A new isoflavanone from the trunk of *Horsfieldia pandurifolia*

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A new isoflavanone, 2,2′-epoxy-4′-methoxy-3,7-dihydroxyisoflavanone (1), and a new natural coumaranone, 2-hydroxy-2-(4′-methoxybenzyl)-6-methoxy-3-coumaranone (2), along with 26 known compounds, were first isolated from the trunk of *Horsfieldia pandurifolia*. Their structures were elucidated by the means of spectroscopic analysis. Compound 1 was assessed for its cytotoxicity against five human tumour lines (HL-60, SMMC-7721, A-549, MCF-7 and SW-480), and the result showed that it has no activity.

Keywords: *Horsfieldia pandurifolia*; Myristicaceae; isoflavanone

1. Introduction

The genus *Horsfieldia* belongs to the family Myristicaceae and comprises about 90 species distributed in South Asia from India to Papua New Guinea, among which five are growing in China (Jiang & Li 1979). Literature survey showed that most phytochemical investigations were focused on the seeds of those species, as the seeds of *Horsfieldia glabra*, *Horsfieldia pandurifolia* and *Horsfieldia tetraptera* are good resources for industrial oil (Xu et al. 2012). Previous phytochemical investigation of non-oil constituents on the genus led to the isolation of arylalkanones, lignans, chromones, flavones, alkaloids and steroids, and some of the compounds demonstrated antimalarial and cytotoxic activities (Gunatilaka et al. 1982; Tillekeratne et al. 1982; Pinto et al. 1988; Jossang et al. 1991; Gonzalez et al. 2002; Al-Mekhlafi et al. 2013; Lu et al. 2014). *H. pandurifolia* is endemic to the subtropical area of Yunnan, China. However, its non-oil chemical constituents have never been reported up to date. As part of a research of structurally unique and biologically active compounds from medicinal plants of Yunnan, China, a new isoflavanone, 2,2′-epoxy-4′-methoxy-3,7-dihydroxyisoflavanone (1), and a new natural coumaranone, 2-hydroxy-2-(4′-methoxybenzyl)-6-methoxy-3-coumaranone (2), as well as 26 known compounds including 21 flavonoids from the trunk of *H. pandurifolia* (Figure 1) have been isolated and identified. Compound 2 has been synthesised (Chopin 1965), but it is new from natural sources.

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2. Results and discussion

Compound 1 was obtained as a white amorphous powder. The HR-EI-MS of 1 exhibited an [M]+ peak at 300.0634 (calcd for 300.0634), which suggested the molecular formula of C_{16}H_{12}O_{6}, indicating 11 degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl (3415 cm\(^{-1}\)), carbonyl (1658 cm\(^{-1}\)), and aromatic moieties (1497 and 1465 cm\(^{-1}\)). The \(^1\)H

Figure 1. Structures of compounds 1–28.
NMR spectrum of 1 displayed signals characteristic of a methoxyl [δ 3.79 (s)], a unique methine [δ 6.34 (s)] and two ABX patterns [δ 7.68 (d, J = 8.5 Hz, H-5), 6.64 (dd, J = 8.5 and 2.0 Hz, H-6), and 6.47 (d, J = 2.0 Hz, H-8); 6.56 (s, H-3)] and 12 aromatic carbons, suggesting that 1 is an isoflavanone. Assignments of the 1H and 13C NMR spectra of 1 were supported by a series of 2D NMR (1H-1H COSY, HSQC, and HMBC) experiments. HMBC correlation from methoxyl to C-4′ (δ 162.8) suggested the location of OMe at C-4, correlations from the methine proton to C-3, C-4, C-1′ (δ 119.2), C-9 (δ 160.4) and C-2′ (δ 161.3) revealed the location of the methine proton at C-2 and a hydroxyl at C-3 (Figure S1). Hence, the structure of 1 was assigned as 2,2′-epoxy-4′-methoxy-3,7-dihydroxyisoflavanone.

