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Bioactive Prenylated Flavonoids from the Stem Bark of *Artocarpus kemando*

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Four known prenylated flavonoids, artonins E (**1**) and O (**2**), artobiloxanthone (**3**), and cycloartobiloxanthone (**4**), were isolated from the stem bark of *Artocarpus kemando* by bioassay-guided fractionation using the DNA strand-scission and the KB cytotoxicity assays as monitors. Compounds **1** and **3** exhibited strong DNA strand-scission activity, and all four compounds were found to be cytotoxic.

Key words: *Artocarpus kemando*, Moraceae, Flavonoids, LC-MS dereplication, Cytotoxicity, DNA strand-scission activity.

INTRODUCTION

Previous phytochemical work on the genus *Artocarpus* (Moraceae) has resulted in the isolation of various types of prenylated flavonoids (Fujimoto *et al.*, 1990; Hakim *et al.*, 1999; Ko *et al.*, 1998; Kumar *et al.*, 1977; Lin and Shieh, 1992; Makmur *et al.*, 2000; Shimizu *et al.*, 2000a; Shimizu *et al.*, 2000b). Some of the flavonoids isolated from this genus have been shown to inhibit K^+-dependent amino acid transport (Parenti *et al.*, 1998), 5-lipoxygenase (Koshihara *et al.*, 1988), and 5α-reductase (Shimizu *et al.*, 2000a; Shimizu *et al.*, 2000b), and to exhibit antioxidant (Ko *et al.*, 1998) and cytotoxic effects (Fujimoto *et al.*, 1990). As part of our ongoing program for the discovery of anticancer agents from plants (Kinghorn *et al.*, 1999), a chloroform-soluble extract of the stem bark of *A. kemando* Miq. (Jarrett, 1959), which was collected in Indonesia, exhibited DNA strand-scission activity, [which is reported to be the mode of action of bleomycin (Sugiyama *et al.*, 1985)], as well as cytotoxicity against KB cells. No previous biological or phytochemical investigations on this species have been reported.

In the present investigation, the bioassay-linked HPLC-ESIMS dereplication (Constant and Beecher, 1995) and the bioassay-guided chromatographic separation of a chloroform-soluble extract of the stem bark of *A. kemando* using the *in vitro* DNA strand-scission and the KB cell cytotoxicity assays, led to the isolation of four known prenylated flavonoids, artonins E (**1**) (Fujimoto *et al.*, 1990; Hano *et al.*, 1990) and O (**2**) (Hano *et al.*, 1993), artobiloxanthone (**3**) (Fujimoto *et al.*, 1990; Sultanbawa and Surendrakumar, 1989), and cycloartobiloxanthone (**4**) (Hano *et al.*, 1993; Hano *et al.*, 1990). Artonin E (**1**) and artobiloxanthone (**3**) exhibited DNA strand-scission activity, whereas all four compounds were found to be cytotoxic against KB cells. This communication deals with the isolation, identification, and biological evaluation of compounds **1**-**4**.

MATERIALS AND METHODS

Plant material

The stem bark of *A. kemando* Miq. was collected at

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Muara Tebo, Jambi, Sumatra, Indonesia in August, 1997. A voucher specimen (accession number ISJB6) has been deposited at the Herbarium Bogoriense, Indonesian Institute of Science, Bogor, Indonesia.

Extraction and isolation

The dried plant material (450 g) was ground and extracted with MeOH (1.5 L×2) for 24 h by percolation. The MeOH extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract was suspended in MeOH:H_2O (1:9) and partitioned with *n*-hexane (300 mL). The aqueous MeOH fraction (300 mL) was further partitioned with $CHCl_3$ (300 mL×2). The $CHCl_3$ fraction was washed with 1% saline solution, and evaporated, affording a $CHCl_3$-soluble extract (9.0 g). The $CHCl_3$-soluble extract displayed significant activity both in a DNA strand-scission (100% relaxation at 25 μg/mL) and a KB cell cytotoxicity (0% survival at 20 μg/mL) assays.

Fractionation of the $CHCl_3$-soluble extract (8.5 g) was initiated by Silica gel column chromatography using a $CHCl_3$-MeOH gradient (100:0 → 10:1) as mobile phase to afford eleven fractions (F001-F011). Fraction F003 (1.5 g), eluted with % MeOH in $CHCl_3$, was subjected to Sephadex LH-20 column chromatography using $CHCl_3$-MeOH (3:1), resulting in pooled fractions F012-F015. Fraction F014 was purified by HPLC [Waters 3000 system; Inertsil ODS 3 column (MetaChem, Torrance, CA), 250×25 mm i.d., 3 μm; Inertsil ODS 3 guard column, 50×10 mm i.d., 8 μm], leading to the isolation of artonin O {**2**, 12.4 mg, 80% MeOH in H_2O, flow rate 10 mL/min, t_R 43.4; mp 131-133 °C; $[\alpha]_D$ 0° (*c* 0.09, MeOH)} {lit. mp 200°C (decomp.); $[\alpha]_D$ 0° (*c* 0.07, MeOH)} (Hano *et al.*, 1993). Fractions F004 (0.5 g, 1.2% MeOH in $CHCl_3$) and F006 (0.4 g, 2% MeOH in $CHCl_3$) were chromatographed on Sephadex LH-20 (MeOH) and HPLC, resulting in the purification of cycloartobiloxanthone {**4**, 45.7 mg, 85% MeOH in H_2O, flow rate 10 mL/min, t_R 17.6; mp 284-285°C; $[\alpha]_D$ 0° (*c* 0.19, MeOH)} (lit. mp 285-287°C) (Hano *et al.*, 1993; Hano *et al.*, 1990) and artonin E (**1**, 20.0 mg, 85% MeOH in H_2O, flow rate 15 mL/min, t_R 16.5; mp 240-242°C) (lit. mp 243-245°C) (Fujimoto *et al.*, 1990; Hano *et al.*, 1990), respectively. Fraction F005 (1.7 g, 1.5% MeOH in $CHCl_3$) was sequentially subjected to passage over Sephadex LH-20 (MeOH), Silica gel (*n*-hexane-acetone, 5:1), and finally HPLC to afford artobiloxanthone {**3**, 4.5 mg, 90% MeOH in H_2O, flow rate 10 mL/min, t_R 25.5, mp 158-160°C, $[\alpha]_D$ 0° (*c* 0.1, MeOH)} (lit. mp 160-162°C, $[\alpha]_D$ 0°) (Fujimoto *et al.*, 1990; Sultanbawa and Surendrakumar, 1989).

