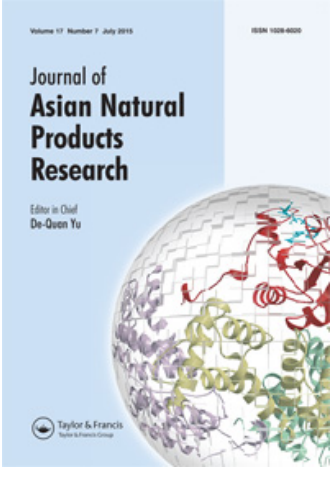

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A new schistosomicidal and antioxidative phenylpropanoid from Astragalus englerianus

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A new schistosomicidal and antioxidative phenylpropanoid from *Astragalus englerianus*

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A new phenylpropanoid, (*E*)-2,3,4-trimethoxy-5-(1-propenyl)phenol (**1**), along with five known aromatic compounds (**2**–**6**), was isolated from the methanol extract of roots of *Astragalus englerianus*. Their structures were elucidated based on the analyses of extensive spectroscopic data and comparison of their physicochemical properties. Compounds **1** and **2** were evaluated schistosomicidal activities, and all the isolated compounds were tested for their antioxidant activities *in vitro*. Compound **1** showed significant schistosomicidal activity with worm mortality rates of 66.7% and 83.3% within 12 and 24 h in a drug-containing (1.16 mM) RPMI 1640 medium, respectively. Also, compound **1** exhibited excellent antioxidant activity (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl free radical-scavenging capability) with an IC_{50} value of 81.3 ± 1.3 μM.

Keywords: *Astragalus englerianus*; phenylpropanoid; schistosomicidal; antioxidant activity

1. Introduction

Radix Astragali (Huang-qi) is a proverbial traditional Chinese medicine and has been used as agents to enhance and regulate immune function and to promote metabolism for quite a long time. It has been verified to possess various functions such as anti-fatigue, anti-anoxia, anti-radiation, and hepatoprotective [1,2]. Traditionally, officinal source of Radix Astragali comes from two *Astragalus* plants only, *Astragalus membranaceus* and *A. membranaceus* var. *mongholicus*. Plants of *Astragalus* are widely distributed in China including 278 species, 2 subspecies, 35 varieties, and 2 forms [3], and at least 51 *Astragalus* species can be found in Yunnan but not including the two species used for Radix Astragali [4]. On the other hand, many *Astragalus* plants in Yunnan can be used as substitutes of Radix Astragali [1]. *Astragalus englerianus* is one of such Radix Astragali-substituted plants. To better understand chemical and bioactive properties of those Astragali substitutes, *A. englerianus* was recently collected from Dali, Yunnan. Phytochemical study of the methanol extract of the roots led to the isolation of a new phenylpropanoid, (*E*)-2,3,4-trimethoxy-5-(1-propenyl)phenol (**1**), together with five known aromatic constituents, isoelemicina (**2**), (*E*)-stearyl ferulate (**3**), (*E*)-hexadecyl *p*-hydroxycinnamate (**4**), 2,4-dimethoxybenzoic acid (**5**), and methyl *p*-hydroxybenzonate (**6**) (Figure 1). Compound **1** also showed significant schistosomicidal and antioxidant activities. Herein, we report the isolation and structural identification of the new phenylpropanoid, as well as the bioassay results of the isolated compounds

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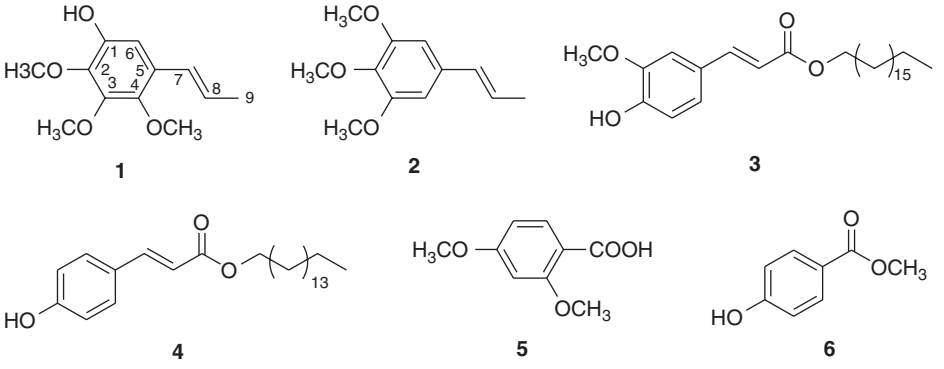

Figure 1. Structures of compounds **1**–**6**.

for schistosomicidal and antioxidant activities.

