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Pongamone A–E, five flavonoids from the stems of a mangrove plant, *Pongamia pinnata*

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Abstract

Chemical investigation of stems of the mangrove plant, *Pongamia pinnata*, resulted in isolation and characterization of five structurally unusual flavonoids pongamones A–E, along with 16 known flavonoid metabolites. Their structures were determined on the basis of spectroscopic analyses and by comparison of their spectroscopic data with those of related compounds reported in the literature. Pongamones A–E were assayed against DHBV RCs DNAP and HIV-1 RT in vitro. A possible biogenetic pathway of the isolated compounds is also proposed.

Keywords: Mangrove plant; *Pongamia pinnata*; Leguminosae; Flavonoids; Pongamones A–E; Structure elucidation

1. Introduction

The mangrove plant *Pongamia pinnata* (Leguminosae.) is a fast growing glabrous, deciduous tree that is widely distributed along Southeast Asia to the sandy coast of the Pacific Ocean; it is also found in limestone shrub forests (Polhill and Raven, 1981). Different parts of the plant have been traditionally used for the treatment of tumors, piles, skin disease, wounds, ulcers, etc in Southeast Asia (Parmar et al., 1976). Previous phytochemical investigation of this plant growing in India and Japan showed a remarkable diversity of flavonoid metabolites involving chalcones (Tanaka et al., 1991), furanoflavonoids (Pavanaram and Row, 1955; Yadav et al., 2004), and pyranoflavonoids (Esperanza et al., 2003, 2004; Kitagawa et al., 1992; Tanaka et al., 1992). Further phytochemical data were reported on *P. glabra* which is a synonym for *P. pinnata* (Zhuang et al., 1997; Wei, 1994). However, the secondary metabolites of *P. pinnata* growing at the coastline of Southern China were rarely investigated (Yin et al., 2005). In the course of a systematic investigation of the metabolic diversity of Chinese mangrove plants, the stems of *P. pinnata* were collected from a mangrove forest on Hainan Island, South China. An examination on the ethanol extract resulted in isolation and characterization of 21 flavonoid derivatives, of which five were new. For the new natural products, the names pongamone A–E (**1**–**5**) are proposed. Additionally 16 known compounds were identified which included 5-hydroxy-4′-methoxy-7-[(3-methyl-2-buthenyl)oxy]-isoflavone (Jain et al., 1970), ovalichromene-B (Gupta and Krishnamurti, 1976), pongachin (Subramanian et al., 1992), ponganone III (Tanaka et al., 1992), 5-hydroxyfurano[7,6:4″,5″]flavone (Talapatra et al., 1980), pongaglabol (Ahmad et al., 1999), karanjin (Talapatra et al., 1980), pongapin (Siddiqui and Zaman, 1998), lancheolatin B (Talapatra et al., 1980), 5′-methoxypongapin (Siddiqui and Zaman, 1998), karanjachromene (Mahmoud and Waterman, 1985), pongachromene (Mukerjee et al.,

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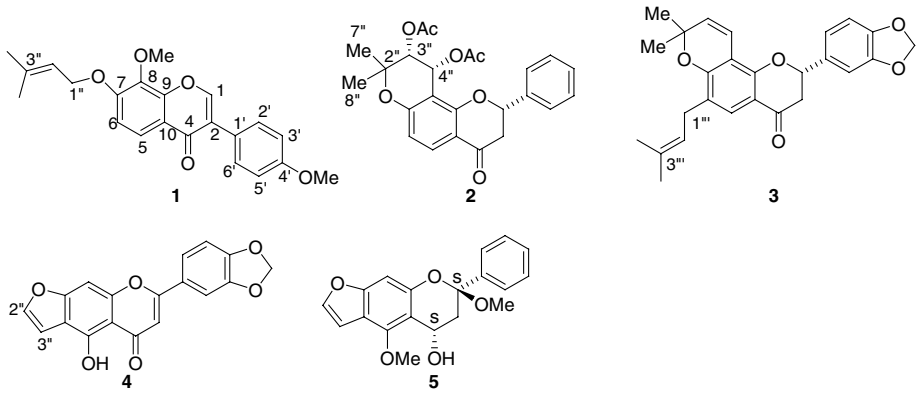

1969), 3′,4′-methylenedioxy- (4″,5″:7,8)-furanoflavanone (Magalhaes et al., 2000), glabrachromene II (Gupta and Krishnamurti, 1976), glabrachromene (Saha et al., 1991), and luteolin. The compounds isolated during this study can be classified as isoflavones, pyranoflavonoids, furanoflavonoids, chalcones, and flavones. With the exception of ovalichromene-B which had been isolated from *Milletia ovalifolia* all other known metabolites had previously been reported for *P. pinnata* growing either in India or Japan.

