Artoindonesianins A and B, Two New Prenylated Flavones from the Root of *Artocarpus champeden*

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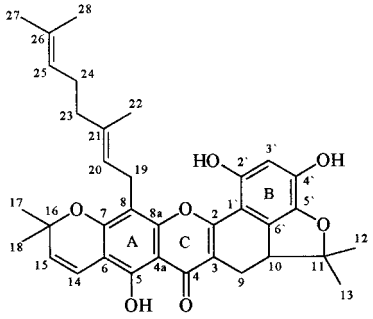
Two new prenylated flavones, named artoindonesianin A (**1**) and artoindonesianin B (**2**), were isolated from the root of *Artocarpus champeden*, together with a known prenylated flavone, artonin A. The structures of artoindonesianins A and B were determined on the basis of spectral evidence (MS, ^{1}H and ^{13}C NMR) and by comparison with known related compounds. Compounds **1** and **2** exhibited cytotoxic activity against murine leukemia (P-388) cells.

In continuation[1−3] of work on the genus *Artocarpus*, two new cytotoxic prenylated flavones, named artoindonesianin A (**1**) and artoindonesianin B (**2**), and a known compound, artonin A, were further isolated from the root of *Artocarpus champeden* Spreng., one of the endemic species of the genus *Artocarpus* (Moraceae). In this paper, we report the isolation and structure elucidation of these compounds on the basis of spectroscopic evidence.



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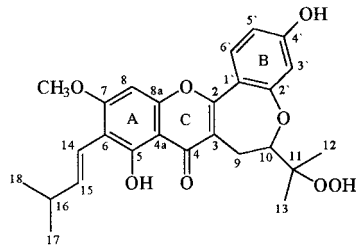

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Artoindonesianin A (**1**) was isolated as a yellow powder. The HRFABMS gave an $[MH]^+$ ion at *m/z* 571.2694, consistent with a molecular formula of $C_{35}H_{38}O_7$. The ^{13}C NMR spectrum revealed the presence of 35 carbons, including seven methyl groups and a carbonyl group (δ 180.2), corresponding to a tetraprenylated flavonoid. The IR spectrum showed absorptions typical of hydroxyl, conjugated carbonyl, and benzene ring functionalities, and the UV spectrum was consistent with the presence of a flavone structure. The analysis of its NMR data, including DEPT, HMQC, and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals. The ^{1}H NMR spectrum had resonances associated with two methyl groups, δ 1.23, 1.58 (each 3H, s) and an ABX spin system δ 2.27, 3.10, 3.34 attributed to the isoprenyl moiety located at the C-3 position, similar to the arrangement found for related compounds, artonin A[4] and cycloartobiloxanthone.[5] In addition to the pyrano-xanthonoid moiety, the ^{1}H NMR spectrum also indicated the presence of two methyl groups at δ 1.38, 1.39 (each 3H, s) and the olefinic protons at δ 5.74 and 6.57 (each 1H, d, J = 10.0 Hz), attributed to the 2,2-dimethylchromene ring. Of the remaining signals in the ^{1}H NMR spectrum, those at δ 1.45, 1.53, 1.79, 1.88, 1.97, 3.35, 3.55, 4.98, and 5.26 indicated the presence of a geranyl group. An aromatic singlet at δ 6.28 was consistent with a 1,2,4,5,6-pentasubstituted B-ring, and a downfield signal at δ 13.70 indicated a chelated hydroxyl group. The order of the substitution on the A and B rings was deduced from HMBC experiments. The HMBC measurements showed long-range correlations between the singlet at δ 10.15 (OH-4′), methine carbon at δ 104.0 (C-3′), and two quaternary carbons at δ 136.2 (C-5′) and 140.5 (C-4′), and between the singlet at δ 9.83 (OH-2′) and two quaternary carbons at δ 103.1 (C-1′) and 151.1 (C-2′), thus establishing the location of the one unsubstituted position on the B ring at carbon C-3′. The HMBC experiments also showed the connectivities between the vinyl proton at δ 6.57 (H-14) and three quaternary carbons at δ 153.5 (C-5), 104.5 (C-6), and 155.6 (C-7). Long-range correlations were observed for the methylene protons at δ 3.4−3.6 (H-19) to three quaternary carbons at δ 155.6 (C-7), 107.0 (C-8), and 152.9 (C-8a). These results provided support for the presence of the geranyl substituent at C-8, and the 2,2-dimethylchromene ring fused to C-6 and C-7. Further evidence for the structure assigned to **1** came from comparison of the ^{13}C NMR spectrum, assigned with the aid of HMQC and HMBC data, to that reported for the related compound artonin A,[4] and from UV and MS data.[6−9] Consequently, artoindonesianin A was assigned structure **1**.

