Cytotoxic prenylated bibenzyls and flavonoids from *Macaranga kurzii*

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**Article info**

**Abstract**

One unique prenylated bibenzyl, kurzphenol A (1), two new prenylated flavonoids, kurzphenols B and C (2 and 3), as well as fourteen known compounds (4–17) were isolated from the twigs of *Macaranga kurzii*. Compound 1 was the first example of prenylated bibenzyl which possesses a benzofuran ring. All the known compounds were isolated from *M. kurzii* for the first time. Their structures were elucidated on the basis of extensive spectroscopic interpretation. Compounds 1–17 were tested for their cytotoxicity against A-549 and Hep G2 cancer cell lines and showed IC\(_{50}\) values in the range of 9.76–30.14 \(\mu\)g/mL.

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*Macaranga kurzii*  
Euphorbiaceae  
Prenylated flavonoids  
Prenylated bibenzyls  
Cytotoxicity

1. Introduction

The genus *Macaranga* is one of the largest genera of the Euphorbiaceae, and many of the plants in this genus were characteristics of secreting red juice after injury. An overview of the literature indicated that prenylated stilbenes [1–4] and flavonoids [5–9] are their typical secondary metabolites, and the biological activities of those metabolites encompass virtually all fields of pharmacological sciences [10,11]. Due to the differences in the prenylation position on the aromatic rings, various lengths of prenyl chain, and further modifications of the prenyl moiety such as cyclization and hydroxylation, prenylation contributes strongly to the structural diversity of aromatic products [12]. Studies have showed that the presence of isoprenoid chains is a major determinant of the bioactivity of prenylated aromatic compounds and made them exhibit much higher biological activities than their mother compounds without derivatization or decoration [13].

*Macaranga kurzii* was a small arbor distributed in tropical rainforests. Previous phytochemical investigations on *M. kurzii* showed that it contained flavonoids and stilbenes, together with their prenylated derivatives [14]. In the course of an investigation to identify anticancer bioactive compounds from the genus *Macaranga*, a phytochemical investigation on *M. kurzii* led to the isolation and characterization of one unique prenylated bibenzyl (1) and two new prenylated flavonoids (2 and 3), coupled with fourteen known compounds (4–17) including one prenylated bibenzyl (4), eight flavonoids (5–12, seven prenylated ones), two polyacetylenes (13 and 14), one ionone (15), one coumarin (16) and one phenolic compound (17) (Fig. 1). Although numbers of prenylated stilbenes have been reported in genus *Macaranga*, bibenzyls which were the reduction products of the stilbene were reported in this genus for the first time, and compound 1 was the first example of prenylated bibenzyl which possesses a benzofuran ring. Herein, we report the isolation and structure elucidation of those...
compounds, as well as their cytotoxicity against A-549 and Hep G2 cancer cell lines.

2. Experimental

2.1. General

ORD spectra were recorded on a Horiba SEPA-300 polarimeter. CD spectra were obtained on an Automated Circular Dichroism spectrometer (Applied Photophysics). UV data were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR experiments were recorded on Bruker AM-400, DRX-500 or Avance III 600 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ESI-MS was performed on an API QSTAR time-of-flight spectrometer. HR-EI-MS was performed on a Waters AutoSpec Premier P776. EI-MS was performed on a Shimadzu UV-2401PC spectrophotometer. IR data were obtained on a Shimadzu IR Prestige-21. CD spectra were obtained on an Automated Circular Dichroism spectrometer (Applied Photophysics). UV data were obtained on a Shimadzu UV-2401PC spectrophotometer. 2.1. General

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2.2. Plant material

The twigs of M. kurzii were collected from Xishuangbanna of Yunnan province, PR China, in August 2012. A voucher specimen (Yangyp–20120806) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, which was identified by one of the authors (Prof. Yong-Ping Yang).

2.3. Extraction and isolation

The air-dried and powdered twigs of M. kurzii (10 kg) were extracted with 90% EtOH (4 × 25 L) for 24 h at room temperature and concentrated in vacuo to give a crude extract. The extract was suspended in H2O and then extracted with 90% EtOH (4 × 25 L) for 24 h at room temperature and concentrated in vacuo to give a crude extract. The extract was suspended in H2O and then extracted with 90% EtOH (4 × 25 L) for 24 h at room temperature and concentrated in vacuo to give a crude extract. The extract was suspended in H2O and then extracted with 90% EtOH (4 × 25 L) for 24 h at room temperature and concentrated in vacuo to give a crude extract.

2.4. Spectroscopic data

2.4.1. Kurzphenol A (1)

Colorless oil; [α]D20 = −2.2 (c 0.22, MeOH); UV (MeOH) λmax (log ε) 208 (4.62), 251 (4.06), 294 (3.81) nm; IR (KBr) νmax 3442, 2965, 2924, 2858, 1616, 1537, 1495, 1452, 1376, 1370, 1245, 1209, 1135, 1070, 1045, 830, 770, 751, 734, 699 cm−1; 1H NMR (CDCl3, 100 MHz) data, see Table 1; negative ESI-MS m/z 305 [M – H]+, 611 [2M – H]+; HR-ESI-MS m/z 306.1613 [M]+ (calcd for C21H22O2, 306.1620).

