

A New Alkaloid from *Ormosia hosiei* Hemsl. et WilsLijun Zheng ^{1,#}, Linjing Zhang ^{2,#}, Jianyong Huang ¹,Xiaoqin Zhang ³, Mingqing Huang ³, Huiyou Xu ² and Lin Ni ^{2,*}¹ Department of Pharmacy, Fujian Medical University Union Hospital, Fuzhou 350001, People's Republic of China² College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, People's Republic of China³ College of Pharmacy, Fujian Key Laboratory of Chinese Materia Medica, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, People's Republic of China

(Received January 29, 2022; Revised March 22, 2022; Accepted April 01, 2022)

Abstract: A new alkaloid, hositisine E (**1**) and nine known compounds (**2-10**) were isolated from the seeds of *Ormosia hosiei* Hemsl. et Wils. Compound **1** was identified on the basis of a combination of UV, IR, NMR, CD and HRESIMS data. The structures of the known compounds were determined as isoprunitin (**2**), biochanin A (**3**), ononin (**4**), 4',8-dimethoxy-7-*O*- β -D-glucopyranosylisoflavone (**5**), sphaerobioside (**6**), ambocin (**7**), rutin (**8**), kaempferol-3-rutinoside (**9**) and narcissin (**10**). In the anti-inflammatory activity assay, compounds **1-3,7** displayed inhibitory effects against lipopolysaccharide-induced interleukin-6 release in RAW264.7 macrophages, exhibiting IC₅₀ values of 19.4 – 58.9 μ M.

Keywords: *Ormosia hosiei*; cytosine-like alkaloid; hositisine; flavonoid; anti-inflammatory; IL-6. © 2022 ACG Publications. All rights reserved.

1. Introduction

The genus *Ormosia* (Leguminosae) has been used for a long time as traditional Chinese medicines and decorations with important medicinal and commercial value [1]. *Ormosia hosiei* Hemsl. et Wils, which is mainly distributed in Fujian, Yunnan, and Sichuan Provinces of People's Republic of China (PRC), has been extensively used in folk medicine to treat rheumatic arthralgia, injuries from falls and irregular menstruations [2]. Previous researches on the *Ormosia* have led to the isolation of alkaloids [3-5], flavonoids [6, 7], lignans [8, 9], triterpenes [5], and volatile oils [10]. And these constituents have been indicated to have anti-inflammatory, anti-tumor, and antifungal effects. In our previous search, some new cytosine-like alkaloids and their biological activities have been investigated from the *Ormosia hosiei* [4, 11]. In our continuing phytochemical study, a new alkaloid (**1**) and nine flavonoids (**2-10**) were isolated (Figure 1) from the seeds of *O. hosiei*, and their anti-inflammatory activities were evaluated.

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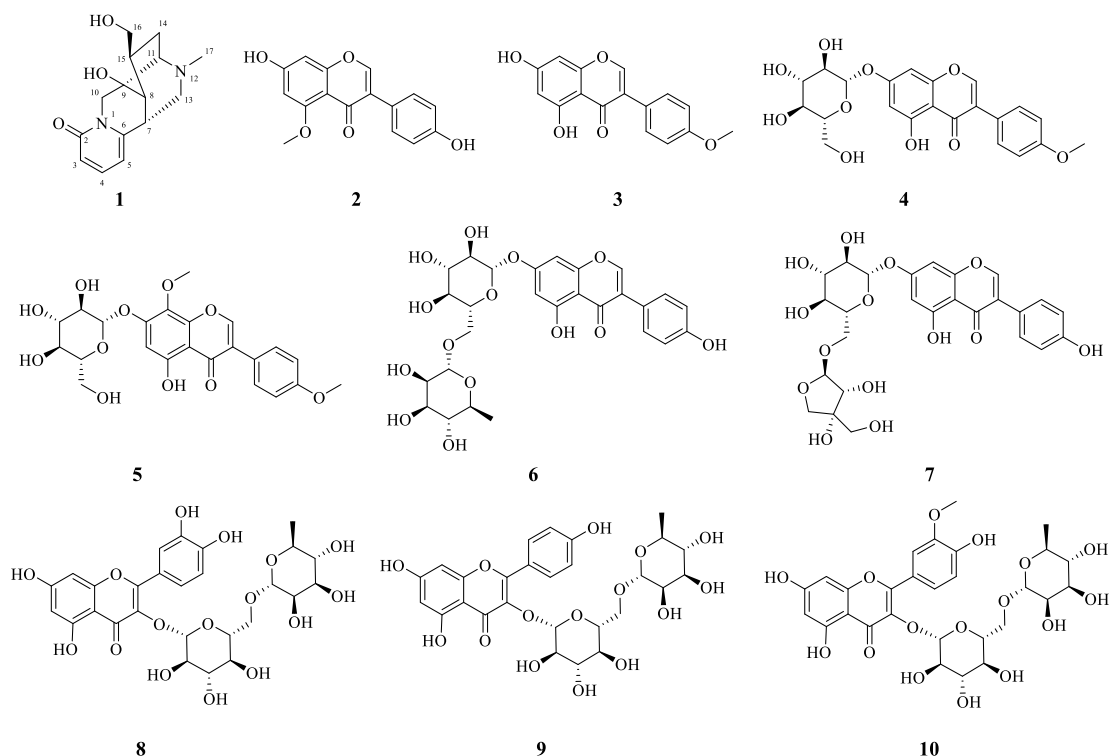
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Figure 1 The structures of compounds **1-10**

