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Flavonoids from Twigs of *Millettia leptobotrya* Dunn.

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Abstract: A new furanoisoflavone, 2'-methoxy-4',5'-methylenedioxy-[2",3":7,8] furanoisoflavone, leptobotryanone (**1**), and a new natural *O*-prenylated isoflavone, 4'-*γ*,*γ*-dimethylallyloxy-5,7-dihydroxyisoflavone (**2**), were isolated from the twigs of *Millettia leptobotrya*, together with twelve known flavonoids, 4'-*γ*,*γ*-dimethylallyloxy-5-hydroxy-7-methoxyisoflavone (**3**), 2',6,7-trimethoxy-4',5'-methylenedioxy-isoflavone (**4**), 2',7-dimethoxy-4',5'-methylenedioxyisoflavone (**5**), maximaisoflavone B (**6**), medicarpin (**7**) , maackiain (**8**), genistein (**9**), biochanin A (**10**), prunetin (**11**), chrysoeriol (**12**), kaempferol (**13**) and desmoxyphyllin A (**14**) The structures of new compounds were elucidated on the basis of spectroscopic data interpretation, including 1D and 2D NMR and HREIMS. This is the first phytochemical investigation of this plant.

Keywords: *Millettia leptobotrya* Dunn; Leguminosae; leptobotryanone; isoflavone.

1. Introduction

About two hundred species of *Millettia* (Leguminosae /Fabaceae) were distributed in subtropical and tropical Africa, Asia and Australia, and many species were used as medicinal drugs, insecticide, or for stupefying fish in China [1]. Plants of the genus *Millettia* are well known for elaborating prenylated flavones and isoflavones with annellated furan and pyran rings [2]. *Millettia leptobotry* Dunn is a tree distributed in south of Yunnan Province, China. The roots and leaves of this plant have been used by the local people for the treatment of fracture, traumatic injury and rheumatoid arthritis [3]. There have been no reports on the chemical composition of this plant so far. As part of our continuing studies on bioactive compounds from tropical medicinal plants, we have first examined the twigs of the title plant and isolated a new furanoisoflavone, leptobotryanone (**1**), and a new natural product, 4'-*γ*,*γ*-dimethylallyloxy-5,7-dihydroxyisoflavone (**2**), along with twelve known analogues. In the present paper, we report the isolation and structure elucidation of the new compounds.

2. Materials and Methods

2.1. General

TLC was preformed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China), and the spots were detected with a UV_{254} lamp and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. Column chromatography was performed using silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), reverse-phase C18 silica gel (40-63 μm, Merck, Darmstadt Germany) and Sephadex LH-20 (GE healthcare, Sweden), MCI-gel CHP 20P (75–150 μm;

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Mitsubishi Chemical Co. Japan). UV spectra were measured with a Shimadzu UV-2401 PC spectrophotometer. IR spectra were recorded on a Bruker Tensor-27 infrared spectrophotometer with KBr pellets. All NMR experiments were performed on a Bruker AM-400 and DRX-500 instruments with TMS as the internal standard. HREIMS spectra were recorded on a Waters AutoSpec Premier P776 instrument.

2.2. Plant Material

The twigs of *Millettia leptobotrya* were collected from Xishuangbanna, Yunnan Province, P.R. China in February 2012, and authenticated by Prof. Hong Wang, herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No 20120202) was deposited in the ethnobotany research group of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

