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# Artoindonesianins Q–T, four isoprenylated flavones from *Artocarpus champeden* Spreng. (Moraceae)

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**Abstract**

Four isoprenylated flavones, artoindonesianins Q–T, were isolated from the heartwood of *Artocarpus champeden* Roxb. The structures of these compounds were elucidated on the basis of their spectroscopic data.

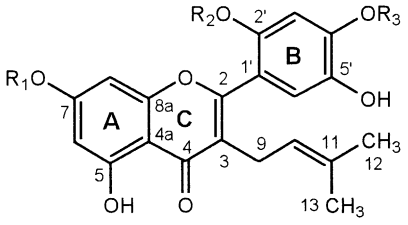

*Keywords:* *Artocarpus champeden* Spreng.; Moraceae; Artoindonesianins Q–T; Isoprenylated flavones

## 1. Introduction

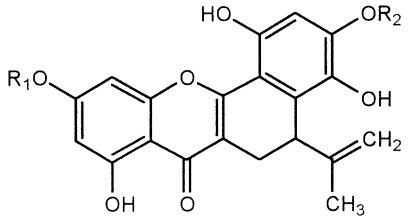
Previously, we reported the isolation and structure determination of some new isoprenylated flavones, namely cyclochampedol (Achmad et al., 1996) and artoindonesianins A, B (Hakim et al., 1999) and M (Syah et al., 2002), from *Artocarpus champeden* Spreng. (Moraceae), locally known as 'Cempedak' (Indonesia). Cyclochampedol was shown to be toxic to brine shrimps (*Artemia salina*) and to inhibit $K^+$-dependent amino acid transport in *Bombyx mori* midgut (Achmad et al., 1996; Parenti et al., 1998), whereas artoindonesianins A and B exhibited cytotoxic effects against P-388 cells (Hakim et al., 1999). In continuation of our investigation on Indonesian moraceaous plants, we now report the isolation and structure determination of four isoprenylated flavones, for which we propose the names artoindonesianins Q–T (**1**–**4**), from the heartwood of *A. champeden*. The structures of these compounds were elucidated on the basis of spectroscopic evidence.

## 2. Results and discussion

Fractionation of a $CHCl_3$ extract of the heartwood of *A. champeden* by silica gel chromatography yielded four fractions. The fraction eluted with *n*-hexane–EtOAc (7:3) was repeatedly purified by radial chromatography to give four methylated isoprenylflavones, artoindonesianins Q–T (**1**–**4**).




(**1**) $R_1 = R_3 = CH_3$, $R_2 = H$
(**2**) $R_1 = H$, $R_2 = R_3 = CH_3$




(**3**) $R_1 = R_2 = CH_3$
(**4**) $R_1 = H$, $R_2 = CH_3$

Artoindonesianin Q (**1**) was isolated as a yellow powder and a molecular formula of $C_{22}H_{22}O_7$ was assigned based on its HR-EIMS and NMR data. The UV spec-

