Chemical constituents from the roots of *Ampelopsis delavayana* and their antibacterial activities

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**ABSTRACT**

Two new aromatic glycosides, 2-methylphenyl\textsuperscript{O}β-d-xylopyranosyl-(1→6)-O-β-d-glucopyranoside (1) and 2-methylphenyl\textsuperscript{O}α-arabinofuranosyl-(1→6)-O-β-glucopyranoside (2), together with eight known compounds were isolated from the roots of *Ampelopsis delavayana*. Their structures were elucidated on the basis of extensive spectroscopic analysis. Furthermore, the *in vitro* antibacterial activities of 1 and 2 were investigated using serial twofold dilution in three bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**ARTICLE HISTORY**

Received 21 June 2016
Accepted 3 August 2016

**KEYWORDS**

*Ampelopsis delavayana*; aromatic glycosides; antibacterial activities

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1. Introduction

The *Ampelopsis* genus, with about 30 species, is widely distributed in Asia, North America and Central America (Editorial Committee of Flora Reipublicae Popularis Sinicae. 1998). Phytochemical investigation of this genus have led to the isolation of different types of compounds, such as flavonoids (Wang et al. 1998; He et al. 2007), phenols and tannins (Yu et al. 1995a, 1995b), oligostilbenes (Oshima et al. 1990, 1993), triterpenoids (Lu 2012; Mi et al. 2014) and anthraquinones (Zou et al. 2000). These compounds have attracted great interests for their broad biological activities, such as anti-inflammatory, analgesic (Zhang et al. 2005), hepaprotective (Yang et al. 1998; Yabe & Matsui 1997; Dong et al. 2012), antibacterial (Mi et al. 2013; Xie et al. 2014), anticancer (Park et al. 2000), antihyperglycemic, hypotensive and hypolipidemic properties (Chen & Liang 2009). *Ampelopsis delavayana* Planch. is mainly distributed in Fujian, Guangdong, Guangxi, Hainan, Sichuan, Guizhou and Yunnan province. The folk medicine uses its roots extensively as antirheumatic, antifracture, detumescence...
and analgesic agent (Chinese Pharmacopoeia Commission 2005). However, rare study on the secondary metabolites of A. delavayana has been previously presented in the literature. In the course of identifying bioactive constituents from A. delavayana, two new aromatic glycosides, 2-methylphenyl O-β-d-xylopyranosyl-(1→6)-O-β-d-glucopyranoside (1) and 2-methylphenyl O-α-arabinofuranosyl-(1→6)-O-β-glucopyranoside (2), together with eight known compounds, were isolated and characterised by extensive spectroscopic analysis (Figure 1). The eight known compounds were determined as catechin (Nahrstedt et al. 1987), vulgarsaponin A (Tian et al. 2000), 3,3′-di-O-methyllellagic acid 4-O-glucoside (Pakulski & Budzianowski 1996), progallin A (Liu et al. 2008), epicatechin-3-O-gallate (Davis et al. 1996), resveratroloside (Oshima et al. 1993), 3-O-galloylprocyanidin B-1 (Nonaka et al. 1981) and procyanidin B-1 (Nonaka et al. 1981). Herein, the isolation and structure elucidation of new compounds and their antibacterial activities were reported in this paper.

