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New Xanthone from *Millettia pachyloba* Drake

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Abstract: A new xanthone, 1,7-dihydroxy-2,3,5,6-tetramethoxyxanthone (**1**), together with eight known compounds including one xanthone (**2**) and seven isoflavones (**3-9**) has been isolated from the leaves of *Millettia pachyloba*. This is the first report on the isolation of xanthone from the genus *Millettia*. The structure of the compound **1** was elucidated on the basis of spectroscopic data interpretation, including 1D and 2D NMR and HREIMS. Compound **1** exhibited weak cytotoxicity against five human cancer cell lines, HL-60, SMMC-7721, A-549, MCF-7 and SW480.

Keywords: *Millettia pachyloba* Drake; Leguminosae; xanthone.

1. Introduction

The vine stems of several *Millettia* species (Leguminosae), locally known in Chinese herbal medicineas "*ji-xue-teng*," are useful in promoting blood circulation and relieving stasis [1]. Plants of the genus *Millettia* are well known for elaborating prenylated flavones and isoflavones with annellated furan and pyran rings [2], some of which have shown significant biological activities [3-5]. *M. pachyloba* is a climb vine distributed in Guangdong, Guangxi, Hainan and Yunnan Province, P. R. China [6]. Previous investigation on *M. pachyloba*, isoflavone, pterocarpan and rotenone were the main compositions of the plant and exhibited cytotoxicity against KB cells [7-8]. In an earlier report, we described the isolation and elucidation of several flavones, isoflavones and pterocarpan from the vine stem of this plant [9]. During further investigation on active substances, we isolated a new xanthone, 1,7-dihydroxy-2,3,5,6-tetramethoxyxanthone, along with eight known compounds from the leaves of *M. pachyloba*. To the best of our knowledge, this is the first report on the isolation of xanthone from the genus *Millettia*. Herein, we describe the isolation, structural determination and cytotoxicity potential of the new metabolite.

2. Materials and Methods

2.1. General

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. The chemical shifts were given in δ (ppm) scale with reference to the solvent signal. HREIMS spectra were recorded on a Waters AutoSpec Premier P776 instrument. Column chromatography was performed using

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silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), reversed-phase C_{18} silica gel (40-63 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (GE healthcare, Sweden). Fractions monitored by TLC, and the spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

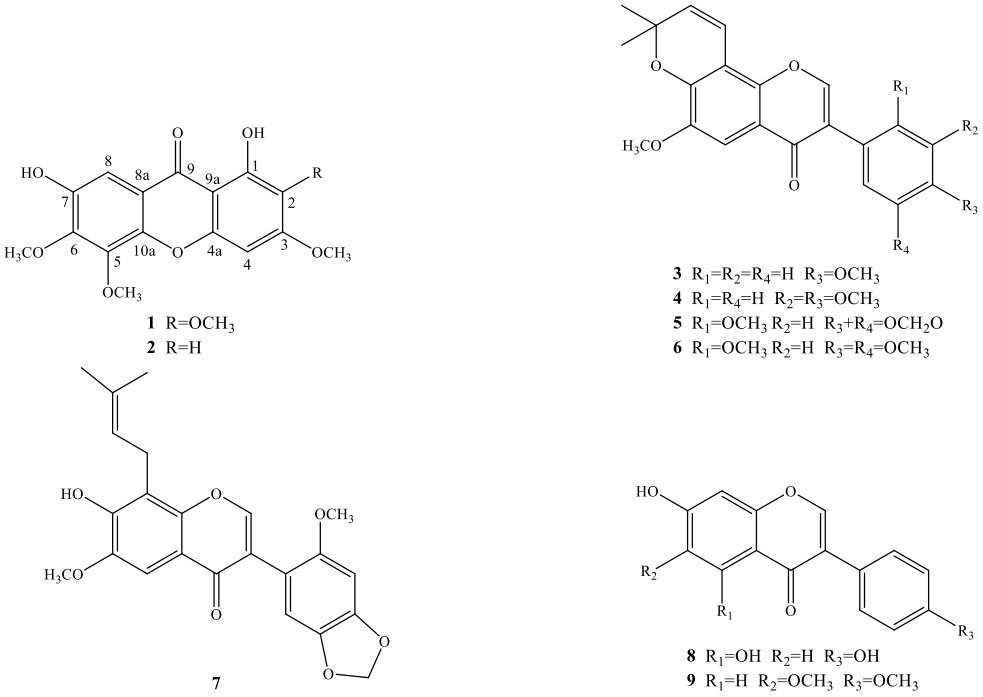

Figure 1. Structures of compounds **1-9**.

