DOI: 10.3969/j.issn.1000-3142.2014.02.001
黄永林,刘金磊,陈月圆,等. 红背山麻杆叶的化学成分研究(Ⅱ)——黄酮和苯乙醇苷类化合物[J]. 广西植物,2014,34(2):143－147
Huang YL, Liu JL, Chen YY, *et al*. Chemical constituents from the leaves of *Alchornea trewioides* (2). Flavonoids and phenylethanoid glycosides[J]. Guihaia, 2014, 34(2):143－147

Chemical constituents from the leaves of *Alchornea trewioides* (2). Flavonoids and phenylethanoid glycosides

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Abstract: The 80% acetone extracts of the fresh leaves of *Alchornea trewioides* was successively separated by Sephadex LH-20, MCI gel CHP 20P, ODS, and Toyopearl Butyl-650C column chromatography to yield seven flavonoids and three phenylethanoid glycosides. Their structures were elucidated by spectroscopic analyses as: quercetin (**1**), quercetin-3-rhamnoside (**2**), quercetin-3-*O*-β-D-glucopyranoside (**3**), rutin (**4**), apigenin-6-C-β-D-glucopyranoside (**5**), apigenin-8-C-β-D-glucopyranoside (**6**), luteolin-7-*O*-a-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**7**), 2-phenylethyl β-D-glucopyranoside (**8**), icariside D_1 (**9**), and 2-phenylethyl D-rutinoside (**10**). Compounds **1**－**3**, **5**－**6**, **8**－**10** were isolated from the *Alchornea* for the first time.

Key words: *Alchornea trewioides*; chemical constituents; flavonoid; phenylethanoid glycoside
CLC number: Q946.8 **Document code**: A **Article ID**: 1000-3142(2014)02-0143-05

红背山麻杆叶的化学成分研究(Ⅱ)——黄酮和苯乙醇苷类化合物

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摘 要: 采用80%丙酮提取物的水萃取部位,利用凝胶、MCI、反相碳18、及Toyopearl Butyl-650C柱色谱进行分离纯化得到7个黄酮和3个苯乙醇苷类化合物。根据化合物的波谱数据分析鉴定为槲皮素(**1**)、槲皮苷(**2**)、异槲皮苷(**3**)、芦丁(**4**)、异牡荆素(**5**)、牡荆素(**6**)、木犀草素-7-*O*-α-L-鼠李糖(1→6)-β-D-葡萄糖苷(**7**)、2-phenethyl β-D-glucoside(**8**)、icariside D_1(**9**)、2-苯乙基-D-芸香甙(**10**)。其中化合物**1**－**3**、**5**－**6**、**8**－**10**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; 黄酮; 苯乙醇苷

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, lumbo-

收稿日期: 2013-10-15 **修回日期**: 2013-12-12
基金项目: 广西自然科学基金(2011GXNSFD018038);广西科技合作与交流计划项目(桂科合1298014-10);广西植物研究所基本业务费(桂植业13002);广西植物功能物质研究与利用重点实验室开放基金(ZRJJ2013-7)。
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crural pain and many other diseases (Jiangsu New Medical College, 1977). The *A. trewioides* belongs to 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; Huang, 2014). To further research for the material basis of pharmacological effects from the species *A. trewioides*, seven flavonoids and three phenylethanoid glycosides were isolated from the 80% acetone extracts of the fresh leaves of *Alchornea trewioides*. Compounds **1**—**3**, **5**—**6**, **8**—**10** were isolated from the *Alchornea* for the first time.

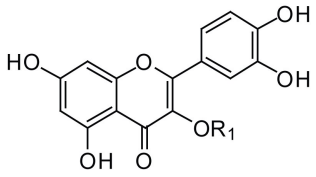
1 Materials and methods

^{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 were expressed in Hz and chemical shifts are given on a δ (ppm) 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), Chromatorex ODS (100-200 mesh, Fuji Silysia Chemical Ltd., kasugai, Japan), 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 a 2% ethanolic $FeCl_3$ and 10% sulfuric acid reagent, followed by heating.

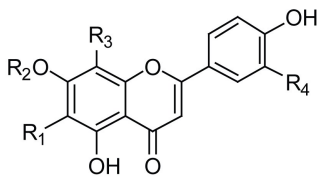
The leaves of *A. trewioides* were collected at Guangxi Institute of Botany, Guangxi, China, in August 2011, and identified by Prof. Wei Fanan. The voucher specimen (2011 0920N) was deposited in the Guangxi key laboratory of functional phytochemicals research and utilization, Guangxi Institute of Botany.

