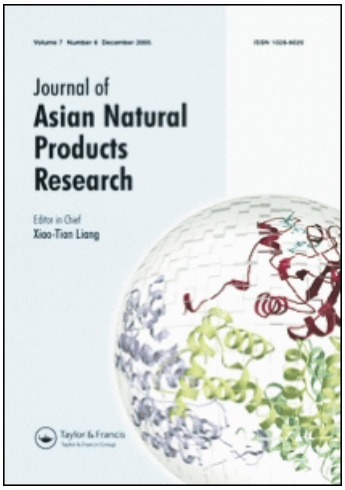

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Three new Diels–Alder type adducts from the stem bark of *Morus cathayana*

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Three new Diels–Alder type adducts cathayanons C (**1**), D (**2**), and E (**3**), together with one known compound sanggenon C (**4**), were isolated from the stem bark of *Morus cathayana.* Their structures were fully elucidated by spectroscopic and chemical methods. Compound **2** showed good anti-oxidation activity with the inhibitory rate of malondialdehyde being 88% at a concentration of 10^{-6} mol/l.

Keywords: *Morus cathayana*; cathayanon C; cathayanon D; cathayanon E; anti-oxidation activity

1. Introduction

The mulberry trees had been important plants in agricultural and medical fields for thousands of years in China, because its leaves were an indispensable feed for silk worm, and its root barks were used to treat diabetes, arthritis, and rheumatism in Chinese herbal medicine. In 11 species of *Morus* found in China, the root bark of *Morus alba* was the classical medical materials, while the root bark of *Morus cathayana* was used as an important alternative in many places [1]. In our previous works, many novel compounds were isolated from *Morus macroura*, *Morus mongolica*, and *Morus australis*. In the continuous phytochemical investigation of *M. cathayana*, three new compounds of cathayanons C–E (**1–3**), together with a known compound sanggenon C (**4**), were isolated from its EtOH extract. This paper deals with the isolation and structure elucidation of these compounds, as well as the evaluation of their anti-oxidation effects.

2. Results and discussion

Cathayanon C (**1**) was obtained as a yellow amorphous powder. The molecular formula was established as $C_{34}H_{25}O_8$ by HR-ESI-MS at *m/z* 561.1548 $[M+H]^+$. The UV spectrum exhibited maxima at 208, 220, 284, 310, and 352 nm revealed the presence of a complicated chromophore. IR spectrum showed absorption bands at 3357.9, 1693, 1603, and 1510 cm^{-1} assignable to hydroxy, C=C, and benzene ring moieties. ^{1}H NMR spectrum exhibited proton signals as follows: one set of ABX-type aromatic protons at δ_H 7.42 (1H, d, $J = 8.4$ Hz, H-6), 6.38 (1H, dd, $J = 8.4$, 2.4 Hz, H-5), and 6.43 (1H, d, $J = 2.4$ Hz, H-3), a pair of *trans*-coupling olefinic protons at δ_H 7.39 (1H, d, $J = 16.5$ Hz, H-α) and 6.88 (1H, d, $J = 16.5$ Hz, H-β), as well as a singlet of two aromatic protons at δ_H 6.80 (2H, s, H-2′, 6′) assignable to a stilbene moiety; two sets of ABX-type aromatic protons at δ_H 6.11 (1H, d, $J = 8.4$ Hz, H-14″), 5.91 (1H, dd, $J = 8.4$, 2.4 Hz, H-13″), 6.29 (1H, d,

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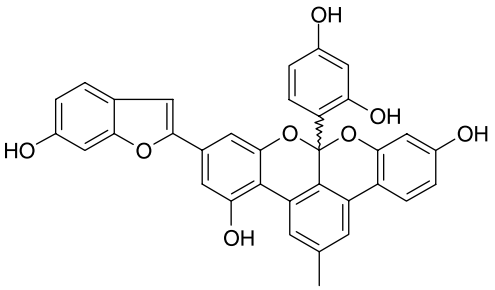
Table 1. ^{13}C NMR spectral data of **1** and **2** (acetone-d_6, 100 MHz).

