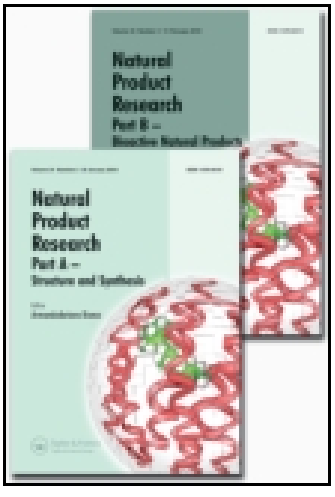

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A new alkaloid glycoside from the rhizomes of Aristolochia fordiana

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A new alkaloid glycoside from the rhizomes of *Aristolochia fordiana*

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A new alkaloid glycoside named fordianoside (**1**), together with three known compounds arabinothalictoside (**2**), 6-*O*-*p*-coumaroyl-β-fructofuranosyl-(2 → 1)-α-D-glucopyranoside (**3**) and 4-[formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl] butanoic acid (**4**), was isolated from the rhizomes of *Aristolochia fordiana*. The structure of **1** was established as (1*S*)-1,2,3,4-tetrahydro-7-hydroxy-1-[(4-hydroxybenzyl) methyl]-2,2-dimethyl-8-*O*-isoquinolinyl β-D-glucopyranoside by using chemical and spectroscopic methods including HR-ESI-MS, 1D and 2D NMR.

Keywords: Aristolochiaceae; *Aristolochia fordiana*; alkaloid glycoside

1. Introduction

The genus *Aristolochia* is distributed in wide areas from the tropics to temperate zones of the world, various species of which have been commonly used as folk medicines (Wu et al. 2005; Kuo et al. 2012). Previous studies carried out on *Aristolochia* species have revealed the presence of several classes of bioactive natural products such as aristolochic acids and their esters (Chen et al. 2010), aristolactams (Shi et al. 2004), alkaloids (Capasso et al. 2006), amides (Chung et al. 2011), flavonoids (Machado & Lopes 2005), lignans (Zhai et al. 2004) and steroids (Navarro-García et al. 2011). *Aristolochia fordiana* Hemsl. is widely found in southwestern China, and its rhizome is used for the treatment of stomach ache, abdominal pain, rheumatism and skin diseases in the Mainland China (Jiangsu New Medicine College 1977; Lopes et al. 1990; Yu et al. 2007; León-Díaz et al. 2010). Previous phytochemical and biological investigations of this plant have led to the isolation of some neolignans (Zhou et al. 2013). In a continuation of our phytochemical survey on the rhizomes from *A. fordiana*, a new alkaloid glycoside named fordianoside (**1**), together with three known compounds, was isolated. In this article, we describe the isolation and structural elucidation of the new compound.

2. Results and discussion

Fordianoside (**1**) was obtained as yellow amorphous solid with $[\alpha]_D^{25} + 34.9$ (*c* 0.06, CH_3OH). The positive HR-ESI-MS revealed a molecular ion peak at *m/z* 462.2119 $[M]^+$ (calcd for 462.2122), consistent with the molecular formula of $C_{24}H_{32}NO_8$. The IR spectrum exhibited the absorptions of hydroxyl group (3552 and 3478 cm^{-1}) and aromatic rings (1638 and 1617 cm^{-1}). The ^1H NMR spectrum revealed signals of two mutually coupled protons (δ_H 7.02 and 6.99; both d, $J = 9.0$ Hz) due to a 1,2,3,4-tetrasubstituted benzene ring, two doublets (δ_H 7.10 and 6.73;

