Dalvelutinoside, a new isoflavone glycoside from the methanol extract of *Dalbergia velutina* roots

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ABSTRACT

A new isoflavone glycoside, dalvelutinoside (1), together with one known isoflavone (2) and five known isoflavone glycosides (3–7) were isolated from the methanol extract of the roots of *Dalbergia velutina*. Their structures were determined by spectroscopic analysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines.

1. Introduction

*Dalbergia velutina* is a perennial climbing tree (Leguminosae), mainly distributed in the north-eastern of Thailand. The previous phytochemistry of this genus resulted in the isolation of various compounds classified as isoflavones (Nagarajan et al. 2006), isoflavanes, isoflavanones, neoflavones, pterocarpsans, anthraquinones, triterpenes, cinnamyl esters (Vasudeva et al. 2009) and phenolic compounds (Inui et al. 2014). Many of these compounds have demonstrated several interesting biological activities such as anticancer, antimicrobial, antioxidant, antiulcerogenic, antiplasmodial (Zheng et al. 2012 and Mutai et al. 2013), analgesic, anti-inflammatory and antibacterial (Narayanan et al. 2007).

In our previous studies on *D. velutina* (Kaennakam et al. 2015), we reported six pterocarpsans with cytotoxicity against KB and HeLa cell lines from CH$_2$Cl$_2$ extract of the roots of this plant. Our continuous study on the methanol extract of this plant led to the isolation of a new isoflavone glycoside, dalvelutinoside (1) along with one known isoflavone, olibergin.
A (2) (Ito et al. 2003) and five known isoflavone glycosides, 2',5'-dimethoxy-genistein 7-O-β-D-apiofuranosyl-(1″→6″)-O-β-D-glucopyranoside (3) (Marzouk et al. 2008), caviunin 7-O-β-D-apiofuranosyl-(1″→6″)-β-D-glucopyranoside (4) (Dixit et al. 2012), genistein-8-C-β-D-glucopyranoside (5), orobol-8-C-β-D-glucopyranoside (6) (Zapesochnaya & Laman 1978) and dalpanitin (7) (Adinarayana & Rajasekhara 1972) (Figure 1). In addition, cytotoxicity result against KB and HeLa cells of all isolated compounds are also reported. Their structures were identified by interpretation of their spectroscopic data as well as comparison with those reported in the literature.

2. Results and discussion

The MeOH extract from the roots of D. velutina was separated by silica gel (Merck Art 7730) and Sephadex LH-20 column chromatography to obtain the compounds 1–7. Their structures were elucidated on the basis of detailed spectroscopic analysis including 1D and 2D NMR spectroscopy and mass spectrometry techniques.

Dalvelutinoside (1) was obtained as a white amorphous powder, \([\alpha]_{D}^{20}=−62.8\) (c 1.0, DMSO). Its molecular formula was determined as \(\text{C}_{23}\text{H}_{24}\text{O}_{12}\) by HRESIMS measurement through the pseudomolecular ion peak at \(m/z\) 515.1161 \(\text{[M + Na]}^{+}\) (calcd. for \(\text{C}_{23}\text{H}_{24}\text{O}_{12}\text{Na}, 515.1165\)). The UV spectrum displayed absorption bands at \(\lambda_{max}\) 244, 262 and 309 nm. The IR spectrum
showed absorption bands for hydroxyl groups (3410 cm$^{-1}$), a carbonyl group (1665 cm$^{-1}$) and aromatic moieties (1540 and 1500 cm$^{-1}$). The $^1$H NMR spectrum had signals of four aromatic protons at $\delta_H$ 6.47 (1H, $J = 2.0$ Hz, H-6), 6.59 (1H, s, H-3′), 6.72 (1H, $J = 2.4$ Hz, H-8), 6.88 (1H, s, H-6″), two phenolic protons at $\delta_H$ 9.25 (1H, s, OH-4′) and 12.92 (1H, s, OH-5, chelating to the carbonyl) which were consistent with a 5,7-disubstituted aromatic ring A and a 2,4,5-trisubstituted aromatic ring B. The four hydroxyl groups of sugar unit at $\delta_H$ 4.61 (1H, $J = 5.4$ Hz, OH-6″), 5.06 (1H, $J = 5.6$ Hz, OH-3″), 5.13 (1H, $J = 4.4$ Hz, OH-4″), 5.41 (1H, $J = 4.8$ Hz, OH-2″), and two methoxy at $\delta_H$ 3.64 (3H, s, OCH$_3$–2′) and 3.72 (3H, s, OCH$_3$–5′). In addition, one olefinic proton at $\delta_H$ 8.28 (1H, s, H-2) is the characteristic of isoflavone skeleton.