2. Results and discussion

The petroleum ether part of the EtOH extract was subjected to chromatography on silica gel followed by separation on Sephadex LH-20, thereby yielding a total of 21 compounds including five new natural products (1–5). The structures of the new compounds were elucidated on the basis of UV, IR, CD, MS and 2D NMR spectroscopic analyses, and by comparison of their spectroscopic data with those reported in the literature.

The molecular formula of pongamone A (**1**) was determined as $C_{22}H_{22}O_5$ by positive HRFABMS (*m/z* 367.1537 $[M+1]^+$, calcd. 367.1539). The UV absorptions at 257 and 305 nm, in association with NMR spectroscopic data analyses, were indicative of the presence of an isoflavone nucleus. The ^{1}H spectrum of **1** showing the aromatic protons at δ_H 7.52 (2H, *d*, $J = 8.5$ Hz, H-2′,6′), 7.00 (2H, *d*, $J = 8.5$ Hz, H-3′,5′) was attributed to a *para*-substituted ring B, and an AB spin system at δ_H 8.03 (1H, *d*, $J = 9.0$ Hz) and 7.07 (1H, *d*, $J = 9.0$ Hz) was in accordance with H-5 and H-6 of ring A, which implied C-7 and C-8 to be substituted. The singlet at δ_H 8.02 (1H, *s*) was characteristic of H-2 of an isoflavone. Furthermore, compound **1** possessed two methoxyl groups at δ_H 4.03 (3H, *s*) and 3.87 (3H, *s*). These ^{1}H NMR signals were in correspondence with the data of 7-hydroxy-4′,8-dimethoxyisoflavone (Lu et al., 2004). The presence of a γ,γ-dimethylallyloxyl unit was indicated by the resonances at δ_H 1.84 (3H, *s*, H-5″), 1.81 (3H, *s*, H-4″), 5.55 (1H, *t*, $J = 7.5$ Hz, H-2″), and 4.75 (2H, *d*, $J = 7.5$ Hz, H-1″) in the ^{1}H NMR spectrum, as well as the respective carbon signals in the HMQC spectrum. The HMBC correlation between H_2-1″ and δ_C 157.0 (*s*, C-7) and the NOE correlation between H_2-1″ and H-6 in NOESY spectrum allowed the assignment of the γ,γ-dimethylallyloxyl subunit at C-7. The remaining two methoxyl groups were thus assigned to positions C-8 and C-4′, respectively. Accordingly, the structure of **1** was determined as 7-(γ,γ-dimethylallyloxy)-8,4′-dimethoxy-isoflavone.

