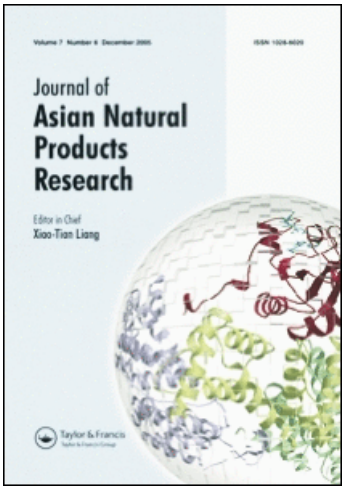

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ORIGINAL ARTICLE

Chemical constituents of the stem bark of *Morus cathayana*

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Phytochemical investigation of the stem bark of *Morus cathayana* led to the isolation and identification of six new compounds, cathayanons F–J (**1**–**5**) and cathayanin A (**6**), and two known compounds, cathayanins B–C (**7**–**8**). Their structures were elucidated by spectroscopic methods. Compounds **1**, **2**, **3**, **5**, and **7** exhibited weak activities against five human cancer cell lines, with IC_{50} values ranging from 4.7 to 9.8 μg/ml.

Keywords: *Morus cathayana*; flavonoids; cytotoxic activities

1. Introduction

The genus *Morus* comprises 16 species, and some of its members have been used in Chinese traditional medicine for the treatment of diabetes, hypertension, and rheumatism [1,2]. Previous phytochemical investigations on this genus revealed the presence of isoprenylated flavonoids, stilbenes, Diels–Alder type adducts, triterpenoids, and alkaloids. The chemical constituents of *Morus* plants have been studied extensively in our laboratory. In our continuing search for biologically active natural products from the stem bark of *Morus cathayana*, six new compounds (**1**–**6**) were isolated from its EtOH extract, together with two known compounds (**7**–**8**). Here, we describe the isolation, structure elucidation, and cytotoxicity of these compounds.

2. Results and discussion

Compound **1**, a yellow amorphous powder, showed a positive reaction to the ferric chloride test. The molecular formula was determined to be $C_{25}H_{26}O_5$ by HR-FAB-MS at *m/z* 407.1835 $[M + H]^+$. The UV spectrum exhibited absorption maxima at 204 and 297 nm. Its IR spectrum disclosed absorption bands assignable to OH (3356 cm^{-1}), carbonyl (1631 cm^{-1}), and benzene ring (1589 and 1514 cm^{-1}) moieties. The ^1H NMR spectrum of compound **1** exhibited the following proton signals: one set of A_2B_2 type aromatic protons at δ 6.82 (2H, d, $J = 8.0$ Hz), 7.17 (2H, d, $J = 8.0$ Hz), which were assigned to a *para*-disubstituted benzene ring (B ring); a singlet at δ 5.99 (1H, s, H-8), assignable to the proton of an A ring; three protons at δ 3.92–3.98 (1H, m) and 4.49–4.60 (2H, m), attributed to H-3 and H-2 of an isoflavanone system; two sets of olefinic protons at δ 4.49–4.60 (2H, m) and 5.20 (1H, s); two sets of methyl protons at δ 1.65 (3H, s) and 1.66 (3H, s); two sets of methylene protons at δ 1.76 (2H, m), 1.97 (1H, m), and 2.19

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(1H, m); and two sets of methine protons at δ 3.04 (1H, m) and 3.92–3.98 (1H, m), which were assigned to a 1,8-*p*-menthadiene moiety. The ^{1}H NMR spectrum of compound **1** also showed three downfield resonances at δ 8.39 (1H, br s), 9.39 (1H, br s), and 12.72 (1H, s), attributed to hydroxyl protons. The ^{13}C NMR and DEPT spectra revealed 25 carbon signals for compound **1**, comprising 13 aliphatic carbons and 12 aromatic carbons. The ^{1}H and ^{13}C NMR spectra of compound **1** were similar to those of a known compound, ficusin A [3], except for the absence of signal due to the 2,3-double bond, which was replaced by hydrogenation signals of the 2,3-double bond. However, detailed analysis of HMBC correlations revealed that the 1,8-*p*-menthadiene moiety was connected to C-6. In the spectrum, the hydrogen-bonded hydroxyl group at δ 12.72 showed long-range correlation with C-6 (111.7) and C-4a (103.0). The absolute configuration of compound **1** at C-3 was determined to be *R* on the basis of the negative Cotton effect at 313 nm in the CD spectrum [4,5]. The *trans* relationship of H-3″ and H-4″ was supported by the absence of NOESY correlation between H-3″ and H-4″. Thus, the structure of compound **1** was assigned as shown in Figure 1.

Compound **2**, a yellow amorphous powder, showed a positive reaction to the ferric chloride test. The molecular formula was determined to be $C_{25}H_{26}O_6$ by HR-FAB-MS at *m/z* 423.1799 $[M + H]^+$. The UV spectrum exhibited absorption maxima at 206, 255, and 376 nm, characteristic of a flavonol system. Its IR spectrum disclosed absorption bands assignable to OH (3535 and 3382 cm^{-1}), carbonyl (1655 cm^{-1}), and benzene ring (1616, 1599, and 1503 cm^{-1}) moieties. The ^{1}H NMR spectrum of compound **2** displayed the following signals: two singlets at δ 6.25 (1H, s) and 6.45 (1H, s) assignable to *meta*-protons in the A ring, two identical singlets at δ 7.93 (2H, s) assignable to two symmetric *meta*-protons in the B ring, and proton resonances corresponding to two symmetrical prenyl groups at δ 1.74 (6H, s), 1.75 (6H, s), 3.43 (4H, d, $J = 7.2$ Hz), and 5.30 (2H, br t, $J = 7.2$ Hz). Additionally, the resonances at δ 7.68 (1H, br s), 7.89 (1H, br s), 9.76 (1H, br s), and 12.18 (1H, s) were attributable to protons in the phenolic hydroxyl groups. The ^{13}C NMR spectrum (Table 1) revealed the presence of 25 carbons including those for a carbonyl carbon, aromatic carbons, and those of the prenyl moieties. In the HMBC spectrum, the long-range correlations of H-1″/C-4′ and H-2″/C-3′ (Figure 2) indicated that two prenyl groups were connected to C-3′ and C-5′. Assignments of ^{1}H and ^{13}C NMR signals were accomplished by a combination of HMQC and HMBC spectra. Based on the above-mentioned analysis, the structure of compound **2** was determined as shown in Figure 2.

