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Coumaronochromones and flavanones from *Euchresta formosana* roots

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Abstract

Four coumaronochromones, formosanatins A–D (**1**–**4**), and a flavanone, euchrenone a_{16} (**5**), along with four known compounds, euchretins E and G, euchrestaflavanone A, and daidzein, were isolated from the roots of *Euchresta formosana*. The structures of **1**–**5** were established by spectroscopic analyses. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Euchresta formosana*; Leguminosae; Roots; Coumaronochromones; Formosanatins A–D; Flavanone; Euchrenone a_{16}

1. Introduction

Euchresta formosana is an evergreen perennial shrub occurring in shady and humid places, and is especially distributed in Taiwan, Java, and the Philippines (Mizuno et al., 1989a,b). The roots of this plant have been used in Taiwanese folk medicine as a painkiller, particularly for throat and snake wounds (Mizuno et al., 1989a,b). Previous studies on this species indicated the presence of 17 flavonoids, including five coumaronochromones (Mizuno et al., 1989a,b, 1991), 11 flavanones, and one pterocarpan (Matsuura et al., 1995). To further understand the chemotaxonomy of this species, and to continue the search for novel agents from Leguminosae plants, the roots of *E. formosana* were chosen for a phytochemical analysis. In this paper, we report the isolation and structure determination of nine compounds, including six coumaronochromones, formosanatin A (**1**), formosanatin B (**2**), formosanatin C (**3**), formosanatin D (**4**), euchretin E (Mizuno et al., 1991), and euchretin G (Mizuno et al., 1992), and two flavanones, euchrenone a_{16} (**5**) and euchrestaflavanone A (Shirataki et al., 1981), and the isoflavone, daidzein (Murthy et al., 1986). Among them, **1**–**5** are new compounds, whereas euchrestaflavanone A and daidzein were isolated for the first time from this species. The structures of compounds **1**–**5** were established on the basis of NMR and MS spectroscopic data interpretation.

2. Results and discussion

Compound **1** was isolated as colorless needles. The HR–EIMS showed an $[M]^+$ ion at m/z 520.2103 (calc. 520.2097), corresponding to the molecular formula, $C_{30}H_{32}O_8$. The coumaronochromone skeleton was suggested by the UV spectroscopic absorptions at 262 and 350 nm and the IR absorptions at 1646 and 3446 cm^{-1} (C=O and OH) (Raju et al., 1981). The ^1H NMR spectrum (Table 1) showed the presence of a two-proton doublet at δ 3.45, a one-proton triplet at δ 5.14, and two methyl singlets at δ 1.59 and 1.78, indicating the substitution of a γ,γ-dimethylallyl group. The spectrum also exhibited the signals of two one-proton doublets at δ 5.72 and 6.65 ($J=10$), and two methyl groups at δ 1.42 (6H), suggesting the existence of a 2,2-dimethyl-2H-pyran ring moiety (Ghirtis et al., 2000). Furthermore, two two-proton multiplets at δ 1.65 and 2.69, and two equivalent three-proton singlets overlapping at δ 1.19 determined the presence of a 3-hydroxy-3-methylbutyl group (Mizuno et al., 1990).

In addition, the ^{13}C NMR and DEPT experiments (Table 2) of **1** showed 30 resonance lines consisting of six methyl groups (δ 17.6, 25.5, 27.2, 27.2, 28.9, and 28.9), three methylenes (δ 16.7, 21.9, and 41.0), four methines (δ 106.1, 115.0, 121.6, and 132.0), and 17 quaternary

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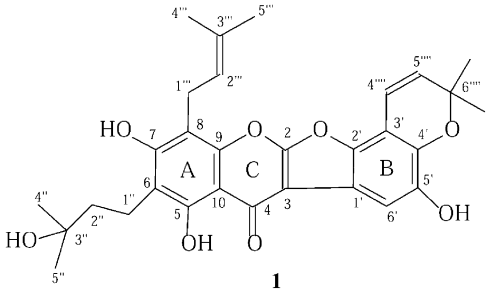

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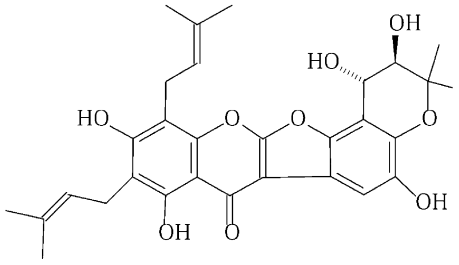

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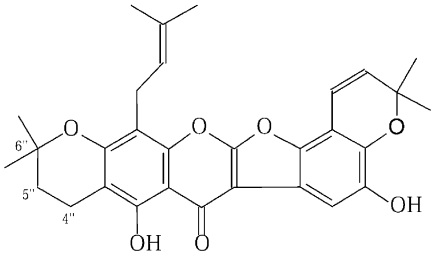