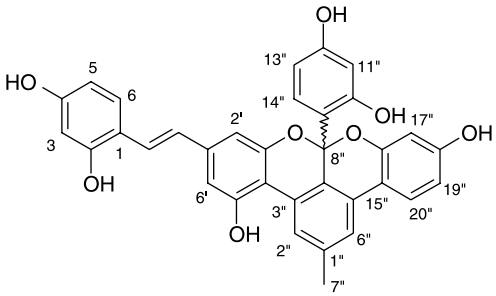
No.	**1**	**2**	No.	**1**	**2**
1	116.9	117.0	4″	121.6	121.4
2	157.1	157.0	5″	129.6	117.3
3	103.6	103.4	6″	120.9	151.4
4	159.5	159.3	7″	22.2	17.6
5	108.6	108.4	8″	106.3	106.5
6	128.5	128.4	9″	115.4	114.9
α	125.7	125.1	10″	158.1	158.1
β	124.9	125.1	11″	104.8	104.6
1′	141.1	139.8	12″	160.8	160.6
2′	107.6	107.4	13″	106.3	106.2
3′	152.9	152.0	14″	130.9	131.1
4′	109.8	110.2	15″	115.1	115.1
5′	156.6	155.7	16″	152.2	152.2
6′	109.3	109.2	17″	105.2	105.2
1″	140.5	127.1	18″	159.9	158.6
2″	126.2	128.4	19″	111.5	110.6
3″	129.6	125.6	20″	125.3	130.5

$J = 2.4$ Hz, H-11″), 7.70 (1H, d, $J = 8.4$ Hz, H-20″), 6.57 (1H, dd, $J = 8.4$, 2.4 Hz, H-19″), and 6.54 (1H, d, $J = 2.4$ Hz, H-17″) ascribable to two 2,4-dioxysubstituted phenyl; one methyl singlet at δ_H 2.50 (3H, s, H-7″), and two singlets at δ_H 8.42 (1H, s, H-2″) and 7.59 (1H, s, H-6″) resembled to those of the aromatized methylcyclohexene ring of albanol B [2], which indicated that **1** was a Diels–Alder type adduct with an aromatized methylcyclohexene ring.

The ^{13}C NMR spectrum of **1** showed 34 carbon signals that were equal to that of a Diels–Alder adduct of a prenyl stilbenoid and a chalcone moiety. The absence of carbonyl and most aliphatic carbon signals confirmed the presence of an aromatized methylcyclohexene ring compared with those carbon signals of albanol B [2]. Two olefinic carbon signals at δ_C 125.7 (C-α) and 124.9 (C-β), which correlated to two *trans*-coupling olefinic protons at δ_H 7.39 (1H, d, $J = 16.5$ Hz, H-α) and 6.88 (1H, d, $J = 16.5$ Hz, H-β) in HSQC spectrum, confirmed the presence of a stilbene moiety. In the HMBC spectrum, the long-range correlations of H-2′, 6′ to C-4′, H-2″ to C-4′, 7″ indicated that stilbene and aromatized methylcyclohexene moieties connected at C-4′ and C-3″. The carbon resonances (Table 1) were assigned using HSQC and HMBC experiments. Thus, the structure of **1** was determined as shown in Figure 1, which was



albanol B



1

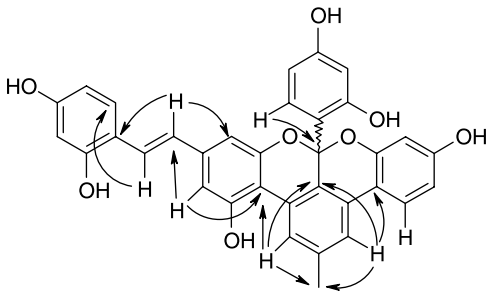


Figure 1. The structure of albanol B and **1**, and the key HMBC correlations (H → C) of **1**.

the first aromatized Diels–Alder adduct with a stilbene moiety. The absolute configuration of C-8″ for **1** had not been determined based on current experiments.

Cathayanon D (**2**) was obtained as a yellow amorphous powder. The molecular formula was established as $C_{34}H_{25}O_9$ by HR-ESI-MS at *m/z* 577.1472 $[M+H]^+$. The UV spectrum exhibited maxima at 206, 284, 308, and 352 nm, which were similar to those of **1**. IR spectrum showed absorption bands at 3374, 1693, 1611, and 1509.6 cm^{-1} assignable to hydroxy, C=C, and benzene ring moieties.

