Two Prenylated Flavones from the Tree Bark of *Artocarpus lanceifolius*

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Two new prenylated flavones, artoindonesianins *Z*-1 (**1**) and *Z*-2 (**2**), together with two other known prenylated flavones, artobiloxantone (**3**) and cycloartobiloxanthone (**4**), had been isolated and identified from the chloroform extract of the tree bark of *Artocarpus lanceifolius*. The structures of these compounds were determined based on spectroscopic data, including UV, IR, 1-D and 2-D NMR, and mass spectra. The significance of the presence of the isolated compounds to the chemotaxonomy of *Artocarpus* is briefly discussed.

Key words: Artoindonesianins Z-1 and Z-2, *Artocarpus lanceifolius*, Chemotaxonomy

Introduction

The genus of *Artocarpus* (Moraceae) consists of approximately 50 species and is widely distributed in tropical and subtropical regions, including in Indonesia [1]. Phytochemical studies show that this genus is an exceptionally rich source of prenylated flavonoids, notably the unique C-3 prenylated flavone derivatives containing oxygenated functionalities at C-2' and C-4' or C-2', C-4', and C-5'. One of the endemic species is *Artocarpus lanceifolius* Roxb., found mainly in the western part of Indonesia and locally known as "Keledang" [2]. Previous phytochemical investigations on this plant revealed a number of prenylated flavones [3, 4] which showed significant cytotoxic effects against murine leukemia P-388 cells. Recently, investigators of Singapore isolated from the same species a rare prenylated flavone, 12-hydroxyartonin E (**5**), containing an alcohol group at C-12 [5]. In this paper, we report the isolation two new prenylated flavones, artoindonesianins *Z*-1 (**1**) and *Z*-2 (**2**), together with the two known compounds artobiloxanthone (**3**) and cycloartobiloxanthone (**4**) (Fig. 1). Structures of these new compounds were determined based on spectroscopic evidence. Chemotaxonomy of *Artocarpus* is briefly discussed.

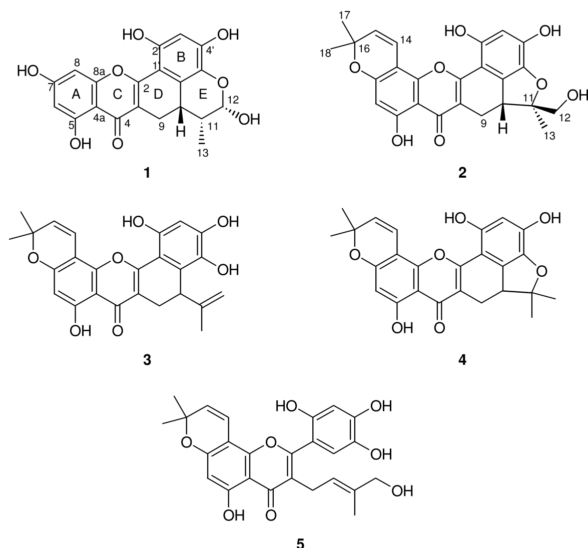

Fig. 1. Compounds isolated from *Artocarpus lanceifolius*.

Results and Discussion

The powdered dried tree barks of *A. lanceifolius* were macerated with methanol at r. t. After solvent evaporation under reduced pressure the concentrated methanol extract was diluted with water, and was partitioned into benzene and $CHCl_3$ fractions. The $CHCl_3$ fraction was fractionated using vacuum liquid chromatography technique on silica gel to give six major fractions A – F. Fractions D and E were separately refractionated using the same method and from TLC analysis the fractions rich in compounds **1** and

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Table 1. NMR data of compounds **1** and **2**.

