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# Isocoumarins from American cockroach (*Periplaneta americana*) and their cytotoxic activities

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ABSTRACT

Four new isocoumarins (**1**–**4**), along with three known ones (**5**–**7**), were isolated from the 70% ethanol extract of the whole body of the traditional Chinese insect medicine, American cockroach (*Periplaneta americana*). The structures with absolute configurations of new compounds were elucidated by extensive spectroscopic methods in combination with X-ray diffraction experiment and CD analyses. Compounds **3–5** showed significant cytotoxic activities in HepG2 and MCF-7 cells with $IC_{50}$ values in the ranges 6.41–23.91 μM and 6.67–39.07 μM, respectively.



## 1. Introduction

The American cockroach, *Periplaneta americana*, is the largest species of pest insect in family Blattidae. *P. americana* is a well-known worldwide domestic pest, which is native to Africa and has spread throughout world especially in the tropical and subtropical regions [1]. In China, the ethanol extract of the dried whole body of *P. americana* has been used as traditional Chinese medicine for the treatment of blood-stasis syndrome, acne and abdominal mass for hundred years [2]. Recent pharmacological studies demonstrated that the crude extract of *P. americana* showed significant anticancer, anti-inflammation and promoting tissue regeneration activities [3–5]. Previous chemical investigations on *P. americana* mainly focused on the bioactive peptides and enzymes [6–11]. Up to now, however, there is scarcely any literature about its small molecule chemical ingredient. In order to search for the significant bioactivity compounds from *P. americana*, we carried out a systematical isolation on the 70% ethanol extract of the whole body of *P. americana*. As a result, four new isocoumarins, periplanetins A–D (**1**–**4**), along with three known ones, (3*R*)-ethyl-6,8-dihydroxy-7-methyl-3,4-dihydroisocoumarin (**5**) [12], (*R*)-6-hydroxymellein (**6**) [13] and (3*R*)-methyl-7-hydroxymethyl-8-hydroxy-3,4-dihydroisocoumarin-6-*O*-β-D-glucopyranoside (7) [14], were isolated (Fig. 1). Their structures with absolute configurations were established by a combination of NMR, HR-ESI-MS, CD spectra and X-ray diffraction methods. Furthermore, the cytotoxic activities of all isolated compounds on HepG2 and MCF-7 cells were evaluated with the MTT assay. Among them, compounds **3–5** showed significant cytotoxic activities on HepG2 and MCF-7 cells. Herein, the isolation and structural elucidation of these new compounds, as well as the cytotoxic activities of all isolated compounds were described.

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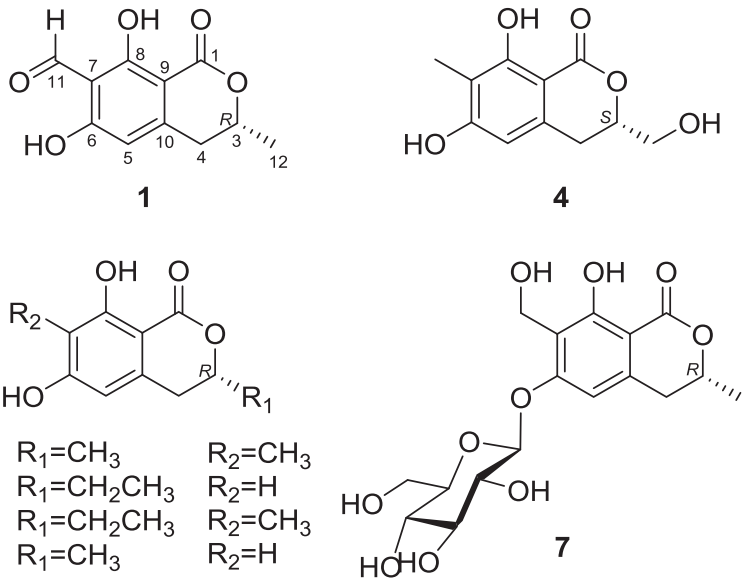