2.3. Extraction and Isolation

The sun dried and powdered twigs of *M. leptobotrya* (6.5 kg) were extracted three times by maceration with 95% EtOH at room temperature, to afford crude extract after evaporation under vacuum. The crude extract was suspended in water and successively extracted with chloroform ($CHCl_3$) and ethyl acetate (EtOAc). The combined $CHCl_3$ extract was evaporated to give a deep-brown gum (148 g), which was separated on a silica gel column chromatography (CC) using petroleum ether (PE)-EtOAc step-gradient elution (9:1→ 4:6) to yield 5 fractions (C1, C2, C3, C4, C5). The fraction C1 (2.5 g 9:1) was further separated by reverse-phase C18 silica gel (RP-18) chromatography (eluted with 80–90% MeOH) and obtained the compound **3** (47 mg). The fraction C2 (18 g 8:2) was subjected to a silica gel CC again using PE - EtOAc (9:1, 85:15, 8:2, 6:4). The fraction (85:15) was applied to RP-18 CC (eluted with 70–90% MeOH/H_2O) and yielded the compounds **1** (17 mg) and **2** (22 mg). The part (8:2) was recrystallized to yield compounds **6** (32 mg). The part (6:4) was further purified by Sephadex LH-20 (MeOH) to afford compounds **7** (21 mg) and **8** (11 mg). The fraction C3 (15 g 7:3) was submitted to silica gel CC using PE-EtOAc (4:1–7:3–4:6) as elute. Compound **5** (25 mg) was obtained from the part (4:1) and the mixture of **10** and **11** (51 mg) was obtained from the part eluted (4:6) and further separated by RP-18 CC (eluted with 70–80% MeOH/H_2O). The fraction C4 (12 g 6:4) was subjected to silica gel CC eluted with $CHCl_3$-MeOH (25: 1) to yield **4** (19 mg) and **9** (24 mg). The fraction C5 (11 g 4:6) was applied to silica gel CC eluted with $CHCl_3$-MeOH (15: 1→10: 1) to afford **12** (19 mg), **13** (36 mg) and **14** (15 mg).

leptobotryanone (**1**): Pale yellow amorphous powder, UV (MeOH): λ_{max} nm (log ε): 304 (3.92), 234 (4.37); IR (KBr):υ_{max}: 1641, 1584, 1504, 1461, 1405, 1346, 1268, 1194 cm^{-1}. HREIMS: *m/z* 336.0629 (calc. for $C_{19}H_{12}O_6$, 336.0634). ^1H and ^{13}C NMR see the Table 1.

4'-γ,γ-dimethylallyloxy-5,7-dihydroxyisoflavone (**2**): Colorless amorphous powder; UV (MeOH): λ_{max} nm (log ε): 326 (sh) (3.49), 266 (4.40); IR (KBr): υ_{max} : 3440, 2854, 1652, 1617, 1577, 1516, 1436, 1370, 1284, 1245, 1181 cm^{-1}. HREIMS: *m/z* 338.1161 (calc. for $C_{20}H_{18}O_5$, 338.1154). ^1H and ^{13}C NMR see the Table 1.

3. Results and Discussion

Repeated column chromatography (including normal-phase silica gel, RP-18 silica gel and Sephadex LH-20) of the EtOH extract of the twigs of *M. leptobotry* has led to the isolation of a new furanoisoflavone, leptobotryanone (**1**), and a new natural product, 4'-*γ*,*γ*-dimethylallyloxy-5,7-dihydroxyisoflavone (**2**), together with twelve known flavonoids, 4'-*γ*,*γ*-dimethylallyloxy-5-hydroxy-7-methoxyisoflavone (**3**) [4], 2',6,7-trimethoxy-4',5'-methylenedioxyisoflavone (**4**) [5], 2',7-dimethoxy-4',5'-methylenedioxyisoflavone (**5**) [6], maximaisoflavone B (**6**) [7], medicarpin (**7**) [8], maackiain (**8**) [9], genistein (**9**) [10], biochanin A (**10**) [11], prunetin (**11**) [12], chrysoeriol (**12**) [13], kaempferol (**13**) [13] and desmoxyphyllin A (**14**) [14].The structures of the known compounds (**3-14)**

were characterized on the basis of spectral data and comparison with those reported in the literature. All compounds (Fig. 2) were isolated from *Millettia leptobotrya* for the first time, and compound **2** was the first example as natural product.