The ^{1}H NMR spectrum of **2** exhibited 14 aromatic proton signals and a methyl singlet as follows: one set of ABX-type aromatic protons at δ_H 7.40 (1H, d, $J = 8.4$ Hz, H-6), 6.37 (1H, dd, $J = 8.4$, 2.4 Hz, H-5), and 6.44 (1H, d, $J = 2.4$ Hz, H-3), a pair of *trans*-coupling olefinic protons at δ_H 7.35 (1H, d, $J = 16.5$ Hz, H-α) and 6.86 (1H, d, $J = 16.5$ Hz, H-β), as well as two singlets at δ_H 6.75 (1H, s, H-2′) and 6.78 (1H, s, H-6′) assignable to a stilbene moiety; one set of ABX-type aromatic protons at δ_H 6.22 (1H, d, $J = 8.4$ Hz, H-14″), 5.90 (1H, dd, $J = 8.4$, 2.4 Hz, H-13″), and 6.27 (1H, d, $J = 2.4$ Hz, H-11″) ascribable to a 2,4-dihydroxyphenyl; another set of ABX-type aromatic protons at δ_H 8.43 (1H, d, $J = 8.4$ Hz, H-20″), 6.51 (1H, dd, $J = 8.4$, 2.4 Hz, H-19″), and 6.53 (1H, d, $J = 2.4$ Hz, H-17″) ascribable to a 2,4-dioxysubstituted phenyl moiety; two singlets at δ_H 8.42 (1H, s, H-2″) and 2.50 (3H, s, H-7″), together with the absence of the signal of H-6″ compared with **1**, indicated that H-6″ was substituted by a hydroxy group in **2**, which were similar to those of mulberrofuran P [3].

The ^{13}C NMR spectrum of **2** showed 32 unsaturated and 2 saturated carbon signals like those of **1**, and confirmed the presence of a stilbene moiety and an aromatized methylcyclohexene ring with an oxygenated aromatic carbon at δ_C 151.4 (C-6″) compared with **1**. In the HMBC spectrum, the long-range correlations of H-2″ to C-4′ determined that two mentioned moieties connected at C-4′ and C-3″, and H-7″ to C-6″ confirmed that a hydroxyl connected to C-6″. Thus, the structure of **2** was elucidated as shown in Figure 2. Its absolute configuration at C-8″ was not determined like compound **1**.

Cathayanon E (**3**) was obtained as a yellow amorphous powder. The molecular formula was established as $C_{40}H_{36}O_{12}$ by HR-ESI-MS at *m/z* 707.2109 $[M-H]^-$. The UV spectrum exhibited maxima at 206, 282, and 306 nm, which were similar to those of sanggenon-type Diels–Alder adducts [4]. IR spectrum showed absorption bands at 3345, 1697, and 1594 cm^{-1} assignable to hydroxyl, C=O, and benzene ring moieties.

In the ^{1}H NMR spectrum of **3**, which was tested at 24°C, some protons showed such broad signals that the spectrum could not be analyzed satisfactorily. When tested at 80°C in DMSO-d_6, these protons displayed better signals as following: one singlet at δ_H 12.77 (1H, s, OH-10″) due to a chelated hydroxyl group; one set of ABX-type aromatic protons at δ_H 7.19 (1H, d, $J = 8.0$ Hz, H-6′), 6.38 (1H, brd, $J = 8.0$ Hz, H-5′), and 6.22 (1H, brs, H-3′),

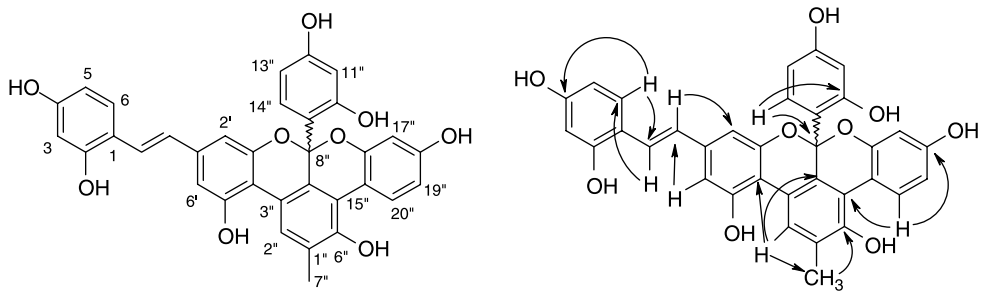


Figure 2. The structure and key HMBC correlations (H → C) of **2**.