2. Materials and Methods

2.1. Plant Material

The seeds of the plant *Ormosia hosiei* Hemsl. et Wils were collected from Fuzhou of Fujian province, PRC in Apr. 2017. The voucher specimen (access number: 20170416) was identified by Professor Shuangquan Zou of Fujian Agriculture and Forestry University (FAFU), PRC, and has been stored in College of Plant Protection, FAFU.

2.2. General Experimental Procedures

Chromatography columns were used with polyamide (60-100 mesh, Luqiao Sijia Biochemical Plastic Factory, Taizhou, PRC), PRP-512A resin (Sunflower Technology Development Co., Ltd., Beijing, PRC), Sephadex LH-20 (GE, USA), and silica gel (100-300 mesh, Jiangyou Silica gel Development Co., Ltd., Yantai, PRC). The preparative HPLC was used by a Shimadzu LC-20AP system with a C₁₈ reversed-phase silica gel chromatography column (20 × 250 mm, 5 μm) using an SPD-20A detector. A Fourier Transform Infrared Spectrometer worked for IR spectrum (KBr). The 1D and 2D NMR were carried out by a Bruker-Ascend-400MHz instrument. The HR-ESI-MS were measured using a Thermo Scientific LTQ Orbitrap XL.

2.3. Extraction and Isolation

The seeds of *O. hosiei* (2.9 kg) were dried and crushed. The powder of seeds was sequentially extracted with petroleum ether and ethyl acetate at 90 °C for 3 × 2 h. And the extracts were concentrated under reduced pressure to obtain a petroleum ether extract (317.4 g) and an ethyl acetate extract (47.1 g), respectively. The ethyl acetate crude extract was fractionated by polyamide with EtOH-H₂O (0:1 to 95:5, v/v) to give six parts (Hd1 – Hd6). Hd1 (13.9 g) was subjected to a PRP-512A column using a EtOH-H₂O (0:1 to 1:0, v/v) gradient to give eight fractions (Hd1.1 – Hd1.8). Hd1.2 (682.4 mg) was subjected to a Sephadex LH-20 column eluted with MeOH and separated using preparative HPLC (CH₃CN-H₂O, 5:95 to 60:40, v/v, 3 mL/min, 300 nm) to obtain four subfractions (Hd1.2.4.1 – Hd1.2.4.4). Hd1.2.4.3 (86.4 mg) was purified by preparative TLC using CH₂Cl₂-EtOH

(5:1) mixture to afford compound **1** (10.3 mg, R_f0.489). Hd3 (812.8 mg) was categorized into four fractions (Hd3.1 – Hd3.4) using a Sephadex LH-20 column with MeOH. Hd3.2 (333.5 mg) was chromatographed by preparative HPLC (CH₃CN-H₂O, 19:81, v/v, 8 mL/min, 254 nm) to generate compound **6** (11.8 mg, *t_R* 22.412 min), compound **7** (18.1 mg, *t_R* 23.524 min), compound **8** (10.0 mg, *t_R* 19.063 min), compound **9** (7.2 mg, *t_R* 29.706 min) and compound **10** (5.3 mg, *t_R* 32.153 min). Hd3.3 (164.3 mg) was purified by preparative HPLC (CH₃CN-H₂O, 22:78, v/v, 8 mL/min, 254 nm) to yield compound **4** (21.1 mg, *t_R* 42.315 min) and compound **5** (11.8 mg, *t_R* 56.706 min). Hd4 (1.1 g) was purified by a Sephadex LH-20 CC with MeOH to obtain compound **2** (26.1 mg). Hd5 (1.3 g) was separated and purified to obtain compound **3** (49.7 mg) by Sephadex LH-20.

