Water-soluble Constituents from Callicarpa pedunculata

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=ABSTRACT> AIM: To explore the water-soluble constituents from Callicarpa pedunculata. METHOD: The water-soluble constituents were isolated and purified by column chromatography, and their structures were identified by spectroscopic analyses, including 1D- and 2D-NMR data. RESULTS: Four caffeoyl phenylethanoid glycosides were purified from the water-soluble extracts of Callicarpa pedunculata R. Br. Brown and were identified to be forsythoside B (1), arenarioside (2), acteoside (3) and isoacteoside (4), respectively. CONCLUSION: Compounds 1-4 were obtained from this plant for the first time.

=KEY WORDS> Callicarpa pedunculata; Caffeoyl phenylethanoid glycosides; Forsythoside B; Arenarioside; Acteoside; Isoacteoside

=CLC number> R284.1 =Document code> A =Article ID> 1672-3651(2008)03-0176-03

doi: 10.3724/SP.J.1009.2008.00176

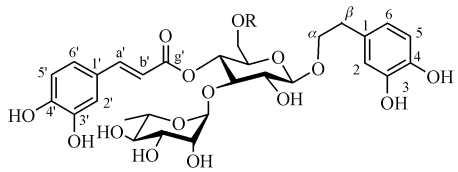
Callicarpa pedunculate R. Brown (Verbenaceae) is a small shrub widely distributed in southeast and southern China to the Philippines, and has been used in traditional Chinese medicine for the treatment of hemostasis, and as anti-inflammatory and antibacterial drugs[1]. In previous papers, we reported the structures of four types of diterpenoids, flavones, and triterpenoids[2-4]. Our continuing phytochemical investigation on water-soluble constituents of this plant, four phenylpropanoids were isolated and their structures were elucidated as forsythoside B (1), arenarioside (2), acteoside (3) and isoacteoside (4), respectively.

1 Apparatus and Material

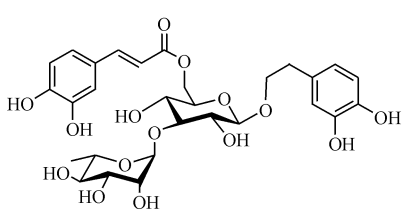
Column chromatography (CC): Qingdao silica gel (200-300 mesh); macrosporous resin D101: (Nankai University); Sephadex LH-20: Pharmacia products; TLC: Qingdao precoated plates; silica GF_{254} plates; NMR Spectra: Bruker DRX-500 spectrometer with TMS as internal standard; ESI-MS: Thermo-Finnigen LCQ-Advantage spectrometer.

2 Plant material

The aerial parts of C. pedunculata R. Brown were collected in Jiangxi Province, China and a voucher specimen was deposited in the herbarium of the Kunming institute of Botany, Chinese Academy of Sciences (No. 0300993).



1 R = β-*D*-Api
2 R = β-*D*-Xyl
3 R = H



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3 Extraction and Isolation

The air-dried aerial parts of this plant (150 g) were extracted five times with boiling water. The aqueous solution was concentrated under vacuum at 45℃ by co-evaporation with n-butanol. The concentrate solution (cal. 400 mL) was subjected to column chromatography over macrosporous resin D101 (250 g) eluted with water (4 L), 20% ethanol (V/V, 4 L) and ethanol (3 L), respectively. The 20% ethanol eluate was collected with 500 mL per fraction. The fractions 2-28 were combined according to the TLC results (TLC plates were precoated with silica gel F_{254}, and developed with n-BuOH-H_2O-FA, 8:1:1, V/V/V) to afford 1.13 g extract after the removal of solvents under vacuum. This extract was subjected to column chromatography over reversed-phase C_{18} silica

=Received on> 2007-07-13

=Foundation Item> This project was supported by the National Natural Science Foundation for Outstanding Youth (No. 30325044) to SHEN Yue-Mao.

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gel (145 g) eluted with gradient water2methanol (H_2O 2018 L; 30% MeOH 2215 L; 60% MeOH 2110 L; MeOH) and collected as 500 mL/fraction1 The 60% MeOH eluate were combined according to the TLC results and further subjected to column chroma2 tography over Sephadex LH220 (120 g) eluted with 85% MeOH (V/V) to yield 1 (8 mg yield: cal 01005 3%) and 2 (27 mg yield: cal 01018%)1

In order to prepare more amounts of compounds 1 and 2, larger scale extraction with more plant mate2 rials was carried out in pharmaceutical factory1 The isolation procedure was the same as described above1 However, compounds 3 and 4 were obtained as a mixture (the ratio of 3 to 4 was estimated to be 1B3 based on the integrations of proton NMR signals, yield: cal 1/10 of 1) besides 1 and 2 this time1

