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Prenylated Stilbenes and Their Novel Biogenetic Derivatives from *Artocarpus chama*

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Two new prenylated stilbenes, artochamins F (**1**) and G (**2**), and their four novel derivatives, artochamins H–K (**3**–**6**), were isolated from the stems of *Artocarpus chama*. Their structures were elucidated mainly by NMR spectroscopy and mass spectrometry. The structure of **3** was confirmed by X-ray crystallographic analysis. The origin of **3**–**6** could be assumed biogenetically from **2**. The cytotoxicity of these compounds against HepG2 cells was evaluated.

Introduction

Artocarpus species (Moraceae) provide a variety of prenylated flavonoids and a limited number of stilbenoids with interesting biological activities, such as cytotoxicity, antibacterial effects against cariogenic bacteria, and cyclooxygenase and tyrosinase inhibitory activities.[1–5] In a program searching for bioactive prenylated phenols from this genus, we have investigated the chemical constituents of the roots of *Artocarpus chama* Buch.-Ham., some cytotoxic prenylated flavones being reported.[6] HPLC and TLC analysis of the chloroform-soluble extracts from the stems and roots of this plant showed that except for these flavones in both extracts, there are some unknown phenols in the stems. Therefore, continuing separation of the chloroform-soluble extract from the stems yielded two new stilbenes with two isoprenoid groups, artochamins F (**1**) and G (**2**), and their four novel derivatives, artochamins H–K (**3**–**6**) (Figure 1). Their structures were elucidated by spectroscopic methods. The structure of **3** was confirmed by X-ray crystallographic analysis. Compounds **3**–**6** possess a unique carbon skeleton, which could be assumed biogenetically arising from **2**. The cytotoxicity of compounds **1**–**6** against HepG2 cells was also tested. In this paper, we describe the structure elucidation, the biogenetic relationship, and cytotoxicity evaluation of **1**–**6**.

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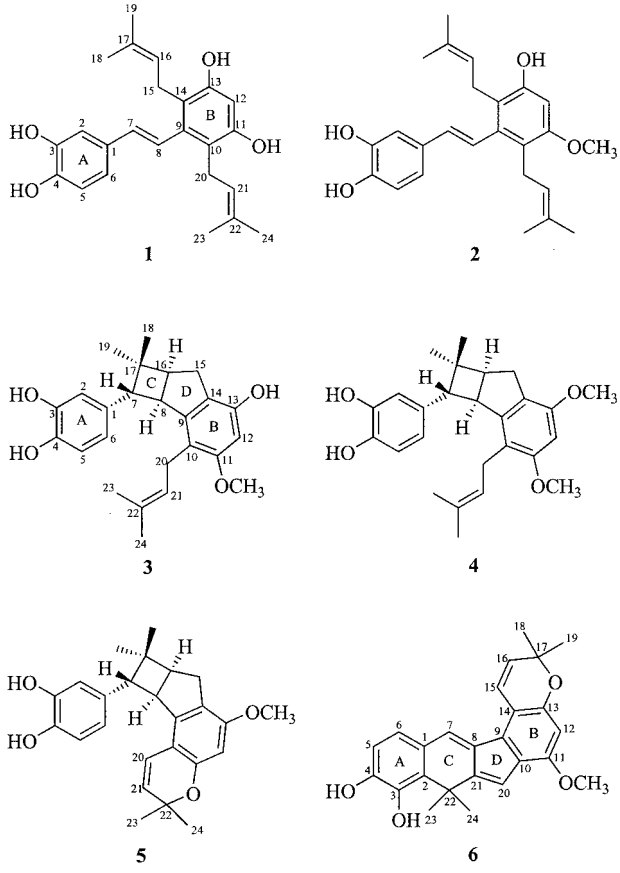


Figure 1. Structures of compounds isolated from *Artocarpus chama*.

Results and Discussion

The EtOH (95%) extract of the stems of *A. chama* was suspended in H_2O and partitioned successively with petro-

leum ether, $CHCl_3$, EtOAc, and *n*BuOH. The $CHCl_3$ fraction was separated by repeated column chromatography on silica gel and RP_{18} gel and by preparative TLC and HPLC to afford compounds **1**–**6**.

Artochamin F (**1**), a white amorphous powder, was assigned a molecular formula of $C_{24}H_{28}O_4$ by HREIMS. The IR spectrum showed absorptions for hydroxy groups ($\tilde{\nu}$ = 3375 cm^{-1}) and benzene rings ($\tilde{\nu}$ = 1592, 1514, and 1442 cm^{-1}). The ^1H NMR spectrum suggests the presence of four hydroxy groups [δ_H = 7.90 (br. s, 1 H), 7.88 (br. s, 1 H), and 7.78 (s, 2 H) ppm] and two symmetrical 3,3-dimethylallyl (prenyl) side chains [δ_H = 5.18 (br. t, J = 6.6 Hz, 2 H), 3.32 (br. d, J = 6.6 Hz, 4 H), 1.64 (br. s, 6 H), and 1.62 (br. s, 6 H) ppm]. Moreover, it also exhibits signals of an ABX spin system at δ_H = 7.05 (d, J = 1.6 Hz, 1 H), 6.85 (dd, J = 1.6, 8.1 Hz, 1 H), and 6.82 (d, J = 8.1 Hz, 1 H) ppm, an (*E*) double bond at δ_H = 6.92 (d, J = 16.6 Hz, 1 H) and 6.37 (d, J = 16.6 Hz, 1 H) ppm, and an aromatic singlet at δ_H = 6.42 (s, 1 H) ppm. The ^{13}C NMR and HMQC spectra revealed ten quaternary sp^2, eight methine sp^2, two methylene sp^3, and four methyl carbon signals. These NMR spectroscopic data suggest that **1** is a diprenylated and tetrahydroxylated (*E*)-stilbene.

