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DITERPENOIDS AND STEROIDS FROM GORGONIAN *Subergorgia mollis*

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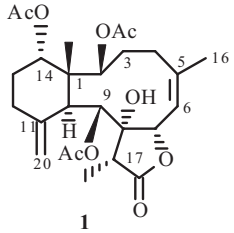
Gorgonian corals of the genus *Subergorgia* are distributed widely in the tropical and subtropical waters of the Indo-Pacific Ocean [1]. Previous studies on the chemical constituents of the genus *Subergornia*, especially *S. reticulate* and *S. suberosa*, have led to the isolation of a number of compounds, such as sesquiterpenes [2–4], diterpenoids [5, 6], steroids [1, 7], and alkaloids [8, 9], some of which showed cytotoxicity toward various cell lines [2, 4, 6] and anti-settlement activity against the larva of *Bugula neritina* [5]. There was only one report on the chemical study of *S. mollis*, which afforded two pregnane derivatives from this species occuring in Taiwanese tropical waters [10]. In a further search for bioactive compounds from the same species, we carried out an investigation of the secondary metabolites of *S. mollis* from the South China Sea, resulting in the isolation of 10 compounds including three briarane-type diterpenoids (**1**–**3**), four rare 24-ketal steroids (**4**–**7**), and three common steroids (**8**–**10**). Most of the isolated compounds showed significant activities, including antifouling activity against barnacle larval settlement, toxicity towards zebrafish embryo, and microalgae growth-inhibition activity.

On the basis of spectral data and by comparison with those reported in the literatures, the structures of compounds **1**–**10** were established as umbraculolide A (**1**) [11], reticulolide (**2**) [5], junceellin A (**3**) [12], suberoretisteroid A (**4**) [13], suberoretisteroid B (**5**) [13], suberoretisteroid C (**6**) [13], 3,22,25-trihydroxy-16-24,20-24-bisepoxy-(3β,16β,20*R*,22*R*,24*S*)-cholest-5-ene (**7**) [1, 14], (22*E*,24*R*)-cerevisterol (**8**) [15], (24*R*)-24-methyl-5α-cholest-7-ene-3β,5α,6β-triol (**9**) [16], and cholesterol (**10**) [17]. Compounds **4**–**7**, which are attributed to polyoxygenated steroids with a 24-ketal function group, are infrequent in marine sources and are found only in *S. reticulate* from the Subergorgiidae family and *Gorgonella umbraculum* from the Ellisellidae family [1, 13, 14]. Therefore, it was deduced that a similar metabolic pathway may exist in some species between these two families. Furthermore, the results of our analysis of chemical constituents differed from those of the same species collected from a different area, the Taiwanese Sea area, which may be due to differences in the biosynthetic system in different sea areas and season changes.

Assessment of Bioactivities. Previous study on the defensive substances from gorgonians have indicated that diterpenoids and steroids are important secondary metabolites of gorgonians playing a significant role in chemical defensive functions. Moreover, these defensive substances can be the source of new drug lead compounds from marine organisms [18]. Therefore, the promising ecological activities of diterpenoids and steroids inspired us to evaluate the antifouling activity, toxicity towards zebrafish embryo, and microalgae growth-inhibition activity of isolated compounds.



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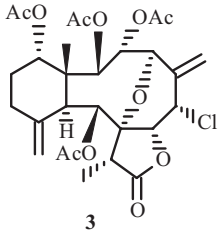

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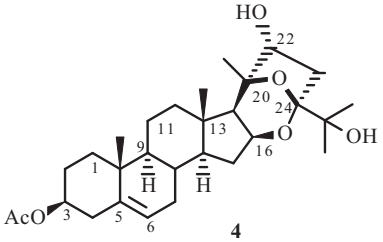

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TABLE 1. Toxicological Endpoints for Compounds on the Embryo of Zebrafish *D. rerio* (EC_{50} μg·mL^{-1})

Compound	Toxicological endpoints					
	Coagulation of egg	Spontaneous movement	Tail malformation	Heart malformation	Death	Delayed hatching
	24 h		48 h		72 h	
4	37.42	–	–	–	18.78	–
5	42.58	11.30	12.66	20.92	7.87	13.30
6	–	32.74	–	–	19.30	23.54
8	–	–	–	–	43.82	43.73

–: No such toxicological endpoinds.

