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Bioactive components from the heartwood of *Pterocarpus santalinus*

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ABSTRACT

One new phenanthrenedione, pterolinus K (**1**), and one new chalcone, pterolinus L (**2**) were isolated from the heartwood extract of *Pterocarpus santalinus*. The structures were elucidated by spectroscopic methods. Both **1** and **2** showed inhibitory effect on elastase release by human neutrophils in response to fMLP with an IC_{50} value of 4.24 and 0.95 μM, and compound **1** also inhibited superoxide anion generation with IC_{50} value of 0.99 μM. In addition, compound **1** showed selective cytotoxicity against HepG2 with IC_{50} value of 10.86 μM, while compound **2** showed a moderate cytotoxicity against KB with IC_{50} values of 17.18 μM.

Pterocarpus santalinus L. (Fabaceae), also named 'red sanders', belongs to the Legume family. It is a rare, commercial tree, which is distributed exclusively in well-defined forest tracts of Andhra Pradesh in Southern India. It is valuable in the international market and is most notably exported from India to Japan and other countries.[1] *P. santalinus* has been used as a folk remedy for the treatment of inflammatory conditions, such as chronic bronchitis, chronic cystitis, fever, headaches, mental aberrations, ulcers, cancers, etc.[2,3] The previous phytochemical investigations of this plant revealed the presence of, six sesquiterpenes,[4] one isoflavone,[5] two lignans,[2] and two aurone glycosides.[6] In preliminary studies, we found the methanolic extract of the heartwood exhibited potent cytotoxicity (IC_{50} <20 μg/mL) against three cancer cell lines (HepG2, Hep3B, A549) and anti-inflammatory activity (IC_{50} <10 μg/mL). These preliminary results encouraged us to peruse further detailed investigation. One new phenanthrenedione (**1**) and one new chalcone (**2**), together with two known compounds, dalbergin (**3**)[7] and cearoin (**4**),[8] (Fig. 1) were isolated from the heartwood extract of this species according to bioactivity-guided fractionation. Cytotoxic and anti-inflammatory activities of the isolated compound were also investigated in the current study.

The heartwood was powdered and extracted with MeOH (5 × 20 L). After solvent evaporation, the crude extract (ca. 480 g) was partitioned between CH_2Cl_2 and 50% MeOH aq. The CH_2Cl_2 layer was concentrated in vacuo, and the CH_2Cl_2 extract (ca. 300 g) was subjected to column chromatography (CC) on celite 545 and eluted with *n*-hexane (8 L), CH_2Cl_2 (40 L), EtOAc (20 L), acetone (10 L), and MeOH (8 L) leading to collect six fractions (PS-C1 to PS-C6). Among them, PS-C2 and PS-C3 showed significant cytotoxic (IC_{50} <20 μg/mL) and anti-inflammatory activity (IC_{50} <10 μg/mL). Therefore, PS-C3 (ca. 100 g) was subjected to silica gel CC and eluted with hexane–EtOAc–MeOH gradient solvent system (3/1/0, 1/1/0, 0/1/0, 0/40/1, 0/20/1, 0/10/1, 0/6/1), to give 11 fractions (PS-C3.1 to PS-C3.11). Compound **1** (162.42 mg) was an abundant reddish precipitate and filtered from sub-fraction PS-C3.8. In addition, PS-C3.5 (31.21 g) was subjected to repeated silica gel CC with a CH_2Cl_2 and MeOH gradient solvent system, and eleven fractions (PS-C3.5.1 to PS-C3.5.11) were collected. PS-C3.5.6 (20.70 g) was subjected to CC on Sephadex LH-20 and eluted

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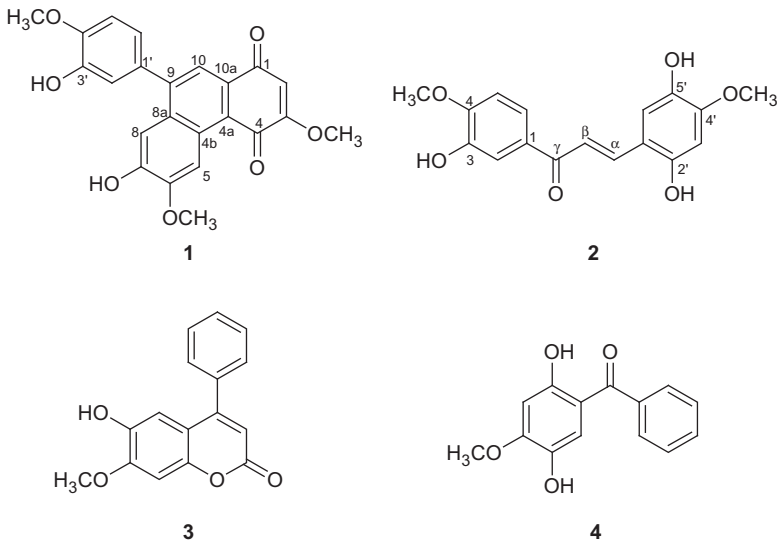

Figure 1. The structures of compounds **1**–**4**.