Compound **3** gave a molecular formula of $C_{25}H_{28}O_6$ by HR-FAB-MS at *m/z* 425.1991 $[M + H]^+$. The UV spectrum of compound **3** exhibited absorption maxima at 206, 291, and 330 nm. The ^{1}H

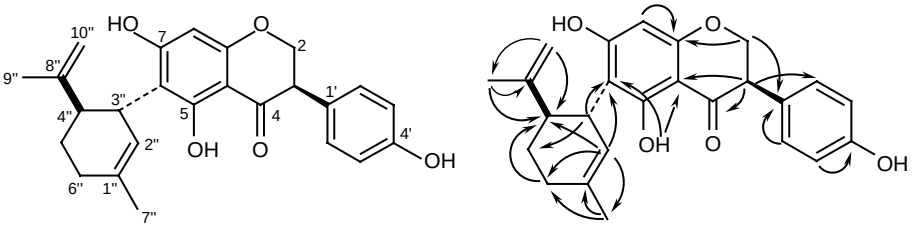

Figure 1. The structure and key HMBC correlations of compound **1**.

Table 1. ^{13}C NMR spectral data (δ) of compounds **1**–**5**.

Position	1	2	3	4	5
2	72.0	147.4	84.7	79.7	76.4
3	51.0	136.8	72.9	72.9	42.4
4	198.0	176.5	197.9	198.0	197.6
4a	103.0	104.2	101.3	101.5	103.2
5	161.8	162.3	164.2	165.0	165.3
6	111.7	99.1	97.1	97.1	96.8
7	161.8	164.8	168.5	167.8	167.2
8	95.8	94.4	96.2	96.1	95.9
8a	161.8	157.7	164.9	164.3	164.5
1′	127.7	123.5	129.8	116.8	118.1
2′	130.6	128.1	127.9	152.9	152.0
3′	116.3	128.9	128.6	117.5	117.2
4′	157.7	155.3	153.7	154.3	154.3
5′	116.3	128.9	128.6	120.8	120.9
6′	130.6	128.1	127.9	126.5	125.9
1″	36.0	29.4	29.3	23.5	23.4
2″	126.2	123.0	123.3	123.8	123.4
3″	130.6	133.7	133.1	131.9	136.4
4″	23.6	17.9	17.9	17.9	40.4
5″	31.2	25.9	25.9	25.8	16.3
6″	30.8				27.3
7″	45.3				125.0
8″	149.8				131.8
9″	110.6				17.7
10″	19.2				25.8
1‴				29.2	29.1
2‴				123.7	123.6
3‴				132.9	133.0
4‴				17.8	17.8
5‴				25.8	25.9

NMR spectrum of compound **3** was similar to that of compound **2**, with the major difference being the resonances for a pair of doublets at δ 4.98 (1H, d, $J = 11.4$ Hz, H-2) and 4.60 (1H, d, $J = 11.4$ Hz, H-3). Furthermore, the ^{13}C NMR spectrum of compound **3** showed two oxygenated carbon resonances at δ 84.7 and 72.9, and a carbonyl resonance at δ 197.9. All these observations were indicative of a

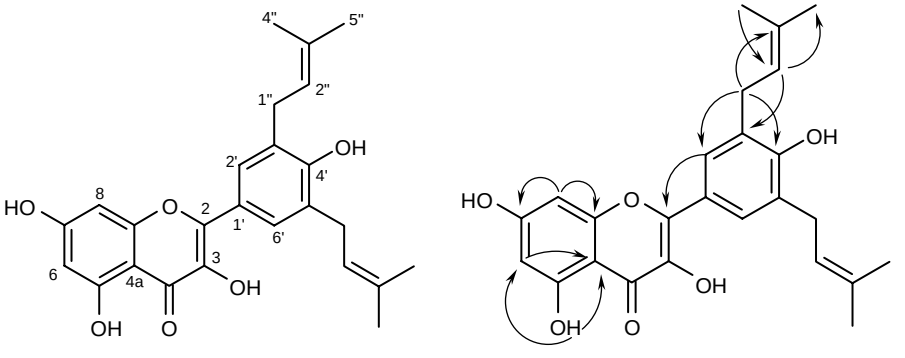

Figure 2. The structure and key HMBC correlations of compound **2**.

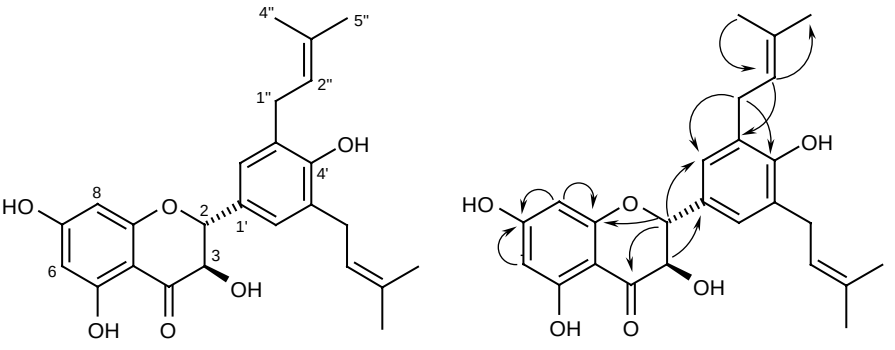

Figure 3. The structure and key HMBC correlations of compound **3**.

flavanol skeleton for compound **3**. The positions of two prenyl groups were confirmed by long-range correlations in the HMBC spectrum (Figure 3). In the spectrum, the long-range correlations of H-1″/C-4′ and H-2″/C-3′ indicated that two prenyl groups were connected to C-3′ and C-5′. The absolute configuration of compound **3** was assigned to be 2*R*, 3*R* by its CD spectrum, in which the negative and positive Cotton effects were exhibited at 294 and 330 nm, respectively [4,6]. Based on the above-mentioned analysis and the literature values [7], the structure of compound **3** was determined as shown in Figure 3.

