

Flavonol Galactoside Caffeate Ester and Homoisflavones from *Caesalpinia millettii* HOOK. et ARN.

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Chemical examination of the stems of *Caesalpinia millettii* HOOK. et ARN led to the isolation of new flavonol glycoside caffeate ester (1) and homoisflavone (2), along with four known homoisflavones: eucomin (3), bonducellin (4), 8-methoxybonducellin (5) and intricatinol (6). The structures of 1 and 2 were established to be tamarixetin 3-O-(6''-O-E-caffeoyl)- β -D-galactopyranoside (1) and (Z)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl) chroman-4-one (2) on the basis of detailed analyses of physical, chemical, and spectral data. Compounds 3–6 were isolated from this plant for the first time.

Key word *Caesalpinia millettii*; tamarixetin; homoisflavone; 8-methoxyisobonducellin; flavonol glycoside caffeate ester

Seventeen species of the *Caesalpinia* genus (Leguminosae) are discovered in southwestern China,¹⁾ and they possess similar morphologic characteristics. However, the reports on the chemical structure of the *Caesalpinia* genus indicate they have different chemical components showing various bioactivities. Thus, further investigation of the chemical constituent of *Caesalpinia* genus is worthwhile.

Caesalpinia millettii HOOK. et ARN. is a thorny liana of the family Leguminosae, which is widely distributed in Yunnan Province, Guangxi Province, Guangdong Province of China. Up to now, there is no any report regarding the constituents of it. In this paper we describes the isolation and structural elucidation of the new flavonol glycoside caffeate ester (1) and homoisflavone (2), as well as four known homoisflavones (3–6) from the acetone extract of the stems of *Caesalpinia millettii* HOOK. et ARN. The structures of the known homoisflavones (3–6) were identified as (*E*)-5,7-dihydroxy-3-(4-methoxybenzyl) chroman-4-one (3), (*E*)-7-hydroxy-3-(4-methoxybenzyl) chroman-4-one (4), (*E*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl) chroman-4-one (5), (*E*)-7,8-dihydroxy-3-(4-methoxybenzyl) chroman-4-one (6),

respectively.

Results and Discussion

Compound 1 was obtained as yellow powder, gave a positive coloration in the Mg–HCl test. The molecular formula was determined as C₃₁H₂₈O₁₅, on the basis of the ion peak [M+Na]⁺ at *m/z* 663.1311 (Calcd 663.1325) in HR-FAB-MS. The UV spectrum showed maximum absorption at 295 and 330 nm, and the IR spectrum exhibited absorption bands ascribed to hydroxyl (3250 cm⁻¹), ester carbonyl (1645 cm⁻¹), and aromatic ring (1604, 1508 cm⁻¹). Thus, 1 was suggested to be flavonol glycoside from its characteristic color reaction and spectral properties. Analysis of the ¹H- and ¹³C-NMR spectra of compound 1 (Table 1; all assignments based on HMQC, HMBC experiments) showed the presence of a tamarixetin core, a *trans*-caffeoyl moiety, and a galactopyranosyl unit. The ¹H-NMR resonances of two *meta* coupled doublets at δ 6.10 (1H, d, *J*=2.0 Hz, H-6) and 6.34 (1H, d, *J*=2.0 Hz, H-8), correlated with the carbons at 99.35 and 94.13 ppm respectively in the HMQC spectrum, characterized the 6- and 8-protons of a flavonol 5, 7 dihydroxy A-ring.⁹⁾ And δ 6.80 (1H, d, *J*=8.5 Hz, H-5'), 7.50 (1H, d, *J*=2.0 Hz, H-2'), and 7.65 (1H, dd, *J*=8.5, 2.0 Hz, H-6'), correlated with the carbons at 115.88, 116.57 and 122.65 ppm respectively in the HMQC spectrum, characterized the ABX system due to 3',4'-disubstituted B-ring. A singlet signal of three protons at δ 3.80 correlated with the carbons at 149.17 ppm and 7.50 (H-2'), 7.65 (H-6') correlated with the carbons at 149.17 ppm in the HMBC (see Fig. 2) indicated the presence of the flavonol 3'-hydroxy-4'-methoxy substituted B-ring. Thus, the aglycone part of 1 was analyzed as tamarixetin. In the aromatic region of the spectrum, the remaining three protons occurred as an ABX spin system at δ 6.76 (1H, d, *J*=8.0 Hz, H-5'''), 6.91 (1H, d, *J*=8.0 Hz, H-6''') and 7.19 (1H, br s, H-2'''), and the double bond protons (δ 6.23, 1H, *J*=16 Hz, H-8'''), (δ 7.34, 1H, *J*=16 Hz, H-7''') for the *trans*-caffeoyl moiety. In the HMBC spectrum, the caffeoyl carbonyl group (δ 166.93) correlated not only with the double bond protons (δ 6.23, 7.34) but also with the 6-methylene protons (δ 4.08), suggesting that the caffeoyl moiety is connected to the 6-methylene carbon of the galactose. Moreover, the cross peak observed between C-3 (δ 134.11) and the anomeric proton (δ 5.39, d, *J*=8.0 Hz) showed that the

