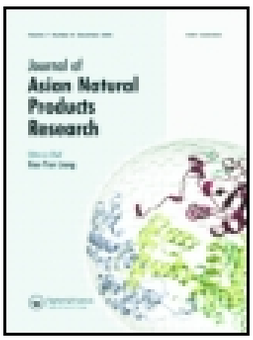

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A new lignan glycoside from *Astragalus yunnanensis*

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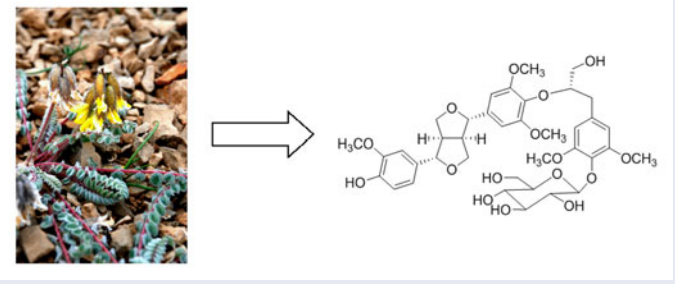
ABSTRACT

A new lignan glycoside, astrayunoside A (**1**), along with eight known compounds (**2–9**), were obtained from the methanol extract of roots of *Astragalus yunnanensis*. All the compounds were obtained from *A. yunnanensis* for the first time. Their structures were elucidated by extensive spectroscopic analysis (1D and 2D-NMR, MS, UV, CD, and IR). The weak antibacterial activities of the crude extracts of *A. yunnanensis* against *Staphyloccocus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi* H901, *Candida albicans*, *Streptococcus mutans,* and *Actinomyces viscosus* were observed.



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Leguminosae; *Astragalus yunnanensis*; lignan glycoside; antibacterial activity

1. Introduction

Astragalus yunnanensis Franch is a perennial flowering herb, which is mainly distributed in west Yunnan, Szechwan and Tibet, China [1]. Just like most *Astragalus* (Leguminosae or Fabaceae) plants, *A. yunnanensis* can be used as folk medicine as well [2], and even used as a substitute for traditional Chinese medicine "Huang-qi" (*A. membranaceus* and *A. membranaceus* var. *mongholicus*) in Tibet [3]. Similar with "Huang-qi", *A. yunnanensis* was mainly used to treat deficiency of *qi* with lack of strength, prolapse of the rectum, spontaneous sweating due to weakened superficial resistance, edema, and anemia as well [2]. During the past several decades, a large

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **1** (in $CDCl_3$).

No.	δ_H (multi, *J* in Hz)	δ_C	No.	δ_H (multi, *J* in Hz)	δ_C
1		132.7 (s)	1″		136.0 (s)
2	6.89 (d, 1.9)	108.9 (d)	2″	6.55 (brs)	106.8 (d)
3		146.8 (s)	3″		152.4 (s)
4		145.3 (s)	4″		133.8 (s)
5	6.88 (d, 8.2)	114.4 (d)	5″		152.4 (s)
6	6.81 (dd, 8.2, 1.9)	118.9 (d)	6″	6.55 (brs)	106.8 (d)
7	4.75 (d, 4.9)	85.7 (d)	7″	3.21 (dd, 13.8, 5.9) 2.99 (dd, 13.8, 7.7)	38.1 (t)
8	3.12 (overlap)	54.0 (d)	8″	4.20–4.23 (m)	83.7 (d)
9	4.29 (overlap) 3.92 (overlap)	72.1 (t)	9″	3.87 (overlap) 3.81 (overlap)	62.3 (t)
1′		137.5 (s)	1‴	4.56 (d, 7.5)	106.3 (d)
2′	6.58 (brs)	102.9 (d)	2‴	3.67 (overlap)	74.2 (d)
3′		153.5 (s)	3‴	3.33-3.36 (m)	76.0 (d)
4′		134.7 (s)	4‴	3.63 (overlap)	70.1 (d)
5′		153.5 (s)	5‴	3.57-3.62 (m)	76.5 (d)
6′	6.58 (brs)	102.9 (d)	6‴	3.59 (overlap) 3.42-3.47 (m)	62.5 (t)
7′	4.73 (d, 5.1)	86.0 (d)	3-OMe	3.89 (3H, s)	56.0 (q)
8′	3.08 (overlap)	54.5 (d)	3′,5′-OMe	3.83 (6H, s)	56.2 (q)
9′	4.25 (overlap) 3.90 (overlap)	71.6 (t)	3″,5″-OMe	3.83 (6H, s)	56.3 (q)