Compound **4** was assigned a molecular formula of $C_{25}H_{28}O_7$ by HR-FAB-MS at *m/z* 441.1913 $[M + H]^+$. When compared with compound **3**, its molecular formula was 16 mass units higher, consistent with the addition of one oxygen atom. The UV spectrum exhibited absorption maxima at 210, 291, and 330 nm, suggesting this compound to be a flavanol. The ^{1}H NMR spectrum of compound **4** was similar to that of compound **3**. Comparison of the NMR spectroscopic data of compounds **4** and **3** indicated that compound **4** was not as symmetrically substituted as compound **3** due to the presence of two hydroxyl groups in ring B. This result was corroborated by the HMBC experiment (Figure 4). The HMBC correlations from the 2′-OH signal to C-2′, and from 4′-OH signal to C-4′, supported the attachment of two hydroxyl groups at C-2′ and C-4′, respectively, and correlations from H-1″ to C-2′,4′, from H-2″ to C-3′, from H-1‴ to C-6′, and from H-2‴ to C-5′ indicated that two prenyl groups were connected to C-3′ and C-5′, respectively. The absolute

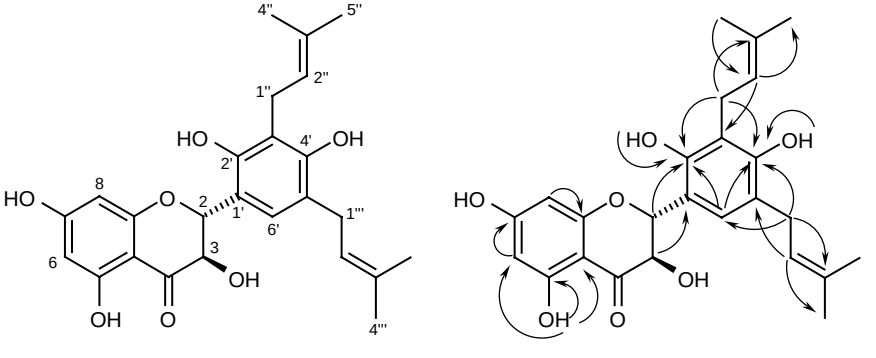

Figure 4. The structure and key HMBC correlations of compound **4**.

configuration of compound **4** was assigned to be 2*R*, 3*R* by its CD spectrum, in which the negative and positive Cotton effects were exhibited at 295 and 326 nm, respectively [4,6]. Thus, the structure of compound **4** was assigned as shown in Figure 4.

Compound **5**, a yellowish powder, was assigned a molecular formula of $C_{30}H_{36}O_6$ by HR-FAB-MS at *m/z* 493.2594 $[M + H]^+$. The UV spectrum exhibited absorption maxima at 210, 228 (sh), 289, and 330 (sh) nm, which was similar to those of flavanones. The ^{1}H NMR spectrum of compound **5** displayed the characteristic signals for a flavanone at δ 2.72 (1H, dd, $J = 3.2$, 17.2 Hz, H-3_{eq}), 3.23 (1H, dd, $J = 12.8$, 17.2 Hz, H-3_{ax}), and 5.75 (1H, dd, $J = 3.2$, 12.8 Hz, H-2), for one prenyl group at δ 1.70 (3H, s, H-4‴), 1.72 (3H, s, H-5‴), 3.32 (2H, d, $J = 6.8$ Hz, H-1‴), and 5.32 (1H, brt, $J = 6.8$ Hz, H-2‴), and for one geranyl group at δ 1.57 (3H, s, H-9″), 1.63 (3H, s, H-10″), 1.79 (3H, s, H-4″), 1.99 (2H, m, H-5″), 2.05–2.10 (2H, m, H-6″), 3.49 (2H, d, $J = 6.8$ Hz, H-1″), 5.08 (1H, br t, $J = 6.8$ Hz, H-7″), and 5.24 (1H, br t, $J = 6.8$ Hz, H-2″). The ^{1}H NMR spectrum further revealed the signals of two identical singlets at δ 5.95 (2H, s, H-6, 8) assignable to *meta*-protons in the A ring, one singlet at δ 7.07 (2H, s, H-6′, and OH-4′) assignable to a proton in the B ring and a proton of OH-4′, and proton resonances at δ 7.25 (1H, br s, OH-2′), 9.63 (1H, br s, OH-7), and 12.20 (1H, s, OH-5) corresponding to the phenolic hydroxyl groups. The ^{13}C NMR spectrum (Table 1) revealed the presence of 30 carbons including those for a carbonyl carbon, aromatic carbons, and those of the prenyl moieties. In the HMBC spectrum, the long-range correlations of H-1″/C-2′, H-2″/C-3′ indicated that the geranyl group was connected to C-3′. The correlations of H-1‴/C-6′ and H-2‴/C-5′ suggested that the prenyl group was connected to C-5′. The HMBC correlations from the 2′-OH signal to C-1′,2′,3′ and from 4′-OH signal to C-3′,4′,5′ supported the attachment of two hydroxyl groups at C-2′ and C-4′, respectively. The correlations of H-1″/H-4″ in the NOESY spectrum (Figure 5) indicated geometry of the C-2″/3″ double bond in compound **5**. Assignments of ^{1}H and ^{13}C NMR signals were accomplished by NOESY, HMQC, and HMBC spectra. The absolute configuration of compound **5** was assigned to be 2*R* by its CD spectrum, in which the negative and positive Cotton effects were exhibited at 279.5 and 289.5 nm, respectively [4,6,8]. Based on the above-mentioned results,