On the basis of HMQC and HMBC spectral analysis, we were able to fully assign all ^1H and ^{13}C NMR signals (Tables 1 and 3) and determine the substitution pattern. The HMBC correlations from 15,20-H_2 (δ_H = 3.32 ppm) to C-9 (δ_C = 140.8 ppm), C-10,14 (δ_C = 118.9 ppm), and C-11,13 (δ_C = 154.7 ppm), as well as those from 11,13-OH (δ_H = 7.78 ppm) to C-10,14, C-11,13, and C-12 (δ_C = 102.7 ppm) (Figure 2), indicate that the two prenyl and two hydroxy groups are located at C-10,14 and C-11,13, respectively. The HMBC correlations from 8-H (δ_H = 6.92 ppm) to C-10,14 and C-1 (δ_C = 131.6 ppm), from 7-H (δ_H = 6.37 ppm) to C-2 (δ_C = 114.0 ppm), C-6 (δ_C = 120.0 ppm), and C-9, along with those shown in Figure 2, support the stilbene skeleton and the constitution of ring A. Thus, the structure of artochamin F (**1**) was elucidated as (*E*)-4-[3,5-dihydroxy-2,6-bis(3-methylbut-2-enyl)styryl]benzene-1,2-diol.

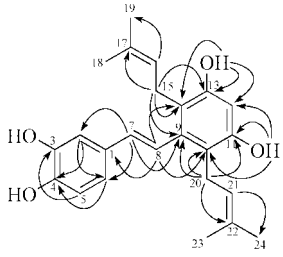


Figure 2. Key HMBC (H→C) correlations of **1**.

Artochamin G (**2**), a white amorphous powder, was assigned a molecular formula of $C_{25}H_{30}O_4$ by HREIMS. The ^1H NMR spectrum shows signals for three hydroxy groups, an *O*-methyl group, two prenyl side chains, an ABX spin system, an (*E*) double bond, and an aromatic singlet. Comparison of the NMR spectroscopic data of **2** and **1** (Tables 1 and 3) indicates that **2** is not as symmetrically substituted as **1** due to the presence of an *O*-methyl group on ring B. This result is corroborated by the HMBC correlation between the *O*-methyl protons at δ_H = 3.74 ppm and C-11 at δ_C = 157.5 ppm. Thus, the structure of artochamin G (**2**) was elucidated as (*E*)-4-[3-hydroxy-5-methoxy-2,6-bis(3-methylbut-2-enyl)styryl]benzene-1,2-diol.

Artochamin H (**3**), colorless needles, gave a positive reaction with ferric chloride reagent, indicating the presence of a phenolic moiety. The molecular formula of $C_{25}H_{30}O_4$ was established by HREIMS. The IR spectrum shows absorptions for hydroxy moieties ($\tilde{\nu}$ = 3373 cm^{-1}) and benzene rings ($\tilde{\nu}$ = 1598, 1518, and 1446 cm^{-1}). The ^1H NMR spectrum exhibits signals for three hydroxy groups at δ_H = 7.74, 7.63, and 7.61 (3 br. s, 1 H each) ppm, which disappeared with the addition of D_2O. It also shows the evidence for an *O*-methyl group [δ_H = 3.66 (s, 3 H) ppm], a prenyl group [δ_H = 4.87 (br. t, J = 5.8 Hz, 1 H), 2.96 (dd, J = 5.8, 14.5 Hz, 1 H), 2.83 (dd, overlap, 1 H), and 1.46, 1.37 (2 br. s, 3 H each) ppm], an aromatic ABX spin system (ring A) [δ_H = 6.75 (d, J = 8.0 Hz, 1 H), 6.73 (d, J = 1.6 Hz, 1 H), and 6.57 (dd, J = 1.6, 8.0 Hz, 1 H) ppm], and an aromatic proton (ring B) [δ_H = 6.31 (s, 1 H) ppm]. Moreover, the following ^1H and ^{13}C NMR signals could be hypothesized to derive from a bicyclic moiety (rings C and D) in consideration of 11 degrees of unsaturation: δ_H = 2.77 (d, J = 5.7 Hz, 1 H, 7-H), 3.90 (t, J = 5.7 Hz, 1 H, 8-H), 3.01 (dd, J = 1.5, 16.6 Hz, 1 H, 15-Hβ), 2.84 (dd, J = 9.5, 16.6 Hz, 1 H, 15-Hα), 2.69 (m, 1 H, 16-H), 0.97 (s, 3 H, 18-H_3), 0.70 (s, 3 H, 19-H_3) ppm; δ_C = 58.9 (C-7), 45.9 (C-8), 30.7 (C-15), 45.7 (C-16), 39.3 (C-17), 26.4 (C-18), and 27.7 (C-19) ppm.