2 Extraction and separation

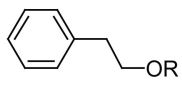
The fresh leaves of *A. trewioides* (5.35 kg) were cut into small pieces and extracted with acetone-H_2O (8 ∶ 2, v/v) by maceration at room temperature. After filtration, the plant debris remaining on the filter paper was extracted with the same solvent a further two times. The filtrate was combined 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 extract (610 g) was partitioned between H_2O (3 L) and Et_2O (1 L) 3 times. The aqueous layer was fractionated by Sephadex LH-20 column chromatography (10 cm i.d. × 40 cm) with water containing increasing proportions of MeOH (0—100%, 10% stepwise elution, each 2 L) and finally with 60% acetone, to yield 9 fractions (Fr. 1—9). Fraction 1 (18.7 g) was separated by a combination of column chromatography over MCI gel CHP 20P (8 cm i.d. × 40 cm) with 0—100% MeOH (10% stepwise elution, each 1 L), Toyopearl 650C (2 cm i.d. × 30 cm) with 0—100% MeOH (10% stepwise elution, each 300 mL), and Chromatorex ODS (2 cm i.d. × 30 cm) with 0—100% MeOH (10% stepwise elution, each 300 mL), to afford **8** (285 mg), **9** (23 mg), **10** (91 mg). Fraction 5 (35.6 g) was separated by a combination of column chromatography over MCI gel CHP 20P (8 cm i.d. × 40 cm) with 0—100% MeOH (10% stepwise elution, each 1 L), and Sephadex LH-20 (2 cm i.d. × 30 cm) with 0—100% MeOH (10% stepwise elution, each 300 mL), to afford **1** (275 mg). Fraction 6 (36.0 g) was further fractionated by MCI gel CHP 20P column chromatography (8 cm i.d. × 40 cm) with 0—100% MeOH (10% stepwise elution, each 1 L) to give nine fractions: frs. 6-1 (6.30 g), 2 (1.02 g), 3 (1.36 g), 4 (2.45 g), 5 (6.28 g), 6 (6.10 g), 7 (4.12 g), 8 (2.56 g), 9 (1.25 g). Fraction 6-3 (1.36 g) was successively applied to a Sephadex LH-20 column chromatography (3 cm i.d. × 30 cm) with 0—100% MeOH (10% stepwise elution, each 200 mL) to yield **2** (20 mg). Fraction 6-8 was subjected to a Sephadex



1 R_1=H
2 R_1=Rha
3 R_1=Glc
4 R_1=Glc(6→1)Rha



5 R_1=Glc, R_2=R_3=R_4=H
6 R_1=R_2=R_4=H, R_3=Glc
7 R_1=R_3=H, R_2=Glc(6→1)Rha, R_4=OH



8 R=Glc
9 R=Glc(6→1)Api
10 R=Glc(6→1)Rha

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

LH-20 column chromatography(3 cm i. d.×30 cm) with 0—100% MeOH(10% stepwise elution, each 200 mL) to yield **4**(25 mg), and **5**(36 mg). Fraction 6-9 was further fractionated by Sephadex LH-20 column chromatography(6 cm i. d.×40 cm) with 10—100% MeOH(10% stepwise elution, each 200 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 compound **3**(6 mg), **6**(10 mg), and **7**(648 mg).

3　Results and analysis

Quercetin (**1**)　Yellow amorphous powder, $C_{15}H_{10}O_7$. ^{1}H-NMR(500 MHz, CD_3OD) δ: 6.16(1H, d, J=2.2 Hz, H-6), 6.36(1H, d, J=2.2 Hz, H-8), 6.87(1H, d, J=8.5 Hz, H-5′), 7.61(1H, dd, J=2.2, 8.5 Hz, H-6′), 7.72(1H, d, J=2.2 Hz, H-2′); ^{13}C-NMR(125 MHz, CD_3OD) δ: 94.4(C-8), 99.2(C-6), 104.5(C-10), 116.0(C-2′), 116.2(C-5′), 121.7(C-6′), 124.1(C-1′), 137.2(C-3), 146.2(C-3′), 147.3(C-2), 148.7(C-4′), 158.2(C-5), 162.5(C-9), 165.5(C-7), 177.3(C-4)(Xiao, *et al.*, 2006; Markham *et al.*, 1976).