Artoindonesianin B (**2**) was isolated as a yellow powder. The HRFABMS gave an $[MH]^+$ ion at *m/z* 469.1883, consistent with a molecular formula of $C_{26}H_{28}O_8$. The ^{13}C NMR spectrum indicated the presence of 26 carbons,

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including four methyl groups, a methoxyl group, and a carbonyl group (δ 182.0) corresponding to a diprenylated flavonoid. The UV spectrum was consistent with a flavone structure. The IR spectrum had absorption bands characteristic of hydroxyl, conjugated carbonyl, and benzene groups. NMR spectroscopy, including HMQC and HMBC spectra, was used to assign all proton and carbon signals. The ^{1}H NMR spectrum of **2** exhibited a set of signals at δ 1.08, 2.43, 6.59, and 6.71, indicating the presence of a 3-methyl-but-1-enyl group; δ 3.99 assignable to a methoxyl group; δ 6.77 for an aromatic proton; and δ 13.83 for a hydrogen-bonded hydroxyl group. The chemical shifts and coupling patterns of these aliphatic and aromatic protons were closely comparable to the relevant protons in ring A of artocarpin.[2] Compound **2** also showed a set of peaks at δ 6.62, 6.75, 8.06 for an AMX system in ring B. Signals at δ 2.63, 3.49, and 4.36 for an ABX system and two methyl protons at δ 1.30 and 1.45 could be accommodated by an oxepin ring fused to rings B and C, similar to the arrangement found for the related compound morusin hydroperoxide.[10] The presence of a hydroperoxide group was supported by the chemical shift (δ 83.4) of the quatenary carbon (C-11), comparable to that of the relevant carbon of similar known hydroperoxide compounds, artonin R and cudraxanthone O.[11] The location of the methoxyl group in ring A was determined with the aid of NOE difference experiments; irradiation of the proton resonance at δ 3.99 (7-OCH_3) caused an NOE enhancement of the aromatic proton at δ 6.77 (H-8). The HMBC measurements showed long-range correlations between the vinyl proton at δ 6.59 (H-14) and two quaternary carbons at δ 159.6 (C-5) and 164.0 (C-7).

Long-range correlations were also observed for the singlet at δ 13.83 (OH-5) to three quaternary carbons at δ 104.4 (C-4a), 159.6 (C-5), and 110.0 (C-6), and the methoxyl protons at δ 3.99 to a quaternary carbon at δ 164.0 (C-7). The HMBC experiments also showed connectivities between the singlet at δ 6.77 (H-8) and four quaternary carbons at δ 104.4 (C-4a), 110.0 (C-6), 164.0 (C-7), and 156.7 (C-8a). These results provided support for the presence of the 3-methyl-but-1-enyl substituent at C-6 and the methoxyl group at C-7, and the location of the one unsubstituted position on ring A at C-8. The HMBC measurements also showed long-range correlations between the doublet at δ 6.62 (H-3′), methine carbon at δ 112.1 (C-5′), and two quaternary carbons at δ 114.6 (C-1′) and 161.5 (C-2′), and between the doublet at δ 8.06 (H-6′) and three quaternary carbons at δ 114.6 (C-1′), 161.5 (C-2′), and 162.5 (C-4′), thus establishing the three unsubstituted positions on ring B at carbons C-3′, C-5′ and C-6′. Unequivocal evidence for the dihydrooxepin ring was obtained from the HMBC experiments in which correlations were observed between the methylene proton at δ 3.49 (H-9) and three quaternary carbons at δ 158.6 (C-2), 117.6 (C-3), and 182.0 (C-4) and a tertiary carbon at δ 86.2 (C-10), as well as between the methyl protons at δ 1.45 (H-12) and 1.30 (H-13) and the oxygenated carbons δ 86.2 (C-10) and 83.4 (C-11). Further evidence for structure **2** came from comparison of the ^{1}H and ^{13}C NMR data to those reported for the related compound morusin hydroperoxide.[10] The NMR data described above suggested that compound **2** was an artocarpin hydroperoxide, a new compound we have named artoindonesianin B. It may be assumed that compound **2** is biogenetically derived from artocarpin through oxidative cyclization reactions.[10]

Dihydrobenzoxanthone-type flavonoids, such as artonin A, have been isolated from Moraceae species.[12,13] However, artoindonesianin A (**1**) represents the first tetraprenylated dihydrobenzoxanthone-type flavone to be isolated from plants. Artoindonesianin B (**2**) also represents the first natural flavone hydroperoxide structurally analogous to morusin hydroperoxide, a product obtained from the photooxidative cyclization of morusin.[10] As far as the genus *Artocarpus* is concerned, *A. heterophyllus* is the only other species yielding a hydroperoxide, the xanthone artonin R.[11] Both **1** and **2** exhibit cytotoxic activity (IC_{50} 21.0 and 3.9 μg/mL, respectively) against murine leukemia (P-388) cells, comparable to other prenylated flavonoids isolated from *A. communis*, which have been reported to exhibit strong cytotoxic activity.[14]

