Supporting Information to:

A New Chalcone Glycoside, a New Tetrahydrofuranoid Lignan, and Antioxidative Constituents from the Stems and Leaves of

**Viburnum propinquum**

Xiao-Yu Wang¹, Hai-Ming Shi¹, Li Zhang², Xiao-Bo Li¹

**Affiliation**

¹ School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P.R. China
² Shanghai Mass Spectrometry Center, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, P.R. China

**Correspondence**

_Prof. Dr. Xiao-Bo Li_

School of Pharmacy
Shanghai Jiao Tong University
No. 800 Dongchuan Road
Minhang District
Shanghai 200240
People’s Republic of China
Tel.: +86-21-3420-4806
Fax: +86-21-3420-4804
xbli@sjtu.edu.cn
Animals
Male Kunming mice (20–25 g), purchased from the Animal Center of Shanghai Medical College, Fudan University, were used. The animals were housed under standard environmental conditions and had free access to standard pellet diet and water ad libitum.

Extraction and isolation
The dried stems and leaves (20 kg) were milled and extracted three times (3 × 2 L) with 85% EtOH for 3 h each time, with EtOH removed under reduced pressure. The 85% ethanolic extract was suspended in water and then partitioned with petroleum ether (3 × 2 L), EtOAc (3 × 2 L), and n-BuOH (3 × 2 L) successively. The petroleum ether–soluble fraction (225 g) was concentrated and subjected to a silica gel (1.0 kg) column (8 × 80 cm) eluting with a petroleum ether (PE) (60–90 °C)–acetone (AC) gradient system (100:1, 50:1, 20:1, 10:1, 2:1; each 10.0 L) to yield fractions P1–P6. After recrystallization of fraction P3 (0.5 g) with PE–AC (1:1, 10 mL), 4 (210 mg) was obtained. Similarly, 5 and 6 (200 mg) were obtained from fraction P4 (2.0 g). Fraction P5 (1.2 g) was further purified by Sephadex LH-20 column (2.5 × 50 cm) chromatography eluted with CHCl3–CH3OH (1:1, 1 L) to yield 7 (100 mg). The EtOAc-soluble fraction (210 g) was concentrated and subjected to a silica gel (1.0 kg) column (8 × 80 cm) eluting with a PE–AC gradient system (50:1, 20:1, 10:1, 2:1, 1:1 each 10.0 L) to yield fractions E1–E8. Fraction E1 (2.5 g) was further purified by Sephadex LH-20 column (3.0 × 60 cm) chromatography eluted with CH3OH (1 L) to yield 8 (300 mg). Fraction E4 (4.0 g) was chromatographed on a silica gel column (3.0 × 70 cm) eluting with PE–AC (5:1, 1 L) and then on a Sephadex LH-20 column (2.0 × 60 cm) eluting with CH3OH (1 L) to afford 9 (200 mg). Fraction E6 (2.5 g) was chromatographed on a silica gel column (3.0 × 70 cm) eluting with PE–AC (4:1, 2 L) to give 10 (100 mg). Fraction E8 (5.5 g) was purified by silica gel chromatography (3.0 × 70 cm) with PE–AC (4:1, 3 L) to afford fractions E8-1 to E8-5. Fraction E8-1 (1.0 g) was further purified by a Sephadex LH-20 column (2.0 × 60 cm) eluted with CH3OH (1 L) to afford 12 (40 mg). Fraction E8-5 (0.5 g) was further purified by HPLC (CH3CN:H2O, 30:70) to afford 13 (15 mg), and 14 (120 mg) was gained from fraction E8-4 (1.0 g) by eluting with CH3OH (1 L) on a Sephadex LH-20 column (2.0 × 60 cm). The n-BuOH-soluble fraction (243 g) was concentrated and subjected to a silica gel (1 kg) column
(8 × 80 cm) eluting with a CHCl3–CH3OH gradient system (100:1, 50:1, 20:1, 10:1, 5:1, 2:1; each 10.0 L) to yield fractions B1–B10. Fraction B1 (0.5 g) was purified by a Sephadex LH-20 column (2.0 × 60 cm) eluted with CH3OH (1 L) to afford 1 (50 mg). Fraction B3 (1.5 g) was purified by a Sephadex LH-20 column (2.0 × 60 cm) eluted with CH3OH (1 L) to afford 11 (200 mg).

