A new ent-kaurane diterpenoid from *Ixora amplexicaulis*

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A new ent-kaurane diterpenoid, 6α,16α-dihydroxy-ent-kaurane (1), was isolated from the stems of *Ixora amplexicaulis*, together with (24R)-6β-hydroxy-24-ethyl-cholest-4-en-3-one (2), 7β-hydroxysitosterol (3), maslinic acid (4), 3,30-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (5) and protocatechuric acid (6). Their structures were established by extensive spectroscopic analysis, including 2D NMR techniques. Compounds 2–5 were isolated from the genus *Ixora* for the first time and 6 obtained originally from *I. amplexicaulis*.

**Keywords:** *Ixora amplexicaulis*; Rubiaceae; ent-kaurane diterpenoid

1. **Introduction**

*Ixora* is a genus of about 400 species in the family Rubiaceae, mainly distributing in tropical Asia, Africa and Oceania, with 19 species being endemic to South China (Lo et al. 1999). The plants of the genus usually have small beautiful flowers, being easily cultured as common roadside and garden trees. The leaves, flowers, stems and roots of the genus have also been used in traditional medicine to treat various ailments. For example, the leaves and stems of *Ixora coccinea* are widely used in traditional Sudanese and Ayurvedic medicinal systems for the treatment of diarrhoea, fever, headache, skin diseases, eye trouble, wounds, sores and ulcers (Jaiswal et al. 2014). Previous investigations led to the isolation of many compounds including phenolics, peptides, terpenoids and sterols from plants of the genus (Ragasa et al. 2004; Idowu et al. 2010; Lee et al. 2010; Ikram et al. 2013). Pharmacological studies showed that extracts of the genus and the isolated compounds displayed extensive activities, such as antitumour, chemoprotective and antioxidant activities (Latha & Panikkar 1998; Wen et al. 2011; Wickramasinghe et al. 2014). But there was no report on *Ixora amplexicaulis*, which is a small shrub endemic to China. As a part of our research of structurally unique and biologically active compounds from medicinal plants of Yunnan, China, we have isolated and identified a new ent-kaurane diterpenoid, 6α,16α-dihydroxy-ent-kaurane (1), as well as five known compounds,

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(24R)-6β-hydroxy-24-ethyl-cholest-4-en-3-one (2), 7β-hydroxysitosterol (3), maslinic acid (4), 3,3′-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (5) and protocatechuic acid (6) from the stems of I. amplexicaulis (Figure 1). Compounds 2–5 were isolated from the genus Ixora for the first time and 6 obtained originally from I. amplexicaulis.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder. It had the molecular formula C_{20}H_{34}O_{2} by HR-EI-MS at m/z 306.2558 [M]+ (calcld for C_{20}H_{34}O_{2}+, 306.2559), implying four degrees of unsaturation. The infrared (IR) spectrum showed a broad absorption band (3386 cm\(^{-1}\)) due to OH groups. In \(^1\)H NMR spectrum of 1, an oxygenated methine [\(\delta\) 3.91 (1H, td, \(J = 10.5\) and 3.6 Hz, H-6)], and four tertiary methyls [\(\delta\) 1.36, 1.14, 1.04 and 0.98 (each 3H, s)] were observed. The \(^{13}\)C NMR and DEPT spectrum displayed 20 carbon signals corresponding to four methyls, eight methylenes, four methines and four quaternary carbons, which was assigned to be a diterpenoid skeleton. Two downfield signals at \(\delta\) 79.2 and 69.6 suggested the existence of an oxygenated quaternary carbon and an oxygenated methine. The diagnostic signals of three methines [\(\delta\) 61.0 (C-5), 56.4 (C-9) and 49.1 (C-13)] and three quaternary carbons [\(\delta\) 33.7 (C-4), 45.5 (C-8) and 41.1 (C-10)] indicated ent-kaurene skeleton of the diterpenoid, where the C-20 methyl is predicted to be \(\alpha\)-oriented on biogenetic grounds (Jahan et al. 2004; Yan et al. 2011). \(^1\)H–\(^1\)H COSY and HSQC analyses disclosed the oxygenated methine (\(\delta\) 69.6) was at C-6, and the presence of three proton sequences of CH (5)–CH (6)–CH\(_2\) (7), CH\(_2\) (1)–CH\(_2\) (2)–CH\(_2\) (3) and CH (9)–CH\(_2\) (11)–CH\(_2\) (12)–CH (13)–CH\(_2\) (14) (Figure S1). Careful inspection in the \(^1\)H and \(^{13}\)C NMR spectra revealed that 1 was similar to suremulol A (7) previously isolated from Suregada multiflora (Jahan et al. 2004). Compound 1

![Figure 1. Structures of compounds 1–6.](image)
differs from 7 only in the deficiency of an additional hydroxyl group at C-17. HMBC correlation from H-5 and H-7 to C-6 further supported C-6 was linked with OH group. The location of the oxygenated quaternary carbon was verified to be at C-16 by the correlation peaks from H-12, H-14, H-15 and CH$_3$-17 to C-16 in HMBC spectrum. Thus, the planar structure of 1 was elucidated as 6,16-dihydroxy-ent-kaurane. ROESY correlations from H-18, H-6 and H-9 to H-5 suggested H-6 were β-oriented (Figure S1); thus, OH at C-6 was in α-orientation. Cross peak between Me-17 and H-9 also displayed OH at C-16 was α-oriented. Finally, 1 was unambiguously identified as 6α,16α-dihydroxy-ent-kaurane, which is the third ent-kaurane diterpenoid isolated from the genus Ixora. Previously, only two ent-kaurane diterpenoids were isolated from I. coccinea (Lee et al. 2010).