The molecular formula of pongamone B (**2**) was established as $C_{24}H_{24}O_7$ based on positive HRFABMS (*m/z* 425.1588 $[M+1]^+$, calcd. $C_{24}H_{25}O_7$, 425.1594), indicating 13 degrees of unsaturation. In the ^{1}H NMR spectrum, an aliphatic ABX coupling system at δ_H 5.51 (1H, *dd*, $J = 3.0$, 10.5 Hz, H-2), 3.06 (1H, *dd*, $J = 10.5$, 17.0 Hz, H-3a), and 2.90 (1H, *dd*, $J = 3.0$, 17.0 Hz, H-3b), and the corresponding carbons at δ_C 79.9 (*d*, C-2) and 43.8 (*t*, C-3), as well as the signal of a conjugated ketone carbon at δ_C 190.1 (*s*, C-4) and 12 aromatic carbons (Table 2) were indicative of a flavanone skeleton. The ^{1}H NMR spectrum displayed seven aromatic protons, of which an AB spin system at δ_H 7.90 (1H, *d*, $J = 8.5$ Hz) and 6.60 (1H, *d*, $J = 8.5$ Hz) was assigned to H-5 and H-6. The signals at δ_H 7.38–7.43 (5H, *m*, H-2′-H6′) originated from the unsubstituted ring B. Besides, two vicinally coupled oxygenated methine doublets at δ_H 6.36 (1H, *d*, $J = 4.5$ Hz, H-4″) and 5.22 (1H, *d*, $J = 4.5$ Hz, H-3″), four methyls at δ_H 2.09 (3H, *s*), 1.99 (3H, *s*), 1.48 (3H, *s*, H-8″), and 1.44 (3H, *s*, H-7″) were observed in the ^{1}H NMR spectrum. The methyl signals at δ_H 2.09 (3H, *s*) and 1.99 (3H, *s*) were assigned to two acetyl groups based on the HMBC correlations with carbonyl carbons at δ_C 169.8 (*s*) and 169.9 (*s*), respectively. Detailed 2D NMR analyses and comparison of the NMR spectroscopic data with those of 5-methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavanone isolated from the same plant (Esperanza et al., 2003, 2004), suggested that both compounds shared the same substructure of the 3″,4″-dihydro-3″,4″-diacetoxy-2″,2″-dimethylpyrano ring, in which H-3″ and H-4″ have β-configurations. The absolute stereochemistry at C-2 was assumed to be *S* according to a positive CE at 331

(+3.02) nm for n → π* absorption band and a negative CE at 286 (−4.63) for π → π* absorption band in the CD spectrum (Slade et al., 2005). Consequently, the structure of **2** was determined as (2*S*) 3″,4″-dihydro-4″,5″-diacetoxy-6″-dimethylpyrano-[5″,6″:7,8] flavanone.

The molecular formula $C_{26}H_{26}O_5$ of pongamone C (**3**) was established on the basis of positive HRFABMS (*m/z* 419.1855, calcd. 419.1853) and NMR spectroscopic data. The ^{1}H and ^{13}C NMR spectroscopic data were very similar to those of ovalichromene-B, indicative of the presence of a pyranoflavanone nucleus. The ^{1}H resonances at δ_H 5.38 (1H, *dd*, $J = 3.0$, 13.0, H-2), 3.00 (1H, *dd*, $J = 13.0$, 16.5 Hz, H-3a), 2.79 (1H, *dd*, $J = 3.0$, 16.5 Hz, H-3b), and the corresponding carbons at δ_C 77.6 (*d*, C-2) and 44.5 (*t*, C-3), as well as a conjugated ketone at δ_C 191.0 (*s*, C-4) were attributed to ring C. An ABX spin system at δ_H 7.01 (1H, *d*, $J = 1.5$ Hz, H-2′), 6.92 (1H, *dd*, $J = 8.0$, 1.5 Hz, H-6′), 6.86 (1H, *d*, $J = 8.0$ Hz, H-5′) was assigned to a trisubstituted ring B. A methylenedioxy group [δ_H 6.03 (2H, *s*) and δ_C 101.5 (*t*)] was attached to C-3′ and C-4′ on the basis of HMBC correlations from the methylenedioxy protons and from H-5′ and H-2′ to the carbons overlapping at δ_C 148.0 (*s*, C-3′, C-4′). Two olefinic protons δ_H 6.65 (1H, *d*, $J = 10.0$ Hz, H-4″) and 5.59 (1H, *d*, $J = 10.0$ Hz, H-3″) and two methyl groups at δ_H 1.46 (*s*, H-7″) and 1.49 (*s*, H-8″) were indicative of a dimethylpyrano ring. Moreover, an aromatic proton at δ_H 7.62 (1H, *s*) showing HMBC correlation with the ketone carbon C-4 was attributed to H-5. The remaining signals at δ_H 5.26 (1H, *t*, $J = 7.5$ Hz, H-2‴), 3.38 (2H, *d*, $J = 7.5$ Hz, H-1‴), 1.75 (6H, *s*, H-4‴, H-5‴) and their corresponding carbons (Table 2) were due to a γ,γ-dimethylallyl moiety. The presence of these subunits was further supported by HMQC, HMBC, and DQFCOSY analyses. Since H-5 showed a long range COSY correlation with the methylene protons H-1‴ and HMBC correlation with δ_C 28.0 (*t*, C-1‴), the γ,γ-dimethylallyl moiety had to be positioned at C-6. Further HMBC correlations from H-5, H-4″ and H_2-1‴ to δ_C 160 (*s*, C-7) supported the assignment of the oxygen atom of the pyrano ring at C-7. The *J* values (13.1, 3.0 Hz) of H-2 and optical rotation of **3** were both similar to those of ovalichromene-B, indicating a C-2 equatorial aryl group. The CD absorptions at 345 (+2.15) nm (n → π* transition) and 307 (−6.68) μm (π → π* transition) were in agreement with those of compound **2** having a *S* configuration at C-2. The structure of **3** was thus assigned as (2*S*)-3′,4′-methylenedioxy-6-γ,γ-dimethylallylpyrano[5″,6″:7,8]-flavanone.