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both d, $J = 8.5$ Hz) because of a *p*-substituted benzene ring, and a singlet of six protons at δ_H 3.15 assigned to two *N*-methyl groups, which were characteristic chemical shifts and multiplicities for a benzylisoquinoline alkaloid previously synthesised by Dwuma-Badu et al. (1983). Other signals were attributed to the partial structures —CH_2—CH_2— and ⟩CH—CH_2— at δ_H 5.74 (H, dd, $J = 7.0$, 3.0 Hz), 3.83 (1H, m), 3.45 (1H, m), 3.06 (1H, dd, $J = 18.0$, 6.0 Hz), 3.40 (1H, m) and 3.23 (2H, dd, $J = 18.0$, 3.0 Hz). Moreover, an anomeric proton at δ_H 4.70 (1H, d, $J = 8.0$ Hz) together with six oxygenated protons at δ_H 3.38 (1H, t, $J = 8.0$ Hz), 3.47 (1H, t, $J = 8.0$ Hz), 3.53 (1H, d, $J = 8.0$ Hz), 3.37 (1H, overlap), 3.98 (1H, dd, $J = 12.0$, 1.5 Hz) and 3.74 (1H, dd, $J = 12.0$, 6.5 Hz) suggested that **1** was a glycosidic compound. The presence of D-glucose was revealed by hydrolysis of **1** with 1 M HCl, which yielded a D-glucose as determined by reversed-phase HPLC of its aldose thiocarbamate derivatives. In the ^{13}C NMR spectrum, 24 carbon signals were observed, including those ascribable to a glucopyranosyl moiety (δ_C 107.7, 79.2, 78.0, 75.6, 71.9, 63.2), two aromatic rings, four aliphatic carbons and two *N*-methyl groups. The analysis of its 2D NMR data, including HSQC and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals. The β-configuration of the anomeric proton of the glucopyranosyl was assigned based on its $J_{H1—H2}$ value ($J = 8.0$ Hz). The ROESY correlation between δ 7.02 (H-6) and 3.06 (H-4) indicated that C5—C6 cannot be interchangeable with C7—C8. The HMBC cross-peaks (Figure 1) from H-1 (δ_H 5.74) and H-13 (δ_H 7.10) to C-11 (δ_C 38.5) revealed the connectivity of the 1,4-disubstituted benzene ring to C-1; cross-peaks from H-1′ (δ_H 4.70) and H-1 (δ_H 5.74) to C-8 (δ_C 143.4) confirmed the attachment of the glucosyl group on C-8; cross-peaks from H-3 (δ_H 3.81 and 3.45) and H-1 (δ_H 5.74) to C-Me (δ_C 54.5 and 52.1) indicated the presence of the two *N*-methyl groups. Thus, the planar structure for **1** is depicted in Figure 1. The absolute stereochemistry at C-1 was determined using the electronic circular dichroism experiment. The observed maxima for a positive Cotton effect at 242 (+1.62) nm and a negative Cotton effect at 227 (−7.61) nm (Craig & Roy 1965; Ringdahl et al. 1981; Yan et al. 2013) indicated that compound **1** had an *S*-configuration at C-1. Therefore, the structure of **1** was established as (1*S*)-1,2,3,4-tetrahydro-7-hydroxy-1-[(4-hydroxybenzyl) methyl]-2,2-dimethyl-8-*O*-isoquinolinyl β-D-glucopyranoside and named fordianoside (Figure 1).

The structures of known compounds were identified as arabinothalictoside (**2**) (Yoshikawa et al. 1993), 6-*O*-*p*-coumaroyl-β-fructofuranosyl-(2 → 1)-α-D-glucopyranoside (**3**) (Georgopoulou et al. 2005) and 4-[formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl] butanoic acid (**4**) (Chin et al. 2003), by comparing their spectroscopic data with previously reported values (Figure 2).

All isolated compounds were tested for their cytotoxic activities on three human cancer cell lines (human breast adenocarinoma cell line MCF-7, human hepatoma cell lines HepG2 and human osteosarcoma cell line MG-63), and the inhibitory activities of acetylcholinesterase and lipopolysaccharide-induced NO production in RAW 264.7 macrophages. Unfortunately, the isolated compounds exhibited no activity in all biological evaluations.

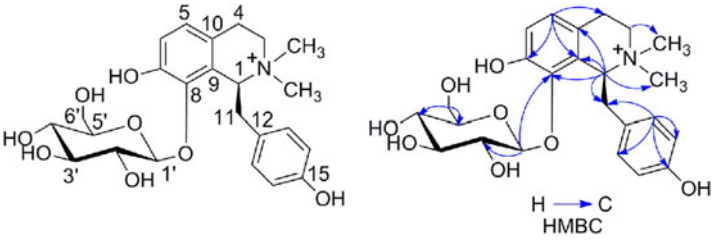

Figure 1. Structure and selected HMBC correlations of compound **1**.

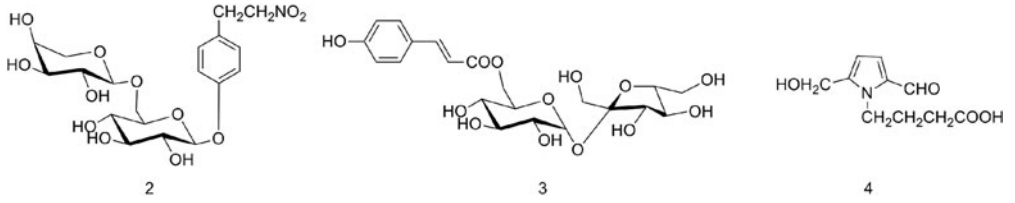

Figure 2. Structures of compounds **2**–**4**.