one singlet at δ_H 5.61 (1H, s, H-8), and nine aliphatic proton signals for a γ,γ-dimethylallyl moiety at δ_H 5.20–5.30 (1H, m, H-10), 2.93 (1H, dd, $J = 14.5$, 8.0 Hz, H-9), 2.65 (1H, dd, $J = 14.5$, 6.0 Hz, H-9), 1.59 (3H, s, H-12), and 1.68 (3H, s, H-13) were similar to those of the flavanonol moiety of sanggenon C [5]; one set of ABX-type aromatic protons at δ_H 7.98 (1H, d, $J = 8.5$ Hz, H-14″), 6.35 (1H, brd, $J = 8.5$ Hz, H-13″), and 6.14 (1H, d, $J = 2.0$ Hz, H-11″) were assignable to a 2,4-dihydroxybenzoyl moiety; one set of ABX-type aromatic protons at δ_H 6.69 (1H, d, $J = 8.5$ Hz, H-20″), 5.97 (1H, dd, $J = 8.5$, 2.0 Hz, H-19″), and 6.15 (1H, d, $J = 2.0$ Hz, H-17″) were assignable to a 2,4-dihydroxyphenyl moiety; compared with sanggenon C, the surplus 10 aldyl proton signals at δ_H 4.43 (1H, d, $J = 11.0$ Hz, H-4″), 3.47 (1H, s, H-3″), 3.20–3.40 (1H, brs, H-5″), 2.28 (1H, brd, $J = 12.0$ Hz, H-2″), 1.60–1.70 (1H, overlapped, H-2″), 2.00–2.12 (1H, m, H-6″), 1.80–1.90 (1H, m, H-6″), and 1.38 (3H, s, H-7″), together with the MS data, indicated that **3** was an isomer of sanggenon C with the double bond of methylcyclohexene moiety transformed through an intramolecular additive reaction [4,6].

In the ^{13}C NMR (DMSO-d_6, 125 MHz, 80°C) spectrum of **3**, seven aldyl carbon signals at δ_C 75.7 (C-1″), 49.4 (C-4″), 45.1 (C-6″), 40.0 (C-5″), 34.7 (C-2″), 30.0 (C-3″), and 28.0 (C-7″), except for the signals of the flavanonol, 2,4-dihydroxybenzoyl and 2,4-dihydroxyphenyl moieties, were assigned to a methylcyclohexane moiety. The oxygenated carbon signal at $\delta_{C\text{-}1''}$ 75.7 indicated the double bond reacted with a hydroxyl group. According to reference, the etherification of 5-OH of flavonoids resulted in upfield shift of $\delta_{C\text{-}4}$ [7]. $\delta_{C\text{-}4}$ 180.5 of **3** was less than that of sanggenon C ($\delta_{C\text{-}4}$ 188.3) [4], which indicated that the methylcyclohexane moiety connected C-5 through an ether band. The HMBC correlations of H-2″/C-6, H-7″/C-1″, 2″, 6″ confirmed that the methylcyclohexane moiety connected to flavanonol moiety at C-6/C-3″, and C-1″ connected C-5 through an oxygen atom (Figure 3).

It was reported that the absolute configuration of C-2, 3 of sanggenon-type flavanonols was stable under acid condition [8,9], while a γ,γ-dimethylallyl group can react with its *ortho*-hydroxyl group through intramolecular addition under this condition [10]. Sanggenon C (**4**), which was obtained from the titled plant, provided a possible precursor of **3**. Sanggenon C was catalyzed by F_3CCOOH to give compound **3a** (Scheme 1). Compounds **3** and **3a** had the same R_t and R_f in HPLC and TLC tests, respectively. Compounds **3** and **3a** had the same IR, MS, ^{1}H NMR spectra, and the same Cotton effects in CD spectra, which indicated that they had the same structure. As 5-OH of sanggenon C reacted with the C═C bond stereospecifically, the absolute configuration of **3** can be deduced as 2*R*, 3*S*, 1″*S*, 3″*S*, 4″*R*, 5″*S* from those of sanggenon C (2*R*, 3*S*, 3″*S*, 4″*R*, 5″*S*) [9]. Thus, the structure of **3** was elucidated as shown in Figure 3.

