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# Two new steroids with NO inhibitory effects from *lansium domesticum*

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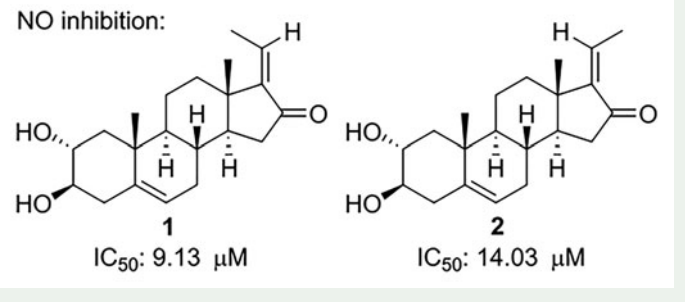
**ABSTRACT**

Chemical investigation of *Lansium domesticum* has led to the isolation of two undescribed compounds, namely 17(20)*E*-dyscusin B (**1**) and 17(20)*Z*-dyscusin B (**2**), as well as three known ones (**3** – **5**). Structural elucidation was accomplished by the analysis of NMR, MS and IR data. Compounds **1** and **2** were a pair of $\Delta^{17(20)}$ geometric isomers of pregnane steroids and showed the significant nitric oxide (NO) inhibitory activities.

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Lansium domesticum; steroids; NO inhibition; Meliaceae

## 1. Introduction

*Lansium domesticum*, one of Meliaceae plants with edible fruits, mainly distributed in Southeastern Asia. Triterpenoids were reported to the bioactive components in *L. domesticum* and those including onoceranoid-, cycloartane- and tetranor-triterpnoids, exhibiting ileum contractive inhibition (Nishizawa et al. 1983), shrimp toxicity (Tanaka et al. 2002), antibacterial (Ragasa et al. 2006; Dong et al. 2011), antimalarial (Saewan et al. 2006), antifeedant (Mayanti et al. 2011), and antimutagenic (Matsumoto et al. 2018) activities, of which the onoceranoid triterpenes were also isolated from the closely related plant *L. parasiticum* (Ramadhan et al. 2018; Potipiranun et al. 2018). To seek the bioactive metabolites of Meliaceous plants from Xishuangbanna area of

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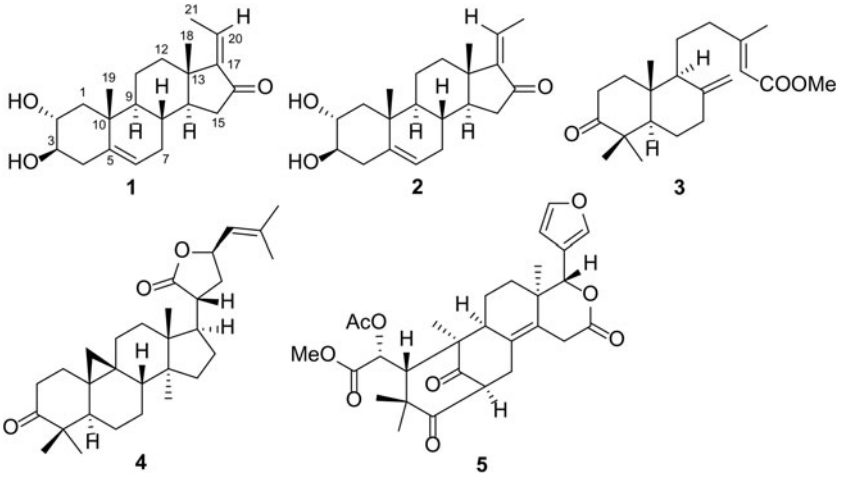


Figure 1. Structures of compounds **1** – **5**.

China (Ji et al. 2014, 2015), the ethanol extract of *L. domesticum* leaves was investigated. As a result, two new compounds (**1** and **2**), along with three known compounds (**3** – **5**) were isolated (Figure 1). Structures of those isolates were elucidated by the analysis of the spectroscopic data and comparison with reported data. All compounds were tested for the NO inhibitory effects against LPS-stimulated RAW264.7 cells and the results showed that compounds **1** and **2** displayed the significant inhibition. Detailed isolation, structure elucidation and biological evaluation of those isolates were reported herein.

