A new sesquiterpenoid from *Polyalthia petelotii*

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**ABSTRACT**

A new sesquiterpenoid (−)-ethyl dihydrophaseate (1) was isolated from the stems and leaves of *Polyalthia petelotii*, together with two clerodane diterpenoids, 16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide (2), 15-hydroxy-cis-ent-cleroda-3,13(E)-diene (3), a eudesmane sesquiterpenoid, eudesm-4(15)-ene-7α,11-diol (4), an aromatic aldehyde, vanillin (5), a bisisoquinolinines alkaloid, spinosine (6) and an aporphine alkaloid, (−)-oliveroline-β-N-oxide (7). Their structures were established by extensive spectroscopic analysis, including 2D-NMR techniques. Compounds 3, 4 and 6 were isolated from the genus *Polyalthia* for the first time and the others obtained originally from *P. petelotii*. The isolates were assessed for their cytotoxicity against five human tumour lines (HL-60, SMMC-7721, A-549, MCF-7 and SW-480), and the result showed that only 2 displayed weak inhibitory activity.

**1. Introduction**

*Polyalthia* is a genus of about 120 species in the family Annonaceae, mainly distributing in tropical and subtropical eastern hemisphere, with 17 species being endemic to South China (Jiang et al. 1979). Various parts of the genus have been used in traditional medicine to treat ailments such as stomach ache, helminthiasis, dysmenorrhoea, pharynx neurosis, fever, skin disease, diabetes and hypertension (Sari et al. 2013; Wang et al. 2014; Bhattacharya et al. 2015). Previous investigations led to the isolation of many compounds including alkaloids,
diterpenoids, triterpenoids, sesquiterpenoids, flavonoids, styryllactones, furans, polyacetylenes, acetogenins and essential oils from plants of the genus (Kanokmedhakul et al. 2007; Wang, Ji, Shu, Chen, et al. 2012; Dai et al. 2014; Liu et al. 2014; Wang et al. 2014; Wu et al. 2014). Pharmacological studies showed that extracts of the genus and the isolated compounds displayed extensive activities, such as cytotoxic (Chen et al. 2000; Sashidhara et al. 2010), antiplasmodial (Kofi et al. 2015), anti-HIV (Li et al. 1993), antineoplastic (Lee et al. 2009), antibacterial (Wang, Ji, Shu, Song, et al. 2012), antioxidant (Sashidhara et al. 2011; Chen et al. 2014) and antityrpanosomal (Ngantchou et al. 2010). But there was no report on *P. petelotii*, which is a small shrub distributed in Vietnam and China. As a part of our research on structurally unique and bioactive compounds from medicinal plants of Yunnan, China, we have isolated and identified a new sesquiterpenoid, (−)-ethyl dihydrophaseate (1), as well as six known compounds, 16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide (2), 15-hydroxy-cis-ent-cleroda-3,13(E)-diene (3), eudesm-4(15)-ene-7α,11-diol (4), vanillin (5), spinosine (6) and (−)-oliveroline-β-N-oxide (7), from the stems and leaves of *P. petelotii* (Figure 1). Compounds 3, 4 and 6 were isolated from the genus *Polyalthia* for the first time and the others obtained originally from *P. petelotii*.

2. Results and discussion

Compound 1 was obtained as a colourless oil. It had the molecular formula C_{17}H_{26}O_{5} by HR-ESI-MS at m/z 333.1677 [M + Na]^{+}, implying five degrees of unsaturation. The IR spectrum showed the absorptions of hydroxyl groups, unsaturated ester carbonyl and C=C double bonds at 3444, 1698 and 1633 cm⁻¹, respectively. In ¹H NMR spectrum of 1, signals of a trans double bond at δ 8.02 (1H, d, J = 16.0 Hz, H-8) and 6.42 (1H, d, J = 16.0 Hz, H-7), a trisubstituted