The proposed structure of **3** was deduced from ^1H, ^1H-COSY, HMQC, and HMBC spectroscopic data. The moiety of ring A was elucidated by the HMBC correlations from 5-H (δ_H = 6.75 ppm) to C-1 (δ_C = 134.4 ppm) and C-3 (δ_C = 145.8 ppm), from 2-H (δ_H = 6.73 ppm) to C-4 (δ_C = 144.3 ppm), C-6 (δ_C = 120.2 ppm), and C-7 (δ_C = 58.9 ppm), together with those from 6-H (δ_H = 6.57 ppm) to C-2 (δ_C = 116.3 ppm), C-4, and C-7 (Figure 3). The HMBC correlations are observed from 20-H_2 (δ_H = 2.96 and 2.83 ppm) to C-9 (δ_C = 150.3 ppm), C-10 (δ_C = 117.3 ppm), and C-11 (δ_C = 158.7 ppm), from the *O*-methyl protons (δ_H = 3.66 ppm) to C-11, and from the aromatic singlet (δ_H = 6.31 ppm) to C-10, C-11, C-13 (δ_C = 152.9 ppm), and C-14 (δ_C = 123.3 ppm). These data assign the aromatic singlet to 12-H and determine the prenyl and methoxy groups at C-10 and C-11, respectively. Thus, the partial structure of two aromatic rings (A and B) was established. Analysis of the ^1H, ^1H-COSY and HMQC spectra suggests the fragment of –CH(7)–CH(8)–CH(16)–CH_2(15)–. Furthermore, the following HMBC correlations are detected: from 7-H to C-8, C-9, C-17, C-18, and C-19, from 8-H to C-1, C-7, C-9, C-14, and C-17, from 18-H_3 and 19-H_3 to C-7, C-16, and C-17, and from 15-Hα,β to C-13, C-14, C-16, and C-17; this confirms the connectivity of rings C and D and indicates ring D to be attached at C-9 and C-14. The HMBC cross-peaks from 7-H to C-1, C-2, and C-6 show that C-7 is bonded directly to C-1.

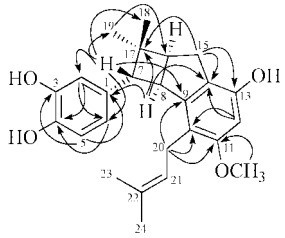


Figure 3. Key HMBC (H→C) correlations of **3**.

The NOESY correlations of 16-H with 8-H, 15-Hα, and 19-H_3 suggest the synperiplanar relationship between 16-H and 8-H, shown in α-configuration. Whereas, the NOESY correlations of 18-H_3 with 7-H and 15-Hβ indicate 7-H is β-oriented. The X-ray crystallographic analysis corroborates the structure of artochamin H as **3** (Figure 4).

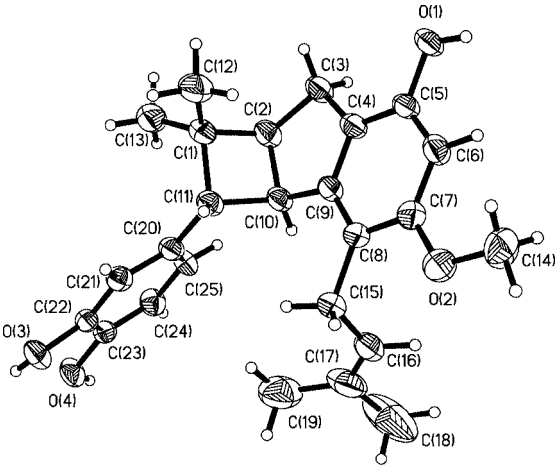


Figure 4. X-ray crystal structure of **3**.

Artochamin I (**4**), a white amorphous powder, was assigned a molecular formula of $C_{26}H_{32}O_4$ by HREIMS. The NMR spectroscopic data of **4** suggest that it should have the same carbon skeleton as compound **3**. Comparison of the ^{13}C NMR spectroscopic data of **3** and **4** (Table 3) shows that 13-OH in **3** is methylated in **4**, which is supported by the HMBC correlation between 13-OCH_3 (δ_H = 3.81 ppm) and C-13 (δ_C = 155.6 ppm) and the NOESY cross-peaks of 12-H (δ_H = 6.45 ppm) with 11-OCH_3 (δ_H = 3.76 ppm) and 13-OCH_3. Thus, the structure of artochamin I was elucidated as **4**.

Artochamin J (**5**), a white amorphous powder, had a molecular formula of $C_{25}H_{28}O_4$, as established by HREIMS. The NMR spectroscopic characteristics of **5** are very similar to those of compounds **3** and **4**. However, **5** contains a 2,2-dimethylpyran moiety attached at ring B rather than the prenyl side chain in **3** and **4**, as deduced by the following ^1H and ^{13}C NMR signals: δ_H = 5.95 (d, *J* = 10.0 Hz, 1 H, 20-H), 5.33 (d, *J* = 10.0 Hz, 1 H, 21-H), and 1.29, 1.27 (2 s, 3 H each, 23,24-H_3) ppm; δ_C = 121.0 (C-20), 128.4 (C-21), 76.8 (C-22), 28.7 (C-23), and 28.1 (C-24) ppm. The HMBC correlations from 20-H to C-9 (δ_C = 146.9 ppm), C-10 (δ_C = 110.8 ppm), and C-11 (δ_C = 155.0 ppm) and from 21-H to C-10 show that the pyranoid ring is fused at C-10 and C-11. The relative configuration of **5** was further confirmed by the NOESY spectroscopic data. Thus, the structure of artochamin J was elucidated as **5**.

