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Flavonoids from the roots of *Millettia erythrocalyx*

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Abstract

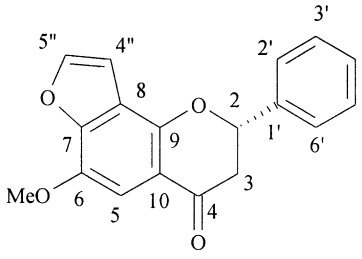
From the roots of *Millettia erythrocalyx*, 6-methoxy-[2″,3″:7,8]-furanoflavanone, 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan, and 3,4-methylenedioxy-2′,4′-dimethoxychalcone were isolated, along with ten other known flavonoids. Their structures were elucidated on the basis of analyses of their spectroscopic data.

Keywords: *Millettia erythrocalyx*; Leguminosae; Flavonoids; Flavanones; Flavan-4-ols; Chalcones; 6-Methoxy-[2″,3″:7,8]-furanoflavanone; 2,5-Dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan; 3,4-Methylenedioxy-2′,4′-dimethoxychalcone

1. Introduction

Several plants in the genus *Millettia* (Leguminosae) are well known for their fish poisoning and insecticidal properties (Kumar et al., 1989). In an earlier report (Sritularak et al., 2002) we described the isolation of several flavones from the stem bark of *Millettia erythrocalyx* Gagnep. In the present paper, we report the isolation, from the roots of this plant, of two new flavonoids, i.e. 6-methoxy-[2″,3″:7,8]-furanoflavanone (**1**), 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan (**2**) and a new natural compound (**3**) (Salem et al., 2000) along with ten known compounds, i.e. 1-(4-hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one (Saxena et al., 1987), derricidin (do Nascimento et al., 1972), purpurenone (Rao and Raju, 1984), pongaglabol (Talapatra et al., 1980), ponganone I, ovalitenone (Tanaka et al., 1991), pongamol (Parmar et al., 1989), milletenone (Mahmoud and Waterman, 1985), ponganone V and lanceolatin B (Tanaka et al., 1992). To our knowledge, 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan (**2**) is the first example of a flavan-4-ol bearing a methoxyl group at C-2 position.

2. Results and discussion



1

Compound **1**, colorless needles, gave a molecular ion [M^+] at m/z 294.08423 in the HREIMS, indicating a molecular formula of $C_{18}H_{14}O_4$ (calcd 294.08920). The IR spectrum showed absorption bands for conjugated carbonyl (1679 cm^{-1}) and ether (1246, 1212 cm^{-1}) functionalities. The UV absorptions at 234, 247 and 341 were indicative of a flavanone skeleton (Markham, 1982). In the ^1H NMR spectrum (Table 1), the aliphatic proton signals at δ 2.90 (*dd*, $J = 16.8$ and 3.0 Hz), δ 3.12 (*dd*, $J = 13.2$ and 16.8 Hz) and δ 5.57 (*dd*, $J = 3.0$ and 13.2 Hz) are typical for H-3eq, H-3ax and H-2, respectively. This was confirmed by the HMQC spectrum in which the two former protons correlated with a carbon (Table 1) at δ 44.3 ppm and the latter (δ 5.57) exhibited a cross peak with a carbon at δ 80.4 ppm. The ^1H NMR spectrum of 1 also revealed the presence of a methoxyl

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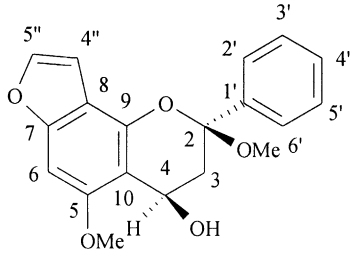
Table 1
NMR spectral data of compounds 1 and 2 (in $CDCl_3$)