Compound **1** was obtained as pale yellow amorphous powder. The HREIMS exhibited a molecular ion peak at *m/z* 336.0629 (calcd. 336.0634), suggesting the molecular formula of $C_{19}H_{12}O_6$. The IR spectrum exhibited strong absorption band of a conjugated carbonyl at 1641 cm^{-1}. The UV spectrum showed strong absorptions at λ 304 and 234 nm. The ^{13}C NMR and DEPT spectrum revealed nineteen carbon signals corresponding to one methoxyl, one methylene, seven methine and ten quaternary carbons (including one carbonyl).The ^{1}H and ^{13}C NMR spectra exhibited characteristic signals at δ_H 8.04 for (1H, *s*, H-2) and δ_C 153.7 (C-2), suggesting the existence of isoflavone skeleton [15] (Table 1). In addition, ^{1}H and ^{13}C NMR signals also revealed the presence of a furan ring at δ_H 7.14 (1H, *d*, *J* = 1.2 Hz, H-4")/δ_C 104.2 (C-4") and δ_H 7.75 (1H, *d*, *J* = 2.4 Hz, H-5")/δ_C 145.6 (C-5") as well as one methoxyl group at δ_H 3.74 (3H, *s*) /δ_C 56.8 and a methylenedioxy moiety at δ_H 5.97 (2H, *s*) /δ_C 101.4.

The appearance of H-5 and H-6 as doublets at δ_H 8.23 (1H, *d*, *J* = 8.8 Hz) and 7.56 (1H, *d*, *J* = 8.8 Hz) and the HMBC correlations of H-5 with C-4 (δ_C 176.2) and C-7 (δ_C 158.1) indicated that the furan ring should be fused in an angular position at C-7 (oxygenated) and C-8 [16], which was supported by the HMBC correlations of H-4" and H-5" with C-7 and H-6 and H-5" with C-8 (δ_C 117.0). The relative positions of substituted groups on ring B were determined on the basis of the HMBC spectrum (Fig. 1). The methoxyl group was placed at C-2′ as its proton (δ_H 3.74) showed ^{3}J correlation with C-2′ (δ_C 152.9) in the HMBC spectrum. The methylenedioxy unit should be located at C-4′, C-5′, which was confirmed by the HMBC ^{3}J correlations of two protons (OCH_2O) at δ_H 5.97 with C-4' (δ_C 148.5) and 5' (δ_C 141.2). Based on the above spectral evidence, compound **1** was assigned as 2'-methoxy-4',5'-methylenedioxy-[2",3":7,8] furanoisoflavone and has been given the trivial name leptobotryanone.

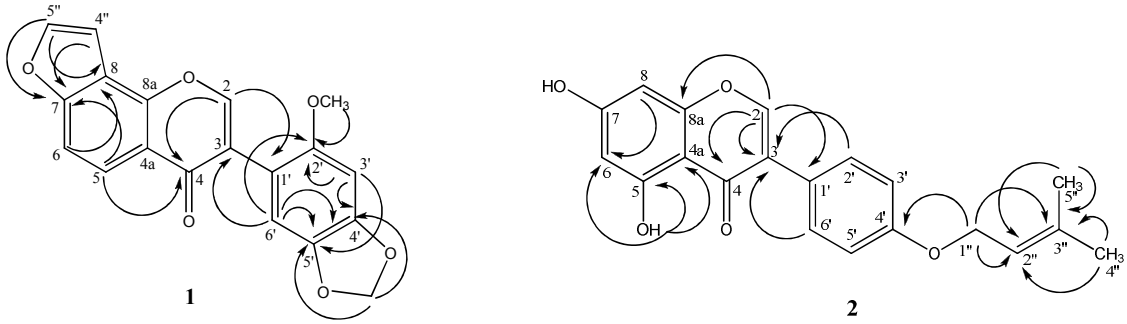

Figure 1. Key HMBC (H→C) correlations of compounds **1** and **2**.

Compound **2** was isolated as colorless amorphous powder. The molecular formula of **2** was determined to be $C_{20}H_{18}O_5$ by HREIMS: *m/z* 338.1161 $[M]^+$, calcd. 338.1154. Its IR spectrum showed the presence of a chelated hydroxyl (3440 cm^{-1}) and a conjugated carbonyl (1652 cm^{-1}) The UV spectrum showed absorptions at λ_{max} 326 (sh) and 266 nm. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) exhibited low-field resonances at δ_H 8.19 (1H, *s*) and δ_C 154.4, which were characteristic of H-2 and C-2, respectively, of an isoflavone nucleus [15]. The HMBC spectrum (Fig. 1) showed the correlations of H-2 (δ_H 8.19) with C-3 (δ_C 124.0), C-4 (δ_C 181.5), C-8a (δ_C 159.0) and C-1' (δ_C 123.7), confirming its isoflavone structure.

