· 化学成分 ·

Caffeoylquinic acid derivatives from *Bidens parviflora* and their antihistamine release activites

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Abstract: Objective Based on the activities of antihistamine release to study the compounds from *Bidens parvi-flora* and find biological active compounds. **Methods** The chemical constituents from *B. parviflora* were isolated by silica gel and Sphadex LH-20 column chromatographies and purified by preparative HPLC. The chemical structures had been identified by physiochemical properties and spectroscopic methods. **Results** Six caffeoylquinic acid derivatives were identified as 3, 5-di-*O*-caffeoylquinic acid (Ⅰ), 3, 4-di-*O*-caffeoylquinic acid (Ⅱ), 4, 5-di-*O*-caffeoylquinic acid (Ⅲ), 4-*O*-caffeoylquinic acid (Ⅳ), 5-*O*-caffeoylquinic acid (Ⅴ), 4-[3-(3, 4-dihydroxy-phenyl)-acryloyloxy]-2, 3-dihydroxy-2-methyl-butyric acid (Ⅵ). **Conclusion** Compounds Ⅰ — Ⅵ are first obtained from *B. parviflora* and Ⅵ is new one. Some of the compounds exhibit the activities in antiallergic assays. Moreover, the structure-activity relationships of these compounds have been also discussed in this paper.

Key words: *Bidens parviflora* Willd.; caffeoyl quinic acid; anti-histamine release

小花鬼针草中咖啡酰奎宁酸类成分及其抑制组胺释放活性

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摘 要: 目的 研究小花鬼针草 *Bidens parviflora* 全株的化学成分, 并通过抑制组胺释放活性方法寻找生物活性化合物。**方法** 采用硅胶、Sephadex LH-20 和 ODS 柱色谱分离化合物, 运用 1D NMR, 2D NMR 等波谱法鉴定了化学结构, 通过组胺抑制实验探讨抗炎活性。**结果** 分离鉴定 6 种咖啡酰奎宁酸类化合物及其甲酯, 分别是 3,5-二氧咖啡酰奎宁酸(3, 5-di-*O*-caffeoylquinic acid, Ⅰ)、3,4-二氧咖啡酰奎宁酸(3, 4-di-*O*-caffeoylquinic acid, Ⅱ)、4,5-二氧咖啡酰奎宁酸(4, 5-di-*O*-caffeoylquinic acid, Ⅲ)、4-氧-咖啡酰奎宁酸(4-*O*-caffeoylquinic acid, Ⅳ)、5-氧-咖啡酰奎宁酸(5-*O*-caffeoylquinic acid, Ⅴ)、4-[3-(3,4-二羟基苯基)-丙烯酰氧基]-2,3-二羟基-2-甲基-丁酸{4-[3-(3, 4-dihydroxy-phenyl)-acryloyloxy]-2, 3-dihydroxy-2-methyl-butyric acid, Ⅵ}。**结论** 所有化合物均为首次从该植物中分得, 化合物Ⅵ为新化合物。这些化合物显示一定的抑制组织胺释放活性。

关键词: 小花鬼针草; 咖啡酰奎宁酸; 组胺抑制活性

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In our screening of bioactivity on Compositae plants, the 60% EtOH extracts of *Bidens parviflora* Willd. have been shown to have higher activities against histamine release from rat mast cells induced by Compound 48/80. For the purpose of hinding biological active compounds, the chemical constituents of this plant have been further studied. During our previous studies, five polyacetylene glucosides[1], three sucrose coumaroyl esters, and one neolignan, which were thought to contribute to the inhibition of histamine release, have been reported[2]. In this paper the six caffeoyl quinic acid derivatives were identified as 3, 5-di-*O*-caffeoylquinic acid (Ⅰ), 3, 4-di-*O*-caffeoylquinic acid (Ⅱ), 4, 5-di-*O*-caffeoylquinic acid (Ⅲ), 4-*O*-caffeoylquinic acid (Ⅳ), 5-*O*-caffeoylquinic acid

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(Ⅴ), 4-[3-(3, 4-dihydroxy-phenyl)-acryloyloxy]-2, 3-dihydroxy-2-methyl-butyric acid (Ⅵ) which were first isolated from the whole plant of *B. parviflora*. Their structures were esbablished on the basis of spectral data (UV, FAB-MS, ^{1}H-NMR, ^{13}C-NMR, and NOE). The further biological test assessed their antiallergic activities on different models, including inhibition on nitric oxide (NO) production in LPS and IFN-γ activated murine macrophages (RAW 264.7), histamine release from rat mast cell stimulated by antigen-antibody reaction and reduction of 2, 2′-diphenyl-β-picrylhydrazyl (DPPH) radical.