Position	^1H[a]		^{13}C[b]		HMBC[c] (correlation with ^1H)	
	1	**2**	**1**	**2**	**1**	**2**
2	5.57 (*dd*, 13.2, 3.0)	–	80.4 (*d*)	101.9 (*s*)	2′ and 6′	4, 2′, 6′ and MeO
3	3.12 (*dd*, 16.8, 13.2)	2.79 (*dd*, 14.8, 1.3)	44.3 (*t*)	42.4 (*t*)	–	–
	2.90 (*dd*, 16.8, 3.0)	2.18 (*dd*, 14.8, 4.5)	–	–	–	–
4	–	5.00 (*m*)	191.2 (*s*)	59.7 (*d*)	2, 3 and 5	3
5	7.27 (*s*)	–	102.1 (*d*)	157.4 (*s*)	–	4, 6* and MeO
6	–	6.81 (*s*)	141.5 (*s*)	88.8 (*d*)	MeO	–
7	–	–	149.8 (*s*)	156.7 (*s*)	5, 4″ and 5″	6*, 4″ and 5″
8	–	–	119.2 (*s*)	110.9 (*s*)	5″	6 and 5″
9	–	–	151.6 (*s*)	144.4 (*s*)	5 and 4″	4 and 4″
10	–	–	115.3 (*s*)	109.0 (*s*)	3	3 and 6
1′	–	–	138.8 (*s*)	140.1 (*s*)	3, 3′ and 5′	3′ and 5′
2′	7.51 (*m*)	7.72 (*m*)	126.2 (*d*)	126.4 (*d*)	4′ and 6′	5′ and 6′
3′	7.41 (*m*)	7.48 (*m*)	128.8 (*d*)	128.8 (*d*)	5′	5′
4′	7.41 (*m*)	7.42 (*m*)	128.8 (*d*)	128.8 (*d*)	2′ and 6′	2′ and 6′
5′	7.41 (*m*)	7.48 (*m*)	128.8 (*d*)	128.8 (*d*)	3′	3′
6′	7.51 (*m*)	7.72 (*m*)	126.2 (*d*)	126.4 (*d*)	2′ and 4′	2′ and 4′
4″	6.91 (*d*, 2.1)	6.91 (*d*, 2.0)	105.3 (*d*)	103.8 (*d*)	5″	5″
5″	7.61 (*d*, 2.1)	7.52 (*d*, 2.0)	145.3 (*d*)	143.1 (*d*)	4″	4″
MeO-6	3.99 (*s*)	–	53.3 (*q*)	–	–	–
MeO-2	–	3.13 (*s*)	–	50.7 (*q*)	–	–
MeO-5	–	3.97 (*s*)	–	56.4 (*q*)	–	–
HO-4	–	4.07 (*d*, 9.8)	–	–	–	–

[a] Coupling constants (J in Hz) for ^1H.
[b] Multiplicities for ^{13}C are in parentheses.
[c] Protons correlating with carbon resonance (optimized J_{C-H} at 8 Hz).
* Two-bond coupling.

group (δ 3.99, *s*, 3H), and a furan ring, as evidenced by two one-proton doublets ($J=2.1$ Hz) at δ 6.91 (H-4″) and δ 7.61 (H-5″). A two-proton multiplet centred at δ 7.51 and a three-proton multiplet centred at δ 7.41 suggested that ring B was unsubstituted. In the EIMS, the fragment ions at m/z 190 and 104 resulting from *retro*-Diels–Alder cleavage of ring C suggested the placement of the furan ring and the methoxyl on ring A (Drewes, 1974). The methoxyl should be situated at C-6, as shown by its NOESY interaction with the proton at δ 7.27 (1H, *s*, H-5) and the HMBC (Table 1) correlation of H-5 with C-4 (δ 191.2). The position of the furan ring on ring A was determined by the HMBC connection between H-5 and C-7. The CD spectrum showed a positive Cotton effect at 350 nm and a negative one at 281 nm, consistent with the 2*S*-configuration (Gaffield, 1970; Yenesew et al., 1998). Based on above spectral evidence, compound **1** was identified as (−)-(2*S*)-6-methoxy-[2″,3″:7,8]-furanoflavanone.

