Antimalarial Activity of Biflavonoids from Ochna integerrima

Abstract

During the screening of antimalarial substances, the 80% EtOH extract from the outer bark of Ochna integerrima Merr. (Ochnaceae) was shown to have a good anti-malarial activity (IC₅₀ value: 6.5 µg/mL) whereas extracts from the inner barks of O. integerrima showed no antimalarial activity. Biflavonone (1), which had not been found previously from a natural plant source, was isolated as a potent antimalarial active ingredient (IC₅₀ value: 80 ng/mL) from the extract of the outer barks. The stereoisomer of 1 ( = compound 2) was also isolated from this plant; however, its activity was significantly lower than that of 1.

Key words
Antimalarial · Ochnaceae · Ochna integerrima Merr. · biflavonones

Introduction

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally among African children) out of 400 million cases each year [1] and to major consequent impacts on economic productivity and livelihood [2]. The incidence of malaria is now increasing because of the appearance of multi-drug resistant Plasmodium falciparum, therefore new and more effective antimalarial drugs are urgently required.

Ochna integerrima Merr. (Ochnaceae) is a tree that is widely distributed in Thailand [3], and the bark of O. integerrima Merr. has been used for digestive disorders as a folk medicine in Thailand [4]. In phytochemical studies on O. integerrima Merr, many flavonoids have been isolated [5], [6], [7]. During a screening for antimalarial active sources among Thai plants, it was found that the 80% EtOH extract from the outer bark of O. integerrima Merr. showed significant anti-malarial activity. The active ingredients in the outer bark were clarified in the present study.

Materials and Methods

Apparatus

Optical rotations were measured on a JASCO polarimeter. ¹H- and ¹³C- NMR spectra were determined on a Varian Mercury-300. Mass spectra (MS) were obtained on JEOL MXA-AM505HA and JMS-700 MStation spectrometers. Chromatographic separations were carried out by column chromatography on Wakogel C-200 (75 - 150 µm, Wako; Osaka, Japan). Preparative reverse phase HPLC was carried out on PEGASIL (250 × 20 mm i.d., Senshu Co. Ltd.; Tokyo, Japan).

Affiliation

1 Kitsato Institute for Life Sciences, Kitasato University, Tokyo, Japan
2 Oriental Medicine Research Center, The Kitasato Institute, Tokyo, Japan
3 Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
4 Research Center for Tropical Diseases, Institute for Basic Research, The Kitasato Institute, Tokyo, Japan

Correspondence

Prof. Dr. Haruki Yamada · Kitasato Institute for Life Sciences · Kitasato University · 5–9–1 Shirokane · Minato-ku · Tokyo 108–8641 · Japan · Phone: +81-3-3444-6164 · Fax: +81-3-3445-1351 · E-mail: yamada@lisci.kitasato-u.ac.jp

Received December 1, 2005 · Accepted January 17, 2006

Bibliography

DOI 10.1055/s-2006-931569 · Published online May 29, 2006
ISSN 0032-0943
Analytical methods
Analytical HPLC experiments were performed on an Agilent 1100 series HPLC instrument (Agilent Technologies Japan, Ltd.; Tokyo, Japan) equipped with a column of PEGASIL 250×4.6 mm i.d., Senshu Co. Ltd.; Tokyo, Japan). The solvent system used was a linear gradient of acetonitrile from 30% to 60% during 30 minutes in 10 mM phosphoric acid. Flow rate was 1 mL/min. Injection volume was 10 µL for MeOH solution of all the samples. Concentrations of the samples were 10 mg/mL for extracts and 1 mg/mL for the isolated compounds.

Plant material
The outer and inner barks of O. integerrima Merr. were collected from Ubon Ratchathani in Thailand. The plants were identified by Associate Professor Wongsasit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. Voucher specimens are kept at the Herbarium of Faculty of Pharmacy, Mahidol University.

Extraction and isolation
The outer barks of O. integerrima (485 g) were extracted with 80% EtOH at room temperature for 3 days, and the extract was evaporated under vacuum to yield 150 g of the crude EtOH extract. The EtOH extract was chromatographed on silica gel (500 g) and eluted with CHCl₃/MeOH (10:1, 1:1, 0:1; 2 L each) to give three fractions (Fr. 1 – 3). Fr. 1 (50 g, CHCl₃/MeOH, 10:1) was further fractionated by silica gel column chromatography (1000 g) using CHCl₃/MeOH containing 0.05% TFA (50:1 (3 L), 20:1 (2 L), 10:1 (2 L), 5:1 (4 L), 1:1 (2 L)) to give eight fractions (Fr. 1/1 – 1/8). Fr. 1/4 (0.5 g of 16.5 g, CHCl₃/MeOH, 5:1) was separated by a reverse phase HPLC (40% CH₃CN containing 0.05% TFA, flow rate, 8.0 mL/min; UV detector, 254 nm) to give the active fraction (Fr. 1/4 – 3, 266.68 mg, tₚ = 19.2 min). Fr. 1/4 – 3 (133.4 mg) was further purified by the same reverse phase HPLC (solvent A, 40% MeOH containing 0.05% TFA; B, MeOH; A/B, 7:1 → 6:2 for 40 minutes; flow rate 8.0 mL/min; UV detector; 254 nm) to give 1 (97.9 mg, tₚ = 38.0 min) and 2 (7.3 mg, tₚ = 41.8 min).