The molecular formula of pongamone D (**4**) was identified as $C_{18}H_{10}O_6$ on the basis of EIMS (*m/z* 322 $[M]^+$), and NMR spectroscopic data and by HREIMS (*m/z* 322.0475, calcd. 322.0477), indicating 14 degrees of unsaturation. The UV absorptions at 260 and 335 nm are characteristic of a flavonoid nucleus (Yadav et al., 2004). The ^{1}H NMR spectrum displayed signals for a hydroxyl proton at δ_H 13.64 (*s*, OH-5), an ABX spin system at δ_H 7.40 (1H, *d*, $J = 1.8$ Hz, H-2′), 6.98 (1H, *d*, $J = 8.5$ Hz, H-5′) and 7.54 (1H, *dd*, $J = 8.5$, 1.8 Hz, H-6′) for ring B, two aromatic singlets at δ_H 6.62 (1H, *s*, H-3) and 7.14 (*d*, $J = 0.8$ Hz, H-8), a methylenedioxy group at δ_H 6.03 (2H, *s*), and two olefinic protons at δ_H 7.04 (1H, *dd*, $J = 0.8$, 2.0 Hz, H-3″) and 7.62 (1H, *d*, $J = 2.0$ Hz, H-2″) for a disubstituted furan ring. These data were further supported through HMBC correlations. The methylenedioxy unit located at C-3′/C-4′ of ring B was established by HMBC correlations from H-2′, H-5′, and the methylenedioxy protons to carbons overlapping at δ_C 150.0 (*s*, C-3′, C-4′). The furan ring was fused at C-6/C-7, rather than at C-7/C-8, as deduced from the HMBC correlation between H-3″ and C-5 (δ_C 155.5, *s*), as well as comparable NMR spectroscopic data between **4** and 5-hydroxyfurano(6,7:4″,5″)flavone in ring A (Talapatra et al., 1980). The small *J* value between H-3″ and H-8 was due to a long range COSY correlation, which occurs also in related known compounds (Talapatra et al., 1980) thereby supporting the position of the ring fusion. Thus, the structure of **4** was determined to be 5-hydroxy-3′,4′-dioxymethylenefurano [4″,5″:6,7] flavone.