The $^{13}$C NMR spectra showed 23 signals comprising 2 methyl, 1 methylene, 10 methine and 10 quaternary carbons. Compound 1 was isoflavone glucoside with anomeric proton signal at $\delta_H$ 5.06 (1H, $J = 7.2$ Hz, H-1″) and anomeric carbon resonance at $\delta_C$ 99.7 (C-1″) of glucose indicated the β-configuration. In the HMBC correlations of 1 (Figure S6), the anomeric proton at H-1″ showed cross peaks with C-3″ ($\delta_C$ 77.0) and C-7 ($\delta_C$ 162.7), hydroxy proton at $\delta_H$ 12.92 (1H, s, OH-5) had the correlations with C-5 ($\delta_C$ 161.3), C-6 ($\delta_C$ 99.3) and C-10 ($\delta_C$ 105.7) and aromatic proton at $\delta_H$ 6.72 (1H, $J = 2.4$ Hz, H-8) correlated with C-7 ($\delta_C$ 162.7), C-9 ($\delta_C$ 157.0) and C-10. The above data indicated that one glucoside unit was located at C-7 of ring A, while those H-2 to C-3 ($\delta_C$ 120.4), C-4 ($\delta_C$ 180.1), C-9 ($\delta_C$ 157.0) and C-1′ ($\delta_C$ 108.8) established that ring C was attached to C-1′ of ring B and two methoxyl groups connected to C-2′ and C-5′ showed cross peak with $\delta_C$ 151.8 and 140.9, respectively. In addition, a hydroxyl group at position 4′ related to C-3′ ($\delta_C$ 100.7), C-4′ ($\delta_C$ 147.7) and C-5′ ($\delta_C$ 140.9). The $^1$H and $^{13}$C NMR spectroscopic data were shown to be quite similar to those of the known isoflavone glycoside, 2′,5′-dimethoxy-genistein 7-O-β-D-apiofuranosyl-(1″→6″)-O-β-D-glucopyranoside (3), except for compound 1 comprised only one glucose unit. The appearance of a glucopyranosyl group was confirmed by TLC analysis of the hydrolysis of 1 with 1 M HCl, and the absolute configuration of this sugar moiety was identified as D-glucopyranoside by HPLC analysis. Thus, the complete assignment of dalvelutinoside (1) was determinated as 2′,5′-dimethoxy-genistein 7-O-β-D-glucopyranoside. The in vitro cytotoxic activities of these compounds are shown in Table 1, compounds 2 and 4 showed weak cytotoxicity against KB and HeLa cells with IC$_{50}$ values of 48.06, 63.77 μM and 59.82, 86.36 μM, respectively, and other compounds were inactive.

3. Experimental

3.1. General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker 400 AVANCE spectrometer. Melting points were obtained using Fisher-Johns Melting Point apparatus. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data were obtained using a Nicolet 6700 FT-IR spectrometer using KBr discs. UV-visible absorption spectra were taken on a UV-2550 UV–vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotation was recorded by Jasco P-1010 Polarimeter. HPLC analysis were obtained using a Alltech System equipped with model 626 binary gradient pumps, select TM degasser, 580 autosampler, 2000 ES evaporative light scattering detector (Alltech) and peak simple chromatography data system software.
3.2. Plant material

The roots of *D. velutina* were collected from Sahatsakhan district, Kalasin province, Thailand, in October 2014. The plant material was identified by Ms Suttira Khumkratok, and a voucher specimen was deposited as a reference (Khumkratok No. 4-12) at the Walai Rukhavej Botanical Research Institute, Mahasarakham University.

3.3. Extraction and isolation

The air-dried roots of *D. velutina* (5.5 kg) were extracted with CH$_2$Cl$_2$ and MeOH over a period of 3 days at room temperature, respectively (3 × 15 L). Removal of the solvent under reduced pressure provided MeOH (80.5 g) crude extracts. The MeOH crude extract was further separated by column chromatography over silica gel (Merck Art 7730) and eluted with a gradient of EtOAc-MeOH (100% EtOAc, 90, 80 and 70% EtOAc-MeOH each 5 L, respectively) to give five fractions (A–E). Fraction A (4.5 g) was purified by Sephadex LH-20 column (150 g) with 100% MeOH (1.5 L) to afford compound 1 (12.5 mg) and compound 2 (20.5 mg). Fraction B (6.5 g) was separated by Sephadex LH-20 column (150 g) eluted with 100% MeOH (1.5 L) to give compound 5 (8.5 mg), compound 6 (10.5 mg) and compound 7 (14.5 mg). Finally, fraction C (5.0 g) was subjected to a Sephadex LH-20 column (150 g) eluted with 100% MeOH (1 L) to yield compound 3 (15.0 mg) and compound 4 (16.5 mg).