By comparison of the physical and spectral data with literature values, the 27 known compounds (2–28) were identified, respectively, as 2-hydroxy-2′-(4′′-methoxybenzy)-6-methoxy-3-coumarane (2) (Chopin 1965), α,2′-dihydroxy-4,4′-dimethoxiydihydrochalcone (3) (Xu et al. 2007), α,4,2′,4′-tetrahydroxydihydrochalcone (4) (Kulesh et al. 2008), rhusopolyphenol E (5) (Kim et al. 2013), 1-(2-hydroxy-4-methoxy-phenyl)-3-(3,4-methylenedioxyphenyl)-propan-2-ol (6) (Talukdar et al. 2000), 1-(2-hydroxy-4-methoxy-phenyl)-3-(3-methoxy-4-hydroxyphenyl)-propan-2-ol (7) (Rotz et al. 1990), isoliquiritigenin (8) (Aida et al. 1990), butein (9) (Wang & Ma 2009), sulfuretin (10) (Li et al. 2006), 7-hydroxyflavonone (11), liquiritigenin (12) (Yang et al. 2009), butin (13) (Kitanaka & Takido 1992), fustin (14) (Kim et al. 2010), (-)-festidinol (15) (Suresh et al. 2012), formononetin (16) (Zhang et al. 2003), iristrectorigenin A (17) (Huang et al. 2010), 2′-hydroxyformononetin (18) (Omobuwajo et al. 1992), tectorigenin (19) (Jeong et al. 2007), genistin (20) (Durango et al. 2002), dihydrogenistenin (21) (Heinonen et al. 1999), luteolin (22) (Yousfi et al. 2009), 3,7,4′-trihydroxyflavone (23) (Innok et al. 2010), ferulylaldehyde (24) (Haruna et al. 1982), sinapaldehyde (25) (Chen et al. 2008), syringic acid (26) (Zuo et al. 2013), 3,4-dihydroxybenzoic acid (27) (Enders & Milovanovic 2007) and β-sitosterol (28) (Kitajima & Tanaka 1993).

The new compound 1 was assessed for its 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), but it showed no activity.

3. Experimental
3.1. Apparatus and reagents
Optical rotations were measured with a JASCO DIP-370 digital polarimeter (JASCO Corporation, Tokyo, Japan). A BioRad Fis-135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets (Bio-Rad Corporation, Hercules, CA, USA). 1D and 2D NMR spectra were recorded on a Bruker DRX-AV-500 spectrometer (Bruker BioSpin Group, Rheinstetten, Germany) with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. MS data were measured on a VG Auto Spec-3000 spectrometer (VG PRIMA, Birmingham, England). Column chromatography was performed on silica gel (80–100 and 200–300 mesh) (Qingdao Marine Chemical Factory, Qingdao, China), and Sephadex LH-20 (20–80 μm; Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden). Fractions were monitored by TLC (Si gel GF254) (Qingdao Marine Chemical Factory, Qingdao, China) and spots were visualised by heating silica gel plates sprayed with 10% H2SO4 in EtOH. Solvents were of industrial purity and distilled prior to use.
3.2. **Plant material**

The trunk of *H. pandurifolia* was collected in January 2007 from Mengla of Yunnan, China, and identified by Mr Chao-Zhong Peng, a botanist of Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. A voucher specimen (No. 070120) has been deposited at the School of Chemistry and Chemical Engineering, Yunnan Normal University.

3.3. **Extraction and isolation**

The trunk of *H. pandurifolia* (8.5 kg) was extracted with MeOH (30 L) for four times at room temperature. Evaporation of the solvent under reduced pressure gave an extract (280 g), which was partitioned between EtOAc and H$_2$O. The EtOAc-soluble portion (105 g) was subjected to a silica gel CC (450 g, 100-200 mesh) using a gradient of petroleum ether–EtOAc (50:1 to 0:1) to yield 11 fractions (A–K).