Pongamone E (**5**) was identified, based on its molecular formula $C_{19}H_{18}O_5$, as determined by EIMS (*m/z* 326 $[M]^+$), and analyses of ^{1}H and ^{13}C NMR (DEPT) spectroscopic data. The ^{1}H NMR spectrum displayed signals for an unsubstituted aromatic B ring at δ_H 7.66 (2H, *d*, $J = 7.0$ Hz, H-2′, 6′), 7.47 (2H, *t*, $J = 7.0$ Hz, H-3′, 5′), and 7.42 (1H, *t*, $J = 7.0$ Hz, H-4′), a disubstituted furan ring at δ_H 6.93 (1H, *dd*, $J = 0.6$, 2.3 Hz, H-3″) and 7.51 (1H, *d*, $J = 2.3$ Hz, H-2″), an aromatic singlet at δ_H 6.94 (1H, *d*, $J = 0.6$ Hz, H-8), an ABX spin system at δ_H 2.13 (1H, *dd*, $J = 10.1$, 13.6 Hz, H-3a), 2.75 (1H, *dd*, $J = 7.4$, 13.6 Hz, H-3b), and 5.47 (*dd*, $J = 7.4$, 10.1 Hz, H-4), and two methoxyl groups at δ_H 3.04 (3H, *s*) and 4.27 (3H, *s*). The ^{1}H and ^{13}C NMR spectroscopic data of **5** (Table 2) were similar to those of 2,5-dimethoxy-4-hydroxy-[4″,5″:6,7]-furanoflavan, a unusual flavan-4-ol with a methoxyl group at C-2 (Boonchoo et al., 2002). The HMBC, HMQC and DQFCOSY correlations indicated that both compounds shared the same gross structure. However, the *J* values of H-4 (1.3, 4.5 Hz) in the latter differed from those of H-4 in **5** (7.4, 10.1 Hz) with regard to the stereochemical orientation. The weak NOE correlation observed between H-4 and OMe-2 (δ_H 3.04, *s*) (Fig. 1) indicated a *cis*-orientation. This evidence implied the OH-4 group in **5** to be in an α-configuration when ring C adopts a semi-chair conformation. An additional NOE correlation between H-6′ (or H-2′) and the geminal protons H_2-3 suggested ring B to be in α-orientation. The CD spectrum (Fig. 2) showing CE at 229 (−1.17), 257 (−1.96), and 299

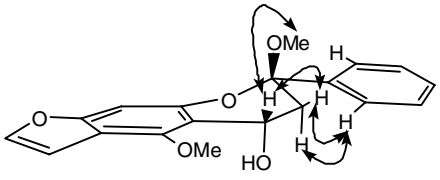

Fig. 1. Main NOE correlations of **5**.

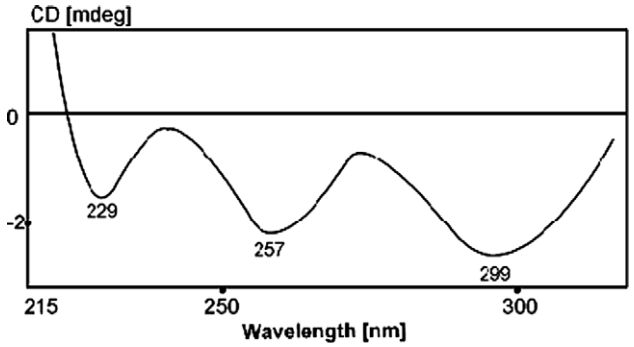

Fig. 2. CD spectrum of **5**.

(−2.46) was consistent with a *S* configuration at C-4 (Slade et al., 2005; Snatzke and Znatzke, 1973), which likewise implied a *S* configuration for C-2. Accordingly, the structure of **5** was determined as (2*S*,4*S*) 2,5-dimethoxy-4-hydroxy-[4″,5″:6,7]-furanoflavan.

Pongamones A–E were assayed against DHBV RCs DNAP and HIV-1 RT in vitro, but showed IC_{50} more than 10 μg/ml.

In the presumed biogenetic pathway to the isolated compounds, it is considered that a chalcone is a precursor of the flavan and isoflavan compounds through ring C formation and via ring B migration (Barron and Ibrahim, 1996; Crombie and Whiting, 1998; Maurya and Yadav, 2005). Flavone and isoflavones are obviously obtained by dehydrogenation of flavan and isoflavan precursors, respectively. Pyrano rings in positions 6/7 or 7/8 are generated from the introduction of a C-prenyl unit at C-6 or C-8 and then via cyclization with *ortho*-phenolic hydroxyl group at C-7 (Crombie and Whiting, 1998), while furano rings are followed by the same step as mentioned above but removing an isopropyl unit in the following step (Barron and Ibrahim, 1996). O-prenylated flavonoids like **1** and 5-hydroxy-4′-methoxy-7-[(3-methyl-2-butenyl)oxy]-isoflavone are uncommon in nature.