Compound **4** was identified as sanggenon C by the comparison of its physical and spectroscopic data ($[\alpha]_D^{20}$, MS, UV, IR, ^{1}H, and ^{13}C NMR) with those reported [4,5,8].

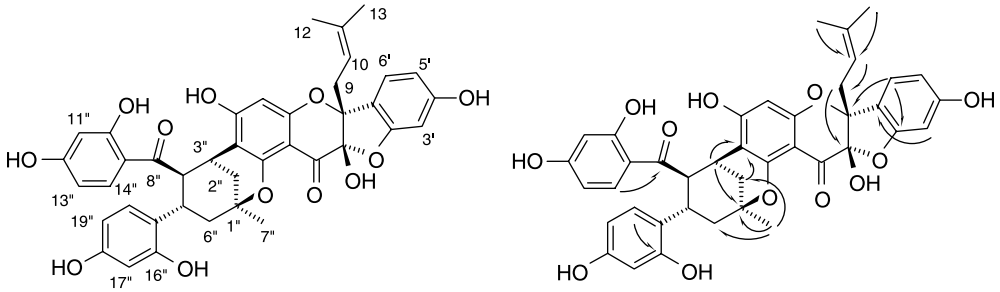
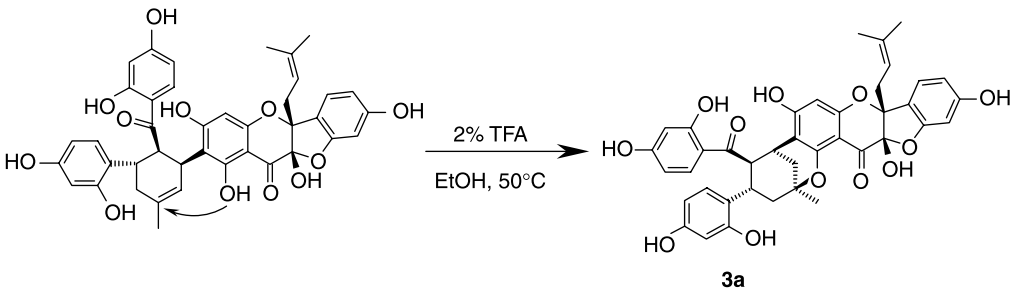


Figure 3. The structure and key HMBC correlations (H → C) of **3**.



Scheme 1. Biomimetic synthesis of **3a** from sanggenon C (**4**).

With respect to the anti-oxidation effects of compounds **1**, **2**, and Vit E, the inhibitory rates of malondialdehyde (MDA) were 95, 103, and 62.6% at concentrations of 10^{-5} mol/l, respectively; and were −14, 88, and 57.3% at concentrations of 10^{-6} mol/l, respectively. Thus, it indicated that compound **2** had good anti-oxidation activity.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. The IR spectra were recorded on a Nicolet IMPACT-400 spectrophotometer as KBr disks. UV spectra were recorded on a Shimadzu UV-241 spectrophotometer. HR-ESI-MS were obtained on an AccuTOF CS mass spectrometer. NMR spectra were recorded with Varian Mercury-300, Varian Mercury-400, and Varian Unity INOVA 500 spectrophotometers. Silica gel for column chromatography and silica gel GF254 for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Precoated plates of silica gel GF254 and silica gel RP-18 F254s (Merck, Darmstadt, Germany) were used for TLC. The HPLC experiments were carried out on a Shimadzu LC10ATvp chromatograph with a UV detector at 220 nm and the MPLC experiments on a Gilson 302 chromatograph with a UV detector at 254 nm.