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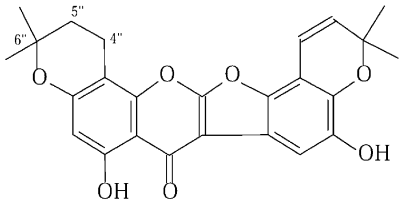

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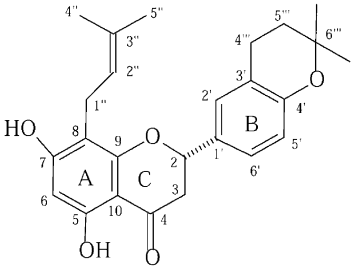

5

carbons (δ 71.0, 76.7, 97.5, 103.2, 107.0, 107.7, 113.0, 114.3, 132.4, 138.2, 138.8, 143.3, 150.3, 157.5, 158.4, 164.9, and 179.4). These spectral data suggested the presence of coumaronochromono skeleton with a γ,γ-dimethylallyl group, a 3-hydroxy-3-methylbutyl group, and a 2,2-dimethyl-2H-pyran ring moiety. According to literature, the coumaronochromones are isoflavone derivatives and the lack of a C-2 proton signal is characteristic of this skeleton (Hanawa et al., 1991a,b; Huang and Yen, 1996); this phenomenon was observed in the NMR spectrum of **1**. Significant NOE correlations between H-1″/H-2″, H-2″/H-4″, H-1‴/H-2‴, and H-2‴/H-5‴, in addition to H-4⁗/H-5⁗ and H-5⁗/Me-6⁗ (Fig. 1), confirmed the substitution pattern in **1**. In the HMBC experiments (Fig. 2), the signal at δ 2.69 (H-1″) revealed ^{2}J correlations to δ 41.0 (C-2″) and 113.0 (C-6), and ^{3}J correlations to δ 157.5 (C-5), 158.4 (C-7), and 71.0 (C-3″). In turn, the response at δ 3.45 (H-1‴) showed ^{2}J correlations to δ 121.6 (C-2‴) and 107.7 (C-8), and ^{3}J correlation to δ 158.4 (C-7), 150.3 (C-9), and 132.4 (C-3‴). It was observed that C-9 had a correlation only with H-1‴ and not with H-1″. Therefore, these correlations confirmed that the γ,γ-dimethylallyl and the 3-hydroxy-3-methylbutyl groups should be attached to C-8 and C-6, respectively. Furthermore, the signal at δ 6.65 (H-4⁗) showed ^{3}J correlations to δ 138.8 (C-2′), 138.2 (C-4′) and 76.7 (C-6⁗), while the signal at δ 5.72 (H-5⁗) displayed a ^{2}J correlation to δ 76.7 (C-6⁗), and a ^{3}J correlation to δ 107.0 (C-3″), which supported that the γ,γ-dimethyl-2H-pyrano group was fused to C-3′ and 4′. Thus, **1** was 5,7,5′-trihydroxy-6-(3-hydroxy-3-methylbutyl)-8-(γ,γ-dimethylallyl)-[6⁗,6⁗-dimethylpyrano-(2⁗,3⁗:4′,3′)]-coumaronochromone, which was named formosanatin A.

Compound **2** was isolated as yellow needles $[\alpha]_{D}^{24}$: −40°($CHCl_3$, *c* 0.05). The molecular formula of $C_{30}H_{32}O_9$ was confirmed by HR–FABMS, and the structure of **2** was established to be a coumaronochromone by comparison with the UV, IR, and NMR spectral data of **1** and euchretin A (Mizuno et al., 1989a). The ^{1}H NMR spectrum (Table 1) showed two methylene doublets at δ 3.41 and 3.49, two olefinic triplets at δ 5.17 and 5.21, and four methyl singlets at δ 1.68, 1.71, 1.79, and 1.81, that indicated the existence of two γ,γ-dimethylallyl groups. The ^{1}H NMR resonances of two methyl singlets at δ 1.25 and 1.52, and an AB spin system at δ 4.89 and 3.72, combined with the ^{13}C NMR signals of two methyl carbons at δ 25.7 and 18.9, two oxygenated methine carbons at δ 67.2 (C-4⁗) and 75.6 (C-5⁗), and one quaternary carbon at δ 79.9 (C-6⁗), suggested the