Quercetin-3-rhamnoside (**2**)　Yellow amorphous powder, $C_{21}H_{20}O_{11}$. ^{1}H-NMR(400 MHz, CD_3OD) δ: 0.93(3H, d, J=5.9 Hz, H-6″), 3.34(1H, t, J=5.8 Hz, H-4″), 3.40(1H, m, H-5″), 3.73(1H, dd, J=3.2, 9.3 Hz, H-3″), 4.21(1H, br s, H-2″), 5.34(1H, br s, H-1″), 6.18(1H, d, J=2.0 Hz, H-6), 6.36(1H, J=2.0 Hz, H-8), 6.90(1H, d, J=8.3 Hz, H-5′), 7.30(1H, dd, J=1.9, 8.3 Hz, H-6′), 7.33(1H, J=1.9 Hz, H-2′); ^{13}C-NMR(100 MHz, CD_3OD) δ: 17.8(C-6″), 71.9(C-2″), 72.1(C-5″), 72.2(C-3″), 73.3(C-4″), 94.7(C-8), 99.8(C-6), 103.5(C-1″), 105.9(C-10), 116.4(C-5′), 116.9(C-2′), 122.9(C-6′), 123.1(C-1′), 136.2(C-3), 146.5(C-4′), 149.8(C-3′), 158.7(C-2), 159.5(C-9), 163.3(C-5), 165.9(C-7), 179.7(C-4)(Fossen *et al.*, 1999).

Quercetin-3-*O*-β-D-glucopyranoside (**3**)　Yellow amorphous powder, $C_{21}H_{20}O_{12}$. ^{1}H-NMR(500 MHz, CD_3OD) δ: 3.21-3.83(6H, m, H-2‴, 3‴, 4‴, 5‴, 6ax‴, 6eq‴), 5.10(1H, d, J=7.5 Hz, H-1‴), 6.18(1H, d, J=2.0 Hz, H-6), 6.36(1H, d, J=2.0 Hz, H-8), 6.88(1H, d, J=8.5 Hz, H-5′), 7.61(1H, dd, J=2.5, 8.5 Hz, H-6′), 7.68(1H, d, J=2.5 Hz, H-2′); ^{13}C-NMR(125 MHz, CD_3OD) δ: 62.5(C-6‴), 71.0(C-4‴), 75.6(C-2‴), 78.2(C-3‴), 78.4(C-5‴), 95.2(C-8), 98.2(C-6), 101.2(C-1‴), 105.3(C-10), 116.1(C-2′), 116.3(C-5′), 123.2(C-1′), 123.3(C-6′), 135.9(C-3), 146.2(C-3′), 150.1(C-4′), 159.0(C-2), 159.2(C-9), 163.1(C-5), 166.4(C-7), 179.4(C-4)(Liu *et al.*, 2010).

Rutin (**4**)　Yellow amorphous powder, $C_{27}H_{30}O_{16}$. ^{1}H-NMR(500 MHz, CD_3OD) δ: 1.15(3H, d, J=5.8 Hz, H-6‴), 3.27-3.54(10H, m, H-2″, 3″, 4″, 5″, 6ax″, 6eq″, 2‴, 3‴, 4‴, 5‴), 5.18(1H, br s, H-1‴), 5.13(1H, d, J=8.0 Hz, H-1″), 6.22(1H, br s, H-6), 6.41(1H, br s, H-8), 6.89(1H, d, J=8.5 Hz, H-5′), 7.65(1H, dd, J=2.0, 8.5 Hz, H-6′), 7.68(1H, d, J=2.0 Hz, H-2′); ^{13}C-NMR(125 MHz, CD_3OD) δ: 16.5(C-6‴), 67.2(C-6″), 68.3(C-5‴), 70.0(C-3‴), 70.7(C-2‴), 70.9(C-4″), 72.6(C-4‴), 74.3(C-2″), 75.8(C-5″), 76.8(C-3″), 93.5(C-8), 98.6(C-6), 101.0(C-1‴), 103.3(C-1″), 104.3(C-10), 114.7(C-5′), 116.3(C-2′), 121.8(C-6′), 122.2(C-1′), 134.2(C-3), 144.4(C-3′), 148.4(C-

4′),157.1(C-9),157.9(C-2),161.7(C-5),164.6(C-7),178.0(C-4)(Sang *et al.*,2001).

