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New lignans from the roots of *Schisandra sphenanthera*

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ABSTRACT

Nine new lignans (**1**–**8**, **13**) and five known ones (**9**–**12**, **14**) have been isolated from the roots of *Schisandra sphenanthera* and were tested for their capacity to scavenge 2,2-diphenyl-1-Picryl-hydrazyl (DPPH). Of these lignans tested, compounds **1**, **7**, **8** and **13** exhibited noteworthy antioxidant activity with IC_{50} values of 92, 115, 35 and 48 μg/mL, respectively. The anti-oxidative haemolysis of human red blood cells (RBCs) activity of the most active compound **8**, which is similar to that of vitamin C, was evaluated.

1. Introduction

The genus *Schisandra* belonging to the plant family *Schisandraceae* has long been used in traditional Chinese medicine for the treatment of hepatitis, diabetes, diarrhea, cough, etc [1,2]. It has been reported that many kinds of lignans had been isolated from this genus, such as dibenzocyclooctadiene, dibenzylbutane and tetrahedrofurane types, and some of them exhibited antimicrobic, antiviral, herbicidal, antifeedant and insulin sensitivity-improving activities [3–9]. *Schisandra sphenanthera* is widely distributed in the southwest region of China [10]. Previous phytochemical studies on the fruits, stems and leaves of *S. sphenanthera* have led to the isolation of a variety of lignans and triterpenoids [11,12], while the chemical components of the roots of *S. sphenanthera* have never been reported. In this paper, we conducted a systematic bioactivity-guided isolation of the 70% aqueous acetone extract from the roots of *S. sphenanthera* and new compounds with natural anti-oxidative activity were discovered for the first time. The systematic isolation led to the identification of eight new tetrahydrofuran lignans (**1**–**8**), one new butane-type lignan (**13**) and five known ones (schiglaucin B, **9** [13]; schiglaucin A, **10** [12]; epoxyzuonin A, **11** [14]; talaumidin, **12** [15]; myristargenol A, **14** [16]) from the anti-oxidative active EtOAc-soluble fraction of the 70% aqueous acetone extract. The antioxidant activity of these compounds was assessed by DPPH scavenging experiments. Of these lignans tested, compound **8** exhibited noteworthy antioxidant activity and its capacity to protect human red blood cells (RBCs) from oxidative haemolysis was evaluated, which is similar to that of vitamin C.

2. Experimental

2.1. General

Column chromatography: silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China); macroporous resin (HP-20, Kabuskiki Kaisha); Sephadex LH-20 (Amersham Pharmacia Biotech). TLC (Thin layer chromatography): silica gel GF_{254} plates (10–40 μm; Qingdao Marine Chemical Factory, China). A Waters 1525 series instrument equipped with a YMC-Pack ODS-A column (250 × 10 mm, 5 μm) was used for semipreparative

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HPLC. ^{1}H and ^{13}C NMR Spectra: Bruker AM-400BB (400 MHz) and a Varian Mercury-600BB (600 MHz) NMR spectrometer; recorded in $CDCl_3$; δ in ppm relative to TMS; J values in Hz. HR-ESI-MS: Bruker APEX-II mass spectrometer; in m/z. Optical rotations: Perkin-Elmer 341 polarimeter. IR Spectra: Nicolet FT-IR-360 spectrometer. CD spectra were obtained on an Olis DSM 1000 spectrometer.

2.2. Plant material

Roots of *S. sphenanthera* were collected from Taibai Mountain in Shaanxi Province of China, in the summer of 2011. The plant was identified by Guo-Liang Zhang, at the School of Life Sciences, Lanzhou University, China. A voucher (No. 11-07) specimen was deposited with the Natural Organic Academy of Lanzhou University.

2.3. Extraction and isolation

The air-dried and powdered roots of (7 kg) were extracted three times (each time for 7 days) with 70% aqueous acetone at room temperature. The solvent was evaporated under reduced pressure, producing an extract (685 g), which was found to have antioxidant effect in the DPPH scavenging assay. The crude extract was dissolved in hot H_2O (60 °C, 2 L) to accelerate dissolution and then partitioned with petroleum ether (PE), EtOAc and *n*-BuOH at room temperature, respectively. The EtOAc-soluble fraction was found to be more anti-oxidative than the PE and *n*-BuOH-soluble fraction, exhibiting that the EtOAc-soluble fraction included the majority of the active components. Thus, the EtOAc-soluble fraction was further purified and investigated. Initially, the EtOAc-soluble fraction was applied to macroporous resin (HP-20,3 L) with a gradient of H_2O: EtOH (70: 30, 50: 50, 20: 80, v:v) as eluent, and three fractions (A, B, and C) were collected according to the TLC analysis.

