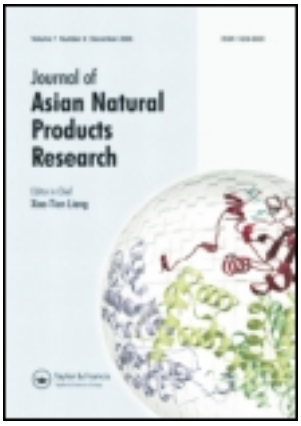

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A novel degraded sesquiterpene from the fresh stem of *Aquilaria sinensis*

Ke Peng [a b c], Wen-Li Mei [b], You-Xing Zhao [b], Le-He Tan [a], Qing-Huang Wang [a] & Hao-Fu Dai [a b]

[a] National Center of Important Tropical Crops Engineering and Technology Research, Chinese Academy of Tropical Agricultural Sciences, Danzhou, 571737, China

[b] Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, China

[c] Luzhou Monitoring Station of Environment Protection, Luzhou, 646000, China

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A novel degraded sesquiterpene from the fresh stem of *Aquilaria sinensis*

Ke Peng[abc1], Wen-Li Mei[b1], You-Xing Zhao[b], Le-He Tan[a], Qing-Huang Wang[a]* and Hao-Fu Dai[ab]*

[a]*National Center of Important Tropical Crops Engineering and Technology Research, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, China;* [b]*Hainan Key Laboratory for Research and Development of Natural Products from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;* [c]*Luzhou Monitoring Station of Environment Protection, Luzhou 646000, China*

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A novel degraded sesquiterpene, named aquilarin B (**1**), together with two known compounds (**2** and **3**), was isolated from the EtOH extract of the fresh stem of *Aquilaria sinensis* (Lour.) Gilg. Their structures were elucidated by spectroscopic methods including 1D and 2D NMR (HMQC, $^{1}H-^{1}H$ COSY, HMBC, and ROESY). The cytotoxic activities of the three compounds against three human tumor cell lines K562, SMMC-7721, and SGC-7901 were evaluated, and compound **3** exhibited obvious cytotoxic activity.

Keywords: *Aquilaria sinensis*; aquilarin B; cytotoxic activity

1. Introduction

Aquilaria sinensis (Lour.) Gilg., belonging to the genus *Aquilaria* of the family Thymelaeaceae, is the only plant resource for Chinese eaglewood, a kind of resinous wood formed in response to injury of cutting, holing, burning, or incursion of moth and microorganism [1,2]. Chinese eaglewood is known as a famous spice, as well as folk medicine used as a sedative, analgesic, and digestive agent [2]. Characteristic sesquiterpenes and 2-(2-phenylethyl) chromone derivatives were the main classes isolated from Chinese eaglewood [3–6], which seemed quite different from the chemical constituents, such as flavones and benzophenone glycosides isolated from the fresh plant of *A. sinensis* [7,8]. Previously, a new benzenoid, several lignans, and flavones were isolated by us from the fresh stem of *A. sinensis* [9,10]. In our continuous effort to search for bioactive components from *A. sinensis*, a novel degraded sesquiterpene, named aquilarin B (**1**), together with two known compounds (**2** and **3**), was isolated, and its structure was elucidated by chemical and spectral means. Compounds **1**–**3** were tested for cytotoxic activities against three tumor cell lines K-562, SMMC-7721, and SGC-7901. In this paper, we describe the isolation, structural elucidation of the new degraded sesquiterpene, as well as their cytotoxic activities against three tumor cell lines.

2. Results and discussion

Compound **1** was obtained as oil, and its molecular formula was assigned to be $C_{13}H_{18}O_3$ from its HR-ESI-MS

*Corresponding authors. Email: hfdai2001@yahoo.com.cn; wqh3687@163.com

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Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectral data of **1** in $CDCl_3$ (δ in ppm; J in Hz).

No.	δ_C	δ_H	No.	δ_C	δ_H
2	69.4 (t)	4.66 (1H, m) 4.40 (1H, m)	9	76.0 (d)	4.22 (1H, m)
3	119.5 (d)	5.93 (1H, d, $J = 2.0$)	10	49.1 (t)	2.83 (2H, d, $J = 5.8$)
4	166.3 (s)		11	206.0 (s)	
5	198.3 (s)		12	31.1 (q)	2.24 (3H, s)
6	53.2 (t)	2.29 (1H, d, $J = 16.4$) 2.21 (1H, d, $J = 16.4$)	13	20.2 (q)	0.97 (3H, s)
7	36.2 (s)		14	29.8 (q)	1.13 (3H, s)
8	56.1 (d)	2.59 (1H, m)			