3. Experimental

3.1. *General experimental procedures*

Optical rotation was measured using a JASCO P-1020 digital polarimeter (Jasco, Tokyo, Japan). CD spectrum was determined on a JASCO 810 spectropolarimeter (Jasco). UV spectrum was measured with a UV-2450 UV–visible spectrophotometer (Shimadzu, Tokyo, Japan). A Bruker Tensor 27 spectrometer (KBr discs, Bruker, Karlsruhe, Germany) was used to acquire the IR spectrum. NMR spectra were performed in MeOD at 303 K on a Bruker AV-500 NMR spectrometer (^{1}H: 500 MHz; ^{13}C: 125 MHz; Bruker) with TMS as internal standard. ESI-MS and HR-ESI-MS data were obtained on an Agilent 1100 series (Agilent Technologies, Santa Clara, CA, USA) LC/MSD Trap mass spectrometer (ESI-MS) (Agilent Technologies, Santa Clara, CA, USA) and a Mariner ESI TOF spectrometer (HR-ESI-MS) (Agilent Technologies, Santa Clara, CA, USA), respectively. Silica gel (Qingdao Marine Chemical Co., Ltd, Qindao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), MCI gel CHP-20 (Mitsubishi, Tokyo, Japan) and ODS (40–63 μm, Fuji, Tokyo, Japan) were used for open column chromatography (CC). Preparative HPLC was performed on an Agilent 1100 Series (Agilent Technologies) coupled with a Shim-pack RP-C_{18} column (200 mm × 20 mm i.d., Shimadzu) and an Agilent 1100 Series multiple wavelength detector (Agilent Technologies).

3.2. *Plant material*

The rhizomes of *A. fordiana* Hemsl. were collected at the Guangxi Zhuangzu Autonomous Region, People's Republic of China, in November 2011, and identified by Prof. Mian Zhang, Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. AF-201111) has been deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. *Extraction and isolation*

The dried and powdered rhizomes (3.9 kg) of *A. fordiana* were extracted with MeOH (3 × 16 L) for 2 h under reflux. After concentration under reduced pressure, the combined MeOH extract (395.0 g) was subjected to a silica gel CC eluted with petroleum ether, EtOAc, acetone and MeOH to obtain four subfractions (SF_I–SF_{IV}). Eighty-one grams of SF_{IV} (MeOH) was chromatographed on MCI gel eluted with a MeOH–H_2O solvent system in gradient (0%, 10%, 30%, 50%, 70% and 100% MeOH) to obtain six subfractions ($SF_{IV\text{-}1}$–$SF_{IV\text{-}6}$). $SF_{IV\text{-}3}$ (2.4 g, 30% MeOH) was subjected to ODS CC eluted with MeOH–H_2O (25% and 100% MeOH) to yield four subfractions ($SF_{IV\text{-}3A}$–$SF_{IV\text{-}3D}$). $SF_{IV\text{-}3A}$ (0.63 g) was further isolated by passage over a Sephadex LH-20 column using MeOH as eluent to obtain six subfractions ($SF_{IV\text{-}3A1}$–$SF_{IV\text{-}3A6}$). $SF_{IV\text{-}3A3}$ (47 mg) was further purified by preparative HPLC (MeOH–H_2O–formic acid 25:75:0.1) to afford **1** (22 mg). $SF_{IV\text{-}3A4}$ (26 mg) was further purified by preparative HPLC (MeOH–H_2O 20:80) to yield **2** (6 mg). $SF_{IV\text{-}3B}$ (1.23 g) was subjected to ODS CC eluted with MeOH–H_2O (25% and 100%

MeOH) to yield three subfractions ($SF_{IV\text{-}3B1}$–$SF_{IV\text{-}3B3}$). $SF_{IV\text{-}3B1}$ (0.85 g) was further isolated by passage over a Sephadex LH-20 column and preparative HPLC (MeOH–H_2O 20:80) to yield **3** (2 mg). $SF_{IV\text{-}2}$ (1.71 g) was subjected to ODS CC eluted with MeOH–H_2O (10%, 30% and 100% MeOH) to yield five subfractions ($SF_{IV\text{-}2A}$–$SF_{IV\text{-}2E}$). $SF_{IV\text{-}2C}$ (744 mg) was further purified by preparative HPLC (MeOH–H_2O 20:80) to afford **4** (245 mg).

