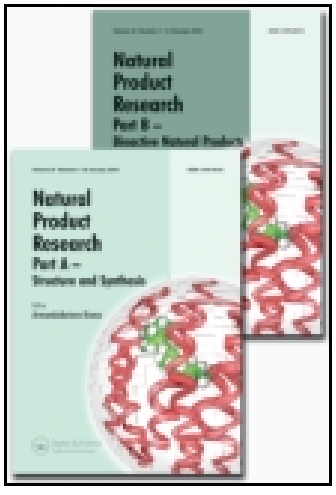

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Two new glycosides from Vitex negundo

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Two new glycosides from *Vitex negundo*

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Two new glycosides, 2-methyl pyromeconic acid 3-*O*-β-D-glucopyranoside-6′-(*O*-4″-hydroxybenzoate) (**1**), 6′-*O*-*p*-hydroxybenzoyl-gardoside (**2**) and four known iridoid glycosides (**3**–**6**) were isolated from the whole plant of *Vitex negundo*. Their structures were elucidated on the basis of spectroscopic methods including HR-ESI-MS, 1D and 2D NMR.

Keywords: *Vitex negundo*; Verbenaceae; pyromeconic acid derivative; iridoid glycoside

1. Introduction

The plant *Vitex negundo* Linn. is a shrub belonging to the family Verbenaceae, mainly distributed in Southern Asia (Watt 1972). Traditionally, the extract of *V. negundo* is widely used for the treatment of some female disorders (Tiwari & Tripathi 2007). Previous phytochemical studies of this plant had led to the isolation of terpenoids (Vishnoi et al. 1983; Chawla et al. 1991, 1992a), volatile oil (Singh et al. 1999), flavonoids (Misra & Subramanian 1980; Achari et al. 1984), iridoid glycosides (Sehgal et al. 1983), stilbenes (Banerji et al. 1988) and lignans (Chawla et al. 1992b). This paper reports the isolation and structural elucidation of two new compounds (**1**–**2**), along with four known iridoid glycosides (**3**–**6**).

2. Results and discussion

Compound **1** was isolated as colourless needles. The molecular formula of **1** was established as $C_{19}H_{20}O_{10}$ by the HR-ESI-MS at *m/z* 431.0988 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_{10}Na$, 431.0954). The IR spectrum indicated the presence of hydroxyl (3317 cm^{-1}), carboxyl (1696 cm^{-1}) and aromatic ring (1608 and 1516 cm^{-1}). The ^1H NMR spectrum showed the presence of two olefinic protons at δ_H 7.86 (1H, d, $J = 5.6$ Hz) and 6.34 (1H, d, $J = 5.6$ Hz), a 1,4-disubstituted benzene ring at δ_H 7.82 (2H, d, $J = 7.8$ Hz) and 6.81 (2H, d, $J = 7.8$ Hz), an anomeric proton of sugar unit at δ_H 4.89 (1H, d, $J = 7.3$ Hz) and a methyl at δ_H 2.29 (3H, s). The ^{13}C NMR and DEPT spectra displayed the signals of a benzene ring at δ_C 163.6, 132.8 × 2, 122.2 and 116.4 × 2; a pyranone moiety at δ_C 176.9, 164.5, 156.9, 143.1 and 117.2; a glucopyranosyl unit at δ_C 104.6, 77.9, 76.0, 75.4, 71.8 and 64.4 and a methyl at δ_C 15.6.

Comparison of ^1H, ^{13}C NMR data of **1** with those of pyromeconic acid 3-*O*-β-D-glucopyranoside-6′-(*O*-4″-hydroxybenzoate) (Ahmed & Mohamed 2002) revealed that most

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signals of the two compounds were similar except that **1** had the signal of an extra methyl at δ_H 2.29 (3H, s)/δ_C 15.6, and the downfield shift of C-3 from δ_C 146.5 to δ_C 164.5, as well as the upfield shift of C-2 from δ_C 147.8 to δ_C 143.1. All the above data suggested that the methyl was located at C-2 position, which was confirmed by the HMBC correlations between H-7 (δ_H 2.27) and C-2 (δ_C 143.1)/C-3 (δ_C 164.5) (Figure 1). Acid hydrolysis of **1** afforded D-glucose, which was identified by GC analysis. The β-configuration of D-glucose was determined by the coupling constant ($J = 7.3$ Hz) of the anomeric proton. Consequently, compound **1** was established as 2-methyl pyromeconic acid 3-*O*-β-D-glucopyranoside-6′-(*O*-4″-hydroxybenzoate).