By comparison of the physical and spectral data with literature values, the five known compounds (2–6) were identified, respectively, as (24R)-6β-hydroxy-24-ethyl-cholest-4-en-3-one (Arai et al. 1998), 7β-hydroxysitosterol (Liu et al. 2007), maslinic acid (Liu et al. 2006), 3,3′-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (Saleem et al. 1997) and protocatechuric acid (Zhang et al. 1994). Compounds 2–5 were isolated from the genus Ixora for the first time and 6 obtained originally from I. amplexicaulis.

3. Experimental

3.1. Apparatus and reagents

Optical rotations were determined on a Horiba SEAP-300 spectropolarimeter. IR was measured on a Perkin-Elmer 241 polarimeter. Mass spectrometry (MS) was obtained on a VG Auto Spec-3000 spectrometer. NMR spectra were recorded on a Bruker DRX-400 spectrometer at 400 MHz for $^1$H and 100 MHz for $^{13}$C using standard pulse sequence programs. All chemical shifts were recorded with respect to tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China), MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan) and RP-18 (40–75 μm, Fuji Chemical Industrial Co., Ltd, Tochigi, Japan). Thin layer chromatography (TLC) was performed on silica gel GF$_{254}$ (Yantai Jiangyou Silica Gel Co. Ltd, Yantai, China). Solvents were of industrial purity and distilled before use.

3.2. Plant material

The stems of I. amplexicaulis were collected from Mengla County of Yunnan Province, China in June, 2006 and identified by Mr Chaozhong Peng, a botanist of Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, where a voucher specimen (no. 0606005) was deposited.

3.3. Extraction and isolation

The stems of I. amplexicaulis (7.5 kg) were extracted with MeOH (three times, each 20 L) at ambient temperature. The MeOH extract was concentrated in vacuo to yield a dark brown residue (400 g). The MeOH extract was partitioned between EtOAc and H$_2$O to get the EtOAc extract (200 g), which was subjected to silica gel chromatography eluting with gradient petroleum ether: EtOAc (20:1–1:1) to provide six fractions. Fr. 3 (15 g) was further purified on column chromatography (MCI-gel CHP 20P, MeOH:H$_2$O, 8:2, 9:1 and 0:1) to afford fractions A–C. Separation of fraction A (2 g) by reversed phase RP-18 chromatography (MeOH:H$_2$O, 8:2 and 9:1) to afford A1 (80 mg) and A2 (30 mg). Compound 1 (20 mg) was obtained from fraction A1 by Sephadex LH-20 column chromatography (CHCl$_3$:MeOH, 3:2). Fraction B (3 g) was isolated by silica gel column chromatography (CHCl$_3$:MeOH, 50:1 → 0:1) to yield 2 (10 mg), 3 (25 mg),
and 4 (38 mg). Fraction C (1 g) afforded 5 (80 mg) and 6 (65 mg) by chromatography over silica gel with CHCl₃:MeOH (50:1 → 0:1) and then on Sephadex LH-20 column chromatography (CHCl₃:MeOH, 3:2).

3.3.1. 6α,16α-dihydroxy-ent-kaurane (1)
White amorphous powder; [α]D²¹⁷ — 63.25 (c 0.123, CHCl₃); IR (KBr) νmax cm⁻¹: 3386, 2927, 2850, 1445, 1038; EI-MS: 306 [M]+, HR-EI-MS m/z 306.2558 [M]+ (calcd for C₂₀H₃₄O₂⁺, 306.2559); ¹H NMR (CDCl₃, 400 MHz): δ 3.91 (1H, td, J = 10.5 and 3.6 Hz, H-6), 1.90 (1H, m, H-14a), 1.86 (1H, m, H-7a), 1.83 (1H, m, H-13), 1.70 (1H, m, H-14b), 1.62 (1H, m, H-2a), 1.60 (1H, m, H-1a), 1.59 (2H, m, H-15), 1.58 (1H, m, H-12a), 1.57 (1H, m, H-7b), 1.54 (1H, m, H-11a), 1.50 (1H, m, H-11b), 1.39 (1H, m, H-12b), 1.37 (1H, m, H-2a), 1.32 (1H, m, H-3a), 1.36 (3H, s, H-7), 1.20 (1H, m, H-3b), 1.14 (3H, s, H-18), 1.04 (3H, s, H-20), 0.98 (3H, s, H-19), 0.97 (1H, m, H-9), 0.87 (1H, d, J = 10.5 Hz, H-5), 0.75 (1H, m, H-1b); ¹³C NMR (CDCl₃, 100 MHz): δ 79.2 (s, C-16), 69.6 (d, C-6), 61.0 (d, C-5), 58.0 (t, C-15), 56.4 (d, C-9), 52.7 (t, C-7), 49.1 (d, C-13), 45.5 (s, C-8), 43.8 (t, C-3), 40.5 (t, C-1), 41.1 (s, C-10), 38.3 (t, C-14), 36.7 (q, C-18), 33.7 (s, C-4), 26.9 (t, C-12), 24.6 (q, C-17), 22.3 (q, C-19), 19.2 (q, C-20), 18.6 (t, C-2), 18.0 (t, C-11).

4. Conclusion
In our research, chemical constituents of *I. amplexicaulis* were first investigated and six compounds were isolated from the stems of *I. amplexicaulis*. 6α,16α-dihydroxy-ent-kaurane (1) is a new ent-kaurane diterpenoid. Compounds 2–5 were isolated from the genus *Ixora* for the first time and 6 obtained originally from *I. amplexicaulis*.

Supplementary material
Supplementary material relating to this article is available online.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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