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trum of **1** showed absorptions at 205 (4.55), 230 (4.31), 254 (4.31), 296 (3.91) and 331 (3.90) nm, typical for C-3 isoprenylated flavone (Nomura, 1988). The UV spectrum also showed a bathochromic shift on addition of NaOH, but it was unchanged on addition of NaOAc, indicating the presence of free hydroxyl groups and an alkylated oxygen functionality at C-7 of the flavone structure (Mabry et al., 1970). The presence of an hydroxyl group at C-5 was deduced from a sharp singlet observed in the $^1$H-NMR spectrum of 1 at $\delta$ 13.10, as well as from a bathochromic shift in the UV spectrum on addition of $AlCl_3$ (Mabry et al., 1970). These features of structure **1** were supported by the IR data which showed absorptions typical of hydroxyl (3316 $cm^{-1}$), conjugated carbonyl (1658 $cm^{-1}$), and benzene ring functionalities (1608 $cm^{-1}$). More structural detail was obtained from analysis of the $^1$H and $^{13}$C-NMR spectroscopic data, including 2D-NMR spectra (HMQC and HMBC). The $^1$H NMR of **1** (Table 1) disclosed the presence of AB signals at $\delta$ 6.29 and 6.45 ($J = 2.3$ Hz) for the protons at C-6 and C-8, two singlets at $\delta$ 6.67 and 6.84 characteristic for the protons in the ring B of a flavone substituted at C-2′, C-4′ and C-5′ (Hano et al., 1994). A set of signals were assignable to an isoprenyl side chain ($\delta$ 1.45, *brd*, 3H; 1.57, *brd*, 3H; 3.13, *brd*, 2H; 5.13, *t sept*., 1H). In addition, two singlets at $\delta$ 3.87 and 3.88 were attributed to two methoxyl groups and two broad singlets at $\delta$ 7.41 and 8.29 to two additional hydroxyl groups. The positions of the methoxyl groups were established from $^1$H–$^{13}$C long range correlations (HMBC) (Table 1) between the protons at $\delta$ 3.87 and 3.88 and carbon signals at 151.1 (C-4′) and 166.4 (C-7), respectively. The $^1$H–$^{13}$C long range correlations also showed connectivities between methylene protons at $\delta$ 3.13 ($H_2$-9) and carbons at $\delta$ 121.9 (C-3), 162.0 (C-2) and 183.1 (C-4), confirming the position of the isoprenyl group at C-3. Further support for the structure assigned to **1** came from comparison of the $^{13}$C NMR spectrum (Table 1), assigned with the aid of DEPT, HMQC and HMBC spectra, to that reported for related compounds (Hano et al., 1994; Lin et al., 1995).

Artoindonesianin R (**2**), also isolated as a yellow powder, has a molecular formula of $C_{22}H_{22}O_7$ based on the HR-EIMS and NMR data. The UV, IR and NMR spectroscopic properties of **2** were very similar to that of **1**, suggesting they are isomers differing only in the position of methoxyl groups. In the $^1$H NMR spectrum of 2 (Table 1) the signals for H-6/H-8 and H-3′/-6′ were slightly more upfield and downfield, respectively, than those of the corresponding protons in **1**. Also, unlike compound **1**, the UV spectrum of **2** showed bathochromic shift on addition of NaOAc. These data indicated that both methoxyl groups were located in the ring B. Evidence for assignment of these groups at C-2′

Table 1
$^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectroscopic data compounds **1** and **2** in $d_6$-acetone[a]

