Collagenase Inhibitory Quinic Acid Esters from *Ipomoea pes-caprae*

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Two new quinic acid esters (**1** and **2**) were isolated from the extract of *Ipomoea pes-caprae* together with six known quinic acid esters (**3**−**8**). The structures of compounds **1** and **2** were elucidated by spectroscopic data interpretation. These new compounds exhibited collagenase inhibitory activity and showed almost no cytotoxicity.

To maintain the elasticity of the skin it is important to prevent reduction of collagen with collagen decomposition enzyme (collagenase). The discovery and development of compounds with collagenase inhibitory activity is therefore an effective method for preventing aging of the skin. Collagenase is also known to be a member of matrix metalloproteinases (MMPs), which have a crucial role in normal physiological processes such as embryogenesis and wound healing, and aberrant expression of MMPs is associated with many pathological abnormalities such as tumor invasion.[1] As a result of our screening program against collagenase inhibitory activity using extracts of a number of medicinal tropical plants, we selected *Ipomoea pes-caprae* collected in Thailand as an active material for the investigation of its active components. *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae) grows at the seashore, and this plant is used in some parts of the world to treat fatigue, strain, arthritis, and rheumatism.[2] Previous chemical investigations have led to the isolation of several isoprenoids[3] and lipophilic pentasaccharides.[4] Herein, we describe the isolation and structure elucidation of two new quinic acid esters (**1** and **2**), together with isolation of the related caffetannins (**3**−**8**) and their collagenase inhibitory activities.

The leaves of *I. pes-caprae* were extracted with MeOH, and the extract was separated by solvent partitioning to afford hexane-, EtOAc-, *n*-BuOH-, and water-soluble fractions. The EtOAc-soluble fraction, which was the most active in the collagenase inhibitory activity test, was subjected to Sephadex LH-20 column chromatography, followed by purification by reversed-phase HPLC on ODS to give two new quinic acid esters (**1** and **2**), together with six known caffetannins (**3**−**8**); the known compounds were identified by comparison with reported spectroscopic data.[5−9] The methyl esters (**4**, **6**, and **8**) could be formed during extraction by using methanol.

Compound **1**, obtained as an amorphous solid, was shown to have the molecular formula $C_{34}H_{30}O_{14}$ from its HRFABMS data (*m/z* 663.1695, $[M + H]^+$, Δ −1.9 mmu). The UV spectrum of **1** showed absorption maxima at 323, 330 (sh), and 232 nm, which were similar to those of **3**−**8** having caffeic acid ester moiety. In turn, the IR absorption bands observed at 3410, 1700, and 1630 cm^{-1} implied the presence of hydroxyl and conjugated carbonyl groups. The ^1H NMR spectrum of **1** in CD_3OD (Table 1) showed signals for six doublets with coupling constants of 15.9 Hz due to the trans olefinic protons of H-2′/H-3′, H-2″/H-3″, and H-2‴/H-3‴. In the aromatic proton region, signals for two ABX systems [δ_H 7.05 (d, J = 2.1 Hz), 6.76 (d, J = 7.9 Hz), and 6.94 (dd, J = 7.9 and 2.1 Hz); δ_H 7.01 (d, J = 1.9 Hz), 6.75 (d, J = 7.8 Hz), and 6.92 (dd, J = 7.8 and 1.9 Hz)] and one A_2B_2 system [δ_H 7.36 (2H d, J = 8.6 Hz) and 6.72 (2H, d, J = 8.6 Hz)] were observed and were assigned to two 1,3,4-trisubstituted aromatic units and one 1,4-disubstituted benzene ring moiety. From these observations, along with the analysis of the ^{13}C NMR spectroscopic data (Table 1), two caffeic acid units and one *p*-coumaroic acid moiety were inferred to be present in the molecule of **1**. The presence of a quinic acid moiety was suggested by the characteristic ^{13}C NMR signals due to three oxymethines (δ_C 69.1, 70.0, and 74.7), two sp^3 methylenes (δ_C 36.6 and 38.6), one oxygenated quaternary carbon (δ_C 74.7), and one carboxyl carbon (δ_C 177.1), as well as the ^1H NMR signals of three oxymethine protons [one equatorial (H-3) and two axial (H-4 and H-5)] and two pairs of sp^3 methylene protons (H_2-2 and H_2-6) as shown in Table 1. These were assignable according to their multiplicity and their spin−spin coupling constants ($J_{3,4}$ = 3.5 Hz and $J_{4,5}$ = 8.3 Hz). These assignments were further supported by the analysis of the ^1H-^1H COSY (H_2-2/H-3, H-3/H-4, H-4/H-5, and H-5/H_2-6) and HMBC spectra (H_2-2/C-1, C-3, C-4, and C-7; H-3/C-1, C-2, and C-4; H-4/C-3, C-5, and C-6; H-5/C-1, C-3, C-4, and C-6; H_2-6/C-1, C-2, C-4, C-5, and C-7) of **1**. The low-field resonances of three oxymethine protons at H-3 (δ_H 5.67), H-4 (δ_H 5.32), and H-5 (δ_H 5.67) implied that two caffeoyl ester units and one coumaroyl ester group are attached at these positions, and the location of the coumaroyl group on C-4 was deduced by the HMBC correlation from H-4 to coumaroyl ester carbonyl carbon (C-1″, δ_C 168.0). This C-1″ carbon was discriminated from two caffeoyl ester carbonyl carbons (C-1′ and C-1‴) since the C-1″ carbon showed an HMBC correlation with H-3″ (δ_H 7.57), and this H-3″ proton in turn showed HMBC cross-peaks to C-4″ (δ_C 127.0) and C-5″ (C-9″) (δ_C 131.4 (2C)), which were assignable to the 1,4-disubstituted benzene ring carbons (*p*-coumaroyl group). From these results, the structure of compound **1** was concluded to be 3,5-di-*O*-caffeoyl-4-*O*-coumaroylquinic acid.