	δ_H (multiplicity, J in Hz)		δ_C	
C-No.	**1**	**2**	**1**	**2**
2	–	–	160.5	160.6
3	–	–	111.2	111.4
4	–	–	178.9	179.9
4a	–	–	103.4	104.0
5	–	–	161.3	160.8
6	6.14 (d, 2.1)	6.17 (s)	98.4	98.8
7	–	–	163.4	157.9
8	6.30 (d, 2.1)	–	93.6	100.6
8a	–	–	156.6	150.5
1'	–	–	103.9	102.6
2'	–	–	150.8	150.8
3'	6.37 (s)	6.26 (s)	103.2	104.0
4'	–	–	150.8	146.0
5'	–	–	132.4	137.0
6'	–	–	124.7	132.6
9	1.82 (t, 15.3)	2,48 (t, 15,2)	21.7	19.5
	3.11 (dd, 15.3, 5.5)	3,15 (dd, 15.2, 7.0)		
10	2.53 (m)	3,38 (dd, 15.2, 7.0)	31.3	45.6
11	1.76 (m)	–	35.7	93.2
12	5.30 (dd, 4.3, 2.1)	3.45 (dd, 11.3, 5.2)		
		3.52 (dd, 11.3, 5.5)	93.1	63.4
13	1.06 (d, 6.8)	1.52 (s)	14.6	23.6
14		6.80 (d, 10.1)		114.5
15		5.73 (d, 10.1)		127.5
16		–		78.0
17		1.41 (s)		27.9
18		1.43 (s)		27.7
5-OH	13.24 (s)	13.38 (s)		
12-OH	6.88 (d, 4.3)	4.88 (dd, 5.2, 5.5)		
2'-OH	*	9.80 (s)		
4'-OH	*	10.13 (brs)		

* 7-, 2'-, 4'-OH appeared as very broad singlets at δ_H = 9.44, 9.85, 10.63.

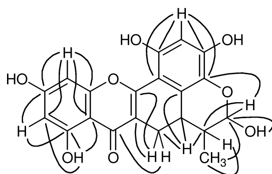

Fig. 2. Selected important HMBC correlations in **1**.

2 were combined. The combined fraction was repeatedly purified using gravitational column chromatography and crystallization to give compounds **1** and **2**. Using the same procedures, fraction C gave compounds **3** and **4** [6].

Compound **1** was isolated as a yellow solid, $[\alpha]_D$ + 1.8 (MeOH, *c* 0.7). The HRFABMS of compound **1** showed a $[M+H]^+$ ion at *m/z* 385.0930 corresponding to a molecular formula $C_{20}H_{16}O_8$. The UV and IR spectra of **1** showed typical absorptions (λ_{max} 261, 313, 380 nm; ν_{max} 1649, C=O) for a flavone containing free hydroxyl groups, including those at C-5, as shown by absorption shifts in the UV spectrum on addition of NaOH and $AlCl_3$. These spectroscopic data suggested that compound **1** was a flavone containing one prenyl unit. Support for a flavone skeleton in **1** was shown by its NMR spectra (Table 1), including a DEPT 135 spectrum, showing a singlet proton signal (δ_H = 13.38 ppm) of a chelated –OH group at C-5 and a quarternary carbon signal (δ_C = 178.9 ppm) for a conjugated carbonyl group at C-4. Furthermore, the presence of carbon signals of oxygenated C-sp^2 atoms (δ_C = 132.4, 150.8 (2C), 156.6, 160.5, 161.3, 163.4 ppm) accounted for seven oxygen atoms in **1**. Taken together with three aromatic proton signals at δ_H = 6.14, 6.30, and 6.37 ppm, this indicated that the pattern of oxygenated functionalities is the same to those of artoindonesianins U and V [7], *i. e.* at C-5 and C-7 in the ring A and at C-2', C-4' and C-5' in the ring B of the flavone skeleton, as well as a prenyl group at C-3. The remaining oxygen atom in **1** was found to be a hemiacetal group as indicated by the carbon and proton signals of a methine group at δ_C = 93.1 and δ_H = 5.30 ppm, respectively. This hemiacetal group must be originating from the prenyl group contained in **1**, and together with the remaining aliphatic proton signals at δ_H = 1.06, 1.76, 1.82, 2.53, and 3.11 ppm and a hydroxyl signal at δ_H = 6.88 ppm, it formed a spin system of -CH_2-CH(CH_3)-CH(O)-OH. The coupling relationship of these aliphatic proton and hydroxyl signals was confirmed by ^1H-^1H COSY spectrum. From these NMR data, structure **1** for artoindonesianin *Z*-1 could be formulated. Support for structure **1**, particularly the connectivities of the structural units involving the rings B – E, was provided by the HMQC and HMBC spectra as shown in Fig. 2. The relative stereochemistry of the aliphatic hydrogens in **1** was determined from the NOE difference spectra, showing that irradiation of the methyl signals at δ_H = 1.06 ppm (H_3-13) enhanced the intensities of the proton signals at δ_H = 1.76 (H-11) and δ_H = 3.11 ppm (H-9b). In addition, irradiation of the methine signal at δ_H = 5.30 ppm (H-12) enhanced the intensities of the proton signals at δ_H = 6.88 (12-OH), 1.76 (H-11), and 2.53 ppm (H-10). These NOE relationships indicated that H-10/H-11/H-12 are in *cis* relationship. Structure **1** was, therefore, assigned to artoindonesianin *Z*-1. The attempt to crystallize compound **1** to obtain a crystal suitable for X-ray analysis was unsuccessful, and, therefore the absolute stereochemistry of **1** was not determined.

