Purine and Pyrimidine Derivatives from the South China Sea Gorgonian *Subergorgia suberosa*

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Three new purine derivatives, namely, 4-caryboxy-5,6-dihydro-4*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10(9*H*)-dione (1), 7,9-dihydro-1-(3-oxobutyl)-1*H*-purine-6,8-dione (2), and 7-hydro-9-(3-oxobutyl)-1*H*-purine-6,8-dione (3) together with six known purine and pyrimidine derivatives were isolated from the EtOH/CH_2Cl_2 extracts of the South China Sea gorgonian *Subergorgia suberosa.* The structures of 1—3 were determined on the bases of extensive spectroscopic analysis, including 1D and 2D NMR data.

Key words *Subergorgia suberosa*; gorgonian; purine derivative

Marine invertebrates such as sponges, soft corals, gorgonians, molluscs, coelenterates, and ascidians produce secondary metabolites of unprecedented structures; sponges and ascidians, in particular, produce nitrogen-containing compounds. However, there were few reports about nitrogen-containing compounds from gorgonians. In order to obtain novel compounds from gorgonians, we investigated on the South China Sea gorgonian *Subergorgia suberosa*. Previous chemical researches on *S. suberosa* have resulted in the isolation of sesquiterpenes,[1—5] sterols,[6—8] and a sesquiterpene-alkaloid.[9] Now, in our further chemical investigation on the EtOH/CH_2Cl_2 extract of *S. suberosa*, three new purine derivatives, namely, 4-caryboxy-5,6-dihydro-4*H*,8*H*-pyrimido[1,2,3-*cd*]-purine-8,10(9*H*)-dione (**1**), 7,9-dihydro-1-(3-oxobutyl)-1*H*-purine-6,8-dione (**2**), and 7-hydro-9-(3-oxobutyl)-1*H*-purine-6,8-dione (**3**) together with six known purine and pyrimidine derivatives guanosine (**4**),[10] adenosine (**5**),[10] 3,7,9-tri-Me-6,8-purinediol (**6**),[11] thymidine (**7**),[10] thymine (**8**),[12] and uracil (**9**)[12] were obtained. This paper deals with the isolation and structural elucidation of **1**—**3**.

Results and Discussion

Compound **1** had the molecular formula of $C_9H_8N_4O_4$ as deduced from NMR spectra and positive HR-FAB-MS. Its ^{1}H-NMR spectrum displayed two methylenes at δ_H 4.36 (2H, d, J=5.45 Hz), 4.03 (1H, dd, J=6.5, 12.75 Hz) and 3.95 (1H, dd, J=4.30, 12.75 Hz), one methine at δ_H 3.51 (1H, m), one proton at δ_H 8.21 (1H, s) and one proton at δ_H 11.6 (1H, s). The ^{13}C-NMR spectrum showed two methylenes at δ_C 39.9 and 42.6, one methine at δ_C 36.5, one carboxyl group at δ_C 170.9, and five deshielded sp^2 hybridized carbons at [δ_C 111.6 (s), 136.1 (d), 139.2 (s), 149.5 (s), 156.3 (s)]. Together with characteristic UV absorptions (262, 210 nm), the above data were consistent with purine derivatives that had previously been isolated from many marine invertebrates, such as gorgonian,[13] ascidians,[14] and sponges.[15,16]

In the HMBC spectrum of **1**, δ_H 8.21 (1H, s) showed correlations with δ_C 139.2 (s) and 111.6 (s), while no correlation with δ_C 156.3 (s), accompanying with the NMR data comparison of the five deshielded sp^2 hybridized carbons between **1** and C^2-α-D-mannosylpyranosyltryptophan,[15] which suggested the presence of purine-2,6-dione, and allowed the assignments of δ_C 111.6 (s, C-5), 136.1 (d, C-8), 139.2 (s, C-4), 149.5 (s, C-2) and 156.3 (s, C-6). HMBC correlations of δ_H 4.36, 4.03, 3.95 with δ_C 170.9 (s), 36.5 (d), and δ_H 3.51 with δ_C 170.9 (s), and ^{1}H–^{1}H COSY correlations of δ_H 4.36 with δ_H 3.51, and δ_H 3.51 with δ_H 4.03, 3.95 in the ^{1}H–^{1}H COSY spectrum of **1** together with the present of one proton at δ_H 11.6 (1H, s) suggested the presence of a –CH_2–CH(COOH)–CH_2– group. Meanwhile, HMBC correlations of δ_H 4.36 with δ_C 139.2 (C-4), 149.5 (C-2), and δ_H 4.03, 3.95 with δ_C 136.1 (C-8), 139.2 (C-4) suggested that the 2′-carboxylisopropyl group was attached to the purine-2,6-dione substructure by two C–N bonds, C-1′ with N(3), and C-3′ with N(9). So, the structure of **1** was determined to be 4-caryboxy-5,6-dihydro-4*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10(9*H*)-dione.