Compound **2** was assigned the molecular formula $C_{43}H_{36}O_{16}$ according to its HRFABMS data [*m/z* 809.2117, $(M + H)^+$, Δ +3.5 mmu], representing one $C_9H_6O_2$ unit

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Table 1. ^1H and ^{13}C NMR Data of Compounds **1** and **2** in CD_3OD

1			**2**		
positions	δ_H/Hz	δ_C	positions	δ_H/Hz	δ_C
1		74.7	1		80.4
2	2.17 dd 14.0, 5.4	36.6	2	2.57 brd 16.0	33.0
	2.45 dd 14.0, 4.0			2.94 dt 16.0, 3.7	
3	5.67 m	70.0	3	5.69 dt 3, 7, 3.3	70.1
4	5.32 dd 8.3, 3.5	74.7	4	5.37 dd 10.7, 3.3	73.6
5	5.67 m	69.1	5	5.87 td 11.1, 2.9	68.3
6 (eq)	2.28 dd 13.6, 3.6	38.6	6 (eq)	2.20 dd 13.0, 2.9	38.4
(ax)	2.34 dd 13.6, 8.7		(ax)	2.75 dd 13.0, 11.1	
7		177.1	7		173.7
caffeoyl (1)[a]			coumaroyl (1)[a]		
1′		168.5	1′		168.1
2′	6.31 d 15.9	115.1	2′	6.38 d 15.9	114.2
3′	7.59 d 15.9	147.7	3′	7.67 d 15.9	147.6
4′		127.8	4′		126.8
5′	7.05 d 2.1	115.2	5′, 9′	7.37 d 8.7 (2H)	131.3 (2C)
6′		146.8	6′, 8′	6.72 d 8.7 (2H)	116.7 (2C)
7′		149.7	7′		161.4
8′	6.76 d 7.9	116.5	caffeoyl (1)[b]		
9′	6.94 dd 7.9, 2.1	123.1	1″		167.7
coumaroyl[b]			2″	6.18 d 15.9	114.9
1″		168.0	3″	7.54 d 15.9	148.0
2″	6.27 d 15.9	114.4	4″		127.6
3″	7.57 d 15.9	147.6	5″	6.88 d 2.0	116.1
4″		127.0	6″		146.7
5″, 9″	7.36 d 8.6 (2H)	131.4 (2C)	7″		149.8
6″, 8″	6.72 d 8.6 (2H)	116.8 (2C)	8″	6.54 d 8.1	116.6
7″		161.4	9″	6.66 dd 8.1, 2.0	122.3
caffeoyl (2)[c]			caffeoyl (2)[c]		
1‴		168.5	1‴		168.5
2‴	6.21 d 15.9	114.6	2‴	6.23 d 15.9	114.7
3‴	7.53 d 15.9	147.6	3‴	7.57 d 15.9	147.8
4‴		127.6	4‴		127.3
5‴	7.01 d 1.9	115.1	5‴	7.00 d 2.0	115.2
6‴		146.8	6‴		146.6
7‴		149.7	7‴		149.5
8‴	6.75 d 7.8	116.4	8‴	6.74 d 8.3	116.5
9‴	6.92 dd 7.8, 1.9	123.2	9‴	6.90 dd 8.3, 2.0	123.2
			coumaroyl (2)[d]		
			1⁗		168.2
			2⁗	6.21 d 16.2	114.4
			3⁗	7.53 d 16.2	147.8
			4⁗		126.9
			5⁗, 9⁗	7.29 d 8.6 (2H)	131.4 (2C)
			6⁗, 8⁗	6.68 d 8.6 (2H)	116.9 (2C)
			7⁗		161.3

[a] Attached on C-3. [b] Attached on C-4. [c] Attached on C-5. [d] Attached on C-1. Signals of the same positions of caffeoyl (1) and caffeoyl (2) as well as those of coumaroyl (1) and coumaroyl (2) may be reversed.