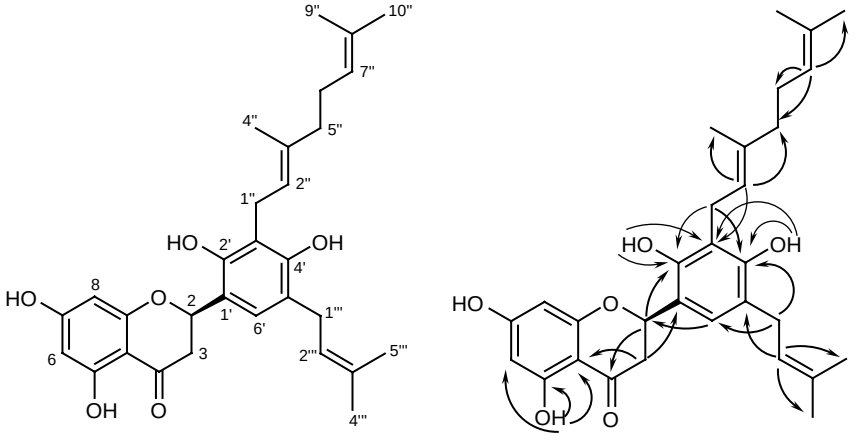

Figure 5. The structure and key HMBC correlations of compound **5**.

the structure of compound **5** was assigned as shown in Figure 5.

Compound **6**, a yellow amorphous powder, was assigned a molecular formula of $C_{25}H_{28}O_4$ by HR-EI-MS at *m/z* 392.1977 $[M]^+$. The UV spectrum exhibited absorption maxima at 206, 228, 285, and 311 nm, which was very similar to those of artochamin J. The IR spectrum showed absorptions for hydroxyl groups (3371.8 and 3242.4 cm^{-1}) and benzene rings (1605.7, 1522.1, and 1458.2 cm^{-1}). The ^1H NMR spectrum of compound **6** exhibited signals for one set of ABX aromatic protons at δ 7.29 (1H, d, $J = 8.0$ Hz), 6.48 (1H, d, $J = 2.5$ Hz), and 6.32 (1H, m), which suggested the presence of a trisubstituted aromatic ring. A singlet at δ 6.23 (1H, s), a pair of doublets at δ 5.95 and 5.28 (each 1H, d, $J = 9.5$ Hz), two signals at δ 1.35 and 1.39 (each 3H, s), and OCH_3 signal at δ 3.80 (3H, s) were assignable to a 2,2-dimethylchromene moiety with a 13-methoxy substituted group. In addition, the ^1H NMR spectrum also showed signals for one set of methylene protons at δ 2.91 (1H, dd, $J = 16.6$, 9.5 Hz) and 3.05 (1H, br d, $J = 16.5$ Hz), three sets of methine protons at δ 4.08 (1H, br t, $J = 8.0$, 6.0 Hz), 3.17 (1H, d, $J = 6.0$ Hz), and 2.74 (1H, br t, $J = 9.5$, 8.0 Hz), and two sets of methyl protons at δ 1.15 and 0.75 (each 3H, s), which were assigned to a bicyclic moiety (rings C and D). The ^{13}C NMR spectrum displayed 25 carbon signals including 13 aliphatic carbons and 12 aromatic carbons. These data closely resembled those of artochamin J [9]. However, compound **6** and artochamin J differed in the chemical shifts of A-ring protons and carbons. The HMBC correlations observed between H-7 and C-2,6 indicated that ring A in compound **6** had substituents at C-2 and C-4, instead of C-3 and C-4 in artochamin J (Figure 6). The HMBC correlations from the OCH_3 signal to C-13 supported the attachment of the methoxy group at C-13, and correlations from H-7 to C-8, C-9, C-16, C-17, and C-19, from H-8 to C-1, C-7, C-10, C-14, C-15, C-16, and C-17, as well as from H-15 to C-8, C-13, C-16, and C-17 revealed the location of rings C and D. All the NMR assignments were obtained using HMQC and HMBC correlations. The relative configuration of compound **6** was confirmed by the NOE difference experiment and comparison with the literature values [9]. In the NOE difference spectrum of compound **6**, H-8 and H-19 were enhanced by irradiation of H-16, while H-18 was enhanced by irradiation of H-7. These data indicated the synperiplanar relationship between H-16 and H-8, as shown in β-configuration and α-orientation of H-7. The absolute configuration of compound **6** was assigned to be 7*R*, 8*S*, 16*R* by applying the exciton chirality CD method [10] (Figure 7). The sign of the first Cotton effect was positive, while that of the second one was negative, indicating that the chirality between the two phenyl groups in compound **6** was a right-handed screw, as shown in Figure 8. Thus, the structure of compound **6** was elucidated as shown in Figure 6.

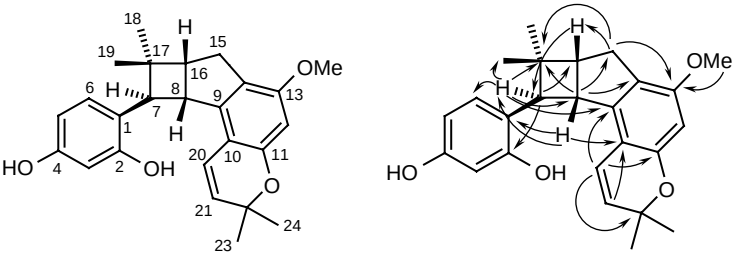

Figure 6. The structure and key HMBC correlations of compound **6**.