1 Apparatus and materials

Plant materials: The whole plant of *B. parviflora* was collected at Da-Hei-Shan Country of Liaoning Province in China, in July, 1999 and was identified by Prof. Weichun Wu (Department of Medicinal Plants, Shenyang Pharmaceutical University, China). A voucher specimen is deposited in the Department of Natural Products Chemistry of Shenyang Plarmaceutical University.

2 Extraction and isolation

The air-dried whole plant (5.5 kg) was extracted twice with 60% ethanol (ten each) under reflux for 1 h. The extract was dissolved and partitioned with hexane, ethyl acetate, and *n*-butanol, respectively, then the butanol phase under reduced pressure below 40 ℃, yielded butanol extract (176 g). The crude fractions were tested for anti-histamine release activity. The *n*-butanol extract was subjected to silica gel column chromatography (SiO_2, 500 g, eluted with $CHCl_3$ and MeOH in increasing polarity to obtain 12 frs. The fr.7 (2.45 g) was applied to a Sphadex LH-20 column eluted with 50% MeOH to obtain frs. 3—6 and was purified by preparative HPLC (Sunshu park, PEGASIL ODS, 25% MeOH) to give compounds 24 mg of Ⅰ, 45 mg of Ⅱ, 16 mg of Ⅲ, 18 mg of Ⅳ, 20 mg of Ⅴ, and 13 mg of Ⅵ. The extraction and isolation of *B. parviflora* were fini-shed in 1999 at College of Pharmacy, Nihon University.

3 Antiallergic assays

3.1 Reduction of DPPH radical: Reduction of radical was determined according to Cavin, *et al*[3]. The mixture contained 0.3 mL of 1.0 mmol/L DPPH redical solution, 2.4 mL of 99% ethanol, and 0.3 mL of sample solution. The solution was rapidly mixed and the scavenging capacity was measured electrophotometrically by monitoring, the decrease in absorbance at 517 nm was determined after 10 min and the scavenging activity was calculated as percentage of the radical reduction. Quercetin 3-*O*-glucoside was used as a reference compound.

3.2 Inhibitory activity on histamine release: All isolated compounds were assayed using the HPLC-fluorometry method as previously described with modifications[4,5]. Male Wistar rats (Japan SLC, Shizuoka) weighting 180—200 g were exsanguinated and ip injected with 10 mL of Tyrode solution. The abdominal region was gently massaged for 3 min and then the peritoneal exudate was collected. The peritoneal cavity fluid containing mast cells was suspended in phosphate-buffered saline (PBS), then layered on BSA (d=1.068) in a test tube at room temperature for 20 min. After centrifugation at 300×g and 4 ℃ for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 3 mL of PBS (pH 7.0) and suspended in the medium. Cell viability was determined using trypan blue. Mast cells (1.0×10^6 cells/mL) were pre-incubated with test samples (10 μL) at 37 ℃ for 10 min, followed by addition of histamine releasers, either Compound 48/80 (5 μg/mL) or anti-DNP-IgE (5 μg/mL) and phosphatidyl serine (100 μg/mL). The mixtures were incubated again for 10 min, the quantity of histamine released was expressed in peak height and inhibitory percentage was calculated.

