SHORT REPORT

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Neoflavonoids from the Heartwood of *Dalbergia melanoxylon*

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Abstract: A new neoflavonoid, (1*R*, 8*R*, 9*R*)-pterolinuse K (**1**) and six known neoflavonoids (**2-7**) were obtained from the heartwood of *Dalbergia melanoxylon*. The structure of the new neoflavonoid was elucidated by extensive NMR investigation, and X-ray crystallographic analysis. Compounds **3** and **6** showed anti-inflammatory activity with IC_{50} values 23.14 ± 0.30 and 19.46 ± 1.02 μM, respectively. Compounds **2-4, 6, 7** were showed cytotoxicity on Caco-2, MDA-MB-468, MDA-MB-231, CT26 cell lines. Moreover, compounds **2**, **4** exhibited the significant activity in MDA-MB-231 cell lines with IC_{50} values 7.54 ± 1.50 and 7.23 ± 0.40 μM, respectively.

Keywords: *Dalbergia melanoxylon;* neoflavonoids; anti-inflammatory activity; anti-tumor activity.

1. Plant Source

The heartwoods of *Dalbergia melanoxylon* Guill. & Perr. (*D. melanoxylon*) were purchased from Fang Cheng Gang market, Guangxi Province, China, in July 2014 and identified by Professor Feng Xu at the product quality inspection center of Guangxi University. A voucher specimen (No. Liu-20140702) was deposited in the Key Laboratory of Innovation Drug and Efficient Energy-saving Pharmaceutical Equipment, Jiangxi University of Traditional Chinese Medicine.

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2. Previous Studies

Neoflavonoids were belong to the flavonoids class with the structural of C6-C3-C6, it contains 4-arylcoumarins, 4-arylchromanes, dalbergiones, and dalbergiquinols [1]. The neoflavonoids were reported to display a variety of pharmacologicalactivities，for example anti-osteoporosis [2]，anti-inflammatory [3]，anti-tumor [4]，anti-androgen [5] and cardioprotective effects [6-9]. *D. melanoxylon* belongs to the family Leguminosae and subfamily Papilionaidae, is a heavily branched deciduous tree [10]. It has a wide range of occurrence in sub-Saharan Africa [11]. *D. melanoxylon* have been used for treating abdominal pain, gonorrhoea, joint pain and bronchitis [12-13].

3. Present Study

The powdered heartwood of *D. melanoxylon* (50.0 kg) was extracted by infusion with 70% ethanol at roomtemperature (24h, 3 times). Next, the extraction was filtered and the solvent was evaporated under reduced pressure in a rotary evaporation equipment (Buchi, Switzerland). And then, the obtained extract (13.9 kg) was dissolved in distilled H_2O and successively partitioned with CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 portion (8.5 kg) was subjected to silica gel CC (column chromatography) using petroleum ether-EtOAc (from 50:1 to 1:5, *v*/*v*) as the elution to yield 22 fractions (Frs.1-Frs.22). Frs.7 (447.4 g) was purified by silica gel column to give six fractions (Frs.7.A-Frs.7.F), through gradient elution with changing ratios of CH_2Cl_2-MeOH from 100:1-10:1(*v*/*v*). Frs.7.C (196.9g) was purified by Sephadex LH-20 CC eluted with CH_2Cl_2-MeOH (1:1, *v*/*v*) to yield three fractions (Frs.7.C.1-Frs.7.C.3). Frs.7.C.2 (7.5 g) was separated by silica gel column (petroleum ether-acetone, 20:1-5:1) to yield **3** (1.7 g). Frs.7.C.3 (7.5 g) was separated by silica gel column (petroleum ether-acetone, 20:1-2:1) to yield **4** (11.2 g). Frs.9 (96.9 g) was fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 400:1-10:1, *v*/*v*) to yield three fractions (Frs.9.A-Frs.9.C). Frs.9.B (45.8 g) was further fractionated *via* silica gel CC eluted with petroleum ether-acetone (from 10:1-2:1, *v*/*v*) to obtain **5** (16.1 mg). Frs.13 (227.6 g) was fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 100:1-10:1, *v*/*v*) to yield three fractions (Frs.13.A-Frs.13.C). Frs.13.B (89.3 g) was further fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 200:1-100:1, *v*/*v*) to obtain **2** (20.1 g). Frs.14 (303.7 g) was loaded ODS column chromatography with MeOH-H_2O gradient elution to give (from 30:70 to 50:50, *v*/*v*) to yield 9 fractions (Frs.14.A-Frs.14.I). Frs.14.C (5.4 g) was further fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 1000:1-100:1, *v*/*v*) to obtain **6** (56.8 mg). Frs.14.I (23.6 g) was fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 100:1-10:1, *v*/*v*) to yield 4 fractions (Frs.14.I.1-Frs.14.I.4). Frs.14.I.2 (953.5 mg) was further fractionated *via* silica gel CC eluted with CH_2Cl_2-MeOH (from 1000:1-200:1, *v*/*v*) to obtain **7** (58.2 mg).Frs.15 (83.2 g) was loaded ODS column chromatography with MeOH-H_2O gradient elution to give (from 30:70 to 50:50, *v*/*v*) to yield 5 fractions (Frs.15.A-Frs.15.E). Frs.15.A(1.8 g) was purified by Sephadex LH-20 CC eluted with CH_2Cl_2-MeOH (1:1, *v*/*v*) to yield 2 fractions (Frs.15.A.1-Frs.15.A.2). Frs.15.A.1 (452.7 mg) was separated by preparative HPLC eluted with MeOH-H_2O (32:68, *v*/*v*) to yield **1** (20.5 mg, t_R = 36.4 min).