Two known compounds, cathayanins B and C (Figure 9 and 10), were also isolated in this study. Their structures were identified by spectroscopic data and by comparison with those of the published values [11].

Compounds **1**–**5** and **7**–**8** were assayed for their cytotoxic activities against five tumor cell lines (A549, Bel7402, BGC-823, HCT-8, and A2780) using the MTT assay. The results demonstrated that compounds **1**, **2**, **3**, **5**, and **7** exhibited weak activities against five human cancer cell lines, with IC_{50} values ranging from 5.0 to 9.7 μg/ml, respectively, while compounds **4** and **8** were inactive (IC_{50} values >10 μg/ml). The results of these studies are presented in Table 2. Compounds **3** and **4**, due to hydrogenation of the 2,3-double bond, were less active than compound **2**, indicating that the 2,3-double bond may be essential for the activity. Compound **4** with one more hydroxyl group substituent at C-2′, exhibited a lower cytotoxic activity than compound **3**, suggesting that addition of a hydroxyl group to C-2′ in the B ring may be responsible for a loss of activity. However, compound **3** was less active than compound **5**. The presence of a hydroxyl group at C-3 may significantly decrease the activity in compound **3**.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded in MeOH on a JASCO P-2000 polarimeter. CD spectra were recorded in MeOH on a JASCO J-815 spectrometer. UV spectra were measured with a JASCO V-650 spectrophotometer. IR spectra were recorded on a Nicolet Impact-400 spectrometer. NMR spectra were obtained on a Varian Mercury-Plus 300, 400, or 500 MHz spectrometer. MS data were recorded on a VG Autospec-300 mass spectrometer. Column chromatography (CC) was conducted on silica gel (60–100 or 200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and ODS (40–60 μm, Merck, Darmstadt, Germany). TLC was performed on GF254 plates (Qingdao Marine Chemical Factory) or TLC plates precoated with PR-18 F254s (Merck). HPLC was carried out using a LUMTECH K-501 pump equipped with a LUMTECH K-2501 UV detector and YMC-Pack ODS-A (20 × 250 mm, 5 μm).

3.2 Plant material

The stem bark of *M. cathayana* was collected from Mountain Lu, Jiangxi Province, China, in July 2005, and identified by Prof. Ce-Ming Tan, Jiujiang Institute of Forest Botany. A voucher specimen (No. 21037) has been deposited at the Herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

3.3 Extraction and isolation

The dried stem bark of *M. cathayana* (9 kg) was powdered and extracted with

Table 2. Cytotoxicities of compounds **1**, **2**, **3**, **5**, and **7** against five human cancer cell lines.

	IC_{50} (μg/ml)				
Compound	A549	Bel 7402	BGC-823	HCT-8	A2780
1	>10	9.32	>10	9.82	>10
2	5.02	5.95	5.55	5.84	5.79
3	>10	>10	>10	>10	6.72
5	4.67	5.95	6.70	5.86	5.79
7	6.61	>10	9.70	>10	7.03

95% aq. EtOH three times at reflux. The solvent was removed under reduced pressure to give 700 g of residue. The residue was submitted to silica gel CC (60–100 mesh, 1.0 kg), eluting with petroleum ether (PE, 60–90°C), $CHCl_3$, EtOAc, CH_3COCH_3, and MeOH, successively, and the fractions were concentrated to dryness. The $CHCl_3$-soluble fraction (100 g) was subjected to silica gel CC (200–300 mesh, 10 × 130 cm, 3 kg) and eluted with PE–acetone as gradient eluent [(10:1–8:1–6:1–4:1–2:1–1:1, v/v)]. The fractions were combined according to TLC profiles into five main fractions. Fraction C (31 g) was separated by silica gel CC (200–300 mesh, 6 × 100 cm, 800 g) eluted with PE–EtOAc (8:1–6:1–4:1–2:1–1:1, v/v) to give eight sub-fractions. Fraction C-6 (2 g) was purified by MPLC (ODS, 40–60 μm, MeOH–H_2O) and HPLC (YMC C-18, 20 × 250 mm, CH_3CN–H_2O, 55:45) to yield compounds **1** (7 mg) and **6** (3 mg). Fraction D (19 g) was separated by silica gel CC (200–300 mesh, 6 × 100 cm, 800 g) eluted with PE–EtOAc (8:1–6:1–4:1–2:1–1:1, v/v) to give five sub-fractions. Fractions D-2 (2.5 g) and D-3 (3.7 g) were separated by silica gel CC eluted with PE–EtOAc (6:1–5:1–3:1–1:1, v/v), purified by MPLC (ODS, 40–60 μm, MeOH–H_2O), and then by silica gel CC (PE–EtOAc), to yield compounds **2** (15 mg), **3** (50 mg), **4** (10 mg), and **5** (15 mg).

The EtOAc-soluble fraction (290 g) was subjected to silica gel chromatography (200–300 mesh, 10 × 130 cm, 3 kg) and eluted with $CHCl_3$–MeOH as gradient eluent [(98:2–95:5–9:1–85:15–75:25–7:3, v/v)]. The fractions were combined according to TLC profiles into 10 main fractions. Fraction D (12 g) was purified by MPLC (ODS, 40–60 μm, MeOH–H_2O) and HPLC (YMC C-18, 20 × 250 mm, CH_3CN–H_2O, 55:45) to yield compounds **7** (16 mg) and **8** (25 mg).