Experimental Section

General Experimental Procedures. Melting points were determined on a micro-melting point apparatus and are uncorrected. UV and IR spectra were measured with Beckman DU-7000 and Shimadzu FT-IR 8501 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded either with a Bruker ARX-500 spectrometer, operating at 500.1 MHz (^{1}H) and 125.8 MHz (^{13}C) or a JEOL JNM EX-400 FTNMR spectrometer, operating at 399.7 MHz (^{1}H) and 100.4 MHz (^{13}C), using TMS as internal standard. MS were obtained with either a VG Autospec or a JEOL JMS-700 mass spectrometer, using the FAB mode. VLC was carried out using Merck Si gel 60 GF_{254}, flash chromatography with Merck Si gel 60 (230−400 mesh), and TLC analysis on precoated Si gel plates (Merck Kieselgel 60 F_{254}, 0.25 mm).

Plant Material. Samples of the root bark and root trunk of *A. champeden* were collected in August 1996, from plantation trees growing in the region of Cipanas, Lebak District, West Java, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia, and a voucher specimen has been deposited at the herbarium.

Extraction and Isolation. The milled, dried root bark (1.5 kg) was extracted exhaustively with hexane and then with MeOH. The MeOH extract, on removal of solvent under reduced pressure, gave a light brown residue (101.7 g, 6.8%). This residue was solubilized in a mixture of H_2O−Me_2CO (75:25) and the soluble portion extracted with EtOAc. A portion (20 g) of the total EtOAc extract (50 g) was fractionated by Si gel VLC (95 g, 6.5 × 7.5 cm, hexane, hexane−EtOAc, EtOAc, in order of increasing polarity) to give 16 fractions. This chromatographic step was repeated twice on portions of 15 g each of the EtOAc extract. Five fractions were ultimately obtained on combining the eluates on the basis of TLC. The second fraction (5.4 g) was further fractionated by Si gel VLC (55 g, 4.5 × 5.5 cm, hexane, hexane−EtOAc, EtOAc, in order of increasing polarity) to afford six major fractions. From the fourth fraction (fractions 55−59) a precipitate was obtained, which was further purified by Si gel column chromatography [30 g, 2.5 × 15 cm, hexane−EtOAc (85:15)] to yield a yellow crude compound (100 mg), which was crystallized from EtOAc to afford **1** (21 mg). A known compound, artonin A (26 mg),[4] was identified from the fifth fraction (fractions 60−67). Using the same methods, the dried root trunk (4.3 kg) afforded, after crystallization from hexane−EtOAc, **2** (25 mg).

Artoindonesianin A (1): obtained as a yellow powder; mp 236−237 °C; $[\alpha]^{25}_D$ +3.5° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3560 (OH), 1649 (C=O, ketone), 1600, 1546, 1475 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 208 (4.24), 244 (3.62), 296 (3.78), 320 (3.70), 388 (3.62) nm; (MeOH + NaOH) 210 (5.02), 278 (4.51), 422 (4.32) nm; ^{1}H NMR (DMSO-d_6, 500.1 MHz) δ 13.70 (1H, s, OH-5), 10.15 (1H, s, OH-4′), 9.83 (1H, s, OH-2′), 6.57 (1H, d, J = 10.0 Hz, H-14), 6.28 (1H, s, H-3′), 5.74 (1H, d, J = 10.0 Hz, H-15), 5.26 (1H, t, J = 7.0 Hz, H-20), 4.98 (1H, t, J = 7.3 Hz, H-25), 3.55 (1H, dd, J = 8.0, 13.8 Hz, H-19), 3.35 (1H, partly obscured, H-19), 3.34 (1H, dd, J = 7.1, 15.2 Hz, H-10), 3.10 (1H, dd, J = 7.1, 15.2 Hz, H-9), 2.27 (1H, t, J = 15.2 Hz, H-9), 1.97 (2H, m, H_2-23), 1.88 (2H, m, H_2-24), 1.79 (3H, s, Me-22), 1.58 (3H, s,