| No | Compound 1 | | | Compound 2 | | |
|---|---|---|---|---|---|---|
| | $\delta_H$ (multiplicity, $J$ in Hz) | $\delta_C$ | HMBC (H⇒C) | $\delta_H$ (multiplicity, $J$ in Hz) | $\delta_C$ | HMBC (H⇒C) |
| 2 | – | 162.0 | – | – | 161.8 | – |
| 3 | – | 121.9 | – | – | 121.4 | – |
| 4 | – | 183.1 | – | – | 182.8 | – |
| 4a | – | 105.8 | – | – | 105.1 | – |
| 5 | – | 162.9 | – | – | 163.3 | – |
| 6 | 6.29 (*d*, 2.3) | 98.3 | C4a, C5, C7, C8 | 6.24 (*d*, 2.1) | 99.3 | C4a, C5, C7, C8 |
| 7 | – | 166.4 | – | – | 164.7 | – |
| 8 | 6.45 (*d*, 2.3) | 92.4 | C4a, C6, C7, C8a | 6.32 (*d*, 2.1) | 94.1 | C4a, C6, C7, C8a |
| 8a | – | 159.1 | – | – | 159.1 | – |
| 9 | 3.13 (*brd*, 7.1) | 24.6 | C2, C3, C4, C10, C11 | 3.03 (*brd*, 7.0) | 24.5 | C2, C3, C4, C10, C11 |
| 10 | 5.13 (*t sept*, 7.1, 1.4) | 122.4 | C12, C13 | 5.08 (*t sept*, 7.0, 1.4) | 122.5 | C12, C13 |
| 11 | – | 132.2 | – | – | 132.0 | – |
| 12 | 1.57 (*brd*, 1.4) | 25.8 | C10, C11, C13 | 1.57 (*brd*, 1.4) | 25.8 | C10, C11, C13 |
| 13 | 1.45 (*brd*, 1.4) | 17.6 | C10, C11, C12 | 1.41 (*brd*, 1.4) | 17.6 | C10, C11, C12 |
| 1′ | – | 112.2 | – | – | 114.2 | – |
| 2′ | – | 149.2 | – | – | a151.8 | – |
| 3′ | 6.67 (*s*) | 101.4 | C1′, C2′, C4′, C5′ | 6.88 (*s*) | 98.3 | C2′, C4′, C5′ |
| 4′ | – | 151.1 | – | – | a150.9 | – |
| 5′ | – | 140.5 | – | – | 141.0 | – |
| 6′ | 6.84 (*s*) | 116.5 | C2, C2′, C4′, C5′ | 6.89 (*s*) | 117.0 | C2, C2′, C4′, C5′ |
| 5-OH | 13.10 (*s*) | – | C4a, C5, C6 | 13.13 (*s*) | – | C4a, C5, C6 |
| 7-$OCH_3$/-OH | 3.88 (*s*)a | a56.3 | C7 | 9.67 (*s*) | – | C6, C7, C8 |
| 2′- $OCH_3$/-OH | 8.29 (*s*) | – | C1′, C2′, C3′ | 3.96 (*s*)a | b56.5 | C2′ |
| 4′-$OCH_3$/-OH | 3.87 (*s*)a | a56.2 | C4′ | 3.81 (*s*)a | b56.8 | C4′ |
| 5′-OH | 7.41 (*s*) | – | C4′, C5′, C6′ | 7.60 (*s*) | – | C6′ |

[a] Signals with the same letters in the same column can be interchanged.

and C-4′ was deduced from $^{1}H$–$^{13}C$ long range correlations between the two singlets at $\delta$ 3.81 and 3.96 and carbon signals at $\delta$ 150.9 (C-4′) and 151.8 (C-2′), respectively. The structure of artoindonesianin R was assigned, therefore, as shown in **2**. A complete list of NMR data of **2**, assigned with the aid of DEPT, HMQC and HMBC spectra, is summarized in Table 1.

Artoindonesianin S (**3**), isolated as a yellow powder, has a molecular formula of $C_{22}H_{20}O_7$ based on the HR-EIMS and NMR spectroscopic data; indicative of a dehydro derivative of either **1** or **2**. The UV, IR and NMR spectrum properties of **3** were similar to that found for artonin B (Hano et al., 1989) and artoindonesianin B (Hakim et al., 1999). The $^{1}H$ NMR spectrum of **3** (Table 2) disclosed a signal of a chelated hydroxyl group at $\delta$ 13.18 (*s*), a pair of *meta*-coupled aromatic protons at $\delta$ 6.30 and 6.69 for the protons at H-6 and H-8 positions in ring A. A singlet for an aromatic proton at $\delta$ 6.56 was indicative of a 1,2,4,5,6-pentasubstituted ring B (Hano et al., 1989; Hakim et al., 1999). The $^{1}H$ NMR also showed a set of signals at $\delta$ 1.77 (3H, *brm*), 2.45, 3.40 (each 1H, *dd*), 4.17 (1H, *brd*), 4.27 and 4.64 (each 1H, *brs*), assignable to a $-CH_2-CH-C(CH_3)=CH_2-$ array, typical for dihydrobenzoxanthone-type flavone (Hano et al., 1989; Hakim et al., 1999). The $^{1}H$ NMR spectrum of **3** also indicated the presence of two methoxyl groups at $\delta$ 3.90 (*s*) and 3.91 (*s*), in addition to two hydroxyl groups at $\delta$ 7.47 (*s*) and 8.20 (*brs*). These $^{1}H$ NMR data were consistent with a dihydrobenzoxanthonoid flavone derivative having the structure shown in **3**. Support for the location of methoxyl groups at C-4′ and C-7 positions came from HMQC and HMBC experiments. The HMBC measurements showed $^{1}H$–$^{13}C$ longe-range correlations between the methoxyl protons with carbon signals at $\delta$ 152.6 (C-4′) and 166.0 (C-7). Further evidence for the structure assigned to **3** came from comparison of the $^{13}C$ NMR data, assigned with the aid of DEPT, HMQC and HMBC data, to that of **1** and related compounds (Hano et al., 1989; Hakim et al., 1999).