2. Results and discussion

Compound 1 was obtained as an optically active yellowish amorphous powder (\([\alpha]_D^{21} = -103.0, \text{c} 0.218, \text{MeOH}\) possessing the molecular formula C\textsubscript{18}H\textsubscript{26}O\textsubscript{10} by HREIMS ([M]+, m/z 402.1521; calc. for C\textsubscript{18}H\textsubscript{26}O\textsubscript{10} 402.1526). The UV spectrum showed absorption maximum at 203 and 268 nm. The IR spectrum showed absorptions at 3440 and 1633 cm\(^{-1}\), attributed to hydroxy and olefin groups, respectively. The UV NMR spectrum displayed signals for an aromatic ring at \(\delta_H\) 7.11 (d, \(J = 7.3\) Hz, H-3), \(\delta_H\) 6.89 (m, H-4), \(\delta_H\) 7.14 (d, \(J = 7.3\) Hz, H-5) and \(\delta_H\) 7.14 (d, \(J = 7.3\) Hz, H-6); a methyl group at \(\delta_H\) 2.26 (s, Me), and two anomeric H-atoms at \(\delta_H\) 4.89 (d, \(J = 8.2\) Hz, H-1′) and \(\delta_H\) 4.31 (d, \(J = 7.4\) Hz, H-1″). The \(^{13}\)C NMR spectrum of 1 (Table S1) displayed signals for an aromatic ring at \(\delta_C\) 157.1, 128.8, 131.6, 123.3, 116.3 and 128.1, and for two anomeric C-atoms at \(\delta_C\) 102.4 and 105.3, respectively. The H and C-atom signals of sugar moiety were fully assigned by the analysis of the HSQC and HMBC spectral data. Similarities of the chemical shifts and coupling constants of 1 with that of known compound 3-methylphenyl O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside (Chen et al. 2008) revealed that 1 possessed an aromatic glycoside skeleton. The main difference between 1 and 3-methylphenyl-O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside was that the methyl group at C-3 in the latter changed to C-2 in 1, which was further confirmed by the HMBC correlations from \(\delta_H\) 4.31 (d, Me) to \(\delta_C\) 157.1 (C-1), \(\delta_C\) 128.8 (C-2) and \(\delta_C\) 131.6 (C-3) (Figure S1). The positions of the sugar chain and inter glycosidic linkage were established by the HMBC experiment, in which the anomeric H-atom signals at \(\delta_H\) 4.89 (glucose) and 4.31 (xylose) were correlated with \(\delta_C\) 157.1 (C-1) of the aglycone and \(\delta_C\) 69.6 (C-6′) of the glucose, respectively. The configurations for two anomeric H-atoms were determined to be β from the large coupling constants of anomeric H-atoms (8.2 and 7.4 Hz). The absolute configurations of sugars were elucidated as d-xylose and d-glucose based on the results of acid hydrolysis and HPLC analysis.

![Figure 1](image-url)
of the sugar’s derivatives. Thus, the structure of 1 was determined as 2-methylphenyl O-β-d-xlyopyranosyl-(1→6)-O-β-d-glucopyranoside.

Compound 2 has the same molecular formula C_{18}H_{26}O_{10} as 1 according to its HREIMS at m/z 402.1532 [M]^+. The \(^1\)H and \(^13\)C NMR spectra of 2 (Table S1) were very similar to those of 1, except that the chemical shift values of methines moiety (C-2″ and C-4″) in 1 were shifted to a lower field in 2, indicating the absence of xylose. By comparing \(^13\)C NMR data of 1 and 2, we inferred that the xylose group in 1 was substituted by an arabinofuranose unit in 2. This assumption was further supported by the HMBC correlations of \(\delta_H 4.92 (d, J = 1.5 \text{ Hz}, H-1″)\) with \(\delta_C 68.2 (C-6′)\) and \(\delta_C 83.3 (C-2″)\) (Figure S1). The configurations for anomeric H-atoms of glucose and arabinose were determined to be β and α, respectively, based on coupling constants (7.4 and 1.5 Hz, respectively). Unfortunately, the absolute configuration of glucose and arabinose cannot be determined because the sample amount of 2 is too little. Therefore, the structure of 2 was determined as 2-methylphenyl O-α-arabinofuranosyl-(1→6)-O-β-glucopyranoside.

Compounds 1 and 2 were evaluated for their antibacterial activity against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus using serial twofold dilution. However, compounds 1 and 2 had the minimal inhibitory concentrations (MICs) exceeding 100 μg/mL.

3. Experimental

3.1. General experimental procedures

Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), C\(_{18}\) reversed-phase silica gel (60–80 mesh, Merck), macroporous adsorptive resins D101 (250–300 mm; Tianjin Pesticide Factory, P. R. China), MCI-gel CHP-20P (Mitsubishi Chemical Industry Co. Ltd.) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC (Merck, silica gel GF\(_{254}\)) in combination with reversed phase HPLC (Agilent 1100, endcapped-C\(_{18}\) column, 4.6 × 250 mm, 5 μm). Optical rotations were obtained with a Jasco P-1020 Automatic Digital Polariscope. UV spectrum was measured with a Shimadzu UV2401PC in MeOH solution. IR spectra (KBr) were obtained on a Bruker Tensor-27 infrared spectrophotometer. \(^1\)H, \(^13\)C and 2D NMR spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal standard at room temperature. MS data were obtained on a Waters Autospec Premier P776.

3.2. Plant material

The roots of A. delavayana was collected from Chuxiong in Yunnan Province, China, in June 2011 and were identified by Professor Bin Qiu of Yunnan Institute of Materia Medica. A voucher specimen (2011-YPTG-001) was deposited at herbarium of Yunnan Institute of Materia Medica.