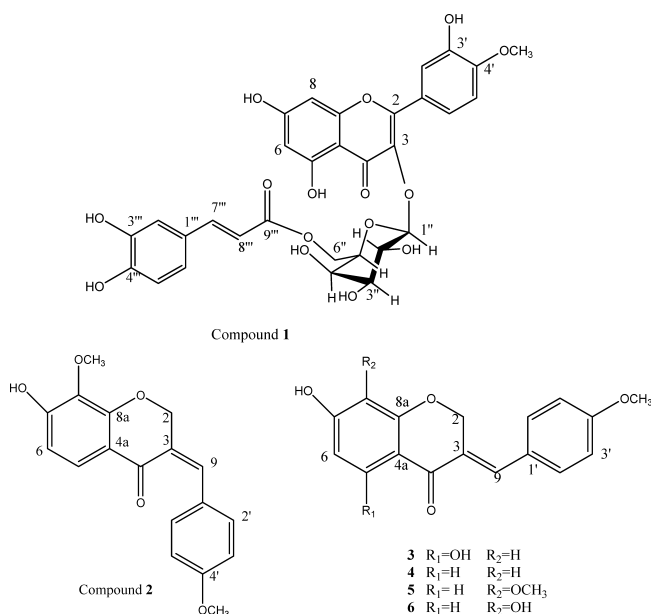


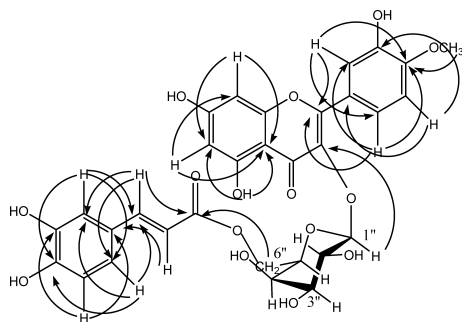
Fig. 1. Structures of Compounds 1–6

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Table 1. ^{13}C - and ^1H -NMR (500 MHz, 125 MHz, $\text{DMSO-}d_6$) Data of Compound **1**

No.	Isorhamnetin 3- <i>O</i> -(6''- <i>O</i> - <i>E</i> -caffeoyl)- β - <i>D</i> -galactopyranoside ^{a)}		1	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
2	157.3	—	156.93	—
3	133.7	—	134.11	—
4	177.7	—	178.12	—
5	161.4	—	161.84	—
6	99.2	6.12 br s	99.35	6.10 (d, 2.0 Hz)
7	166.3	—	164.78	—
8	93.9	6.27 br s	94.13	6.34 (d, 2.0 Hz)
9	157.0	—	156.93	—
10	103.7	—	104.50	—
1'	121.5	—	121.74	—
2'	113.0	7.82 (d, 1.5 Hz)	116.57	7.50 (d, 2.0 Hz)
3'	146.9	—	145.55	—
4'	149.4	—	149.17	—
5'	112.8	6.80 (m)	115.88	6.80 (d, 8.5 Hz)
6'	122.5	7.54 (dd, 8.5, 1.5 Hz)	122.65	7.65 (dd, 8.5, 2.0 Hz)
1''	102.7	5.24 (d, 7 Hz)	102.29	5.39 (d, 8.0 Hz)
2''	74.4	3.47 (m)	71.71	3.57 (1H, m)
3''	74.4	3.50 (m)	73.55	3.43 (1H, m)
4''	70.4	3.33 (m)	68.99	3.66 (1H, m)
5''	76.6	3.47 (m)	73.67	3.68 (1H, m)
6''	62.8	4.27 (dd, 2, 11.8 Hz) 4.22 (dd, 7, 11.8 Hz)	63.82	4.08 (2H, m)
1'''	126.2	—	126.14	—
2'''	113.0	6.94 (br s)	111.70	7.19 (d, 2.0 Hz)
3'''	145.3	—	148.55	—
4'''	148.2	—	150.00	—
5'''	115.1	6.78 (m)	116.13	6.76 (d, 8.0 Hz)
6'''	121.8	6.78 (m)	123.73	6.91 (dd, 8.0, 2.0 Hz)
7'''	145.7	7.30 (d, 16 Hz)	145.75	7.34 (d, 16 Hz)
8'''	113.7	5.98 (d, 16 Hz)	114.60	6.23 (d, 16 Hz)
9'''	167.5	—	166.93	—
OMe	55.2	3.89 (s)	56.33	3.79 (s)