Artoindonesianin T (**4**) was isolated as a yellow powder and has a molecular formula of $C_{21}H_{18}O_7$ based on the HR-EIMS and NMR spectroscopic data. The UV, IR and NMR spectra of **4** (Table 2) were almost identical to that of **3**, except that **4** contained only one methoxyl group. The location of this group at C-4′ position was determined with the aid of $^{1}H$-$^{13}C$ long-range correlation observed between the singlet of the methoxyl protons at $\delta$ 3.90 and carbon at $\delta$ 152.5 (C-4′). Moreover, consistent with structure **4**, the $^{1}H$-NMR

Table 2
$^{1}H$ (500 MHz) and $^{13}C$ NMR (125 MHz) spectroscopic data compounds **3** and **4** in $d_6$-acetone

| No | Compound **3** | | | Compound **4** | | |
|---|---|---|---|---|---|---|
| | $\delta_H$ (multiplicity, *J* in Hz) | $\delta_C$ | HMBC (H⇒C) | $\delta_H$ (multiplicity, *J* in Hz) | $\delta_C$ | HMBC (H⇒C) |
| 2 | – | 161.4 | – | – | 161.2 | – |
| 3 | – | 112.1 | – | – | 111.9 | – |
| 4 | – | 181.0 | – | – | 180.9 | – |
| 4a | – | 105.5 | – | – | 104.8 | – |
| 5 | – | 162.8 | – | – | 163.1 | – |
| 6 | 6.30 (*d*, 2.3) | 98.6 | C4a, C5, C7, C8 | 6.24 (*d*, 2.1) | 99.5 | C4a, C5, C7, C8 |
| 7 | – | 166.0 | – | – | 164.3 | – |
| 8 | 6.69 (*d*, 2.3) | 93.1 | C4a, C6, C7, C8a | 6.50 (*d*, 2.1) | 94.7 | C4a, C6, C7, C8a |
| 8a | – | 157.5 | – | – | 157.6 | – |
| 9 | 2.45 (*dd*, 16.0, 6.6)<br>3.40 (*dd*, 16.0, 1.7) | 22.2 | C2, C3, C4, C6′, C11 | 2.43 (*dd*, 16.0, 6.6)<br>3.39 (*dd*, 16.0, 1.6) | 22.2 | C2, C3, C4, C10, C11, C6′ |
| 10 | 4.17 (*brd*, 6.6) | 37.6 | C3, C6′, C11, C12 | 4.00 (*brd*, 6.6) | 37.7 | C3, C11, C12, C1′, C5′, C6′ |
| 11 | – | 145.3 | – | – | 145.3 | – |
| 12 | 4.27 (*brs*) 4.64 (*brs*) | 111.7 | C10, C13 | 4.26 (*m*) 4.63 (*m*) | 111.7 | C13 |
| 13 | 1.77 (*brm*) | 21.9 | C10, C11, C12 | 1.76 (*m*) | 21.9 | C10, C11, C12 |
| 1′ | – | 106.8 | – | – | 106.9 | – |
| 2′ | – | 151.0 | – | – | 150.9 | – |
| 3′ | 6.56 (*s*) | 100.5 | C1′, C2′, C4′, C5′ | 6.55 (*s*) | 100.4 | C1′, C2′, C4′, C5′ |
| 4′ | – | 152.6 | – | – | 152.5 | – |
| 5′ | – | 137.6 | – | – | 137.6 | – |
| 6′ | – | 127.9 | – | – | 127.9 | – |
| 5-OH | 13.18 (*s*) | – | C4a, C5, C6 | 13.21 (*s*) | – | C4a, C5, C6 |
| 7-OH/$OCH_3$ | 3.90 (*s*) | 56.3 | C7 | n.o.[a] | – | |
| 2′-OH | 7.47 (*s*) | – | C1′, C2′, C3′ | n.o. | – | |
| 4′-OH/$OCH_3$ | 3.91 (*s*) | 56.3 | C4′ | 3.90 (*s*) | 56.3 | C4′ |
| 5′-OH | 8.20 (*brs*) | – | C4′, C5′, C6′ | n.o. | – | |