![Figure 1. Structures of compounds 1–7.](image-url)
double bond at δ 5.75 (1H, s, H-10), a carboxylic acid ethyl ester at δ 1.29 (3H, t, J = 7.1 Hz) and 4.14 (2H, q, J = 7.1 Hz), an oxygenated methine [δ 4.28 (1H, m, H-3)], an alkenyl methyl [δ 2.04 (3H, d, J = 1.5 Hz, H-15)] and two tertiary methyls [δ 1.18, 0.96 (each 3H, s)] were observed. The 13C NMR and DEPT spectra of 1 showed 17 carbon signals corresponding to 4 methyls, 4 sp3 CH2, 3 sp3 CH, and 2 sp2 and 3 sp3 quaternary carbons, which was assigned to be a sequiterpenoid skeleton. 1H–1H COSY and HSQC analysis disclosed the oxygenated methine (δ 65.4) at C-3 and the presence of three proton sequences of CH2 (2)-CH (3), CH (7)-CH (8) and OCH2CH3 (Figure S2). Careful inspection of the 1H, 13C NMR and HMBC spectra revealed that 1 was similar to (−)-methyl dihydrophaseate (8) previously isolated from Astragalus complanatus and Carthamus tinctorius (Cui et al. 1993; Li et al. 2012). Compound 1 differs from 8 only in the presence of an ethoxy instead of a methoxy at C-11. HMBC correlation from OCH2 (δ 4.18) to C-11 (δ 166.2) further supported 1 was a carboxylic acid ethyl ester (Figure S2). Cross peaks from CH3-15 (δ 2.04) to H-7 (δ 6.42) and H-10 (δ 5.75) in ROE spectra of 1 suggested the configuration. Thus, 1 was unambiguously identified as (−)-ethyl dihydrophaseate.

By comparing the physical and spectral data with the literature values, the six known compounds (2–7) were identified respectively as 1α-hydroxy clerod-3,13(14)Z-dien-15,16-olide (2) (Misra et al. 2010), 15-hydroxy-cis-ent-cleroda-3,13(E)-diene (3) (Blas et al. 2004), eudesm-4(15)-ene-7α,11-diol (4) (Wang et al. 2008), vanillin (5) (Wang, Ji, Shu, Chen, et al. 2012), spinosine (6) (Queiroz et al. 1996) and (−)-oliveroline-β-N-oxide (7) (Wu 1989) from the stems and leaves of P. petelotii. Compounds 3, 4 and 6 were isolated from the genus Polyalthia for the first time and the others obtained originally from P. petelotii.

The isolates were assessed for their cytotoxicity against five human tumour lines (promyelocytic leukaemia cell line HL-60, liver cancer cell line SMMC-7721, lung cancer cell line A-549, breast adenocarcinoma cell line MCF-7 and colon carcinoma cancer cell line SW-480), and the result showed that only 2 displayed weak inhibitory activity, with IC50 of 21.28–34.89 μM against the tested human tumour lines.

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. MS was obtained on a VG Auto Spec-3000 spectrometer. NMR spectra were recorded on a Bruker DRX-AV-500 spectrometer at 500 MHz for 1H and 125 MHz for 13C using standard pulse sequence programs. All chemical shifts were recorded with respect to 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 Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden). TLC was performed on silica gel GF254 (Yantai Jiangyou Silica Gel Co. Ltd, Yantai, China). Solvents were of industrial purity and distilled prior to use.

3.2. Plant material

The stems and leaves of P. petelotii were collected from Jinghong County of Yunnan Province, China, in June, 2013 and identified by Mr Shuncheng Zhang, a botanist of Xishuangbanna
Tropical Botanical Garden, Chinese Academy of Sciences, where a voucher specimen (No. 1306018) was deposited.

3.3. Extraction and isolation

The air-dried stems and leaves of *P. petelotii* (11.0 kg) were powdered and extracted with MeOH (five times, each 60 L) at room temperature. After evaporation of the solvent, the MeOH extract was partitioned between EtOAc and 1% aq. H₂SO₄ to provide the EtOAc extracts (326 g), and the acidic aqueous phase was basified with aq. NaOH to pH 10 and then extracted with CHCl₃ to yield the crude alkaloids (4.0 g). The EtOAc extracts were applied to silica gel chromatography eluting with gradient petroleum ether:EtOAc (1:0 to 0:1) to provide seven fractions (A–G). Fr. B (45 g) was further purified on column chromatography (MCI-gel CHP 20, MeOH:H₂O 8:2) to afford fractions B₁–B₆. Separation of fraction B₁ (900 mg) was done by silica gel chromatography (CHCl₃:acetone 20:1) and Sephadex LH-20 column chromatography (MeOH) to afford 4 (22 mg) and 5 (12 mg). Fraction B₂ (640 mg) was isolated on Sephadex LH-20 column chromatography (CHCl₃:MeOH 3:2) to furnish 3 (35 mg). Fraction B₃ (260 mg) afforded 2 (13 mg) by chromatography (silica gel, CHCl₃:acetone 20:1, and then petroleum ether:acetone 3:1). The crude alkaloids (4.0 g) were subjected to column chromatography over silica gel eluting with gradient CHCl₃:MeOH (1:0 to 0:1) to yield four fractions (Fr. 1–Fr. 4). Fr. 1 (2 g) was purified on Sephadex LH-20 column chromatography (CHCl₃:MeOH 3:2) to produce two fractions (Fr. 1a and Fr. 1b). Fr. 1a (178 mg) was isolated on column chromatography (silica gel, petroleum ether:acetone:diethylamine 4:1:1) to furnish 1 (4 mg) and 6 (8 mg). Fr. 4 (90 mg) was separated on silica gel column chromatography (CHCl₃:MeOH 10:1) and then on Sephadex LH-20 (CHCl₃:MeOH 3:2) to give 7 (10 mg).

