Fitoterapia 83 (2012) 161–165

Contents lists available at SciVerse ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

# Polyprenylated isoflavanone and isoflavonoids from *Ormosia henryi* and their cytotoxicity and anti-oxidation activity

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ARTICLE INFO

*Article history:*
Received 25 July 2011
Accepted in revised form 12 October 2011
Available online 24 October 2011

*Keywords:*
Ormosia
*Ormosia henryi* Prain
Isoflavonoids
Cytotoxicity
Anti-oxidation activity

ABSTRACT

A rare naturally-occurring polyprenylated isoflavanone, designated ormosinol (**1**), and a new isoflavonoid glycoside, named ormosinoside (**2**), along with 21 known compounds were isolated from the root bark of *Ormosia henryi* Prain. The structures of compounds **1** and **2** were determined as 5,7,2′,4′-tetrahydroxyl-6,8,5′-tri-(γ,γ-dimethylallyl)isoflavanone and isoprunetin-7-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside on the basis of a combination of 1D-, 2D-NMR and mass spectroscopic measurements. Compound **1** showed significant anti-oxidation activity against DPPH radicals ($IC_{50}$ 28.5 μM) and cancer cell line (A549, LAC, and HepG2) growth inhibitory activity with $IC_{50}$ ranging from 4.25 to 7.09 μM, while compound **2** found to be inactive to both testing systems.



## 1. Introduction

The genus *Ormosia* (Leguminosae), comprising about 120 species, is mainly distributed in the topical areas of the word with 35 species being native in China. Many plants of this genus are used for features or ornamental, some species have applications in folk medicine [1]. Previous phytochemical studies of this genus reported the isolation of a variety of secondary metabolites such as flavonoids, alkaloids, and triterpenoids [2–6]. *Ormosia henryi* Prain, a tall tree, mainly grows in southern provinces of China, with its root, leaves and stem bark used as Chinese folk medicine for the treatment of swallow, pain and inflammation [7]. With an aim to search for new anti-inflammatory natural products, we initiated an intensive phytochemical study on the roots of *O. henryi*, resulting in the isolation of two new compounds, ormosinol (**1**) and ormosinoside (**2**), as well as 21 known compounds (**3**–**23**). Herein, we describe the isolation, structure elucidation and biological evaluation of the two new compounds **1** and **2**.

## 2. Experimental

### 2.1. General

Melting points were determined on a Yanagimoto Seisakusho MD-S2 and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH and DMSO as solvent. The UV spectra were recorded in MeOH on a Perkin Elmer Lambda 25 UV–VIS Spectrophotometer. The IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer. The $^{1}H$ (400 MHz), $^{13}C$ (100 MHz) and 2D NMR spectra were recorded on a Bruker DRX-400 instrument using TMS as an internal standard. The chemical shifts are given in δ (ppm) and coupling constants in Hz. ESI-MS were collected on MDS SCIEX API 2000 LC/GC/MS instrument. HRESI-MS data were obtained on an API QSTAR mass spectrometer. For column chromatography, silica gel 60 (100—200 mesh) and polyamide (80–100 mesh), were produced by Qingdao Marine Chemical Ltd., (Qingdao, People's Republic of

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doi:10.1016/j.fitote.2011.10.007

China), Sephadex LH-20 and Diaion HP-20 porous resin were produced by Mitsubishi Chemical Holdings of Japan.

### 2.2. Plant material

The root bark of *Ormosia henryi* Prain was collected from Nankang County of Jiangxi Province, People's Republic of China, in October 2007. The plant was identified by Professor Fuwu Xin of SCBG and a voucher was deposited at Laboratory of Phytochemistry, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China.

### 2.3. Extraction and isolation

The air-dried material (34 kg) was smashed into small pieces and extracted 3 times by 95% EtOH at room temperature. The combined extracts were filtered and concentrated under vacuum to obtain a crude residue (2800 g). The residue was suspended in $H_2O$ and partitioned in sequence using petroleum ether (60–90 °C), EtOAc, and *n*-BuOH, respectively, to afford a petroleum ether extract (220 g), EtOAc extract (1150 g), and an *n*-BuOH extract (280 g).

The petroleum ether-soluble extract was dissolved in acetone and a white precipitate was obtained, which was filtered and washed by MeOH and Acetone successively to obtain compound **3** (100 g). The supernatant was subjected to a silica gel column chromatography (CC) and eluted with a gradient petroleum ether–acetone (from 10:0 to 6:4) to yield eight fractions (1–8). Fraction 2 (10.0 g) had white residue, which was filtered and washed by MeOH to get compound **6** (500 mg). Fraction 4 (5.0 g) was further chromatographed on silica gel CC and eluted with $CHCl_3$–MeOH (98:2) to obtain compound **21** (100 mg).