Artochamin K (**6**), a red amorphous powder, had a molecular formula of $C_{25}H_{24}O_4$, as determined by HREIMS. The positive reaction with ferric chloride reagent suggested the presence of a phenolic moiety, consistent with the absorptions for hydroxy groups ($\tilde{\nu}$ = 3386 cm^{-1}) and benzene rings ($\tilde{\nu}$ = 1601 and 1493 cm^{-1}) in the IR spectrum. The ^1H NMR spectrum shows signals of two hydroxy groups [δ_H = 9.18 and 7.42 (2 br. s, 1 H each) ppm], a 2,2-dimethylpyran ring [δ_H = 7.09 (d, *J* = 10.0 Hz, 1 H), 5.77 (d, *J* = 10.0 Hz, 1 H), and 1.42 (s, 6 H) ppm], two *ortho*-coupled aromatic protons (ring A) [δ_H = 6.98 (d, *J* = 8.0 Hz, 1 H) and 6.85 (d, *J* = 8.0 Hz, 1 H) ppm], an aromatic singlet (ring B) [δ_H = 6.34 (s, 1 H) ppm], and an *O*-methyl group [δ_H = 3.86 (s, 3 H) ppm]. Evidence for the isoprenoid moiety, the methoxy group, and two aromatic rings are also apparent in the ^{13}C NMR spectrum. The remaining ^1H NMR signals for a pair of long-range-coupled protons [δ_H = 7.54 (d, *J* = 1.6 Hz, 1 H, 7-H) and 6.75 (d, *J* = 1.6 Hz, 1 H, 20-H) ppm] and two methyl groups [δ_H = 1.74 (s, 6 H, 23,34-H_3) ppm], together with the corresponding ^{13}C NMR signals [δ_C = 131.8 (C-7), 136.2 (C-8), 119.2 (C-20), 147.3 (C-21), 38.3 (C-22), and 30.3 (C-23,24) ppm], could be assumed to originate from the other two conjugated rings (C and D) in consideration of 14 degrees of unsaturation.

By detailed analysis of the HMQC and HMBC spectra, the carbon skeleton of **6** was established. In the HMBC spectrum (Figure 5), 15-H (δ_H = 7.09 ppm) correlates with C-9 (δ_C = 131.4 ppm), C-13 (δ_C = 153.4 ppm), C-14 (δ_C = 112.4 ppm), and C-17 (δ_C = 76.4 ppm), and 16-H (δ_H = 5.77 ppm) couples with C-14, indicating the attachment of the 2,2-methylpyran ring at C-13 and C-14 (Figure 5A). The HMBC correlations from the aromatic singlet at δ_H = 6.34 ppm to C-10 (δ_C = 125.9 ppm), C-11 (δ_C = 153.6 ppm), C-13, and C-14 and from the *O*-methyl protons at δ_H = 3.86 ppm to C-11 assign the singlet to 12-H and the methoxy group to C-11. The HMBC correlations from 20-H to C-8, C-9, and C-10 indicate the presence of a five-member unsaturated ring. Thus, the substructure of rings B and D was deduced. Furthermore, the HMBC correlations from 7-H to C-1 (δ_C = 127.5 ppm), C-2 (δ_C = 131.8 ppm), C-6 (δ_C = 124.3 ppm), C-9, and C-21, from 23, 24-H_3 to C-2, C-21, and C-22, and from 6-H (δ_H = 6.98 ppm) to C-2 and C-7 suggest the presence of ring C with two methyl groups (Figure 5B). The substitution of ring A is further supported

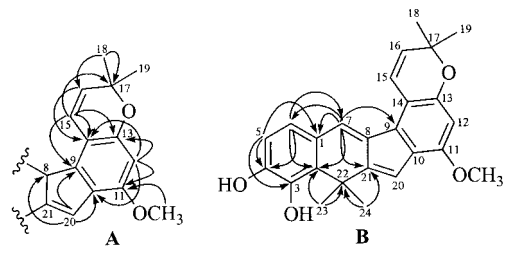


Figure 5. Key HMBC (H→C) correlations of **6**.

by the HMBC correlations shown in Figure 5B. Thus, the structure of artochamin K was elucidated as **6**, which is further confirmed by the NOESY correlations (Figure 6).

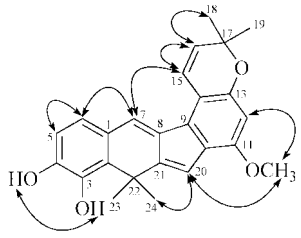
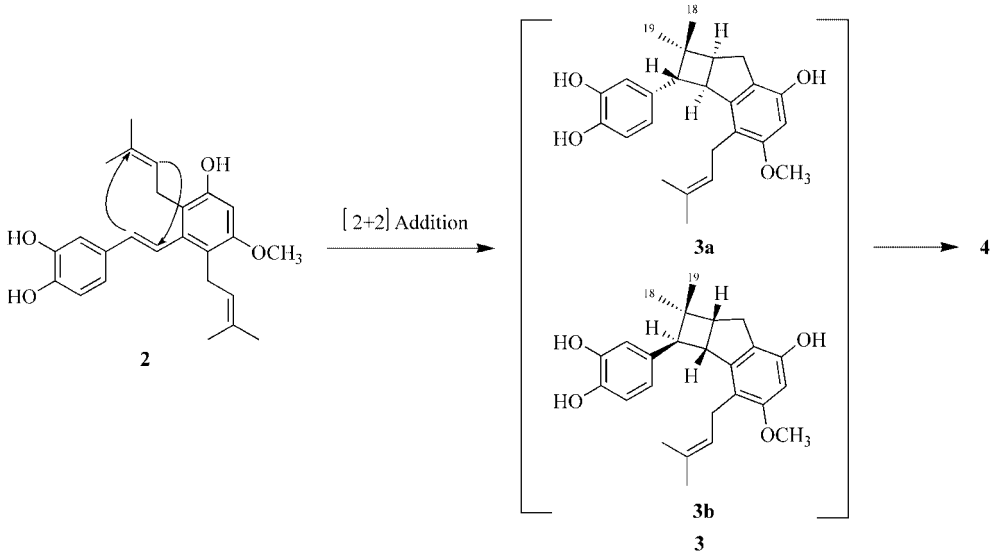