In the ^{1}H NMR spectrum, a downfield signal at δ_H 13.01 was assigned to OH-5 by the HMBC correlations of δ_H 13.01 with C-4a (δ_C 106.0), C-5 (δ_C 163.8) and C-6 (δ_C 99.8); two *meta*-coupled aromatic protons at δ_H 6.28 and 6.41 (each 1H, *d*, *J*=1.6 Hz) were attributed to H-6 and H-8 in ring A, respectively. Moreover, two sets of *ortho*-coupled doublets (*J* = 8.4 and 8.8 Hz) at δ_H 6.98 and 7.52, integrating for two protons each, were attributed to H-3', H-5' and H-2', H-6', respectively, indicating the presence of a *para*-disubstituted ring B in compound **2**. The signals at δ_H 5.47 (1H, *t*, *J* = 6.7 Hz), 4.59 (2H, *d*, *J* = 6.4 Hz), 1.77 (3H, *s*) and 1.75 (3H, *s*), assigned to methine, methylene and *gem*-

dimethyl protons, respectively, revealed the presence of an oxyprenyl residue in **2** [17]. The oxyprenyl moiety was connected to C-4' as the oxygenated methylene protons at δ_H 4.59 (CH_2-1") correlated to C-4' (δ_C 159.8) of the isoflavone nucleus in the HMBC spectrum. Beside the partial structures mentioned above, the molecular formula $C_{20}H_{18}O_5$ requires a hydroxyl group which should be connected with C-7, which was a quaternary carbon and chemical shift appeared at δ_C 165.1 ppm in the low magnetic field. Therefore, compound **2** was identified as 4'-*γ*, *γ*-dimethylallyloxy-5,7-dihydroxyisoflavone.

Although compound **2** has been reported synthetically [18], this is the first time that it has been found as a naturally occurring compound and fully characterized. Prior to this study, the ^1H and ^{13}C NMR data of **2** have not been reported.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** (in $CDCl_3$) and **2** in (Me_2CO-d_6).

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2	8.04 (1H, *s*)	153.7	8.19 (1H, *s*)	154.4
3		122.7		124.0
4		176.2		181.5
4a		119.9		106.0
5	8.23 (1H, *d*, *J* = 8.8)	122.5		163.8
6	7.56 (1H, *d*, *J* = 8.8)	110.1	6.28 (1H, *d*, *J* = 1.6)	99.8
7		158.1		165.1
8		117.0	6.41 (1H, *d*, *J* = 1.6)	94.4
8a		156.3		159.0
1'		112.6		123.7
2'		152.9	7.52 (1H, *d*, *J* = 8.8)	131.0
3'	6.64 (1H, *s*)	95.4	6.98 (1H, *d*, *J* = 8.4)	115.2
4'		148.5		159.8
5'		141.2	6.98 (1H, *d*, *J* = 8.4)	115.2
6'	6.86 (1H, *s*)	111.2	7.52 (1H, *d*, *J* = 8.8)	131.0
1"			4.59 (2H, *d*, *J* = 6.4)	65.3
2"			5.47（1H, *t*, *J* = 6.7）	121.0
3"				137.8
4"	7.14 (*d*, *J* = 1.2)	104.2	1.75 (3H, *s*)	25.8
5"	7.75 (*d*, *J* = 2.4)	145.6	1.77 (3H, *s*)	18.2
5-OH			13.01 (1H, *s*)	
2'-OMe	3.74 (3H, *s*)	56.8		
OCH_2O	5.97 (2H, *s*)	101.4		

Repeated column chromatography (including normal-phase silica gel, RP-18 silica gel and Sephadex LH-20) of the EtOH extract of the twigs of *Millettia leptobotry* has led to the isolation of a new furanoisoflavone, leptobotryanone and a new natural product, 4'-*γ*,*γ*-dimethylallyloxy-5,7-dihydroxyisoflavone, together with twelve known flavonoids, including two *O*-prenylated isoflavones, five simple isoflavones, two simple flavones, two pterocarpans and one coumaronochromone. This study reveals that isoflavone is the main type of flavonoid compound that could be considered chemomarker of the genus *Millettia*.