**Fig. 1.** Chemical structures of **1**–**7**.

## 2. Experimental

### 2.1. General

Optical rotations were measured in $CH_3OH$ on a JASCO P-1020 digital polarimeter at room temperature. Melting point was measured on an X-5 melting point apparatus. UV spectra were measured in $CH_3OH$ on a JASCO V-550 UV/VIS spectrophotometer with a 1 cm length cell. IR spectra were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer using KBr pellets. HR-ESI-MS data were obtained on an Agilent 6210 ESI/TOF mass spectrometer and a Waters XeVO G2 Q-TOF mass spectrometer. $^1H$, $^{13}C$, and 2D NMR spectra were measured on Bruker AV-400 and AV-500 spectrometers. CD spectra were obtained on a JASCO J-810 spectropolarimeter at room temperature. Column chromatographic separations were performed on silica gel (300–400 mesh, Qingdao Marine Chemical Group Corporation, Qingdao, P. R. China), macroporous resin Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), reverse-phase $C_{18}$ and $C_8$ gel (Merck, Darmstadt, Germany). TLC analyses were carried out using precoated silica gel $GF_{254}$ plates (Yantai Chemical Industry Research Institute, Yantai, P. R. China). Analytic HPLC was performed on an Agilent chromatography equipped with a G1311C pump and a G1325D diode-array detector (DAD) with a Cosmosil $5C_{18}$-MS-II column (4.6 × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan). Preparative HPLC separations were performed on an Agilent instrument equipped with a G1310B pump and a G1365D UV/VIS detector with a Cosmosil $5C_{18}$-MS-II column (10 × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan).

### 2.2. Insect material

The dried whole bodies of *P. americana* (killing in high-temperature sterilization conditions) were purchased from Weishan American Cockroach Breeding Base in Dali city, Yunnan province of P. R. China. A voucher specimen (No. 2011052501) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, P. R. China.

### 2.3. Extraction and isolation

The dried whole bodies of *P. americana* (2.5 kg) were powdered and extracted with 70% (v/v) EtOH under percolation twice (2 × 25 L, 24 h each) at room temperature. The solution was concentrated under vacuum to yield a residue (210 g), which was suspended in $H_2O$ and subsequently partitioned between $CH_2Cl_2$ and $H_2O$. The $CH_2Cl_2$ extract was evaporated to give a residue (53 g), which was then subjected to silica gel column (10 × 80 cm) eluted with cyclohexane–EtOAc mixtures (100:0 → 0:100, v/v) to afford six major fractions (Fr. A–Fr. F). Fr. D (12 g) was subjected to a reverse-phase $C_{18}$ gel column (3 × 20 cm) eluted with gradient mixtures of MeOH–$H_2O$ (15:85; 30:70; 50:50; 70:30; 85:15, v/v) to afford five subfractions (Fr. D-1–Fr. D-5). Fr. D-2 (232 mg) was then purified by preparative HPLC on a reversed-phase $C_{18}$ column (10 × 250 mm, 5 μm) using MeCN–$H_2O$ (64: 36, 3 mL/min) as eluent to yield **1** (16 mg, $t_R$ = 16.0 min), **3** (9 mg, $t_R$ = 19.3 min), and **4** (19 mg, $t_R$ =18.5 min). Compounds **2** (7 mg, $t_R$ = 18.0 min), **5** (16 mg, $t_R$ = 20.5 min), and **6** (24 mg, $t_R$ = 22.0 min) were obtained from Fr. D-4 (152 mg) by preparative HPLC using MeOH–$H_2O$ (73:27, 3 mL/min) as mobile phase. The $H_2O$ soluble fraction (145 g) was subjected to macroporous resin HP-20 column (15 × 60 cm) eluted with EtOH–$H_2O$ (0:100; 35:65; 70:30; 90:10, v/v) to yield four fractions (Fr. a–Fr. d). Fr. b (43 g) was subjected to reverse-phase $C_8$ gel column (10 × 80 cm) eluted with gradient mixtures of MeOH–$H_2O$ (15:85; 30:70; 50:50, v/v) to afford four subfractions (Fr. b-1–Fr. b-5). Fr. b-3 (2 g) was separated by a Sephadex LH-20 column (2 × 80 cm, MeOH) to afford **7** (9 mg).