number of phytochemical studies have been conducted on "Huang-qi", which leads to the isolation of a lot of cycloartane-type triterpene glycosides and flavonoids that has been thought to be the major bioactive constituents of "Huang-qi" [4–7]. However, these principal components had not been found in most *Astragalus* plants collected from Yunnan including *A. yunnanensis*. Instead, four lignan glycosides (**1–4**) including a new lignan glycoside astrayunoside A (**1**), along with other five known compounds (**5–9**), were obtained from the methanol extract of roots of *A. yunnanensis* in this paper. Their structures were elucidated by extensive analysis of their spectral data. Herein, we report the isolation and structural elucidation of the new lignan glycoside, as well as antibacterial activities of the crude extracts of *A. yunnanensis*.

2. Results and discussion

Compound **1** was isolated as yellow amorphous powder, and assigned the molecular formula $C_{38}H_{48}O_{16}$ (fifteen unsaturation degrees) from its HR-ESI-MS (*m/z*: 783.2826 $[M+Na]^+$) and ^{1}H and ^{13}C NMR spectra (including DEPT, Table 1). According to ^{1}H NMR spectrum (Table 1), **1** contained five methoxyls at δ_H 3.89 (s) and 3.83 (12H, s), an ABX coupling system protons at δ_H 6.89 (1H, d, $J = 1.9$ Hz, H-2), 6.88 (1H, d, $J = 8.2$ Hz, H-5) and 6.81 (1H, dd, $J = 8.2$, 1.9 Hz, H-6), and other four olefinic protons at δ_H 6.58 (2H, brs), and 6.55 (2H, brs). Analysis of the ^{13}C NMR spectrum with the aid of DEPT experiments (Table 1) revealed the existence of 38 carbon resonances including five methoxyls (δ_C 56.0, 56.2, 56.2, 56.3, and 56.3), a sugar moiety (δ_C 106.3, 74.2, 76.0, 70.1, 76.5, and 62.5), 18 olefinic carbons, 6 sp^3 methylenes (including three oxy-methylenes at δ_C 72.1, 71.6, and 62.3), and three sp^3 methines (including an oxy-methine at δ_C 83.7). These spectroscopic data obviously indicated a lignan glycoside for **1**, which high similarity with **1a** (Figure 1) [8]. The obvious

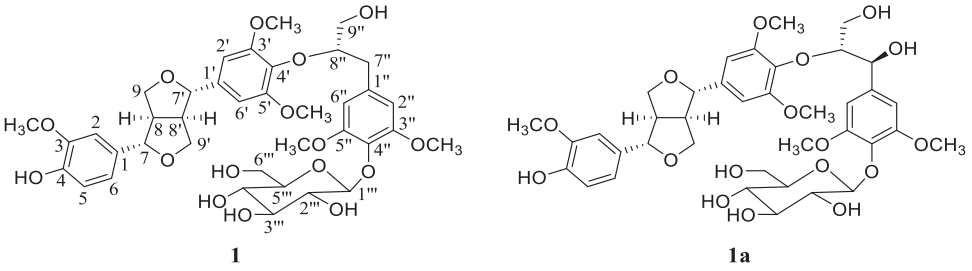

Figure 1. Structures of compounds **1** and **1a**.

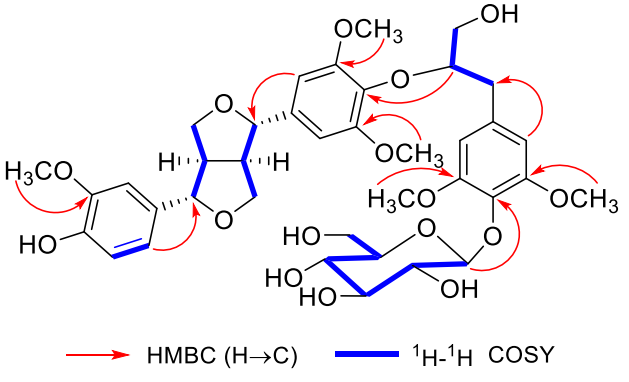

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of compound **1**.

difference between the two compounds was that an oxygenated methine (δ_C 72.6) in **1a** changed to a methylene (δ_C 38.1) in **1**, suggesting that compound **1** should be a reduction product of **1a**.

