33 (1H, dd, $J = 5.6$, 15.1 Hz, H-23), 4.72 (2H, br s, H-26), 2.82, 2.13 (each 1H, d, $J = 18.0$ Hz, H-4), 2.74 (1H, m, H-24), 2.49, 2.42 (each 1H, m, H-16), 2.21 (2H, m, H-11), 2.19, 1.36 (each 1H, m, H-7), 1.99 (1H, br m, H-20), 1.86, 1.40 (each 1H, m, H-12), 1.74 (1H, m, H-17), 1.69 (3H, s, H-27), 1.51 (1H, m, H-6), 1.11 (3H, d, $J = 7.0$ Hz, H-21), 1.10 (3H, s, H-19), 1.07 (3H, d, $J = 6.6$ Hz, H-28), 0.97 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 158.5 (d, C-1), 128.4 (d, C-2), 201.7 (s, C-3), 41.1 (t, C-4), 71.5 (s, C-5), 25.7 (t, C-6), 27.6 (t, C-7), 126.6 (s, C-8), 143.7 (s, C-9), 39.0 (s, C-10), 24.7 (t, C-11), 41.0 (t, C-12), 43.7 (s, C-13), 144.8 (s, C-14), 116.0 (d, C-15), 32.4 (t, C-16), 55.3 (d, C-17), 18.2 (q, C-18), 20.7 (q, C-19), 44.6 (d, C-20), 21.0 (q, C-21), 134.8 (d, C-22), 132.1 (d, C-23), 45.1 (d, C-24), 149.4 (s, C-25), 108.8 (t, C-26), 20.8 (q, C-27), 18.9 (q, C-28); ESI-MS (+) *m/z*: 407 $[M+H]^+$; HR-ESI-MS: 407.2865 $[M+H]^+$ (calcd for $C_{28}H_{39}O_2$, 407.2871).

*3.3.2 1-(1H-indol-3-yl)-2,3-dihydroxy-5-methyl-hexane (**2**)*

White powder; $[\alpha]_D^{20} + 25.1$ ($c = 0.56$, $CHCl_3$); UV (MeOH) λ_{max}: 216, 225, 278, 286; IR (KBr) ν_{max}: 3416, 1668, 1648, 1453 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H: 8.09 (br s, NH), 7.63 (1H, d, $J = 7.9$ Hz, H-8), 7.39 (1H, d, $J = 7.9$ Hz, H-5), 7.23 (1H, t, $J = 7.9$ Hz, H-6), 7.15 (1H, t, $J = 7.9$ Hz, H-7), 7.11 (1H, br s, H-2), 3.91 (2H, overlap, H-2′ and H-3′), 3.02 (1H, dd, $J = 15.0$, 2.5 Hz, H-1′a), 2.92 (1H, dd, $J = 15.0$, 9.5 Hz, H-1′b), 1.90 (1H, m, H-5′), 1.59, 1.39 (each 1H, m, H-4′), 1.02 and 0.97 (each 3H, d, $J = 6.6$ Hz, Me-6′, Me-7′); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 122.9 (d, C-2), 111.9 (s, C-3), 127.4 (s, C-4), 111.3 (d, C-5), 122.4 (d, C-6), 119.6 (d, C-7), 118.8 (d, C-8), 136.5 (s, C-9), 26.9 (t, C-1′), 74.1 (d, C-2′), 71.8 (d, C-3′), 40.8 (t, C-4′), 24.7 (d, C-5′), 23.8 (q, C-6′), 21.8 (q, C-7′); ESI-MS (+) *m/z*: 248 $[M+H]^+$; HR-ESI-MS *m/z*: 248.1564 (calcd for $C_{15}H_{22}NO_2$, 248.1572).

*3.3.3 Valcyl-leucine (**5**)*

White powder; ^1H NMR (500 MHz, $CDCl_3$) δ_H: 0.95, 0.97, 1.00, 1.06 (each 3H, $J = 8.0$ Hz, H-5, H-6, H-10, H-11), 1.67, 1.86 (each 1H, m, H-3), 1.79 (1H, m, H-4), 2.46 (1H, m, H-9), 3.87 (1H, s, H-8), 4.02 (1H, m, H-2), 6.66, 6.76 (each 1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 168.9 (s, C-1), 167.6 (s, C-7), 60.3 (d, C-8), 52.9 (d, C-2), 42.5 (t, C-3), 32.2 (d, C-9), 24.2 (d, C-4), 23.2, 21.2 (each q, C-5, C-6), 18.6, 16.2 (each q, C-10, C-11).

*3.3.4 3,5-Dibromo-1-hydroxy-4,4-dimethoxy-2,5-cyclohexadiene-1-acetamide (**6**)*

White powder; ^1H NMR (500 MHz, Pyr-d_6) δ_H: 8.58 (br s, NH), 8.23 (br s, NH), 7.39 (2H, s, H-2), 3.23 (3H, s, H-9), 3.18 (3H, s, H-10), 2.98 (2H, s, H-7); ^{13}C NMR (125 MHz, Pyr-d_6) δ_C: 172.2 (s, C-8), 143.3 (d, C-2, C-6), 122.3 (s, C-3, C-5), 97.7 (s, C-4), 71.1 (s, C-1), 51.2 (q, C-9, C-10), 47.1 (t, C-7); ESI-MS (+) *m/z*: 392.2 $[M+Na]^+$:396.0 (2:1), 470.2 $[M+Na+2K]^+$:472.2:474.1 (1:2:1).

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