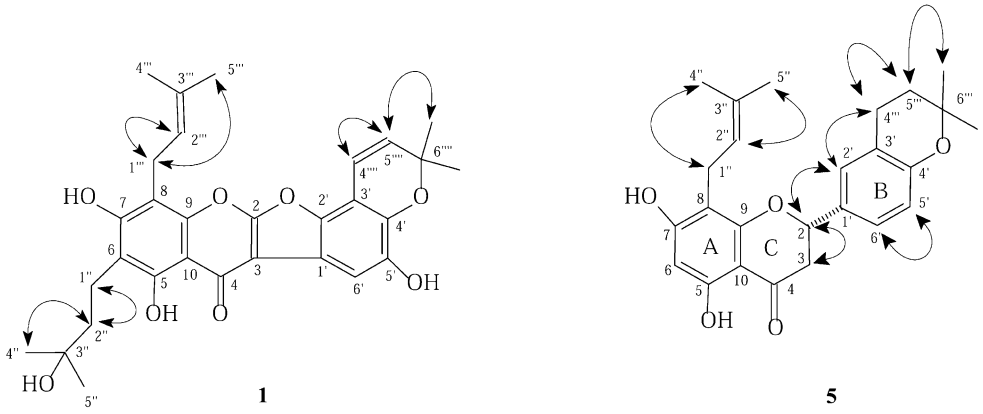

Fig. 1. NOESY correlations of compounds **1** and **5**.

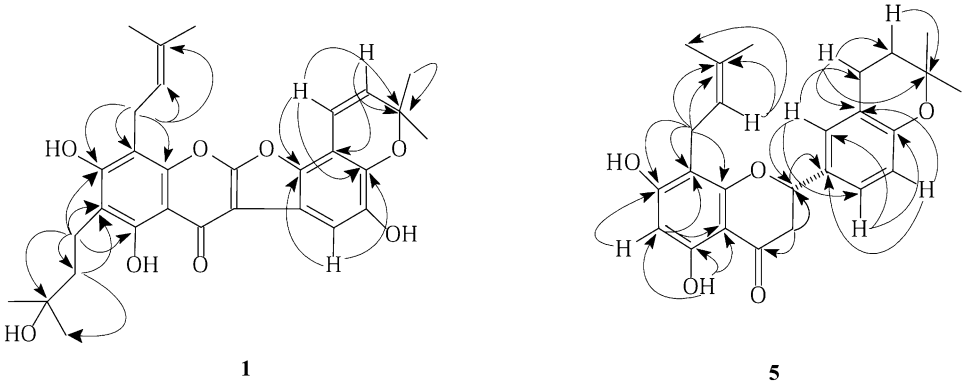

Fig. 2. HMBC correlations of compounds **1** and **5**.

Table 1
^{1}H NMR (400 MHz) spectral data of **1**–**5** in $CDCl_3$[a]

Position	1	2	3	4	5
2					5.31 (*dd*, 12.9, 2.8)
3α					2.81 (*dd*, 16.8, 2.8)
3β					3.03 (*dd*, 16.8, 12.9)
6				6.34 (*s*)	6.06 (*s*)
2′					7.14 (*d*, 2.4)
5′					6.81 (*d*, 8.4)
6′	7.29 (*s*)	7.40 (*s*)	7.46 (*s*)	7.47 (*s*)	7.17 (*dd*, 8.4, 2.4)
1″	2.69 (*t*, 6.8)	3.49 (*d*, 7.0)			3.30 (*d*, 7.0)
2″	1.65 (*t*, 6.8)	5.17 (*t*, 7.0)			5.20 (*t*, 7.0)
4″	1.19 (*s*)	1.81 (*s*)	2.73 (*t*-like, 6.8)	2.86 (*t*-like, 6.8)	1.77 (*s*)
5″	1.19 (*s*)	1.68 (*s*)	1.84 (*t*-like, 6.8)	1.88 (*t*-like, 6.8)	1.68 (*s*)
6″-CH_3			1.19 (*s*)	1.38 (*s*)	
6″-CH_3			1.19 (*s*)	1.38 (*s*)	
1‴	3.45 (*d*, 7.0)	3.41 (*d*, 7.0)	3.46 (*d*, 7.0)		
2‴	5.14 (*t*, 7.0)	5.21 (*t*, 7.0)	5.34 (*t*, 7.0)		
4‴	1.78 (*s*)	1.79 (*s*)	1.61 (*s*)	6.73 (*d*, 10.0)	2.78 (*t*-like, 6.8)
5‴	1.59 (*s*)	1.71 (*s*)	1.82 (*s*)	5.80 (*d*, 10.0)	1.83 (*t*-like, 6.8)
6‴-CH_3				1.52 (*s*)	1.35 (*s*)
6‴-CH_3				1.52 (*s*)	1.25 (*s*)
4⁗	6.65 (*d*, 10.0)	4.89 (*d*, 7.5)	6.74 (*d*, 10.0)		
5‴	5.72 (*d*, 10.0)	3.72 (*d*, 7.5)	5.79 (*d*, 10.0)		
6⁗ -CH_3	1.42 (*s*)	1.52 (*s*)	1.51 (*s*)		
6⁗-CH_3	1.42 (*s*)	1.25 (*s*)	1.51 (*s*)		
5-OH	12.94 (*s*)	13.26 (*s*)	13.13 (*s*)	12.65 (*s*)	12.02 (*s*)
5′-OH				5.47 (*s*)	