3. Concluding remarks

From our investigation and the data from the literature, it is noted that all parts of this plant are rich in phenylpropanoid derivatives. Whereas the roots mainly contain chalcones, the stem bark affords various types of flavonoids including pyranoflavonoids, furanoflavonoids, simple flavanes and flavones, while the seeds and flowers are rich in furanoflavones. It is furthermore of interest to note that different collections of this plant from India, Japan or China are largely similar with regard to flavonoid derivatives indicating a remarkable chemical conformity.

Table 1
^{1}H NMR spectroscopic data of pongamone A–E (**1**–**5**)[a]

Position	1	2	3	4	5
2	8.02 *s*	5.51 *dd* (10.5, 3.0)	5.38 *dd* (3.0, 13.0)		
3		3.06 *dd* (10.5, 17.0)	3.00 *dd* (13.0, 16.5)	6.62 *s*	2.13 *dd* (10.1, 13.6)
		2.90 *dd* (3.0, 17.0)	2.79 *dd* (3.0, 16.5)		2.75 *dd* (7.4, 13.6)
4					5.47 *dd* (7.4, 10.1)
5	8.03 *d* (9.0)	7.90 *d* (8.5)	7.62 *s*		
6	7.07 *d* (9.0)	6.60 *d* (8.5)			
8				7.14 *d* (0.8)	6.94 *d* (0.6)
2′	7.52 *d* (8.5)	7.48 *m*	7.01 *d* (1.5)	7.40 *d* (1.8)	7.66 *d* (7.0)
3′	7.00 *d* (8.5)	7.43 *m*			7.47 *t* (7.0)
4′		7.38 *m*			7.42 *t* (7.0)
5′	7.00 *d* (8.5)	7.43 *m*	6.86 *d* (8.0)	6.98 *d* (8.5)	7.47 *t* (7.0)
6′	7.52 *d* (8.5)	7.48 *m*	6.92 *dd* (1.5, 8.0)	7.54 *dd* (1.8, 8.5)	7.66 *d* (7.0)
1″	4.75 *d* (7.5)				
2″	5.55 *t* (7.5)			7.62 *d* (2.0)	7.51 *d* (2.3)
3″		5.22 *d* (4.5)	5.59 *d* (10.0)	7.04 *dd* (0.8, 2.0)	6.93 *dd* (0.6, 2.3)
4″	1.81 *s*	6.36 *d* (4.5)	6.65 *d* (10.0)		
5″	1.84 *s*				
7″		1.44 *s*	1.46 *s*		
8″		1.48 *s*	1.49 *s*		
1‴			3.38 *d* (7.5)		
2‴			5.26 *t* (7.5)		
4‴			1.75 *s*		
5‴			1.75 *s*		
5-OH				13.64 *s*	
OCH_2O			6.03 *s*	6.03 *s*	
OCH_3	4.03 *s*				3.04 *s*
	3.87 *s*				4.27 *s*
OAc		1.99 *s*			
		2.09 *s*			

[a] Measured in $CDCl_3$, coupling constants (*J* in Hz).

4. Experimental

4.1. General

Optional rotations were measured on a JASCO DIP-370 polarimeter. The IR spectra were recorded on a Perkin–Elmer Nicol FTIR NEXUS-470 spectrometer. The CD spectrum was measured on a JASCO J-810 spectropolarimeter. The ^{1}H and ^{13}C NMR, as well as 2D, spectra were taken on a Bruker Avance DRX 500 NMR spectrometer using TMS as internal standard. EIMS was performed on a Bruker APEXII mass spectrometer, while HRFABMS spectra were obtained on a VG Atospec spectrometer and HREIMS spectrum was measured on a GCT-MS Micromass spectrometer. Column chromatography was carried out on Merck silica gel (200–400 mesh), and the HF_{254} silica gel for TLC was provided by Sigma Co. Ltd. Sephadex LH-20 (18–110 cm) was obtained from Pharmacia Co. High pressure liquid chromatography (HPLC) was performed on an Alltech-426 apparatus using a Kromasil prepacked column (ODS, 10 × 250 mm, for reversed phase) and monitored by UV detection (254 nm).