2. Results and discussion

Compound **1** was isolated as a pale brownish red oil and assigned the molecular formula $C_{12}H_{16}O_4$ (five unsaturation degrees) from its HR-EI-MS, showing the molecular ion at *m/z* 224.1054 $[M]^+$. The IR spectrum indicated the presence of hydroxyl (3417 cm^{-1}), conjugated *trans*-double bond (3037, 1652, and 968 cm^{-1}), methyl (2936, 2880, 1448, and 1368 cm^{-1}), and aromatic ring (3037, 1581, 1487, and 887 cm^{-1}). The absorption maxima in UV spectrum (194, 218, 258, and 304 nm) also exhibited the existence of a benzene ring. ^{1}H NMR spectrum of **1** showed three methoxyls at δ_H 3.90 (6H, s) and 3.76 (3H, s), one aromatic proton at δ_H 6.76 (1H, s, H-6), and a hydroxyl at δ_H 5.89 (1H, brs, 1-OH). ^{1}H NMR spectrum of **1** also exhibited two alkene protons at δ_H 6.57 (1H, brd, $J = 15.7$ Hz, H-7) and 6.10 (1H, dq, $J = 15.7$, 6.7 Hz, H-8), which further confirmed the presence of a conjugated *trans*-double bond. Analyses of ^{13}C NMR spectrum with the aid of the DEPT experiment revealed the existence of 12 carbons (Table 1) including 3 methoxyls (δ_C 62.8, 62.6, and 62.3), 1 methyl (δ_C 20.3), and 8 olefinic carbons (including 3 sp^2 methines at δ_C 127.8, 125.9, and 107.3, and 5 sp^2 quaternary carbons at δ_C 147.5, 146.8, 145.3, 140.7, and 128.8). The spectroscopic data mentioned above indicated that the structure of compound **1** was in close resemblance with that of 2,3,6-

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

Position	δ_H (400 MHz)	δ_C (100 MHz)
1		146.8
2		140.7
3		147.5
4		145.3
5		128.8
6	6.76 (s)	107.3
7	6.57 (brd, $J = 15.7$ Hz)	125.9
8	6.10 (dq, $J = 15.7$, 6.7 Hz)	127.8
9	1.86 (brd, $J = 6.7$ Hz)	20.3
$-OCH_3$	3.90(s), 3.90 (s), 3.76 (s)	62.8, 62.6, 62.3
1-OH	5.89 (brs)	

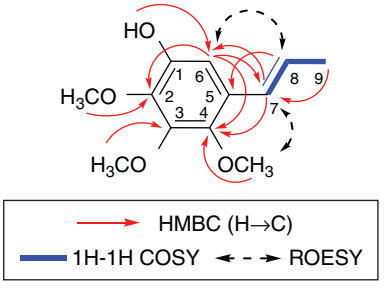

Figure 2. Key $^{1}H-^{1}H$ COSY, HMBC, and ROESY correlations of compound **1**.

trimethoxy-5-(1-propenyl)phenol given in the literature [5]. Then, the structure assembly of **1** was achieved by 2D NMR experiments. The correlations from $^{1}H-^{1}H$ COSY spectrum suggested one segment (bold shown in Figure 2) according to the spin system of H-7/H-8/H_3-9. HMBC correlations of methoxyls (δ_H 3.90, 6H, s and 3.76, 3H, s) to C-2 (δ_C 140.7), C-3 (δ_C 147.5), and C-4 (δ_C 145.3), respectively, indicated that three methoxyls were substituted at positions C-2, 3, and 4, respectively. Correlations of δ_H 1.86 (brd, $J = 6.7$ Hz, Me-9) to C-7 (δ_C 125.9), δ_H 6.10 (dq, $J = 15.7$, 6.7 Hz, H-8) to C-5 (δ_C 128.8), δ_H 6.57 (brd, $J = 15.7$ Hz, H-7) to C-4 (δ_C 145.3) and C-6 (δ_C 107.3), δ_H 5.89 (brs, 1-OH) faintly to C-6, as well as the other correlations shown in Figure 2, and comparing its spectral data with those of 2,3,6-trimethoxy-5-(1-propenyl)phenol [5] demonstrated compound **1** with a hydroxyl at position C-1 and one propenyl at C-5. Thus, compound **1** was characterized as (*E*)-2,3,4-trimethoxy-5-(1-propenyl)phenol.