3.3.1. (−)-Ethyl dihydrophaseate (1)

Colourless oil; [α]D₂⁰ = −19.52 (c 0.0027, MeOH); IR (KBr) νmax cm⁻¹: 3444, 2932, 1698, 1633, 1602, 1382, 1230, 1160; ESI-MS m/z: 310 [M⁺]; HR-ESI-MS m/z 333.1677 [M + Na⁺] (Calcd for C₁₇H₂₆O₅Na, 333.1672); ¹H NMR (CDCl₃, 400 MHz): δ 8.02 (1H, d, J = 16.0 Hz, H-8), 6.42 (1H, d, J = 16.0 Hz, H-7), 5.75 (1H, s, H-10), 4.28 (1H, m, H-3), 4.14 (2H, q, J = 7.1 Hz, OCH₂CH₃), 3.80 (1H, dd, J = 7.8, 2.0 Hz, H-12a), 3.75 (1H, d, J = 7.8 Hz, H-12b), 2.17 (1H, m, H-4a), 2.04 (3H, d, J = 1.5 Hz, H-15), 1.91 (1H, m, H-2a), 1.74 (1H, m, H-4b), 1.69 (1H, m, H-2b), 1.29 (3H, t, J = 7.1 Hz, OCH₂CH₃), 1.18 (3H, s, H-14), 0.96 (3H, s, H-13); ¹³C NMR (CDCl₃, 100 MHz): δ 166.2 (s, C-11), 149.4 (s, C-9), 132.9 (d, C-7), 130.7 (d, C-8), 118.5 (d, C-10), 86.0 (s, C-5), 82.4 (s, C-6), 76.3 (t, C-12), 65.4 (d, C-3), 60.0 (t, OCH₂CH₃), 48.5 (s, C-1), 45.0 (t, C-4), 43.6 (t, C-2), 21.1 (q, C-15), 19.1 (q, C-14), 16.0 (q, C-13), 14.3 (q, OCH₂CH₃).

4. Conclusion

In our research, chemical constituents of *P. petelotii* were first investigated and seven compounds were isolated from the stems and leaves of *P. petelotii*. (−)-Ethyl dihydrophaseate (1) is a new sesquiterpenoid. Compounds 3, 4 and 6 were isolated from the genus *Polalthia* for the first time and the others obtained originally from *P. petelotii*. Compound 2 showed weak inhibitory activity towards five human tumour cell lines.
Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Bhattacharya AK, Chand HR, John J, Deshpande MV. 2015. Clerodane type diterpene as a novel antifungal agent from *Polyalthia longifolia* var. *pendula*. Eur J Med Chem. 94:1–7.

Blas B, Zapp J, Becker H. 2004. *ent*-Clerodane diterpenes and other constituents from the liverwort *Adelanthus lindenberghianus* (Lehm.) Mitt. Phytochemistry. 65:127–137.

Chen CY, Chang FR, Shih YC, Hsieh TJ, Chia YC, Tseng HY, Chen HC, Chen SJ, Hsu MC, Wu YC. 2000. Cytotoxic constituents of *Polyalthia longifolia* var. *pendula*. J Nat Prod. 63:1475–1478.

Chen XX, Liang G, Chai WM, Feng HL, Zhou HT, Shi Y, Chen QX. 2014. Antioxidant and antityrosinase proanthocyanidins from *Polalthia longifolia* leaves. J Biosci Bioeng. 118:583–587.

Cui B, Nakamura M, Kinjo J, Nohara T. 1993. Studies on the constituents of leguminous plants. Part XXXV. Chemical constituents of Astragalus species. Chem Pharm Bull. 41:178–182.

Dai DN, Thang TD, Ogunwande IA. 2014. Chemical composition of essential oils from the leaves and stem barks of Vietnamese species of *Polalthia harmandii*, *Polyalthia jucunda* and *Polyalthia thorelii*. Nat Prod Res. 28:555–562.