3.3 Inhibitory effect on NO production by activated macrophages[6]: The cells were seeded at 1.2×10^6 cells/mL onto a 35 mm Petri dish and incubated at 37 ℃ for 2 h. Then, test compounds were added to the culture simultaneously with both 100 ng/mL LPS and 10 U/mL INF-γ, and the cells were incubated at 37 ℃ usually for 8 h. After incubation, the cells were chilled on ice, scraped

from the dish with a cell scraper, collected in a microfuge tube and then immediately centrifuged at 7 000 r/min at 4 ℃. A 7 000 μL volume of the supernatant was placed in a new micro-fuge tube stored at 4 ℃ before assaying for nitrite (NO_2^-). The cells were washed twice with PBS without divalent cations by repeated centrifugation at 7 000 r/min. The finalcell pellet was extracted with 40 ℃ of a lysis buffer, comprising 1% Triton X-100, 0.1 mmol/L DETA, and 1% Aprotinin (Sigma) in 20 mmol/L HEPES NaOH buffer, pH 7.5, at 4 ℃ for 30 min. The cell lysate was centrifuged at 10 000 r/min at 4 ℃ for 1 min, and 30 μL of the resultant supernatant was placed in a new microfuge tube. The final cell extract was stored at −80 ℃ until use.

4 Results and discussion

The extraction and separation were carried out as described in the experimental section.

Compound Ⅰ: yellow oil, $[\alpha]_D^{25}$ − 287.6° (c=0.50, MeOH), UV λ_{max}^{MeOH} nm (lgε): 332 (4.656), 286 sh, 245 (4.383). IRν_{max}^{KBr} (cm^{-1}): 3 386, 1 689, 1 600, 1 521, 1 446, 1 274, 1 170, 1 116. FAB-MS *m/z*: 531 $[M+H]^+$, 163 (100); HR-FAB-MS found 531.334 67, calcd for $C_{26}H_{27}O_{12}$ ($[M+H]^+$ 531.480 17). From ^{1}H-NMR spectra (Table 1), quinic acid moiety was observed between δ 4−5.5. Of the double doublet signals, the signal that appeared at the lowest frequency region was assigned to H-4 (δ 3.97), which was bonded to the sp^3 carbon that was also connected to a hydroxyl group. The other two signals appeared at an analogous frequency region because H-3 and H-5 had different stereochemical configuration (*ax* or *eq*) given by the chirality of Ⅰ, they were able to be distingushed by their coupling patterns. The broad signal due to 1, 2-axial coupling was assigned to H-30 (δ 5.43) and the other signal without axial coupling was assigned to H-5 (δ 5.39). The location of caffeoyl substitution on the quinic acid moiety was deduced from the HMBC (Table 1 and 2), thus indicating esterification at C-3 and C-5[7,8]. Meanwhile, the full assignments of all signals in the ^{13}C-NMR spectrum were performed by 2D NMR spectroscopy and compound Ⅰ was confirmed as 3, 5-*O*-dicaffeoylquinic acids.

Compound Ⅱ: yellow oil, $[\alpha]_D^{25}$ − 297.1° (c=0.50, MeOH), UV λ_{max}^{MeOH} nm (lgε): 334 (4.629), 286 sh, 245 (4.353). IRν_{max}^{KBr} (cm^{-1}): 3 386, 1 692, 1 600, 1 521, 1 446, 1 170, 1 116. FAB-MS *m/z*: 531 $[M+H]^+$, 163 (100); HR-FAB-MS found 531.333 06, calcd for $C_{26}H_{27}O_{12}$ ($[M+H]^+$ 531.480 17). The protons H-3 and H-4 were shifted downfield at about δ 5.63 and 5.12, respectively, thus indicating esterification at C-3 and C-4. Compound Ⅱ was identified as 3, 4-di-*O*-caffeylquinic acid by ^{1}H-NMR and ^{13}C-NMR data (Table 1 and 2).

Compound Ⅲ: yellow oil, $[\alpha]_D^{25}$ − 252.9° (c=0.52, MeOH), UV λ_{max}^{MeOH} nm (lgε): 332 (4.650), 286 sh, 245 (4.378). IRν_{max}^{KBr} (cm^{-1}): 3 386, 1 692, 1 600, 1 521, 1 446, 1 274, 1 170, 1 096. FAB-MS *m/z*: 531 $[M+H]^+$, 163 (100); HR-FAB-MS found 531.356 07, calcd for $C_{26}H_{27}O_{12}$ ($[M+H]^+$ 531.480 17). From ^{1}H-NMR spectra (Table 1), two caffeoyl residues esterified at position C-4 and C-5 of quinic acid were deduced. The protons H-4 and H-5 were shifted downfield at about δ 5.04 and 5.61, respectively, thus indicating esterification at C-4 and C-5. Further evidence for 4, 5-di-*O*-caffeoylquinic acid[8] was provided by its ^{13}C-NMR spectra (Table 2).