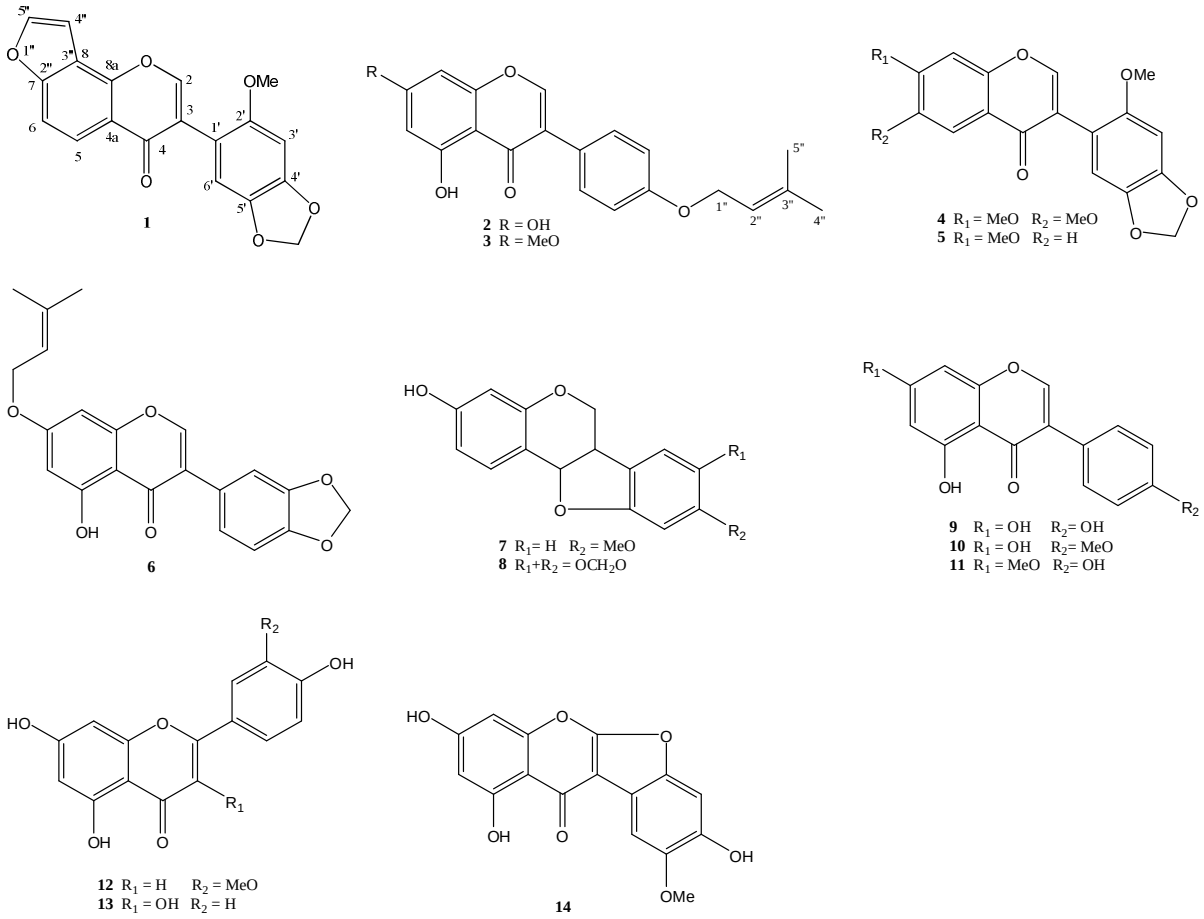

Figure 2. Structures of compounds **1-14**.

