Identification of a New Cytotoxic Biflavanone from *Selaginella doederleinii*

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A new biflavanone, 2,2′,3,3′-tetrahydrodorboaflavone 7,4′,7′-trimethyl ether (1) was isolated from the whole plant of *Selaginella doederleinii* HieRoon. (Selaginellaceae) together with the known biflavonoid, robustaflavone 7,4′,7′-trimethyl ether (2) as the cytotoxic constituents against the three human cancer cell lines, HCT, NCI-H358, and K562. The structure of the new compound 1 was elucidated by spectral analysis including various 1D- and 2D-NMR experiments.

**Key words** *Selaginella doederleinii;* Selaginellaceae; biflavanone; cytotoxicity

The genus *Selaginella* is composed of about 700 species and belongs to the family Selaginellaceae. In particular, *Selaginella doederleinii* HieRoon, has been used as a traditional Chinese medicine a which is a well-known perennial Pteridophyte plant growing in South and Southwestern China at low altitude. b Several biflavonoids, ligans, and alkaloids have been reported from this plant c–f together with some biological activities such as cytotoxicity, g anti-human immunodeficiency virus (HIV) activity, inhibition of human DNA polymerase, and anti-inflammatory effects. d–f In the present study, a new biflavanoid 1 was isolated from the whole plant of *S. doederleinii* with the known biflavonoid, robustaflavone 7,4′,7′-trimethyl ether (2). g Compound 2 was isolated from the family Selaginellaceae for the first time. They were evaluated for their cytotoxic activity to

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<th>Compounds</th>
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<td>HCT116</td>
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<tr>
<td>1</td>
<td>19.1</td>
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<td>2</td>
<td>15.6</td>
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<tr>
<td>3</td>
<td>&gt;100</td>
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<td>Ellipticine</td>
<td>1.0</td>
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a) Results are expressed as EC50 values in μM. b) Cell lines: HCT=cancerous colorectal carcinoma; NCI-H358=brachialveolar carcinoma (non-small cell lung cancer cells); K562=chronic myelogenous leukemia. c) Ellipticine was used as a positive control.
CD spectrum of 1 exhibited a positive cotton effect at 328 nm and a negative cotton effect at 290 nm, respectively, indicating the typical “S” configuration at C-2 in flavanone skeleton. Therefore, the structure of 1 was determined as a new biflavonane, 2,2′,3,3′-tetrahydrorobustaflavone 7,4′,7′-trimethyl ether. Compounds 2 and 3 were identified as the known biflavonoids, robustaflavone 7,4′,7′-trimethyl ether and robustaflavone 4′-methyl ether, respectively.

**Experimental**

General. Optical rotations were measured with a P-1010 polarimeter (JASCO, Japan) at 25°C. UV spectra (λmax) were recorded on a U-3000 spectrophotometer (Hitachi, Japan). IR spectra (νmax) were determined on a FTS 135 FT-IR spectrometer (Bio-Rad, CA, U.S.A.). Circular dichroism measurements were performed using JASCO J-715 CD/ORD spectropolarimeter. The 1D and 2D NMR experiments were conducted on a UNITY INOVA 400 MHz FT-NMR (Varian, CA, U.S.A.), and TMS was used as an internal standard. ESI-MS, HR-ESI-MS and FAB-MS were obtained on a Semiconductor Analyzer (Milford, MA, USA). The FT-NMR spectra of compounds 1 and 2 were measured on a Bruker Avance 400 MHz FT-NMR spectrometer (Billerica, MA, USA).

**Plant Material**

The whole plants of *S. debeleiniana* Hiera., collected at Batu Meda Centre, East Java, Indonesia, in May 2005 and were identified by Prof. Adam Wiryawan (Brawijaya University, Malang 65145, Indonesia). A voucher specimen has been deposited at the Batu Herba Medica Centre.

**Extraction and Isolation**

The dried and milled plant material (335 g) was extracted with MeOH (3×815 ml) by maceration. The MeOH extracts (25 g) were separated by liquid column chromatography (glass column (6.5 cm, 40 cm), using gradient Elution: 0:0:1 to 0:1:0) as mobile phases to give 13 fractions (F1—F13) (14.4 mg, 0.0026% w/w). Fraction F12 (14.4 mg, 0.0026% w/w) was further fractionated by flash silica gel column chromatography (250 g) using CHCl3–MeOH (gradient from 49:1 to 0:0:1) as mobile phases to give 13 subfractions (F1201—F1213) (14.4 mg, 0.0026% w/w).

**In Vitro Cytotoxicity Assay**

Cytotoxic potential was determined as described previously. Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/ml with fresh medium, and added to 96-well microtiter plates (190 μl/well) containing test materials (10 μl in 10% aqueous DMSO). Test plates were incubated for 3 d at 37°C in CO2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μl of cold 50% aqueous trichloroacetic acid (4°C for 30 min), washed 4—5 times with tap water, and air-dried. The fixed cells were stained with sulfonfluoradamine B (SRB) (0.4% w/v in SRB in 1% aqueous acetic acid) for 30 min. Free SRB solution was then air-dried, the bound dye was solubilized with 200 μl of 10% tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and EC50 values were calculated using non-linear regression analyses (percent survival versus concentration). Ellipsoïd was used as a positive control.

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