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Chemical constituents from the leaves of *Alchornea trewioides*(1). Phenolic acids and related compounds

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Abstract: 80% acetone extracts of the fresh leaves of *Alchornea trewioides* was successively separated by Sephadex LH-20, MCI gel CHP 20P, and Toyopearl Butyl-650C column chromatography to yield ten phenolic acids and related compounds. Their structures were elucidated by spectroscopic analyses as: salicylic acid(**1**), p-hydroxybenzoic acid(**2**), 2,5-dihydroxybenzoic acid(**3**), 3,4-dihydroxybenzoic acid(**4**), *trans*-p-coumaric acid(**5**), *cis*-p-coumaric acid(**6**), caffeic acid(**7**), caffeic acid methyl ester(**8**), gallic acid(**9**), and methyl gallate(**10**). Compounds **1**－**8**, **10** were isolated from the *Alchornea* for the first time.

Key words: *Alchornea trewioides*; chemical constituents; phenolic acid
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红背山麻杆叶的化学成分研究(I)——酚酸类及相关化合物

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摘 要: 采用80%丙酮提取石油醚萃取部位,利用凝胶、MCI及Toyopearl Butyl-650C柱色谱进行分离纯化得到10个酚酸类及相关化合物。根据化合物的波谱数据分析鉴定为水杨酸(**1**)、对羟基苯甲酸(**2**)、2,5-二羟基苯甲酸(**3**)、3,4-二羟基苯甲酸(**4**)、反-对香豆酸(**5**)、顺-对香豆酸(**6**)、咖啡酸(**7**)、咖啡酸甲酯(**8**)、没食子酸(**9**)、没食子酸甲酯(**10**)。其中化合物**1**～**8**、**10**均为首次从本属植物中分离得到。

关键词: 红背山麻杆; 化学成分; 酚酸

The genus *Alchornea* belongs to the family Euphorbiaceae and contains approximately 70 species. Over 6 species have been recorded in China(Editorial Committee in Flora of China, 1996), many of which have been used for treating inflammation of the prostate gland, hematuria, shigella, inflammation, lumbocrural pain and many other diseases (Jiangsu New Medical College, 1977). The *A. trewioides* belongs to

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the family *Alchornea*, it was used as traditional medicines to alleviate disease and discomfort. Previously, flavonoid glycosides, phenolic acids and antioxidant activity have been reported from the species (Lu, 2012; Qin, 2012; Lu, 2011). To further research for the material basis of pharmacological effects from the species *A. trewioides*, ten phenolic acids and related compounds were isolated from 80% acetone extracts of the fresh leaves of *A. trewioides*. Compounds **1-8**、**10** were isolated from the Alchornea for the first time.

1 Materials and methods

^{1}H- and ^{13}C-NMR spectra were measured in CD_3OD or acetone-d_6 at 27 ℃ using a Bruker Avance 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) (Bruker Biospin AG, Faellanden, Switzerland) or a JEOL JNM-AL 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) (JEOL Ltd., Tokyo, Japan). Coupling constants are expressed in Hz and chemical shifts are given on a δ (mg/L) scale. Column chromatography was performed using MCI gel CHP 20P (75－150 μm; Mitsubishi Chemical, Tokyo, Japan), Sephadex LH-20 (25－100 mm; GE Healthcare Bio-Science AB, Uppsala, Sweden), and Toyopearl Butyl-650C (TOSOH Co., Tokyo, Japan) columns. TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick; Merck, Darmstadt, Germany) with $CHCl_3$-MeOH-H_2O (9∶1∶0.1, 8∶2∶0.2, or 7∶3∶0.5, v/v) and toluene-ethyl formate-formic acid (1∶7∶1, v/v) as the solvent, and spots were detected by UV illumination (254 nm) and by spraying with 2% ethanolic $FeCl_3$ and 10% sulfuric acid reagent, followed by heating.

The leaves of *A. trewioides* were collected at Guangxi Institute of Botany, Guangxi, China, in August 2011, and identified by Prof. WEI Fa-Nan. The voucher specimen (20110920N) was deposited in the Guangxi key laboratory of functional phytochemicals research and utilization, Guangxi Institute of Botany.

2 Extraction and separation

The fresh leaves of *A. trewioides* (5.35 kg) were cut into small pieces and extracted with acetone-H_2O (8∶2, v/v) by maceration at room temperature. After filtration, the plant debris remaining on the filter paper was extracted with the same solvent a further two times. The filtrate was combined and concentrated under reduced pressure to give an aqueous solution with dark green precipitates. The precipitant was mainly composed of chlorophylls and waxes, and removed by filtration. The filtrate was defatted by partitioning with Et_2O, to give an Et_2O fraction (Fr. E 5.46 g).