[a] Not observed.

spectrum showed a pair of *meta*-coupled aromatic protons which were more upfield in **4** ($\delta$ 6.24 and 6.50) than those found in **3** ($\delta$ 6.30 and 6.69). Artoindonesianin T, therefore, was assigned structure **4**. The $^{1}H$ and $^{13}C$ NMR spectral data of **4**, assigned with the aid of DEPT, HMQC and HMBC spectra, are summarized in Table 2.

A number of mono-, di- and trimethylated derivatives of isoprenylated flavonoids has been isolated from a limited number of *Artocarpus* plants (Nomura, 1988; Nomura and Hano, 1994; Nomura et al., 1998). The isolation of compounds 1–4 in *A. champeden* provides further examples of the occurrence of these types of compounds in this genus. Artoindonesianins Q–S (**1**–**3**) represent the first cases of dimethylated isoprenylated flavones to be isolated from Moraceous plants.

## 3. Experimental

### 3.1. General

UV and IR spectra were measured with a Varian Conc.100 instrument and Perkin Elmer Spectrum One FTIR spectrometer respectively. $^{1}H$ and $^{13}C$ NMR spectra were recorded with a Bruker AM 500 operating at 500 ($^{1}H$) 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 and radial chromatography were carried out using Merck silica gel 60 $GF_{254}$ and for TLC analysis, precoated silica gel plates (Merck Kieselgel 60 $GF_{254}$, 0.25 mm) were used.

### 3.2. Plant material

Samples of the heartwood of *A. champeden* were collected in May 1997 from the village 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 had been deposited at the herbarium.

### 3.3. Extraction and isolation

The dried powdered heartwood (2.6 kg) of *A. champeden* was macerated successively in *n*-hexane, $CHCl_3$ and MeOH. The $CHCl_3$ extract (30 g) was fractionated by VLC (silica gel, *n*-hexane–EtOAc) into four major fractions (A-D). Fraction B (5 g) was further fractionated using the same method (silica gel, light petroleum-diisopropyl ether, diisopropyl ether, diisopropyl ether–acetone) to afford three fractions. Repeated purification of the third fraction (0.5 g) by radial chromatography (silica gel, light petroleum–$CHCl_3$, $CHCl_3$, $CHCl_3$–acetone) yielded **1** (45 mg), **2** (25 mg), **3** (7 mg) and **4** (17 mg).

#### 3.3.1. Artoindonesianin Q (*1*)

Yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 205 (4.55), 230 (4.21), 254 (4.31), 296 (3.91), 331 (3.90) nm; (MeOH + NaOH): 206 (4.96), 257 (4.33), 316 (3.91), 437 (3.40) nm; (MeOH + $AlCl_3$): 205 (4.58), 268 (4.41), 309 (3.89), 380 (3.93) nm; (MeOH + $AlCl_3$/HCl): unchanged from those in (MeOH + $AlCl_3$); (MeOH + NaOAc): unchanged; IR $\nu_{max}$ 3316, 3014, 2978, 2938, 2853, 1658, 1635, 1608, 1581, 1504, 1446, 1353, 1284, 1207, 1196, 1162, 1077, 1036, 1025, 866, 834, 810 $cm^{-1}$; $^{1}H$ NMR ($d_6$-acetone, 500 MHz) see Table 1; $^{13}C$-NMR ($d_6$-acetone, 125 MHz) see Table 1; EIMS *m*/*z*: [$M^+$] 398 (82), 381 (39), 355 (100), 341 (51), 325 (24), 311 (24); HR-EIMS *m*/*z*: [$M^+$] 398.1358 (calc. for $C_{22}H_{22}O_7$, 398.1365).