3.3.1 *Cathayanon F (**1**)*

Yellowish, amorphous powder. $FeCl_3$ test (brown). $[\alpha]_D^{20}$ + 90 (*c* 0.15, MeOH). UV λ_{max} (MeOH, log ε): 204 (4.56), 227 (sh) (4.50), 297 (4.35), 353 (3.56) nm. CD (MeOH) $\Delta\varepsilon_{313\,nm}$ +3.90, $\Delta\varepsilon_{347\,nm}$ −1.08. IR ν_{max}: 3356, 2962, 2923, 1631, 1589, 1514, 1442 cm^{-1}. ^1H NMR (acetone-d_6, 500 MHz): δ 1.65 (3H, s, H-7″), 1.66 (3H, s, H-9″), 1.76 (2H, m, H-5″), 1.97 (1H, m, H-6″), 2.19 (1H, m, H-6″), 3.04 (1H, m, H-4″), 3.92–3.98 (2H, m, H-3, 3″), 4.49–4.60 (4H, m, H-2, 10″), 5.20 (1H, s, H-2″), 5.99 (1H, s, H-8), 6.82 (2H, d, J = 8.0 Hz, H-2′,6′), 7.17 (2H, d, J = 8.0 Hz, H-3′,5′), 8.39 (1H, br s, OH-4′), 9.39 (1H, br s, OH-7), 12.72 (1H, s, OH-5). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data are presented in Table 1. HR-FAB-MS: *m/z* 407.1835 $[M + H]^+$ (calcd for $C_{25}H_{27}O_5$, 407.1858).

3.3.2 *Cathayanon G (**2**)*

Yellowish, amorphous powder. $FeCl_3$ test (green). UV λ_{max} (MeOH, log ε): 206 (4.57), 255 (4.23), 376 (4.14), 424 (sh) (3.94) nm. IR ν_{max}: 3535, 3381, 2973, 2922, 1655, 1616, 1599, 1503 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz): δ 1.74 (6H, s, H-4″), 1.75 (6H, s, H-5″), 3.43 (4H, d, J = 7.2 Hz, H-2″), 5.30 (2H, br t, J = 7.2 Hz, H-1″), 6.25 (1H, s, H-6), 6.45 (1H, s, H-8), 7.68 (1H, br s, OH-3), 7.89 (1H, br s, OH-4′), 7.93 (2H, s, H-2′,6′), 9.76 (1H, br s, OH-7), 12.18 (1H, s, OH-5). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data are presented in Table 1. HR-FAB-MS: *m/z* 423.1799 $[M + H]^+$ (calcd for $C_{25}H_{29}O_6$, 423.1808).

3.3.3 *Cathayanon H (**3**)*

Pale yellowish, powder. $FeCl_3$ test (reddish brown). $[\alpha]_D^{20}$ + 12 (*c* 0.15, MeOH). UV λ_{max} (MeOH, log ε): 206 (4.81), 291 (4.25) nm. CD (MeOH) $\Delta\varepsilon_{294\,nm}$ −6.28, $\Delta\varepsilon_{329\,nm}$ +2.70. IR ν_{max}: 3444, 3075, 2975, 2928, 1630, 1470 cm^{-1}. ^1H NMR (acetone-d_6, 300 MHz): δ 1.67 (12H, s, H-4″,5″), 3.35

(4H, d, $J = 7.2$ Hz, H-1″), 4.60 (1H, $J = 11.4$ Hz, H-3), 4.98 (1H, $J = 11.4$ Hz, H-2), 5.30 (2H, br t, $J = 7.2$ Hz, H-2″), 5.88 (1H, $J = 2.1$ Hz, H-6), 5.93 (1H, $J = 2.1$ Hz, H-8), 7.13(2H, s, H-2′,6′). ^{13}C NMR (acetone-d_6, 125 MHz) spectral data are presented in Table 1. HR-FAB-MS: *m/z* 425.1991 $[M + H]^+$ (calcd for $C_{25}H_{29}O_6$, 425.1964).

3.3.4 *Cathayanon I (**4**)*

Yellowish, amorphous powder. $FeCl_3$ test (reddish brown). $[\alpha]_D^{20}$ + 4.7 (*c* 0.1, MeOH). UV λ_{max} (MeOH, log ε): 208 (4.45), 291 (3.94), 336 (sh) (3.39) nm. CD (MeOH) $\Delta\varepsilon_{295\,nm}$ −3.17, $\Delta\varepsilon_{326\,nm}$ +0.91. IR ν_{max}: 3411, 2971, 2919, 1637, 1504, 1474 cm^{-1}. ^{1}H NMR (acetone-d_6, 300 MHz): δ 1.65 (3H, s, H-4″), 1.70 (6H, s, H-5″,4‴), 1.76 (3H, s, H-5‴), 3.20 (2H, d, $J = 6.9$ Hz, H-1‴), 3.46 (2H, d, $J = 6.9$ Hz, H-1″), 4.74 (1H, d, $J = 11.7$ Hz, H-3), 5.19 (1H, br t, $J = 6.9$ Hz, H-2″), 5.33 (1H, br t, $J = 6.9$ Hz, H-2‴), 5.48 (1H, d, $J = 11.7$ Hz, H-2), 5.96 (1H, d, $J = 2.1$ Hz, H-6), 5.99 (1H, d, $J = 2.1$ Hz, H-8), 7.12 (1H, s, H-6′), 7.07 (1H, s, OH-4′), 7.33 (1H, s, OH-2′), 9.76 (1H, br s, OH-7), 11.70 (1H, s, OH-5). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data are shown in Table 1. HR-FAB-MS: *m/z* 441.1917 $[M + H]^+$ (calcd for $C_{25}H_{29}O_7$, 441.1913).

