Notes

Flavonoids and Benzene Derivatives from the Flowers and Fruit of *Tetrapanax papyriferus*

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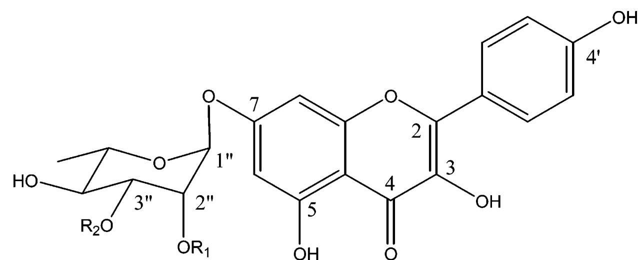
Two new flavonoids, kaempferol 7-*O*-(2-*E*-*p*-coumaroyl-α-L-rhamnoside) (**1**) and kaempferol 7-*O*-(2,3-di-*E*-*p*-coumaroyl-α-L-rhamnoside) (**2**), together with 10 known compounds were isolated from the flowers and fruit of *Tetrapanax papyriferus.* Compounds **1** and **2** showed cytotoxicity by brine shrimp lethality bioassay with LC_{50} values of 0.57 and 0.40 mM, respectively.

Tetrapanax papyriferus (Hook) K. Koch (Araliaceae) is a shrub that is widely distributed throughout the hills of Taiwan and southern China.[1] Since ancient times, in Asia, the pith of *Tetrapanax papyriferus* has been dried, peeled, and pressed into "rice paper". *T. papyriferus* is used as a traditional medicine in China to treat inflammation and dysentery.[2] Studies have shown that CH_2Cl_2 and MeOH extracts of *T. papyriferus* exhibit antithrombin activity.[3]

Triterpenoids and saponins have been isolated from the leaves and roots of *T. papyriferus*.[4,5] Our previous work reported the structure, cytotoxicity, and anti-HIV activity of oleanane-type triterpenes from the leaves, pith, flowers, and fruit of this plant.[6] This study discusses the isolation and structure of two new flavonoids, kaempferol 7-*O*-(2-*E*-*p*-coumaroyl-α-L-rhamnoside) (**1**) and kaempferol 7-*O*-(2,3-di-*E*-*p*-coumaroyl-α-L-rhamnoside) (**2**), four known flavonoids, 3,7,4′-tri-*O*-acetylkaempferol (**3**),[7] kaempferol (**4**),[8] astragalin (**5**),[9] and afzelin (**6**),[10] and six known benzene derivatives, 1,2,3-trimethoxybenzene (**7**),[11] *trans*-cinnamic



1 R_1=*E*-*p*-coumaroyl R_2=H
2 R_1=R_2=*E*-*p*-coumaroyl

acid (**8**),[12] cinnamyl alcohol (**9**),[13] 5-formylbenzofuran (**10**),[13] coumarin (**11**),[14] and dihydrocoumarin (**12**),[14] from the flowers and fruit of *T. papyriferus*. Compounds **1**−**12** were from the flowers, and **3**, **5**, and **6** were from the fruit. The

Table 1. ^1H NMR Data for Compounds **1** and **2** (δ, ppm, in acetone-d_6)

no.	1	2
kaempferol moiety		
6	6.36, d (1.2)	6.31, d (1.2)
8	6.51, d (1.2)	6.52, d (1.2)
2′, 6′	7.86, d (8.8)	7.98, d (8.6)
3′, 5′	7.03, d (8.8)	7.10, d (8.6)
5-OH	13.06, br.s	12.55, br.s
rhamnopyranosyl moiety		
1″	5.65, d (1.2)	5.61, d (1.3)
2″	5.58, dd (3.3, 1.2)	5.92, dd (3.2, 1.3)
3″	3.95, dd (9.3, 3.3)	5.40, dd (9.7, 3.2)
4″	3.43, dd (9.4, 9.3)	3.78, dd (9.7, 9.5)
5″	3.35, dd (9.4, 6.1)	3.58, dd (9.5, 6.1)
6″	0.96, d (6.1)	1.10, d (6.1)
E-*p*-coumaroyl moiety		
2‴, 6″	7.52, d (8.5)	7.58, d (8.6)
		7.48, d (8.5)
3‴, 5″	6.87, d (8.5)	6.92, d (8.6)
		6.86, d (8.5)
7‴	7.63, d (15.6)	7.68, d (17.1)
		7.64, d (16.3)
8‴	6.34, d (15.6)	6.32, d (17.1)
		6.43, d (16.3)

cytotoxicity of compounds **1**−**3** determined by brine shrimp lethality bioassay is also reported.