[a] d_H (mult., *J* Hz).

presence of a 2,2-dimethyl-3,4-dihydro-3,4-dihydroxy-2H-pyrano moiety (Takemura et al., 1996). An aromatic proton at δ 7.40 was assigned to H-6′, which was deshielded diamagnetically by the carbonyl group at C-4. The absolute configuration of **2** was established by comparing the optical rotation value with literature data of (−)-(3*R*,4*S*)-*trans*-2,2-dimethylchroman-3,4-diol (Boyd et al., 1996). Based on the coupling constant 7.5 Hz between H-4′′′′ and H-5′′′′, the conformation was determined as *threo* (4′′′′*S*/5′′′′*R* or 4′′′′*R*/5′′′′*S*). Because of its negative optical rotation, the stereochemistry of C-4′′′′ and C-5′′′′ was defined as 4′′′′*R*/5′′′′*S*. Unambiguous assignments for the ^{1}H and ^{13}C NMR signals were made by combination of COSY, HETCOR, NOESY and HMBC spectra. In the HMBC spectrum, the resonance at δ 3.49 (H-1′′) showed ^2J correlations to δ 121.2 (C-2′′) and 111.2 (C-6), and ^3J correlations to δ 159.9 (C-5), 158.7 (C-7), and 135.0 (C-3′′). Further, the signal at δ 3.41 (H-1′′′) displayed ^2J correlations to δ 121.3 (C-2′′′) and 106.6 (C-8), and ^3J correlations to δ 158.7 (C-7), 150.3 (C-9), and 133.0 (C-3′′′). The presence of two correlations between C-7 and each H-1′′ and H-1′′′ proves that two γ,γ-dimethylallyl groups are located at C-6 and C-8, respectively. Moreover, the signal at δ 4.89 (H-4′′′′) displayed a ^2J correlation to δ 109.3 (C-3′), and ^3J correlations to δ 141.1 (C-2′) and 135.7 (C-4′), and the signal at δ 3.72 (H-5′′′′) revealed ^2J correlations to δ 67.2 (C-4′′′′) and 79.9 (C-6′′′′), and a ^3J correlation to δ 25.7 (C_6-CH_3), which indicated that the hydroxylation of chromeno group is such as to give a 2,2-dimethyl-3,4-dihydro-3,4-dihydroxy-2H-pyrano group at C-4′′′′ and C-5′′′′. Thus, **2** was 5,7,5′,4′′′′,5′′′′-pentahydroxy-6,8-bis-(γ,γ-dimethylallyl)-[6′′′′,6′′′′-dimethyl-4′′′′,5′′′′-dihydropyrano-(2′′′′,3′′′′:4′,3′)]-coumaronochromone, named formosanatin B.

Compound **3** was isolated as pale yellow needles. The HR–EIMS confirmed the molecular formula as $C_{30}H_{30}O_7$, whereas the UV and IR spectra suggested the presence of a coumaronochromone skeleton. The ^{1}H and ^{13}C NMR spectra of **3** (see Tables 1 and 2) are similar to those of **1**. In comparison with the ^{1}H NMR spectra of **1**, the spectrum of **3** showed that the two methylene signals at δ 1.65 and 2.69 in **1** were shifted to δ 1.84 and 2.73 in **3**, which indicated that a 3-hydroxy-3-methylbutyl group was cyclized to form a 2,2-dimethyl-3,4-dihydro-2H-pyrano ring (Ahluwalia et al., 1983). Significant NOE correlations and HMBC spectra established the presence of a coumaronochromano skeleton, along with substitutions of the 2,2-dimethylallyl group, 2,2-dimethyl-2H-pyrano ring, and γ,γ-dimethyl-3,4-dihydro-2H-pyrano ring. The signal at δ 13.13 (C_5–OH) displayed a ^2J correlation to δ 157.8 (C-5), and ^3J correlations to δ 106.1 (C-6) and 102.8 (C-10) in the HMBC spectrum, which confirmed the hydroxyl group was attached to C-5. Thus, **3** was 5,5′-dihydroxy-8-(γ,γ-dimethylallyl)-[6′′,6′′-dimethyl-4′′,5′′-dihydropyrano-(2′′,3′′:7,6)]-[6′′′′,6′′′′-dimethylpyrano-(2′′′′,3′′′′:4′,3′)]-coumarnochromone, named formosanatin C.