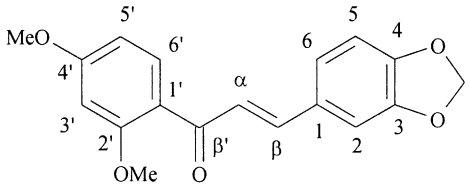
Compound **2**, colorless crystals, gave a molecular ion [M^+] at 326.11934 in the HREIMS, corresponding to the molecular formula $C_{19}H_{18}O_5$ (calc: 326.11542). The IR spectrum demonstrated the presence of a hydroxyl (3447 cm^{-1}) but not a carbonyl group. The UV maximal absorptions at 214 and 250 nm were suggestive of a flavan skeleton (Gómez et al., 1985). The presence of a one-proton multiplet at δ 5.00 (H-4) and two one-proton



2

doublets of doublets at δ 2.18 ($J=4.5$ and 14.8 Hz, H-3eq) and δ 2.79 ($J=1.3$ and 14.8 Hz, H-3ax), together with the appearance of the quaternary carbon signal at δ 101.9 (C-2) in the HMQC spectrum indicated that compound **2** should be a flavan with oxygenation at C-2 and C-4. Four substituents were attached to the flavan skeleton, as indicated by signals for two methoxyls at δ 3.97 (3H, *s*) and δ 3.13 (3H, *s*), for a furan ring at δ 7.52 (*d*, $J=2.0$ Hz, H-5″) and δ 6.91 (*d*, $J=2.0$ Hz, H-4″), and for a hydroxyl group at δ 4.07 (*br d*, $J=9.8$ Hz, exchangeable with D_2O) in the ^1H NMR spectrum (Table 1). The presence of an AA′BB′C spin system at δ 7.72 (2H, *m*, H-2′ and H-6′), δ 7.48 (2H, *m*, H-3′ and H-5′) and δ 7.42 (1H, *m*, H-4′) indicated an unsubstituted B ring. The first methoxyl and the furan ring should be on ring A and the second methoxyl should be

at C-2, as evident from the fragment ions at *m*/*z* 192 and 134 caused by RDA cleavage of ring C in the mass spectrum (Drewes, 1974). On ring A, a NOESY cross peak between the methoxyl at δ 3.97 and H-4, suggested the location of this methoxyl at C-5. This was confirmed by the 3-bond HMBC (Table 1) correlation of C-5 (δ 157.4) with this methoxyl protons and with H-4. The location of the second methoxyl (δ 3.13) at C-2 was confirmed by its NOESY correlation peak with H-2′/H-6′, together with the HMBC correlations of C-2 (δ 101.9) with the methoxyl protons, H-2′/H-6′ and H-4. The furan ring was fused in an angular position at C-7 and C-8, as established by the NOESY interaction of H-4″ with H-2′/H-6′ and MeO-2, and two-bond coupling of H-6 with C-5 and C-7 in the HMBC spectrum. The relative configuration of compound **2** was established by the NOESY interaction between the OH and OMe groups, indicating their *cis*-orientation. Thus, structure **2** was established as 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan, the first representative of flavan-4-ols with a methoxyl group at C-2.



3

Compound **3**, with a [M^+] at *m*/*z* 312 in the EI mass spectrum, was obtained as a yellow powder. The IR bands at 1653 (C=O) and 1602 (C=C) cm^{-1} and the UV absorption at 351 nm were suggestive of a chalcone skeleton (Markham, 1982). The ^{1}H NMR signals for a set of *trans*-olefinic protons at δ 7.36 and δ 7.24 (each *d*, J = 15.9 Hz) confirmed the existence of the chalcone nucleus. The ^{13}C NMR and HMQC spectra showed 19 signals, corresponding to two methoxyls, one methylene, eight methines and seven quaternary carbons. Three substituents were attached to the chalcone nucleus, as indicated by signals for two methoxyls at δ 3.91 and δ 3.94 (each 3H, *s*) and for a methylenedioxy at δ 6.05 (2H, *s*) in the ^{1}H NMR spectrum. The two methoxyls should be located on ring A and the methylenedioxyl on ring B, as shown by the fragment ions at *m*/*z* 147 and 165 in the EIMS (Drewes, 1974). On ring A, an ABM splitting system consisting of two doublets at δ 6.53 (J = 1.8 Hz, H-3′) and δ 7.78 (J = 8.7 Hz, H-6′) and a broad doublet at δ 6.59 (J = 8.7, H-5′), together with the HMBC correlation of H-6′ with C-β′ (δ 141.9) suggested the location of the two methoxyls at C-2′ and C-4′ positions. This was confirmed by NOESY interactions of MeO-2′ (δ 3.94, *s*) with H-3′ and MeO-4′ (δ 3.91, *s*) with H-3′ and H-5′, respectively. On ring B, the ^{1}H NMR ABM spin system at δ 7.16 (1H, *br s*, H-2), 7.10 (1H, *br d*, J = 8.1 Hz, H-6) and 6.85 (1H, *d*, J = 8.1 Hz, H-5), together with the HMBC correlation of C-β (δ 141.9) with H-2 and H-6, indicated the placement of the methylenedioxyl at C-3 and C-4. Thus, structure **3** was identified as 3,4-methylenedioxy-2′,4′-dimethoxychalcone. Although **3** has been earlier synthesized (Salem et al., 2000), this is the first time it has been found as a naturally occurring compound. Prior to this study, the ^{13}C NMR data of **3** have not been reported.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. Melting points were obtained on Fisher–Johns Melting Point Apparatus. CD spectra were recorded on a JASCO J-715 spectropolarimeter. UV spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer, and IR spectra were recorded with a Perkin-Elmer FT-IR 1760X spectrophotometer. EI (70 eV) and HREI mass spectra were obtained with a Varian MAT 311A. NMR spectra were recorded on a Varian Unity Inova 500 or 300 MHz.

