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Three new sesquiterpenoids from agarwood of *Aquilaria crassna*

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ABSTRACT

Three new sesquiterpenoids (**1**–**3**), together with two known ones were isolated from the EtOAc extract of agarwood originating from *Aquilaria crassna*. The new compounds were elucidated on the basis of spectroscopic techniques (UV, IR, MS, 1D and 2D NMR). Compounds **1**–**5** were isolated from agarwood of *A. crassna* for the first time. In the acetylcholinesterase inhibition experiment of **2**–**5**, compound **3** showed acetylcholinesterase inhibition activity (IR 42.9 ± 0.6%). Compound **5** expressed antibacterial activities against *Staphylococcus aureus* and *Ralstonia solanacearum* with diameter of the inhibition zones of 12.35 ± 0.11 mm and 16.90 ± 0.09 mm, respectively.

1. Introduction

Agarwood, the resin of the plant from *Aquilaria* species in Thymelaeaceae family [1], has been used to relieve gastric problems, coughs, rheumatism and high fever [2] as traditional Chinese medicine. Besides, it has a series of pharmacological functions, such as sedative [3], antibacterial [4], antitumor [5], neuroprotective [6], and anti-inflammatory [7]. So far, some sesquiterpenoids and 2-(2-phenylethyl)chromone derivatives as predominant constituents of agarwood especially from agarwood of *Aquilaria sinensis* (Lour.) Gilg and *Aquilaria malaccensis* Lam. have been reported [4,8–10]. However, natural products from wild agarwood originating from *Aquilaria crassna* Pierre ex Lecomte were rarely reported. Previously, three new diepoxy tetrahydrochromones were isolated from intentionally injured *A. crassna* wood [11]. Our previous chemical investigation on the wild agarwood sample originating from *A. crassna* have led to the isolation of ten known 2-(2-phenylethyl)chromone derivatives, such as 6-methoxy-2-(2-phenylethyl)chromone, 2-[2-(4-methoxyphenyl)ethyl]chromone and 6,7-dimethoxy-2-(2-phenylethyl)chromone [12]. In an effort to search for new bioactive compounds from wild agarwood, a follow-up chemical study on this sample led to the discovery of three new compounds (1–3), along with two known ones (7α-H-9(10)-ene-11,12-epoxy-8-oxoeremophilane (4) [13] and 5-desoxylongilobol (5) [14]), which were identified by comparison of spectroscopic data with those reported in the literatures. Compounds **1**–**5** were isolated from agarwood originating from *A. crassna* for the first time. All the compounds except **1** were tested *in vitro* for the acetylcholinesterase inhibition activities and antibacterial activities. Herein, the details of the isolation, structure elucidation and bioactivity of the compounds were presented (Fig. 1).

2. Experimental

2.1. General experimental procedures

UV spectra were recorded on Shimadzu UV-2550 spectrometer (Beckman, America) and optical rotations were obtained on Rudolph Autopol III polarimeter (Rudolph, Flanders, America). CD spectra were recorded with a J-815 spectrometer (JASCO, Tokyo, Japan). IR absorptions were measured by a Nicolet 380 FT-IR instrument (Thermo, America) using KBr pellets. 1D and 2D NMR spectra were performed on Bruker AV III 500 spectrometers. HRESIMS were determined by API Qstar Pulsar mass spectrometer (Bruker, Germany). Column chromatography were used with silica gel (60–80, 200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China), ODS gel (20–45 mm, Fuji Silysia

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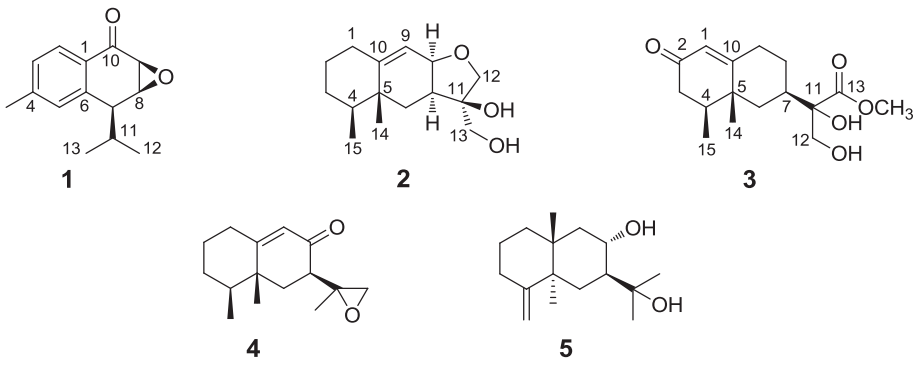

Fig. 1. The structures of compounds **1**–**5**.

Chemical Co. Ltd.), as well as Sephadex LH-20 (Merck, Darmstadt, Germany). TLC were carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.).

2.2. Plant material

A. crassna trees, light-green leaves, alternate, shiny but not reflecting sunshine, elliptic with a marked tip, and by branches which bend 45° upwards, were further distinguished from other species by smooth bark, often with spots, and small open crown [15]. The agarwood chips of *A. crassna* were collected from three trees with the diameter of 67.32 cm, 52.86 cm and 39.91 cm, respectively, in Laos, in September 2014. Based on physical properties, trunks and leaves feature of *A. crassna* tree, the identification of agarwood originating from *A. crassna* has been done by Dr. Jun. Wang , Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 20140909) was deposited.