4 Structure Elucidation

The structures of 124 were elucidated by compari2 son of the spectral data (^{1}H NMR and ^{13}C NMR, Ta2 bles 1 and 2) with those reported data and 2D2NMR spectrum to be forsythoside B[5], arenarioside[6], acte2 oside[7,8] and isoacteoside[9], respectively1

Table 1 ^{1}H NMR data of compounds 1 2 4[a] (500 MHz in MeOD)

Position of aglycone	1 ^{1}H	2 ^{1}H[b]	3 ^{1}H	4 ^{1}H[b]
1	-	-	-	-
2	6170 (d, 210)	6161 (d, 210)	6171 (d, 210)	6168 (d, 210)
3	-	-	-	-
4	-	-	-	-
5	6170 (d, 810)	6173 (d, 810)	6173 (d, 810)	6165 (d, 810)
6	6158 (dd, 210, 810)	6147 (dd, 210, 810)	6157 (dd, 210, 810)	6153 (dd, 210, 810)
A	3171 (m), 4108 (m)	3163 (m); 3190 (m)	3170 (m); 4102 (m)	3170 (m); 4102 (m)
B	2182 (dt, 217, 710)	2168 (m, 2H)	2177 (m, 2H)	2177 (m, 2H)
caffeoyl moiety				
1c	-	-	-	-
2c	7107 (d, 210)	6196 (d, 210)	7107 (d, 210)	7104 (d, 210)
3c	-	-	-	-
4c	-	-	-	-
5c	6179 (d, 810)	6169 (d, 810)	6180 (d, 810)	6178 (d, 810)
6c	6197 (dd, 210, 810)	6186 (dd, 210, 810)	6196 (dd, 210, 810)	6188 (dd, 210, 810)
Ac	6129 (d, 1519)	6119 (d, 1518)	6129 (d, 1518)	6129 (d, 1518)
Bc	7161 (d, 1519)	7151 (d, 1518)	7161 (d, 1518)	7156 (d, 1518)
Cc	-	-	-	-
glucose				
1	4137 (d, 717)	4127 (d, 719)	4138 (d, 719)	4134 (d, 718)
2		3131 (t, 812)	3153[c]	3136[c]
3		3171 (m)	[c]	3160 (m)
4	4197	4190 (t, 915)	4190 (m)	[c]
5		3163 (m)	3153[c]	3136[c]
6		3149 (m); 3177 (m)	3156[c]; 3170[c]	4138 (m); 4151 (m)
rhamnose				
1	5117 (d, 113)	5107 (br s)	5119 (br s)	5119 (br s)
2		3183 (dd, 118, 310)	[c]	[c]
3		3149 (m)	[c]	[c]
4		3121 (t, 916)	3132[c]	3145[c]
5		3149 (m)	[c]	[c]
6	1109 (d, 611)	0198 (d, 612, 3H)	1110 (d, 612, 3H)	1127 (d, 612, 3H)
apiose		xyl		
1	4191 (d, 212)	4114 (d, 715)		
2	3168 (d, 212)	3113 (dd, 716, 819)		
3	-	3123 (t, 819)		
4	3160231 56	3137 (m)		
5	3131	3106 (t, 1019); 3174 (m)		

a ^{1}H NMR spectra were obtained at 500 MHz and recorded in MeOD at room temperature1

b Coupling constants are presented in Hertz1 Unless otherwise indicated, all proton signals integrate to 1H1

c The proton signals overlapped severely1

Tabele 2 ^{13}C NMR data of compounds 1 2 4 (125 MHz in MeOD)

Position	1	2	3	4
of aglycone	^{13}C	^{13}C	^{13}C	^{13}C
1	131.4	131.4	131.4	131.3
2	115.3	117.1	117.1	117.1
3	146.0	145.9	145.9	145.9
4	144.5	144.5	144.4	144.4
5	116.3	116.3	116.4	116.4
6	121.3	121.3	121.3	121.3
A	72.2	72.3	72.3	72.2
B	36.5	36.4	36.3	36.4
caffeoyl moiety				
1c	127.6	127.5	127.6	127.6
2c	114.7	115.3	115.6	115.3
3c	146.7	146.6	146.5	146.5
4c	149.6	149.7	149.5	149.4
5c	116.5	116.5	116.5	116.5
6c	123.2	123.3	123.2	123.2
Ac	117.1	114.5	114.6	114.7
Bc	148.0	148.2	148.1	147.2
Cc	168.2	168.4	168.3	169.7
glucose				
1	104.1	104.0	103.9	104.1
2	81.6	75.9	76.0	75.5
3	76.0	81.6	81.7	83.8
4	70.4	70.3	70.4	70.2
5	74.4	74.6	75.7	75.1
6	68.3	69.2	62.2	64.5
rhamnose				
1	103.0	102.9	102.9	102.5
2	72.2	72.2	72.3	72.1
3	72.0	71.9	71.9	72.1
4	73.7	73.6	73.7	73.8
5	70.8	70.3	70.3	69.9
6	18.4	18.4	18.4	17.8
apiose		xyl		
1	110.9	105.1		
2	78.0	74.7		
3	80.6	77.4		
4	75.0	71.0		
5	65.6	66.7		