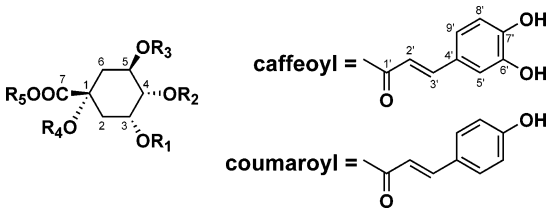
more than **1**. This $C_9H_6O_2$ unit corresponded to a coumaroyl group, and the presence of two caffeoyl and two *p*-coumaroyl residues esterified to quinic acid nucleus was deduced by interpretation of the ^1H and ^{13}C NMR data of **2** (Table 1), aided by its $^1H-^1H$ COSY and HMBC spectroscopic data. The caffeoyl groups were assigned to the hydroxyls at C-4 and C-5, from the HMBC correlations observed from H-4 (δ_H 5.37) to C-1″ (δ_C 167.7) and from H-5 (δ_H 5.87) to C-1‴ (δ_C 168.5). The C-1″ and C-1‴ carbons were correlated by the HMBC spectrum with H-3″ (δ_H 7.54) and H-3‴ (δ_H 7.57), respectively, which in turn showed HMBC connectivities with C-5″ (δ_C 116.1) and C-5‴ (δ_C 115.2), respectively. These C-5″ and C-5‴ carbons bore meta coupled protons, since they showed HMQC correlations with H-5″ (δ_H 6.88, d, J = 2.0 Hz) and H-5‴ (δ_H 7.00, d, J = 2.0 Hz), respectively, and were part of 1,3,4-trisubstituted benzene rings (caffeoyl groups). The H-3 (δ_H 5.69) signal of the quinic acid nucleus showed an HMBC correlation with C-1′, which was assigned to the ester carbonyl of one coumaroyl group [HMBC correlations: H-3′/C-1′ and H-3′/C-5′(C-9′)], thus indicating that one coumaroyl residue was esterified at the C-3 position of quinic acid. Another remaining coumaroyl group was attached on the tertiary hydroxyl group on C-1, since the ^{13}C NMR chemical shift of the C-1 oxygenated quaternary carbon was at a lower value (δ_C 80.4) compared with that of quinic acid derivatives with free OH groups on C-1 such as compound **1** (δ_C 74.7). This fact was previously described for 1-acylquinic acid derivatives (e.g., δ_C 80.7, C-1 of 1,4,5-tri-*O*-caffeoylquinic acid).[10,11] Thus, compound **2** was assigned as 4,5-di-*O*-caffeoyl-1,3-di-*O*-coumaroylquinic acid.

Table 2. Collagenase Inhibitory Activities of Quinic Acid Esters from *I. pes-caprae* (IC_{50} Values, μM)

compounds	IC_{50} (μM)
1	19.1
2	14.2
3	37.2
4	26.6
5	31.7
6	16.2
7	23.6
8	5.8
caffeic acid	82.7
quinic acid	>500

Collagenase inhibition activity of the eight quinic acid esters (**1**−**8**) isolated from *I. pes-capre* was examined, and all these compounds proved to be active with the IC_{50} values shown in Table 2, with the methyl ester **8** found to be the most active. As shown in Table 2, caffeic acid itself showed weak collagenase inhibitory activity (IC_{50}, 82.7 μM),

while quinic acid was inactive (IC_{50}, >500 μM).[12,13] In addition, cytotoxicity of compounds **1**−**4** was examined against Jurkat human T-cell leukemia cells to show that all these compounds were almost noncytotoxic (each IC_{50} value, >35 μM/mL).



	R_1	R_2	R_3	R_4	R_5
1	caffeoyl	coumaroyl	caffeoyl	H	H
2	coumaroyl	caffeoyl	caffeoyl	coumaroyl	H
3	H	caffeoyl	caffeoyl	H	H
4	H	caffeoyl	caffeoyl	H	Me
5	caffeoyl	caffeoyl	H	H	H
6	caffeoyl	caffeoyl	H	H	Me
7	caffeoyl	H	caffeoyl	H	H
8	caffeoyl	H	caffeoyl	H	Me

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained on a Hitachi U-3400 spectrometer, and IR spectra were measured on a JASCO FT-IR 230 spectrophotometer. NMR spectra were recorded on JEOL ECP 600 and A500 spectrometers. HRFABMS was obtained on a JMS HX-110 mass spectrometer. HPLC separations were carried out by using Develosil ODS-UG-5 (10 × 250 mm; flow rate, 2.0 mL/min; detection, UV at 254 nm).

