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Two novel aromatic valerenane-type sesquiterpenes from the Chinese green alga *Caulerpa taxifolia*

Shui-Chun Mao, Yue-Wei Guo* and Xu Shen

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Zu Chong Zi Rd. 555, Zhangjiang Hi-Tech Park, Shanghai 201203, PR China

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Abstract—Caulerpal A (**2**) and B (**3**), two novel sesquiterpenes possessing an uncommon aromatic valerenane-type carbon skeleton, along with one known metabolite, caulerpin (**4**), have been isolated from the Chinese green alga *Caulerpa taxifolia* (Vahl) C. Agardh. Their structures and relative stereochemistry were elucidated on the basis of extensive spectroscopic analysis. Compounds **2**–**4** were evaluated for their inhibitory activity against hPTP1B and the result showed that only compound **4** had a strong PTP1B inhibitory activity with an IC_{50} value of 3.77 μM.

The green alga *Caulerpa taxifolia*, one of a few toxic seaweeds, is widely distributed in tropical and subtropical waters. The metabolite pattern of the alga was extensively characterized by a suite of unusual sesqui- (exemplified by caulerpenyne, **1**[1]) and monoterpenes which were found to be responsible for antimicrobial, cytotoxic, and ichthyotoxic activities.[2–4] Similar metabolites were also isolated from three Mediterranean sacoglossan opisthobranch molluscs[5,6] suggesting the possible prey-predator relationship between the molluscs and the alga.

Recently, in the course of our systematic investigations toward the isolation of bioactive metabolites from Chinese marine organisms,[7–10] we carried out a chemical study on the seaweed *C. taxifolia*, collected along the coast of the East China Sea, since no phytochemical investigation has been done previously on this Chinese species. Careful chromatographic separation of the Et_2O-soluble portion of acetone extract of the alga resulted in the isolation of two novel sesquiterpenes, caulerpals A (**2**) and B (**3**), both possessing an uncommon aromatic valerenane-type carbon skeleton, together with one known metabolite (**4**).[11,12] This paper deals with the isolation and structure elucidation of two novel sesquiterpenes (**2**, **3**) and the biological evaluation of compounds **2**–**4** (Fig. 1).

The algal material was collected from Nanji Island, Zhejiang Province, China, in June 2000, and kept frozen prior to extraction. The fresh alga (150 g dry weight) was exhaustively extracted with acetone (3 × 1 L) in room temperature. The acetone extract was partitioned between Et_2O and H_2O, the organic layer (19.0 g) was subjected to separation by silica gel and Sephadex

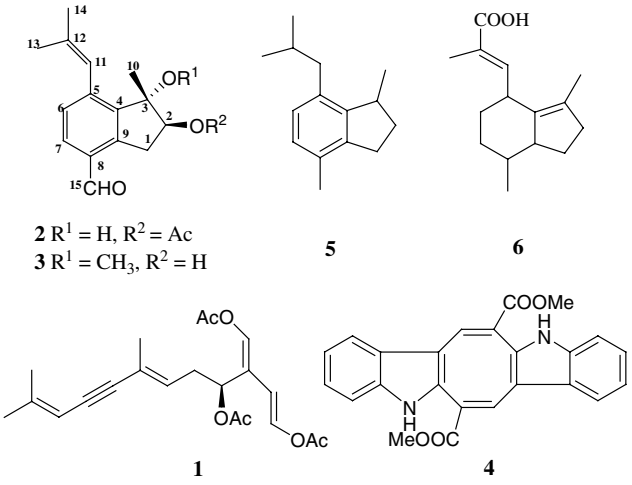

Figure 1. Chemical structures of **2**–**4**.

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* Corresponding author. Tel./fax: +86 21 50805813; e-mail: ywguo@mail.shcnc.ac.cn

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LH-20 column chromatography, followed by purification with C_{18} HPLC to afford two novel sesquiterpenens, named caulerpals A (**2**, 3.2 mg, 0.002% dry weight) and B (**3**, 4.3 mg, 0.003% dry weight), respectively, along with one known metabolite, caulerpin (**4**, 79.3 mg, 0.05% dry weight).[11,12]