Compound **2** was isolated as a yellow solid, $[\alpha]_D$ – 2.0 (MeOH, *c* 0.25). The molecular formula $C_{25}H_{22}O_8$

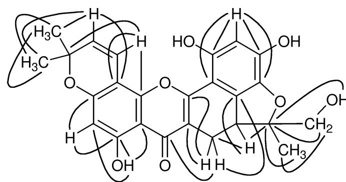

Fig. 3. Selected important HMBC correlations in **2**.

was assigned to compound **2** from its $[M+H]^+$ ion at *m/z* 451.1410, suggesting that the compound is a hydroxyl derivative of cycloartobiloxanthone (**4**) [6], which is also isolated in this plant. The UV and IR spectra of **2** resembled to those of compound **4**, indicating that compound **2** contains also a furanodihydrobenzoxanthone skeleton. The NMR data of compound **2** (Table 1) were also very similar to those of compound **4**, with the exception that one of the methyl group has changed to a hydroxymethylene ($\delta_H = 3.45$ and 3.52, each 1H and *dd*, H-12a/12b; 4.88, 1H, *dd*, 12-OH; $\delta_C = 63.4$). From these spectroscopic data, artoindonesianin *Z*-2 was formulated as 12-hydroxycycloartobiloxanthone (**2**). Support for the structure **2** was provided by the HMQC and HMBC spectra. The HMBC correlations, shown in Fig. 3, confirmed the connectivities of structural units involving rings B – E and the hydroxymethylene group, as well as a fused ring between the dihydropyran moiety and the ring A at C-7 and C-8. The relative stereochemistry of the chiral carbon atoms was determined from NOE difference spectra. Irradiation at the methyl signal at $\delta_H =$ 1.52 ppm (H_3-13) enhanced only one of the methylene proton signal at $\delta_H = 2.48$ ppm (H-9a), which means that the hydrogen atoms H_3-13/H-10 should be in a *trans* relationship. Considering the high value of the coupling constants of H-9a signal, *i. e.* as a triplet of 15.2 Hz separation, implied that both the H-9a/H-10 and H-10/H3-13 should be in diaxial conformations. Consequently, the relative stereochemistry as shown in the structure **2** was assigned to artoindonesianin *Z*-2. The absolute stereochemistry of **2** was also not determined.

The presence of compounds **1** – **2** and **5** in *A. lanceifolius* represents the ability of the plant to hydroxylate the methyl group at C-12 of the C-3 prenylated flavone derivatives, and therefore has a significance to the chemotaxonomy of *Artocarpus*. Jarrett has divided the genus into two subgenera, *Artocarpus* and *Pseudojaca* [8, 9]. In the subgenus *Artocarpus* he further divides into two sections. Within this taxonomic division, *A. lanceifolius* is in the same section as *A. rigidus* (section *Duricarpus*), while *A. heterophyllus* and *A. champeden* belongs to another section (*Artocarpus*). Based on the content of prenylated flavones, *A. heterophyllus* and *A. champeden* [10 – 12] are dominantly characterized by the presence of a linier fused dihydropyran ring at the ring A, while *A. lanceifolius* and *A. rigidus* [10, 11] are dominant with the angular counterpart. The chemotaxonomic difference between these two pairs of *Artocarpus* species is comparable to the results of molecular phylogenetic study by Kanzaki *et al.* [13], and placed *A. lanceifolis* in the posisition more advance than *A. rigidus*. The ability of *A. lanceifolius* to modify further the C-3 prenylated flavones at C-12 of the prenyl unit, which has not happened in *A. rigida*, supported the molecular phylogenetic results. Thus, in general, the results of chemotaxonomy and molecular phylogeny are in agreement with Jarrett's division of *Artocarpus* species.

Experimental Section

General experimental procedures

All melting points were determined on a micro-melting point apparatus and were uncorrected. UV spectra were measured with a Varian Conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM A500 spectrometer, operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards. Mass spectra was obtained with a VG Autospec mass spectrometer (FAB mode). Vacuum liquid (VLC) and column chromatography were carried out using Merck silica gel 60 GF_{254} and silica gel G_{60} 35 – 70 mesh. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used.