Compound **2** was obtained as amorphous powder. The molecular formula of **2** was determined as $C_{23}H_{26}O_{12}$ by its HR-ESI-MS at *m/z* 493.1332 $[M - H]^+$ (calcd for $C_{23}H_{25}O_{12}$, 493.1346). Acid hydrolysis of **2** also afforded D-glucose. The IR spectrum showed the presence of hydroxyl (3378 cm^{-1}), carboxyl (1697 cm^{-1}) and aromatic ring (1608, 1516 cm^{-1}). The ^1H NMR spectrum showed two olefinic protons at δ_H 5.23 (1H, t, $J = 1.8$ Hz) and 5.15 (1H, br s), suggesting the presence of an exo-methylene group. The protons of a 1,4-disubstituted benzene ring at δ_H 7.87 (2H, d, $J = 7.8$ Hz) and 6.81 (2H, d, $J = 7.8$ Hz), as well as an anomeric proton of sugar unit at δ_H 4.69 (1H, d, $J = 7.9$ Hz), were also displayed in the ^1H NMR spectrum. The ^{13}C NMR spectrum showed 23 carbon signals including a glucopyranosyl unit at δ_C 100.2, 77.9, 75.6, 74.7, 71.9 and 64.6; a *p*-hydroxybenzoyl moiety at δ_C 167.9, 163.8, 132.8 × 2, 122.2 and 116.2 × 2 and an iridoid aglycone at δ_C 172.1, 152.8, 152.0, 113.5, 113.2, 96.8, 73.9, 44.7, 41.1 and 32.5. All the above data indicated that **2** was an iridoid glycoside with a benzoyl unit.

The NMR data of **2** were very similar to those of the known compound 6′-*O*-benzoyl-gardoside (Harput et al. 2004). The difference was the presence of a *p*-hydroxybenzoyl (δ_C 167.9, 163.8, 132.8 × 2, 122.2, 116.2 × 2) in **2** instead of a benzoyl (δ_C 167.8, 134.4, 131.4, 130.6 × 2, 129.7 × 2) in 6′-*O*-benzoyl-gardoside. The HMBC correlation between H-6′ (δ_H 4.58) and C-7″ (δ_C 167.9) confirmed that the *p*-hydroxybenzoyl group was connected to C-6′ position of glucose. Thus, Compound **2** was elucidated as 6′-*O*-*p*-hydroxybenzoyl-gardoside.

In addition, four known compounds (**3**–**6**) (Figure 2) were elucidated as 6′-*p*-hydroxybenzoylmussaenosidic acid (**3**) (Sehgal et al. 1983), 2′-*p*-hydroxybenzoylmussaenosidic acid (**4**) (Sehgal et al. 1982), 2′-*O*-*trans*-*p*-coumaroylloganic acid (**5**) (Wu et al. 2009) and 2′-*O*-*trans*-*p*-hydroxybenzoyl-8-epiloganic acid (**6**) (Sridhar & Subbaraju 2004), respectively, by comparison of their spectral data with those of the literatures.

3. Experimental

3.1. General methods

Melting points were obtained on an X-5 micro-melting point detector (uncorrected). Optical rotation values were measured on a JASCO P-1020 polarimeter. UV spectra were recorded by a JASCO V-550 UV/Vis spectrophotometer. IR spectra were measured on a JASCO FT/IR-480 plus FT-IR spectrometer. HR-ESI-MS data were carried out on an Agilent 6210 ESI/TOF mass

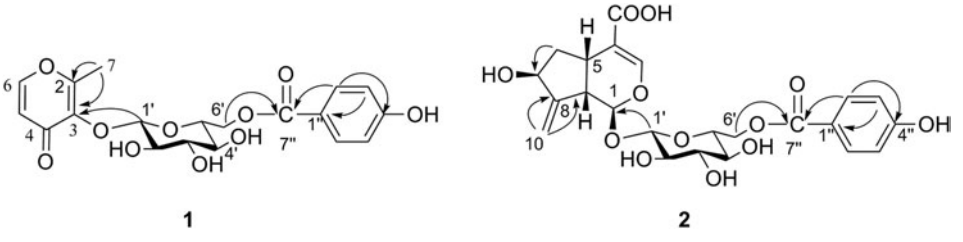
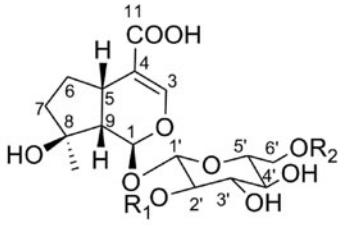
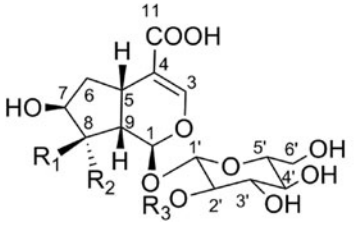


Figure 1. The chemical structure and key HMBC of compounds **1** and **2**.