Apigenin-6-C-β-D-glucopyranoside(**5**) Yellow amorphous powder, $C_{21}H_{20}O_{10}$. ^{1}H-NMR(500 MHz, CD_3OD)δ:3.45-3.52(3H,m,H-3″,4″,5″),3.78(1H,dd,J=6.5,12.0 Hz,H-6ax″),3.92(1H,d,J=12.0 Hz,H-6eq″),4.21(1H,t,J=9.8 Hz,H-2″),4.61(1H,d,J=9.8 Hz,H-1″),6.42(1H,s,H-8),6.49(1H,s,H-3),6.89(2H,d,J=8.0 Hz,H-3′,5′),7.42(2H,d,J=8.0 Hz,H-2′,6′);^{13}C-NMR(125 MHz,CD_3OD)δ:61.5(C-6″),70.4(C-2″),71.3(C-4″),73.9(C-1″),78.7(C-3″),81.2(C-5″),93.9(C-8),102.4(C-3),103.8(C-10),107.7(C-6),115.6(C-3′,5′),121.6(C-1′),128.0(C-2′,6′),157.2(C-9),160.5(C-5),161.3(C-4′),163.4(C-7),164.6(C-2),182.5(C-4)(Talita *et al.*,2012;Maatooq *et al.*,1997).

Apigenin-8-C-β-D-glucopyranoside(**6**) Yellow amorphous powder, $C_{21}H_{20}O_{10}$. ^{1}H-NMR(500 MHz, CD_3OD)δ:3.46-3.52(3H,m,H-3″,4″,5″),3.78(1H,dd,J=6.5,12.0 Hz,H-6ax″),3.92(1H,d,J=12.0 Hz,H-6eq″),4.21(1H,t,J=9.8 Hz,H-2″),4.61(1H,d,J=9.8 Hz,H-1″),6.21(1H,s,H-6),6.45(1H,s,H-3),6.90(2H,d,J=8.0 Hz,H-3′,5′),7.98(2H,d,J=8.0 Hz,H-2′,6′);^{13}C-NMR(125 MHz,CD_3OD)δ:61.3(C-6″),70.6(C-2″),70.9(C-4″),73.4(C-1″),78.7(C-3″),81.8(C-5″),98.2(C-6),102.5(C-3),104.1(C-8),104.6(C-10),115.8(C-3′,5′),121.1(C-1′),128.9(C-2′,6′),156.0(C-5),160.4(C-9),161.1(C-4′),162.7(C-7),164.0(C-2),181.1(C-4)(Talita *et al.*,2012).

Luteolin-7-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside(**7**) Yellow amorphous powder, $C_{27}H_{30}O_{15}$. ^{1}H-NMR(500 MHz, acetone-d_6)δ:1.20(3H,d,J=6.5 Hz,H-6‴),3.43-3.48(10H,m,H-2″,3″,4″,5″,6ax″,6eq″,2‴,3‴,4‴,5‴),4.80(1H,br s,H-1‴),5.16(1H,d,J=7.5 Hz,H-1″),6.53(1H,br s,H-6),6.68(1H,br s,H-8),6.87(1H,br s,H-3),7.04(1H,d,J=8.5 Hz,H-5′),7.47(1H,dd,J=2.0,8.5 Hz,H-6′),7.50(1H,d,J=2.0 Hz,H-2′);^{13}C-NMR(125 MHz,acetone-d_6)δ:17.1(C-6‴),66.2(C-6″),68.5(C-5‴),69.8(C-4″),70.6(C-3‴),71.2(C-2‴),72.5(C-4‴),73.3(C-2″),75.7(C-5″),76.3(C-3″),95.2(C-8),100.0(C-6),100.4(C-1‴),100.6(C-1″),103.3(C-3),105.8(C-10),113.3(C-5′),116.0(C-2′),119.5(C-6′),122.2(C-1′),145.6(C-3′),149.7(C-4′),157.4(C-9),161.3(C-5),163.3(C-7),165.3(C-2),182.5(C-4)(Petrovic *et al.*,1999).