Fr. C (14.4 g) was washed with EtOAc and recrystallised from CHCl$_3$ to afford 28 (1.5 g). Compound 3 (49 mg) was obtained from fr. D (2.4 g) by Sephadex LH-20 CC eluting with CHCl$_3$–MeOH (3:2), and further purified on a silica gel with petroleum ether–acetone (5:1). Fr. E (1.0 g) was separated by Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2), and silica gel column eluting with CHCI$_3$–MeOH (100:1) to give 6 (182 mg) and fr. E$_1$ (77 mg), which was isolated by Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2) to give 11 (25 mg). Fr. F (6.1 g) was subjected to CC over Sephadex LH-20 eluting with CHCl$_3$–MeOH (3:2) to produce five fractions (F$_1$–F$_5$).

F$_2$ (575 mg) was subjected to silica gel column eluting with CHCl$_3$–MeOH (60:1), and then isolated on Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2) to furnish 2 (11 mg) and 16 (10 mg). Fr. F$_3$ (490 mg) was fractionated by silica gel column, eluting with CHCl$_3$–MeOH (60:1) to give F$_3$–1 (190 mg), F$_3$–2 (46 mg) and F$_3$–3 (170 mg), respectively. From F$_3$–2, 24 (88 mg) was obtained by Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2), and silica gel column eluting with petroleum ether–EtOAc (3:1). Compound 7 (16 mg) was afforded from F$_3$–2 by CC over silica gel eluting with petroleum ether–acetone (4:1). Fr. G (3.9 g) was subjected to Sephadex LH-20 column chromatography and eluted with CHCl$_3$–MeOH (3:2) to yield three fractions (G$_1$–G$_3$). G$_1$ (293 mg) was isolated on silica gel eluting with CHCl$_3$–MeOH (60:1) to produce three subfractions (G$_{1-1}$–G$_{1-3}$). Compound 25 (6 mg) was obtained from G$_{1-1}$ (37 mg) on a silica gel column, eluting with petroleum ether–acetone (4:1). Fr. H (6.2 g) was separated over Sephadex LH-20 CC (CHCl$_3$–MeOH: 3:2) into four fractions (H$_1$–H$_4$). 12 (125 mg) was obtained by CC over silica gel eluting with petroleum ether–EtOAc (2:1) and Sephadex LH-20 of H$_2$ (1.6 g) with MeOH. H$_3$ (750 mg) was subjected to CC over silica gel eluting with a gradient of CHCl$_3$–MeOH (50:1 to 30:1) to yield four subfractions (H$_{3-1}$–H$_{3-4}$). From H$_3$–3 (76 mg), 4 (13 mg), 20 (10 mg) and 21 (3 mg) were furnished by isolating on Sephadex LH-20 CC with MeOH and CC over silica gel with petroleum ether–EtOAc (2:1). In the same way, 5 (14 mg) and 8 (49 mg) were yielded from H$_3$–4 (40 mg) and H$_4$ (70 mg), respectively. Fr. I (6.2 g) was subjected to Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2) to give five fractions (I$_1$–I$_5$). Compound 26 (25 mg) was obtained from I$_2$ (1.6 g) by Sephadex LH-20 CC with CHCl$_3$–MeOH (3:2). I$_4$ (975 mg) was subjected to silica gel column eluting with CHCl$_3$–MeOH (20:1) and petroleum ether–acetone (3:2), and then isolated on Sephadex LH-20 CC with MeOH to give 10 (11 mg), 13 (31 mg), 15 (64 mg), 23 (19 mg) and 27 (45 mg). Compound 9 (8 mg) was obtained from I$_5$ (35 mg) by silica gel column
eluting with CHCl$_3$–MeOH (20:1). From fr. J (7.2 g), 14 (43 mg) and 22 (8 mg) were purified by silica gel column eluting with CHCl$_3$–MeOH (15:1), petroleum ether–acetone (5:4) and Sephadex LH-20 CC with MeOH.

