



An Oxepinoflavone from *Artocarpus elasticus* with Cytotoxic Activity Against P-388 Cells

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A new oxepinoflavone, artoindonesianin E1 (**1**), was isolated from the wood of *Artocarpus elasticus*, along with four known prenylated flavones: artocarpin (**2**), cycloartocarpin (**3**), cudraflavones A (**4**) and C (**5**). The structure of the new compound was identified by spectroscopic methods. Upon cytotoxic evaluation against murine leukemia P-388 cells, the new compound showed IC₅₀ 5.0 µg/mL.

Key words: *Artocarpus elasticus*, Flavonoids, Oxepinoflavone, Artoindonesianin E1, Cytotoxicity, P-388 cells

INTRODUCTION

Artocarpus plants (Moraceae) are known to be rich sources of C-3 prenylated flavones containing a unique oxygenation pattern at C-2'/C-4' and C-2'/C-4'/C-5' in ring B of the flavone skeleton (Nomura and Hano, 1994; Nomura et al., 1998). The occurrence of these plants in Indonesia is well-known and some are endemic including *Artocarpus elasticus* Reinw. ex Blume. In this region, particularly in the west part of Java, this plant has been used to treat inflammation, female contraception (bark), dysentery (latex), and tuberculosis (young leaves) (Heyne, 1987). A literature survey disclosed that a number of chemical and biological studies have been carried out on this plant (Kijjoa et al., 1996; 1998; Nascimento et al., 1997; Cidade et al., 2001; Pedro et al., 2005; Ko et al., 2005; Cerqueira et al., 2008), and revealed that 3-prenylflavones, pyranoflavones, furanodihydrobenzoxanthone-type of flavones, and oxepinoflavones are the major flavonoids isolated. In continuation of our work aimed at finding new cytotoxic metabolites of *Artocarpus* (Hakim et al., 2006; Syah et al., 2006a; 2006b, 2006c), we have examined wood samples of this plant and have isolated a

new oxepinoflavone, which we named artoindonesianin E1 (**1**) (Fig. 1), together with four known prenylated flavones: artocarpin (**2**), cycloartocarpin (**3**), cudraflavones A (**4**) and C (**5**). This paper reports the isolation and structure elucidation of compound **1** and its cytotoxic activity against murine leukemia P-388 cells.

MATERIALS AND METHODS

General

UV and IR spectra were measured with a Varian Conc. 100 instrument and Perkin Elmer Spectrum One FTIR spectrometers (KBr), respectively. ¹H and ¹³C NMR spectra were recorded with a JEOL ECP400 operating at 400 (¹H) and 100 (¹³C) MHz, using residual (δ_H 2.04) and deuterated solvent (δ_C 29.8) peaks of acetone-*d*₆ as reference standards. Mass spectra were measured with a VG Autospec mass spectrometer (EI mode). VLC (vacuum liquid chromatography) and radial chromatography were carried out using Merck silica gel 60 GF₂₅₄; for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF₂₅₄, 0.25 mm thickness) were used. Solvents used for extraction and preparative chromatography were of technical grade and distilled before use.

Plant material

The wood of *A. elasticus* was collected in August 2004 from the Malangbong District, West Java, Indonesia. The