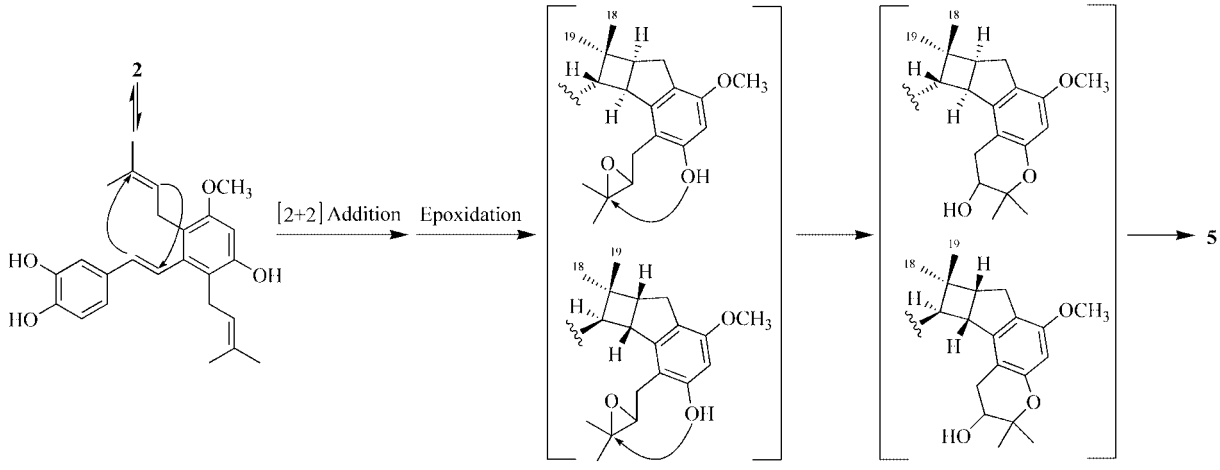
Figure 6. Key NOESY correlations of **6**.

The biogenetic origins of artochamins H–K (**3**–**6**) could be traced back to compound **2**. Two enantiomers (**3a** and **3b**) produced by an intramolecular [2+2] addition of **2** could show why **3** and **4** are not optically active (Scheme 1). The biogenetic origin of **5** resembles that of **3** and **4**. After intramolecular [2+2] addition, epoxidation, cyclization, and dehydrolysis, **2** could be transformed into **5**, a racemic mixture (Scheme 2). A possible biosynthetic pathway for **6** is proposed in Scheme 3.

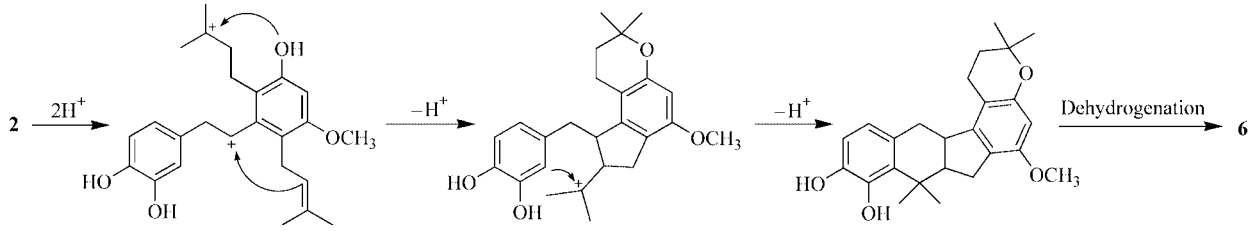
Compounds **1**–**6** were evaluated for cytotoxicity against HepG2 cells. Compounds **1**, **4**, **5**, and **6** showed weak inhibitory activity (IC_{50} = 87.66, 49.04, 48.99, and 63.56 μM, respectively), while **2** and **3** were noncytotoxic.



Scheme 1. Plausible biogenetic pathway for **3** and **4**.



Scheme 2. Plausible biogenetic pathway for **5**.



Scheme 3. Plausible biogenetic pathway for **6**.