3.2 Plant material

M. Cathayana was collected in Lushan, Jiangxi Province, China, in July 2005, and identified by Prof. Ce-Ming Tan, Jiujiang Institute of Forest Botany, Jiangxi Province, China. A voucher specimen (ID-21037) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The air-dried stem bark (9.0 kg) of *M. cathayana* was finely cut and extracted with refluxing 95% EtOH. After the solvent was evaporated under vacuum, the residue (700 g) was submitted to chromatography over a silica gel column (60–100 mesh, 1.2 kg) and eluted with petroleum ether (PE, 60–90°C), $CHCl_3$, EtOAc, CH_3COCH_3, and MeOH, successively.

The EtOAc fraction (200 g) was subjected to a silica gel column (160–200 mesh, 10 × 80 cm) and eluted with $CHCl_3$–MeOH mixtures (98:2–95:5–90:10–85:15–75:25–70:30, v/v) to provide 10 fractions. Fraction I (16 g) was subjected to a Sephadex LH-20 column (2.5 × 90 cm) and eluted with MeOH to give six fractions. Fraction I-6 (1.5 g) was purified by silica gel column chromatography (200–300 mesh, 2.5 × 25 cm), eluted with PE–acetone 2:1 to give **1** (220 mg) and two fractions. Fraction I-6-2 (70 mg) was further separated by preparative HPLC (Rainin C-18, 20 × 300 mm, eluted by MeOH–H_2O, 60:40) to give **2** (30 mg).

Fraction G (23 g) was separated by silica gel column chromatography (160–200 mesh, 6 × 100 cm), eluted with PE–acetone mixtures (3:1–2:1–1:1, v/v) to give five fractions.

Fraction G-4 (1.3 g) was subjected to a Sephadex LH-20 column (2.5 × 90 cm, eluted with MeOH) to give seven fractions. Fraction G-4-3 (800 mg) was further separated by MPLC (ODS, 40–60 μm, 2.5 × 24 cm, eluted by MeOH–H_2O, 55:45) and preparative HPLC (Rainin C-18, 20 × 300 mm, eluted by MeOH–H_2O, 60:40) to give **3** (15 mg). Fraction G-1 (5 g) was subjected to a Sephadex LH-20 column (2.5 × 90 cm, eluted with MeOH) to give four fractions. Fraction G-1-2 (1.5 g) was purified by MPLC (ODS, 40–60 μm, 2.5 × 24 cm, eluted by MeOH–H_2O, 60:40) to yield **4** (800 mg).

3.3.1 *Cathayanon C* (**1**)

Yellow amorphous powder; $[\alpha]_D^{20}$ + 11.4 (c = 0.40, MeOH). UV λ_{max} (MeOH, log ε): 208 (4.78), 220 (4.71), 284 (4.34), 310 (4.37), 352 (4.40) nm. IR (KBr) ν_{max}: 3357.9, 2922, 1693, 1603, 1510, 1455, 1409 cm^{-1}. ^1H NMR (acetone-d_6, 300 MHz): δ_H 7.42 (1H, d, J = 8.4 Hz, H-6), 6.38 (1H, dd, J = 8.4, 2.4 Hz, H-5), 6.43 (1H, d, J = 2.4 Hz, H-3), 6.11 (1H, d, J = 8.4 Hz, H-14″), 5.91 (1H, dd, J = 8.4, 2.4 Hz, H-13″), 6.29 (1H, d, J = 2.4 Hz, H-11″), 7.70 (1H, d, J = 8.4 Hz, H-20″), 6.57 (1H, dd, J = 8.4, 2.4 Hz, H-19″), 6.54 (1H, d, J = 2.4 Hz, H-17″), 7.39 (1H, d, J = 16.5 Hz, H-α), 6.88 (1H, d, J = 16.5 Hz, H-β), 6.80 (1H, s, H-2′, 6′), 8.42 (1H, s, H-2″), 7.59 (1H, s, H-6″), and 2.50 (3H, s, H-7″). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data, see Table 1. HR-ESI-MS: m/z 561.1548 $[M+H]^+$ (calcd for $C_{34}H_{25}O_8$, 561.1549).

