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Artoindonesianins X and Y, two isoprenylated 2-arylbenzofurans, from *Artocarpus fretessi* (Moraceae)

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Abstract

Two isoprenylated 2-arylbenzofurans, artoindonesianins X and Y (**1**–**2**), together with seven known flavonoids, have been isolated from the roots and tree bark of *Artocarpus fretessi*. Their structures were established on the basis of spectral analysis. Compounds **1** and **2** showed moderate activity against the brine shrimp *Artemia salina*.

Keywords: *Artocarpus fretessi* Hassk.; Moraceae; Natural products chemistry; Isoprenylated 2-arylbenzofuran; Artoindonesianins X and Y; *Artemia salina*

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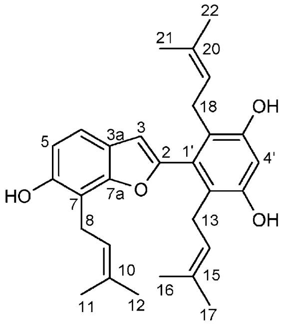
1. Introduction

The *Artocarpus* genus (Moraceae) is noted as an abundant source of isoprenylated flavonoids, a class of compounds whose chemistry and biological properties has attracted much attention (Nomura and Hano, 1994; Nomura et al., 1998). The biosynthetically related stilbene and 2-arylbenzofuran derivatives are also found in this genus although their distribution is more limited (Boonlaksiri et al., 2000; Christensen et al., 1988; Christensen and Lam, 1989; Lien et al., 1998; Likhitwitayawuid and Stritularak, 2001; Lin and Lu, 1993; Shimizu et. al., 1997; Shimizu at al., 1998; Su et al., 2002). Some of these compounds show interesting biological activities, such as antimalarial activity (Boonlaksiri et al., 2000), inhibition of tyrosinase and melanin biosynthesis (Likhitwitayawuid and Stritularak, 2001; Shimizu at al., 1998) and of 5α-reductase (Shimizu et al., 2000). In continuation of our studies on Indonesian moraceaous plants (Achmad et al., 1996; Ersam et al., 2002; Makmur et al., 2000; Hakim et al., 1999; Hakim et al., 2002a; b; Suhartati et al., 2001; Syah et al., 2000, 2001, 2002a, b), we now report the isolation and structure determination of two new isoprenylated arylbenzofurans, named artoindonesianins X and Y (**1**–**2**) from *A. fretessi* Hassk., along with seven known flavonoids. Compounds **1** and **2** showed moderate activity against the brine shrimps *Artemia salina*.



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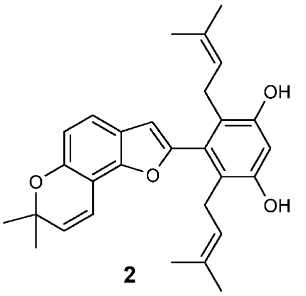

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2. Results and discussion

The dried, coarsely powdered, root bark of *A. fretessi* was extracted with MeOH and the MeOH extract was sequentially partitioned with *n*-hexane, CH_2Cl_2 and EtOAc, respectively. The CH_2Cl_2 fraction was further

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fractionated using VLC to give six major fractions A–F. Repeated purification of fraction C by radial chromatography yielded the new compounds artoindonesianins X (**1**) and Y (**2**). Using the same methods, five known flavonoids were isolated from the EtOAc fraction, and two other known flavone derivatives from the CH_2Cl_2 extract of the tree bark. The identity of the known compounds was established by comparison of their spectral data with those reported for mulberrin (Desphane et al., 1968; Wenkert and Gottlieb, 1977), norartocarpetin (Radhakrishnan and Rao, 1966), (±)-catechin (Achmad et al., 1998), (−)-afzelechin (Achmad et al., 1998), (−)-afzelechin-3-*O*-rhamnoside (Achmad et al., 1998), mulberrochromene (Desphane et al., 1968; Wenkert and Gottlieb, 1977), and artonin A (Hano et al., 1989), respectively.