3.3. Extraction and isolation

The dried roots (10 kg) of A. delavayana Planch. were powdered and extracted with 70% EtOH three times under room temperature. The 70% EtOH extract was concentrated in
vacuum to afford a residue that was subjected to CC (macroporous adsorptive resins D101, EtOH/H₂O 0:100, 30:70, 50:50 and 95:5). The 50% EtOH eluate was fractionated by CC (silica gel, CHCl₃/MeOH 100:1–10:1) to get Fractions A-F. Fraction C was separated to MPLC with RP-18 CC (MeOH/H₂O, 3:7–8:2), then followed by Sephadex LH-20 (Me₂CO/MeOH, 1:1) and MCI gel (MeOH/H₂O, 3:7–8:2) to afford 1 (882 mg). Fraction E, was separated on silica gel (CHCl₃/MeOH, 30:1–10:1) to give four subfractions E1-E4. Subtraction E4 was further isolated and purified by Sephadex LH-20, MCI gel, and then silica gel (EtOAC/MeOH) to afford 2 (4 mg). Other fractions were further purified by RP-18 and MCI-gel CHP-20P chromatographies developing with aq. MeOH, and Sephadex LH-20 chromatography developing with Me₂CO/MeOH (1:1) to yield catechin (1080 mg), vulgarsaponin A (48 mg), 3,3′-di-O-methyl-ellagic acid 4-O-glucoside (61 mg), progallin A (53 mg), epicatechin-3-O-gallate (1352 mg), resveratroloside (138 mg), 3-O-galloylprocyanidin B-1 (2820 mg) and procyanidin B-1 (41 mg).

3.3.1. 2-methylphenyl O-β-D-xylopyranosyl-(1→6)-O-β-D-glucopyranoside (1)
Yellowish amorphous powder; [α]D²¹ +103.0 (c 0.218, MeOH); UV (MeOH) λ max: 203, 268 nm; IR (KBr) ν max 3440, 2922, 1633, 1494, 1373, 1238, 1043 cm⁻¹; HREIMS: m/z 402.1521 (calc. for C₁₈H₂₆O₁₀, 402.1526). ¹H NMR (400 MHz, CD₃OD): δ 7.14 (1H, d, J = 7.3 Hz, H-5), 7.14 (1H, d, J = 7.3 Hz, H-6), 7.11 (1H, d, J = 7.3 Hz, H-6), 6.89 (1H, m, H-4), 4.89 (1H, d, J = 8.2 Hz, H-1′), 4.31 (1H, d, J = 7.4 Hz, H-1″), 4.09 (1H, m, H-6′a), 3.81 (1H, m, H-6′b), 3.80 (1H, m, H-5″a), 3.11 (1H, d, J = 11.0 Hz, H-5″b), 3.62 (1H, m, H-5′), 3.50 (1H, m, H-2′), 3.49 (1H, m, H-4″), 3.47 (1H, m, H-3″), 3.42 (1H, m, H-4′a), 3.28 (1H, t, J = 8.8 Hz, H-3″), 3.21 (1H, t, J = 7.6 Hz, H-2″), 2.26 (3H, s, 2-Me). ¹³C NMR (100 MHz, CD₃OD): δ 157.1 (C-1), 131.6 (C-3), 128.8 (C-2), 128.0 (C-6), 123.3 (C-4), 116.3 (C-5), 105.3 (C-1″), 102.4 (C-1′), 78.0 (C-3′), 77.6 (C-3″), 77.2 (C-5″), 74.9 (C-2″), 74.9 (C-2″), 71.3 (C-4″), 71.2 (C-4′), 69.6 (C-6′), 66.8 (C-5″), 16.6 (2-Me).