(*m/z* 245.1160 $[M + Na]^+$, calculated 245.1154) and NMR spectral data (Table 1). The IR spectrum of **1** showed absorption bands due to carbonyl groups (1712 cm^{-1}). The ^{1}H NMR spectrum of **1** suggested the presence of an olefinic proton (δ 5.93, d, $J = 2.0$ Hz) and three methyl singlets at δ 2.24 (3H, s), 1.13 (3H, s), and 0.97 (3H, s) (Table 1). The ^{13}C NMR and DEPT spectra of **1** presented 13 carbon signals for 4 quaternary carbons (δ 36.2, 166.3, 198.3, and 206.0) including 2 carbonyls, 3 methines (δ 56.1, 76.0, 119.5) including 1 oxygenated carbon, 3 methylenes (δ 49.1, 53.2, and 69.4) including 1 oxygenated carbon, and 3 methyl groups (δ_C 20.2, 29.8, and 31.1; Table 1). The corresponding proton resonances were assigned from HMQC spectrum. In the $^{1}H-^{1}H$ COSY spectrum, the correlations of H-9 at δ 4.22 with H-8 at δ 2.59 and H-10 at δ 2.83 determined the chain of C-8 to C-10. In addition, the cross peak from H-2 at δ 4.66 and 4.40 to H-3 at δ 5.93 indicated that C-2 was connected with C-3. In the HMBC spectrum, the methyl proton signal at δ 2.24 (12-Me, s) was correlated with the carbon signals at δ 206.0 (C-11, s) and 49.1 (C-10, t), which suggested that the methyl group (C-12) was connected to C-10 through the carbonyl group (C-11). Furthermore, the pyranoid ring was deduced by the HMBC cross peaks from the methylene protons at δ 4.66 and 4.40 (H-2) to the oxygenated C-9 at δ 76.0 and C-4 at δ 166.3, and from the methine proton at δ 5.93 (H-3) to C-8 at δ 56.1. In addition, both the methyl proton signals at δ 0.97 (13-Me, s) and 1.13 (14-Me, s) were correlated with the carbon signals at δ 53.2 (C-6, t), 36.2 (C-7, s), and 56.1 (C-8, d), which suggested that the two methyl groups, together with C-6 and C-8, were attached to the same quaternary carbon (C-7). The HMBC cross peaks from the methylene protons at δ 2.29 and 2.21 (H-6) to the quaternary carbon at δ 166.3 (C-4) and from the methine proton at δ 2.59 (H-8) to the quaternary carbon at δ 198.3 (C-5) suggested a cyclopentanone structure unit consisting of the five carbons from C-4 to C-8. Thus, the primary structure of **1** was obtained (Figure 1). The relative configuration at the chiral centers in **1** was supported by the ROESY spectrum (Figure 2). The NOE between H-13 (δ 0.97) and H-9 (δ 4.22) indicated that H-13 and H-9 were at the same side, whereas the NOE between H-14 at δ 1.13 and H-8 at δ 2.59 indicated that H-14 and H-8 were β-oriented, and thus the *trans* configuration at C-8 and C-9 was deduced. On the basis of the above evidence, the structure of compound **1** was elucidated and named aquilarin B.

On the basis of the comparison of the ^{1}H and ^{13}C NMR spectral data with those reported in the literatures [11,12], compounds **2** and **3** were determined as phorbol 13-acetate and dihydrocucurbitacin F, respectively.

Compounds **1–3** were evaluated for their cytotoxic activities against K-562,

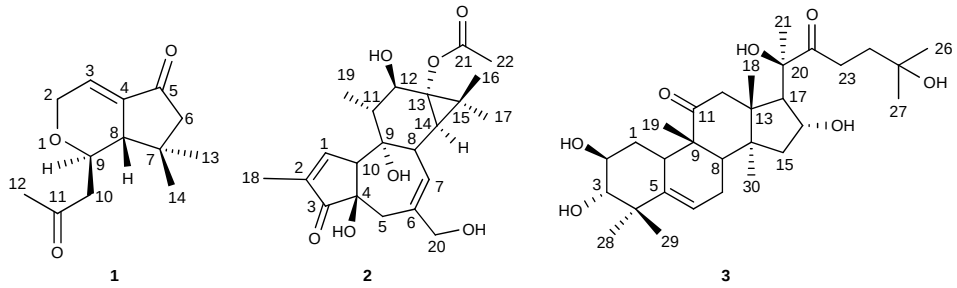

Figure 1. Structures of compounds **1**–**3**.

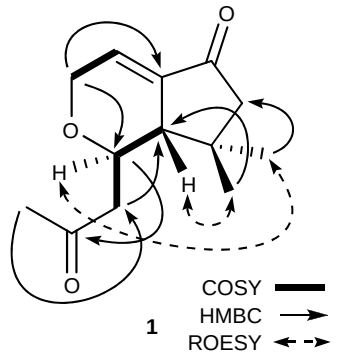

Figure 2. The Key $^{1}H-^{1}H$ COSY, HMBC, and ROESY correlations of compound **1**.