2-Phenylethyl β-D-glucopyranoside(**8**) Amorphous powder, $C_{14}H_{20}O_6$. ^{1}H-NMR(400 MHz, CD_3OD)δ:2.93(2H,t,J=7.8 Hz,H_2-β),3.20(1H,dd,J=7.8,8.5 Hz,H-2′),3.29-3.42(3H,m,H-3′,4′,5′),3.67(1H,dd,J=5.1,11.7 Hz,H-6ax′),3.72(1H,m,H-αax),3.87(1H,dd,J=3.2,11.7 Hz,H-6eq′),4.09(1H,m,H-αeq),4.31(1H,d,J=7.8 Hz,H-1′),7.18(1H,s,H-4),7.25(4H,m,H-2,3,5,6);^{13}C-NMR(100 MHz,CD_3OD)δ:37.2(C-β),62.7(C-6′),71.3(C-α),71.7(C-4′),75.0(C-2′),77.8(C-5′),78.0(C-3′),104.6(C-1′),127.2(C-4),129.3(C-3,5),129.9(C-2,6),140.0(C-1)(Miyase *et al.*,1988;Kaoru *et al.*,1988).

Icariside D_1(**9**) Colorless syrup, $C_{19}H_{28}O_{10}$. ^{1}H-NMR(400 MHz,CD_3OD)δ:2.93(2H,t,J=6.9 Hz,H_2-β),3.17(1H,dd,J=7.8,9.0 Hz,H-2′),3.29-3.37(3H,m,H-3′,4′,5′),3.32-3.36(2H,m,H-5ax″,5eq″),3.59(1H,dd,J=6.1,11.2 Hz,H-6ax′),3.74(1H,d,J=9.8 Hz,H-4ax″),3.75(1H,m,H-αax),3.89(1H,d,J=2.4 Hz,H-2″),3.89(1H,d,J=9.8 Hz,H-4eq″),3.97(1H,dd,J=2.2,11.2 Hz,H-6eq′),4.04(1H,m,H-αeq),4.28(1H,d,J=7.8 Hz,H-1′),5.00(1H,d,J=2.4 Hz,H-1″),7.16(1H,s,H-4),7.25(4H,m,H-2,3,5,6);^{13}C-NMR(100 MHz,CD_3OD)δ:37.2(C-β),65.6(C-5″),68.7(C-6′),71.7(C-α),71.8(C-4′),75.0(C-2′),75.1(C-4″),76.9(C-5′),78.0(C-3′),78.1(C-2″),80.5(C-3″),104.4(C-1′),111.0(C-1″),127.2(C-4),129.3(C-3,5),130.0(C-2,6),140.0(C-1)(Miyase *et al.*,1987).

2-Phenylethyl D-rutinoside(**10**) Colorless syrup, $C_{20}H_{30}O_{10}$. ^{1}H-NMR(400 MHz, CD_3OD)δ:1.20(3H,d,J=6.5 Hz,H-6″),2.92(2H,t,J=7.0 Hz,H_2-β),3.19(1H,dd,J=7.6,8.5 Hz,H-2′),3.28(1H,t,J=9.2 Hz,H-4″),3.30-3.41(3H,m,H-3′,4′,5′),3.61(1H,dd,J=5.5,11.2 Hz,H-6ax′),3.67-3.69(2H,m,H-3″,5″),3.72(1H,m,H-αax),3.82(1H,dd,J=1.7,3.1 Hz,H-2″),3.89(1H,dd,J=2.2,11.2 Hz,H-6eq′),4.04(1H,m,H-αeq),4.29(1H,d,J=7.6 Hz,H-1′),4.73(1H,d,J=1.7 Hz,H-1″),7.17(1H,s,H-4),7.25(4H,m,H-2,3,5,6);^{13}C-NMR(100 MHz,

CD_3OD)δ:18.0(C-6″),37.2(C-β),68.1(C-6′),69.8(C-5″),71.6(C-α),71.8(C-4′),72.1(C-2″),72.3(C-3″),74.0(C-4″),75.0(C-2′),76.7(C-5′),78.0(C-3′),102.2(C-1″),104.4(C-1′),127.2(C-4),129.3(C-3,5),130.0(C-2,6),140.0(C-1)(Kaoru *et al*.,1988,Hase *et al*.,1995).

Acknowledgements The authors are grateful to Mr. NING De-Sheng (Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization) for NMR measurements.

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(上接第 247 页 Continue from page 247)

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