In the ^{1}H–^{1}H COSY spectrum, the correlations of H_2-7″/H-8″/H_2-9″ (Figure 2), demonstrated that the reduction site at C-7″ (δ_C 38.1). According to HMBC spectrum, a long-range correlation between H-1‴ (δ_H 4.56, d, $J = 7.5$ Hz) and C-4″ (δ_C 133.8) indicated that a sugar moiety was bound to C-4″. H-8″ (δ_H 4.22, m) correlated to C-4′ (δ_C 134.7), which suggested the phenylpropanoid glycoside group connect to C-4′. Based on the further analyses of ^{1}H–^{1}H COSY and HMBC spectra (Figure 2), the planar structure of **1** was confirmed as shown in Figure 1.

The relative configuration of **1** was determined by the chemical shift differences of the two pairs of diastereotopic methylene protons of H_2-9 and H_2-9′ ($\Delta\delta_{H-9} = \delta_{H\text{-}9a} - \delta_{H\text{-}9b}$ and $\Delta\delta_{H-9'} = \delta_{H\text{-}9'a} - \delta_{H\text{-}9'b}$). The approximately equal value of $\Delta\delta_{H-9}$ (0.37) and $\Delta\delta_{H-9'}$ (0.35) suggested that the H-7 and H-7′ were in the same orientation, and the H-8 and H-8′ were in the opposite direction [9]. Comparing the specific rotation data of compound **1** with those of **1a** [8], as well as the circular dichroism data of **1** with those of reported furofuran lignans [10], compound **1** was finally determined as shown in Figure 1, and named astrayunoside A.

By analyses of their spectral data, as well as comparing physicochemical properties with those reported in the literature, the eight known compounds were identified as hedyotol C-4″-*O*-*β*-D-glucopyranoside (**2**) [11], eleutheroside D (**3**) [12],

Table 2. Antibacterial activities of crude extracts from *A. yunnanensis*.

	MIC (mg/ml)				
Bacterial strain	EtOAc soluble portion	*n*-BuOH soluble portion	Water soluble portion	CHL	AMB
S. aureus	1.56	12.50	1.56	0.007	–
E. coli	6.25	6.25	6.25	0.008	–
P. vulgaris	3.13	25.00	6.25	0.005	–
P. aeruginosa	6.25	12.50	25.00	0.150	–
S. typhi H901	1.56	25.00	1.56	0.004	–
S. dysenteriae	6.25	6.25	1.56	0.004	–
S. mutans	1.56	3.13	12.50	0.130	–
A. viscosus	6.25	NA	12.50	0.150	–
C. albicans	6.25	25.00	NA	–	0.095

NA, No activity; CHL, Chloramphenicol; AMB, Amphotericin B.

syringaresinol-4-*O*-β-D-glucopyranoside (**4**) [13], glucosyringic acid (**5**) [14], (24*R*)-24-ethyl-cholestane-3β,5α,6β-triol (**6**) [15], 5α,6β-dihydroxydaucosterol (**7**) [16], lupenone (**8**) [17], and taraxasterol (**9**) [18], respectively.

In addition, the *in vitro* antibacterial activities of crude extracts of *A. yunnanensis* were evaluated. Unfortunately, all of them only showed a weak inhibition activity (Table 2).

Chemical substances are the foundation of biological activity, and the activity of one compound generally unlike the others. Triterpenoid saponins and flavonoids are the major active ingredients for Radix Astragali [4–7]. According to the *Chinese Pharmacopoeia*, the cycloartane saponin astragaloside IV and the isoflavonoid glycoside calycosin-7-glucoside are the officially designated marker compounds to monitor the quality of Radix Astragali [19]. In this study, chemical investigation of *A. yunnanensis* had not discovered the two markers even the two types of active ingredients, but four lignan glycosides. Therefore, from the chemical perspective, *A. yunnanensis* should not be used as a substitute for Radix Astragali.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a SGW-3 automatic polarimeter (Shanghai INESA Physico optiacal instrument Co., Ltd, Shanghai, China). UV data were obtained on a Shimadzu UV 2401PC UV/Vis spectrophotometer (Shimadzu, Kyoto, Japan). CD spectrum was obtained on a Chirascan spectropolarimeter (Applied Photophysics, Leatherhead, Surrey, UK). IR spectra were recorded by a Nicolet 380 FT-IR spectrophotometer (Thermo Scientific, Madison, WI, USA) with KBr pellet. NMR spectra (1D and 2D NMR) were recorded on a Bruker Avance III-400 instrument (Bruker, Faellanden, Switzerland) with TMS as an internal reference. EI-MS data were obtained on a Waters AutoSpec Premier P776 mass spectrometer (Waters Co., Milford, MA, USA). HR-ESI-MS data were obtained on an Agilent G6230 TOF-MS spectrometer (Agilent Technologies Inc., Santa Clara, USA). Column chromatography (CC) was performed on D101 macroporous adsorption resin (Tianjin Bohong Resin Technology Co., Ltd., Tianjin, China), silica gel (200-300 or 300-400 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 (Amersham