The five known compounds were identified as isoelemicina (**2**) [6], (*E*)-stearyl ferulate (**3**) [7], (*E*)-hexadecyl *p*-hydroxycinnamate (**4**) [8], 2,4-dimethoxybenzoic acid (**5**) [9], and methyl *p*-hydroxybenzonate (**6**) [10] by comparing their spectral data and physicochemical properties with those reported in the literature.

Compounds **1** and **2** were evaluated schistosomicidal activities, and all the isolated compounds (**1**–**6**) were screened with antioxidant activities, *in vitro*. In the schistosomicidal assay, compound **1** showed significant schistosomicidal activity with worm mortality rates of 66.7% and 83.3% within 12 and 24 h in a drug-containing (1.16 mM) RPMI 1640 medium, respectively, and the other results are listed in Table 2.

Antioxidant activities were evaluated by 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) free radical-scavenging assay. As a result, compound **1** exhibited excellent antioxidant activity with IC_{50} value of 81.3 ± 1.3 μM (positive control

Table 2. *In vitro* effect of compounds **1** and **2** against adult *S. japonicum*.

Group and concentration (mM)	Period of incubation in drug-containing medium							
	12 h		24 h		36 h		48 h	
	MR (%)	VR (%)	MR (%)	VR (%)	MR (%)	VR (%)	MR (%)	VR (%)
2.5% DMSO	0	0	0	0	0	0	0	0
PZQ								
0.12	100	100	66.7	91.7	50.0	87.5	33.3	83.3
1								
1.16	66.7	91.7	83.3	95.8	83.3	95.8	83.3	95.8
0.23	0	0	0	0	0	25	0	25
2								
1.15	0	50	0	50	0	75	50	87.5
0.23	0	0	0	25	0	50	0	50

Note: MR, worm mortality rate;VR, worm vigor reduction rate.

vitamin C (Vc): $IC_{50} = 48.9 \pm 1.1\,\mu M$), while compounds **3** and **6** showed moderate capability ($IC_{50} = 126.2 \pm 7.2$, and $348.7 \pm 5.9\,\mu M$, respectively) of DPPH radical scavenging, and compounds **2**, **4**, and **5** did not show antioxidation.

3. Experimental

3.1 General experimental procedures

UV data were obtained on a Shimadzu UV2401PC UV/Vis spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded by a Bruker Tensor 27 FT-IR spectrophotometer (Bruker, Bremen, Germany) 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 and HR-EI-MS data were obtained on a Waters AutoSpec Premier P776 mass spectrometer (Waters Co., Milford, MA, USA). Optical density values in the antioxidant activity assay were analyzed on a BioTek Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Silica gel (Qingdao Marine Chemical Ltd, Qingdao, China), RP-18 (Fuji Silysia Chemical Ltd, Nagoya, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for open column chromatography (CC). Silica gel GF_{254} plates (Qingdao Marine Chemical Ltd) were used for TLC analyses. Rabbit hosts burden *Schistosoma japonicum* worms were kindly provided by Prof. Yi-Mei Yang's Research Group (School of Basic Medicine, Dali University, China). Gibco RPMI 1640 medium (Invitrogen Co., Grand Island, NY, USA) and praziquantel (Aldrich Chem. Co., St. Louis, MO, USA) were used for schistosomicidal activity experiments. DPPH (Aldrich Chem. Co.) and Vc (Shanghai Chemical Reagent Ltd, Shanghai, China) were used for antioxidant activity assay.