Experimental Section

General Experimental Procedures: Melting points were measured with an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer polarimeter 341. UV spectra were obtained with a Shimadzu UV-2401PC spectrophotometer. IR spectroscopic data were recorded with a Nicolet Avatar 360 spectrometer with KBr pellets. NMR spectra were obtained with a Bruker DRX-500 instrument. Chemical shifts are reported with respect to [D_6]acetone (δ_H = 2.04, δ_C = 206.0 ppm). EIMS and HREIMS data were recorded with a Finnigan MAT 95 mass spectrometer. Column chromatography was performed on silica gel H (200–300 mesh and 10–40 μm, Yantai Institute of Chemical Technology, China) and Lichroprep RP_{18} gel (40–63 μm, Merck, Darmstadt, Germany). Preparative HPLC was carried out with Waters 1525 EF chromatography spectrometer with column ZORBAX SB-C_{18} (9.8 × 250 mm, 5 μm, Agilent, USA). Preparative and analytical TLC was run on precoated silica gel GF_{254} plates (10–40 μm, Yantai Institute of Chemical Technology, China).

Collection, Extraction, and Isolation: The stems of *Artocarpus chama* Buch.-Ham. were collected in Xishuangbanna, Yunnan, P. R. China, in July 1998, and air-dried. The plant was identified by Professor Han-Dong Sun (Kunming Institute of Botany, Chinese Academy of Sciences), and a voucher specimen (TCM 98-07-01s Hou) was deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University. The dried and powdered stems of *A. chama* (6.2 kg) were extracted with 95% EtOH under reflux three times and then filtered. The filtrate was concentrated in vacuo to give a residue (680 g), which was suspended in H_2O and partitioned successively with petroleum ether, $CHCl_3$, EtOAc, and *n*BuOH. The $CHCl_3$ extract (190 g) was subjected to column chromatography (CC) on silica gel eluted with petroleum ether/Me_2CO (4:1, 3:1, 2:1, and 1:1) and MeOH to yield fractions 1–8. Fraction 3 (5 g) was purified by CC on silica gel with petroleum ether/Me_2CO (4:1) to give fractions 3a–3d. Fraction 3b was separated by CC on silica gel with $CHCl_3$/EtOAc (9:1), and further purified by preparative TLC with $CHCl_3$/EtOAc (9:1) to provide **5** (10 mg). Fraction 3c was separated by CC on silica gel with petroleum ether/$CHCl_3$ (2:1) and Lichroprep RP_{18} gel with MeOH/H_2O (7:3), followed by preparative HPLC with ZORBAX SB-C_{18} column (MeOH/H_2O, 75:25), to give **4** (53 mg). Fraction 6 (27 g) was isolated by CC on silica gel with petroleum ether/EtOAc (4:1) to afford fractions 6a–6d. Fraction 6b was separated by CC on silica gel with petroleum ether/$CHCl_3$ (1:1), and further purified by preparative TLC with petroleum ether/Me_2CO (4:1), to provide **2** (6 mg). Fraction 6d was fractionated by CC on silica gel with petroleum ether/Me_2CO (4:1) and petroleum ether/$CHCl_3$ (1:1) to yield **3** (150 mg) as needle crystals and **6** (3 mg). Fraction 8 (3.7 g) was separated by CC on silica gel with $CHCl_3$/EtOAc (4:1) and petroleum ether/2-propanol (9:1), then further purified by preparative TLC with $CHCl_3$/Me_2CO (6:1) to afford **1** (14 mg).

Artochamin F (1): White amorphous powder. UV (MeOH): λ_{max} (ε) = 205 (38000), 285 (12300) nm. IR (KBr): $\tilde{\nu}$ = 3375, 2996, 2911, 1592, 1514, 1442, 1375, 1271, 1158, 1082 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 3. EIMS: *m*/*z* (%) = 380 (8) $[M]^+$, 365 (6), 325 (7), 281 (18), 269 (15), 257 (9), 216 (66), 164 (100), 123 (16). HREIMS: calcd. for $C_{24}H_{28}O_4$ 380.1988, found 380.1973.

Artochamin G (2): White amorphous powder. UV (MeOH): λ_{max} (ε) = 206 (29500), 284 (6700) nm. IR (KBr): $\tilde{\nu}$ = 3375, 2964, 2924, 1600, 1516, 1444, 1364, 1281, 1195, 1111 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 3. EIMS: *m*/*z* (%) = 394 (5) $[M]^+$, 379 (5), 339 (4), 321 (5), 295 (10), 271 (6), 230 (100), 215 (19), 187 (4), 175 (38), 164 (29), 123 (8). HREIMS: calcd. for $C_{25}H_{30}O_4$ 394.2144, found 394.2136.

Table 1. ^1H NMR spectroscopic data of compounds **1**, **2** and **6** [500 MHz, $(CD_3)_2CO$; δ in ppm; *J* in Hz].

Proton	1	2	6
2	7.05 (d, 1.6)	7.05 br. s	–
5	6.82 (d, 8.1)	6.82 (d, 8.3)	6.85 (d, 8.0)
6	6.85 (dd, 1.6, 8.1)	6.84 (dd, 1.6, 8.3)	6.98 (d, 8.0)
7	6.37 (d, 16.6)	6.36 (d, 16.6)	7.54 (d, 1.6)
8	6.92 (d, 16.6)	6.91 (d, 16.6)	–
12	6.42 s	6.48 s	6.34 s
15	3.32 (br. d, 6.6)	3.35 (br. d, 6.4)	7.09 (d, 10.0)
16	5.18 (br. t, 6.6)	5.17 (br. t, 6.4)	5.77 (d, 10.0)
18	1.62 br. s	1.63 br. s	1.42 s
19	1.64 br. s	1.64 br. s	1.42 s
20	3.32 (br. d, 6.6)	3.30 (br. d, 6.4)	6.75 (d, 1.6)
21	5.18 (br. t, 6.6)	5.10 br. t, 6.4	–
23	1.62 br. s	1.61 br. s	1.74 s
24	1.64 br. s	1.63 br. s	1.74 s
3-OH	7.90 br. s[a]	7.96 br. s	9.18 br. s[a]
4-OH	7.88 br. s[a]	7.96 br. s	7.42 br. s[a]
11-OH	7.78 s	–	–
11-OCH_3	–	3.74 s	3.86 s
13-OH	7.78 s	7.96 br. s	–

[a] The assignment may be interchanged.