3.3.2 *Cathayanon D* (**2**)

Yellow amorphous powder; $[\alpha]_D^{20}$ − 5.7 (c = 0.08, MeOH); UV λ_{max} (MeOH): 206, 284, 308, 352 nm. IR (KBr) ν_{max}: 3374, 1693, 1611, 1509.6, 1464.6, 1407 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz): δ_H 7.40 (1H, d, J = 8.4 Hz, H-6), 6.37 (1H, dd, J = 8.4, 2.4 Hz, H-5), 6.44 (1H, d, J = 2.4 Hz, H-3), 6.22 (1H, d, J = 8.4 Hz, H-14″), 5.90 (1H, dd, J = 8.4, 2.4 Hz, H-13″), 6.27 (1H, d, J = 2.4 Hz, H-11″), 8.43 (1H, d, J = 8.4 Hz, H-20″), 6.51 (1H, dd, J = 8.4, 2.4 Hz, H-19″), 6.53 (1H, d, J = 2.4 Hz, H-17″), 7.35 (1H, d, J = 16.5 Hz, H-α), 6.86 (1H, d, J = 16.5 Hz, H-β), 6.75 (1H, s, H-2′), 6.78 (1H, s, H-6′), 8.42 (1H, s, H-2″), and 2.50 (3H, s, H-7″). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data, see Table 1. HR-ESI-MS: m/z 577.1472 $[M+H]^+$ (calcd for $C_{34}H_{25}O_9$, 577.1500).

3.3.3 *Cathayanon E* (**3**)

Yellow amorphous powder; $[\alpha]_D^{20}$ + 189.1 (c = 0.10, MeOH). UV λ_{max} (MeOH, log ε): 206 (4.73), 282 (4.33), 306 (4.26) nm. IR (FT-IR microscope transmission) ν_{max}: 3345, 2928, 1697, 1594, 1507, 1451 cm^{-1}. CD (c = 0.052, MeOH) $\Delta\varepsilon$ (λ, nm): − 4.46 (239), + 6.48 (315). ^1H NMR (DMSO-d_6, 500 MHz, 80°C): δ_H 12.77 (1H, s, OH-10″), 7.19 (1H, d, J = 8.0 Hz, H-6′), 6.38 (1H, brd, J = 8.0 Hz, H-5′), 6.22 (1H, brs, H-3′), 5.61 (1H, s, H-8), 5.20–5.30 (1H, m, H-10), 2.93 (1H, dd, J = 14.5, 8.0 Hz, H-9), 2.65 (1H, dd, J = 14.5, 6.0 Hz, H-9), 1.59 (3H, s, H-12), 1.68 (3H, s, H-13), 7.98 (1H, d, J = 8.5 Hz, H-14″), 6.35 (1H, d, J = 8.5 Hz, H-13″), 6.14 (1H, d, J = 2.0 Hz, H-11″), 6.69 (1H, d, J = 8.5 Hz, H-20″), 5.97 (1H, dd, J = 8.5, 2.0 Hz, H-19″), 6.15 (1H, d, J = 2.0 Hz, H-17″), 4.43 (1H, d, J = 11.0 Hz, H-4″), 3.47 (1H, s, H-3″), 3.20–3.40 (1H, brs, H-5″), 2.28 (1H, brd, J = 12.0, H-2″), 1.60–1.70 (1H, overlapped, H-2″), 2.00–2.12 (1H, m, H-6″), 1.80–1.90 (1H, m, H-6″), and 1.38 (3H, s, H-7″). ^{13}C NMR (acetone-d_6, 100 MHz) spectral data, see Table 2. HR-ESI-MS: m/z 707.2109 $[M+H]^+$ (calcd for $C_{40}H_{35}O_{12}$, 707.2129).

3.3.4 *Synthesis of* **3a**

Sanggenon C (50 mg) dissolved in anhydrous EtOH 5 ml, catalyzed with 2% F_3CCOOH, churned 24 h at 50°C. The products were isolated by preparative TLC (PE–acetone, 1:1, v/v) to yield **3a** (8 mg). Compounds **3** and **3a** showed the same R_f (0.4) on TLC (PE–acetone, 1:1, v/v) and R_t (10.2 min) on HPLC (Alltima, ∅ 4.6 × 150 mm, eluted by MeOH–H_2O, 55:45, 1 ml/min). IR (FT-IR microscope transmission) ν_{max}: 3365, 2924, 1697, 1589,

Table 2. ^{13}C NMR spectral data of **3** and **4**.

