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Short communication

Laurene-type sesquiterpenes from the Red Sea red alga *Laurencia obtusa* as potential antitumor–antimicrobial agents

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ABSTRACT

Three new laurene-type sesquiterpenes, 12-hydroxy isolaurene (**1**), 8,11-dihydro-12-hydroxy isolaurene (**2**) and isolauraldehyde (**3**) were isolated from the organic extract of the red alga *Laurencia obtusa*. The chemical structures of isolates were determined by interpretation of their spectral data 1D and 2D NMR, UV, IR and MS. The newly isolated compounds were tested for their antimicrobial and antitumor activities. Compounds (**1**–**3**) exhibited potent activity against the Gram-positive *Bacillus subtilis* and *Staphylococcus aureus*, where **3** proved to be the most active (MIC 35 and 27 μg/mL, respectively). Moreover, compound 3 exhibited a significant activity against *Candida albicans* (MIC of 70 μg/mL) and revealed to have very promising activity in an *in vitro* model of Ehrlich ascites Carcinoma.

1. Introduction

Bioactive metabolites originated from marine organisms exhibited different effects on many diseases other than that of the terrestrial counterparts, which may lead to the discovery of new efficient bioactive metabolites with different modes of action [1]. The different locations with diversity of atmosphere led to production of different active substances as well as structures [2].

Laurene-type sesquiterpenes are aryl cyclopentanes substituted with three methyl groups in 1, 2 and 3 fashion. In addition to laurenes, two closely similar sesquiterpene families; cuparenes and laurokamurenes, differ from each other only in the methylation pattern (1, 2, 2 and 2, 2, 3 for cuparenes and laurokamurenes, respectively). On the contrary to the laurenes, the biological activities of several members of the cuparenes family have been examined as antifungal, antibiotic, neurotrophic and anti-lipidperoxidation agents [3].

Among all marine algal flora, species of the genus *Laurencia* are always expected to be a source of novel secondary metabolites, especially sesquiterpenes, diterpenes and halogenated C_{15} acetogenins [4,5]. Several metabolites of *Laurencia* showed noticeable antibacterial [6,7] insecticidal [8] antifungal [9], antiviral activity [10], tyrosine inhibitor [11] and apoptosis inducing or suppressing activity [5], this tempted us to investigate some more chemical constituents of this organism. Laurene sesquiterpenes have been isolated from the red algae of the genus *Laurencia* (Rhodomelaceae, Ceramiales) [12,13]. These were first isolated from the sea hare *Aplysia kurodai* [14] which suggests that this sea hare may consume *Laurencia* sp. and concentrate these sesquiterpenoid compounds in its body [15].

Ehrlich Ascites Carcinoma (EAC) is a common tumor. It is an undifferentiated carcinoma with high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor-specific transplantation antigen (TSTA) [16]. Three methods are applied for cancer therapy; chemotherapy, radiotherapy and surgery. Recently, chemotherapy is the most widely used therapy [17]. The main principle of chemotherapy, which serves as a drug treatment in cancer, is to prevent the growth and progression of tumor cells or to destroy

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them by the effect it has on tumor cells more than the normal cells of the patient without or with minimum side effects [18].

As a part of systematic endeavors to isolate bioactive compounds from the Saudi Red Sea organisms, we investigated constituents of *Laurencia obtusa* collected off the coast of Jeddah, KSA. We report the isolation and characterization of three new laurenes (**1**–**3**) along with one known chamigrene sesquiterpene (**4**), in addition to one known steroid, cholest-4-en-3-one (**5**) obtained from the pet-ether extract of the red alga *L. obtusa*.

2. Results and discussion

2.1. Chemistry

The sequential use of separation techniques including column and preparative TLC of the petroleum ether extract of the red alga *L. obtusa* afforded three new sesquiterpenes **1**, **2** and **3**, in 0.005%, 0.0015% and 0.002% yield (based on dry weight of the algal material), respectively, in addition to known α-chamigrene (**4**) and a steroidal ketone compound (**5**) (Fig. 1).

Compound **1** was obtained as an optically active colorless oil $[\alpha]_D = +41.7$ ($CHCl_3$; $c = 0.01$). The molecular formula of **1**, $C_{15}H_{20}O$ (corresponding to 6 degrees of unsaturation), was deduced from the HRESIMS analysis, *m/z* 215.1423 $[M - H]^+$ in negative-ion mode. The EIMS of **1** showed a molecular ion peak at *m/z* 216. The parent peak at *m/z* 201 corresponding to the formula $C_{14}H_{17}O^+$ arises from the expulsion of a methyl group, and the *m/z* 109, 91 and 77 peaks would be from an aromatic group. The existence of a substituted benzene ring was concluded from UV absorption spectrum, which showed maxima at 273 and 279 nm, supported by IR absorption at 1510 cm^{-1}. Moreover, the IR bands at ν_{max} 3298 and 1637 cm^{-1} were attributed to a hydroxyl and to an isolated double bond, respectively.

