

Neoflavonoids from the Heartwood of *Dalbergia melanoxylon*

Zhangjun Xu¹, Yang Liu¹, Xiaowei Meng², Li Yang¹, Feng Shao¹,
Ronghua Liu^{2*} and Lanying Chen^{3*}

¹ Key Laboratory of Modern Preparation of TCM, Ministry of Education
Jiangxi University of Chinese Medicine, Nanchang, Jiangxi 330004, P. R. China

² The College of Pharmacy, Jiangxi University of Chinese Medicine,
Nanchang, Jiangxi 330004, P. R. China

³ National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Jiangxi
University of Chinese Medicine,
Nanchang, Jiangxi 330004, P. R. China

(Received May 10, 2021; Revised June 25, 2021; Accepted June 26, 2021)

Abstract: A new neoflavonoid, (1R, 8R, 9R)-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 IC₅₀ 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 IC₅₀ values 7.54 ± 1.50 and 7.23 ± 0.40 μM , respectively.

Keywords: *Dalbergia melanoxylon*; neoflavonoids; anti-inflammatory activity; anti-tumor activity. © 2021 ACG Publications. All rights reserved.

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.

* Corresponding author: E- Mail: rhliu@163.com (R. -H. Liu) and ely5831@163.com (L. -Y. Chen)

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 dalbergiquinolins [1]. The neoflavonoids were reported to display a variety of pharmacological activities, 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 Papilionoidae, 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].

2. Present Study

The powdered heartwood of *D. melanoxylon* (50.0 kg) was extracted by infusion with 70% ethanol at room temperature (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 H₂O and successively partitioned with CH₂Cl₂, EtOAc and *n*-BuOH. The CH₂Cl₂ 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 CH₂Cl₂-MeOH from 100:1-10:1(*v/v*). Frs.7.C (196.9g) was purified by Sephadex LH-20 CC eluted with CH₂Cl₂-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 CH₂Cl₂-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 CH₂Cl₂-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 CH₂Cl₂-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-H₂O 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 CH₂Cl₂-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 CH₂Cl₂-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 CH₂Cl₂-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-H₂O 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 CH₂Cl₂-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-H₂O (32:68, *v/v*) to yield **1** (20.5 mg, *t*_R = 36.4 min).

(1*R*, 8*R*, 9*R*)-*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} ($\Delta\epsilon$) 234 (+1.41), 283 (+1.34); HR-ESI-MS *m/z* 303.1224 ([*M*+*H*]⁺ calcd for C₁₇H₁₉O₅,

303.1227). $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, 600 MHz) and $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$, 150 MHz): see Table S1 in supporting information.

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

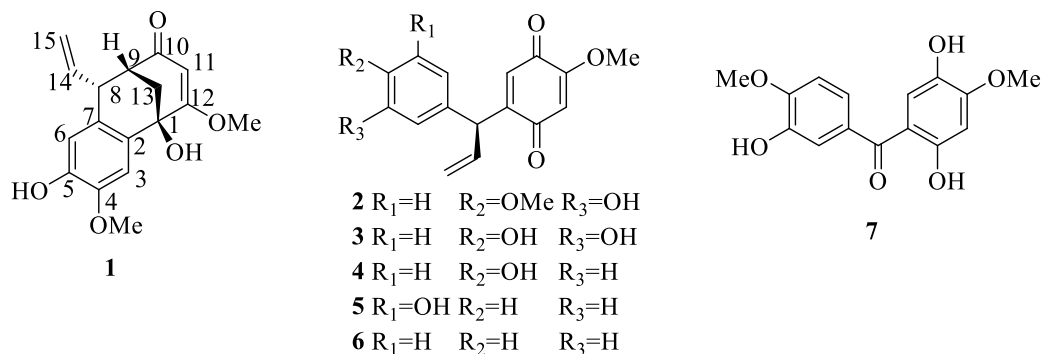


Figure 1. Structures of compounds **1-7**

Compound **1** was a colorless crystals, with a molecular formula of $\text{C}_{17}\text{H}_{18}\text{O}_5$ as deduced from the (+)-HR-ESI-MS m/z 303.1224 ($[\text{M}+\text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{O}_5$, 303.1227), inferring 9 degree of unsaturation. The $^1\text{H-NMR}$ 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-OCH₃) and 3.73 (3H, s, 4-OCH₃), 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 $^{13}\text{C-NMR}$ spectra exhibited 17 carbon resonances assignable to two methoxy groups at δ_{C} 56.9 (4-OCH₃) and 56.2 (12-OCH₃), 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-OCH₃ (δ_{H} 3.73) was located at C-4 (δ_{C} 146.4) and 12-OCH₃ (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],

Neoflavonoids from *Dalbergia melanoxylon*

(*S*)-3'-hydroxy-4-methoxydalbergione (5)[16], (*S*)-4-methoxydalbergione (6)[17], melanoxoin (7)[18], by comparing the observed and reported NMR data.