3.2 Plant material

Roots of *A. englerianus* were collected in November 2011 from Cangshan Mountain, Dali, Yunnan Province, China. The plant material was identified by Dr Chun-Lei Xiang (Kunming Institute of Botany, Chinese Academy of Sciences) to be *A. englerianus* Ulbr. A voucher specimen (No. 20100928-1b) has been deposited at Prof. Bei Jiang's Research Group, College of Pharmacy and Chemistry, Dali University.

3.3 Extraction and isolation

The air-dried roots (6.1 kg) were milled and extracted six times with methanol (6 × 25 l) at room temperature. After the extract solutions were combined and concentrated under reduced pressure, the resulting residue (480 g) was suspended in water and partitioned with ethyl acetate and *n*-BuOH successively. The ethyl acetate soluble portion (100 g) was chromatographed on a silica gel (200–300 mesh) column eluted with gradient of $CHCl_3$–CH_3COCH_3 (1:0 to 0:1) to yield 10 fractions (Frs A–J). Fr. A (5.0 g) was purified by a silica gel CC with petroleum ether (PE)–EtOAc (10:1 to 1:1) to yield 10 fractions (A_1–A_{10}). Fraction A_2 (50 mg) was subjected to silica gel CC (PE) and Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1) to yield compound **5** (15.9 mg). Fraction A_4 (600 mg) was performed on silica gel CC (PE–EtOAc, 40:1) and Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1) to yield compound **2** (272.3 mg). Fraction A_5 (1.0 g) was subjected to silica gel CC (PE–acetone, 20:1), RP-18 CC (MeOH–H_2O, 80–100%), and Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1) to yield compound **1** (500.0 mg). Fr. B (9.4 g) was purified by silica gel CC with PE–acetone (30:1 to 0:1) to yield 10 fractions (B_1–B_{10}). Fraction B_3 (350 mg) was rechromatographed on silica gel with PE–EtOAc (10:1) and on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1), and then was further purified

by repeated silica gel CC ($CHCl_3$–MeOH, 480:1; PE–EtOAc, 10:1 and PE–EtOAc, 8:1) to afford compounds **3** (15.0 mg) and **4** (118.9 mg). Fraction B_4 (200 mg) was subjected to repeated silica gel CC (PE–EtOAc, 10:1 and $CHCl_3$–MeOH, 480:1) and Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1) to yield compound **6** (31.4 mg).

*3.3.1 (E)-2,3,4-Trimethoxy-5-(1-propenyl)phenol (**1**)*

Pale brownish red oil; UV (MeOH) λ_{max} (log ε): 194 (4.19), 218 (4.45), 258 (4.08), 304 (3.57) nm; IR (KBr) ν_{max}: 3417, 3037, 2936, 2880, 2836, 1652, 1581, 1487, 1448, 1419, 1368, 1296, 1233, 1201, 1071, 968, 887 cm^{-1}; for ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* (%): 224 $[M]^+$ (100), 209 (40), 177 (95), 149 (80); HR-EI-MS *m/z*: 224.1054 $[M]^+$ (calcd for $C_{12}H_{16}O_4$, 224.1049).

3.4 Schistosomicidal assay

Schistosomicidal assay was performed according to a previous method [11,12]. Briefly, *S. japonicum* worms harvested from rabbits were washed in RPMI 1640 medium. Worms were incubated in a 24-well culture plate (Corning Inc.) containing 1.9 ml of the same medium with 10% fetal bovine serum at 37°C in a 5% CO_2 atmosphere, six worms per well. After 2 h, 0.1 ml of different concentration samples solution was added to the culture. Samples were dissolved in RPMI 1640 containing 2.5% dimethyl sulfoxide (DMSO). The control worms were assayed in RPMI 1640 medium with 2.5% DMSO as a negative control group and in 0.12 mM Praziquantel (PZQ) as a positive control group. Worm mortality rate and vigor reduction rate were monitored 12 h per day for 2 days using an inverted microscope and a stereomicroscope. Worm mortality rate (MR%) and vigor reduction rate (VR%) were according to the following formulas:

$$\mathrm{MR}\% = \frac{N_D}{N_A} \times 100,$$

where N_D and N_A are the numbers of dead worms and of all worms, respectively.