Jiang Y, Li BT, Li YH. 1979. Flora Reipublicae Popularis Sinicae. Vol. 30(2), Beijing: Science Press. p. 83–107.

Kanokmedhakul S, Kanokmedhakul K, Lekphrom R. 2007. Bioactive constituents of the roots of *Polalthia cerasoides*. J Nat Prod. 70:1536–1538.

Kofer A, Edmund E, Cindy A, Kwame S, Dominik P, Lukas O, Ben AG, Michael O. 2015. Antiplasmodial constituents from the stem bark of *Polyalthia longifolia* var. *pendula*. Phytochem Lett. 11:28–31.

Lee TH, Wang MJ, Chen PY, Wu TY, Wen WC, Tsai FY, Lee CK. 2009. Constituents of *Polyalthia longifolia* var. *pendula*. J Nat Prod. 72:1960–1963.

Li HY, Sun NJ, Kashiwada Y, Sun L, Snider JV, Cosentino LM, Lee KH. 1993. Anti-AIDS agents. 9. Suberosol, a new C<sub>31</sub> lanostane-type triterpene and anti-HIV principle from *Polalthia suberosa*. J Nat Prod. 56:1130–1133.

Li XF, Hu XR, Dai Z, Zhang Y, Liang H, Lin RC. 2012. Isolation and identification of two sesquiterpenes from flowers of *Carthamus tinctorius*. Chin Tradit Herb Drugs. 43:1685–1687.

Liu BJ, Jian L, Chen GY, Song XP, Han CR, Wang J. 2014. Chemical constituents and in vitro anticancer cytotoxic activities of *Polalthia plagioneura*. Chem Nat Compd. 49:1172–1174.

Misra P, Sashidhara KV, Singh SP, Kumar A, Gupta R, Chaudhaery S, Gupta SS, Majumder HK, Saxena AK, Dube A. 2010. 16α-Hydroxyclerodera-3,13(14)Z-dien-15,16-olide from *Polalthia longifolia*: a safe and orally active antileishmanial agent. Br J Pharmacol. 159:1143–1150.

Nkantchou I, Nyasse B, Denier C, Blonski C, Hannaert V, Schneider B. 2010. Antityranososomal alkaloids from *Polalthia suaveolens* (Annonaceae): their effects on three selected glycolytic enzymes of *Trypanosoma brucei*. Bioorg Med Chem Lett. 20:3495–3498.

Queiroz EF, Roblot F, Cavé A. 1996. Pessoine and spinosine, two catecholic berbines from *Annona spinescens*. J Nat Prod. 59:438–440.

Sari DP, Ninomiya M, Efdi M, Santoni A, Ibrahim S, Tanaka K, Koketsu M. 2013. Clerodane diterpenes isolated from *Polalthia longifolia* induce apoptosis in human leukemia HL-60 Cells. J Oleo Sci. 62:843–848.

Sashidhara KV, Singh SP, Sarkar J, Sinha S. 2010. Cytotoxic clerodane diterpenoids from the leaves of *Polalthia longifolia*. Nat Prod Res. 24:1687–1694.

Sashidhara KV, Singh SP, Srivastava A, Puri A. 2011. Identification of the antioxidant principles of *Polyalthia longifolia* var. *pendula* using TEAC assay. Nat Prod Res. 25:918–926.
Wang HX, Liu CM, Liu Q, Gao K. 2008. Three types of sesquiterpenes from rhizomes of *Atractylodes lancea*. Phytochemistry. 69:2088–2094.

Wang JH, Ji MH, Shu HM, Chen GY, Song XP, Wang J. 2012. Chemical constituents from the roots of *Polyalthia obliqua*. Chin J Nat Med. 10:303–306.

Wang JH, Ji MH, Shu HM, Song SP, Xiao XD. 2012. Antioxidation and antibacterial activity of extracts from root of *Polyalthia consanguinea*. Chin Tradit Pat Med. 34:617–620.

Wang LK, Zheng CJ, Li XB, Chen GY, Han CR, Chen WH, Song XP. 2014. Two new lanostane triterpenoids from the branches and leaves of *Polyalthia oblique*. Molecules. 19:7621–7628.

Wu TH, Cheng YY, Chen CJ, Ng LT, Chou LC, Huang LJ, Chen YH, Kuo SC, El-Shazly M, Wu YC, Chang FR, Liaw CC. 2014. Three new clerodane diterpenes from *Polyalthia longifolia* var. *pendula*. Molecules. 19:2049–2060.

Wu YC. 1989. Azafluorene and aporphine alkaloids from *Polyalthia longifolia*. Heterocycles. 29:464–475.