3.4. *Fordianoside (1)*

Yellow amorphous solid; $[\alpha]_D^{25} + 34.9$ (*c* 0.06, CH_3OH); CD $\Delta\varepsilon$ (nm, $c = 3.0 \times 10^{-4}$, MeOH): + 1.25 (322), − 2.11 (271), + 1.62 (242), − 7.61 (227), + 30.76 (208); UV (MeOH) λ_{max} nm (log ε) 280 (3.55), 227 (4.12), 204 (4.48); IR (KBr) ν_{max} (cm^{-1}): 3552, 3478, 3415, 3235, 1638, 1617, 1400, 1077, 621, 478; ^{1}H NMR (500 MHz, CD_3OD): δ 5.74 (1H, dd, $J = 7.0$, 3.0 Hz, H-1), 3.83 (1H, m, H-3) and 3.45 (1H, m, H-3), 3.06 (1H, dd, $J = 18.0$, 6.0 Hz, H-4), 6.99 (1H, d, $J = 9.0$ Hz, H-5), 7.02 (1H, d, $J = 9.0$ Hz, H-6), 3.40 (1H, m, H-11), 3.23 (2H, dd, $J = 18.0$, 3.0 Hz, H-11, H-4), 7.10 (2H, d, $J = 8.5$ Hz, H-13, 17), 6.73 (2H, d, $J = 8.5$ Hz, H-14, 16), 3.15 (6H, s, 2 × *N*—CH_3), 4.70 (1H, d, $J = 8.0$ Hz, H-1 × —Glc), 3.38 (1H, t, $J = 8.0$ Hz, H-2 × —Glc), 3.47 (1H, t, $J = 8.0$ Hz, H-3 × —Glc), 3.53 (1H, d, $J = 8.0$ Hz, H-4 × —Glc), 3.37 (1H, overlap, H-5 × —Glc), 3.98 (1H, dd, $J = 12.0$, 1.5 Hz, H-6 × —Glc) and 3.74 (1H, dd, $J = 12.0$, 6.5 Hz, H-6 × —Glc). ^{13}C NMR (500 MHz, CD_3OD): δ 70.5 (C-1), 55.3 (C-3), 24.0 (C-4), 119.1 (C-5), 127.6 (C-6), 150.2 (C-7), 143.4 (C-8), 128.3 (C-9), 121.0 (C-10), 38.5 (C-11), 128.7 (C-12), 131.6 (C-13, 17), 116.7 (C-14, 16), 158.0 (C-15), 54.5 (C—*N*—CH_3), 52.1 (C—*N*—CH_3), 107.7 (C-1 ×), 79.2 (C-2 ×), 78.0 (C-3 ×), 75.6 (C-4 ×), 71.9 (C-5 ×), 63.2 (C-6 ×); ESI-MS: *m/z* 462 $[M]^+$, 300 $[M - Glc]^+$; HR-ESI-MS: *m/z* 462.2119 $[M]^+$ (Calcd for $C_{24}H_{32}NO_8$, 462.2122).

3.4.1. *Acid hydrolysis of **1***

Compound **1** (2.0 mg) was treated in 1 M HCl (2 mL) at 100°C for 2 h. The aqueous solution was concentrated under reduced pressure to yield a residue, which was dissolved in pyridine (2 mL) containing L-cysteine methyl ester hydrochloride (2 mg) and heated at 60°C for 1 h. And then 10 μL of *o*-tolylisothiocyanate was added to the mixture, which was heated at 60°C for another 1 h. The reaction mixture was dissolved in MeOH after drying *in vacuo* and directly analysed by reversed-phase HPLC (acetonitrile–H_2O–formic acid 25:75:0.1, 0.8 mL/min). The peak at 19.11 min coincided with the derivative of D-glucose.

4. Conclusion

In this article, a new alkaloid glycoside named fordianoside, together with three known compounds, was isolated from the rhizomes of *A. fordiana*. The structure of fordianoside was identified as (1*S*)-1,2,3,4-tetrahydro-7-hydroxy-1-[(4-hydroxybenzyl) methyl]-2,2-dimethyl-8-*O*-isoquinolinyl β-D-glucopyranoside by 1D, 2D NMR and MS spectra analyses.

Supplementary material

Supplementary material relating to this article is available online, including 1D NMR, selected 2D NMR, HR-ESI-MS, CD spectrum and HPLC analysis of monosaccharide derivative of **1** (Figures S1–S8).

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