$$\mathrm{VR}\% = \frac{S_C - S_T}{S_C} \times 100,$$

where S_C is the total score for negative control group worms incubated at the 0 h and S_T is the total score for trial group worms incubated in drug-containing medium at certain periods. The scoring criteria are as follows: worm is very active, whose body is natural, deft and hyaline (4 points); worm is active, whose body is weakly inflexible and translucence (3 points); worm is inactive, whose body is inflexible and translucence (2 points). For worm, only the head and tail or sucker has some motions, whose body is stiff and opaque with white (1 point); worm has no motion within 2 min, whose body is stiff and opaque with white, namely worm is dead (0 point).

This study was conducted according to protocols approved by the institutional ethical committee of Dali University (approval no.: 2012-016). All animals were handled in strict accordance with good animal practice as defined by the Animals Use Ethics Committee of the Dali University and the Institute of Materia Medica; the study was conducted adhering to the institution's guidelines for animal husbandry.

3.5 DPPH free radical-scavenging capability assay

Antioxidant activity was tested by the DPPH method previously described [13]. In short, the reaction mixture containing 50 μl of sample solution (different concentrations in DMSO) and 150 μl of DPPH (100 μM) in methanol was taken in a 96-well plate and incubated at 37°C for

30 min. Then, the absorbance (A) was measured at 517 nm using an automated microplate reader. Percent radical-scavenging activity was determined by comparison with a DMSO-containing control. Inhibition percentage ($I\%$) was derived from the following equation:

$$I\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100,$$

where A_{blank} and A_{sample} are the absorbencies of the control and of the test sample, respectively. IC_{50} values represent the concentration of compounds to scavenge 50% of DPPH radicals and are expressed as means ± SD of four separate experiments. Vc was used as a positive control.

Acknowledgments

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References

[1] Editorial Board of China Herbal, and State Administration of Traditional Chinese Medicine, *China Herbal* (Scientific and Technical Publishers, Shanghai, 1999), Vol. 11.

[2] Chinese Pharmacopeia Committee, *Pharmacopoeia of China* (China Medical Science Press, Beijing, 2010), Vol. 1.

[3] Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, *Flora Reipublicae Popularis Sinicae*. 42(1) (Science Press, Beijing, 1997).

[4] Kunming Institute of Botany, and Chinese Academy of Sciences, *Flora Yunnanica* (Science Press, Beijing, 2006), Vol. 10.

[5] M. Sairafianpour, O. Kayser, J.Christensen, M. Asfa, M. Witt, D. Stærk, and J.W. Jaroszewski, *J. Nat. Prod.* **65**, 1754 (2002).

[6] F.R. Garcez, W.S. Garcez, L. Hamerski, and C.H. Miguita, *Quim. Nova.* **32**, 407 (2009).

[7] L.Q. Hai, Q.Y. Zhang, Y.Y. Zhao, H. Liang, and N.S. Du, *China J. Chin. Mater. Med.* **29**, 432 (2004).

[8] J. Jin, C.F. Zhang, and M. Zhang, *Mod. Chin. Med.* **10**, 20 (2008).

[9] J.W. Zhang, J.R. Guo, F. Tang, X.Q. Zhang, J.T. Xu, G.M. Yao, and Y.H. Zhang, *J. Chin. Med.* **33**, 1725 (2010).

[10] D.D. Zhao, D.Y. Li, J.X. Wei, H.M. Hua, and Z.L. Li, *J. Shenyang Pharm. Univ.* **29**, 514 (2012).

[11] S.-H. Xiao, J. Keiser, J. Chollet, J. Utzinger, Y. Dong, Y. Endriss, J.L. Vennerstrom, and M. Tanner, *Antimicrob. Agents Chemother.* **51**, 1440 (2007).

[12] J.D. Moraes, C. Nascimento, P.O.M. V. Lopes, E. Nakano, L.F. Yamaguchi, M.J. Kato, and T. Kawano, *Exp. Parasitol.* **127**, 357 (2011).

[13] L.B. Zhang, J.L. Lv, and H.L. Chen, *Fitoterapia* **87**, 89 (2013).