SMMC-7721, and SGC-7901 cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [13], and compound **2** showed cytotoxic activity against SGC-7901 cell lines. Compound **3** showed strong cytotoxic activity against K-562, SMMC-7721, and SGC-7901 cell lines, whereas compound **1** was inactive ($IC_{50} > 100$ $\mu g\,ml^{-1}$; Table 2).

Table 2. *In vitro* cytotoxicities of compounds **1**, **2**, and **3** (IC_{50} values, $\mu g\,ml^{-1}$).

	Compounds			
Cell lines	**1**	**2**	**3**	Mitomycin C[a]
K-562	–	–	0.7	1.6
SMMC-7721	–	–	0.5	2.2
SGC-7901	–	31.2	1.0	8.8

[a] MMC was used as a positive control.

3. Experimental

3.1 General experimental procedures

Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The UV spectrum was measured on a Shimadzu UV-2550 spectrometer. The IR spectrum was obtained on a Nicolet 380 FT-IR instrument from KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS spectrum was measured with an API QSTAR Pulsar mass spectrometer. Column chromatography (CC) was carried out with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). Thin-layer chromatography was carried out with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Plant material

Fresh stems of *A. sinensis* (Lour.) Gilg. were collected in Ding'an county, Hainan province, China, in November 2008, and the plant was identified by Associate Professor Zheng-Fu Dai. A voucher specimen (No. AS200811) has been deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

3.3 Extraction and isolation

The fresh and crushed stems of *A. sinensis* (66.0 kg) were extracted with 95% EtOH

three times at room temperature. After removal of EtOH by evaporation, the extract was suspended in water (10 l) and partitioned with petroleum ether (PE), EtOAc, and *n*-BuOH successively. The EtOAc fraction (66.0 g) was subjected to vacuum liquid chromatography over silica gel, eluting with gradient elution of $CHCl_3$–MeOH (1:0–0:1, v/v) to afford 10 fractions (Fr. 1–Fr. 10). Fr. 1 (22.0 g) was chromatographed on a silica gel column using a step gradient elution of PE–acetone (1:0–0:1, v/v) to afford eight fractions (Fr. 1-1–Fr. 1-8). Fraction 1-5 (2.4 g) was subjected to CC over silica gel using PE–acetone (2:1, v/v) as eluent and further separated by CC over Sephadex LH-20 using $CHCl_3$–MeOH (1:1, v/v) as eluent to afford compound **1** (8.1 mg). Fr. 2 (25.0 g) was chromatographed on a silica gel column using a step gradient elution of PE–acetone (1:0–0:1, v/v) to afford eight fractions (Fr. 2-1–Fr. 2-8). Fr. 2-4 (6.5 g) was separated by CC over Sephadex LH-20 to yield compound **2** (18.1 mg). Fr. 4 (2.2 g) was further separated by silica gel using $CHCl_3$–MeOH (25:1, v/v) and Sephadex LH-20 using $CHCl_3$–MeOH (1:1, v/v) to obtain compound **3** (19.0 mg).

*3.3.1 Aquilarin B (**1**)*

Oil; $[\alpha]_D^{25} + 26.4$ ($c = 0.8$, $CHCl_3$); UV (MeOH): λ_{max} ($\log \varepsilon_{max}$): 226 (1.30), 214 (1.09) nm; IR ν_{Max}^{KBr}: 3626, 2946, 2257, 1712 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 245. 1160 $[M + Na]^+$ (calculated for $C_{13}H_{18}O_3Na$, 245.1154).

3.4 Cell cultures and in-vitro cytotoxicity assay

Each compound was dissolved in dimethyl sulfoxide to make a stock solution having the concentration of 10 mM, which was then diluted to the required concentrations with RPMI 1640 medium (Beijing Xin Jing Ke Biotechnology Co. Ltd, Beijing, China). Cytotoxicities of compounds **1**–**3** against three human tumor cell lines K562 (human myeloid leukemia cell line), SMMC-7721 (human hepatoma cell line), and SGC-7901 (human gastric cell line) were measured. Cells were plated in 96-well plates for 24 h with an initial density of 5000 cells/well and continuously exposed to different concentrations of compounds **1**–**3** for 72 h, with Mitomycin C (MMC; Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) as the positive control. Inhibition rates of cell proliferation after treatment were determined by MTT method [13]. IC_{50} was calculated as the concentration (μg/ml) of samples causing 50% inhibition of cell viability. The IC_{50} values are listed in Table 2.

Acknowledgements

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Note

1. Co-first author.

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