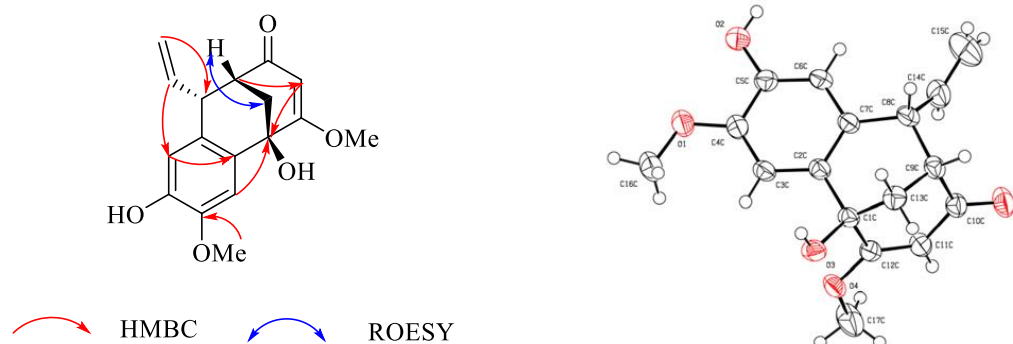


Figure 2. Selected HMBC and ROESY correlations of compound **1** **Figure 3.** ORTEP drawing of compound **1**

The isolates **1-7** were assessed anti-inflammatory properties against lipopolysaccharide (LPS)-activated RAW 264.7 cells in *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 μ M, respectively (Table 1). 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 1. Cytotoxicities and anti-inflammatory activities (IC_{50} in μ M) 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 2. Anti-tumor activities (IC_{50} in μ M) 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** showed potent cytotoxic activities against four above cell lines with IC₅₀ 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 IC₅₀ values ranging from 16.60 ± 2.98 to 52.38 ± 16.51 μM (Table 2), 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.

Acknowledgments

The work was partially supported by the National Natural Science Foundation of China (81660676 and 81360629), the National Key R&D Program of China (2018YFC1706102) and the Natural Science Foundation of Jiangxi Province (20202BABL216074).

Supporting Information

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

ORCID 

Zhangjun Xu: [0000-0002-8430-0806](https://orcid.org/0000-0002-8430-0806)

Yang Liu: [0000-0002-0959-3004](https://orcid.org/0000-0002-0959-3004)

Xiaowei Meng: [0000-0003-2956-8460](https://orcid.org/0000-0003-2956-8460)

Li Yang: [0000-0001-9098-694X](https://orcid.org/0000-0001-9098-694X)

Feng Shao: [0000-0002-4306-6451](https://orcid.org/0000-0002-4306-6451)

Ronghua Liu: [0000-0001-5623-9000](https://orcid.org/0000-0001-5623-9000)

Lanying Chen: [0000-0001-8115-8114](https://orcid.org/0000-0001-8115-8114)

References

- [1] M. M. Garazd, Y. L. Garazd and V. P. Khilya (2003). Neoflavones.1. Natural distribution and spectral and biological properties, *Chem. Nat. Compd.* **39**, 54 - 121.
- [2] P. Kumar, P. Kushwaha, V. Khedgikar, J. Gautam, D. Choudhary, D. Singh, R. Trivedi and R. Maurya (2014). Neoflavonoids as potential osteogenic agents from *Dalbergia sissoo* heartwood, *Bioorg. Med. Chem. Lett.* **24**, 2664 - 2668.
- [3] C. Lee, J. W. Lee, Q. Jin, D. S. Jang, S. Lee, D. Lee, J. T. Hong, Y. Kim, M. K. Lee and B. Y. Hwang (2013). Inhibitory constituents of the heartwood of *Dalbergia odorifera* on nitric oxide production in RAW 264.7 macrophages, *Bioorg. Med. Chem. Lett.* **23**, 4263 - 4266.
- [4] K. R. Park, H. M. Yun, T. H. Quang, H. Oh, D. S. Lee, Q. S. Auh and E. C. Kim (2016). 4-Methoxydalbergione suppresses growth and induces apoptosis in human osteosarcoma cells *in vitro* and *in vivo* xenograft model through down-regulation of the JAK2/STAT3 pathway, *Oncotarget* **7**, 6960 - 6971.