Compound Ⅳ: yellow oil, $[\alpha]_D^{25}$ − 318° (c=0.15, MeOH), UV λ_{max}^{MeOH} nm (lgε): 328 (4.56), 265 (3.95), 218 (4.46). IRν_{max}^{KBr} (cm^{-1}): 3 376, 1 686, 1 624, 1 526, 1 450, 1 289, 1 260, 1 169. FAB-MS *m/z*: 355 $[M+H]^+$, HR-FAB-MS found 355.186 81, calcd for $C_{16}H_{19}O_9$ 355.310 22 ($[M+H]^+$). From ^{1}H-NMR spectra (Table 1), one caffeoyl residue esterified at position C-4 of quinic acid was deduced. The proton H-4 was shifted downfield at about δ 5.12, thus indicating esterification at C-4. Further evidence for 4-*O*-caffeoylquinic acid was provided by its ^{13}C-NMR spectra (Table 2).

Table 1 ^{1}H-NMR Data of dicaffeoylquinic acid derivatives

Position	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ
Quinic acid moiety						
2 ex	2.23 dd (12.9,4.2)	2.32 dd (13.7,3.7)	2.18 dd (14.4,4.7)	2.26 dd (14.7,4.3)	2.31 dd (13.4,4.2)	
2 ax	2.21 dd (12.8,4.3)	2.16 dd (13.7,4.3)	2.20 dd (14.4,5.5)	2.21 dd (14.7,4.5)	2.25 dd (13.4,5.7)	
3 ex	5.43 ddd (4.3,2.2,4.0)	5.63 ddd (4.3,3.7,3.8)	4.32 ddd (5.5,4.7,4.3)	4.30 ddd (4.5,4.3,3.9)	5.55 ddd (5.7,4.2,3.4)	4.03 t (6.1)
4 ex	3.97 dd (7.5,4.0)	5.12 dd (7.5,3.8)	5.04 dd (10.4,4.3)	5.12 dd (7.8,3.9)	4.12 dd (7.5,3.4)	4.43,4.14 t (6.1)
5 ax	5.39 ddd (10.9,4.6,7.5)	4.37 ddd (10.3,4.5,7.5)	5.61 ddd (10.9,4.6,10.4)	4.39 ddd (10.1,4.6,7.8)	4.30 ddd (10.2,3.9,7.5)	1.45 s
6 ex	2.33 dd (14.1,4.6)	2.26 dd (14.7,4.5)	2.35 dd (13.9,4.6)	2.34 dd (16.2,4.6)	2.33 dd (13.8,3.9)	
6 ax	2.15 dd (14.1,10.9)	2.18 dd (14.7,10.3)	2.11 dd (13.9,10.9)	2.14 dd (16.2,10.1)	2.09 dd (13.9,10.2)	
Caffeoyl moiety						
2	4.06 d (1.9)	7.06 d (1.9)	7.0 d (2.2)	7.01 d (2.2)	7.01 d (2.2)	7.04 d (2.2)
5	6.76 d (8.2)	6.75 d (7.9)	6.79 d (8.1)		6.75 d (8.2)	6.77 d (8.1)
6	6.95 dd (8.2,1.9)	6.92 dd (7.9,1.9)	6.87 dd (8.1,2.2)	6.98 dd (8.1,2.2)	6.92 dd (8.2,1.9)	6.95 dd (8.1,2.2)
7	7.56 d (16.1)	7.59 d (15.9)	7.54 d (15.9)	7.62 d (15.9)	7.60 d (15.9)	7.55 d (15.9)
8	6.62 d (16.1)	6.28 d (15.9)	6.25 d (15.9)	6.26 d (15.9)	6.30 d (15.9)	6.23 d (15.9)
2′	7.06 d (1.9)	7.00 d (1.9)	7.02 d (2.2)			
5′	6.79 d (8.2)	6.74 d (7.9)	6.79 d (8.1)			
6′	6.79 dd (8.2,1.9)	6.90 dd (7.9,1.9)	6.90 dd (8.1,2.2)			
7′	7.62 d (16.1)	7.52 d (15.9)	7.55 d (15.9)			
8′	6.32 d (16.1)	6.19 d (15.9)	6.25 d (15.9)			

a-assigned by ^{1}H-^{1}HCOSY, HMQC, and HMBC spectra b-500 MHz, TMS as internal standard, (in MeOH-d_4)