Acknowledgments

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] Editorial Committee of Flora Reipublicae Popularis Sinicae (1994). Flora of China, Science Press, Beijing, Vol. **40**, pp.135.

[2] F. A. Bisby, J. Buckingham and J. B. Harborne (1994). Phytochemical Dictionary of the Leguminosae, Chapman & Hall, London, pp. 587.

[3] Z. Y. Wu, T. Y. Zhou and P. G. Xiao (1998). Xin Hua Compendium of Materia Medica, Shanghai Science & Technology Press, Shanghai, Vol. **2**, pp. 165.

[4] Z. J. Huang, J. X. Yang, Z. G. She and Y. C. Lin (2012). A new isoflavone from the mangrove endophytic fungus *Fusarium* sp. (ZZF60), *Nat. Prod. Res.* **26**, 11-15.

[5] D. D. Marquesa, M. I. L. Machadoa, M. G. de Carvalhob, L. A. C. Meleirab and R. Braz-Filhoc (1998). Isoflavonoids and triterpenoids isolated from *Pterodon polygalaeflorus*, *J. Braz. Chem. Soc.* **9**, 295-301.
[6] E. Galina and O. R. Gottlieb (1974). Isoflavones from *Pterodon apparicioi*, *Phytochemistry* **13**, 2593-2595.
[7] E. V. Rao and M. S. R. Murthy (1985). Further studies on the isoflavones of *Tephrosia maxima*, *Phytochemistry* **24**, 875-876.
[8] K. Rayanil, P. Bunchornmaspan and P. Tuntiwachwuttikul (2011). A new phenolic compound with anticancer activity from the wood of *Millettia leucantha*, *Arch. Pharm. Res.* **34**, 881-886.
[9] E. Bedira, I. Çalis, R. Aquino, S. Piacente and C. Pizza (1999). Trojanoside H: a cycloartane-type glycoside from the aerial parts of *Astragalus trojanus*, *Phytochemistry* **51**, 1017-1020.
[10] J. Feng, C. Xiang and H. Liang (2007). Chemical constituents of isoflavones from vine stems of *Millettia nitita* var. *hirsutissima*, *Chin. J. Chin. Mat. Med*. **32**, 321-322.
[11] Y. R. Deng, T. Wang and Y. Z. He (2008). Studies on chemical constituents of *Caragana spinifera*, *Chin. J. Chin. Mat. Med*. **33**, 775-777.
[12] S. Y. Huang and P. F. Tu (2007). Isolation and identification of isoflavones from *Trifolium pratense*, *Acta Sci. Natur. Univ. Pekinensis.* **40**, 544-549.
[13] A. Liu, L. Z. Xu, Z. M. Zhou and S. L. Yang (2009). Studies on chemical constituents from leaves of *Cassia alata*, *Chin. J. Chin. Mat. Med.* **34**, 861–863.
[14] M. Mizuno, K. Baba, M. Linuma and T. Tanaka (1992). Coumaronochromones from leaves of *Desmodium oxyphyllum*, Phytochemistry. **31**, 361-363.
[15] C. Ito, M. Itoigawa, N. Kojima, H. Tokuda, T. Hirata, H. Nishino and H. Furukawa (2004). Chemical constituents of *Millettia taiwaniana*: Structure elucidation of five new isoflavonoids and their cancer chemopreventive activity, *J. Nat. Prod.* **67**, 1125-1130.
[16] B. Sritularak, K. Likhitwitayawuid, J. Conrad, B. Vogler, S. Reeb, I. Klaiber and W. Kraus (2002). New flavones from *Millettia erythrocalyx*, *J. Nat. Prod.* **65**, 589-591.
[17] B. Sritularak and K. Likhitwitayawuid (2006). Flavonoids from the pods of *Millettia erythrocalyx*, Phytochemistry. **67**, 812-817.
[18] T. Ozaki, S. Mishima, M. Nishiyama and T. Kuzuyama (2009). NovQ is a prenyltransferase capable of catalyzing the addition of a dimethylallyl group to both phenylpropanoids and flavonoids, *J. Antibiot* **62**, 385-392.