3.3.2. 2-methylphenyl O-α-L-arabinofuranosyl-(1→6)-O-β-D-glucopyranoside (2)
Colourless amorphous powder; [α]D²¹ +165.5 (c 0.134, MeOH); UV (MeOH) λ max: 203, 268 nm; IR (KBr) ν max 3441, 2927, 1632, 1494, 1373, 1238, 1069 cm⁻¹; HREIMS: m/z 402.1532 (calc. for C₁₈H₂₆O₁₀, 402.1526). ¹H NMR (400 MHz, CD₃OD): δ 7.15 (1H, m, H-6), 7.14 (1H, m, H-5), 7.12 (1H, m, H-3), 6.90 (1H, m, H-4), 4.92 (1H, d, J = 1.5 Hz, H-1″), 4.88 (1H, d, J = 7.4 Hz, H-1′), 4.04 (1H, d, J = 13.9 Hz, H-6′a), 3.62 (1H, m, H-6′b), 4.01 (1H, m, H-4″), 3.96 (1H, m, H-2″), 3.83 (1H, m, H-3″), 3.73 (1H, m, H-5″a), 3.60 (1H, m, H-5″b), 3.60 (1H, m, H-5′), 3.49 (1H, m, H-2″), 3.46 (1H, m, H-3″), 3.40 (1H, m, H-4″), 2.27 (3H, s, 2-Me). ¹³C NMR (100 MHz, CD₃OD): δ 157.1 (C-1), 131.6 (C-3), 128.8 (C-2), 128.0 (C-6), 123.3 (C-4), 116.3 (C-5), 105.3 (C-1″), 102.4 (C-1′), 78.0 (C-3′), 77.6 (C-3″), 77.2 (C-5″), 74.9 (C-2″), 74.9 (C-2″), 71.3 (C-4″), 71.2 (C-4′), 69.6 (C-6′), 66.8 (C-5″), 16.6 (2-Me).

3.4. Acid hydrolysis and HPLC analysis
The absolute configuration of the sugar moieties in compound 1 were determined by the method of Tu et al (Tu et al. 2011). Compound 1 (5 mg) was hydrolyzed with 2 M HCl for 2 h at 90 °C. The mixture was evaporated to dryness under a vacuum, and then the residue was dissolved in H₂O and extracted with CHCl₃. The aqueous layer was collected. After drying in vacuum, the residue was dissolved in pyridine (1 mL) containing L-cysteine methyl ester (1 mg) and heated at 60 °C for 1 h. Then, o-tolylsithiocyanate (5 mL) was added to the
mixture, which was heated at 60 °C for 1 h. The reaction mixture was directly analysed by reversed-phase HPLC. Analytical HPLC was performed on a Thermo C18 column (250 × 4.6 mm, 5 μm) at 30 °C with 20% CH3CN at a flow rate 1.0 mL/min. Peaks were detected by a UV detector at 254 nm. The mixture of standard monosaccharides, D-glucose and D-xylose, were subjected to the same method. The peaks of the standard monosaccharide derivatives were recorded at $t_R$ 15.8 (D-Glc), 17.0 (D-Xyl) min. Following the above procedure, the derivatives of 1 gave two peaks at $t_R$ 15.9 (D-Glc) and 17.1 (D-Xyl) min, respectively.

### 3.5. Bacterial culture condition

Single *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa* colony from viable, growing agar plates were transferred to 1 L of sterile liquid Mueller-Hinton (MH) broth containing 600 mL of veal infusion broth, 17.5 g of casein hydrolysate, 1.5 g of soluble starch, and 400 mL of Distillated water or 1 L of sterile liquid Luria-Bertani (LB) broth containing 10 g of tryptone, 10 g of NaCl, and 5 g of yeast and then cultivated aerobically in 50 mL volumes at 35 °C or 37 °C in a heated, shaking environmental chamber overnight. Then the activated bacteria were transferred to a 50 mL fresh MH or LB broth for another 8 h at 35 or 37 °C.

### 3.6. Drug susceptibility assay

Cells in log phase (1 × 10⁵ CFU/mL) were cultivated in 96-well plates. Respectively, the MICs were determined by serial twofold dilutions in MH or LB broth containing various agents in accordance with National Committee for Clinical Laboratory Standards 2006.

### 4. Conclusion

Phytochemical investigation on the 70% EtOH extract of the roots of *A. delavayana* resulted in the isolation of two aromatic glycosides, 2-methylphenyl O-β-D-xylopyranosyl-(1→6)-O-β-D-glucopyranoside (1) and 2-methylphenyl O-α-arabinofuranosyl-(1→6)-O-β-glucopyranoside (2). The antibacterial activities of compounds 1 and 2 were evaluated *in vitro* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, however, compounds 1 and 2 showed no obvious antibacterial activities.

### Acknowledgements

The authors are grateful to State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for the measurements of NMR and MS spectroscopic data.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Funding

This work was financially supported by the Training Program for Science and Technology Talents of Yunnan Province [grant number 2014HA001, Program 1512].
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