Table 2
^{13}C NMR (100 MHz) spectral data of compounds **1**–**5** in $CDCl_3$[a]

Position	1	2	3	4	5
2	164.9 (*s*)	164.7 (*s*)	164.9 (*s*)	164.7 (*s*)	79.0 (*d*)
3	97.5 (*s*)	97.4 (*s*)	97.5 (*s*)	98.3 (*s*)	43.2 (*t*)
4	179.4 (*s*)	179.3 (*s*)	179.3 (*s*)	178.9 (*s*)	196.7 (*s*)
5	157.5 (*s*)	159.9 (*s*)	157.8 (*s*)	160.4 (*s*)	162.3 (*s*)
6	113.0 (*s*)	111.2 (*s*)	106.1 (*s*)	101.2 (*d*)	96.8 (*d*)
7	158.4 (*s*)	158.7 (*s*)	157.1 (*s*)	160.2 (*s*)	163.6 (*s*)
8	107.7 (*s*)	106.6 (*s*)	107.9 (*s*)	100.4 (*s*)	106.1 (*s*)
9	150.3 (*s*)	150.3 (*s*)	150.0 (*s*)	153.3 (*s*)	159.9 (*s*)
10	103.2 (*s*)	103.7 (*s*)	102.8 (*s*)	104.2 (*s*)	103.2 (*s*)
1′	114.3 (*s*)	114.7 (*s*)	115.3 (*s*)	114.9 (*s*)	129.6 (*s*)
2′	138.8 (*s*)*	141.1 (*s*)*	138.7 (*s*)*	138.9 (*s*)*	127.5 (*d*)
3′	107.0 (*s*)	109.3 (*s*)	106.5 (*s*)	106.2 (*s*)	121.1 (*s*)
4′	138.2 (*s*)*	135.7 (*s*)*	137.2 (*s*)*	137.5 (*s*)*	154.5 (*s*)
5′	143.3 (*s*)	143.6 (*s*)	143.0 (*s*)	143.1 (*s*)	117.5 (*d*)
6′	106.1 (*d*)	105.8 (*d*)	106.3 (*d*)	106.2 (*d*)	125.4 (*d*)
1′′	16.7 (*t*)	21.8 (*t*)			21.8 (*t*)
2′′	41.0 (*t*)	121.2 (*d*)			121.7 (*d*)
3′′	71.0 (*s*)	135.0 (*s*)			134.9 (*s*)
4′′	28.9 (*q*)	25.8 (*q*)	16.2 (*t*)	16.2 (*t*)	17.8 (*q*)
5′′	28.9 (*q*)	17.7 (*q*)	31.5 (*t*)	31.6 (*t*)	25.8 (*q*)
6′′			76.0 (*s*)	75.9 (*s*)	
6′′-CH_3			26.8 (*q*)	26.2 (*q*)	
6′′-CH_3			26.8 (*q*)	26.2 (*q*)	
1′′′	21.9 (*t*)	21.5 (*t*)	21.6 (*t*)		
2′′′	121.6 (*d*)	121.3 (*d*)	121.9 (*d*)		
3′′′	132.4 (*s*)	133.0 (*s*)	131.9 (*s*)		
4′′′	25.5 (*q*)	25.6 (*q*)	25.7 (*q*)	115.1 (*d*)	22.6 (*t*)
5′′′	17.6 (*q*)	18.9 (*q*)	17.9 (*q*)	132.0 (*d*)	32.7 (*t*)
6′′′				77.9 (*s*)	74.6 (*s*)
6′′′-CH_3				27.7 (*q*)	26.9 (*q*)
6′′′-CH_3				27.7 (*q*)	26.9 (*q*)
4′′′′	115.0 (*d*)	67.2 (*d*)	115.1 (*d*)		
5′′′′	132.0 (*d*)	75.6 (*d*)	131.7 (*d*)		
6′′′′	76.7 (*s*)	79.9 (*s*)	77.7 (*s*)		
6′′′′-CH_3	27.2 (*q*)	25.7 (*q*)	27.1 (*q*)		
6′′′′-CH_3	27.2 (*q*)	18.9 (*q*)	27.1 (*q*)		

* These assignments may be interchangeable.
[a] ^{13}C (mult.).