The EtOAc extraction was dissolved by 80% EtOH and had white deposition, which was filtered and recrystallized with 80% EtOH to get white crystals of compound **12** (250 g). Then the supernatant was separated by a silica gel CC using $CH_3Cl$–MeOH (from 100:0 to 60:40) to give eight fractions (9–16). Fraction 9 was further subjected to silica gel CC and eluted with petroleum ether–acetone (3:1) to give six subfractions (9a–9f). Subfraction 9a (1.0 g) was purified by $CHCl_3$–MeOH (98:2) on a silica gel CC to yield compounds **3** (130 mg) and **4** (150 mg). Subfraction 9b (21 g) and 9f (100 g) both had white deposition, which was filtered and recrystallized with 80% EtOH to obtain compounds **5** (5 g) and **9** (31 g), respectively. Subfraction 9c (3 g) was subjected to recrystallization with MeOH to yield compound **7** (30 mg); while the supernatant was further purified by silica gel CC eluting with $CH_3Cl$–MeOH (92:8) to get compound **1** (160 mg). Fraction 10 (5.5 g) was applied to silica gel CC using $CHCl_3$–acetone (3:1) and purified by Sephadex LH-20 CC using MeOH to obtain compounds **16** (56 mg), **18** (39 mg) and **20** (89 mg). Fraction 11 (20.6 g) was purified with the same method to get compounds **8** (1.0 g), **11** (40 mg), **10** (300 mg), **19** (200 mg) and **17** (393 mg). Fraction 12 (21.8 g) had white needles, which was recrystallized with 80% EtOH to yield compound **22** (13.5 g); the supernatant was subjected to silica gel CC eluting with $CHCl_3$–MeOH–$H_2O$ (10:3:1) and purified by Sephadex LH-20 (MeOH) to obtain compound **13** (80 mg). Fr.13 (5.5 g) was subjected to silica gel CC eluting with $CHCl_3$–MeOH–$H_2O$ (7:3:1) and purified by Sephadex LH-20 (MeOH) to yield compound **14** (80 mg).

The *n*-BuOH-soluble extract (280 g) was chromatographed on a Diaion HP-20 resin column and eluted successively with $H_2O$, 50% EtOH and finally EtOH. The 50% EtOH eluent was collected and concentrated under vacuum to afford a residue (150 g) which was then subjected to a silica gel CC and eluted with $CHCl_3$–MeOH–$H_2O$ (7:3:1) to give four fractions (17–20). Fraction 19 was separated by polyamide CC eluting with 20% MeOH, and further purified by Sephadex LH-20 CC (MeOH) to yield compounds **23** (120 mg), **15** (45 mg) and **2** (50 mg).

Ormosinol (**1**): white power, mp 99–104 °C; $[\alpha]^{20}_D + 1°$ (*c* 2.0, MeOH). UV/Vis $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 203 (2.90), 295 (2.40). IR $\nu_{max}$ (KBr) ($cm^{-1}$): 3380, 2969, 2913, 1700, 1631, 1509, 1442 and 1378. $^1$H NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C NMR (DMSO-$d_6$, 100 MHz) data see Table 1. HR-ESI-MS *m/z* 493.2609 $[M+H]^+$ (calcd for $C_{30}H_{37}O_6$, 493.2584).

Ormosinosides A (**2**): white power, mp 225–228 °C, $[\alpha]^{20}_D - 57°$ (*c* 1.0, DMSO). UV/Vis $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 202 (3.69), 250 (3.75). IR $\nu_{max}$ (KBr) ($cm^{-1}$): 3390, 2919, 1637, 1515, 1461, 1428 and 1375. $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$: 9.51 (1H, s, OH-4′), 8.11 (1H, s, H-2), 7.30 (2H, d, $J$ = 8 Hz, H-2′ and 6′), 6.78 (2H, d, $J$ = 8 Hz, H-3′ and 5′), 6.73 (1H, d, $J$ = 2 Hz, H-8), 6.55 (1H, d, $J$ = 2 Hz, H-6), 5.04 (1H, d, $J$ = 6.8 Hz, H-1″), 4.16 (1H, d, $J$ = 7.2 Hz, H-1‴), 3.67 (3H, s, 5-$OCH_3$), 2.0–5.0 (10H, m, sugar protons); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) data see Table 2. HR-ESI-MS *m/z* 577.1556 $[M-H]^-$ (calcd for $C_{27}H_{29}O_{14}$, 577.1562).

### 2.4. Cytotoxicity assay

The MTT assay was performed according to literature with slight modification [8]. Human cancer cells were plated at $1\times10^4$ cells per well in 96 well microtiter plates and incubated for 24 h at 37 °C, 5% $CO_2$. Some wells of the plate were added with only 100 μl of culture medium as a background well, different concentrations (50, 25, 12.5, 6.5 and 3.125 μmol) of the compound were added. After 3 days of incubation at 37 °C, 5% $CO_2$, 20 μl MTT reagent (5 mg/ml) was added. After incubating at 37 °C for 4 h, MTT reagent was removed and DMSO (150 μl) was added to each well and shaken for another 10 min. The absorbance was then determined by a CENios microplate reader (TECAN, Genios) at a wavelength of 570 nm. Control wells received only the media without the test samples. The conventional anticancer drug, doxorubicin was used as positive control in this study. The inhibition of cell growth by the samples tested was calculated as percentage anticancer activity and was calculated using the following formula: percentage anticancer activity $(A_c - A_s/A_c)\times100\%$. $A_c$ and $A_s$ refer to the absorbance of control and the sample, respectively.

### 2.5. Antioxidant activities assay

The DPPH-scavenging assay was carried out according to literature [79] with minor modification, namely, 200 μl of reaction mixtures containing 20 μl test samples dissolved in DMSO and 180 μl of DPPH (0.1 mM) were plated in 96-cell plates incubated in the dark for 60 min. After the reaction, absorbance was measured at 515 nm, and percent inhibition was calculated. The antioxidant activity of each sample was