The Et_2O fraction was subjected to Sephadex LH-20 column chromatography with EtOH containing increasing proportions of water (4 cm i. d.×40 cm, 0－50%, 10% stepwise elution, each 400 mL) and finally 100% MeOH (500 mL) to give five fractions: frs. E-1 (2.35 g), 2 (0.15 g), 3 (0.67 g), 4 (0.53 g), 5 (1.28 g). Fraction E-1 was further fractionated by MCI gel CHP 20P column chromatography (3 cm i. d.×40 cm) with 0－100% MeOH (10% stepwise elution, each 300 mL) and the subfractions were separated by column chromatography using the Toyopearl Butyl-650C (2 cm i. d. ×30 cm) with 0－100% MeOH (10% stepwise elution, each 200 ml) to yield compounds **7** (4 mg), **8** (9 mg), **9** (937 mg), and **10** (67 mg). Fraction E-3 was successively applied to a MCI gel CHP 20P column chromatography (2 cm i. d.×30 cm) with 0－100% MeOH (10% stepwise elution, each 100 mL) to yield **3** (7 mg). Fraction E-4 was further fractionated by MCI gel CHP 20P column chromatography (2 cm i. d.×40 cm) with 10%－100% MeOH (10% stepwise elution, each 100 ml) to yield compounds **1** (17 mg), and **6** (6 mg). Fraction E-5 was further fractionated by MCI gel CHP 20P column chromatography (2 cm i. d. ×40 cm) with 10%－100% MeOH (10% stepwise elution, each 150 mL), and the subfractions were purified by Toyopearl Butyl-650C (1 cm i. d.×30 cm) with 0－100% MeOH (10% stepwise elution, each 100 mL) to get compounds **2** (8 mg), **4** (17 mg), and **5** (36 mg).

3 Results and analysis

Salicylic acid (**1**)　White amorphous powder, $C_7H_6O_3$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.85 (1H, dd, J=1.0, 8.3 Hz, H-3), 6.88 (1H, m, H-5), 7.44 (1H, m, H-4), 7.84 (1H, dd, J=1.7, 8.3 Hz, H-6); ^{13}C-NMR

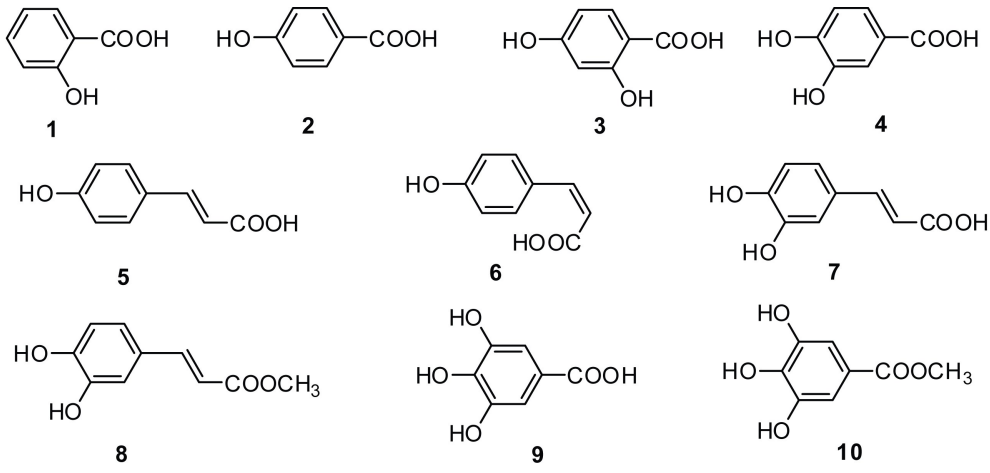

Fig. 1 Chemical structures of compounds **1**—**10**

(100 MHz, CD_3OD) δ: 114.1(C-1), 118.1(C-3), 120.0(C-5), 131.5(C-6), 136.5(C-4), 163.2(C-2), 173.6(C-7)(Milena *et al.*, 2004).

p-Hydroxybenzoic acid (**2**) Brown amorphous powder, $C_7H_6O_3$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.90(2H, d, J = 8.5 Hz, H-3, 5), 7.77 (2H, d, J = 8.5 Hz, H-2, 6); ^{13}C-NMR(100 MHz, CD_3OD) δ: 116.7(C-3, 5), 123.6(C-1), 133.8(C-2, 6), 164.2(C-4), 171.3(C-7)(Penchom *et al.*, 1998).