2.4. Spectroscopic Data

Hositisine E (1): white powder, C₁₅H₂₀N₂O₃, [α]_D²⁰ -18.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max}: 230.8, 305.6 nm; IR (KBr) ν_{max}: 3 448, 3 369, 2 923, 1 642 and 1 536 cm⁻¹; CD (MeOH) λ_{max} (Δε): 235.0 (+8.52), 310.0 (-6.37) nm; HRESIMS *m/z* 277.154 1 [M+H]⁺ (calcd for C₁₅H₂₁N₂O₃, 277.154 7); ¹H NMR (400 MHz, MeOH-*d*₄) and ¹³C NMR (100 MHz, MeOH-*d*₄): Table 1.

Table 1. The ¹H and ¹³C NMR data of compound **1**.

No.	δ _H (<i>mult.</i> , <i>J</i> in Hz) ^a	δ _C (DEPT) ^b
2	-	165.1(C)
3	6.44(1H, dd, 1.2, 8.8)	116.8(CH)
4	7.48(1H, dd, 6.8, 8.8)	141.7(CH)
5	6.29(1H, dd, 1.2, 6.8)	107.0(CH)
6	-	152.9(C)
7	2.96(1H, overlapped)	44.4(CH)
8	2.12(1H, overlapped)	41.7(CH)
9	-	77.8(C)
10	3.84(1H, d, 15.6); 4.24(1H, d, 15.6)	53.5(CH ₂)
11	2.96(1H, overlapped)	73.3(CH)
13	2.44(1H, br d, 11.6); 2.58(1H, dd, 4.4, 11.6)	54.8(CH ₂)
14	1.57(1H, dt, 5.6, 14.0); 2.01(1H, dd, 9.2, 14.0)	24.0(CH ₂)
15	2.13(1H, overlapped)	45.4(CH)
16	3.69(2H, dd, 4.0, 6.8)	67.2(CH ₂)
17	2.15(3H, s)	42.9(CH ₃)

^a In MeOH-*d*₄ (400 MHz); ^b In MeOH-*d*₄ (100 MHz).

2.5. Determination of the Production of IL-6

The inhibitory effects on IL-6 of all compounds were evaluated with Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Cell cytotoxicity was evaluated by MTT (Sigma, USA) assay. RAW264.7 cells (7 × 10⁵ cells/well) were cultured for 18 h in 96-well plate with Dulbecco's modified Eagle's medium (DMEM) and then the LPS (1 μg/mL) and test materials were added to the cultured cells to stimulate for 24 h. The production of IL-6 was determined by the ELSIA method previously described [12].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** exhibited a molecular formula as C₁₅H₂₀N₂O₃ by HRESIMS at *m/z* 277.154 1 [M+H]⁺ (calcd for C₁₅H₂₁N₂O₃, 277.154 7), which implied an unsaturation degree of 7. The IR spectrum revealed the possible attendance of hydroxyl groups (3 448 and 3 369 cm⁻¹) [13] and α, β-unsaturated lactam (1642 and 1536 cm⁻¹) functionalities [14, 15]. The UV spectrum (230.8, 305.6 nm) showed the typical chromophore of cytosine [16, 17].

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The ^1H NMR (Figure S1) data of compound **1** displayed a methyl group at δ 2.15 (3H, s) and indicative group signals at δ 7.48 (1H, dd, J = 6.8, 8.8 Hz), 6.44 (1H, dd, J = 1.2, 8.8 Hz), and 6.31 (1H, dd, J = 1.2, 6.8 Hz). The ^{13}C NMR (Figure S2), DEPT (Figure S3) and HSQC (Figure S4) spectroscopic data displayed signals for all 15 carbons, comprising one methyl (δ 42.9), four methylenes (δ 67.2, 54.8, 53.5 and 24.0), seven methines (δ 141.7, 116.8, 107.0, 73.3, 45.4, 44.4 and 41.7) and three quaternary carbons (δ 165.1, 152.9 and 77.8). These NMR data indicated that compound **1** was closely structurally related to hosieine B [3], and the only change is the existof a hydroxyl group at C-9 (δ 77.8) in compound **1**.