3.3.1 2,2'-Epoxy-4'-methoxy-3,7-dihydroxyisoflavanone (1)
White amorphous powder; $[a]_D^{26}$ $-0.33$ (c = 0.10, acetone); IR (KBr) $\nu_{max} \text{cm}^{-1}$: 33415, 1658, 1620, 1497, 1465, 1376, 1330, 1282, 1195, 1171, 1143, 1111, 1041, 994, 974, 831; HR-EI-MS $m/z$: 300.0634 [M]$^+$ (calcd for C$_{16}$H$_{12}$O$_6$, 300.0634). $^1$H NMR (CD$_3$COCD$_3$, 500 MHz): $\delta$ 7.68 (1H, d, $J$ = 8.5 Hz, H-5), 7.20 (1H, d, $J$ = 9.0 Hz, H-6), 6.56 (s, H-3'), 6.64 (1H, dd, $J$ = 8.5 and 2.0 Hz, H-6), 6.54 (1H, d, $J$ = 9.0 Hz, H-5'), 6.47 (1H, d, $J$ = 2.0 Hz, H-8), 6.34 (1H, s, H-2), 3.79 (3H, s, 4'-OMe); $^{13}$C NMR (CD$_3$COCD$_3$, 125 MHz): $\delta$ 187.9 (s, C-4), 165.3 (s, C-7), 162.8 (s, C-5), 161.3 (s, C-2'), 160.4 (s, C-9), 129.1 (d, C-5), 125.4 (d, C-6'), 119.2 (s, C-1'), 113.3 (d, C-2'), 112.0 (s, C-10), 111.9 (d, C-6), 108.5 (d, C-5'), 103.3 (d, C-8), 96.5 (d, C-3'), 79.5 (s, C-3), 54.4 (q, 4'-OMe).

3.3.2 2-Hyroxy-2-(4'-methoxybenzyl)-6-methoxy-3-coumaranone (2)
Light-yellow oil; $^1$H NMR (500 MHz, CD$_3$COCD$_3$): $\delta$ 7.36 (1H, d, $J$ = 8.0 Hz, H-4), 7.15 (2H, d, $J$ = 8.5 Hz, H-2', 6'), 6.73 (2H, d, $J$ = 8.5 Hz, H-3', 5'), 6.62 (1H, br, 2-OH), 6.54 (1H, dd, $J$ = 8.5 and 2.0 Hz, H-5), 6.52 (1H, d, $J$ = 2.0 Hz, H-7), 3.88 (3H, s, 6-OMe), 3.70 (3H, s, 4'-OMe), 3.19 (1H, d, $J$ = 14.0 Hz, H$_a$CH$_2$), 3.14 (1H, d, $J$ = 14.0 Hz, H$_b$CH$_2$); $^{13}$C NMR (125 MHz, CD$_3$COCD$_3$): $\delta$ 195.8 (s, C-3), 172.8 (s, C-7a), 168.7 (s, C-6), 158.6 (s, C-4'), 131.5 (d, C-2', 6'), 125.8 (s, C-1'), 125.2 (d, C-4), 113.2 (d, C-3', 5'), 113.0 (s, C-3a), 110.6 (d, C-5), 106.0 (s, C-2), 96.0 (d, C-7), 55.5 (q, 6-OMe), 54.4 (q, 4'-OMe), 40.7 (t, CH$_2$).

4. Conclusion
In this study, 28 compounds were first isolated and identified from the trunk of *H. pandurifolia*. 2,2'-Epoxy-4'-methoxy-3,7-dihydroxyisoflavanone (1) is a new isoflavanone, 2-hyroxy-2-(4'-methoxybenzyl)-6-methoxy-3-coumaranone (2) is being isolated for the first time from natural sources, and 3–23 are different types of flavonoids. Compound 1 was assessed for its cytotoxicity against five human tumour lines with no activity.

Supplementary material
Supplementary material relating to this article is available online at http://dx.doi.org/10.1080/14786419.2015.1043554.

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

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