3 R_1 = H R_2 = *p*-hydroxybenzoyl
4 R_1 = *p*-hydroxybenzoyl R_2 = H



5 R_1 = CH_3 R_2 = H R_3 = (*E*)-*p*-coumaroyl
6 R_1 = H R_2 = CH_3 R_3 = *p*-hydroxybenzoyl

Figure 2. The chemical structure of compounds **3**–**6**.

spectrometer. 1D and 2D NMR spectra were recorded on Bruker AV-400 spectrometer using tetramethylsilane (TMS) as internal standard. GC was carried out on a Shimadzu GCMS-QP2010 plus gas chromatograph–mass spectrometer using HP-1701 column (0.25 mm × 30 m). A Waters 1525 pump and a 2487 Dual λ absorbance detector for analytical HPLC using Cosmosil 5C18-MS-II waters column (4.6 mm × 250 mm). A Gilson 360 pump and a UV/Vis-152 detector for preparative HPLC using Cosmosil 5C18-MS-II waters column (20 mm × 250 mm). Column chromatographies were carried out on macroporous resin such as Diaion HP-20 (Mitsubishi Chemical Corporation, Japan), silica gel (200–300 mesh; Qingdao Marine Chemical Group Co. Ltd, Qingdao, China), ODS (50 μm, 120 Å; YMC) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC was performed using precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, Yantai, China) or RP-18 F_{254} plates (Merck, Darmstadt, Germany). D- and L-glucose were purchased from Sigma (St Louis, MO, USA).

3.2. Plant material

The whole plant of *V. negundo* was collected in Guangzhou city, Guangdong Province of P.R. China, and authenticated by Prof. Guang-Xiong Zhou (College of Pharmacy, Jinan University). A voucher specimen (No. 20110910) was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, P.R. China.

3.3. Extraction and isolation

The dried and powdered whole plant of *V. negundo* (9.0 kg) was extracted with 95% EtOH at room temperature for three times (3 × 20 L, each 10 h). The EtOH extract was concentrated *in vacuum* to yield a residue (450 g), which was suspended in water and partitioned using petroleum ether, EtOAc and *n*-BuOH, respectively. The *n*-BuOH soluble fraction (200 g) was subjected to silica gel column (10 × 150 cm, 2000 g) eluting with $CHCl_3$–MeOH (100:0 → 0:100) to afford six fractions (A–F). Fraction C (16 g) was separated by Sephadex LH-20 column (2.5 × 150 cm, $CHCl_3$–MeOH, 50:50) to afford three subfractions (I–III). Subfraction II was subjected to ODS column (3 × 60 cm, MeOH–H_2O) and preparative HPLC (MeOH–H_2O, 40:60) to afford **1** (6.0 mg) and **2** (4.0 mg). Subfraction III was also subjected to

an ODS column and preparative HPLC (MeOH–H_2O, 30:70) to afford **3** (3.4 mg) and **6** (2.9 mg). Fraction E (7 g) was separated by Sephadex LH-20 column (3.0 × 80 cm, $CHCl_3$–MeOH, 50:50) to afford **4** (4.1 mg) and **5** (3.2 mg).

*3.3.1. 2-Methyl pyromeconic acid 3-O-β-D-glucopyranoside-6′-(O-4″-hydroxybenzoate) (**1**)*