No.	**3**[a]	**4**[b]	No.	**3**[a]	**4**[b]
2	88.8	91.9	1″	75.7	135.0
3	102.8	102.4	2″	34.7	122.8
4	180.5	188.3	3″	30.0	35.8
4a	100.3	99.9	4″	49.4	48.2
5	158.6	163.9	5″	40.0	32.7
6	103.4	109.0	6″	45.1	33.7
7	162.0	167.7	7″	28.0	23.7
8	93.7	96.5	8″	203.8	208.7
8a	160.8	161.9	9″	113.2	113.9
9	31.4	32.0	10″	163.7	165.9
10	117.7	118.5	11″	102.7	103.7
11	134.3	136.7	12″	164.2	166.8
12	17.4	25.9	13″	107.3	107.6
13	25.4	19.1	14″	131.4	129.0
1′	119.8	122.2	15″	119.8	121.3
2′	159.6	161.1	16″	155.3	156.4
3′	97.6	99.4	17″	102.2	103.5
4′	159.4	161.1	18″	155.7	157.7
5′	108.0	109.7	19″	105.7	108.7
6′	124.2	125.6	20″	128.0	134.7

[a] DMSO-d_6, 125 MHz, $t = 80$°C.
[b] Acetone-d_6, 100 MHz, $t = 24$°C.

1505, 1450 cm^{-1}. CD ($c = 0.161$, MeOH) $\Delta\varepsilon$ (λ, nm): -6.08 (239), $+8.98$ (315). ^{1}H NMR of **3a** (DMSO-d_6, 300 MHz, 70°C): δ_H 12.64 (1H, s, OH-10″), 7.19 (1H, d, $J = 8.4$ Hz, H-6′), 6.37 (1H, dd, $J = 8.4, 2.4$ Hz, H-5′), 6.21 (1H, d, $J = 2.4$ Hz, H-3′), 5.64 (1H, s, H-8), 5.20–5.30 (1H, m, H-10), 2.93 (1H, dd, $J = 14.4$, 7.8 Hz, H-9), 2.64 (1H, dd, $J = 14.4$, 5.7 Hz, H-9), 1.58 (3H, s, H-12), 1.68 (3H, s, H-13), 7.97 (1H, d, $J = 8.4$ Hz, H-14″), 6.34 (1H, dd, $J = 8.4$, 2.1 Hz, H-13″), 6.13 (1H, d, $J = 2.1$ Hz, H-11″), 6.69 (1H, d, $J = 8.4$ Hz, H-20″), 5.97 (1H, dd, $J = 8.4$, 2.4 Hz, H-19″), 6.14 (1H, d, $J = 2.4$ Hz, H-17″), 4.42 (1H, d, $J = 10.8$ Hz, H-4″), 3.47 (1H, s, H-3″), 3.20–3.40 (1H, m, H-5″), 2.28 (1H, dd, $J = 12.3$, 2.0 Hz, H-2″), 1.60–1.70 (1H, overlapped, H-2″), 2.05–2.14 (1H, m, H-6″), 1.75–1.90 (1H, m, H-6″), and 1.37 (3H, s, H-7″). ESI-MS: m/z 707.3 $[M-H]^-$.

3.4 Anti-oxidation bioassays

The anti-oxidation activities of **1**, **2**, and Vit E were determined by the content of MDA, a compound that is produced during microsomal lipid peroxidation induced by ferrous cysteine. MDA was detected by using the thiobarbituric acid (TBA) method. Briefly, 1 mg/ml microsomal protein, different concentrations of test compound or vehicle and 0.2 mM cysteine in 0.1 M PBS were incubated for 15 min at 37°C, 0.5 mM ferrous ion was added, mixed, and the whole incubated for 15 min at 37°C again. An equal volume of 20% TCA was added to determine the reaction and the mixture was centrifuged for 10 min at 3000 rpm. The supernatants were reacted with 0.67% TBA for 10 min at 100°C. After cooling, the MDA was quantified by determining the absorbance at 532 nm, and then the inhibition rate was calculated.

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