Fraction C (248 g) was subjected to silica gel column chromatography (200–300 mesh, 2000 g) eluted with a gradient of PE (petroleum ether): acetone (100: 0, 50: 1, 20: 1, 10: 1, 5: 1, 2: 1, 1: 1, 0: 100, v:v) to give eight fractions, FrC.1–FrC.8. Using H_2O: MeOH (1: 1, 2: 5, 3: 10, 1: 5, 0: 1, v:v) as eluent, FrC.6 (6.8 g) was separated on a column of reversed-phase C_{18} silica gel, thus resulting in five subfractions. FrC.6.1–FrC.6.5. FrC.6.1 (1.3 g) was separated on a Sephadex LH–20 (CH_3OH) to obtain three fractions (FrC.6.1.1–FrC.6.1.3). FrC.6.1.1 (0.1 g) and FrC.6.1.3 (0.12 g) were further fractionated on semipreparative HPLC (MeOH: H_2O, 1: 3, v:v) to yield compounds **1** (3 mg) and **2** (2.1 mg). Compounds **3** (2.2 mg) and **4** (1.8 mg) were obtained from FrC.6.1.2 (0.15 g) by silica gel column chromatography (PE: acetone, 10: 1 to 5: 1, v:v). FrC.6.3 (2.1 g) was separated on a silica gel column and eluted with gradient mixtures of PE: acetone (from 8: 1 to 5: 1, v:v) to obtain compound **5** (3.2 mg) and FrC.6.3.1 (0.2 g). The separation of FrC.6.3.1 was done on semipreparative HPLC (MeOH: H_2O, 1: 4, v:v) to obtain compounds **6** (2.8 mg) and **7** (1.9 mg). FrC.6.4 (0.15 g) was separated on silica gel column chromatography (PE: acetone, 20: 1 to 5: 1, v:v), and then on Sephadex LH-20 ($CHCl_3$: MeOH, 1: 1, v:v) to give compound **8** (2.3 mg). FrC.7 (2.8 g) was applied to a silica gel column and eluted with various gradients of PE: acetone (15: 1, 10: 1, 5: 1, v:v) to give three fractions (FrC.7.1–FrC.7.3). FrC.7.1 (0.15 g) was separated on semipreparative HPLC (MeOH: H_2O, 1: 3, v:v) to yield compounds **9** (1.3 mg) and **10** (2.5 mg). In the same way, compounds **11** (3.6 mg) and **12** (2.7 mg) were obtained from FrC.7.2 (0.21 g). Further separation of FrC.7.3 (0.13 g) by reversed-phase C18 silica gel column, eluted with MeOH: H_2O (4: 1, v:v), led to the appearance of compounds **13** (2.3 mg) and **14** (4.2 mg).

2.3.1. (7S, 8S, 7′R, 8′S)-7-methoxyl-7-(3,4-methylenedioxyphenyl)-8-hydroxyl-7′-(5′-hydroxy-3′-methoxyphenyl)-8′-methyl-tetrahydrofuran (**1**)

Yellow oil; [α]25 D +60 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 228 (0.15) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 389.1977 $[M + H]^+$ (calcd for $C_{21}H_{24}O_7$).

2.3.2. (7S, 8S, 7′R, 8′S)-7-methoxyl-7-(3,4-methylenedioxy-phenyl)-8-hydroxyl-8′-methyl-7′-(3′,4′,5′-trimethoxyphenyl)-tetrahydrofuran (**2**)

Yellow oil; [α]25 D +50 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 232 (0.42) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 433.1613 $[M + H]^+$ (calcd for $C_{23}H_{28}O_8$).

2.3.3. (7S, 8S, 7′R, 8′S)-7-methoxyl-7-(3,4-methylenedioxyphenyl)-8-hydroxyl-8′-methyl-7′-(3′,4′-methylenedioxyphenyl)-tetrahydrofuran (**3**)

Yellow oil; [α]25 D +50 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 226 (0.25) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 387.1824 $[M + H]^+$ (calcd for $C_{21}H_{22}O_7$).

2.3.4. (7S, 8S, 7′R, 8′S)-(7, 8-trans-8, 8′-trans-7′, 8′-trans)-7-methoxyl-7-(3,4- methylenedioxy-phenyl)-8 methyl -8′-methyl-7′-(3′,4′-dimethoxyphenyl)-tetrahydrofuran (**4**)

Yellow oil; [α]25 D +20 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 232 (0.25) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 409.1712 $[M + H]^+$ (calcd for $C_{22}H_{26}O_6$).

2.3.5. (7S, 8S, 7′R, 8′S)-7-methoxyl-7-(3,4,5-trimethoxyphenyl)-8methyl -8′-methyl-7′-(3′,4′- methylenedioxyphenyl)-tetrahydrofuran (**5**)

Yellow oil; [α]25 D +40 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 227 (0.24) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 417.1823 $[M + H]^+$ (calcd for $C_{23}H_{28}O_7$).

2.3.6. (7S, 8S, 7′R, 8′S)-7,8-epoxide-7-(3,4-methylenedioxyphenyl)-8 methyl -8′-methyl-7′-(3′,4′-dimethoxyphenyl)-tetrahydrofuran (**6**)

Yellow oil; [α]25 D +20 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 227 (0.32) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 371.1629 $[M + H]^+$ (calcd for $C_{21}H_{22}O_6$).

2.3.7. (7S, 8S, 7′R, 8′S)-7,8-epoxide -7-(3,4-methylenedioxyphenyl)-8-methyl -8′-methyl-7′-(4′-hydroxy-3′-methoxyphenyl)-tetrahydrofuran (**7**)

Yellow oil; [α]25 D +40 (*c* 0.1, MeOH); UV (CH_3CH_2OH) λ_{max} (log ε) 229 (0.18) nm; ^{1}H and ^{13}C NMR data, see Tables 1