Caulerpal A (**2**)[13] was isolated as a colorless oil, $[\alpha]_D^{24}$ +6.0° (*c* 0.12, $CHCl_3$). Its molecular formula, $C_{17}H_{20}O_4$, was deduced from its HRESIMS {*m*/*z* 311.1258 $[M+Na]^+$, Δ −0.1 mmu}. The IR spectrum showed the presence of hydroxyl (3442 cm^{-1}), two carbonyls (1724 and 1691 cm^{-1}), and an aromatic ring (1592, 1513, and 1452 cm^{-1}). Inspection of the ^{13}C NMR spectrum data for **2** revealed the presence of one aldehydic carbonyl, three olefinic linkages, five quaternary sp^2 carbons, one oxygen-bearing quaternary carbon, one methylene, one oxygen-bearing methine, three methyl groups, and an acetoxyl group. The total of 15 carbons, except for acetoxyl, including three methyl groups, indicated a probable sesquiterpene. Two carbonyls and one trisubstituted double bond left five sites of unsaturation, which, bearing in mind the typical IR absorptions for the aromatic ring, were attributed to a bicyclic skeleton. From the ^1H NMR spectrum, the olefinic proton, a broad singlet at δ 6.81, had been on the trisubstituted double bond (H-11). The most downfield signal resonating at δ 10.07 was assignable to an aldehydic proton (H-15). The two doublets resonating at δ 7.31 (1H, d, J = 7.9 Hz, H-6) and 7.68 (1H, d, J = 7.9 Hz, H-7), respectively, clearly indicated that the aromatic ring was 1,2,3,4-tetrasubstituted. Two three-proton singlets at δ 1.82 (H_3-13) and 1.97 (H_3-14) were assigned to the methyl groups attached to a quaternary olefinic carbon (C-12). A singlet at δ 2.20 was obviously attributed to the methyl of an acetate moiety. The ^1H NMR spectrum was completed by signals attributable to an AB-type methylene (δ 3.02, dd, J = 17.4, 8.9 Hz, H_a-1; 3.90, dd, J = 17.4, 8.4 Hz, H_b-1), an oxygen-bearing methine (δ 5.29, dd, J = 8.9, 8.4 Hz, H-2), and a tertiary methyl (δ 1.35, H_3-10), that, following the isoprene rule and bearing in mind the presence of a quaternary carbon at δ 82.2 (C-3), were arranged in a five-membered cycle. Finally, all the ^1H and ^{13}C NMR resonances[13] were ambiguously assigned by applying homo- and hetero-nuclear NMR methodologies. Thus, analysis of ^1H–^1H COSY spectrum readily allowed to recognize three spin–spin systems [H_2-1 to H-2 (ABX system); H-6 to H-7; H-11 to H_3-13, H_3-14]. Long-range proton–proton couplings between the olefinic proton H-11 and H_3-13 and H_3-14 indicated the presence of an isobutylene group. The HMBC experiment (Fig. 2a) of **2** further confirmed the presence of the isobutylene group as judged from diagnostic long-range correlations between H_3-13 and C-11 (δ 121.8), C-12 (δ 138.7), and C-14 (δ 26.9); H_3-14 and C-11, C-12, and C-13 (δ 19.8). The isobutylene group attached to C-5 (δ 141.9) was deduced from the HMBC correlations between H-6 and C-4 (δ 143.0), C-5, C-8 (δ 130.0), and C-11. The HMBC correlations between H-7 and C-8 and C-15 (δ 191.8); H-15 and C-8 showed that the aldehyde function was linked to C-8. The hydroxyl group at δ 3.68 (1H, br s) at C-3 was determined by the HMBC correlations between OH-3 (δ 3.68, br s) and C-3 and C-4. The acetoxyl group was assigned at C-2 (δ 85.5) mainly based on HMBC correlations between H-2 and C-1 (δ 33.9), C-3, C-4, and C-9 (δ 138.3); H_3-10 and C-2 (δ 85.5), C-3, and C-4.

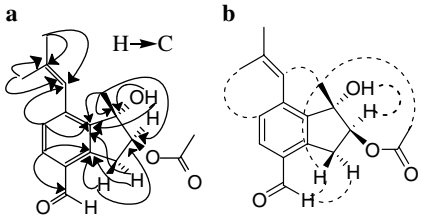

Figure 2. Selected key HMBC correlations (a) and NOESY (---) correlations (b) for caulerpal A (**2**).

The relative stereochemistry at C-2 and C-3 was established by a NOESY experiment (Fig. 2b) running on **2**. The methyl group H_3-10 showed a correlation with the acetyl methyl (OAc-2), while the hydroxyl group (OH-3) was correlated with the methine proton (H-2). These observations indicated that OH-3 and H-2 were α-oriented, while H_3-10 and OAc-2 were consequently β-oriented.

Literature checking revealed that the carbon skeleton of **2** is the same as valerenic acid (**6**),[14] a metabolite isolated previously from the plant *Valeriana officinalis*.[15] However, to the best of our knowledge, sesquiterpene (like compound **2**) possessing an aromatic valerenane skeleton has never been encountered from a natural source though a similar compound **5** was reported as a synthetic intermediate derived in the course of structural determination of **6**.

Caulerpal B (**3**)[16] was obtained as a colorless oil, $[\alpha]_D^{24}$ −6.0° (*c* 0.29, $CHCl_3$). Its molecular formula, $C_{16}H_{20}O_3$, was determined by HRESIMS at *m*/*z* 283.1310 {$[M+Na]^+$, calcd 283.1310}. Like compound **2**, the IR spectrum of **3** showed also absorption bands due to the hydroxyl group (3450 cm^{-1}), an aldehydic carbonyl (1722 cm^{-1}), and aromatic ring (1599, 1543, and 1469 cm^{-1}). The UV absorption pattern of **3** was also the same as that of **2**. Careful comparison of NMR spectra of **2** and **3** revealed that the differences between them occurred only at C-2 (–OAc in **2** and –OH in **3**) and C-3 (–OH in **2** and –OMe in **3**), while the rest of the molecule was the same. Due to deacetylation, the methine proton at C-2 of **3** was reasonably shifted upfield (from δ 5.29 to 4.71), while the methylation of OH-3 caused a downfield shift of C-3 from δ 82.2 to 88.2. Furthermore, the HMBC spectrum showed a correlation between the *O*-methyl (OCH_3-3) and C-3 (δ 88.2), suggesting that the methoxyl group was attached to C-3. The hydroxyl group linked at C-2 (δ 74.6) was confirmed from the HMBC correlations between H-2 (δ 4.71, dd, J = 8.5, 8.0 Hz) and C-1 (δ 33.9), C-3, C-4 (δ 141.9), and C-9 (δ 140.6); H_2-1 (δ 3.84, dd, J = 16.9, 8.0 Hz; 2.84, dd, J = 16.9, 8.5 Hz) and C-2 (δ 74.6) and C-9.