a) ^1H - and ^{13}C -NMR assignments in $\text{MeOH-}d_4$.

Fig. 2. Main HMBC Correlations of Compound **1**

site of glycosidation was at the C-3 position of tamarixetin. Compare with the isorhamnetin 3-*O*-(6''-*O*-*E*-caffeoyl)- β -*D*-galactopyranoside,¹⁰ the difference is the substituted B-ring, and it is flavonol 3'-methoxy-4'-hydroxy substituted B-ring. Therefore, the structure was determined as tamarixetin 3-*O*-(6''-*O*-*E*-caffeoyl)- β -*D*-galactopyranoside.

Compound **2** was obtained as yellow powder. The molecular formula was established as $\text{C}_{18}\text{H}_{16}\text{O}_5$ by HR-EI-MS. The IR spectrum showed characteristic absorption (ν_{max} 3500, 2945, 1600, 1510, 1465 cm^{-1}) of an unsaturated homoisoflavone containing hydroxyl group.¹¹ The ^1H -NMR

Table 2. ^{13}C -NMR (125 MHz, $\text{DMSO-}d_6$) Data of Compounds **2—6**

No.	2	5	3	4	6
2	75.12	67.78	67.23	67.52	67.58
3	127.13	128.63	127.36	128.79	129.13
4	181.83	179.77	184.46	179.47	180.15
4a	116.51	115.27	101.89	114.23	115.08
5	122.96	123.06	164.28	129.37	118.35
6	110.73	111.04	96.26	111.09	110.43
7	156.78	156.94	166.83	164.57	152.34
8	134.90	135.03	95.00	102.39	132.69
8a	155.37	155.10	162.14	162.44	150.34
9	139.11	135.46	136.41	135.19	135.17
1'	126.83	126.45	126.36	126.48	126.54
2', 6'	132.80	132.24	132.70	132.24	132.12
3', 5'	113.33	114.29	114.57	114.28	114.29
4'	160.43	160.31	160.77	160.24	160.23
4'-OCH ₃	55.12	55.33	55.55	55.31	55.32
8-OCH ₃	60.13	60.28	—	—	—

Table 3. ^1H -NMR (500 MHz, $\text{DMSO-}d_6$) Data of Compounds **2—6**

No.	2	5	3	4	6
2	5.05	5.41	5.35	5.35	5.35
5	7.49	7.49	—	7.72	7.25
6	6.59	6.61	5.88	6.52	6.55
8	—	—	5.92	6.31	—
9	7.02	7.64	7.70	7.62	7.63
2', 6'	7.89	7.41	7.43	7.39	7.41
3', 5'	6.93	7.04	7.06	7.03	7.05
4'-OCH ₃	3.78	3.81	3.82	3.81	3.81

spectrum of the compound was very informative regarding its structure. In the aromatic region two protons appearing at δ 7.49 (1H, d, $J=9.0$ Hz), δ 6.59 (1H, d, $J=9.0$ Hz), could be assigned to H-5, H-6 respectively. The signals for two protons doublets ($J=7.5$ Hz) at δ 7.89 were suggested for H-2' and H-6' and δ 6.93 for H-3' and H-5'. The signals at δ 7.02 (1H) and δ 5.05 (2H) were assigned to H-9 and H-2 respectively. These signals confirmed the compound **2** as a derivative of 3-benzylidene-4-chromanone.¹² The position of the vinylic proton was indicative of a *cis* (*Z*) double bond at C₃-C₉ as the corresponding signal for *cis* (*Z*)-isomer appears at (δ 6.97) and (δ 4.99).¹³ And the signal for H-2 at δ 5.05 (2H) showed a cross-peak with the signal of H-9 at δ 7.02 (1H) in the NOESY spectrum. The ^1H -NMR spectra also revealed the presence of a hydroxyl (δ 10.34), two methoxyl groups (δ 3.78, 3.70) in the molecule. The hydroxyl group has been placed at C-7 and the methoxyl group at C-8, C-4' on the basis of ^1H -NMR spectrum along with mass spectrum which showed characteristic signals at m/z 146 and 166 due to retroDiels-Alder cleavage of the molecule. The compound **2** was thus characterized as *cis* (*Z*)-7-hydroxy-8-methoxy-3-(4-methoxybenzyl)chroman-4-one which is the *cis* (*Z*) isomer of 8-methoxybonducellin, the structure was further confirmed from its ^{13}C -NMR (see Table 2) and HMBC experimental data. Thus the compound **2** was unambiguously established and named 8-methoxyisobonducellin.