**Dalvelutoside (1):** White amorphous powder; m.p. 218.0–219.0 °C; [α]$_D$ –62.8 (c 1.0, DMSO); UV $\lambda_{\text{max}}$ (DMSO) 244, 262, 309 nm; IR $\nu_{\text{max}}$ (KBr): 3410, 1665, 1540, 1500 cm$^{-1}$; $^{1}$H NMR (400 MHz, in DMSO-$_d$6) $\delta$H: 12.92 (1H, s, OH-5), 9.25 (1H, s, OH-4′), 8.28 (1H, s, H-2), 6.88 (1H, s, H-6′), 6.72 (1H, d, $J = 2.4$ Hz, H-8), 6.59 (1H, s, H-3′), 6.47 (1H, d, $J = 2.0$ Hz, H-6), 5.41 (1H, d, $J = 4.8$ Hz, OH-2″), 5.13 (1H, d, $J = 4.4$ Hz, OH-4″), 5.07 (1H, d, $J = 5.6$ Hz, OH-3″), 5.06 (1H, d, $J = 7.2$ Hz, H-1″), 4.61 (1H, t, $J = 5.4$ Hz, OH-6″), 3.72 (3H, s, OCH$_3$–5′), 3.70 (1H, m, H-6″), 3.64 (3H, s, OCH$_3$–2′), 3.46 (1H, m, H-3″), 3.32 (1H, m, H-5″), 3.29 (1H, m, H-2″), 3.18 (1H, m, H-4″). $^{13}$C NMR (100 MHz, in DMSO-$_d$6) $\delta$C: 180.1 (C-4), 162.7 (C-7), 161.3 (C-5), 157.0 (C-9), 155.7 (C-2′), 152 (C-2′′), 147.7 (C-4″), 141 (C-5′), 120.4 (C-3), 116.4 (C-5′), 108.8 (C-1′), 105.7 (C-10), 100.7 (C-3′), 99.7 (C-1″), 99.3 (C-6), 94.4 (C-8), 77.0 (C-3″), 76.2 (C-5″), 72.9 (C-2″), 69.4 (C-4″), 60.4 (C-6″), 56.5 (C-5′), OCH$_3$, 55.8 (C-2″, OCH$_3$), HRESIMS m/z: 515.1161 [M + Na]$^+$ (calcd. for C$_{23}$H$_{24}$O$_{12}$Na, 515.1165).

3.4. Acidic hydrolysis and HPLC analysis

A solution of dalvelutoside (1) (2 mg) in 1 M HCl (1.0 mL) was heated at reflux for 1 h and then the reaction mixture was neutralised with an equal volume of 1 M NaOH and extracted with CH$_2$Cl$_2$ (5 mL). The sugar moiety was identified as glucose by co-TLC analysis (EtOAC: MeOH: H$_2$O, 1:8:1) of the aqueous solution in comparison with an authentic glucose. In addition, the glucose was identified as D-glucose by HPLC analysis (column: lichrocart-NH$_2$ (250 × 4.0 mm), carrier: 82% ACN in H$_2$O (1.5 mL/min), retention time: 8.133 min) in comparison with an authentic D-glucose.

3.5. Cytotoxicity assay

All isolated compounds (1–7) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the
colorimetric method (Skehan et al. 1990; Kongkathip et al. 2003; Kaennakam et al. 2015). Adriamycin was used as the reference substance which exhibited activity against KB and HeLa cell lines.

### 4. Conclusion

The MeOH crude extract from the roots of *D. velutina* comprises one new dalvelutinoside (1), one known isoflavone (2) and five known isoflavone glycosides (3–7). Compound 3 was isolated for the first time from this genus. Compounds 2 and 4 showed weak cytotoxicity against KB and HeLa cells with IC₅₀ values of 48.06, 63.77 µM and 59.82, 86.36 µM, respectively. Therefore, we believe that this plant is an important source for the diverse structure of isoflavone glycosides and should be further investigated for other biological activities.

### Supplementary material

Supplementary material relating to this paper is available online, along with Figures S1–S6.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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