5 Discussion

Caffeoyl phenylethanoid glycosides have been found in the families of Scrophulariaceae, Oleaceae, Plantaginaceae, Lamiaceae and Orobanchaceae, and the genera Clerodendron, Lantana, Petrea and Verbena of the family Verbenaceae[10]. Till 2005, Koo et al[11] isolated phenylethanoid glycosides from Callicarpa dichotoma and reported their neuroprotective activities and anti-amnesic activity of acteoside[12]. Our work is the first report about the isolation of caffeoyl phenylethanoid glycosides from the plant C. pedunculata.

References

[1] Jiangsu New Medicinal College. Dictionnary of Chinese Materia Medica [M]. Shanghai: Shanghai Scientific and Technological Press, 1977: 2346.

[2] Hu YM, Shen YM, Gu QX, et al. Studies on chemical constituents of Callicarpa pedunculata [J]. Chin Tradit Herb Drugs, 2001, 32(12): 1063-1065.

[3] Hu YM, Shen YM, Gan FY, et al. Four diterpenes from Callicarpa pedunculata [J]. Biochem Syst Ecol, 2002, 30: 999-1001.

[4] Liu HY, He HP, Gao S, et al. Two new diterpenoids from Callicarpa pedunculata [J]. Helvetica Chimica Acta, 2006, 89 (5): 1017-1022.

[5] Endo K, Takahashi K, Abe T, et al. Structure of forsythoside B, an antibacterial principle of Forsythia koreana stems [J]. Heterocycles, 1982, 19(2): 261-264.

[6] Andary C, Privat G, Wylde R, et al. Phelipo side et arenario side, deux nouveaux esters heterosidiques de l'acide caféique isoles de orobanche arenaria [J]. J Nat Prod, 1985, 48(5): 778-783.

[7] Andary C, Wylde R, Laffite C, et al. Structures of verbascoside and orobanchoside, caffeic and suger esters from orobanche rapum-genistae [J]. Phytochemistry, 1982, 21(5): 1123-1127.

[8] Pettit GR, Numata A, Takemura T, et al. Antineoplastic agents, 107. Isolation of acteoside and isoacteoside from Castilleja linariaefolia [J]. J Nat Prod, 1990, 53(2): 456-458.

[9] Miyase T, Koizumi A, Ueno A, et al. Studies on the acyl glycoside from Leucosceptrum japonicum (Miq.) Kitamura et Murata [J]. Chem Pharm Bull, 1982, 30: 2732-2737.

[10] Jiménez C, Riguera R. Phenylethanoid glycosides in plants: structure and biological activity [J]. Nat Prod Rep, 1994, 11 (6): 591-606.

[11] Koo KA, Sung SH, Park JH, et al. In vitro neuroprotective activities of phenylethanoid glycosides from Callicarpa dichotoma [J]. Planta Med, 2005, 71(8): 778-780.

[12] Lee KY, Jeong EJ, Lee HS, et al. Acteoside of Callicarpa dichotoma attenuates scopolamine-induced memory impairments [J]. Biol Pharm Bull, 2006, 29(1): 71-74.

紫珠的水溶性成分

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=摘　要> 目的: 研究中药紫珠的水溶性成分。方法: 运用多种色谱技术进行分离纯化, 运用光谱技术鉴定化合物结构。结果: 从紫珠的水溶性提取物中分离到 4个苯丙素苷类化合物, 分别鉴定为连翘苷 (forsythoside B, 1), arenarioside (2), 类叶升麻苷 (acteoside, 3)和异类叶升麻苷 (isoacteoside, 4)。结论: 这 4个化合物都是首次从该植物中分离得到。

=关键词> 紫珠; 苯丙素苷; Forsythoside B; Arenarioside; Acteoside; Isoacteoside

=基金项目> 国家杰出青年基金 (No. 30325044)