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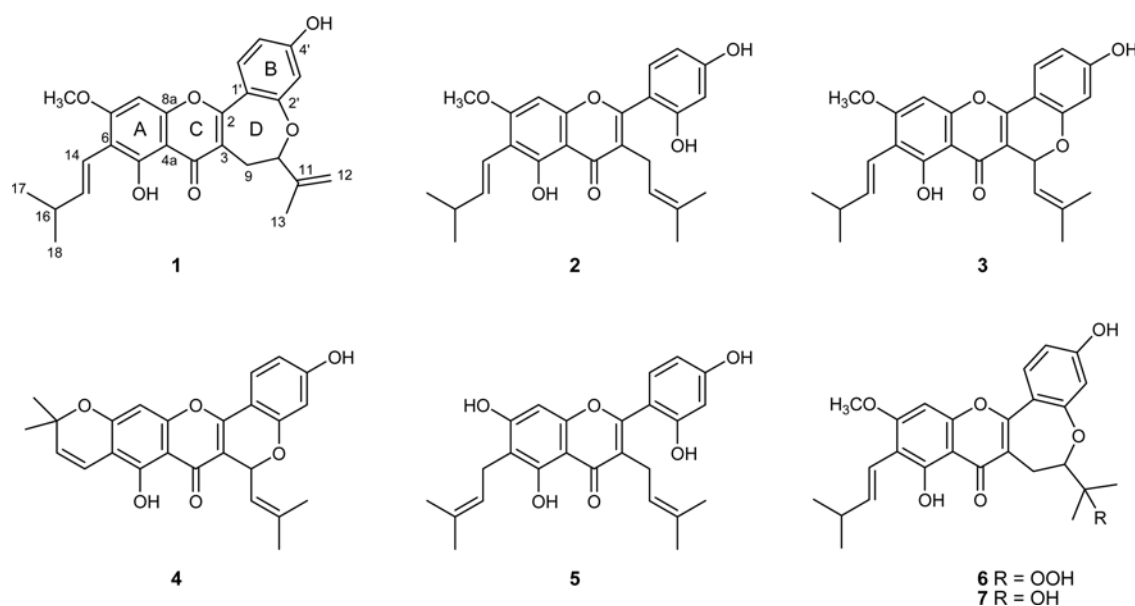


Fig. 1. Structures of prenylated flavone derivatives

plant was identified by staff at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia, and the voucher specimen has been deposited at the herbarium.

Extraction and isolation

The dried and powdered wood of *A. elasticus* (2.5 kg) was macerated in MeOH. The total MeOH extract (80 g) was fractionated by VLC (silica gel, *n*-hexane-EtOAc = 3:1 → 0:10) into four major fractions, A-D. Fraction C (14.9 g) was refractionated using the same method (silica gel, *n*-hexane-EtOAc = 9:1 → 0:10) into another four fractions, C1-C4. Fraction C4 (1.1 g) was purified by centrifugal planar chromatography (silica gel, CHCl₃-EtOAc-MeOH = 12:8:1) to give compounds **1** (10 mg) and **5** (30 mg). Using the same methodology on fraction C-1 (0.24 g) afforded compounds **4** (15 mg) and **3** (85 mg), and from fraction C-3 (2.8 g), compound **2** (175 mg) was obtained.

Artoindonesianin E1 (**1**)

Pale yellow solid; UV (MeOH) λ_{max} nm (log ϵ) 280 (4.40), 328 (4.01); UV (MeOH+NaOH) λ_{max} nm (log ϵ) 277 (4.40), 368 (3.91); IR (KBr) ν_{max} 3403, 2956, 2866, 1651, 1607, 1556, 1482, 1461; ¹H NMR (acetone-*d*₆) see Table I; ¹³C NMR (acetone-*d*₆) see Table I; HREIMS (*m/z*) 434.1721 (calcd. for C₂₆H₂₆O₆ 434.1729).

Cytotoxicity assay

The cytotoxicity of compound **1** against murine leukemia P-388 cells was conducted using MTT assay according to the method previously described (Sahidin et al., 2005).

RESULTS AND DISCUSSION

Maceration of the dried and powdered wood of *A. elasticus* in MeOH yielded a brown extract. Fractionation of the extract by VLC on silica gel gave four major fractions, A-D. From TLC analysis, the flavonoid constituents were concentrated in fraction C which was further fractionated using the same method to give four major fractions, C1-C4. Compounds **1** and **5** were obtained from fraction C4, while compound **2** from fraction C3, and compounds **2** and **4** from fraction C1. Compounds **2-5** were identified, based on the analysis of their ¹H and ¹³C NMR data, as artocarpin (Lin et al., 1995; Wang et al., 2004), cycloartocarpin (Nair et al., 1990; Lu and Lin, 1994), cudraflavones A (Wei et al., 2005) and C (Hano et al., 1990).

