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

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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

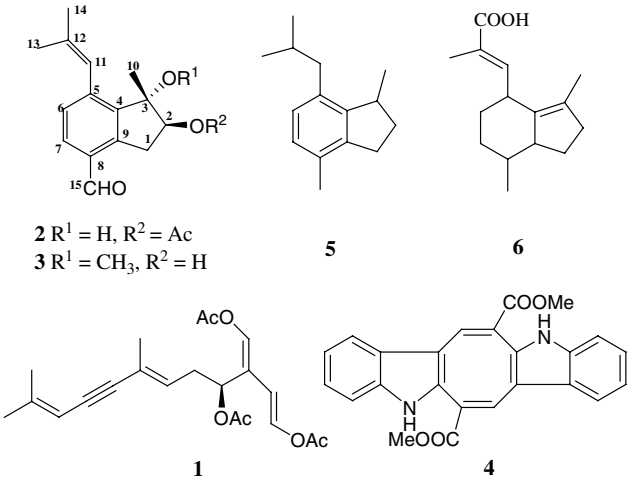

Figure 1. Chemical structures of **2**–**4**.

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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.

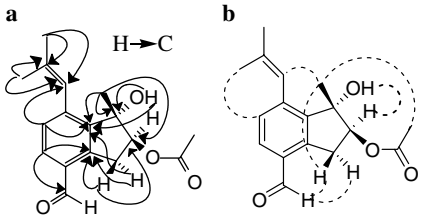


Figure 2. Selected key HMBC correlations (a) and NOESY (---) correlations (b) for caulerpal A (**2**).

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**.

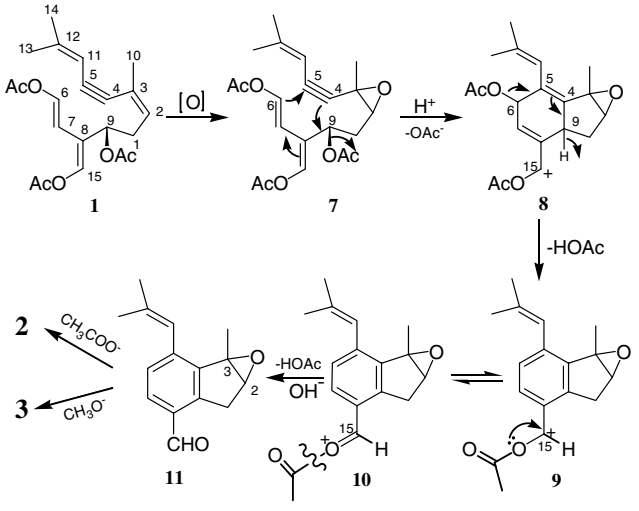
Caulerpal B (**3**)[16] was obtained as a colorless oil, $[\alpha]_D^{24}$ −6.0° (*c* 0.29, $CHCl_3$). Its molecular formula, $C_{16}H_{20}O_3$, was determined by HRESIMS at *m*/*z* 283.1310 {$[M+Na]^+$, calcd 283.1310}. Like compound **2**, the IR spectrum of **3** showed also absorption bands due to 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.

Analogous to **2**, the relative configuration of **3** at C-2 and C-3 was also established by analysis of its NOESY spectrum. The interactions between H-2 and OCH_3-3, and between H_3-10 and H_β-1 (δ 2.84, dd, $J = 16.9$, 8.5 Hz), clearly indicated that OCH_3-3 and H-2 were α-oriented and the methyl group (H_3-10) was consequently assigned as β-configuration. Detailed analysis of its 2D NMR spectra allowed unambiguous assignments of the ^1H and ^{13}C NMR data[16] of **3**.

Sesquiterpenes with an aromatic valerenane-type carbon skeleton are rare in the nature. Compounds **2** and **3** represent the only two examples with such a carbon skeleton from a natural source. It may be worth to point out that the typical enol-acetate terpenes reported previously from this species were not found in this collection. It raises the necessity to check the correctness of taxonomy of the alga, as well as to understand the biosynthetic origin of these metabolites and their real biological role in the life cycle of the alga.

Although compounds **2** and **3** formally displayed a quite different skeleton from that of typical metabolites of *C. taxifolia* (e.g., caulerpenyne **1**[1]), however, they are actually related to each other. To explain the biogenetic origin of caulerpals A (**2**) and B (**3**), a hypothetical pathway is proposed as shown in Scheme 1. Epoxidation of the $\Delta^{2(3)}$ double bond of **1** gives the epoxide **7**. Subsequent cyclization (6–5; 4–9) accompanying the loss of the acetoxyl group at C-9 of **7** leads to intermediate structure **8**. Further loss of second acetoxyl at C-6 of **8** yields aromatic compound **9/10**, which, after cleavage of the bond between carbonyl and oxygen atom at C-15, gives the framework (**11**) of caulerpals. Finally, opening of the epoxide ring by attack of an acetate at C-2 or a methylate at C-3 should produce caulerpals A (**2**) and B (**3**), respectively.

Human protein tyrosine phosphatase 1B (hPTP1B) is regarded as a key target for the treatment of Type-II diabetes and obesity because it could hydrolyze phosphotyrosines on the insulin receptor, deactivating it. For this reason, PTP1B has been the subject of intense study for the past few years.[17] The crude Et_2O-soluble extract of the alga exhibited significant PTP1B inhibitory activity. To trace the responsible compound, **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 cytotoxicity against HL-60 and MCF-7 cell lines was also tested for compounds **2**–**4**, but they showed no significant bioactivity.



Scheme 1. Plausible biogenetic pathway of caulerpals A (**2**) and B (**3**).

Acknowledgments

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–OH), 2.20 (s, –OAc). The chemical shift (ppm) of ^{1}H of $CDCl_3$ was taken as standard at δ 7.26.

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