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Four new bi-phenylethylchromones from artificial agarwood

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ABSTRACT

Four new bi-phenylethylchromones (**1–4**) were isolated from the EtOAc extract of artificial agarwood induced by holing method originating from *Aquilaria sinensis* (Lour.) Gilg. The structures of new compounds were unambiguously elucidated by one- and two-dimensional NMR and HRESIMS measurements, and the absolute configuration was determined by analysis of circular dichroism (CD) spectra. All compounds were tested for acetylcholinesterase (AChE) inhibitory activity using modified Ellman's colorimetric method and α-glucosidase inhibitory activity using PNPG method. Compounds **2–4** exhibited different levels of inhibitory activity against AChE with the inhibition ratios in the range of 10–45%. However, none of the compounds was active against the α-glucosidase.

1. Introduction

Agarwood is the fragrant resinous heartwood harvested from the *Aquilaria* species of the family Thymelaeaceae, which was mostly distributed in China, Japan, India and Southeast Asian countries [1,2] and was widely used in incenses, perfumes, traditional medicines and other products in the world market [3]. *Aquilaria sinensis* (Lour.) is the main plant resource of Chinese agarwood, and the wild agarwood was close to exhausted due to the condition particularity of formation and a long period of time required for agar to accumulate in the wound tissues of trees [4,5], coupled with the increase of commercial demand resulted in the anthropogenic deforestation. Fortunately, the holing method has widely been accepted recently to produce artificial agarwood attributed to the good quality of the product and the lasting aroma of its ether extract [5,6]. Despite the commercial and biological significance of artificial agarwood, only a few researches on chemical constituents was conducted, which led to a series of sesquiterpenoids and 2-(2-phenylethyl)chromones [7–11].

Though 2-(2-phenylethyl)chromones were widely distributed in agarwood, and was considered as one of diagnostic components of agarwood quality [8–9,12–13], the research progress about phenylethylchromone polymers (including dimer and trimer) was slow that no more than eleven analogues have been reported since the first one was isolated from the Kalimantan agalwood named “Jinko” and characterized by Iwagoe et al. in 1986 [14–18].

With the aim to study the chemical constituent of artificial agarwood, as well as to expand the diversity of bioactivities and chemical structures of bi-phenylethylchromones, the ongoing chemical investigation on agarwood induced by artificial holing was carried out, which led to the isolation and identification of four new bi-phenylethylchromone derivatives (**1–4**). Their structures were elucidated by extensive UV, IR, 1D and 2D NMR, and the absolute configuration was determined by electronic circular dichroism (ECD) spectroscopic analysis. All compounds were tested for AChE and α-glucosidase inhibitory activity.

2. Experimental

2.1. General

1D and 2D NMR spectra were recorded on Bruker AV III spectrometer (Bruker) at 500 MHz (^{1}H NMR) or 125 MHz (^{13}C NMR), using TMS as an internal standard. HRESIMS spectra were measured with an API QSTAR Pulsar mass spectrometer (Bruker). CD data were collected using a JASCO J-715 spectrophotometer. Optical rotations were measured on a Rudolph Autopol III polarimeter. Semi-preparative HPLC was carried out using a Agilent Technologies 1260 Infinity equipped with a Agilent DAD G1315D detector (λ = 220 and 254 nm) by a reversed-phased column (YMC-packed C_{18}, 5 μm, 250 mm × 10 mm, 4 mL/min). UV spectra were performed on a Shimadzu UV-2550

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spectrometer (Beckman, America). IR absorptions were obtained on a Nicolet 380 FT-IR instrument (Thermo) using KBr pellets, and a πNAP column (COSMOSIL-packed, 5 μm, 250 mm × 10 mm, 4 mL/min). Silica gel (60–80, 200–300 mesh, Qingdao Marine Chemical Co. Ltd.), ODS gel (20–45 μm, Fuji Silysia Chemical Co. Ltd.) and Sephadex LH-20 (Merck) were used for column chromatography. TLC was conducted on precoated silica gel G plates (Qingdao Marine Chemical Co. Ltd.).

2.2. Plant material

After four years of formation by artificial holing into the trunk of *A. sinensis* tree, the agarwood was collected in November 2012 in Yunnan province, China. The identification of original plant as *A. sinensis* was performed by Dr. Jun Wang. Its voucher specimen (no. 20121108) was deposited at Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science.