Colourless needles, mp 205–207°C. $[\alpha]_D^{25}$ −43 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 208 (1.97), 258 (1.92) nm. IR (KBr) ν_{max}: 3317, 1696, 1638, 1602, 1557, 1455, 1281, 1167, 1125, 1081, 848, 773, 618 cm^{-1}. HR-ESI-MS *m/z*: 431.0988 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_{10}Na$, 431.0954). ^1H NMR (CD_3OD, 400 MHz) δ: 6.34 (1H, d, $J = 5.6$ Hz, H-5), 7.86 (1H, d, $J = 5.6$ Hz, H-6), 2.29 (3H, s, H-7), 4.89 (1H, d, $J = 7.3$ Hz, H-1′), 3.43 (1H, m, H-2′), 3.41 (1H, m, H-3′), 3.40 (1H, m, H-4′), 3.35 (1H, m, H-5′), 4.45 (1H, dd, $J = 11.8$, 6.7 Hz, H-6′α), 4.55 (1H, dd, $J = 11.8$, 2.4 Hz, H-6′β), 7.82 (2H, d, $J = 7.8$ Hz, H-2″, H-6″), 6.81 (2H, d, $J = 7.8$ Hz, H-3″, H-5″). ^{13}C NMR (CD_3OD, 100 MHz) δ: 143.1 (C-2), 164.5 (C-3), 176.9 (C-4), 117.2 (C-5), 156.9 (C-6), 15.6 (C-7), 104.6 (C-1′), 77.9 (C-2′), 75.4 (C-3′), 71.8 (C-4′), 76.0 (C-5′), 64.4 (C-6′), 122.2 (C-1″), 132.8 (C-2″, C-6″), 116.4 (C-3″, C-5″), 163.6 (C-4″), 167.7 (C-7″).

*3.3.2. 6′-O-p-Hydroxybenzoyl-gardoside (**2**)*

Amorphous powder, mp 178–180°C. $[\alpha]_D^{25}$ −54 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 207 (1.82), 255 (1.90) nm. IR (KBr) ν_{max}: 3378, 1697, 1608, 1516, 1282, 1169, 1078, 1013, 771 cm^{-1}. HR-ESI-MS *m/z*: 493.1332 $[M - H]^+$ (calcd for $C_{23}H_{25}O_{12,}$ 493.1346). ^1H NMR (CD_3OD, 400 MHz) δ: 5.14 (1H, d, $J = 5.2$ Hz, H-1), 7.34 (1H, br s, H-3), 3.13 (1H, dt, $J = 7.6$, 7.0 Hz, H-5), 1.93 (1H, m, H-6α), 1.80 (1H, dt, $J = 6.4$, 12.8 Hz, H-6β), 4.28 (1H, m, H-7), 2.88 (1H, m, H-9), 5.15 (1H, br s, H-10a), 5.23 (1H, t, $J = 1.8$ Hz, H-10b), 4.69 (1H, d, $J = 7.9$ Hz, H-1′), 3.25 (1H, m, H-2′), 3.41 (1H, m, H-3′), 3.40 (1H, m, H-4′), 3.60 (1H, m, H-5′), 4.42 (1H, dd, $J = 11.8$, 6.7 Hz, H-6′α), 4.58 (1H, dd, $J = 11.8$, 2.4 Hz, H -6′β), 7.87 (2H, d, $J = 7.8$ Hz, H-2″, H-6″), 6.81 (2H, d, $J = 7.8$ Hz, H-3″, H-5″). ^{13}C NMR (CD_3OD, 100 MHz) δ: 96.8 (C-1), 152.0 (C-3), 113.5 (C-4), 32.5 (C-5), 41.1 (C-6), 73.9 (C-7), 152.8 (C-8), 44.7 (C-9), 113.2 (C-10), 172.1 (C-11), 100.2 (C-1′), 74.7 (C-2′), 77.9 (C-3′), 71.9 (C-4′), 75.6 (C-5′), 64.6 (C-6′), 122.2 (C-1″), 132.8 (C-2″, C-6″), 116.2 (C-3″, C-5″), 163.8 (C-4″), 167.9 (C-7″).

3.4. Acid hydrolysis and GC analysis of 1 and 2

Each solution of compounds **1** and **2** (each 1.5 mg) was hydrolysed with 1.5 mL of 2 N HCl (CH_3OH) for 3 h at 80°C. The reaction mixture was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated and dried by N_2. Then, 1 mL of dried pyridine and 2 mg of L-cysteine methylester hydrochloride were added to the residue. The mixture was heated at 60°C for 2 h and concentrated to dryness with N_2. *N*-(Trimethylsilyl) imidazole (0.2 mL) was added into the mixture, and then kept at 60°C for 1 h. At last, the solution was diluted with H_2O (1 mL) and extracted with hexane (1 mL). The organic layer was analysed under the following conditions: HP-1701 (0.25 mm × 30 m), detector: FID, column temperature: 200–250°C (5°C/min), detector temperature: 280°C, injector temperature: 250°C and carried gas: N_2. The standard D-glucose and L-glucose were subjected to the same reaction and GC analysis under the above conditions [t_R (min): 30.63 (D-glucose), t_R (min): 33.23 (L-glucose)]. As a result, D-glucose [t_R (min): 30.68–30.79] was detected from the hydrolysates of **1** and **2**, respectively.

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