The molecular formula of **2** was determined as $C_9H_{10}N_4O_3$

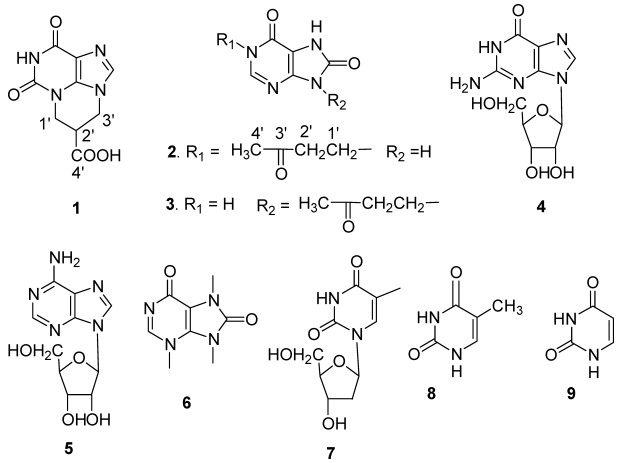

Fig. 1. Structures of Compounds **1**—**9**

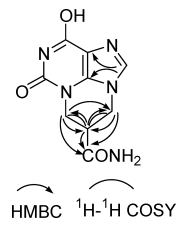

Fig. 2. Key HMBC and ^{1}H–^{1}H COSY Correlations of **1**

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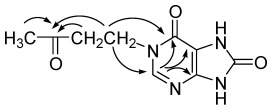

Fig. 3. Key HMBC Correlations of **2**

by analysis of its NMR spectra and ESI-MS. The ^{13}C-NMR spectrum of **2** also showed five low-field carbons [δ_C 143.1 (d), 151.1 (s), 107.4 (s), 157.0 (s), 153.1 (s)], together with one methyl (δ_C 29.7), two methylenes (δ_C 41.8, 43.7), and one carbonyl group (δ_C 205.9, s). The ^{1}H-NMR spectrum displayed one methyl at δ_H 2.04 (3H, s), two methylenes at δ_H 4.58 (2H, t, J=6.45 Hz), 3.22 (2H, t, J=6.45 Hz), and one proton at δ_H 8.09 (1H, s). Comparison of the NMR spectral data of **2** with those of **1**, and HMBC correlations of δ_H 8.09 (1H, s) with δ_C 151.1 (s), 107.4 (s) and 157.0 (s) in the HMBC spectrum suggested the presence of purine-6, 8-dione in **2**. HMBC correlations of δ_H 4.58, 3.22, 2.04 with δ_C 205.9 (s), and ^{1}H–^{1}H COSY correlations of δ_H 4.58 with δ_H 3.22 suggested the presence of CH_3–CO–CH_2CH_2– unit. Meanwhile, HMBC correlations of δ_H 4.58 with δ_C 157.0 (s, C-6), 143.1 (d, C-2) suggested that the CH_3–CO–CH_2CH_2– unit was attached to the purine-6,8-dione substructure by a C–N(1) bond. So, the structure of **2** was determined to be 7,9-dihydro-1-(3-oxobutyl)-1*H*-purine-6,8-dione.

Compound **3** showed the same molecular formula of $C_9H_{10}N_4O_3$ as **2**, which was deduced from the NMR and ESI-MS data of **3**. Comparison of overall ^{1}H- and ^{13}C-NMR spectral data revealed similarity between **3** and **2**. The only obvious difference between them was the chemical shifts of low-field carbons. HMBC correlations of δ_H 4.66 (2H, t, J= 7.5 Hz), 3.15 (2H, t, J=7.5 Hz), 2.09 (3H, s) with δ_C 206.5 (s), and ^{1}H–^{1}H COSY correlations of δ_H 4.66 with δ_H 3.15 also suggested the presence of CH_3–CO–CH_2CH_2– unit. However, HMBC correlations of δ_H 4.66 with δ_C 152.2 (s, C-8), 150.1 (s, C-4) suggested that the CH_3–CO–CH_2CH_2– unit was attached to the N(9) of purine-6,8-dione by a C–N(9) bond. So, the structure of **3** was determined to be 7-hydro-9-(3-oxobutyl)-1*H*-purine-6,8-dione.