Table 2 ^{13}C-NMR Data of dicaffeoylquinic acid derivatives

Position	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ
Quinic acid						
1	74.8 s	76.1 s	75.2 s	75.3 s	75.8 s	76.8 d
2	37.8 t	38.4 t	36.9 t	38.0 t	38.1 t	74.5 d
3	72.2 d	72.3 d	66.1 d	67.2 d	72.1 d	66.1 t
4	70.8 d	75.7 d	69.9 d	73.9 d	70.9 d	23.2 q
5	72.6 d	69.4 d	69.9 d	68.6 d	67.9 d	
6	36.1 t	39.4 t	41.4 t	35.2 t	37.8 t	
-COO-	175.4 s	175.7 s	175.2 s	175.2 s	175.5 s	178.4 s
Caffeoyl						
1	127.8 s	127.7 s	127.7 s	127.7 s	127.7 s	127.8 s
2	115.2 d	114.7 d	114.9 d	115.2 d	115.1 d	115.2 d
3	146.8 s	146.8 s	146.8 s	146.8 s	146.9 s	146.8 s
4	149.5 s	149.7 s	149.6 s	149.7 s	149.7 s	149.7 s
5	116.5 d	116.5 d	116.5 d	116.5 d	116.6 d	116.5 d
6	123.0 d	123.2 d	123.1 d	123.0 d	123.0 d	123.2 d
7	147.1 d	147.3 d	147.3 d	147.1 d	147.2 d	147.3 d
8	115.3 d	115.2 d	115.1 d	115.1 d	115.0 d	115.2 d
9	168.4 s	168.6 s	168.5 s	168.3 s	168.3 s	168.6 s
1′	127.9 s	127.6 s	127.8 s			
2′	115.2 d	115.2 d	115.0 d			
3′	146.8 s	146.8 s	146.9 s			
4′	149.6 s	149.7 s	149.7 s			
5′	116.5 d	116.5 d	116.5 d			
6′	123.1 d	123.1 d	123.3 d			
7′	147.3 d	147.3 d	147.4 d			
8′	115.7 d	115.2 d	115.2 d			
9′	168.9 s	168.3 s	168.6 s			

a-assigned by ^{1}H-^{1}HCOSY, HMQC, and HMBC spectra.

b-500 MHz, TMS as internal standard, (in MeOH-d_4)

Compound Ⅴ: yellow oil, $[\alpha]_D^{25}$ − 228° (c=0.25, MeOH), UV λ_{max}^{MeOH} (nm) (lgε): 326 (4.36), 264 (3.75), 218 (4.46). IRν_{max}^{KBr}(cm^{-1}): 3 376, 1 685, 1 619, 1 526, 1 450, 1 289, 1 260, 1 169. FAB-MS m/z: 355 $[M+H]^+$, HR-FAB-MS found 355.276 78, calcd for $C_{16}H_{19}O_9$ 355.310 22 ($[M+H]^+$). From ^{1}H-NMR spectra (Table 1), one caffeoyl residue esterified at position C-3 of quinic acid was deduced. The proton H-3 was shifted downfield at about δ 5.55, thus indicating esterification at C-5. Further evidence for 5-*O*-caffeoylquinic acid was provided by its ^{13}C-NMR spectra (Table 2).