Compounds **4**–**7** with the 24-ketal function group all showed potent antifouling activity against larval settlement of the barnacle *Balanus amphitrite* at nontoxic concentrations with EC_{50} values of 1.25–2.5, 7.91, 7.31, and 0.81 μg·mL^{-1}, respectively. Compound **2**, belonging to a briarane-type diterpenoid, showed strong inhibition on larval settlement of *B. amphitrite* with an EC_{50} value of 0.35 μg·mL^{-1}. The EC_{50} values of compounds **2**, **4**–**7** were lower than the standard requirement of an EC_{50} of 25 μg·mL^{-1} established as an effective level for natural antifoulants [19], highlighting their potential role as natural antifouling agents. Other compounds did not exhibit obvious antifouling activity at a concentration of 100 μg·mL^{-1}.

According to the results of toxicity toward the embryo of zebrafish *Danio rerio*, the steroids exhibited particular zebrafish toxicity (Table 1). It was noteworthy that compound **5** displayed potent toxicity with EC_{50} values of 7.87 μg·mL^{-1} (72 h death) and 13.3 μg·mL^{-1} (72 h delayed hatching), while compounds **4**, **6**, and **8** showed moderate toxicities, and many toxicological endpoints could also be observed. In contrast to the above compounds, the rest of the compounds were found to be practically inactive at a concentration of 50 μg·mL^{-1}.

Furthermore, compound **4** exhibited moderate inhibition of the growth of microalgae *Karenia mikimotoi* with an EC_{50} value of 40–50 μg·mL^{-1}. However, the remaining compounds showed weaker inhibition activity at a concentration of 50 μg·mL^{-1}.

The preliminary study on structure-activity relationship indicated that the acetoxy groups at C3 or C22 in 24-ketal steroids could increase the toxicity but lessen their antifouling activity. It could be proposed that these two regions might serve as potential positions for fine-tuning biological performance. In addition, compared with compound **1**, compound **2** had an obvious effect on enhancement of antifouling activity, revealing that a chlorine atom conjugated with C16 may be a significant active position.

Animal Material. The gorgonian coral *S. mollis* (Nutting, 1910) was collected from the Sanya coral reef in the South China Sea at a depth of about 10 m in September 2006, and was identified by Hui Huang, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen was deposited at the Key Laboratory of Marine Drugs, Ministry of Education, the School of Medicine and Pharmacy, Ocean University of China.

Extraction and Isolation. The sliced fresh gorgonian coral *S. mollis* (480.0 g) was extensively extracted with 95% ethanol (4000 mL × 5) at room temperature, and the combined solution was evaporated to dryness under vacuum (3.5 g). The residue was subjected to column chromatography on silica, using petroleum ether–EtOAc (from 10:0 to 0:10) as eluent. By combining the fractions with TLC monitoring, eight fractions were obtained. Fractions 2–5 and 7 were then isolated by repeated column chromatography on silica gel and Sephadex LH-20 and purified by semi-preparative HPLC, leading to the isolation of compounds **1** (11.2 mg), **2** (7.8 mg), **3** (3.6 mg), **4** (6.5 mg), **5** (8.9 mg), **6** (4.6 mg), **7** (9.6 mg), **8** (4.2 mg), **9** (3.4 mg), and **10** (212.7 mg).

Umbraculolide A (1). $C_{26}H_{36}O_9$, colorless needle crystals, mp 214–216°C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.65 (1H, d, J = 9.6, H-6), 5.32 (1H, d, J = 6.0, H-9), 5.29 (1H, d, J = 9.6, H-7), 5.04 (1H, s, H-20a), 4.98 (1H, s, H-20b), 4.84 (1H, br.s, H-2), 4.66 (1H, dd, J = 4.3, 1.8, H-14), 3.43 (1H, d, J = 6.0, H-10), 2.59 (1H, m, H-3a), 2.45 (1H, q, J = 7.2, H-17), 2.32 (2H, m, H-13), 2.20 (3H, s, acetate methyl), 2.17 (1H, m, H-3b), 2.05 (2H, m, H-4), 2.04 (3H, d, J = 1.2, H-16), 1.98 (3H, s, acetate methyl), 1.91 (3H, s, acetate methyl), 1.81 (2H, m, H-12), 1.12 (3H, s, H-15), 1.12 (3H, d, J = 7.0, H-18). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 176.1 (C-19), 170.5 (acetate carbonyl), 170.4 (acetate carbonyl), 169.5 (acetate carbonyl), 150.9 (C-11), 145.2 (C-5), 120.3 (C-6), 113.1 (C-20), 82.9 (C-8), 78.0 (C-2), 74.4 (C-14), 74.3 (C-7), 71.3 (C-9), 46.8 (C-1), 42.5 (C-17), 41.8 (C-10), 31.3 (C-13), 29.1 (C-3), 26.7 (C-4), 26.8 (C-16), 26.2 (C-12), 21.7 (acetate methyl), 21.2 (acetate methyl), 21.1 (acetate methyl), 15.6 (C-15), 6.5 (C-18).