Plant material

The tree barks of *A. lanceifolius* were collected from Bukit Tambun Tulang, West Sumatera, Indonesia, in March 1997, and identified by Dr. Rusdi Tamin from the Department of Biology, Andalas University, Padang, Indonesia. The voucher specimen was deposited at the herbarium of the Department.

Extraction and isolation

The powdered dried tree barks (2.8 kg) of *A. lanceifolius* were successively macerated with *n*-hexane and acetone, respectively, at room temperature for 24 h to give *n*-hexane (60 g) and acetone (140 g) extracts. The acetone extract was partitionated into benzene (73 g), $CHCl_3$ (30 g), and ethyl acetate (10 g) fractions. The $CHCl_3$ fraction was fractionated using vacuum liquid chromatography (silica gel; eluted with

n-hexane/ethyl acetate of increasing polarity) to give six major fractions A – F (0.2, 0.6, 2.1, 7.3, 6.4, and 10.4 g, respectively). Refractionation of fraction D using the same method yielded four major fraction D1 – D4 (0.1, 3.2, 1.4, and 0.8 g, respectively). On TLC analysis, fraction D3 showed to contain the same major components as those in the fraction E, and thus both fractions were combined. The combined fraction was refractionated again using vacuum liquid chromatography (silica gel; eluted with *n*-hexane/ethyl acetate of increasing polarity) to give five major fractions E1 – E5 (0.9, 2.4, 1.2, 0.8, and 0.7 g, respectively). Repeated purification of the combined fractions E2 and E3 using gravitational liquid chromatography (silica gel; eluted with benzene/acetone 17 : 3) afforded compound **2** (15 mg) after crystallization in benzene/acetone. The same procedure was also carried out to purify the combined fractions E4 and E5 (silica gel; eluted with benzene/acetone 2 : 1) to give compound **1** (35 mg). Using the same method, fractionation and purification of fraction C yielded compounds **3** (25 mg) and **4** (20 mg).

Artoindonesianin Z-1 (**1**): Yellow needle crystal. – M. p. 295 – 297 °C. – $[\alpha]_D^{20} + 1.8$ (0.7 mg/ml, MeOH). – UV/vis (MeOH): $\lambda_{max}(\log\varepsilon) = 261$ (3.96), 313 (3.47), 380 (3.90) nm; (MeOH+NaOH): 272 (3.93), 322 (3.40), 426 (4.08) nm; (MeOH+$AlCl_3$): 274 (3.94), 332 (3.48), 415 (3.94) nm; (MeOH+NaOAc) and (MeOH+NaOAc+H_3BO_3): no shifts from those in MeOH. – IR (KBr): $\nu = 3500 -$ 3400 (broad), 1649, 1602, 1558, 1485, 1396, 1269, 1164, 810 cm^{-1}. – ^1H NMR (500 MHz, DMSO-d_6): δ = see Table 1. – $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6): δ = see Table 1. – MS (EI, 70 eV): *m/z* (%) = 384 (31) $[M]^+$, 356 (3.6), 326 (100), 297 (8.7), 255 (6). – HRMS (FAB): *m/z* 385.0930 $[M+H]^+$ (calcd. for $C_{20}H_{17}O_8$ 385.0923).

Artoindonesianin Z-2 (**2**): Yellow needle crystal. – M. p. 247 – 249 °C. – $[\alpha]_D - 2.0$ (MeOH, *c* 0.25). – UV/vis (MeOH): $\lambda_{max}(\log\varepsilon) = 228$ (4.04), 273 (4.03), 394 (3.78) nm; (MeOH+NaOH): 205 (4.60), 265 (4.24), 435 (4.10) nm; (MeOH+$AlCl_3$): 201 (4.06), 230 (4.07), 251 (3.83), 286 (4.07), 321 (3.73), 348 (3.62), 430 (3.78) nm; (MeOH+NaOAc): 204 (4.21), 229 (4.05), 265 (4.08), 426 (4.03). – IR (KBr): $\nu = 3400$ (broad), 2972, 1651, 1622, 1594, 1539, 1477, 1350, 1277, 1175, 1033 cm^{-1}. – ^1H NMR (500 MHz, DMSO-d_6): δ = see Table 1. – $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6): δ = see Table 1. – MS (EI, 70 eV): *m/z* (%) = 450 (25) $[M]^+$, 435 (100), 377 (20), 279 (4), 256 (12). – HRMS (FAB): *m/z* 451.1410 $[M+H]^+$ (calcd. for $C_{25}H_{23}O_8$ 453.1393).

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