Experimental

General Experimental Procedures The procedures were the same as previously reported.[9]

Animal Material The material was the same as previously reported.[9]

Extraction and Isolation The frozen specimen was extracted with EtOH/CH_2Cl_2 (2 : 1) three times at room temperature, and the solution was evaporated *in vacuo*. The residue was suspended in H_2O and extracted with $CHCl_3$ and *n*-BuOH three times, respectively. The $CHCl_3$ and *n*-BuOH layers were concentrated *in vacuo* to afford 50 g and 8 g of residues, respectively. The $CHCl_3$ extract was subjected to column chromatography (CC) on silica, using $CHCl_3$/Me_2CO (from 10 : 0 to 0 : 10) as eluent. By combining the fractions with TLC (GF_{254}) monitoring, eight fractions were obtained. Fraction 7 was chromatographed over Sephadex LH-20 eluting with $CHCl_3$/MeOH (1 : 1), then repeatedly subjected to CC on Si gel, eluted with $CHCl_3$/MeOH (from 9 : 1 to 7 : 3) to yield **1** (8 mg), **2** (3 mg) and **3** (4 mg). The *n*-BuOH extract was subjected to CC on Si gel, using $CHCl_3$/MeOH (from 9 : 1 to 0 : 10) as eluent to give five fractions. Fraction 2 was subjected to CC on Si gel, eluted with $CHCl_3$/MeOH (from 8 : 2 to 1 : 1) to yield **6** (10 mg), **8** (19 mg), **9** (22 mg), **4** (8 mg), **7** (9 mg) and **5** (9 mg).

4-Caryboxy-5,6-dihydro-4*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10(9*H*)-dione (**1**): White powder; ^{1}H-NMR (500 MHz, Pyr-d_5) δ_H: 11.6 (1H, s, OH), 8.21 (1H, s, H-8), 4.36 (2H, d, J=5.45Hz, H-1′), 4.03 (1H, dd, J=6.5, 12.75 Hz, H-3′), 3.95 (1H, dd, J=4.30, 12.75 Hz, H-3′), 3.51 (1H, m). ^{13}C-NMR (125 MHz, Pyr-d_5) δ_C: 170.9 (s, C-4′), 156.3 (s, C-6), 149.5 (s, C-2), 139.2 (s, C-4), 136.1 (d, C-8), 111.6 (s, C-5), 42.6 (t, C-1′), 39.9 (t, C-3′), 36.5 (d, C-2′). IR (KBr): 3501, 3115, 1740, 1710, 1670, 1658 cm^{-1}. UV (MeOH) λ_{max} (log ε) 210 (3.87), 262 (3.96). HR-FAB-MS *m/z*: 237.0619 [M+H]$^+$ (Calcd for $C_9H_9N_4O_4$: 237.0623). FAB-MS(+) *m/z*: 236 [M+H]$^+$. $[\alpha]_D^{24}$ 30.7° (c=0.084 in MeOH).

7,9-Dihydro-1-(3-oxobutyl)-1*H*-purine-6,8-dione (**2**): White powder; ^{1}H-NMR (500 MHz, Pyr-d_5) δ_H: 8.09 (1H, s, H-2), 4.58 (2H, t, J=6.45 Hz, H-1′), 3.22 (2H, t, J=6.45 Hz, H-2′), 2.04 (3H, s, H-4′). ^{13}C-NMR (125 MHz, Pyr-d_5) δ_C: 205.9 (s, C-3′), 157.0 (s, C-6), 153.1 (s, C-8), 151.1 (s, C-4), 143.1 (d, C-2), 107.4 (s, C-5), 43.7 (t, C-2′), 41.8 (t, C-1′), 29.7 (q, C-4′). IR (KBr): 3501, 3115, 1740, 1710, 1670, 1658 cm^{-1}. UV (MeOH) λ_{max} (log ε) 212 (3.51), 264 (3.65). HR-FAB-MS *m/z*: 221.0750 [M−H]$^-$ (Calcd for $C_9H_9N_4O_3$ 221.0753). ESI-MS(−) *m/z*: 221 [M−H]$^-$.

7-Hydro-9-(3-oxobutyl)-1*H*-purine-6,8-dione (**3**): White powder; ^{1}H-NMR (500 MHz, Pyr-d_5) δ_H: 8.27 (1H, s, H-2), 4.66 (2H, t, J=7.5 Hz, H-1′), 3.15 (2H, t, J=7.5 Hz, H-2′), 2.09 (3H, s, H-4′). ^{13}C-NMR (125 MHz, Pyr-d_5) δ_C: 206.5 (s, C-3′), 156.3 (s, C-6), 152.2 (s, C-8), 150.1 (s, C-4), 140.6 (d, C-2), 108.7 (s, C-5), 42.0 (t, C-2′), 38.3 (t, C-1′), 29.7 (q, C-4′). IR (KBr): 3501, 3115, 1740, 1710, 1670, 1658 cm^{-1}. UV (MeOH) λ_{max} (log ε) 212 (3.52) , 264 (3.65). HR-FAB-MS *m/z*: 221.0749 [M−H]$^-$ (Calcd for $C_9H_9N_4O_3$ 221.0753). ESI-MS(−) *m/z*: 221 [M−H]$^-$.

Acknowledgment The authors are grateful to the Hi-tech Research and Development Program of China (grant SQ2007AA09Z435255), National Science Foundation of China (grant 20502027), Knowledge Innovation Program of the Chinese Academy of Science (grant KZCX2-YW-216-1), and Science and Technology Foundation of Guangdong Province (grant 2007B030300003) for financial support.

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