2.4.2. Kurzphenol B (2)

Yellow oil; [α]D0 = −1.77 (c 0.28, MeOH); UV (MeOH) λmax (log ε) 204 (4.68), 300 (4.27), 346 (3.74) nm; IR (KBr) νmax 3421, 2968, 2916, 1631, 1452, 1376, 1276, 1230, 1182, 1112, 1003, 766, 698 cm−1; 1H NMR (CDCl3, 100 MHz) and 13C NMR (CDCl3, 100 MHz) data, see Table 1; negative ESI-MS m/z 407 [M – H]+; HR-ESI-MS m/z 408.1913 [M]+ (calcd for C25H26O6, 408.1937).

2.4.3. Kurzphenol C (3)

Yellow oil; [α]D0 = −1.31 (c 0.20, MeOH); UV (MeOH) λmax (log ε) 202 (4.77), 274 (4.36) nm; IR (KBr) νmax 3424, 2924, 2854, 1657, 1611, 1553, 1506, 1452, 1434, 1359, 1300, 1268, 1230, 1178, 1159, 1115, 1067, 1009, 946, 835, 635, 578 cm−1; 1H NMR (acetone-d6, 500 MHz) and 13C NMR (acetone-d6, 100 MHz) data, see Table 1; negative ESI-MS m/z 367 [M – H]+, 735 [2M – H]+; HR-ESI-MS m/z 367.1187 [M – H]+ (calcd for C21H19O6, 367.1182).

2.5. Cytotoxicity assay

Compounds 1–17 were tested for their cytotoxicity against human lung carcinoma (A-549) and human hepatocellular (Hep G2) by the MTT method, 5-FU was used as a positive control. Briefly, 100 µL of cell suspension (1 × 105 cells/mL) was seeded into 96-well microtiter plates and cultured for 24 h before the compound was added. Then, different concentrations of the compounds were added to the plates, the cells were cultivated for 48 h, and 10 µL of MTT (5 mg/mL) was added to each well. After 4 h, the culture medium was removed and the formazan crystals were completely dissolved with 150 µL DMSO in each well by vigorously shaking the plate. Finally, formazan absorbance was assessed by a BioRad microplate reader at 570 nm.

3. Results and discussion

Kurzphenol A (1) was obtained as colorless oil. The molecular formula of 1 was determined as C21H22O2 by HR-ESI-MS, requiring 11 degrees of unsaturation. The IR spectrum showed absorption bands for OH (3432 cm−1) and aromatic ring (1616, 1537 cm−1) moieties. The UV spectrum of 1 showed absorption maxima at 208 nm (4.62) and 294 nm (3.81), which
indicated the presence of an unconjugated aromatic system [15]. Analysis of the $^1$H and $^{13}$C NMR (Table 1) data of 1 aided by HSQC revealed resonances for one prenyl ($\delta$C 25.5 (t), 122.6 (d), 134.2 (s), 25.7 (q), 18.0 (q); $\delta$H 3.44 (2H, d, J = 6.6 Hz), 5.11 (1H, d, J = 6.6 Hz), 1.75 (3H, s), 1.85 (3H, s)], one mono-substituted benzene ring ($\delta$H 7.20 (2H, d, J = 7.3 Hz), 7.31 (2H, t, J = 7.3 Hz), 7.24 (1H, t, J = 7.3 Hz)], one 1,3-diaryloxy substituted benzene ring [$\delta$C 97.0 (d); $\delta$H 6.92 (1H, s), two adjacent benzylic methylenes [$\delta$H 3.15 (2H, dd, J = 9.5, 6.8 Hz), 2.90 (2H, t, J = 9.5, 6.8 Hz)] and one benzofuran ring [$\delta$C 105.1 (d), 143.3 (d); $\delta$H 6.67 (1H, d, J = 2.1 Hz), 7.49 (1H, d, J = 2.1 Hz)]. These signals showed similarities to those of 3,5-diaryloxy-2-(3-methyl-2-butenyl)bienzyl and 6-diaryloxy-4-(2-phenylethyl)benzofuran [16] and revealed that 1 may be a prenylated benzyl which possesses a benzofuran ring. This deduction was confirmed by the $^1$H−$^1$H COSY correlations of H-7/H-8; H-1′/H-2′ and the HMBC correlations of H-5/C-5, C-6; H-1′/C-1, C-2, C-3; Me-4′/C-2′, C-3′, C-5′; Me-5′/C-2′ (Fig. 2). Therefore, the structure of 1 was determined as 3-diaryloxy-2-(3-methyl-2-butenyl)-1-(2-phenylethyl) benzofuran, and it is the first example of prenylated benzyl which possesses a benzofuran ring.