HPLC-ESIMS dereplication

The $CHCl_3$ extract was subjected to dereplication analysis, employing a previously published protocol, using standard chromatographic conditions and the Col2 (human colon cancer), LNCaP (hormone-dependent human prostate cancer), and KB (human oral epidermoid carcinoma) cytotoxicity assays to monitor activity (Constant and Beecher, 1995; Cordell and Shin, 1999).

Biological evaluation

The original $CHCl_3$ extract, chromatographic fractions, and compounds **1-4** were tested in DNA strand-scission and KB cytotoxicity assays using established protocols (Likhitwitayawuid *et al.*, 1993; Seo *et al.*, 1999).

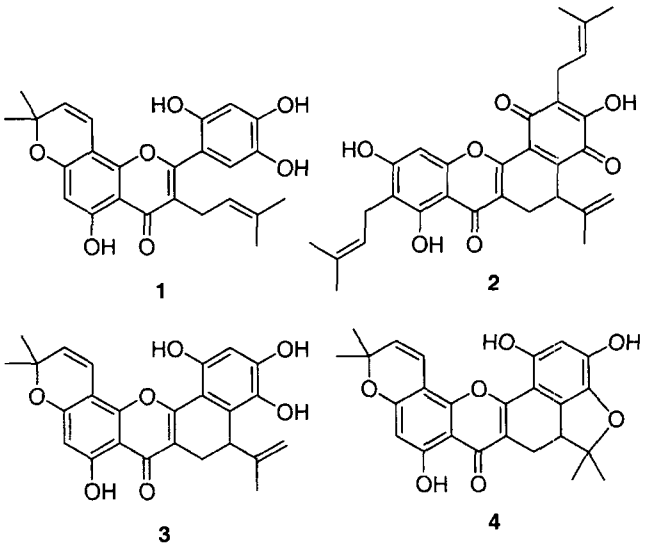
RESULTS AND DISCUSSION

Bioassay-guided fractionation of a chloroform-soluble extract applying successive Silica gel and Sephadex LH-20 column chromatography and HPLC, resulted in the isolation of artonins E (**1**) (Hano *et al.*, 1990) and O (**2**) (Hano *et al.*, 1993), artobiloxanthone (**3**) (Sultanbawa and Surendrakumar, 1989), and cycloartobiloxanthone (**4**) (Hano *et al.*, 1990). The structures of these compounds were identified by comparison of their physical and spectral data with those reported in the literature. Compounds **1-4** were evaluated in DNA strand-scission and the KB (human oral epidermoid carcinoma) cell cytotoxicity assays (Likhitwitayawuid *et al.*, 1993; Seo *et al.*, 1999). The biological profiles of **1-4** are summarized in Table I. Artonin E (**1**) and artobiloxanthone (**3**), which have a catechol moiety in ring B, exhibited significant DNA strand-scission activity (93 and 84% relaxation at 2.5 μg/mL, respectively), whereas artonin O (**2**) and cycloartobiloxanthone (**4**), which lack the catechol moiety, were found to be inactive. In previous reports (Chaudhuri *et al.*, 1995; Huang *et al.*, 1996; Huang *et al.*, 1998; Seo *et al.*, 1999), we have noted that the presence of a catechol moiety has been associated with DNA strand-scission activity, and this idea was supported by the postulation that the catechol moiety chelates Cu^{2+} (Singh *et al.*, 1995). The results of the present study are in good agreement with previous reports (Chaudhuri *et al.*, 1995; Huang *et al.*, 1996; Huang *et al.*, 1998; Seo *et al.*, 1999). Compounds **1-4** showed modest cytotoxic activity against KB cells (Table I), and no direct correlation between DNA

Table I. DNA strand-scission and KB cytotoxicity of compounds 1-4

Compound	DNA strand-scission[a]	KB[b]
1	93	3.0
2	inactive[c]	0.5
3	84	3.5
4	inactive[c]	2.5

[a]% relaxation of supercoiled DNA determined at a concentration of 2.5 μg/mL. [b]IC_{50} (concentration to inhibit cell growth by 50%) values (μg/mL). [c]% relaxation of supercoiled DNA at 2.5 μg/mL <80%.



strand-scission activity and KB cell cytotoxicity was observed, implying alternative mechanism of action.

The presence of **3** and **4** was predicted using a bioassay-linked HPLC-ESIMS dereplication method (Constant and Beecher, 1995; Cordell and Shin, 1999), in which the chloroform-soluble extract of the stem bark of *A. kemando* was evaluated using the Col2 (human colon cancer), LNCaP (hormone-dependent human prostate cancer), and KB cell lines. The ions found in the negative mode extracted ion chromatogram of the active region were at *m/z* 391, 407, and 433, of which that at *m/z* 433 corresponded to the quasimolecular ion of compounds **3** (Sultanbawa and Surendrakumar, 1989) and **4** (Hano *et al.*, 1990).

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