Compounds **3—6** were obtained as yellow needles. Compounds **3—6** gave yellow-orange coloration with a 10% H_2SO_4 reagent on TLC, and showed positive color reactions. They had absorption bands assignable to hydroxyl, conjugated carbonyl groups and aromatic rings in the IR spectra.

The 3-benzylidenechroman-4-one system was established from analyses of their $^1\text{H-NMR}$ spectra and the E -geometry of the $\Delta^{3,9}$ was clearly discerned by the characteristic chemical shifts of the C-9 proton at δ 7.6 and C-2 proton at δ 5.3 ppm.^{11,13} They were characterized as the known compounds: eucomin (**3**),¹¹ bonducellin (**4**),¹³ 8-methoxybonducellin (**5**),¹² and intricatinol (**6**),¹⁴ respectively from their spectral (UV, IR, 1D and 2D NMR and MS) data and by comparison of the values with those reported earlier for the compounds.

Experimental

General Melting points were determined on a Fisher-Johns apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured in DMSO- d_6 on a Bruker AM-500 spectrometer, using TMS as internal standard. NMR experiments included the HMQC, HMBC. Coupling constants (J values) were given in Hz. An Autospec-Ultima ETOF spectrometer was used to record the FAB-MS and HR-FAB-MS. silica gel 60H (400–500 mesh) and silica gel GF₂₅₄ sheets (0.20–0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material The stems of *Caesalpinia millettii* HOOK. et ARN. were collected in Jinghong, Yunnan Province (China) in October 2005, identified by associate Prof. Chao-Zhong PENG. And a voucher specimen (No. 00324971) is deposited at the Institute of Botany, the Chinese Academy of Sciences (CLDIS), Beijing, China.

Extraction and Isolation Dried stems of *C. millettii* HOOK. et ARN. (7 kg) were extracted with acetone under reflux (3 h \times 3), and the extract was evaporated *in vacuo* to afford a crude extract (400g). The crude extract was fractionated by VLC with hexane-CHCl₃-MeOH (1:1:0; 0:1:0; 0:4:1; 0:1:1; 0:0:1) gradient system to give five fractions.

Fr. 2 (15 g) was rechromatographed on silica gel column with hexane-EtOAc (9:1, v/v) to give 85 subfractions. Subfraction 59–69 (87 mg) was further subjected to sephedax LH-20 with MeOH to give **2** (30 mg), **5** (25 mg). Fr. 3 (78 g) was rechromatographed on silica gel column with CHCl₃-MeOH (10:0 \rightarrow 1:1, v/v) to give five subfractions. Fr. 3-2 (500 mg) was subjected to column chromatography with hexane-CHCl₃ (9:1, v/v) to give **3** (46 mg). Similarly, Fr. 3-3 (750 mg) was separated by CC with hexane-EtOAc (9:1, v/v) to give **4** (6 mg). Fr. 3-5 (200 mg) was separated by Sephadex LH-20 (MeOH), followed by column chromatography with hexane-EtOAc (7:3, v/v) to give **6** (37 mg). Fr. 5 (2 g) was separated by Sephadex LH-20 (MeOH) to give **1** (30 mg).