2.3. Extraction and isolation

The agarwood (4.7 kg, dry weight) was exhaustively extracted three times by refluxing with 95% EtOH, and the EtOH extract (510.0 g) was dispersed in H_2O (2.5 L), then partitioned with EtOAc (2.5 L × 3) and n-BuOH (2.5 L × 3), subsequently. The EtOAc extract (310.0 g) was subjected to vacuum-liquid chromatography (VLC) using silica gel employing a gradient of $CHCl_3$/MeOH (v/v, 1:0, 50:1, 25:1, 15:1, 10:1, 5:1, 2:1, 1:1, 0:1, each 8.0 L) to obtain nine fractions (Fr.1–Fr.9). Fr.3 (23.6 g) was applied to a ODS gel column eluting with a stepwise gradient of 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% MeOH in H_2O to yield Fr.A-G.

Fr.C was divided into two parts (Fr.C1–Fr.C2). Fr.·C2 (1.3 g) was subjected to Sephadex LH-20 column eluded with $CHCl_3$/MeOH (v/v, 1:1) to give eight fractions (Fr.C2-1–C2-8). Fr.C2–2 (480.3 mg) was eluted with a gradient of $CHCl_3$/MeOH (v/v, 120:1) by silica gel and followed by Sephadex LH-20 with petroleum ether (PE)/$CHCl_3$/MeOH (v/v/v, 2:1:1) to give three fractions. Fr.C2–2-1 (73.2 mg) was further purified by silica gel (PE/acetone, 2:1) to obtain the mixture (30.3 mg). The following semi-preparative HPLC separation on a πNAP column (MeOH/H_2O, v/v 70:30; flow rate 4.0 mL/min; UV detection at 208 nm) afforded the optical pure compound **1** (t_R, 24.6 min, 9.5 mg).

Fr.E was divided into four parts (Fr.E1–Fr.E4). Fr.E1 (2.0 g) was separated using a Sephadex LH-20 column eluting with $CHCl_3$/MeOH (v/v, 1:1) and then chromatographed on silica gel column with $CHCl_3$/MeOH (v/v, 100:1) as eluent to give 7 fractions (E1-1–E1-7). Fr.E1–5 (353.7 mg) was separated on Sephadex LH-20 column (MeOH), and then purified by semi-preparative HPLC (C_{18} column; MeOH/H_2O, v/v 65:35; flow rate 4.0 mL/min; UV detection at 204 nm) to afford compound **3** (t_R, 31.0 min, 6.1 mg), and a mixture of compounds **2** and **4** (11.5 mg). The following semi-preparative HPLC separation on a πNAP column (MeOH/H_2O, v/v 80:20; flow rate 4.0 mL/min; UV detection at 226 nm) afforded the optical pure compounds **2** (t_R, 29.5 min, 4.3 mg) and **4** (t_R, 20.3 min, 2.4 mg).

2.3.1. (5S,6R,7S,8R)-2-[2-(4-Methoxyphenyl)ethyl]-5,6,7-trihydroxy-5,6,7,8-tetrahydro-8-{6-methoxy-2-[2-(3‴-methoxy-4‴-hydroxyphenyl)ethyl]chromonyl-7-oxy}chromone (1)

Pale yellow amorphous solid; $[\alpha]_D^{25}$ = + 163.15 (*c* 4.0, MeOH); UV ($CHCl_3$) λ_{max} 242.0 nm; IR (KBr) ν_{max} 3414, 2977, 2926, 1636, 1512, 1431, 1400, 1269, 1086, 1049 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 250 (− 8.17), 280 (+ 10.28) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 695.2100 $[M + Na]^+$ (calcd. 695.2099 for $C_{37}H_{36}NaO_{12}$).