Artochamin H (3): Colorless needles. M.p. 191–193° C. $[\alpha]_D^{20}$ = 0 (*c* = 0.35, Me_2CO). UV (MeOH): λ_{max} (ε) = 201 (1600), 218 (24500), 286 (5700) nm. IR (KBr): $\tilde{\nu}$ = 3373, 2959, 2931, 1598, 1518, 1446, 1344, 1281, 1188, 1110, 1058 cm^{-1}. ^1H and ^{13}C NMR: Tables 2 and 3. EIMS: *m*/*z* (%) = 394 (1) $[M]^+$, 379 (2), 295 (5), 230 (100), 215 (21), 174 (11), 164 (20). HREIMS: calcd. for $C_{25}H_{30}O_4$ 394.2144, found 394.2132.

Table 2. ^1H NMR spectroscopic data of compounds **3**–**5** [500 MHz, $(CD_3)_2CO$ + D_2O; δ in ppm; *J* in Hz].

Proton	3	4	5
2	6.73 (d, 1.6)	6.75 (d, 1.8)	6.74 (d, 1.4)
5	6.75 (d, 8.0)	6.76 (d, 8.0)	6.77 (d, 8.0)
6	6.57 (dd, 1.6, 8.0)	6.58 (dd, 1.8, 8.0)	6.58 (dd, 1.4, 8.0)
7	2.77 (d, 5.7)	2.77 (d, 5.7)	2.77 (d, 6.0)
8	3.90 (t, 5.7)	3.94 (t, 5.7)	3.95 (t, 6.0)
12	6.31 s	6.45 s	6.17 s
15β	3.01 (dd, 1.5, 16.6)	3.00 (dd, 1.8, 17.0)	2.95 (dd, 1.6, 16.7)
15α	2.84 (dd, 9.5, 16.6)	2.84 (dd, 9.7, 17.0)	2.81 (dd, 9.8, 16.7)
16	2.69 m	2.71 m	2.70 m
18	0.97 s	0.97 s	0.99 s
19	0.70 s	0.71 s	0.70 s
20a	2.96 (dd, 5.8, 14.5)	2.97 (dd, overlap)	–
20b	2.83 (dd, overlap)	2.86 (dd, 5.7, 14.5)	–
20	–	–	5.95 (d, 10.0)
21	4.87 (br. t, 5.8)	4.88 (br. t, 5.7)	5.33 (d, 10.0)
23	1.37 br. s	1.39 br. s	1.29 s[a]
24	1.46 br. s	1.48 br. s	1.27 s[a]
3-OH	7.74 br. s[a,b]	7.71 br. s[a,b]	7.70 br. s[b]
4-OH	7.63 br. s[a,b]	7.68 br. s[a,b]	7.70 br. s[b]
11-OCH_3	3.66 s	3.76 s	–
13-OH	7.61 br. s[a,b]	–	–
13-OCH_3	–	3.81 s	3.75 s

[a] The assignment may be interchanged. [b] The chemical shift values were obtained from ^1H NMR spectra performed in $(CD_3)_2CO$.

Table 3. ^{13}C NMR spectroscopic data of compounds **1**–**6** [125 MHz, $(CD_3)_2CO$; δ in ppm].

Carbon	1	2	3	4	5	6
1	131.6	131.4	134.4	134.2	134.1	127.5
2	114.0	114.1	116.3	116.3	116.2	131.8
3	146.5	146.6	145.8	145.8	146.1	145.8
4	146.2	146.4	144.3	144.4	144.7	147.5
5	116.6	116.6	115.8	115.8	116.2	114.0
6	120.0	119.9	120.2	120.2	120.3	124.3
7	134.9	135.2	58.9	58.9	58.7	131.8
8	125.6	125.2	45.9	45.9	44.8	136.2
9	140.8	140.8	150.3	150.2	146.9	131.4
10	118.9	120.6	117.3	118.4	110.8	125.9
11	154.7	157.5	158.7	158.9	155.0	153.6
12	102.7	99.2	98.8	95.4	99.0	99.9
13	154.7	154.9	152.9	155.6	157.7	153.4
14	118.9	119.4	123.3	125.0	125.6	112.4
15	27.2	27.2	30.7	30.9	31.0	120.6
16	126.2	125.9	45.7	45.6	46.1	130.8
17	130.3	130.6	39.3	39.3	39.7	76.4
18	18.5	18.5	26.4	26.4	26.4	28.1
19	26.3	26.3	27.7	27.7	27.7	28.1
20	27.2	27.2	26.8	26.8	121.0	119.2
21	126.2	126.0	125.0	124.7	128.4	147.3
22	130.3	130.4	130.6	131.0	76.8	38.3
23	18.5	18.5	18.0	18.0	28.7	30.3
24	26.3	26.3	26.0	26.0	28.1	30.3
11-OCH_3		56.2	56.3	56.7		56.2
13-OCH_3				55.9	55.9	