Compound Ⅵ: yellow oil, $[\alpha]_D^{25}$ − 569° (c=0.12, MeOH), UV λ_{max}^{MeOH} nm (lgε): 327 (4.05), 266 (3.47), 204 (3.95). IRν_{max}^{KBr}(cm^{-1}): 3 404, 2 956, 1 734, 1 697, 1 631, 1 602, 1 522, 1 450. EI-MS m/z: 326 $[M]^+$; HR-FAB-MS found 312.260 15, calcd for $C_{14}H_{17}O_8$ ($[M]^+$) 312.267 69. ^{1}H-NMR (CD_3OD, TMS) δ: 4.03 (1H, d, J=6.1 Hz, H-3), 4.43 (1H, t, J=6.1 Hz, H-4ex), 4.14 (1H, t, J=6.1 Hz, H-4ax). Structure is seen in Fig. 1.

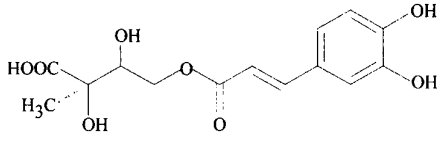

Fig. 1 Structure of compound Ⅵ

The ^{13}C-NMR and DEPT spectra of Ⅵ displayed five carbon signals which comprised of one

carboxylic carbon or ester (δ_C 178.4), an oxygen-bearing methane (δ_C 74.5), an oxygen-bearing quaternary carbon (δ_C 76.8), one oxymethylene (δ_C 66.1), and one methyl group (δ_C 23.2). From ^{1}H-NMR and COSY experiments, H-4 (δ_H 4.43), H-4 (δ_H 4.14) and H-3 (δ_H 4.03) showed an AMX-system, which suggested that C-3 (δ_C 74.5) was connected to C-4 (δ_C 66.1). The carbons C-1 (δ_C 178.4), C-3 (δ_C 74.6), and C-5 (δ_C 23.2) were all linked to C-2 (δ_C 76.8), and H-3 (δ_H 4.03), H-5 (δ_H 1.45) as well as between C-5 (δ_C 23.2) and H-3 (δ_H 4.03) in the HMBC experiment reconfirmed the elucidation of Ⅵ. Thus, compound Ⅵ was established as 4-[3-(3, 4-dihydroxyphenyl)-acryloyloxy]-2, 3-dihydroxy-2-methylbutyric acid.

The above compounds, the methyl signals observed at δ 3.80 in ^{1}H-NMR and δ 53.1 in ^{13}C-NMR spectra, were unequivocally associated with an ester rather than an aromatic ether, otherwise both signals would be shifted downfield[9].

Under the bioassay-guided isolation, six compounds from *B. parviflora* were extracted and purified, whose bioassay activities are shown in Table 3.

Table 3 Antiallergic activities of caffeoylquinic acid derivatives

Compounds	DPPH* Inhibition IC_{50} ($\mu g \cdot mL^{-1}$)	Hise△ Inhibition IC_{50} ($\mu g \cdot mL^{-1}$)	NO# Inhibition IC_{50} ($\mu g \cdot mL^{-1}$)
Ⅰ	16.8	188	300
Ⅱ	16.5	>300	>300
Ⅲ	17.4	44.6	136
Ⅳ	26.9	47.5	172
Ⅴ	37.4	56.7	88
Ⅵ	8.1	>300	>300
Indomethacin^a		88.6	
Q^a	27.8		

* DPPH-reduction scavenging of 2, 2-diphenyl-β-picrylhy drazyl radical inhibitory activity; △His-histamine release inhibitory activity; # NO-nitric oxide; Indomethacin^a-a medicine to diminish inflammation for histamine release inhibitory activity; Q^a-quercetin 3-*O*-glucoside, a medicine to diminish inflammation for DPPH inhibitory activity

These results suggest the dicaffeoyl substitution of quinic acid derivatives whichever 3, 4- or 3, 5- or 4, 3- substitute enhance their activities compared to mono-caffeoylquinic acid derivatives in both of the histamine and DPPH models. However, in contrast, the existence of quinine ring in these compounds was decisive to retain their inhibitory effect on NO production. The methyl ester of the carboxyl group in quinic derivatives would improve the ability of DPPH, inhibition of histamine release, and NO production of these compounds.

In summary, the caffeoylquinic acid derivatives have great prospects in further development of antioxidant and antiinflammatory drugs, and to remove relative diseases.

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