2, 5-Dihydroxybenzoic acid (**3**) Pale brown amorphous powder, $C_7H_6O_4$. ^{1}H-NMR(400 MHz, CD_3OD) δ: 6.74 (1H, d, J = 8.9 Hz, H-3), 6.91 (1H, dd, J = 2.2, 8.9 Hz, H-4), 7.25(1H, d, J = 2.2 Hz, H-6); ^{13}C-NMR(100 MHz, CD_3OD) δ: 113.6 (C-1), 116.2(C-6), 118.4(C-3), 124.2(C-4), 150.1 (C-5), 156.3 (C-2), 173.1 (C-7) (Akiyo *et al.*, 1995).

3, 4-Dihydroxybenzoic acid (**4**) White amorphous powder, $C_7H_6O_4$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.78 (1H, d, J = 8.3 Hz, H-5), 7.41 (1H, dd, J = 2.2, 8.3 Hz, H-6), 7.42(1H, d, J = 2.2 Hz, H-2); ^{13}C-NMR(100 MHz, CD_3OD) δ: 116.2 (C-5), 118.2(C-2), 123.6(C-6), 124.5(C-1), 146.6 (C-3), 151.6(C-4), 170.2(C-7)(Ban *et al.*, 2007).

Trans-p-coumaric acid(**5**) Pale brown amorphous powder, $C_9H_8O_3$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.29 (1H, d, J = 15.8 Hz, H-8), 6.80 (2H, d, J = 8.3 Hz, H-3, 5), 7.41 (2H, d, J = 8.3 Hz, H-2, 6), 7.58 (1H, d, J = 15.8 Hz, H-7); ^{13}C-NMR(100 MHz, CD_3OD) δ: 115.1(C-8), 116.7(C-3, 5), 127.2(C-1), 131.1(C-2, 6), 146.8(C-7), 161.3 (C-4), 171.2(C-9)(An *et al.*, 2008).

Cis-p-coumaric acid (**6**) Pale brown amorphous powder, $C_9H_8O_3$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.67 (1H, d, J = 12.7 Hz, H-8), 6.64 (2H, d, J = 8.5 Hz, H-3, 5), 7.70 (1H, d, J = 12.7 Hz, H-7), 7.50 (2H, d, J = 8.5 Hz, H-2, 6); ^{13}C-NMR(100 MHz, CD_3OD) δ: 116.5 (C-3, 5), 126.3 (C-8), 130.1 (C-7), 130.2 (C-2, 6), 131.5 (C-1), 156.6(C-4), 171.7(C-8)(Kort *et al.*, 1996).

Caffeic acid (**7**) White amorphous powder, $C_9H_8O_4$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.20 (1H, d, J = 15.9 Hz, H-8), 6.77(1H, d, J = 8.1 Hz, H-5), 6.91 (1H, dd, J = 2.2, 8.1 Hz, H-6), 7.03 (1H, d, J = 2.2 Hz, H-2), 7.52(1H, d, J = 15.9 Hz, H-7); ^{13}C-NMR(100 MHz, CD_3OD) δ: 115.1(C-2), 115.6(C-8), 116.5(C-5), 122.8(C-6), 127.8(C-1), 146.7(C-3), 147.0(C-7), 149.4(C-4), 171.1(C-9) (Fukuoka *et al.*, 1982).

Caffeic acid methyl ester (**8**) White amorphous powder, $C_{10}H_{10}O_4$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 3.88 (3H, s, OCH_3), 6.30 (1H, d, J = 15.9 Hz, H-8), 6.79(1H, d, J = 8.1 Hz, H-5), 7.05 (1H, dd, J = 2.2, 8.1 Hz, H-6), 7.15(1H, d, J = 2.2 Hz, H-2), 7.58(1H, d, J = 15.9 Hz, H-7); ^{13}C-NMR (100 MHz, CD_3OD) δ: 56.5 (C-OCH_3), 111.7 (C-8), 116.0(C-2), 116.5(C-5), 124.0(C-6), 127.8(C-1), 146.8(C-7), 147.3(C-3), 150.6(C-4), 167.9(C-9)(Shin *et al.*, 2004).

Gallic acid (**9**) White amorphous powder,

$C_7H_6O_5$. ^{1}H-NMR(500 MHz, acetone-d_6) δ:7.14 (2H, s, H-2, 6); ^{13}C-NMR(125 MHz, acetone-d_6) δ:110.1(C-2, 6), 121.9(C-1), 138.7(C-4), 146.0(C-3, 5), 168.1(C-7)(Lu *et al*., 1999).

Methyl gallate(**10**) White amorphous powder, $C_8H_8O_5$. ^{1}H-NMR(400 MHz, acetone-d_6) δ: 3.77(3H, s, OCH_3), 7.11(2H, s, H-2, 6); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 51.9 (C-OCH_3), 109.8 (C-2, 6), 121.7(C-1), 138.7(C-4), 145.7(C-3, 5), 167.2(C-7)(Ma *et al*., 2005).

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