Compound **1** was isolated as a pale yellow solid. The UV spectrum of **1** showed absorption maxima (λ_{max} 280 and 328 nm) typical for a flavone chromophore (Mabry et al., 1970). The HREIMS of **1** showed a molecular ion at *m/z* 434.1721, consistent with the molecular formula C₂₆H₂₆O₆ (calcd. 434.1729, Δ 0.8 mmu). The ¹³C NMR spectrum of **1** (APT) (Table I) disclosed the presence of 26 carbon resonances, one of them (δ_{C} 56.6) being assignable to a methoxyl carbon atom. These spectroscopic data suggested that compound **1** was a methoxyl derivative of a diprenylated flavone. The ¹H NMR spectrum of **1** (Table I), however, did not show signals characteristic for a 3-methyl-2-buten-1-yl group. Instead, it exhibited proton signals characteristic of an oxepin ring (δ_{H} 2.59, 2.78 and 4.42, each *dd*), a 3-methyl-1-buten-1-yl (δ_{H} 1.08, 6H; 2.44, 6.60 and 6.72, each 1H), a propen-2-yl (δ_{H}

Table I. NMR data (acetone- d_6) of artoindonesianin E1

C No	δ_H (mult., J in Hz)	δ_C	HMBC ($^1H \leftrightarrow ^{13}C$)
2	-	163.3	-
3	-	119.8	-
4	-	183.8	-
4a	-	105.4	-
5	-	159.7	-
6	-	109.8	-
7	-	163.9	-
8	6.58 (s)	90.5	C-4a, C-6, C-7, C-8a
8a	-	157.4	-
9	2.59 (dd, 13.6, 8.4) 2.78 (dd, 13.6, 5.1)	30.3	C-2, C-3, C-4, C-10, C-11
10	4.42 (dd, 8.4, 5.1)	73.8	*
11	-	148.7	-
12	4.67 (s) 4.82 (s)	110.4	C-10, C-13
13	1.58 (s)	17.6	C-10, C-11, C-12
14	6.60 (d, 16.0)	116.9	C-5, C-6, C-7, C-15
15	6.72 (dd, 16.0, 6.9)	142.3	C-6, C-14, C-16, C-17 18
16	2.44 (oct, 6.9)	30.4	C-15, C-17 18
17/18	1.08 (d, 6.9)	23.1	C-15, C-16, C-18 17
1'	-	112.9	-
2'	-	156.9	-
3'	6.53 (dd, 2.6, 8.8)	108.2	C-1', C-2', C-4', C-5'
4'	-	161.5	-
5'	6.54 (d, 2.6)	104.1	C-3', C-1'
6'	7.33 (d, 8.8)	132.8	C-2', C-4', C-2
5-OH	13.80 (s)	-	C-4a, C-6, C-5
7-OCH ₃	3.98 (s)	56.6	C-7

*HMBC correlations from H-10 were not observed.

1.58, 3H; 4.67 and 4.82, each 1H), a chelated phenolic (δ_H 13.80) group, and a methoxyl signal at δ_H 3.98. In the aromatic region, a singlet (δ_H 6.58) as well as signals of an ABM-type (δ_H 6.53, 6.54 and 7.33) were observed. These parameters were very similar to those of artoindonesianin B (**6**) (Hakim et al., 1999), except that the 2-hydroperoxyprop-2-yl group attached to the oxepin ring (ring D) at C-10 in **6** was replaced by the propen-2-yl group in **1**. Structure **1** can thus be formulated for the new compound, artoindonesianin E1. Confirmation of the structure was obtained from 1H - ^{13}C correlations found in the HMQC and HMBC spectra of **1**. The HMBC spectrum, in particular, showed two- or three-bond correlations between the proton signals of the propen-2-yl group and a carbon signal at δ_C 73.8 (C-10); between a doublet signal at δ_H 6.60 (H-14) and carbon signals at δ_C 159.7 (C-5), 109.8 (C-6), and 163.9 (C-7); and between the latter carbon signal and the proton methoxyl signal (δ_H

3.98). Other HMBC correlations, in support of structure **1**, are shown in Table I.

The cytotoxic properties of compounds **2-5** against murine leukemia P-388 cells have been reported in a previous study (Hakim et al., 2006). Using the same assay, compound **1** showed moderate cytotoxicity with IC_{50} 5.0 $\mu g/mL$. A comparison of the cytotoxic properties of compound **1** to those of compound **6** (IC_{50} 3.9 $\mu g/mL$) and chaplashin (**7**) (IC_{50} 2.0 $\mu g/mL$) (Hakim et al., 2006) suggested that the presence of the 2-hydroxyprop-2-yl group at C-10 of the oxepin ring (ring D) could be important for cytotoxicity against P-388 cells. Replacing this group with a 2-hydroperoxyprop-2-yl (as in **6**) or a propen-2-yl group (as in **1**) reduces the cytotoxic properties.

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