## 2. Results and discussion

The ethanol extract of *L. domesticum* leaves was suspended in $H_2O$ and successively partitioned with EtOAc and *n*-BuOH. The EtOAc-soluble fraction was purified by the extensive column chromatography to yield two new compounds (**1** and **2**) and three known ones, 3-oxoanticopalic acid methyl ester (**3**) (Zinkel and Magee 1987), (23*R*)-3-oxo-5$\alpha$-cycloart-24-en-21,23-olide (**4**) (Achenbach and Frey 1992), and ekeberin $C_3$ (**5**) (Murata et al. 2008).

Compound **1** was obtained as the white, colorless powder. Its HR-ESIMS spectrum showed the ion peak with sodium adduct at m/z 353.2089 $[M+Na]^+$ (calcd 353.2087), which determined the molecular formula of $C_{21}H_{30}O_3$, corresponding to 7 indices of hydrogen deficiency (IHDs). The IR absorption spectrum revealed the presence of hydroxyl (3429 $cm^{-1}$) and keto carbonyl (1721 $cm^{-1}$) groups. Analysis of the $^1H$ and $^{13}C$ NMR data (Table S1) and HSQC spectrum indicated compound **1** possessed an $\alpha,\beta$-unsaturated ketone [$\delta_H$ 6.52 q ($J=7.5$); $\delta_C$ 129.7, 147.7, 206.4], two singlet methyls [$\delta_H$ 1.04 s, 1.09 s; $\delta_C$ 17.5, 20.6], one doublet methyl [$\delta_H$ 1.86 d ($J=7.5$); $\delta_C$ 13.4] and resonances for 15 carbons due to six methylenes, six methines (one olefinic and two oxgenated) and three quaternary carbons (one olefinic). The two double bonds and one keto functionalities account for 3 out of 7 IHDs requiring four additional rings.

Those identified characters suggested that compound **1** was a tetracyclic pregnane steroid (Kurimoto et al. 2011).

The planar structure of compound **1** was established by the analysis of the 2D NMR correlations (Figure S1). Three spin-spin coupling fragments as depicted by double bond in Figure S1 were identified by the analysis of $^{1}$H-$^{1}$H COSY spectrum. The connection of these three coupling fragments with quaternary carbons and methyls was established on the basis of HMBC spectrum. The multiple HMBC correlations of $H_3$-19/C-1, C-5, C-9 and C-10; H-4/C-5 and C-6, $H_3$-18/C-12, C-13, C-14 and C-17; $H_2$-15/C-16 and C-17; and H-20/C-16 and C-17, together with the $^{1}$H-$^{1}$H COSY coupling fragments constructed the C-21 steroid skeleton for **1**. In the ROESY spectrum, the correlations of $H_3$-19/H-2, H-8 and H-11$\beta$; and $H_3$-18/H-8, H-11, H-15$\beta$ and $H_3$-21 indicated those protons on the same side of molecule and assigned as $\beta$-direction. The $\alpha$-orientation for H-3, H-9 and H-14 was based on the ROESY cross peaks of H-1$\alpha$/H-3; and H-14/H-9 and H-15$\alpha$. The structure of **1** was thus established as shown and named 17(20)*E*-dyscusin B.

Compound **2** had the same molecular formula with that of compound **1** as established by the analysis of HR-ESIMS spectrum. The $^{1}$H and $^{13}$C NMR data (Table S1) of **2** were highly similar to that of **1** with the difference being the result from C-17 side chain. The gross structure of **2** was finally established by the analysis of the 2D NMR correlations (Figure S2), suggesting the $\Delta^{17(20)}$ geometric isomer of **1**. This deduction was supported by the ROESY correlation of $H_3$-18/H-20 and the higher carbon chemical shift of C-17 vinyl and methyl groups compared with those of **1**, owing to the deshielding effect of the carbonyl group at C-16 (Rogers et al. 1998). Compound **2** was thus assigned as depicted and named 17(20)*Z*-dyscusin B.