3.3.5 *Cathayanon J (**5**)*

Yellowish, amorphous powder. $FeCl_3$ test (reddish brown). $[\alpha]_D^{20}$ −8.3 (*c* 0.11, MeOH). UV λ_{max} (MeOH, log ε): 211 (4.69), 228 (sh) (4.42), 289 (4.20), 336 (sh) (3.52) nm. CD (MeOH) $\Delta\varepsilon_{279.5\,nm}$ −0.50, $\Delta\varepsilon_{290\,nm}$ +0.51. IR ν_{max}: 3433, 2969, 2918, 1635, 1592, 1493 cm^{-1}. ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.57 (3H, s, H-9″), 1.63 (3H, *s*, H-10″), 1.70 (3H, s, H-4‴), 1.72 (3H, s, 5‴), 1.79 (3H, s, H-4″), 1.99 (2H, m, H-5″), 2.05–2.10 (2H, m, H-6″), 2.72 (1H, dd, $J = 3.2$, 17.2 Hz, H-3$_{eq}$), 3.23 (1H, dd, $J = 12.8$, 17.2 Hz, H-3$_{ax}$), 3.32 (2H, d, $J = 6.8$ Hz, H-1‴), 3.49 (2H, d, $J = 6.8$ Hz, H-1″), 5.32 (1H, br t, $J = 6.8$ Hz, H-2‴), 5.08 (1H, br t, $J = 6.8$ Hz, H-7″), 5.24 (1H, br t, $J = 6.8$ Hz, H-2″), 5.75 (1H, dd, $J = 3.2$, 12.8 Hz, H-2), 5.95 (2H, s, H-6, 8), 7.07 (2H, s, H-6′ and OH-4′), 7.25 (1H, s, OH-2′), 9.63 (1H, s, OH-7), 12.20 (1H, s, OH-5). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data are presented in Table 1. HR-FAB-MS *m/z* 493.2594 $[M + H]^+$ (calcd for $C_{30}H_{37}O_6$, 493.2590).

3.3.6 *Cathayanin A (**6**)*

Yellowish, amorphous powder. $FeCl_3$ test (green). $[\alpha]_D^{20}$ −22 (*c* 0.1, MeOH). CD (MeOH) $\Delta\varepsilon_{230\,nm}$ −0.30, $\Delta\varepsilon_{247.5\,nm}$ +0.15, $\Delta\varepsilon_{290\,nm}$ +0.07 (290). UV λ_{max} (MeOH, log ε): 206 (4.91), 228 (4.71), 285 (4.20), 311 (sh) (3.97) nm. IR ν_{max}: 3535, 3381, 2973, 2922, 1655, 1616, 1599, 1503 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.75 (3H, s, H-19), 1.15 (3H, s, H-18), 1.35 (3H, s, H-24), 1.39 (3H, s, H-23), 2.74 (1H, br t, $J = 9.5$, 8.0 Hz, H-16), 2.91 (1H, dd, $J = 16.5$, 9.5 Hz, H$_\beta$-15), 3.05 (1H, br d, $J = 16.5$ Hz, H$_\alpha$-15), 3.17 (1H, d, $J = 6.0$ Hz, H-7), 3.80 (3H, s, 13-OMe), 4.08 (1H, br t, $J = 8.0$, 6.0 Hz, H-8), 5.28, 5.95 (each 1H, d, $J = 9.5$ Hz, H-21, 20), 6.23 (1H, s, H-12), 6.32 (1H, m, H-5), 6.48 (1H, d, $J = 2.5$ Hz, H-3), 7.29 (1H, d, $J = 8.0$ Hz, H-6). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.9 (C-18), 26.9 (C-19),

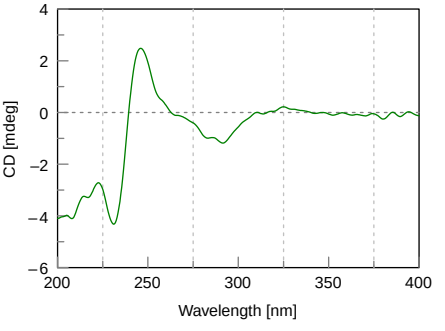

Figure 7. CD spectrum of cathayanin A (**6**).

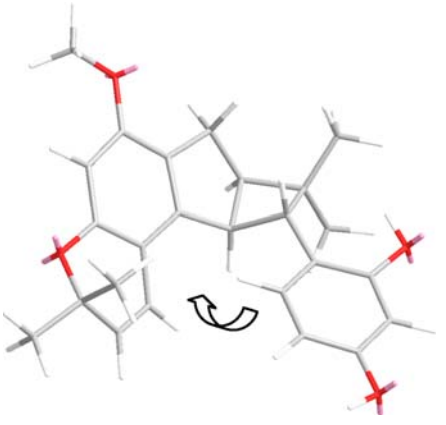

Figure 8. Stereostructure of cathayanin A (**6**) in reference to artochamin J.

27.4 (C-24), 28.2 (C-23), 30.1 (C-15), 38.7 (C-17), 42.0 (C-8), 44.6 (C-16), 50.4 (C-7), 55.3 (OMe-13), 76.0 (C-22), 97.9 (C-12), 102.7 (C-3), 107.2 (C-5), 109.8 (C-10), 119.6 (C-1), 119.9 (C-20), 124.7 (C-14), 127.3 (C-21), 128.2 (C-6), 145.7 (C-9), 154.8 (C-2), 154.8 (C-4), 153.4 (C-11), 156.2 (C-13). HR-EI-MS: *m/z* 392.1977 $[M]^+$ (calcd for $C_{25}H_{28}O_4$, 392.1988).