Neoflavonoids from *Dalbergia melanoxylon*

- [5] M. Kuroyanagi, A. Ueno, Y. Hirayama, Y. Hirayama, T. Gokita, T. Isiiimaru, S. Kameyama, T. Yanagawa, M. Satake and S. Satake (1996). Anti-androgen active constituents from *Dalbergia cochinchinensis* PIERRE, *Nat. Medicin.* **50**, 408 - 412.
- [6] Y. Liu, N. Zhang, J. W. He, L. Y. Chen, L. Yang, X. W. Meng, F. Shao and R. H. Liu (2021), Two new compounds from the heartwood of *Dalbergia melanoxylon* and their protective effect on hypoxia/reoxygenation injury in H9c2, *Nat. Prod. Commun.* **16**, 1 -7.
- [7] X. X. Lai, N. Zhang, L. Y. Chen, Y. Y. Luo, B. Y. Shou, X. X. Xie and R. H. Liu (2020), Latifolin protects against myocardial infarction by alleviating myocardial inflammatory via the HIF-1 α /NF- κ B/IL-6 pathway, *Pharm. Biol.* **58**, 1156 - 1166.
- [8] N. Zhang, B. Y. Shou, L. Y. Chen, X. X. Lai, Y. Y. Luo, X. W. Meng and R. H. Liu (2020), Cardioprotective effects of latifolin against doxorubicin-induced cardiotoxicity by macrophage polarization in mice, *J. Cardiovasc. Pharmacol.* **75**, 564 - 572.
- [9] Y. Liu, J. C. Shu, M. F. Wang, Z. J. Xu, L. Yang, X. W. Meng, W. B. Duan, N. Zhang, F. Shao, R. H. Liu and L. Y. Chen (2021), Melanoxylonin A-G, neoflavonoids from the heartwood of *Dalbergia melanoxylon* and their cardioprotective effects, *Phytochemistry* **189**, 112845.
- [10] M. Jenkins, S. Oldfield and T. Aylett (2002). International trade in African blackwood, *Cambridge, UK: Fauna and Flora International*.
- [11] R. E. Malimbwi, E. J. Luoga, O. Hofstad, A. G. Mugasha and J. S. Valen (2000). Prevalence and standing volume of *Dalbergia melanoxylon* in coastal and inland sites of Southern Tanzania, *J. Trop. For. Sci.* **12**, 336 - 347.
- [12] P. Mutai, M. Heydenreich, G. Thoithi, G. Mugumbate, K. Chibale and A. Yenesew (2013), 3-Hydroxyisoflavanones from the stem bark of *Dalbergia melanoxylon*: Isolation, antimycobacterial evaluation and molecular docking studies, *Phytochem. Lett.* **6**, 671 - 675.
- [13] P. G. Kareru, A. N. Gachanja, J. M. Keriko and G. M. Kenji, (2008). Antimicrobial activity of some medicinal plants used by herbalists in Eastern province, Kenya, *Afr. J. Trad. CAM.* **5**, 51 - 55.
- [14] M. F. Wang, G. Q. Ma, F. Shao, R. H. Liu, L. Y. Chen, Y. Liu, L. Yang and X. W. Meng (2020). Neoflavonoids from the heartwood of *Dalbergia melanoxylon*, *Nat. Prod. Res.* **16**, 1 - 7. DOI: 10.1080/14786419.2020.1800692
- [15] S. F. Wu, F. R. Chang, S. Y. Wang, T. L. Hwang, C. L. Lee, S. L. Chen, C. C. Wu and Y. C. Wu (2011). Anti-inflammatory and cytotoxic neoflavonoids and benzofurans from *Pterocarpus santalinus*, *J. Nat. Prod.* **74**, 989 - 996.
- [16] S. P. Shrestha, Y. Narukawa and T. Takeda (2007). Chemical constituents of Nepalese propolis: isolation of new dalbergiones and related compounds, *J. Nat. Med.* **61**, 73 - 76.
- [17] W. B. Eyton, W. D. Ollis, I. O. Sutherland, O. R. Gottlieb, M. T. Magalhaes and L. M. Jackman (1965). The neoflavanoid group of natural products-I: Dalbegiones-A new class of quinones, *Tetrahedron* **21**, 2683 - 2696.
- [18] V. Pathak, O. Shirota, S. Sekita, Y. Hirayama, Y. Hakamata, T. Hayashi, T. Yanagawa and M. Satake (1997). Antiandrogenic phenolic constituents from *Dalbergiaco chinchinensis*, *Phytochemistry* **46**, 1219 - 1223.