Over-expression of NO in host organism showed the deleterious effects, which is always associated with many diseases including human vascular and cardiac diseases (Farah et al. 2018). All the compounds were evaluated for the NO inhibitory effect in LPS-stimulated RAW264.7 cells (Table S2). Compounds **1** and **2** showed the significant NO inhibition with the $IC_{50}$ values of 9.13 and 14.03 μM, respectively, whereas compounds **3**–**5** were inactive ($IC_{50} > 25$ μM).

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were obtained using the Shimadzu UV-2401A instrument. IR spectra (KBr) were determined on a Bruker Tensor-27 infrared spectrometer. NMR spectra were recorded on the Bruker Avance III 600 spectrometer. ESIMS and HREIMS were collected from an Agilent 6200 series Q-TOF mass spectrometer. Semi-preparative HPLC was carried out using a Waters system consisting of a 600 pump and a 2996 photodiode array detector and an YMC-Pack ODS-A column (10 mm × 250 mm, 5 μm). Silica gel (200 – 300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 gel (40 – 70 μm, Amersham Pharmacia Biotech AB, Sweden), and MCI gel (CHP20/P120, 75 – 150 μm, high porous polymer, Mitsubishi Chemical Corporation, Japan) were used for column

chromatography. All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory, China).

### 3.2. Plant material

The leaves of *Lansium domesticum* were collected from Menglun town of Yunnan province, China, in October 2015, and identified by one of authors Y. K. Xu. A voucher specimen (HITBC_162443) was deposited in the herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

### 3.3. Extraction and isolation

The air-dried powdered plant material (5 kg) was extracted with EtOH/$H_2O$ (95/5, v/v, 30 L) at room temperature and afforded a crude residue (300 g). The residue was suspended in $H_2O$ (2 L) and partitioned with EtOAc, and *n*-BuOH, successively. The EtOAc-soluble fraction (100 g) was subjected to MCI gel column chromatography (CC) (MeOH/$CHCl_3$: 1/1 to 1/0) to produce five fractions (F1–F5). Fraction F3 (15 g) was applied to a silica gel CC (Acetone/Petroleum ether: 1/50 to 2/1) to yield five sub-fractions (F3a–F3e). The F3c was further purified by Sephadex LH-20 (MeOH) to yield compounds **4** (20 mg) and **5** (60 mg). The similar treatment for the fraction F3d with Sephadex LH-20 gave three small fractions (F3d1–F3d3). The major steroids mixture fraction F3d2 was purified by semi-preparative HPLC ($CH_3CN/H_2O$ from 3/2 to 4/1) to produce **1** (8 mg), **2** (5 mg) and **3** (4 mg), respectively.

17(20)*E*-Dyscusin B (**1**): white, amorphous powder; $[\alpha]^{26}_{D}$ −212.6 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 201 (3.71), 243 (3.87) nm; IR (KBr) $\nu_{max}$ 3429, 2933, 2855, 1721, 1645, 1453, 1438, 1418, 1377, 1236, 1200, 1178, 1155, 1127, 1057 $cm^{-1}$; $^1H$ NMR (600 MHZ, $CDCl_3$): $\delta_H$ 1.09 (1H, m, H-1$\alpha$), 2.11 (1H, dd, $J = 12.6$, 4.5 Hz, H-1$\beta$), 3.69 (1H, ddd, $J = 11.9$, 9.0, 4.5 Hz, H-2), 3.34 (1H, ddd, $J = 10.9$, 9.0, 6.0 Hz, H-3), 2.34 (2H, m, H-4), 5.41 (1H, br d, $J = 5.2$ Hz, H-6), 1.65 (1H, m, H-7$\alpha$), 2.00 (1H, m, H-7$\beta$), 1.67 (1H, m, H-8), 1.18 (1H, m, H-9), 1.72 (2H, m, H-11), 1.68 (1H, m, H-12$\alpha$), 2.38 (1H, m, H-12$\beta$), 1.46 (1H, ddd, $J = 14.5$, 10.6, 6.8 Hz, H-14), 2.21 (1H, dd, $J = 17.2$, 6.8 Hz, H-15$\alpha$), 2.03 (1H, m, H-15$\beta$), 1.04 (3H, s, H-18), 1.09 (3H, s, H-19), 6.52 (1H, q, $J = 7.5$ Hz, H-20), 1.86 (3H, d, $J = 7.5$, H-21); $^{13}C$ NMR (150 MHz, $CDCl_3$): $\delta_C$ 44.8 (C-1), 72.6 (C-2), 76.3 (C-3), 39.3 (C-4), 139.6 (C-5), 122.0 (C-6), 31.7 (C-7), 30.1 (C-8), 49.8 (C-9), 38.5 (C-10), 20.9 (C-11), 36.1 (C-12), 43.1 (C-13), 50.2 (C-14), 38.0 (C-15), 206.4 (C-16), 147.7 (C-17), 17.5 (C-18), 20.6 (C-19), 129.7 (C-20), 13.4 (C-21); (+)-HREIMS *m/z* 353.2089 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_3Na$, 353.2087).