3.3.7 *Cathayanin B (**7**)*

Yellowish, amorphous powder. $[\alpha]_D^{20}$ + 380 (*c* 0.11, MeOH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 92.3 (C-2), 103.6 (C-3), 189.1 (C-4), 101.1 (C-4a), 153.0 (C-5), 107.1 (C-6), 163.8 (C-7), 95.9 (C-8), 163.3 (C-8a), 32.2 (C-9), 118.5 (C-10), 137.1 (C-11), 18.1 (C-12), 25.4 (C-13), 122.0 (C-1′), 161.1 (C-2′), 99.4 (C-3′), 161.1 (C-4′), 109.8 (C-5′), 125.6 (C-6′), 134.3 (C-1″), 121.2 (C-2″), 36.7 (C-3″), 28.0 (C-4″), 33.6 (C-5″), 36.2 (C-6″), 23.7 (C-7″), 103.9 (C-8″), 116.5 (C-9″), 161.7 (C-10″), 103.9 (C-11″), 157.8 (C-12″), 106.1 (C-13″), 129.6 (C-14″), 117.1 (C-15″), 157.1 (C-16″), 104.7 (C-17″), 160.1 (C-18″), 110.1 (C-19″), 127.9 (C-20″). ESIMS: *m/z* 713.2 $[M + Na]^+$, 689.2 $[M-H]^-$.

3.3.8 *Cathayanin C (**8**)*

Yellowish, amorphous powder. $[\alpha]_D^{20}$ + 337 (*c* 0.02, MeOH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 92.1 (C-2), 102.4 (C-3), 189.0 (C-4), 101.4 (C-4a), 153.1 (C-5), 107.3 (C-6), 164.1 (C-7), 96.3 (C-8), 163.3 (C-8a), 31.6 (C-9), 118.7 (C-10), 136.7 (C-11), 18.1 (C-12), 25.7 (C-13), 122.0 (C-1′), 161.3 (C-2′), 99.6 (C-3′), 161.3 (C-4′), 109.8 (C-5′), 125.8 (C-6′), 134.2 (C-1″), 121.3 (C-2″), 36.8 (C-3″), 28.2 (C-4″), 33.6 (C-5″), 36.2 (C-6″), 23.8 (C-7″), 103.7 (C-8″), 116.4 (C-9″), 161.5 (C-10″), 104.0 (C-11″), 157.9 (C-12″), 106.4 (C-13″), 129.9 (C-14″), 117.1 (C-15″), 157.1 (C-16″), 104.7 (C-17″), 160.1 (C-18″), 110.2

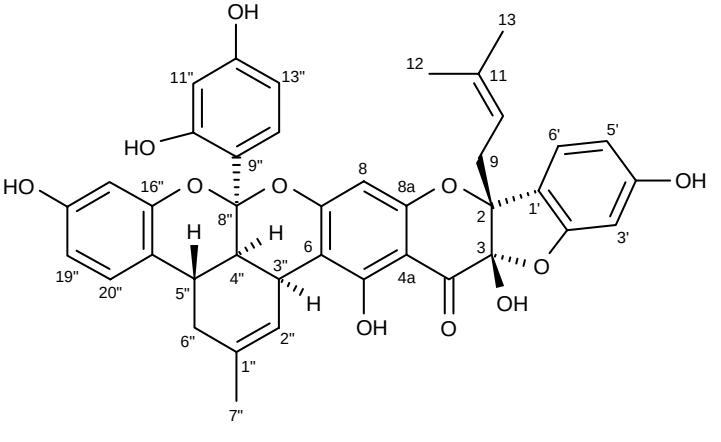

Figure 9. The structure of cathayanin B (**7**).

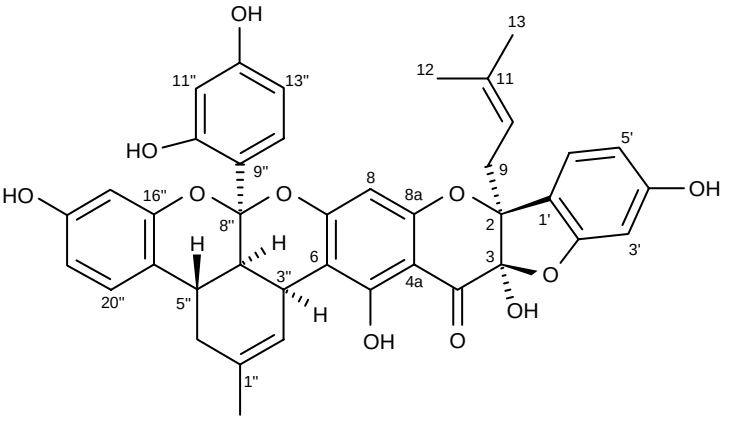

Figure 10. The structure of cathayanin C (**8**).

(C-19″), 127.9 (C-20″). ESIMS: *m/z* 713.2 $[M + Na]^+$, 689.5 $[M\text{-}H]^-$.

3.4 Cytotoxicity assay

Compounds **1**–**5** and **7**–**8** were tested for cytotoxicity against A549 (human lung carcinoma), Bel 7402 (human liver carcinoma), BGC 823 (human stomach carcinoma), HCT-8 (human colon carcinoma), and A2780 (human ovarian carcinoma) cells by means of the MTT assay. Briefly, these cells were plated in 96-well plates and cultured for 24 h. The appropriate test compounds and positive control were added into triplicate wells at concentrations of 0.1, 1.0, and 10.0 μg/ml and incubated for 4 d at 37°C. MTT solution (10 μl, 5 mg/ml) was added into each well, and the plate was incubated for another 4 h. The resulting formazan crystals were dissolved in DMSO (100 μl), and the UV–VIS absorbance (optical density, OD) was determined with a microplate spectrophotometer at 570 nm. The linear dependences between OD and the pre cent cell survival were calculated with Excel (Microsoft), and IC_{50} values were determined graphically as described previously [12]. The reference compound, 5-fluorouracil, exhibited activity toward the A549, Bel7402, BGC-823, HCT-8, and A2780 cell lines with IC_{50} ranges of 0.2–0.7 μg/ml.

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