4.2. Plant material

The stem of *P. pinnata* was collected from Hainan Island, PR China, in 2003, and identified by Professor Peng Lin of Xiamen University. The specimen (HNMP-25) was deposited in The State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

4.3. Extraction and isolation

Air dried and powdered stems of *P. pinnata* (10 kg) were extracted with EtOH–H_2O (95:5) at r.t. (25°C) for 48 h. The EtOH extract (360 g) was then partitioned between H_2O and petroleum ether, EtOAc, and *n*-BuOH successively. The petroleum ether fraction (60 g) was subjected to CC silica gel (200–300 mesh) and eluted with a gradient of petroleum ether–EtOAc (1:0–1:1) to afford eight fractions (F1–F8) monitored by TLC. F3 (1.2 g) was submitted to silica gel CC eluted with petroleum ether–EtOAc (9:1) to afford **1** (5.0 mg), **2** (10.0 mg), **4** (1.0 mg), **5** (25.0 mg), 5-hydroxy-4′-methoxy-7-[(3-methyl-2-butenyl) oxy]-isoflavone (8.0 mg), pongachin (8.0 mg), ponganone III (15.0 mg), 5-hydroxyfurano[7,6:4″,5″] flavone (4.0 mg), pongaglabol (7.5 mg), karanjin (20.0 mg), lancheolatin B (15.0 mg), 5′-methoxypongapin (11.0 mg), glabrachromene II (5.0 mg), glabrachromene (10.0 mg), and luteolin (30.0 mg). F6 (800 mg) was subjected, in the same manner as F3, to silica gel CC eluting with petroleum ether–EtOAc (5:1) to yield two portions P-1 (18.0 mg) and P-2 (9.0 mg), and each was analysed by ^{1}H NMR spectroscopic analyses. Pongapin (10.0 mg) and karanjachromene (5.0 mg) were separated from P-1 on Sephadex LH-20 by eluting with MeOH, while pongachromene (6.0 mg) and 4′-methylenedioxy-(4″,5″:7,8)-furanoflavanone (1.2 mg) were obtained from P-2 in the same manner as P-1. F5 (35 mg) showing one spot on TLC was separated by HPLC using MeOH–H_2O (80:20) as mobile phase at a flow rate of 1.50 ml/min to obtain 3 (1.5 mg) and ovalichromene-B (4.5 mg).

4.3.1. Pongamone A (*1*)

Amorphous powder; UV (MeOH) λ_{max} nm: 257, 305. IR ν_{max} cm^{-1}: 3029, 1642, 1603, 1566, 1510, 1449, 1426, 1374, 1281, 1178, 1054. EIMS *m/z* 366 $[M]^+$, 312, 298, 297, 283, 255, 252, 240, 213, 166, 149, 138, 132, 117. HRFABMS *m/z* 367.1537 (calcd. for $C_{22}H_{23}O_5$, 367.1539). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. Pongamone B (*2*)

Amorphous powder; $[\alpha]_D^{20}$ − 37.0 (*c* 0.08, $CHCl_3$). UV (MeOH) λ_{max} nm: 270, 315. IR ν_{max} cm^{-1}: 3066, 1753, 1687, 1604, 1443, 1373, 1317, 1238, 1148, 1102, 1040, 905. CE (acetonitrile) nm: 286 (−4.64), 331 (+3.02). EIMS *m/z* 424 $[M]^+$, 364, 349, 307, 289, 277, 240, 217, 203, 187, 165, 149, 131. HRFABMS *m/z* 425.1588 (calcd. for $C_{24}H_{25}O_7$, 425.1594). For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

Table 2
^{13}C NMR spectroscopic data of pongamone A–E (**1**–**5**)[a]