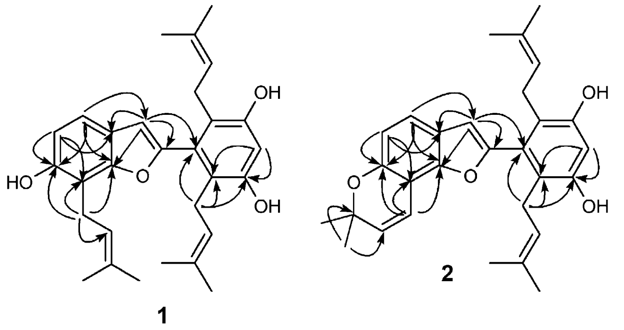
Artoindonesianin X (**1**), isolated as a yellow powder, has a molecular formula of $C_{24}H_{34}O_4$ as determined by HREIMS data. The UV spectrum of **1** showed absorption maxima at 203 and 297 nm, indicating the presence of an extended benzene chromophore. The IR spectrum of **1** exhibited absorptions for hydroxyl (3420 cm^{-1}), aliphatic (2973, 2914 and 2855 cm^{-1}) and aromatic (1596, 1489 and 1421 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) showed signals assignable to a 2,6,7-trisubstituted benzofuran moiety (δ 6.58, 6.81 and 7.29), a pentasubstituted phenyl (δ 6.53) and three isoprenyl groups. Two of the isoprenyl groups (δ 3.20, 4H; 5.23, 2H; 1.65 and 1.71, each 6H) could be assigned as substituents located at C-2′ and C-6′ of the pentasubstituted phenyl group. The other isoprenyl group (δ 3.65, 5.41, 1.76 and 1.84) can be located at C-7 of the 2-arylbenzofuran moiety given the need to accommodate the *ortho*-disposed protons at δ 6.81 and 7.29 ($J_{4,5} = 8.3$ Hz). From the above mentioned data, artoindonesianin X can be formulated as **1**. Further support for structure **1** came from the ^{13}C NMR spectrum of **1** (Table 1), assigned with the aid of DEPT, HMQC, and HMBC methods. The HMBC measurements, in particular, disclosed long-range correlations between the two symmetrical methylene signal at δ 3.20 (H_2-13/18) with carbon signals at δ 131.8 (C-1′), 120.1 (C-2′/6′) and 153.8 (C-3′/5′), and between the latter two carbon signals with the proton signal at δ 6.53, confirming the location of the two isoprenyl and hydroxyl groups at C-2′/6′ and C-3′/5′, respectively. In the HMBC spectrum, the signal assigned to the other methylene protons at δ 3.65 also showed strong correlation with carbon signals at δ 110.6 (C-7), 151.7 (C-6) and 154.0 (C-7a), securing the location of the third isoprenyl group at C-7. Other HMBC correlations were in agreement with structure **1** (Fig. 1). Further support for structure **1** came from comparison of the NMR data with that of related compound (Fukai et al., 1996), isolated from *Morus* species.

Table 1
^1H and ^{13}C NMR data of compounds **1** and **2**

No	δ_H (multiplicity, *J* in Hz)		δ_C	
	1	**2**	**1**	**2**
2	–	–	152.9	152.9
3	6.58 (*s*)	6.53 (*brd*)	106.9	106.8
3a	–	–	121.8	122.0
4	7.29 (*d*, 8.3)	7.28 (*d*, 8.3)	118.3	120.0
5	6.81 (*d*, 8.3)	6.75 (*d*, 8.3)	112.4	116.0
6	–	–	151.7	150.8
7	–	–	110.6	106.3
7a	–	–	154.0	150.2
1′	–	–	131.8	131.9
2′/6′	–	–	120.1	119.9
3′/5′	–	–	153.8	154.0
4′	6.53 (*s*)	6.51 (*s*)	105.3	105.4
8	3.65 (*d*, 7.1)	6.75 (*d*, 9.9)	23.0	112.5
9	5.41 (*m*)	5.68 (*d*, 9.9)	121.1	130.4
10	–	–	134.9	76.3
11	1.76 (*brs*)	1.47 (*s*)	17.9	27.7
12	1.84 (*brs*)	1.47 (*s*)	25.7	27.7
13/18	3.20 (*d*, 6.8)	3.17 (*brd*)	27.4	27.5
14/19	5.23 (*m*)	5.20 (*m*)	122.7	122.5
15/20	–	–	134.0	134.2
16/21	1.65 (*brs*)	1.63 (*brs*)	17.7	17.8
17/22	1.71 (*brs*)	1.70 (*brs*)	25.7	25.7

Artoindonesianin Y (**2**), isolated as a yellow powder, was shown to have a molecular formula $C_{24}H_{32}O_4$ by HREIMS, as expected for the didehydro derivative of **1**. The UV and IR properties of **2** were very similar to that of artoindonesianin X (**1**), except for the absence of the signals attributable to the isoprenyl group at C-7, and the presence of signals assignable to a –CH=CH–C(O–)$(CH_3)_2$ moiety, forming a fused 2,2-dimethylpyran ring at C-6 and C-7 of the benzofuran skeleton. Further evidence for structure **2** came from HMBC correlations, which showed connectivities between a pair of vinylic proton signals at δ 5.68 and 6.75 with carbon signals at δ 76.3 (C-10) and 106.3 (C-7). The second proton signal also correlated with carbon signals at δ 150.2 (C-7a) and 150.8 (C-6). Other HMBC connectivities were in agreement with structure **2** (Fig. 1). Accordingly, the structure of artoindonesianin Y was formulated as **2**.