*(1R, 8R, 9R)-pterolinuse K (**1**)*: Colorless crystals (MeOH); $[\alpha]_D^{24}$ = + 22.7 (*c* = 0.1, MeOH). UV (MeOH) λ_{max}: 290, 250, 240 nm, IR (KBr) ν_{max} 3372.2, 1660.1, 1453.1, 1452.9, 1413.3 cm^{-1}. CD (MeOH) λ_{max} ($\Delta\varepsilon$) 234 (+1.41), 283 (+1.34); HR-ESI-MS *m/z* 303.1224 ($[M+H]^+$ calcd for $C_{17}H_{19}O_5$, 303.1227). ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz): see Table S1 in supporting information.

Neoflavonoids from *Dalbergia melanoxylon*

In our ongoing project of the investigation on the chemical constituents and bioactive of *D. melanoxylon*, one new neoflavonoid (**1**) and six known neoflavonoids (**2-7**) were obtained from the heartwood of *D. melanoxylon* (Figure 1).

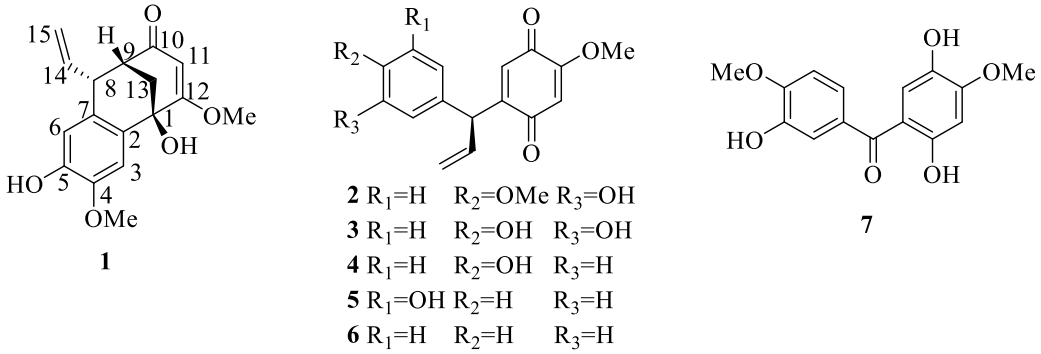
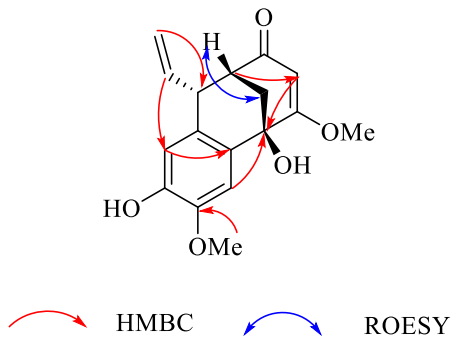