The ^1H, ^{13}C and DEPT NMR spectra of **1** showed the presence of 15 carbon atoms (Table 1), including three methyl groups, three methylenes (one oxygenated at δ_H/δ_C 4.62/65.2), four sp^2 methine (δ_C 2 × 126.7 and 2 × 127.1) and five quaternary carbons (δ_C 54.8, 137.2, 132.1, 148.9 and 137.8). From the previous discussion, the structure suggested to be composed of a 1,4-disubstituted benzene ring, extra double bond, primary hydroxyl group and one more ring (to fulfill 6 degrees of unsaturation).

HSQC spectrum indicates the presence of three tertiary methyl groups signals appeared at δ_H/δ_C: 1.36/24.2, 1.41/10.3 and 1.72/14.3, in addition to a *singlet* signal at δ_H 4.62 that was attributed to two protons linked to a carbon atom at δ_C 65.2. Furthermore, the $^1H-^1H$ COSY spectrum supported the existence of 1,4-disubstituted benzene ring through the $^1H-^1H$ spin system between H-7 (11) and H-8 (10) and the long range correlation between H-8 and H_2-12. This allowed us to indicate the presence of 1,4-disubstituted benzene ring together with a 1,2,3-trimethylcyclopentenyl partial structure (Fig. 1).

The spectral data of compound **1** resembles that was reported for isolaurene (**6**) [19], except the appearance of a hydroxyl function. The absence of any aromatic methyl group in compound **1** compared to isolaurene (**6**) and the presence of a hydroxy methyl group together with HMBC correlations between 2H-12 and C-9 in addition to C-8 and C-10 confirmed the location of the carbinol group as *p*-position to the other substitution. Moreover, the HMBC correlations between H_3-13 (δ 1.36) and C-1, C-2, C-5 and C-6 is a further confirmation of the structure of compound (The down field shift of the Me-13 δ_H 1.36, could be attributed to the anisotropic effect of the benzene ring). Thus, in view of the above-mentioned data and discussion, compound **1** has been identified as 12-hydroxy isolaurene.

Compound **2** was obtained as an optically active colorless oil $[\alpha]_D = +11.5$ ($CHCl_3$; $c = 0.01$). The molecular formula of **2**, $C_{15}H_{22}O$, was deduced from the analysis of the HRESIMS *m/z* 217.1579 $[M - H]^+$, in negative-ion mode. The EIMS of **2** showed a molecular ion peak at *m/z* 218. The parent peak at *m/z* 203 corresponding to the formula $C_{14}H_{19}O^+$ arises from the loss of a methyl group, and the *m/z* 185 corresponding to molecular formula $C_{14}H_{17}^+$ arises from the expulsion of water molecule from the $M^+ - CH_3$. The *m/z* 90 and 77

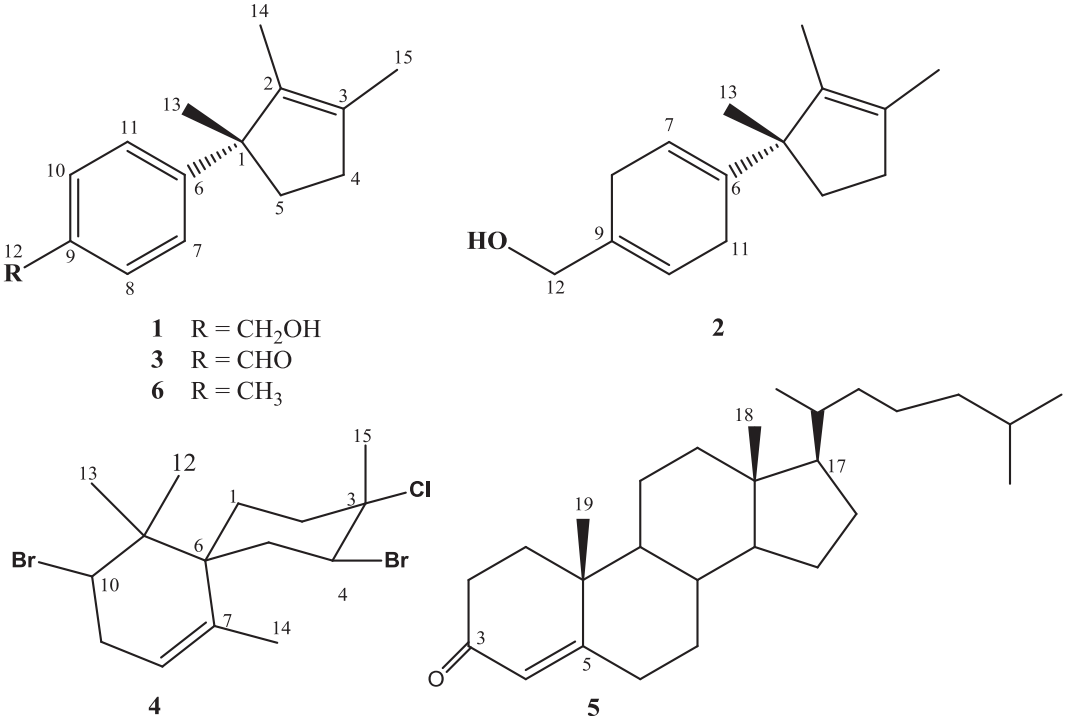

Fig. 1. Structures of compounds **1**–**6**.