2.3.2. (5S,6R,7S,8R)-2-[2-(4-Methoxyphenyl)ethyl]-5,6,7-trihydroxy-5,6,7,8-tetrahydro-8-{2-[2-(4‴-methoxyphenyl)ethyl]chromonyl-6-oxy}chromone (2)

Pale yellow oil; $[\alpha]_D^{25}$ = + 38.0 (*c* 1.1, MeOH); UV ($CHCl_3$) λ_{max} 242.0 nm; IR (KBr) ν_{max} 3423, 2976, 1638, 1512, 1470, 1432, 1384, 1272, 1213, 1181, 1083, 1050 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 218 (− 17.42), 239 (+ 12.30) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 649.2044 $[M + Na]^+$ (calcd. 649.2044 for $C_{36}H_{34}NaO_{10}$).

2.3.3. (5S,6R,7S,8R)-2-(2-Phenylethyl)-5,6,7-trihydroxy-5,6,7,8-tetrahydro-8-[2-(2-phenylethyl)chromonyl-6-oxy]chromone (3)

Yellow oil; $[\alpha]_D^{25}$ = + 78.3 (*c* 2.5, MeOH); UV (MeOH) λ_{max} 204.0 and 239.0 nm; IR (KBr) ν_{max} 3424, 2977, 1638, 1512, 1470, 1432, 1383, 1272, 1213, 1181, 1083, 1049 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 218 (− 19.35), 243 (+ 14.16) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 589.1836 $[M + Na]^+$ (calcd. 589.1833 for $C_{34}H_{30}NaO_8$).

2.3.4. (5R,6R,7R,8S)-2-(2-Phenylethyl)-5,6,7-trihydroxy-5,6,7,8-tetrahydro-8-[2-(2-phenylethyl)chromonyl-6-oxy]chromone (4)

Colorless oil; $[\alpha]_D^{25}$ = + 32.5 (*c* 0.8, MeOH); UV (MeOH) λ_{max} 204.0 nm; IR (KBr) ν_{max} 3423, 2977, 1638, 1512, 1470, 1432, 1384, 1272, 1213, 1181, 1083, 1050 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 219 (+ 7.48), 243 (− 13.11) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 589.1838 $[M + Na]^+$ (calcd. 589.1833 for $C_{34}H_{30}NaO_8$).

2.4. Bioassay for AChE inhibitory activity

Compounds **1–4** dissolved in DMSO respectively, were tested for AChE inhibitory activity using a spectrophotometric method developed by Ellman [19]. Briefly, 200 μL reaction mixture containing phosphate buffer (pH 8.0), 50 μg/mL test compound, 0.02 U/mL acetylcholinesterase, was incubated for 20 min (30 °C). After that 20 μL of 5, 5′-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent) (2.48 mg/mL) and 20 μL *S*-acetylthiocholine iodide (1.81 mg/mL) were added, and the hydrolysis of acetylthiocholine was monitored at 405 nm for 30 min. Tacrine (Sigma-Aldrich 99%) with final concentration of 0.08 μg/mL was served as positive control, and DMSO with final concentration of 0.1% was served as negative control. The percentage of inhibition was calculated by the equation: % inhibition = (E − S) / E × 100 (E is the absorbance at 405 nm of the solution without test compounds and S is the absorbance at 405 nm of solution with test compounds). The reagents involved in this reaction were purchased from Sigma Chemical. All the reactions were conducted in triplicate. The values are expressed as the mean ± standard deviation (SD).

2.5. Bioassay for in vitro α-glucosidase inhibitory activity

Compounds **1–4** were tested for α-glucosidase inhibitory activity using PNPG method [20–21]. The method optimized by Jong [22] was performed *in vitro* to test the α-glucosidase inhibitory activity of compounds **1–4**. 0.25 mg/mL α-glucosidase (100 μL) was premixed with the testing sample (0.5 mL) in 0.1 mM potassium phosphate buffer (pH 6.8, 0.5 mL), and pre-incubated at 37 °C for 5 min. 2.5 mmol/L PNPG (0.5 mL) was added to initiate the reaction, which was then quenched with 0.2 M solution of Na_2CO_3 (0.75 mL) after further incubation at 37 °C for 15 min. The acarbose (National Institutes for Food and Drug Control, China, 99%) was used as the positive control. The absorbance values (OD) of the reaction mixture were determined spectrophotometrically at 405 nm wavelength.

3. Results and discussion

Chemical examination of EtOAc extract of artificial agarwood originating from *A. sinensis* resulted in the isolation and identification of four new bi-phenylethylchromones.

Compound **1** was obtained as a pale yellow amorphous solid. The