Kurzphenol B (2) was obtained as optically active yellow oil ($[\alpha]_D^{20} = 1.77$, $c$ 0.28, MeOH) and the molecular formula was deduced to be $C_{32}H_{32}O_8$ based on its HR-El-MS (m/z 408.1913), suggesting 12 degrees of unsaturation. The IR spectrum of 2 suggested characteristic bands of hydroxyl (3421 cm$^{-1}$) and carbonyl (1631 cm$^{-1}$) groups. The UV spectrum showed the characteristic absorbances for a dihydroflavonol ($\lambda_{\text{max}}$ (log $\varepsilon$) 300 (4.27) and 346 (3.74, sh) nm) [17]. An analysis of the NMR data of 2 (Table 1) suggested the presence of two prenyls [$\delta$C 21.2 (t)], 121.4 (d), 134.9 (s), 25.8 (q), 17.9 (q); $\delta$H 3.34 (2H, d, J = 7.0 Hz), 5.22 (1H, d, J = 7.0 Hz), 1.74 (3H, s), 1.81 (3H, s); $\delta$H 21.7 (t), 121.5 (d), 134.4 (s), 25.8 (q), 17.8 (q); $\delta$H 3.27 (2H, d, J = 7.1 Hz), 5.17 (1H, d, J = 7.1 Hz), 1.70 (3H, s), 1.66 (3H, s), one mono-substituted benzene ring [$\delta$H 7.54 (2H, d, J = 7.1 Hz), 7.44 (2H, t, J = 7.1 Hz), 7.41 (1H, t, J = 7.1 Hz)], two adjacent oxygenated methines [$\delta$H 83.1 (d), 72.5 (d); $\delta$H 5.02 (1H, d, J = 11.9 Hz), 4.49 (1H, d, J = 11.9 Hz)] and one ketone [$\delta$C 196.2]. A comparison of the 1D NMR data (Table 1) of 2 with those of 6,8-diprenylaromadendrin [17] revealed that 2 may be a diprenyldihydroflavonol. The differences between them could be rationalized to the carbon signals corresponding to an oxygenated quaternary carbon (C-4′, $\delta$C 156.4) in 6,8-diprenylaromadendrin, which were replaced by a non-oxygenated methine [$\delta$C 129.1 (d), $\delta$H 7.41 (1H, d, J = 7.1 Hz)] in 2. This deduction was confirmed by the $^1$H−$^1$H COSY correlations of H-3′/H-4′/H-5′ (Fig. 2). The observed HMBC (Fig. 2) correlations of H-1′/C-5, C-6, C-7, H-1′/C-7, C-8, C-9 demonstrated that two prenyls were located at C-6 and C-8, respectively.

The absolute configurations of 2 were elucidated by the comparison of the coupling constant patterns with those reported data and CD spectra. Two AB systems at $\delta$H 5.02 (1H, d, J = 11.9 Hz) and 4.49 (1H, d, J = 11.9 Hz) are characteristic of H-2 and H-3 in the axial conformation of a 2,3-trans-

### Table 1

$^1$H and $^{13}$C NMR data of compounds 1–3 (δ in ppm, J in Hz).

<table>
<thead>
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<th>No.</th>
<th>1 (in CDCl$_3$)</th>
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<td>$\delta$C$^a$</td>
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<td>97.0 d</td>
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<td>10</td>
<td>105.9 s</td>
<td>163.2 s</td>
<td>105.9 s</td>
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$^a$ $^1$H NMR data measured at 500 MHz. 
$^b$ $^{13}$C NMR data measured at 100 MHz. 
$^c$ $^1$H NMR data measured at 600 MHz.
Fig. 1. Structures of compounds 1–17.

Fig. 2. Selected HMBC (\(\sim\)) and \(^1H–^1H\) COSY (\(\rightarrow\)) correlations of compounds 1–3.
The cytotoxicity of compounds 1–2 and C-3 was determined to be both R by analysis of the CD spectrum, in which a positive Cotton effect (+1.24) was observed at 319 nm along with a negative Cotton effect (−7.18) at 294 nm [18]. Therefore, the structure of 2 was determined as (2R, 3R), 6,8-diprenylinobanksin.

Kurzphenol C (3) possessed a molecular formula of C_{21}H_{26}O_{6} as deduced from its HR-ESI-MS ([M + H]^+ m/z 367.1187), suggesting 12 degrees of unsaturation. The IR spectra of 3 indicated the characteristic bands of hydroxyl (3424 cm\(^{-1}\)) and conjugated carbonyl (1657 cm\(^{-1}\)) groups, which was further supported by its NMR data. The 1D NMR data (Table 1) exhibited one 8-substituted kaempferol [34] which was further supported by its NMR data. The 1D NMR data were in agreement with those of icaritin [19]. Therefore, the structure of 3 was determined as 3-O-methyl-8-(3-methyl-2-butenyl)kaempferol.

Table 2

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<th>Hep G2</th>
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Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.10.003.

References