The molecular formula of **1** was determined as $C_{30}H_{26}O_{12}$, using high-resolution (HR) FABMS. The IR spectrum included hydroxy (3300 cm^{-1}), conjugated ester (1695 cm^{-1}), conjugated ketone (1650 cm^{-1}), and aromatic absorptions (1600, 1500 cm^{-1}). The UV spectrum in EtOH exhibited absorptions (λ_{max}) (log ϵ) at 318 (4.46) and 297 (4.25) nm. The ^1H NMR (Table 1), $^1H-^1H$ COSY, ^{13}C NMR (Table 2), and HMQC spectra exhibited characteristic signals of flavonol, sugar, and *p*-coumaroyl moieties.

The ^1H NMR and $^1H-^1H$ COSY spectra showed a hydrogen-bonded hydroxyl group at δ 13.06 (1H, br s, 5-OH), A_2M_2-type proton signals at δ 7.86 (2H, d, J = 8.8 Hz, H-2′, 6′) and 7.03 (2H, d, J = 8.8 Hz, H-3′, 5′), and a pair of doublets at δ 6.36 (1H, d, J = 1.2 Hz, H-6) and 6.51

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Table 2. ^{13}C NMR Data for Compounds **1** and **2** (δ, ppm, in acetone-d_6)

no.	**1**	**2**
2	158.3	158.3
3	135.3	135.3
4	179.3	179.3
5	163.2	163.5
6	100.1	100.0
7	166.0	165.4
8	95.0	95.0
9	158.8	158.6
10	105.8	106.1
1′	122.5	122.6
2′, 6′	131.9	132.0
3′, 5′	116.8	116.7
4′	161.2	161.0
1″	100.0	100.0
2″	71.9	71.0
3″	72.9	73.0
4″	70.8	70.7
5″	72.8	72.2
6″	18.2	18.2
E-*p*-coumaroyl moiety		
1‴	127.0	127.2, 127.1
2‴, 6″	131.4	131.5, 131.3
3‴, 5″	117.0	117.1, 117.1
4‴	161.5	161.2, 161.4
7‴	146.5	147.0, 146.3
8‴	115.5	115.6, 115.0
9‴	167.3	166.8, 167.5

(1H, d, J = 1.2 Hz, H-8). These data and ^{13}C NMR suggest that the flavonol moiety was kaempferol.[8,15] Additionally, the ^1H NMR resonances at δ 5.65 (d, J = 1.2 Hz, H-1″), 5.58 (dd, J = 3.3, 1.2 Hz, H-2″), 3.95 (dd, J = 9.3, 3.3 Hz, 3″), 3.43 (dd, J = 9.4, 9.3 Hz, H-4″), 3.35 (dd, J = 9.4, 6.1 Hz, H-5″), and 0.96 (d, J = 6.1 Hz, Me-6″) suggested that the sugar moiety was rhamnose. The anomeric proton at δ 5.65 (d, J = 1.2 Hz, H-1″) indicated the α-pyranoside configuration.[16] The existence of one *E*-*p*-coumaroyl group in compound **1** was evidenced by the spin system at δ 7.63 (1H, d, J = 15.6 Hz, H-7‴), 7.52 (2H, d, J = 8.5, H-2‴, 6‴), 6.87 (2H, d, J = 8.5 Hz, H-3‴, 5‴), and 6.34 (1H, d, J = 15.6 Hz, H-8‴). Finally, the results of HMBC were used to determine the positions of the attachments. The anomeric proton at δ 5.65 (H-1″) exhibited a ^3J correlation to a carbon in kaempferol at δ 166.0 (C-7), indicating the attachment of the rhamnose unit at C-7. A ^3J interaction between H-2″ of the rhamnose (δ 5.58) and the *p*-coumaroyl carbonyl carbon (δ 167.3, C-9‴) suggested the attachment of the *p*-coumaroyl ester at C-2″ of the rhamnose moiety. These results reveal that **1** was kaempferol 7-*O*-(2-*E*-*p*-coumaroyl-α-L-rhamnoside).

The molecular formula of **2** was determined as $C_{39}H_{32}O_{14}$ using high-resolution (HR) FABMS. The IR spectrum showed hydroxy (3300 cm^{-1}), conjugated ester (1705 cm^{-1}), conjugated ketone (1660 cm^{-1}), and aromatic absorptions (1600, 1505 cm^{-1}). The UV spectrum in EtOH exhibited absorptions (λ_{max}) at (log ϵ) 314 (4.61) and 297 (4.33) nm. The ^1H NMR, $^1H-^1H$ COSY, ^{13}C NMR, and HMQC spectra displayed characteristic signals for a kaempferol, a rhamnose, and two *p*-coumaroyl moieties.