3.2. Plant material

The roots of *M. erythrocalyx* Gagnep were collected from Petchaburi Province, Thailand in April, 2000. Voucher specimens (KL 012543) identifying the sample are on deposit at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

3.3. Extraction and isolation

Air-dried and powdered roots of *M. erythrocalyx* (8 kg) were successively extracted with *n*-hexane, $CHCl_3$ and MeOH at room temp., yielding an *n*-hexane extract (91 g), a $CHCl_3$ extract (87 g) and a MeOH extract (429g), respectively. The *n*-hexane extract was initially subjected to vacuum liquid chromatography (VLC) on silica gel (EtOAc–pet. ether gradient) to give fractions A–E. Fraction B (6.9 g) was separated by CC (silica gel; EtOAc–pet. ether 0.5:9.5) and then by MPLC (silica gel; toluene–pet. ether 3:7) to give 14 fractions (I–XIV). Fraction III (191 mg) was subjected to repeated CC (silica gel; toluene–pet. ether 3:7) to afford 1-(4-hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one (7 mg; R_f 0.63, silica gel, toluene). Fraction VII (44 mg) was purified by CC (silica gel; toluene–pet. ether 4:6) to yield derricidin (3.5 mg; R_f 0.40, silica gel, EtOAc–hexane 1:20). Fraction IX (79 mg) was separated by CC (silica gel; toluene–pet. ether 3:7) to give 1-(4-hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one (4 mg) and

purpurenone (14 mg; R_f 0.44, silica gel, toluene). Separation of fraction XII (390 mg) was performed by CC over silica gel, eluted with toluene to give pongaglabol (21 mg; R_f 0.14, silica gel, $CHCl_3$–toluene 1:2). Fraction C (14.8 g) was separated by CC (silica gel; toluene) to give 38 fractions. Ponganone I (227 mg, R_f 0.27, silica gel, $CHCl_3$–toluene 1:4) was obtained from fractions 29–37. Fractions 11–17 (760 mg) were combined and further subjected to repeated CC over silica gel, eluted with toluene–pet. ether (1:1) to afford pongamol (54 mg; R_f 0.44, silica gel, toluene). Fraction D (36.4 g) was separated by VLC (silica gel; EtOAc–pet. ether gradient) and then by MPLC (silica gel; EtOAc–pet. ether 1:9) to give 9 fractions (A1–A9). Fraction A3 (169 mg) after purification by CC on silica gel (toluene), gave ovalitenone (26 mg; R_f 0.24, silica gel, $CHCl_3$–toluene 1:2) and 1 (16 mg; R_f 0.20, silica gel, $CHCl_3$–toluene 1:1). Fraction A4 (8.3 g) was subjected to MPLC over silica gel using EtOAc–pet. ether (0.5:9.5) as eluent to yield 55 fractions. Pongamol (5 mg), ovalitenone (52 mg) and milletenone (165 mg; R_f 0.38, silica gel, EtOAc–pet. ether 2:3) were obtained from fractions 4, 8–9 and 14–16, respectively. Fractions 29–33 (341 mg) were combined, dried and separated by CC (silica gel; $CHCl_3$–toluene 1:9) and then recrystallized from MeOH to give ponganone V (34 mg; R_f 0.44, silica gel, EtOAc–toluene 1:5). Fractions 37–41 (1.6 g) were further purified by MPLC (silica gel; EtOAc–pet. ether 0.5:9.5) and then by recystallization from MeOH to furnish 2 (183 mg; R_f 0.29, silica gel, $CHCl_3$–toluene 1:1). From fraction A5 (5.8 g), ovalitenone (19 mg), milletenone (131 mg) and 2 (95 mg) were obtained using MPLC on silica gel ($CHCl_3$–toluene 2:8). Separation of fraction A6 (2.0 g) was performed by MPLC over silica gel (EtOAc–toluene 1:9) to give 35 fractions. Ovalitenone (20 mg) and milletenone (155 mg) were obtained from fractions 2 and 6, respectively. Fractions 22–26 were combined, dried and separated by CC (silica gel; toluene) to give 3 (9 mg; R_f 0.53, silica gel, EtOAc–toluene 1:5). Fraction 34 (668 mg) was purified by MPLC (silica gel; EtOAc–toluene 0.5:9.5) and recrystallized from MeOH to give lanceolatin B (16 mg; R_f 0.38, silica gel, EtOAc–toluene 1:5).