Compound 1: brown oil; [α]D²⁵ +62.2° (c 1.00, MeOH); HR-FAB-MS: m/z = 511.1399 [M + H⁺] (calc. for C₂₇H₂₀O₂: 511.1393); LR-FAB-MS: m/z (rel. int.) = 511 (60), 255 (45); ¹H-NMR (acetone-d₆, 300 MHz, 23 °C): δ = 6.29 (2H, d, J = 2.0 Hz, H-8/H-8’); 6.59 (2H, dd, J = 8.5, 2.0 Hz, H-6/H-6’); 6.88 (4H, d, J = 8.5 Hz, H-3’/H-3” and H-5’/H-5”); 7.14 (4H, d, J = 8.5 Hz, H-2’/H-2” and H-6’/H-6”); 7.78 (2H, d, J = 8.5 Hz, H-5/H-5’); ¹³C-NMR (acetone-d₆, 75 MHz, 23 °C): δ = 49.8 (C-3/C-3’), 82.5 (C-2/C-2’), 103.4 (C-8/C-8’), 111.5 (C-6/C-6’), 114.4 (C-4a/C-4’a), 116.2 (C-3’/C-3” and C-5’/C-5”), 129.1 (C-1’/C-1”), 129.8 (C-5/C-5’), 130.1 (C-2’/C-2” and C-6’/C-6”), 159.0 (C-4’/C-4”), 164.2 (C-8a/C-8’a), 165.4 (C-7/C-7’), 190.7 (C-4/C-4’).

Compound 2: brown oil; [α]D²⁵ +83.2° (c 0.25, MeOH); HR-FAB-MS: m/z = 533.1209 [M + H⁺] (calc. for C₃₂H₂₂O₂Na: 533.1212); ¹H-NMR (acetone-d₆, 300 MHz, 23 °C): δ = 2.73 (2H, d, J = 12.0 Hz, H-3/H-3’), 5.98 (2H, d, J = 12.0 Hz, H-2/H-2’), 6.33 (2H, d, J = 1.5 Hz, H-8/H-8’), 6.58 (2H, dd, J = 8.5, 1.5 Hz, H-6/H-6’), 8.68 (4H, d, J = 8.0 Hz, H-3’/H-3” and H-5’/H-5”), 7.02 (4H, d, J = 8.0 Hz, H-2’/H-2” and H-6’/H-6”), 7.75 (2H, d, J = 8.5 Hz, H-5/H-5’); ¹³C-NMR (acetone-d₆, 75 MHz, 23 °C): δ = 51.8 (C-3/C-3’), 84.7 (C-2/C-2’), 103.4 (C-8/C-8’), 111.3 (C-6/C-6’), 115.2 (C-
The present paper is the first report on the isolation of compounds 1 and 2 directly from natural plant extracts. When the 80% extracts from both the inner and outer barks of O. integerrima were compared by HPLC, compounds 1 and 2 could not be detected in the extract from the inner barks, therefore explaining why the extracts of the inner barks were not active (Fig. 2).

Compounds 1 and 2 were evaluated in vitro for antimalarial activity (Table 1). The antimalarial activity of compound 1 against the multidrug-resistant strain (K1) was three times stronger than against the sensitive strain (FCR3), and the selectivity (IC₅₀ value against MRC-5/IC₅₀ value against K1) was 375. However, the antimalarial activity of compound 2 was 65 and 17 times weaker than those of 1 for the K1 and FCR3 strains, respectively, although similar cytotoxic activities against MRC-5 were observed for both compounds 1 and 2.

The antimalarial biflavanones, sikokianins B and C, have been isolated from Wikstroemia indica [11], and sikokianins B and C both showed ten times weaker activity than compound 1, assuming that the stereochemistry of the C3/C3′ coupling bond and methoxy substitution might affect the degree of activity. The monomer of compound 1 is liquiritigenin which has no antimalarial activity, while a chalcone (could be related to 1/2), name-ly isoliquiritigenin, has weaker in vitro antimalarial activity than the present biflavanone (1) [12]. Finally, biflavanone 1 could be a promising compound for investigation of in vivo antimalarial activity in animal models.

**Table 1** Antimalarial and cytotoxic activity of the extracts, fractions and compounds from *O. integerrima* Merr.

<table>
<thead>
<tr>
<th>Extract/Fraction/Compound</th>
<th>K1 [µg/mL]</th>
<th>FCR3 [µg/mL]</th>
<th>MRC-5 [µg/mL]</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% EtOH extract (outer barks)</td>
<td>6.5</td>
<td>4.5</td>
<td>4.5</td>
<td>0.7</td>
</tr>
<tr>
<td>80% EtOH extract (inner barks)</td>
<td>&gt; 12.5</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>5.0</td>
<td>4.5</td>
<td>4.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>40.0</td>
<td>40.0</td>
<td>40.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>50.0</td>
<td>45.0</td>
<td>45.0</td>
<td>0.9</td>
</tr>
<tr>
<td>1</td>
<td>0.08</td>
<td>0.26</td>
<td>30.0</td>
<td>375</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>4.5</td>
<td>27.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Sikokianin B</td>
<td>0.54</td>
<td>0.54</td>
<td>22.5</td>
<td>41.7</td>
</tr>
<tr>
<td>Sikokianin C</td>
<td>0.56</td>
<td>0.34</td>
<td>11.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Artemisinin*</td>
<td>0.007</td>
<td>0.005</td>
<td>N.T.</td>
<td></td>
</tr>
<tr>
<td>Chloroquine*</td>
<td>0.11</td>
<td>0.009</td>
<td>N.T.</td>
<td></td>
</tr>
</tbody>
</table>

* Positive control.
* N.T. = not tested.
* IC₅₀ on MRC-5/IC₅₀ on K1.

**Fig. 2** HPLC profiles at 254 nm of (a) the extract of inner bark of *Ochna integerrima* Merr.; (b) the extract of outer bark of *Ochna integerrima* Merr.; (c) compound 1; (d) compound 2.

**Fig. 1** Antimalarial biflavanones from *Ochna integerrima* Merr.
References