The ^1H NMR spectrum revealed a hydrogen-bonded phenolic hydroxyl resonance (δ 12.55), a pair of doublets at δ 6.52 (1H, d, J = 1.2 Hz, H-8) and 6.31 (1H, d, J = 1.2 Hz, H-6), and A_2M_2-type proton signals at δ 7.98 (2H, d, J = 8.6 Hz, H-2′, 6′) and 7.10 (2H, d, J = 8.6 Hz, H-3′, 5′). The above data revealed the presence of a kaempferol moiety in **2**. Rhamnosyl resonances appeared at δ 5.61 (1H, d, J = 1.3 Hz, H-1″), 5.92 (1H, dd, J = 3.2, 1.3 Hz, H-2″), 5.40 (1H, dd, J = 9.7, 3.2 Hz, 3″), 3.78 (1H, dd, J = 9.7, 9.5 Hz, H-4″), 3.58 (1H, dd, J = 9.5, 6.1 Hz, H-5″), and 1.10 (1H, d, J = 6.1 Hz, Me-6″). The anomeric proton at δ 5.61 (d, J = 1.3 Hz, H-1″) suggested an α-pyranoside configuration. The resonances at δ 7.68 (1H, d, J = 17.1 Hz, H-7‴), 7.64 (1H, d, J = 16.3 Hz, H-7‴a), 7.58 (2H, d, J = 8.6, H-2‴, 6‴), 7.48 (2H, d, J = 8.5, H-2‴a, 6‴a), 6.92 (2H, d, J = 8.6 Hz, H-3‴, 5‴), 6.86 (2H, d, J = 8.5 Hz, H-3‴a, 5‴a), 6.32 (1H, d, J = 17.1 Hz, H-8‴), and 6.42 (1H, d, J = 16.3 Hz, H-8‴a) suggested the existence of two *E*-*p*-coumaroyl groups in compound **2**.

Table 3. Cytotoxicity of the Compounds **1**−**3** by Brine Shrimp Lethality Bioassay

compound	LC_{50} ppm (mM)
1	327 (0.57)
2	289 (0.40)
3	911 (2.21)

The positions of the substituents were confirmed by HMBC. The anomeric proton at δ 5.61 (H-1″) showed a ^3J correlation to a carbon in kaempferol at δ 165.4 (C-7), indicating the attachment of the rhamnoside at C-7. A ^3J interaction between H-2″ (δ 5.92) and H-3″ (δ 5.40) of the rhamnosyl and *p*-coumaroyl carbonyl carbons was evident at δ 166.8 (C-9‴) and 167.4 (9‴a), respectively, suggesting the attachment of two *p*-coumaroyl esters at C-2″ and C-3″ in the rhamnosyl moiety. These results reveal that **2** was kaempferol 7-*O*-(2,3-di-*E*-*p*-coumaroyl-α-L-rhamnoside).

The biological activities of kaempferol glycosides have been reported as anti-inflammatory, antitumor, and antioxidative.[16−18] The kaempferol glycosides may contribute to the traditional anti-inflammatory property of *T. papyriferus*. The cytotoxic activity of the three flavonoids, kaempferol 7-*O*-(2-*E*-*p*-coumaroyl-α-L-rhamnoside) (**1**), kaempferol 7-*O*-(2,3-di-*E*-*p*-coumaroyl-α-L-rhamnoside) (**2**), and 3,7,4′-tri-*O*-acetylkaempferol (**3**), was evaluated using the brine shrimp lethality bioassay. After 24 h, all three exhibited cytotoxic activity. The LC_{50} values were 0.57, 0.40, and 2.21 mM, respectively (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. EIMS was recorded with a JMS-HX-100 instrument and FABMS with a JEOL LMS-SX 102 system. IR spectra were recorded on a JASCO FT-IR-110 infrared spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. All NMR, HMQC, and HMBC spectra were recorded on Bruker AM-400 NMR and Bruker 600 NMR spectrometers. Column chromatography was performed using silica gel (230−400 mesh, Merck), Sephadex LH-20 (Pharmacia Fine Chemicals), and Charcoal (Wako). Thin-layer chromatography (TLC) was conducted on precoated Kiesel gel 60 F_{254} plates (0.25 mm, Merck), and spots were located by ultraviolet illumination and by spraying with $FeCl_3$ or 10% H_2SO_4 followed by heating. MPLC was carried out on a Buchi MPLC system (pump, Buchi 688; detector, KAUER). HPLC was carried out on a Waters 1525 Binary HPLC system (RI detector, Waters 2410; UV detector, Waters 2487).