#### 3.3.2. Artoindonesianin R (*2*)

Yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 205 (4.74), 252 (4.47), 297 (4.17), 330 (4.10) nm; (MeOH + NaOH): 207 (5.05), 266 (4.56), 319 (4.17), 358 (4.15) nm; (MeOH + $AlCl_3$): 205 (4.74), 267 (4.54), 309 (4.10), 376 (4.04) nm; (MeOH + $AlCl_3$/HCl): practically the same as those in (MeOH + $AlCl_3$); (MeOH + NaOAc): 204 (4.88), 260 (4.46), 300 (4.14), 330 (4.06); IR $\nu_{max}$ 3381, 2967, 2933, 2840, 1652, 1620, 1615, 1453, 1359, 1294, 1207, 1165, 1078, 1030, 865, 834, 812 $cm^{-1}$; $^{1}H$-NMR ($d_6$-acetone, 500 MHz) see Table 1; $^{13}C$-NMR ($d_6$-acetone, 125 MHz) see Table 1; EIMS *m*/*z*: [$M^+$] 398 (59), 367 (56), 355 (100), 340 (13), 325 (20), 311 (48); HR-EIMS *m*/*z*: [$M^+$] 398.1369 (calc. for $C_{22}H_{22}O_7$, 398.1365).

#### 3.3.3. Artoindonesianin S (*3*)

Yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 205 (4.58), 261 (4.36), 311 (3.90), 375 (4.21) nm; (MeOH + NaOH): 205 (4.96), 263 (4.30), 299 (*sh*, 4.03), 349 (*sh*, 3.71), 433 (3.42) nm; (MeOH + $AlCl_3$): 203 (4.63), 276 (4.39), 298 (*sh*, 4.01), 333 (3.99), 414 (4.31) nm; (MeOH + $AlCl_3$/HCl): was very close with those in (MeOH + $AlCl_3$); (MeOH + NaOAc): unchanged; IR $\nu_{max}$ 3533, 3446, 3078, 2940, 2909, 2848, 1653, 1606, 1596, 1493, 1440, 1371, 1283, 1208, 1195, 1166, 872, 830 $cm^{-1}$; $^{1}H$ NMR ($d_6$-acetone, 500 MHz) see Table 2; $^{13}C$-NMR ($d_6$-acetone, 125 MHz) see Table 2; EIMS *m*/*z*: [$M^+$] 396 (100), 381 (24), 353 (79), 340 (20), 325 (18), 311 (12); HR-EIMS *m*/*z*: [$M^+$] 396.1197 (calc. for $C_{22}H_{20}O_7$, 396.1209).

#### 3.3.4. Artoindonesianin T (*4*)

Yellow powder; UV (MeOH) $\lambda_{max}$ (log $\epsilon$): 207 (4.37), 276 (4.14), 298 (sh, 3.81), 335 (3.75), 418 (4.06) nm; (MeOH + NaOH): 272 (4.20), 368 (3.69), 426 (3.56) nm; (MeOH + $AlCl_3$): 276 (4.14), 298 (3.81), 335 (3.75), 412 (4.03) nm; (MeOH + NaOAc) 207 (4.37), 276 (4.14), 335 (3.75), 406 (3.99); IR $\nu_{max}$ 3529, 3397, 3079, 2970, 2927, 2848, 1654, 1611, 1570, 1492, 1462, 1440, 1364, 1342,

1282, 1173, 1124, 1034, 958, 893, 869, 855, 832 $cm^{-1}$; $^1H$ NMR ($d_6$-acetone, 500 MHz) see Table 2; $^{13}C$ NMR ($d_6$-acetone, 125 MHz) see Table 2; EIMS $m/z$: [$M^+$] 382 (100), 367 (34), 365 (34), 355 (18), 341 (54), 339 (87), 321 (39), 311 (32), 149 (43); HR-EIMS $m/z$: [$M^+$] 382.1047 (calc. for $C_{21}H_{18}O_7$, 382.1052).

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