3.4. (−)-(2S)- 6-Methoxy-[2″,3″:7,8]-furanoflavanone (*1*)

Colorless needles; mp 190–192 °C; $[\alpha]_D^{28}$ −55.8 ° (MeOH; *c* 0.1); UV (MeOH) λ_{max} (log ϵ): 234 (3.44), 247 (3.32), 341 (2.65) nm; CD (MeOH; *c* 0.1): $[\theta]_{215}$ −4821, $[\theta]_{226.5}$ +1538, $[\theta]_{233}$ −166, $[\theta]_{244}$ +4492, $[\theta]_{281}$ −9871, $[\theta]_{312}$ −12850, $[\theta]_{350}$ +12398; IR (KBr) ν_{max} 1679, 1246, 1212 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1; EIMS *m*/*z* 294 [M^+] (48), 190 (100), 119 (30), 104 (46), 77 (10), 28 (12); HREIMS *m*/*z* 294.08423 (calc. for $C_{18}H_{14}O_4$, 294.08920).

3.5. 2,5-Dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan (*2*)

Colorless crystals; mp 135–136 °C; $[\alpha]_D^{28}$ +42.1 ° (MeOH; *c* 0.3); UV (MeOH) λ_{max} (log ϵ): 214 (3.15), 250 (2.68) nm; CD (MeOH; *c* 0.3): $[\theta]_{209.5}$ −1556, $[\theta]_{223}$ +212, $[\theta]_{233}$ −873, $[\theta]_{248.5}$ +1703, $[\theta]_{282}$ +863; IR (KBr) ν_{max} 3447, 1315, 1278 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; EIMS *m*/*z* 326 [M^+] (20), 277 (65), 192 (100), 174 (50), 146 (60), 134 (10); HREIMS *m*/*z* 326.11934 (calc. for $C_{19}H_{18}O_5$, 326.11542).

3.6. 3,4-Methylenedioxy-2′,4′-dimethoxychalcone (*3*)

Yellow powder; mp 123–125 °C; $C_{18}H_{16}O_5$; UV (MeOH) λ_{max} (log ϵ): 351 (2.91) nm; IR (KBr) ν_{max} 3007, 1653, 1602 cm^{-1}; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 190.4 (*s*, C-β), 164.1 (*s*, C-4′), 160.3 (*s*, C-1′), 149.4 (*s*, C-4), 148.2 (*s*, C-3), 141.9 (*d*, C-β), 132.8 (*d*, C-6′), 129.9 (*s*, C-1), 125.4 (*d*, C-α), 124.8 (*d*, C-6), 122.4 (*s*, C-1′), 108.6 (*d*, C-5), 106.6 (*d*, C-2), 105.1 (*d*, C-5′), 101.5 (*t*,-OCH_2O-), 98.7 (*d*, C-3′), 55.7 (*q*, MeO-2′), 55.5 (*q*, MeO-4′); EIMS *m*/*z* 312 [M^+] (98), 297 (30), 284 (45), 165 (100), 147 (26), 135 (74).

Known compounds were identified by comparison of their physical properties with literature values.

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