Compound (**1**): Yellow powder (MeOH). IR (KBr): 3250 (OH), 1645, 1604, 1508, 1456, 1361, 1298, 1274, 1197, 1170 cm⁻¹. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 12.61 (5-OH), 10.78 (–OH), 9.70 (–OH), 9.55 (–OH), 9.12 (–OH), 7.65 (1H, dd, $J=8.5$, 2.0 Hz, H-6'), 7.50 (1H, d, $J=2.0$ Hz, H-2'), 7.34 (1H, d, $J=16$ Hz, H-7''), 7.19 (1H, d, $J=2.0$ Hz, H-2''), 6.91 (1H, dd, $J=8.0$, 2 Hz, H-6''), 6.80 (1H, d, $J=8.5$ Hz, H-5'), 6.76 (1H, d, $J=8.0$ Hz, H-5''), 6.34 (1H, d, $J=2.0$ Hz, H-8), 6.23 (1H, d, $J=16$ Hz, H-8''), 6.10 (1H, d, $J=2.0$ Hz, H-6), 5.39 (1H, d, $J=8.0$ Hz, H-1''), 5.18 (–OH), 4.93 (–OH), 4.71 (–OH), 4.08 (1H, m, H-6''), 3.80 (OCH₃), 3.68 (1H, m, H-5''), 3.66 (1H, m, H-4''), 3.57 (1H, m, H-2''), 3.43 (1H, m, H-3''). $^1\text{H-}$ (500 MHz) and $^{13}\text{C-NMR}$ (125 MHz) in DMSO- d_6 see Table 1. HR-FAB-MS (positive-ion) m/z : 663.1311 [M+Na]⁺ (Calcd for C₃₁H₂₈O₅Na: 663.1325).

Compound (**2**): Yellow powder. IR (KBr): 3500 (OH), 2945, 1666 (C=O), 1600 (aromatic), 1510, 1465, 1440, 1396, 1284, 1259, 1178, 1083, 1207, 835, 786 cm⁻¹. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 10.34 (1H, s, 7-OH), 7.89 (2H, d, $J=7.5$ Hz, H-2', 6'), 7.49 (1H, d, $J=9$ Hz, 5-H), 7.02 (1H, s, 9-H), 6.93 (2H, d, $J=7.5$ Hz, H-3', 5'), 6.59 (1H, d, $J=7.5$ Hz, 6-H), 5.05 (2H,

s, 2-H), 3.78 (3H, s, OCH₃), 3.70 (3H, s, OCH₃). $^{13}\text{C-NMR}$ (125 MHz) in DMSO- d_6 see Table 2. HR-EI-MS m/z : 312.0998 [M]⁺ (Calcd for C₁₈H₁₆O₅: 312.0997).

Compound (**3**): Yellow needles (CHCl₃/MeOH); mp 199–201 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 7.70 (1H, s, 9-H), 7.43 (2H, d, $J=8.8$ Hz, 2', 6'-H), 7.06 (2H, d, $J=8.8$ Hz, 3', 5'-H), 5.92 (1H, d, $J=2$ Hz, 8-H), 5.88 (1H, d, $J=2$ Hz, 6-H), 5.35 (2H, s, 2-H), 3.82 (3H, OCH₃). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6) see Table 2. EI-MS m/z : 298 [M]⁺.

Compound (**4**): Yellow needles (CHCl₃/MeOH); mp 207 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 10.61 (7-OH), 7.72 (1H, d, $J=8.0$ Hz, 5-H), 7.62 (1H, s, 9-H), 7.39 (2H, d, $J=8.5$ Hz, 2', 6'-H), 7.03 (2H, d, $J=8.5$ Hz, 3', 5'-H), 6.52 (1H, dd, $J=8.5$, 2 Hz, 6-H), 6.31 (1H, d, $J=2.0$ Hz, 8-H), 5.35 (2H, d, $J=1.5$ Hz, 2-H), 3.81 (3H, s, OCH₃). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6) see Table 2. EI-MS m/z : 282 [M]⁺.

Compound (**5**): Yellow needles (CHCl₃/MeOH); mp 124–125 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 10.36 (–OH), 7.64 (1H, s, 9-H), 7.49 (1H, d, $J=9.5$ Hz, 5-H), 7.41 (2H, d, $J=8.5$ Hz, 2', 6'-H), 7.04 (2H, d, $J=8.5$ Hz, 3', 5'-H), 6.61 (1H, d, $J=9.5$ Hz, 6-H), 5.41 (2H, s, 2-H), 3.81 (3H, OCH₃), 3.70 (3H, OCH₃). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6) see Table 2. EI-MS m/z : 312 [M]⁺.

Compound (**6**): Yellow needles (CHCl₃/MeOH); mp 196–197 °C. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ : 7.63 (1H, s, 9-H), 7.41 (2H, d, $J=9$ Hz, 2', 6'-H), 7.25 (1H, d, $J=9$ Hz, 5-H), 7.05 (2H, d, $J=9$ Hz, 3', 5'-H), 6.55 (1H, d, $J=9$ Hz, 6-H), 5.35 (2H, d, $J=1.5$ Hz, 2-H), 3.81 (3H, s, OCH₃). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6) see Table 2. EI-MS m/z : 298 [M]⁺.

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