Biosciences, Uppsala, Sweden). Thin layer chromatography (TLC) was performed on precoated silica gel GF_{254} plates (Qingdao Marine Chemical Ltd.). Strains of *Staphyloccocus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi* H901, *Candida albicans*, *Streptococcus mutans*, and *Actinomyces viscosus* were kindly provided by Senior Lab Technician Tao Wang (School of Basic Medical Sciences, Dali University, China).

3.2. Plant materials

Roots of *Astragalus yunnanensis* were collected in July 2012 from Shangri-La, Yunnan province, China. The plant material was identified by Dr. De-Quan Zhang (College of Pharmacy and Chemistry, Dali University, China) to be *A. yunnanensis* Franch. A voucher specimen (No. 20120712-1A) has been deposited at Prof. Bei Jiang's Research Group.

3.3. Extraction and isolation

Air-dried roots of *A. yunnanensis* (1.4 kg) were milled and extracted six times with methanol (6 × 6.5 L, each for 24 h) at room temperature. After the extract solutions were combined and concentrated under reduced pressure, the resulting residue (179.2 g) was suspended in water and partitioned with EtOAc and *n*-BuOH, successively. The *n*-BuOH soluble portion (52.0 g) was chromatographed on D101 macroporous adsorption resin chromatography eluting with a gradient solvent system of H_2O/MeOH (1:0–0:1) to yield five fractions (A–E). Fr. C (8.0 g) was purified by repeated silica gel CC eluting with $CHCl_3$/acetone (20:1–1:1) and Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to yield compounds **1** (3.8 mg), **2** (4.8 mg), **3** (5.9 mg), **4** (18 mg), **5** (10.3 mg), **6** (20.2 mg), and **7** (8 mg). The EtOAc soluble portion (48.0 g) was chromatographed on a silica gel column chromatography eluting with a gradient of $CHCl_3$/acetone (1:0–0:1) to yield 11 fractions (I–XI). Fr. I (1.0 mg) was subjected to a silica gel column with petroleum ether/acetone (20:1) and a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to give compounds **8** (25 mg) and **9** (6 mg).

3.3.1. Astrayunoside A (1)

Yellow amorphous powder;$[\alpha]_D^{25}$-15.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 217 (4.5), 265 (2.9) nm; CD (MeOH):$\Delta\varepsilon_{206\,nm}$ +2.01, $\Delta\varepsilon_{218\,nm}$ +0.97, $\Delta\varepsilon_{246\,nm}$ +0.65, $\Delta\varepsilon_{273\,nm}$ +0.51; IR (KBr) ν_{max}: 3309, 2930, 2875, 1595, 1509, 1461, 1333, 1235, 1124, 1064, 827, 675 cm^{-1}. ^1H and ^{13}C NMR spectroscopic data see Table 1; EI-MS: *m/z* (rel. int. %): 760 $[M]^+$ (6), 648 (9), 598 (21), 418 (18), 388 (28), 357 (5), 211 (45), 192 (15), 181 (28), 167 (100), 92 (6); HR-ESI-MS *m/z*: 783.2826 $[M + Na]^+$ (calcd for $C_{38}H_{48}O_{16}$, 783.2840).

3.4. Antibacterial activity assay

Antibacterial activity assay was performed according to a previous method [20]. Briefly, samples were prepared in Mueller–Hinton broth (MHB) by serial two-fold

dilutions ranging from 0.39 to 25 mg/ml, and 0.1 ml of each dilution and 0.1 ml of inoculum (10^7 CFU/ml) were distributed in 96-well plates for broth microdilution. A positive growth control without antimicrobial agent but the equivalent amount of MHB and a negative growth control without bacteria were also prepared for each assay. All the treatments were performed in triplicate. After the microplates were incubated for 24 h at 37 °C, the minimum inhibitory concentration (MIC) was determined visually as the lowest antimicrobial agent concentration that inhibiting bacterial growth.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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