Table 1
^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR data of pterolinus K (**1**) in pyridine-d_5

	1	
Position	^{1}H (δ_H)	^{13}C (δ_c)
1		185.7
2	6.34 s	107.6
3		161.5
4		182.8
4a		132.8
4b		127.1
5	9.50 s	106.9
6		153.5
7		150.6
8	7.97 s	110.4
8a		124.0
9		145.6
10	8.37 s	121.8
10a		130.4
1′		133.8
2′	7.54 d (2.0)	118.1
3′		148.2
4′		148.7
5′	7.07 d (8.0)	112.3
6′	7.10 d (8.0, 2.0)	121.0
3-OCH_3	3.78 s	56.4
6-OCH_3	4.03 s	55.7
4′-OCH_3	3.87 s	56.0
OH	11.42 br	
OH	12.50 br	

Table 2
^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR data of pterolinus L (**2**) in MeOH-d_4

	2	
Position	^{1}H (δ_H)	^{13}C (δ_c)
1		133.3
2	7.48 d (1.5)	116.1
3		147.9
4		153.8*
5	7.03 d (8.5)	111.9
6	7.60 d (8.5, 1.5)	123.1
α	8.04 d (15.5)	141.9
β	7.53 d (15.5)	119.5
γ		191.9
1′		115.4
2′		153.6
3′	6.47 s	101.0
4′		153.1*
5′		141.0
6′	7.08 s	114.8
4-OCH_3	3.93 s	56.4
4′-OCH_3	3.86 s	56.6

* May changeable.

with acetone and CH_2Cl_2 (1:1) to give 10 fractions (PS-C3.5.6.1 to PS-C3.5.6.10). Compound **2** (12.97 mg) was isolated from PS-C3.5.6.3 using CC with C18 silica gel (12–100% MeOH aq) and Sephadex LH-20 CC (acetone/CH_2Cl_2 1:1, v/v).

Compound **1** was obtained as a reddish amorphous powder and its molecular formula, $C_{23}H_{18}O_7$, was determined by the HRESI-MS peak at *m/z* 407.1133 $[M+H]^+$ ($C_{23}H_{19}O_7$, calcd for 407.1131), indicated 15° of unsaturation. The IR spectrum of **1** revealed a hydroxyl group at 3418 cm^{-1}, a carbonyl absorption band at 1635 cm^{-1}, as well as an aromatic ring at 1507 cm^{-1}. UV absorption at 248, 307, and 405 nm also indicated an aromatic system. In ^{1}H NMR, compound **1** exhibited an ABX system (δ_H 7.07, d, J = 8, δ_H 7.10, dd, J = 8, 2, δ_H 7.54, d, J = 2), four singlet protons (δ_H 6.34, 7.97, 8.37, 9.50) and three methoxy groups (δ_H 3.78, 3.87, 4.03) (Table 1). Based on ^{13}C NMR and HSQC spectra, 23 carbon signals, including 13 quaternary, six methane, and three methylene carbons were observed. Among the quaternary 13 carbons, two were identified as carbonyl carbons based on the chemical shift δ_c 182.8 and 185.7. Through comparing our spectral data with the previously reported data,[9] the above data indicated the presence of a phenanthrenedione attached to an additional aromatic ring to fulfill the 15° of unsaturation. The additional aromatic ring was attached at C-9 according to the HMBC corrections of H-2′/C-9 and H-6′/C-9. The other olefinic proton at δ_H 6.34 showed HMBC corrections with two carbonyl carbons (δ_c 182.8, 185.7) and C-2 (δ_c 161.5). Proton at δ_H 8.37 exhibited the HMBC corrections of C-1, C-4a, C-8a, C-9, and C-1′. Protons at δ_H 7.97 and 9.50 presented the HMBC corrections of C-4b, C-6, C-7, C-8a as well C-9, and C-4a, C-4b, C-6 as well C-8a, respectively. The methoxy locations at C-3, C-6 and C-4′ were confirmed by the following NOE corrections: δ_H 6.34/3.78,[10] δ_H 7.07/3.87 and δ_H 9.50/4.03. Thus, compound **1** was identified and named as pterolinus K (**1**).[11]