Plant Material. Leaves of *Ipomoea pes-caprae* were collected in Khon Kaen, Thailand, in April 2002 and were identified by T.K. A voucher specimen (6-427) is maintained at the Faculty of Agriculture, Khon Kaen University.

Extraction and Isolation. The air-dried leaves (310 g) were extracted with MeOH, and the MeOH extract (73 g) was partitioned between hexane (800 mL × 3) and 10% aqueous MeOH (800 mL). The aqueous phase was further extracted with EtOAc (800 mL × 3) and *n*-BuOH (800 mL × 2) to give four fractions (hexane phase, 18.1 g; EtOAc phase, 6.1 g; *n*-BuOH phase, 12.1 g; aqueous phase, 35 g). The collagenase inhibition test showed that the EtOAc phase was the most active [inihibition (%) at 100 μg/mL: hexane phase, 55%; EtOAc phase, 90%; *n*-BuOH phase, 82%; aqueous phase, 6%]. The EtOAc phase was subjected therefore to Sephadex LH-20 column chromatography (35 × 550 mm) eluted with 100% MeOH. The fraction (3.7 g) in the 700−1100 mL elution was passed through a Sep-Pak ODS original cartridge (Waters) eluted with 40% CH_3CN, and the eluant was partially (307 mg) purified by HPLC (eluant, 20% CH_3CN with 0.1% trifluoroacetic acid (TFA)) to afford compound **5** (15.4 mg, t_R 28.4 min), compound **7** (86.6 mg, t_R 32.0 min), and compound **3** (32.7 mg, t_R 43.2 min). The fraction (225 mg) of the first Sephadex LH-20 column in the 1100−1400 mL elution was passed through a Sep-Pak ODS original cartridge eluted with 40% CH_3CN, and the eluant (216 mg) was separated by HPLC (eluant, 35% CH_3CN with 0.1% TFA) to afford three fractions: A (58.7 mg, t_R 2.4 min), B (21.2 mg, t_R 14.2 min), and C (55.7 mg, t_R 31.2 min). Fraction A was further purified by HPLC (eluant, 30% CH_3CN with 0.1% TFA) to give compound **3** (19.5 mg, t_R 11.6 min), compound **4** (7.2 mg, t_R 20.4 min), and a crude fraction (23.6 mg, mixture of many peaks) containing several quinic acid esters, which was purified again with HPLC (eluant, 30% CH_3CN with 0.1% TFA) to give compound **5** (2.9 mg, t_R 9.6 min), compound **6** (1.2 mg, t_R 14.4 min), and compound **8** (0.6 mg, t_R 18.4 min). Fraction B (vide supra) was further purified by HPLC (eluant, 30% CH_3CN with 0.1% TFA) to give compound **1** (4.3 mg, t_R 34.0 min), while fraction C (vide supra) was also purified by HPLC (eluant, 35% CH_3CN with 0.1% TFA) to give compound **2** (22.7 mg, t_R 33.2 min).

3,5-Di-*O*-caffeoyl-4-*O*-coumaroylquinic acid (1): amorphous solid; $[\alpha]^{22}_D$ −378° (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 323 (4.9), 300 sh (4.8), 232 (4.7), 219 (4.8) nm; IR (film) ν_{max} 3410, 1700, 1630, 1600, 1520, and 1450 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 663 $(M + H)^+$; HRFABMS *m/z* 663.1695 [calcd for $C_{34}H_{31}O_{14}$, $(M + H)^+$ 663.1714].

4,5-Di-*O*-caffeoyl-1,3-di-*O*-coumaroylquinic acid (2): amorphous solid; $[\alpha]^{22}_D$ −329° (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ϵ) 316 (4.9), 299 sh (4.8), 234 (4.6), 221 (4.7) nm; IR (film) ν_{max} 3390, 1700, 1630, 1600, 1520, and 1450 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 809 $(M + H)^+$; HRFABMS *m/z* 809.2117 [calcd for $C_{43}H_{37}O_{16}$, $(M + H)^+$ 809.2082].

Collagenase Inhibition Activity Test. Collagenase Type V (EC 3.4.21.7), purchased from Sigma-Aldrich (Tokyo, Japan), and the substrate MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2, purchased from Peptide Institute Inc. (Osaka, Japan), were used. Test samples (50 μL, dissolved in 4% MeOH) were added to a well of 96-well microtiter plates. Then, 100 μL of enzyme solution (20 μg/mL) were added to the sample solution, and the sample was preincubated at 37 °C for 10 min. After preincubation, 50 μL of substrate solution (5 μM) was added to the mixture to begin the reaction. The fluorescence values were measured at an excitation of 320 nm and an emission of 405 nm during incubation at 37 °C for 1 h. As a positive control, phosphramidon (Peptide Institute; IC_{50} value, 0.6 μM) was used in all experiments.

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