Me-13), 1.53 (3H, s, Me-27), 1.45 (3H, s, Me-28), 1.39 (3H, s, Me-17), 1.38 (3H, s, Me-18), 1.23 (3H, s, Me-12); ^{13}C NMR (DMSO-d_6, 125.7 MHz) δ 180.2 (s, C-4), 160.7 (s, C-2), 155.6 (s, C-7), 153.5 (s, C-5), 152.9 (s, C-8a), 151.1 (s, C-2′), 140.5 (s, C-4′), 136.2 (s, C-5′), 134.1 (s, C-21), 132.3 (s, C-6′), 130.6 (s, C-26), 128.5 (d, C-15), 124.1 (d, C-25), 122.3 (d, C-20), 115.1 (d, C-14), 110.9 (s, C-3), 107.0 (s, C-8), 104.5 (s, C-6), 104.0 (d, C-3′), 103.8 (s, C-4a), 103.1 (s, C-1′), 92.4 (s, C-11), 77.5 (s, C-16), 46.2 (d, C-10), 39.3 (t, C-23), 27.9 (q, Me-13), 27.8 (q, Me-17), 27.7 (q, Me-18), 26.1 (t, C-24), 25.4 (q, Me-27), 22.6 (q, Me-12), 20.9 (t, C-19), 19.5 (t, C-9), 17.5 (q, Me-28), 16.1 (q, Me-22); FABMS *m*/*z* [MH]$^+$ 571 (36.8), 570 (24.4), 489 (14.0), 467 (12.4), 447 (20.2), 446 (13.6), 316 (23.2), 315 (100), 314 (10.8); HRFABMS *m*/*z* [MH]$^+$ 571.2694 (calcd for $C_{35}H_{39}O_7$, 571.2696).

Artoindonesianin B (2): obtained as yellow powder; mp 165−166 °C; $[\alpha]^{22}_D$ +8.6° (*c* 0.18, MeOH); IR (KBr) ν_{max} 3500 (OH), 1649 (C═O, ketone), 1606, 1548, 1475 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 252 (3.87), 294 (4.26), 346 (4.26) nm; (MeOH + NaOH) 214 (4.63), 273 (4.40), 286 (4.41), 388 (4.57), 486 (3.95) nm; ^{1}H NMR (Me_2CO-d_6, 399.7 MHz) δ 13.83 (1H, s, OH-5), 10.35 (1H, s, OOH-11), 9.20 (1H, s, OH-4′), 8.06 (1H, d, J = 8.8 Hz, H-6′), 6.77 (1H, s, H-8), 6.75 (1H, dd, J = 2.4 and 8.8 Hz, H-5′), 6.71 (1H, dd, J = 7.0, 16.2 Hz, H-15), 6.62 (1H, d, J = 2.4 Hz, H-3′), 6.59 (1H, dd, J = 1.0, 16.2 Hz, H-14), 4.36 (1H, dd, J = 2.0, 10.0 Hz, H-10), 3.99 (3H, s, OMe-7), 3.49 (1H, dd, J = 2.0, 17.1 Hz, H-9), 2.63 (1H, dd, J = 10.0, 17.1 Hz, H-9), 2.43 (1H, m, H-16), 1.45 (3H, s, Me-12), 1.30 (3H, s, Me-13), 1.08 (6H, each d, J = 6.8 Hz, Me-17 and Me-18); ^{13}C NMR (Me_2CO-d_6, 100.4 MHz) δ 182.0 (s, C-4), 164.0 (s, C-7), 162.5 (s, C-4′), 161.5 (s, C-2′), 159.6 (s, C-5), 158.6 (s, C-2), 156.7 (s, C-8a), 142.3 (d, C-15), 131.3 (d, C-6′), 117.6 (s, C-3), 117.0 (d, C-14), 114.6 (s, C-1′), 112.1 (d, C-5′), 110.0 (s, C-6), 108.5 (d, C-3′), 104.4 (s, C-4a), 90.5 (d, C-8), 86.2 (d, C-10), 83.4 (s, C-11), 56.7 (q, OMe-7), 34.0 (d, C-16), 25.9 (t, C-9), 23.1 (q, Me-17), 23.1 (q, Me-18), 22.5 (q, Me-13), 20.1 (q, Me-12); FABMS *m*/*z* [MH]$^+$ 469 (100), 468 (70.8), 453 (24.2), 393 (33.3), 337 (31.7), 321 (18.3), 307 (14.2), 233 (6.7), 179 (8.3), 79 (5.4), 57 (6.8), 41 (3.7); HRFABMS *m*/*z* [MH]$^+$ 469.1883 (calcd for $C_{26}H_{29}O_8$, 469.1863).

Cytotoxicity Assay. Both **1** and **2** were tested against murine leukemia (P-388) cells using established protocols.[15] Compounds **1** and **2** exhibited cytotoxic activity, IC_{50} 21.0 and 3.9 μg/mL, respectively.

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