Plant Material. The fresh flowers (16.0 kg) and the fresh fruit (1.6 kg) of *Tetrapanax papyriferus* were collected from Miaoli County, Taiwan, in August 1995 and then identified by Prof. C. M. Chen. A voucher specimen was deposited at the Department of Chemical and Materials Engineering, Ta-Hwa Institute of Technology, Hsinchu of Taiwan, R.O.C.

Extraction and Isolation. The fresh flowers were extracted with hot MeOH for 6−8 h (30 L ×4) and concentrated

to give a deep brown syrup (250 g). The MeOH layer was chromatographed on a charcoal column, eluted with MeOH and CH_2Cl_2, to afford two fractions. Each fraction was concentrated to give a brown residue. The MeOH portion (150 g) was subjected to silica gel and eluted with CH_2Cl_2−MeOH mixtures of increasing polarities to obtain six fractions. Fractions 2 and 3 were purified by silica gel column chromatography (CH_2Cl_2−MeOH gradient) to obtain compounds **3** (12.3 mg), **7** (8.9 mg), **8** (12.0 mg), **9** (35.6 mg), **10** (4.5 mg), **11** (25.6 mg), and **12** (21.3 mg), successively. Fractions 4−6 were further separated by a combination of Sephadex LH-20 CC (H_2O−MeOH), MPLC (C-18, 50% H_2O−MeOH → MeOH), HPLC (C-18, 30% H_2O−MeOH → MeOH), and preparative TLC (silica gel, CH_2Cl_2−MeOH) to obtain compounds **1** (5.6 mg), **2** (6.6 mg), **4** (18.9 mg), **5** (156.6 mg), and **6** (126.8 mg).

The fresh fruit was extracted with MeOH (three times, each time 6 L) under reflux for 6−8 h and concentrated to give a deep brown syrup (65 g). This syrup was partitioned between 1:1 EtOAc−H_2O. The EtOAc layer was concentrated to give a brown residue (43 g), which was subjected to chromatography on silica gel (CH_2Cl_2−MeOH gradient), followed by MPLC (C-18, 40% H_2O−MeOH → MeOH) and HPLC (C-18, 40% H_2O−MeOH → MeOH) to give **3** (45.0 mg), **5** (6.5 mg), and **6** (9.5 mg).

Kaempferol 7-*O*-(2-*E*-*p*-coumaroyl-α-L-rhamnoside) (1): yellowish prisms (CH_2Cl_2−MeOH); mp 278−279 °C; $[\alpha]_D^{22}$ −78.5° (MeOH, *c* 1.0); HRFABMS (negative) *m/z* 577.1392 ([M − H]$^-$, calcd for $C_{30}H_{25}O_{12}$ 577.1346); FABMS (negative) *m/z* 577 [M − H]$^-$ (12), 367 (22), 286 (69), 285 (100), 284 (64), 255 (38), 25 (34); IR (KBr) ν_{max} 3400, 1695, 1650, 1600, 1500 cm^{-1}; UV λ_{max}^{EtOH} (log ϵ) 318 (4.46), 297 (4.25) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2.

Kaempferol 7-*O*-(2,3-di-*E*-*p*-coumaroyl-α-L-rhamnoside) (2): yellowish prisms (CH_2Cl_2−MeOH); mp 287−288 °C; $[\alpha]_D^{22}$ −86.7° (MeOH, *c* 1.0); HRFABMS (negative) *m/z* 723.1781 ([M − H]$^-$, calcd for $C_{39}H_{31}O_{14}$ 723.1713); FABMS (negative) *m/z* 723 [M − H]$^-$ (14), 285 (100), 255 (32), 151 (25), 25 (40); IR (KBr) ν_{max} 3300, 1705, 1660, 1600, 1505 cm^{-1}; UV λ_{max}^{EtOH} (log ϵ): 314 (4.61), 297 (4.33) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2.

Brine Shrimp Lethality Bioassay. The cytoxic effect of compounds **1**−**3** was evaluated by LC_{50} values of the brine shrimp lethality test. The compounds were dissolved in DMSO, and five graded doses, 62.5, 125, 250, 500, and 1000 μg/mL, respectively, were used for 5 mL of seawater containing 10 brine shrimp nauplius in each group. The number of survivors was counted after 24 h, and LC_{50} was determined by probit analysis described by Meyer.[19] The experiment was carried out in quadruplicate, and mean LC_{50} values were measured.

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