Compound **4** was isolated as yellow needles. The HR-EIMS determined the molecular formula as $C_{25}H_{22}O_7$, whereas the UV and IR spectral data suggested that **4** had a coumaronochromone skeleton as with compound **1**. The ^{1}H NMR spectrum of **4** (Table 1) displayed resonances for benzylic protons at δ 2.86 coupled to a pair of methylene protons at δ 1.88, and the *gem*-dimethyl protons at δ 1.38 (6H, s), indicating the presence of a 2,2-dimethyl-3,4-dihydro-2H-pyrano group. Similarly, the two olefinic protons of an AB spin system at δ 5.80 and 6.73, and the *gem*-dimethyl protons at δ 1.52, revealed the presence of a 2,2-dimethyl-2H-pyrano group. Two aromatic protons appeared at δ 6.34 and 7.47, which were assigned to the H-6 signal on the A ring and the typical C-6′ of the B-ring, respectively (Mizuno et al., 1992). The ^{13}C NMR and DEPT experiments (Table 2) showed 25 carbon signals. The

structure of **4** was further confirmed by 2D NMR spectroscopic techniques. In the HMBC spectrum, the signal at δ 12.65 (C_5–OH) showed a ^2J correlation to δ 160.4 (C-5), and ^3J correlations to δ 104.2 (C-10) and 101.2 (C-6). These HMBC data suggested that the hydroxyl group was located at C-5 on the A-ring. Thus, **4** was confirmed as 5,5′-dihydroxy-8-[6″,6″-dimethyl-4″,5″-dihydropyrano-(2″,3″:7,8)]-[6‴,6‴-dimethylpyrano-(2‴,3‴:4′,3′)]-coumaronochromone, named formosanatin D.

Compound **5** was obtained as a yellow oil, $[\alpha]_D^{24}$: −213° ($CHCl_3$, *c* 0.05). The HR–EIMS determined the molecular formula as $C_{25}H_{28}O_5$, whereas the UV spectrum exhibited absorption maxima at λ 265 and 288 nm and the IR spectrum showed carbonyl and hydroxyl absorption bands at 1640 and 3444 cm^{-1}, which corresponded to a flavanone-type structure (Shirataki et al., 1981). The ^1H NMR spectrum of **5** exhibited a pair of non-equivalent methylene protons at δ 2.81 and 3.03, and a double doublet at δ 5.31, which were assignable to the protons at C-3 and C-2, respectively, of the flavanone skeleton. An aromatic proton at δ 6.06 were assigned to H-6 in the A ring (Mizuno et al., 1988). A two-proton doublet at δ 3.30, a one-proton triplet at δ 5.20, and two methyl singlets at δ 1.68 and 1.77, indicated the presence of a γ,γ-dimethylallyl group. The coupling of benzylic protons at δ 2.78 to the methylene protons at δ 1.83, and to the *gem*-dimethyl group resonances at δ 1.25, and 1.35, suggested the presence of a 2,2-dimethyl-3,4-dihydro-2H-pyrano group. A characteristic ABX system at δ 7.14, 6.81, and 7.17 indicated the presence of a C-3′, 4′ disubstitution on the B ring moiety. The absolute configuration at C-2 was established as *S* by comparing the optical rotation value with literature data of euchrestaflavanone A (Shirataki et al., 1981).

The key NOE correlations are showed in Fig. 1. Twenty-five ^{13}C NMR resonances (Table 2) were attributed to four methyl groups (δ 17.8, 25.8, 26.9, and 26.9), four methylenes (δ 21.8, 22.6, 32.7, and 43.2), six methines (δ 79.0, 96.8, 117.5, 121.7, 125.4, and 127.5), and 11 quaternary carbons (δ 74.6, 103.2, 106.1, 121.1, 129.6, 134.9, 154.5, 159.9, 162.3, 163.6, and 196.7). The HMBC spectrum (Fig. 2) revealed that the doublet at δ 3.30 (H-1″) showed ^2J correlations to δ 121.7 (C-2″) and 106.1 (C-8), and ^3J correlations to δ 163.6 (C-7), 159.9 (C-9), and 134.9 (C-3″). Also, the signal at δ 12.02 (C_5–OH) displayed a ^2J correlation to δ 162.3 (C-5), and ^3J correlations to δ 96.8 (C-6) and 103.2 (C-10). The spectral data confirmed the location of γ,γ-dimethylallyl at C-8 and two hydroxyl at C-5 and C-7. Furthermore, the signal at δ 2.78 (H-4‴) showed ^2J correlations to δ 121.1 (C-3′) and 32.7 (C-5‴), and a ^3J correlation to δ 74.6 (C-6‴), and the signal at δ 7.14 (H-2′) displayed ^3J correlations to δ 79.0 (C-2), 125.4 (C-6′), and 22.6 (C-4‴), which supported the interpretation that the pyrano ring is linked at C-3′ and C-4′. The EIMS spectrum showed fragment ion peaks at *m*/*z* 188 and 220, which were caused by cleavage through Retro–Diels–Alder (RDA) rearrangement fragmentation (Mizuno et al., 1989b; Hanawa et al., 1991b). Based on the above data, **5** was elucidated as (2*S*)-5,7-dihydroxy-8-(γ,γ-dimethylallyl)-[6‴,6‴-dimethyl-4‴,5‴-dihydropyrano-(2‴,3‴:4′,3′)]-flavanone, named euchrenone a_{16}.