Crystal Structure Determination of Artochamin H (3): $C_{25}H_{30}O_4$, M = 394.49, monoclinic, space group $P2(1)/c$, a = 6.1498(8) Å, b = 18.461(3) Å, c = 19.586(3) Å, β = 96.490(2)°, V = 2209.3(5) Å^3, Z = 4, $D_{calcd.}$ = 1.186 mg/m^3, F (000) = 848, T = 213(2) K. Bruker SMART CCD, graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.079 mm^{-1}, colorless needles, size 0.22 × 0.10 × 0.10 mm, 14289 reflections collected, 5236 [R(int) = 0.0710] independent reflections, 1.52° < θ < 27.88°, –8 ≤ h ≤ 7, –24 ≤ k ≤ 15, –24 ≤ l ≤ 25. Completeness to θ = 27.88° 99.5%, data/restraints/parameters 5236/0/382, goodness-of-fit on F^2 1.164, final R indices [$I > 2\sigma(I)$] at R_1 = 0.0721, wR_2 = 0.1484, R indices (all data) at R_1 = 0.0922, wR_2 = 0.1567. The maximum and minimum peaks on final ΔF map corresponded to 0.277 and –0.233 e·Å^{-3}, respectively. Cell parameters were retrieved using SMART[7] software and refined using SAINT on all observed reflections. Data reduction was performed using the SAINT software[8] which corrects for Lp and decay. Absorption corrections were applied using SADABS[9] supplied by G. Sheldrick. The structures were solved by direct methods using the SHELXS-97[10] program and refined by least-squares methods on F^2, SHELXL-97,[11] incorporated in SHELXTL-PC V 6.10.[12] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were found by difference Fourier methods and refined isotropically. The crystal used for the diffraction study showed no decomposition during data collection. The drawing was done with ellipsoids at the 50% probability level. CCDC-602713 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Artochamin I (4): White amorphous powder. $[\alpha]_D^{20}$ = 0 (c = 0.69, Me_2CO). UV (MeOH): λ_{max} (ε) = 205 (38900), 282 (4400) nm. IR (KBr): $\tilde{\nu}$ = 3370, 2925, 1600, 1517, 1437, 1313, 1276, 1201, 1085 cm^{-1}. ^1H and ^{13}C NMR: Tables 2 and 3. EIMS: m/z (%) = 408 (1) $[M]^+$, 393 (2), 377 (7), 309 (3), 285 (2), 244 (100), 229 (31), 213 (19), 188 (19), 164 (14). HREIMS: calcd. for $C_{26}H_{32}O_4$ 408.2300, found 408.2315.

Artochamin J (5): White amorphous powder. $[\alpha]_D^{20}$ = 0 (c = 0.18, Me_2CO). UV (MeOH): λ_{max} (ε) = 205 (27500), 230 (16200), 287 (5100), 313 (2750) nm. IR (KBr): $\tilde{\nu}$ = 3422, 2942, 2532, 1638, 1611, 1523, 1436, 1265, 1118 cm^{-1}. ^1H and ^{13}C NMR: Tables 2 and 3. EIMS: m/z (%) = 392 (10) $[M]^+$, 377 (24), 228 (39), 213 (100), 149 (12). HREIMS: calcd. for $C_{25}H_{28}O_4$ 392.1988, found 392.1996.

Artochamin K (6): Red amorphous powder. UV (MeOH): λ_{max} (ε) = 208 (44600), 286 (5100), 350 (1500), 387 (1700) nm. IR (KBr): $\tilde{\nu}$ = 3386, 2928, 1601, 1493, 1436, 1312, 1283, 1199, 1118, 1084 cm^{-1}. ^1H and ^{13}C NMR: Tables 1 and 3. EIMS: m/z (%) = 388 (19) $[M]^+$, 373 (100), 358 (26), 343 (13), 179 (38). HREIMS: calcd. for $C_{25}H_{24}O_4$ 388.1674, found 388.1660.

Cytotoxicity Assay: The compounds were tested for cytotoxicity against HepG2 cells using the Alamar Blue assay.[13,14] Drug stock solutions were prepared in DMSO and stored at –80 °C. Upon dilution with phosphated-buffered saline (PBS) into culture medium, the final DMSO concentration was < 1% DMSO (v/v). HepG2 cells were maintained in RPMI-1640 medium containing 10% FCS and 1% P/S at 37 °C in a humidified atmosphere with 5% CO_2. The day before the experiments were carried out, HepG2 cells were harvested with trypsin/EDTA, centrifuged (200 × g, 4 min), resuspended in medium, and seeded in 96-well plates ($3 \cdot 10^4$ cells/well and 100 μL/well). After 12 h of incubation, the medium was exchanged and the cells were incubated with different concentrations of the drugs (100–0.412 μM) at 37 °C in a 5% CO_2 atmosphere for 24 h; 0.5 h before the end of incubation, 33% EtOH was added as the positive control. After incubation, 10 μL of Alamar Blue was added to each well, which was incubated for another 2 h. Then, the fluorescence was monitored at 560 nm excitation wavelength and 580 nm emission wavelength with a fluorimeter plate reader Victor (Wallac Instruments). The IC_{50} values were determined using the software GraphPad Prism 4.0.

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