Compound **2** was isolated as a brown amorphous powder. The HRESI-MS of **2** displayed a pseudomolecular ion peak at *m/z* 317.1024 $[M+H]^+$, corresponding to the formula of $C_{17}H_{16}O_6$

Table 3
Inhibitory effects of compounds **1** and **2** isolated from *P. santalinus* on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB

Compounds	IC_{50} (μM)	
	Superoxide anion	Elastase
1	0.99 ± 0.15	4.24 ± 1.33
2	NT[a]	0.95 ± 0.13
LY294002[b]	3.84 ± 0.78	6.48 ± 1.14

± SEM (n = 3).
[a] Not tested.
[b] LY294002, phosphatidylinositol-3-kinase inhibitor, was used as a positive control for superoxide anion generation and elastase release.

($C_{17}H_{17}O_6$, calcd for 317.1025), indicated 10° of unsaturation. The UV absorption maxima at 277, 316 and 398 nm together with the IR absorption bands at 3417 (OH), 1699 (C=O) cm^{-1} and 1610, 1514 cm^{-1} indicated the presence of an aromatic system. In the ^{13}C NMR spectrum, 15 carbons including one carbonyl carbon, two oleflinic carbons, five methines and five quaternary carbons of the main skeleton were detected. The combination of ^{1}H NMR and HSQC spectrum, an ABX system, two *para*-aromatic protons and a pair of *trans* olefinic protons were observed together with two methoxy groups. According to the above data, a chalcone skeleton was presumed. Based on the HMBC data, proton at δ_H 7.47 showed corrections of C-5 and C-γ, proton at δ_H 7.08 showed HMBC correction of C-α and proton at δ_H 8.04 exhibited corrections of C-1′ and C-2′. The location of methoxy groups were confirmed by NOE corrections as the following data: δ_H 7.03/3.93, δ_H 6.47/3.86 and δ_H 7.60/7.53/7.08. Thus, compound **2** was identified and named pterolinus L.[12]

Compounds **1** and **2** were screened for their anti-inflammatory activity (Table 3), compound **1** showed a notable inhibition of superoxide anion generation and elastase release with IC_{50} value 0.99 and 4.24 μM, respectively. Compound **2** also showed a potent inhibition of elastase release with IC_{50} value 0.95 μM, which was 6-folds more potent than the positive control. In addition, compounds **1**–**4** were screened for their cytotoxic activity against six cancer lines (A549, DU145, KB, KBvin, HepG2 and Hep3B) (see Supplementary data). Compound **1** showed a selective activity against Hep3B with IC_{50} value 10.86 μM. Compound **2** exhibited a moderate cytotoxicity (IC_{50}: 17.18–29.81 μM) against six cancer cell lines, while both compounds **3**, neoflavone with a lactone ring, and **4** neoflavone with a ketone group, showed weak or no cytotoxic activity.

Overall, two new compounds were isolated from *P. santalinus* heartwood portion, and were identified as phenanthrenedione and chalcone. The biological data demonstrated that compounds **1** and **2** showed potent anti-inflammatory effect either through the inhibition of superoxide anion generation or elastase release. Therefore, these two new compounds may act as future lead compounds for the anti-inflammatory drug development.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.036.

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11. *Pterolinus K* (***1***): red amorphous powder; IR (Neat) $_{max}$ 3418, 1635, 1507, 1486, 1266, 1231 cm^{-1}; UV(MeOH) $_{max}$ nm (log ε): 248 (3.89), 307 (3.46), 405 (3.07); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) are given in Table 1; HRESI-MS m/z 407.1133 $[M+H]^+$ (calcd for $C_{23}H_{19}O_7$, 407.1131).
12. *Pterolinus L* (***2***): brown amorphous powder; IR (Neat) $_{max}$ 3417, 1699, 1610, 1514, 1443, 1274 cm^{-1}; UV (MeOH) $_{max}$ nm (log ε): 277 (3.62), 316 (3.62), 398 (3.73); ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) are given in Table 2; HRESI-MS m/z 317.1024 $[M+H]^+$ (calcd for $C_{17}H_{17}O_6$, 317.1025).