Four known compounds, euchretins E and G, euchrestaflavanone A, and daidzein, were identified by comparison with their physical and spectral data (UV, IR, and ^1H NMR spectra) with values in the literature (Shirataki et al., 1981; Murthy et al., 1986; Mizuno et al., 1991, 1992).

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Laboratory Devices Mel-Temp II and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 220-20 spectrophotometer in EtOH, whereas IR spectra were measured on a Hitachi 260–30 spectrophotometer. ^1H NMR (400 MHz), using $CDCl_3$ as solvent for measurement ^{13}C, NOESY, DEPT, COSY, HMQC, HMBC NMR spectra were obtained on a Varian NMR spectrometer. FABMS and EIMS were recorded on a Jeol JMS-SX/SX 102A mass spectrometer or a Quattro GC–MS spectrometer having a direct inlet system. HR-EIMS were measured on a Jeol JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230–400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC and precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. Spots were detected by spraying with 50% H_2SO_4 and then heated on a hot plate.

3.2. Plant material

The roots of *E. formosana* were collected from Pingtung Hsieh, in the southern part of Taiwan, in July 1997. A voucher specimen (Legumin 35–1) is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

3.3. Extraction and isolation

The roots of *E. formosana* (3.5 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated under reduced pressure to yield a brownish viscous residue (350.2 g), which was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ portion (110.5 g) was subjected to a Si gel column, this being eluted with *n*-hexane/$CHCl_3$/MeOH mixtures of increasing polarity to obtain 14 fractions. Compounds

1 (10.2 mg) (acetone/*n*-hexane, 1:5, R_f 0.50) and euchrestaflavanone A (20.0 mg) (acetone/*n*-hexane, 1:4, R_f 0.45) were eluted from fraction 3 (10.4 g) by Si gel column chromatography (acetone/*n*-hexane, 1:25). Daidzein (6.1 mg) (acetone/*n*-hexane, 1:4, R_f 0.39) was obtained from fraction 4 (8.2 g), which was eluted with acetone/*n*-hexane (1:20) by Si gel column chromatography. Fraction 5 (10.1 g), eluted with acetone/*n*-hexane (1:15), was further separated and purified by Si gel column chromatography and preparative TLC to afford euchretins E (6.2 mg) (acetone/*n*-hexane, 1:3, R_f 0.48). Fraction 6 (15.5 g), eluted with EtOAc/*n*-hexane (1:5), was separated by Si gel column chromatography and preparative TLC to afford **2** (8.2 mg) (EtOAc/*n*-hexane, 1:3, R_f 0.45) and **3** (8.1 mg) (EtOAc/*n*-hexane, 1:3, R_f 0.40). Further purification from the fraction 7 by preparative TLC yielded compounds **4** (10.0 mg) (EtOAc/*n*-hexane, 1:2, R_f 0.45) and **5** (5.1 mg) (EtOAc/*n*-hexane, 1:2, R_f 0.40). Fraction 10 (13.7 g) was eluted with gradient mixtures of EtOAc/*n*-hexane by Si gel column chromatography to obtain compound euchretins G (5.2 mg) (EtOAc/*n*-hexane, 1:1, R_f 0.39).

3.4.1. Formosanatin A (*1*)

{5,7,5′-Trihydroxy-6-(3-hydroxy-3-methylbutyl)-8-(γ,γ-dimethylallyl)-[6‴, 6‴-dimethylpyrano-(2‴,3‴:4′,3′)]-coumaronochromone}. Colorless needles; mp 221–223 °C; UV (EtOH) λ_{max} nm (log ε): 262 (4.7), 350 (4.4), (EtOH + NaOMe): 250, 364, (EtOH + $AlCl_3$): 260, 355, (EtOH + NaOAc): 264, 355; IR (Neat) ν_{max} cm^{-1}: 3446, 1646; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HR-EIMS *m/z* 520.2103 (calc. for $C_{30}H_{32}O_8$, 520.2097); EIMS (70 eV) *m/z* (rel. int.): 520 $[M]^+$ (35), 502 (12), 487 (21), 459 (21), 447 (21), 431 (31), 403 (27), 391 (65), 243 (8), 215 (16), 208 (31), 188 (44), 177 (15), 109 (19), 95 (30), 81 (38), 69 (63), 55 (74).