2.3. Extraction and isolation

Air-dried agarwood originating from *A. crassna* was refluxed with 95% EtOH (10.0 L × 3). The EtOH extract (296.8 g) was suspended in H_2O (4.0 L) and partitioned with EtOAc (4.0 L × 3) and then n-BuOH (4.0 L × 3). The EtOAc extract (196.0 g) was applied to silica gel (10 × 55 cm) vacuum liquid chromatography and eluted with $CHCl_3$–MeOH (1:0, 50:1, 25:1, 15:1, 10:1, 5:1, 2:1, 1:1, 0:1, 6 L of each, *v*/*v*) to provide 8 fractions (Fr.1–8). Fr.3 (7.3 g) was applied to ODS gel (5 × 40 cm) eluting with MeOH–H_2O (2:3, 1:1, 3:2, 7:3, 4:1, 9:1, 1:0, 2.0 L of each, *v*/*v*) and Sephadex LH-20 gel (3 × 150 cm) with $CHCl_3$–MeOH (1:1, *v*/*v*), followed by silica gel (1.2 × 50 cm) eluting with petroleum ether–EtOAc (50:1, *v*/*v*) to afford compounds **1** (1.0 mg) and **4** (2.0 mg). Then, Fr.4 (25.0 g) was subjected to ODS gel (5 × 40 cm) eluting with MeOH–H_2O (3:7, 2:3, 1:1, 3:2, 7:3, 4:1, 9:1, 1:0, 5.0 L of each, *v*/*v*) to yield Fr.4-1–Fr.4-8. Fr.4-1 (983.3 mg) and Fr.4-2 (1.8 g) were partly purified using Sephadex LH-20 gel (3 × 150 cm) with MeOH, after that Fr.4-1-1 (196.2 mg) purified by silica gel (1.2 × 50 cm) eluting with $CHCl_3$–EtOAc (5:1, *v*/*v*) to attain compounds **2** (8.0 mg) and **3** (5.1 mg), Fr.4-2-1 (112.0 mg) was chromatographed on silica gel (1.2 × 50 cm) eluting with $CHCl_3$ to gain compound **5** (11.5 mg).

2.3.1. (7β,8β,9β)-8,9-Epoxycalamenen-10-one (**1**)

Pale yellow oil; $[\alpha]_D^{28}$ +49 (*c* 0.05, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 240 (4.72), 262 (4.89) nm; IR (KBr) ν_{max} 3481, 1634 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HRESIMS *m*/*z* 217.1225 $[M + H]^+$ (calcd. 217.2863 for $C_{14}H_{17}O_2$).

Table 1
^{13}C NMR (125 MHz) and ^1H NMR (500 MHz) data of compounds **1**–**3** in $CDCl_3$ (δ in ppm, *J* in Hz).

	1		**2**		**3**	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	144.5 C		32.3 CH_2	2.01 m 2.32 d, (4.6)	125.6 CH	5.81 s
2	127.1 CH	7.77 d, (7.9)	30.9 CH_2	1.47 d, (4.4) 2.29 d, (4.6)	199.4 C	
3	128.6 CH	7.18 d, (7.9)	29.8 CH_2	1.85 dd, (4.4, 2.2) 1.88 dd, (4.6, 2.2)	42.3 CH_2	1.77 d, (2.5) 2.32 d, (6.5)
4	128.7 C		38.1 CH	1.55 m	40.0 CH	2.55 dd, (6.5, 2.5)
5	130.3 CH	7.01 s	39.4 C		35.2 C	
6	140.4 C		31.1 CH_2	1.04 d, (13.8) 1.77 dd, (13.8, 4.3)	24.5 CH_2	1.84 d, (10.2) 2.17 dd, (10.2, 5.9)
7	45.7 CH	3.48 d, (3.1)	43.1 CH	2.05 dd, (4.9, 4.3)	35.4 CH	2.19 m
8	54.6 CH	3.79 dd, (4.0, 3.1)	75.1 CH	4.46 t, (4.9)	28.6 CH_2	1.22 d (7.7) 2.47 m
9	55.2 CH	3.66 d, (4.0)	115.0 CH	5.54 d, (4.9)	33.6 CH_2	2.22 dd, (17.5, 7.7) 2.27 d, (17.5)
10	200.7 C		154.6 C		173.8 C	
11	33.5 CH	2.05–2.12 m	84.3 C		80.0 C	
12	19.8 CH_3	0.92 d, (7.0)	75.6 CH_2	3.74 dd, (9.9, 5.3) 3.92 d, (9.9)	66.3 CH_2	3.73 s
13	19.2 CH_3	0.85 d, (7.0)	64.3 CH_2	3.65 dd, (10.8, 5.3) 3.80 d, (10.8)	175.4C	
14	22.0 CH_3	2.39 s	20.3 CH_3	0.95 s	19.3 CH_3	1.05 s
15			15.8 CH_3	0.82 d, (6.6)	15.5 CH_3	0.98 d (6.6)
OCH_3					53.4	3.82 s