Reticulolide (2). $C_{26}H_{35}ClO_9$, white powder, mp 145–148°C, ESI-MS *m/z* 549/551 $[M + Na]^+$.

Junceellin A (3). $C_{28}H_{35}ClO_{11}$, colorless needle crystals, mp 289–291°C. ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.14 (1H, dd, J = 13.2, 7.8, H-3), 5.93 (1H, s, H-9), 5.56 (1H, d, J = 2.4, H-16a), 5.43 (1H, d, J = 7.8, H-2), 5.36 (1H, d, J = 2.4, H-16b), 5.10 (1H, s, H-20a), 5.02 (1H, d, J = 3.0, H-6), 4.97 (1H, br.t, J = 3.0, H-14), 4.77 (1H, s, H-20b), 4.51 (1H, d, J = 3.0, H-7), 4.48 (1H, d, J = 13.2, H-4), 3.11 (1H, s, H-10), 2.78 (1H, q, J = 7.2, H-17), 2.32 (3H, s, acetate methyl), 2.31 (1H, m, H-12a), 2.25 (1H, m, H-12b), 2.07 (3H, s, acetate methyl), 2.05 (3H, s, acetate methyl), 2.00 (3H, s, acetate methyl), 1.82 (1H, m, H-13a), 1.70 (1H, m, H-13b), 1.29 (3H, d, J = 7.0, H-18), 1.12 (3H, s, H-15).

Suberoretisteroid A (4). $C_{29}H_{44}O_6$, colorless needle crystals, mp 271–274°C. ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.38 (1H, d, J = 6.0, H-6), 4.59 (1H, m, H-3), 4.51 (1H, m, H-16), 3.86 (1H, dd, J = 13.2, 6.0, H-22), 2.73 (1H, dd, J = 16.8, 13.2, H-23a), 2.32 (2H, m, H-4), 2.14 (1H, m, H-15a), 2.04 (3H, s, acetate methyl), 2.02 (1H, m, H-12a), 1.98 (2H, m, H-7), 1.87 (1H, m, H-2a), 1.86 (1H, m, H-1a), 1.76 (1H, dd, J = 16.8, 6.0, H-23b), 1.60 (1H, m, H-2b), 1.59 (1H, m, H-8), 1.53 (2H, m, H-11), 1.39 (1H, d, J = 7.8, H-17), 1.38 (1H, m, H-15b), 1.37 (3H, s, H-21), 1.24 (3H, s, H-27), 1.20 (1H, m, H-12b), 1.15 (3H, s, H-26), 1.14 (1H, m, H-1b), 1.06 (3H, s, H-18), 1.04 (3H, s, H-19), 0.99 (1H, m, H-14), 0.86 (1H, m, H-9). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 170.7 (acetate carbonyl), 139.7 (C-5), 122.3 (C-6), 107.6 (C-24), 83.3 (C-20), 79.2 (C-22), 73.9 (C-3), 72.6 (C-16), 72.4 (C-25), 54.8 (C-14), 50.0 (C-9), 49.4 (C-17), 41.6 (C-13), 39.5 (C-12), 38.0 (C-4), 37.2 (C-23), 36.9 (C-1), 36.6 (C-10), 33.4 (C-15), 31.7 (C-7), 31.1(C-8), 27.7 (C-2), 24.0 (C-27), 23.4 (C-26), 23.4 (C-21), 21.4 (acetate methyl), 20.4 (C-11), 19.3 (C-19), 14.4 (C-18).

Suberoretisteroid B (5). $C_{29}H_{44}O_6$, colorless needle crystals, mp 148–151°C.

Suberoretisteroid C (6). $C_{31}H_{46}O_7$, colorless needle crystals, mp 191–194°C.

3,22,25-Trihydroxy-16-24,20-24-bisepoxy-(3*β*,16*β*,20*R*,22*R*,24*S*)-cholest-5-ene (7). $C_{27}H_{42}O_5$, colorless needle crystals, mp 236–238°C.

Biological Assays. Antifouling activity against barnacle *B. amphitrite* larval settlement was assayed by the method described by Harder et al. [20]. Toxicity towards the embryo of zebrafish *D. rerio* was studied in accordance with the procedure of [21]. The growth-inhibition activity on microalgae *K. mikimotoi* was performed according to the standard protocols reported previously [22].

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