Position	1	2	3	4	5
2	152.2 *d*	79.9 *d*	77.6 *d*	162.0 *s*	102.5 *s*
3	124.0 *s*	43.8 *t*	44.5 *t*	103.0 *d*	42.1 *t*
4	176.0 *s*	190.1 *s*	191.0 *s*	184.2 *s*	62.3 *d*
5	121.5 *d*	138.4 *d*	125.0 *d*	155.5 *s*	151.0 *s*
6	111.6 *d*	112.0 *d*	117.0 *s*	113.5 *s*	111.0 *s*
7	157.0 *s*	159.9 *s*	156.8 *s*	152.0 *s*	157.0 *s*
8	137.0 *s*	107.0 *s*	115.0 *s*	91.0 *d*	94.7 *d*
9	151.0 *s*	161.7 *s*	157.0 *s*	160.0 *s*	150.0 *s*
10	119.0 *s*	114.0 *s*	115.6 *s*	108.0 *s*	112.0 *s*
1′	124.0 *s*	138.4 *s*	131.3 *s*	121.0 *s*	140.4 *s*
2′	130.2 *d*	125.8 *d*	107.0 *d*	107.0 *d*	126.2 *d*
3′	114.0 *d*	128.7 *d*	148.0 *s*	150.0*s*	128.3 *d*
4′	160.0 *s*	128.7 *d*	148.0 *s*	150.0 *s*	128.4 *d*
5′	114.0 *d*	128.7 *d*	108.9 *d*	109.0 *d*	128.3 *d*
6′	130.2 *d*	125.8 *d*	120.0 *d*	122.0 *d*	126.2 *d*
1″	66.3 *t*				
2″	119.1 *d*	77.3 *s*	77.5 *s*	145.0 *d*	143.1 *d*
3″	139.0 *s*	71.0 *d*	129.0 *d*	104.0 *d*	104.7 *d*
4″	25.80 *q*	61.2 *d*	115.0 *d*		
5″	18.3 *q*				
7″		21.8 *q*	28.4 *q*		
8″		25.8 *q*	28.4 *q*		
1‴			28.0 *t*		
2‴			121.0 *d*		
3‴			132.0 *s*		
4‴			27.0 *q*		
5‴			17.8 *q*		
OCH_2O			101.5 *t*	102.0 *t*	
2-OCH_3					50.4 *q*
5-OCH_3					59.7 *q*
8-OCH_3	61.5 *q*				
4′-OCH_3	56.4 *q*				
OAc		20.5 *q*; 169.8 *s*			
		20.7 *q*; 169.9 *s*			

[a] Measured in $CDCl_3$.

4.3.3. Pongamone C (**3**)

Pale yellow amorphous powder; $[\alpha]_D^{20}$ − 55.0 (*c* 0.05, $CHCl_3$). UV (MeOH) λ_{max} nm: 234, 260, 340. IR ν_{max} cm^{-1}: 3075, 1710, 1673, 1623, 1465, 1413, 1378, 1196, 1094, 1066, 999, 903. CE (acetonitrile) nm: 307 (−6.68), 345 (+2.15). EIMS *m/z*: 418 $[M]^+$, 403, 377, 363, 255, 215, 148, 147, 115. HRFABMS *m/z* 419.1855 (calcd. for $C_{26}H_{27}O_5$, 419.1853). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. Pongamone D (**4**)

Amorphous yellow powder, UV (MeOH) λ_{max} nm: 260, 335. IR ν_{max} cm^{-1}: 3165, 1641, 1572, 1524, 1448, 1416, 1377, 1267, 1160, 1043, 807. EIMS *m/z*: 322 $[M]^+$, 225, 223, 222, 194, 176, 167, 149, 115, 89. HREIMS *m/z* 322.0475 (calcd. for $C_{18}H_{10}O_6$, 322.0477). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. Pongamone E (**5**)

Colorless powder; $[\alpha]_D^{20}$ − 23.0 (*c* 0.1, $CHCl_3$). UV (MeOH) λ_{max} nm: 230, 250, 280. IR ν_{max} cm^{-1}: 3062, 1662, 1626, 1594, 1469, 1440, 1349, 1275, 1123, 1082, 1043. CE (acetonitrile) nm: 229 (−1.17), 257 (−1.96), 299 (−2.46). EIMS *m/z*: 326 $[M]^+$, 308, 293, 278, 277, 262, 231, 192, 174, 149, 146, 133, 105. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4. Bioassay

The bioassay for anti-DHBV RCs DNAP and anti-HIV-1 RT in vitro was performed by the method described previously (Tao et al., 1998; Wang et al., 2004).

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