2.2. Plant Material

The leaves of *M. pachyloba* were collected from Xishuangbanna, Yunnan Province, P. R. China in August 2016, and authenticated by Dr. Yun-Hong Tan, herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. 20160801) was deposited in the ethnomedicine research group of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

2.3. Extraction and Isolation

The sun dried and powdered leaves of *M. pachyloba* (3.0 kg) were extracted three times by maceration with 95% ethanol at room temperature, to afford crude extract (265 g) after evaporation under vacuum. The crude extract was suspended in water and successively extracted with petroleum ether, chloroform and ethyl acetate, respectively. The petroleum ether extract (75g) and chloroform extract (86g) showed similar thin-layer chromatography; hence they were combined and subjected to silica gel column chromatography (CC) with petroleum ether–ethyl acetate step-gradient elution (v/v 9:1→3:7) to yield compound **3** (75 mg) and six fractions (Fr_1–Fr_6). The Fr_2 (7.8 g) was chromatographed on silica gel column using a gradient solvent petroleum ether–ethyl acetate (4:1, 3:1, 2:1, 6:4) to give four subfractions Fr_{2-1}–Fr_{2-4}. The Fr_{2-1} was subsequently purified by C18 CC eluted with MeOH–H_2O (70→90%) to give compounds **1** (21 mg) and **2** (32 mg). The Fr_{2-3} was further purified by CC on Sephadex LH-20 eluted with MeOH to afford compound **8** (18 mg). The Fr_3 (9.5 g) was purified over columns of silica gel eluted with $CHCl_3$–MeOH (40: 1, 20:1, 10: 1) to afford compound **5** (35 mg) and three subfractions Fr_{3-1}–Fr_{3-3}. The Fr_{3-1} was subjected to C18 CC eluted with MeOH–H_2O (60→90%) to obtain compound **4** (30 mg). The Fr_{3-2} was followed by Sephadex LH-20 CC eluted with MeOH to yield compound **9** (32 mg). The Fr_4 (4.7 g) was further separated by C18 CC eluted with MeOH–H_2O (60→90%) to afford compounds **6** (36 mg) and **7** (24 mg).

2.4. Spectroscopic Data

1,7-dihydroxy-2,3,5,6-tetramethoxyxanthone (**1**): Yellow amorphous powder, UV (MeOH): λ_{max} (log ε): 365 (3.50), 312 (3.89), 260 (4.18), 238 (4.10) nm; IR (KBr):ν_{max}: 3380, 2960, 1658, 1567, 1478 cm^{-1}. EIMS *m/z* (rel. int.): 348 $[M]^+$ (90), 333(100), 305(25), 282(22), 268(48), 132(21). HREIMS: *m/z* 348.0841 (calc. for $C_{17}H_{16}O_8$, 348.0845). ^1H and ^{13}C NMR ($CDCl_3$, 500/125 MHz) see Table 1.

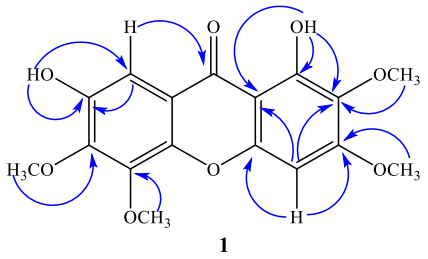

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Figure 2. Key HMBC (H→C) correlations of compound **1**.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as yellow amorphous powder. The HREIMS exhibited a molecular ion at *m/z* 348.0841 (calcd. 348.0845), suggesting the molecular formula $C_{17}H_{16}O_8$. The IR spectrum showed the presence of hydroxyl group at 3380 cm^{-1} and a conjugated carbonyl at 1658 cm^{-1}.

Table 1. ^1H NMR and ^{13}C NMR data of **1** and **2**

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		154.1		163.3
2		131.8	6.35 (1H, *d*, 2.25)	97.2
3		159.7		166.4
4	6.54(1H, *s*)	90.6	6.48 (1H, *d*, 2.25)	92.7
4a		153.3		157.5
5		139.7		139.7
6		145.7		145.7
7		145.6		145.5
8	7.50 (1H, *s*)	103.6	7.49 (1H, *s*)	103.7
8a		116.4		116.6
9		180.4		180.0
9a		103.9		103.5
10a		145.2		145.2
1-OH	12.82 (1H, *s*)		12.88 (1H, *s*)	
7-OH	5.78 (1H, *s*)		5.76 (1H, *s*)	
2-OCH_3	3.92 (3H, *s*)	61.0		
3-OCH_3	3.98 (3H, *s*)	56.4	3.89 (3H, *s*)	55.8
5-OCH_3	4.06 (3H, *s*)	62.0	4.06 (3H, *s*)	61.9
6-OCH_3	4.17 (3H, *s*)	61.5	4.17 (3H, *s*)	61.5

*500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR in $CDCl_3$, δ in ppm, *J* in Hz).