**Acid hydrolysis of 2**

A methanol solution of compound 2 (15 mg) in 0.05 mol/L H2SO4 was kept at 60 °C for 2 h. The reaction mixture was diluted with H2O. The solution was kept at 60 °C for another 1 h and then neutralized with Ba(OH)2 and extracted with EtOAc. The EtOAc extract was purified on a Sephadex LH-20 column using MeOH as eluant to afford the aglycone. The aglycone of 2 was identified as 3,4,2′,4′-tetrahydroxy-trans-chalcone (5.7 mg) by comparing its HPLC [CH3CN/H2O (32:68)] retention time with compound 1 (Rt = 8.1 min). The neutral hydrolysate revealed the presence of glucose by HP-TLC [CHCl3/CH3OH/H2O (8:5:1), lower layer] when compared with authentic samples (Rf = 0.5), and it was further concentrated to dryness and subjected to silica gel chromatography eluting with CHCl3–MeOH (1:1) to give purified d-glucose (4.4 mg): [α]D25: +24.5 (c 0.2, H2O).

**Assay of DPPH radical scavenging activity**

Briefly, the methanolic solution (2 mL) of DPPH (0.2 M, Sigma, St. Louis, Mo., USA) was added to 2 mL of methanolic sample solution, and the mixture was kept at room temperature in the dark for 30 min. Vitamin C (99%, Acros Organics, New Jersey, USA) and vitamin E (98%, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) were selected as positive control. The decrease in the absorbance at 517 nm was measured and the inhibition percentage of radical scavenging activity was calculated as \(1 - (A_1 - A_2) / A_0\) × 100%, where \(A_0\) was the absorbance of the control (without sample), \(A_1\) was the absorbance in the presence of the sample, and \(A_2\) was the absorbance without DPPH.

**Assay of hydroxyl radical scavenging activity**

Briefly, the reaction mixture (3 mL) contained 0.5 mL FeSO4 (9 mM), 0.5 mL H2O2 (1%), 0.5
mL salicylic acid (20 mM), and varying concentrations of sample. After incubation for 0.5 h at 37 °C, the absorbance of the hydroxylated salicylate complex was measured at 510 nm. Vitamin C and vitamin E were selected as positive control. The percentage scavenging effect was calculated as \[1 - \frac{(A_1 - A_2)}{A_0}\times 100\%\], where \(A_0\) was the absorbance of the control (without sample), \(A_1\) was the absorbance in the presence of the sample, and \(A_2\) was the absorbance without reaction mixture.

**Inhibition of lipid peroxidation in rat liver homogenate**

Briefly, mice were sacrificed by cervical dislocation, and the liver was removed immediately and washed with cold saline. Then the liver was cut into pieces and a 10% solution of homogenate in cold saline was prepared. The 10% liver homogenate was incubated with 0.5 mM each of FeCl₂ and H₂O₂ with varying concentrations of sample. After incubation at 37 °C for 60 min, the formation of MDA in the incubation mixture was measured at 532 nm according to the test method of a commercial kit (Jiancheng Institute of Biotechnology, Nanjing, China). Vitamin C and vitamin E were used as positive control. The percentage inhibitory effect was calculated as \[1 - \frac{(A_1 - A_2)}{A_0}\times 100\%\], where \(A_0\) was the absorbance of the control (without sample), \(A_1\) was the absorbance in the presence of the sample, and \(A_2\) was the absorbance without the liver homogenate.
Fig. 1S Structures of the known compounds.