17(20)*Z*-Dyscusin B (**2**): white, amorphous powder; $[\alpha]^{19}_{D}$ −142.6 (*c* 0.03, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (3.63), 243 (3.69) nm; IR (KBr) $\nu_{max}$ 3429, 2972, 2935, 2853, 1716, 1643, 1436, 1376, 1343, 1247, 1201, 1174, 1154, 1129, 1057 $cm^{-1}$; $^1H$ NMR (600 MHZ, $CDCl_3$): $\delta_H$ 1.07 (1H, m, H-1$\alpha$), 2.12 (1H, dd, $J = 12.8$, 4.4 Hz, H-1$\beta$), 3.69 (1H, ddd, $J = 11.2$, 8.9, 4.4 Hz, H-2), 3.34 (1H, ddd, $J = 10.9$, 8.9, 6.0 Hz, H-3), 2.35 (2H, m, H-4), 5.41 (1H, br d, $J = 3.9$ Hz, H-6), 1.65 (1H, m, H-7$\alpha$), 2.01 (1H, m, H-7$\beta$), 1.62 (1H, m, H-8), 1.16 (1H, m, H-9), 1.73 (1H, m, H-11$\alpha$), 1.60 (1H, m, H-11$\beta$), 1.37 (1H, m, H-12$\alpha$), 1.89 (1H, m, H-12$\beta$), 1.41 (1H, m, H-14), 2.21 (1H, dd, $J = 17.3$, 7.0 Hz, H-15$\alpha$), 2.04 (1H,

m, H-15$\beta$), 0.93 (3H, s, H-18), 1.09 (3H, s, H-19), 5.74 (1H, q, $J = 7.3$ Hz, H-20), 2.09 (3H, d, $J = 7.3$, H-21); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta_C$ 44.9 (C-1), 72.6 (C-2), 76.3 (C-3), 39.3 (C-4), 139.7 (C-5), 122.0 (C-6), 31.6 (C-7), 30.4 (C-8), 50.0 (C-9), 38.5 (C-10), 20.8 (C-11), 35.6 (C-12), 43.1 (C-13), 49.7 (C-14), 39.6 (C-15), 208.7 (C-16), 148.1 (C-17), 19.5 (C-18), 20.6 (C-19), 130.7 (C-20), 14.2 (C-21); (+)-HREIMS *m/z* 353.2085 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_3Na$, 353.2087).

### 3.4. NO inhibition assay

The LPS-stimulated RAW264.7 cells were used for the experiment. Cell viability was determined by MTS assay and the NO inhibition of compounds was tested by Griess Reagent System as previously reported (Li et al. 2014).

## 4. Conclusion

In conclusion, two new $\Delta^{17(20)}$ geometric isomers of steroids, 17(20)*E*-dyscusin B (**1**) and 17(20)*Z*-dyscusin B (**2**), together with three known compounds **3** – **5** were isolated and identified from the plant *Lansium domesticum*. Bioassay revealed compounds **1** and **2** showed the significant NO inhibition with the $IC_{50}$ values of 9.13 and 14.03 μM, respectively.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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