The cytosine skeleton was confirmed based on the HMBC correlations (Figure S6) from H-4 (δ 7.48) to C-2 (δ 165.1) and C-6 (δ 152.9); from H-5 (δ 6.31) to C-3 (δ 116.8), C-6 and C-7 (δ 44.4), from H-10 (δ 4.24 and 3.84) to C-2, C-6, C-8 (δ 41.7), C-9 (δ 77.8) and C-11 (δ 73.3); from H-13 (δ 2.44 and 2.58) to C-6; from H-17 (δ 2.15) to C-11 and C-13 (δ 54.8), and the ^1H - ^1H COSY correlations (Figure S5) of H-3 (δ 6.44)/H-4/H-5, H-8 (δ 2.12)/H-7/H-13 and H-11 (δ 2.96)/H-14 (δ 1.07 and 2.57). The HMBC correlations from H-16 to C-8, C-14 (δ 24.0) and C-15 (δ 45.4) confirmed the C-8 was connected to C-15 and form a bridge ring. Similar to the hosieine B, orientations were assigned by the NOESY correlations (Figure S7) of H-15/H-13 and H-15/H-7.

The absolute configuration of compound **1** was consistent with that of Hosieine B. Due to their similarity (Figure S11), the configurations of **1** were assigned as 7*R*, 8*R*, 9*R*, 11*R* and 15*R*.

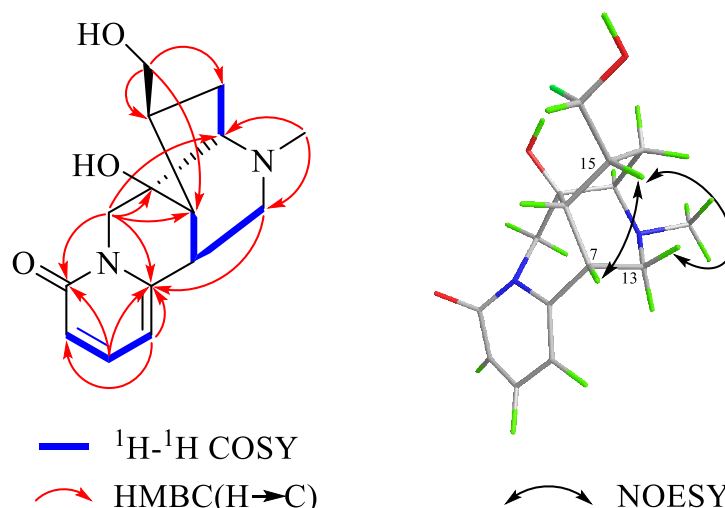


Figure 2 Key HMBC and ^1H - ^1H COSY correlations of compound **1**

The structures of nine isolates (**2-10**) were identified as isopruneitin (**2**)[7], biochanin A (**3**)[18], ononin (**4**)[19], 4',8-dimethoxy-7-*O*- β -*D*-glucopyranosylisoflavone (**5**)[20], sphaerobioside (**6**)[21], ambocin (**7**)[22], rutin (**8**)[23], kaempferol-3-rutinoside (**9**)[24] and narcissin (**10**)[23] by detailed analysis of their ^1H and ^{13}C NMR spectroscopic data (Table S1 and S2) in comparison with those reported in the references.

3.2. Anti-inflammatory Activity

The anti-inflammatory activities about compounds **1-10** were evaluated by measuring their inhibitory effects on IL-6 production on RAW 246.7 cells stimulated by LPS. None of the test materials showed obvious toxicity to RAW 246.7 cells at 0-500 μM . As a result, compounds **1-3**, and **7** moderately exhibited inhibitory effect on LPS-induced IL-6 with IC_{50} values of 19.4 ± 1.8 , 58.9 ± 5.7 , 35.0 ± 3.9 and 41.6 ± 3.6 μM , while the IC_{50} of the positive control hydrocortisone was 44.6 ± 5.1 μM . (Table S3).

Acknowledgments

This research is funded by Scientific Research Project of Education Department of Fujian Province (JAT200166), Outstanding-Young Scientific Research Talents Program of Fujian Agriculture and Forestry University (XJQ202103), and Research Project of National Agricultural Education Steering Committee (2021-NYYB-12).

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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