3.4.2. Formosanatin B (*2*)

{5,7,5′,4‴,5‴-Pentahydroxy-6,8-bis-(γ,γ-dimethylallyl)-[6‴,6‴-dimethyl- 4‴,5‴-dihydropyrano-(2‴,3‴:4′,3′)]-coumaronochromone}. Yellow needles; mp 243–245 °C; $[\alpha]_D^{24}$ −40° ($CHCl_3$, *c* 0.05); UV (EtOH) λ_{max} nm (log ε): 260 (4.7), 348 (4.4), (EtOH + NaOMe): 275, 350, (EtOH + $AlCl_3$): 256, 348, (EtOH + NaOAc): 262, 348; IR (Neat) ν_{max} cm^{-1}: 3402, 1630; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HR–FABMS *m/z* 537.2122 $[M+H]^+$ (calc. for $C_{30}H_{32}O_9$, 537.2125); FABMS (70 eV) *m/z* (rel. int.): 537 $[M+H]^+$ (29), 481 (33), 425 (84), 393 (29), 353 (100), 337 (19), 231 (14), 177 (19), 165 (8), 115 (9), 91 (10), 39 (22).

3.4.3. Formosanatin C (*3*)

{5,5′-Dihydroxy-8-(γ,γ-dimethylallyl)-[6″,6″-dimethyl-4″,5″-dihydropyrano-(2″,3″:7,6)]-[6‴,6‴-dimethylpyrano-(2‴,3‴:4′,3′)]-coumaronochromone}. Pale yellow needles; mp 248–250 °C; UV (EtOH) λ_{max} nm (log ε): 258 (4.7), 348 (4.4), (EtOH + NaOMe): 260, 350, (EtOH + $AlCl_3$): 256, 350, (EtOH + NaOAc): 258, 348; IR (Neat) ν_{max} cm^{-1}: 3440, 1642; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HR–EIMS *m/z* 502.2003 (calc. for $C_{30}H_{30}O_7$, 502.1992); EIMS (70 eV) *m/z* (rel. int.): 502 $[M]^+$ (100), 488 (26), 487 (92), 431 (8), 403 (7), 391 (38), 373 (11), 243 (8), 233 (6), 215 (12), 208 (35), 188 (46), 177 (14), 115 (10), 69 (10), 55 (19), 43 (38).

3.4.4. Formosanatin D (*4*)

{5,5′-Dihydroxy-8-[6″,6″-dimethyl-4″,5″-dihydropyrano-(2″,3″:7,8)]-[6‴,6‴-dimethylpyrano-(2‴,3‴:4′,3′)]-coumaronochromone}. Yellow needles ($CHCl_3$); mp 246–248 °C; UV (EtOH) λ_{max} nm (log ε): 261 (4.7), 348 (4.4), (EtOH + NaOMe) 258, 370, (EtOH + $AlCl_3$): 262, 350, (EtOH + NaOAc): 262, 348; IR (neat) ν_{max} cm^{-1}: 3466, 1656; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HR–EIMS *m/z* $[M]^+$ 434.1365 (calc. for $C_{25}H_{22}O_7$, 434.1366); EIMS (70 eV) *m/z* (rel. int.): 434 $[M]^+$ (49), 420 (26), 419 (100), 389 (21), 363 (14), 203 (41), 182 (60), 177 (22), 165 (50), 149 (33), 81 (31), 69 (76).

3.4.5. Euchrenone a_{16} (*5*)

{5,7-Dihydroxy-8-(γ,γ-dimethylallyl)-[6‴,6‴-dimethyl-4‴,5‴-dihydropyrano-(2‴,3‴:4′,3′)]-flavanone}. Yellow oil; $[\alpha]_D^{24}$ −213° ($CHCl_3$, *c* 0.05); UV (EtOH). (log ε) λ_{max}/nm: 265 (4.6), 288 (4.5), (EtOH + NaOMe): 285, 330, (EtOH + $AlCl_3$): 256, 285, (EtOH + NaOAc): 235, 288; IR (Neat) ν_{max}/cm^{-1}: 3444, 1640; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HR–EIMS *m/z* $[M]^+$ 408.1923 (calc. for $C_{25}H_{28}O_5$, 408.1937); EIMS (70 eV) *m/z* (rel. int.): 408 $[M]^+$ (47), 391 (65), 365 (11), 353 (14), 233 (17), 219 (36), 220 (35), 205 (100), 188 (77), 171 (99), 165 (56), 133 (39), 69 (23), 55 (30), 43 (34).

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