Figure 1. Structures of compounds **1**-**7**

Compound **1** was a colorless crystals, with a molecular formula of $C_{17}H_{18}O_5$ as deduced from the (+)-HR-ESI-MS *m/z* 303.1224 ($[M+H]^+$ calcd for $C_{17}H_{19}O_5$, 303.1227), inferring 9 degree of unsaturation. The ^{1}H-NMR spectrum exhibited the signals (Table 1) of two hydroxyls at δ_H 5.89 (1H, s, 1-OH) and 8.93 (1H, s, 5-OH), two methoxy groups at δ_H 3.62 (3H, s, 12-OCH_3) and 3.73 (3H, s, 4-OCH_3), two aromatic protons at δ_H 7.15 (1H, s, H-3) and 6.51 (1H, s, H-6), four olefinic protons at δ_H 5.14 (1H, s, H-11), 5.35 (1H, ddd, *J* = 16.9, 10.1, 8.1 Hz, H-14), 5.20 (1H, d, *J* = 16.9 Hz, H-15a) and 5.14 (2H, m, H-15b), two methines at δ_H 3.68 (1H, t, *J* = 8.1 Hz, H-8) and 2.83 (1H, s, H-9), diastereotopic methylene protons at δ_H 2.44 (1H, d, *J* = 12.0 Hz, H-13a) and 2.15 (1H, d, *J* = 12.0 Hz, H-13b). Inspection of its ^{13}C-NMR spectra exhibited17 carbon resonances assignable to two methoxy groups at δ_C 56.9 (4-OCH_3)and 56.2 (12-OCH_3), six aromatic carbons at δ_C 132.0 (C-2), 109.2 (C-3), 146.4 (C-4), 146.3 (C-5), 115.8 (C-6) and 127.7 (C-7), two double bonds at δ_C 100.6 (C-11), 183.3 (C-12), 140.0 (C-14) and 117.2 (C-15), four methines at δ_C 69.5 (C-1), 45.0 (C-8), 48.8 (C-9) and 40.7 (C-13), one conjugated ketone at δ_C 197.5 (C-10) (Table 1). These data were similar with those of (1*S*, 8*R*, 9*S*)-1, 5-dihydroxy-4,12-dimethoxy-8-vinyl-tricyclo[7.3.1.0^{2,7}]trideca-2,4,6,11 tetraen-10-one skeleton [14]. The HMBC correlation of H-13a (δ_H 2.44) and H-13b (δ_H 2.15) with C-2 (δ_C 132.0), C-8 (δ_C 45.0), C-10 (δ_C 197.5) and C-12 (δ_C 183.3) have confirmed the methylene was linked to C-1 (δ_C 69.5) and C-9 (δ_C 48.8), two hydroxy groups were attached to C-1 and C-5, respectively. In HMBC spectrum, cross-peaks for 1-OH (δ_H 5.89)/C-1 (δ_C 69.5), C-2 (δ_C 132.0), C-12 (δ_C 183.3) and C-13 (δ_C 40.7), 5-OH (δ_H 5.59)/C-5 (δ_C 146.3) and C-6 (δ_C 115.8). 4-OCH_3 (δ_H 3.73) was located at C-4 (δ_C 146.4) and 12-OCH_3 (δ_H 3.62) was located at C-12 (δ_C 183.3) observed in HMBC and HSQC. The relative configuration was assigned from the ROESY spectrum, in which H-13 [δ_H 3.68 (1H, t, *J* = 8.1 Hz)] showed correlation with H-8 suggesting that H-13 [δ_H 2.44 (1H, d, *J* = 12.0 Hz), 2.15 (1H, d, *J* = 12.0 Hz)] was on the same side with H-8 (δ_H 3.51) (Figure 2). The absolute configuration of compound **1** was also determined to be (1*R*, 8*R*, 9*R*)-pterolinuse K by X-ray crystallography (CCDC: 2052275) (Figure 3).