Some isoprenylated 2-arylbenzofurans have been isolated from a very limited number of moraceous species. These include monoisoprenylated derivatives,



1 **2**

Fig. 1. Selected important HMBC correlations ($^1H \Rightarrow ^{13}C$) in **1** and **2**.

3-prenylmoracin M from *A. dadah* (Su et al., 2002), artotonkin from *A. tonkinensis* (Lien et al., 1998), and moracins A–E, G–I, K–L, N–Y from *Morus* species (Nomura, 1988), and diisoprenylated derivatives, morasin Z, mulberrofurans A, B, D, L and N (=mulberrofuran V) from *Morus* species (Fukai, 1996; Nomura, 1988). The isolation of triprenylated derivatives, artoindonesianins X (**1**) and Y (**2**) from *A. fretessi,* is therefore the first example for moraceous species. It is interesting to note that most of the isoprenylated 2-arylbenzofurans isolated from *Morus* species are considered to be phytoalexins (Nomura, 1988).

The compounds isolated from *A. fretessi* were tested in the brine shrimp (*A. salina*) bioassay (Meyer et al., 1982). Of these, compounds **1** and **2**, mulberrin, mulberrochromene, and artonin A showed moderate activity (LC_{50} 78.7, 294.1, 77.4., 67.8 and 100.6 µg/ml, respectively), while (±)-catechin, (−)-afzelechin, and (−)-afzelechin-3-*O*-rhamnoside were inactive ($LC_{50} > 500$ µg/ml).

3. Experimental

3.1. General experimental procedure

All melting points were determined on a micro-melting point apparatus and are uncorrected. UV and IR spectra were measured with UV/VIS Varian Cary 100 Conc and One Perkin-Elmer spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were recorded with a Bruker AM 500 operating at 500 (^1H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards. MS spectra were obtained with a VG Autospec mass spectrometer (EI mode). VLC was carried out using Merck Si-gel 60 GF_{254}, and TLC analysis on precoated Si-gel plates (Merck Kieselgel 60 F_{254}, 0.25 mm). All solvents were of technical grade and were distilled before used.

3.2. Plant material

Samples of *A. fretessi* were collected in December 1998 from the village of Kalaenakiri, Luwu District, South Sulawesi, Indonesia. The plant (Koba-Mora-Sulsel01) was identified by the staff at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia, and a voucher specimen has been deposited at the herbarium.

3.3. Extraction and isolation

The dried powdered root bark (1.8 kg) of *A. fretessi* was macerated with MeOH, and the MeOH extract was partitioned sequentially into *n*-hexane (20.2 g), CH_2Cl_2 (14.1 g), and EtOAc-soluble (43.0 g) fractions. The CH_2Cl_2 fraction was separated by VLC (silica gel, *n*-hexane–EtOAc of increasing polarity) into six major fractions A-F. Fraction C (1.1 g) was subjected to radial chromatography (silica gel, *n*-hexane–EtOAc = 8.5:1.5 and 8.0:2.0) to give a fraction (470 mg) which, on repeated purification using the same method (silica gel, 0–3% MeOH in $CHCl_3$, and 20–25% acetone in *n*-hexane), yielded compounds **1** (60 mg) and **2** (9 mg). Mulberrin (10 mg), norartocarpetin (15 mg), (±)-catechin (400 mg), (−)-afzelechin (70 mg), and (−)-afzelechin-3-*O*-rhamnoside (60 mg) were isolated from the EtOAc fraction in a similar way, while mulberrochromene (100 mg) and artonin A (5 mg) were obtained from CH_2Cl_2 extract of the tree bark (4.5 kg).

3.3.1. Artoindonesianin X (*1*)

Yellow powder; UV λ_{max} (MeOH) (log ε): 203 (4.43), 297 (3.93) nm; IR ν_{max} (KBr): 3420, 2973, 2914, 2855, 1596, 1489, 1421, 1373, 1280, 1215, 1159, 1119, 1088, 1041, 820 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) see Table 1. ^{13}C NMR (acetone-d_6, 125 MHz) see Table 1; EIMS m/z (rel. int.): [M^+] 446 (100), 431 (15), 429 (24), 403 (21), 391 (19), 375 (16), 347 (40); HREIMS m/z: [M^+] 446.2452 (calc. for $C_{29}H_{34}O_4$, 446.2457).

3.3.2. Artoindonesianin Y (*2*)

Yellow powder; UV λ_{max} (MeOH) (log ε): 203 (4.40), 231 (4.28), 279 (3.98) nm; IR ν_{max} (KBr): 3429, 2975, 2927, 1598, 1478, 1423, 1375, 1290, 1211, 1152, 1116, 1060, 821, 729 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) see Table 1; EIMS m/z (rel. int.): [M+] 444 (83), 429 (19), 401 (24), 389 (15), 373 (18), 345 (10); HREIMS m/z: [M^+] 444.2295 (calc. for $C_{29}H_{32}O_4$, 444.2301).

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