The UV spectrum had maxima absorptions at 238, 260, 312 and 365 nm. The ^1H NMR spectrum of compound **1** (Table 1) showed the presence a chelated hydroxyl at δ_H 12.82, four methoxyl groups at δ_H 3.92, 3.98, 4.06 and 4.17, two singlet aromatic protons at δ_H 6.54 (1H, s) and 7.50 (1H, s), and an additional non-chelated hydroxyl group at δ_H 5.78. The ^{13}C NMR spectrum showed 17 carbon resonances assigned to a carbonyl group (δ_C 180.4), ten quaternary aromatic carbons and two aromatic CH groups (δ_C 90.6 and 103.6); and four methoxyl groups (δ_C 56.4, 61.0, 61.5 and 62.0), The ^1H and ^{13}C NMR data

suggested a xanthone-like structure [10]. In fact, the ^{1}H NMR spectral properties of 1 was very similar to those of 1,7-dihydroxy-3,5,6-trimethoxyxanthone (**2**) except for the presence of an additional methoxy resonance and absence of a singlet proton signal in **1**. As a result, **1** should have a dihydroxy-tetramethoxyxanthone skeleton. To unequivocally corroborate the substitution pattern of **1**, the ^{1}H- and ^{13}C-NMR spectra of the known compound **2** were also listed in Table 1. The additional methoxyl group had to be at the position C-2, C-4 or C-8. The most deshielded aromatic proton (δ_H 7.50) was assigned to C-8 as it showed a HMBC ^{3}J correlation (Figure 2) to the carbonyl carbon. Another aromatic proton at δ_H 6.54 showed four HMBC correlations (^{2}J: C-3, C-4a; ^{3}J: C-2, C-9a), which confirmed C-4 was unsubstituted position. The fourth methoxyl hence had to attach to C-2. This conclusion was further confirmed by a HMBC ^{3}J correlation between the methoxyl protons (δ_H 3.92) and C-2 (δ_C 131.8). Based on the above mentioned evidence, the structure of **1** was determined as 1,7-dihydroxy-2,3,5,6-tetramethoxyxanthone.

Additionally, one known xanthone and seven known isoflavones were also isolated and identified as 1,7-dihydroxy-3,5,6-trimethoxyxanthone (**2**) [11], 6-methoxycalpogonium isoflavone A (**3**) [12], durallone (**4**) [13], ichthynone (**5**) [14], millesianin C (**6**) [12], millesianin D (**7**) [15], genistein (**8**) [16] and afromosin (**9**) [17], respectively. The structures of the known compounds were characterized on the basis of spectral data and comparison with those reported in the literature.

The genus *Millettia* (Leguminosae) is a rich source of flavonoids [2]. However, this is the first isolation of xanthones (**1**-**2**) from the genus *Millettia*. Compounds **1** and **2** have not been previously isolated from any species of *Millettia*, while **2** has ever been reported to occur in *Polygala nyikensis* (Polygalaceae) [11]. Only a few xanthones and anthraquinones have been obtained from several other species of Leguminosae, such as *Caesalpinia sappan* [18], *Cassia fistula* [19], *Cassia sieberiana* [20] *Cyclopia subternata* [21], *Dalbergia sissoides* [22], *Hedysarum denticulatum* [23]. In conclusion, flavonoids are important chemical characteristics of the *Millettia* species. Regarding xanthones, they are rarely found in genus *Millettia*. This phytochemical investigation of *M. pachyloba* has shown that its chemistry differs from other species of *Millettia*. This study acts as foundation for further chemotaxonomic studies on the genus.

3.2. Cytotoxicity

Cytotoxicity of compound **1** was evaluated against human leukemia (HL-60), hepatoma (SMMC-7721), lung carcinoma (A549), breast adenocarcinoma (MCF-7) and colon adenocarcinoma (SW480) cell lines by the MTT assay [24] with *cis*-platinum (MW 300) and taxol as positive reference substance. Compound **1** showed weak cytotoxicity against five cancer cell lines with IC_{50} values over 40 μM (Table S1 in supporting information).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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