The six known neoflavonoids (2-7) were identified as (*S*)-3'-hydroxy-4,4'-dimethoxydalbergione (2) [15], (*S*)-3',4'dihydroxy-4-methoxydalbergione (3)[16], (*S*)-4'-hydroxy-4-methoxydalbergione (4)[17], (*S*)-3 '-hydroxy-4-methoxydalbergione (5)[16], (*S*)-4-methoxydalbergione (6)[17], melanoxoin (7)[18], by comp aring the observed and reported NMR data.



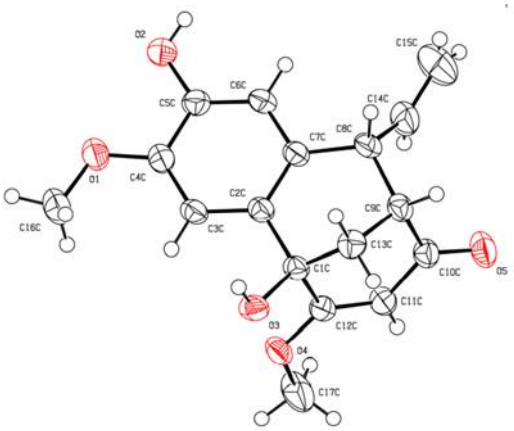


Figure 2. Selected HMBC and ROESY correlations of correlations of compound **1**

Figure 3. ORTEP drawing of compound **1**

The isolates **1-7** were assessed anti-inflammatory properties against lipopolysaccharide (LPS)-activated RAW 264.7 cells in *vitro* assay. Among all tested compounds, **3** and **6** showed moderate anti-inflammatory activity with IC_{50} values 23.14 ± 0.30 and 19.46 ± 1.02 μM, respectively (Table 2). Compounds **3** and **6** have similar chemical structure and **6** showed better activity, which suggested that the hydroxy group in C-3' and C-4' might weaken the anti-inflammatory activity of neoflavonoids.

Table 2. Cytotoxicities and anti-inflammatory activities (IC_{50} in μM) of compounds

Compound	Cytotoxicity	Anti-inflammatory activity
quercetin	>100	17.92 ± 0.92
1	>100	>100
2	26.09 ± 1.99	-
3	>100	23.14±0.30
4	22.24 ± 2.30	-
5	>100	>100
6	>100	19.46 ± 1.02
7	98.48 ± 20.85	89.31 ± 7.51

Table 3. Anti-tumor activities (IC_{50} in μM) of compounds

Compound	Caco-2	MDA-MB-468	MDA-MB-231	CT26
5-FU	190.32 ± 24.13	149.09 ± 21.02	48.84 ± 14.84	61.89 ± 16.35
1	>100	>100	>100	>100
2	15.14 ± 1.13	40.90 ± 7.56	7.54 ± 1.50	23.10 ± 1.20
3	26.46 ± 3.76	37.52 ± 1.70	16.60 ± 2.98	52.38 ± 16.51
4	11.42 ± 1.08	23.66 ± 1.58	7.23 ± 0.40	24.43 ± 0.90
5	>100	>100	>100	>100
6	32.92 ± 2.34	89.00 ± 10.90	21.88 ± 0.63	>100
7	46.89 ± 2.54	22.14 ± 1.07	27.31 ± 1.10	33.83 ± 0.94

All isolated neoflavonoids (**1-7**) from *D. melanoxylon* were evaluated for their cytotoxic activities on

Caco-2, MDA-MB-231, MDA-MB-468 and CT26 cell lines by MTT assays. The results revealed that compound **2** and **4** showd potent cytotoxic activities against four above cell lines with IC_{50} values ranging from 7.23 ± 0.40 to 40.90 ± 7.56 μM, compound **3** and **7** displayed moderate cytotoxic activities against four above cell lines with IC_{50} values ranging from 16.60 ± 2.98 to 52.38 ± 16.51 μM (Table 3), which suggested that the hydroxy group might enhance the anti-cancer activity of neoflavonoids. Compounds **2-6** has similar chemical structure but **5** showed no anti-cancer activity. It indicated the hydroxy group in C-3' of **5** might weaken anti-cancer activity.

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Supporting Information

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