A New Caffeoyl Quinic Acid from *Aster scaber* and Its Inhibitory Activity against Human Immunodeficiency Virus-1 (HIV-1) Integrase

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The phytochemical study of the aerial parts of *Aster scaber* THUNB. (Asteraceae) yielded a new caffeoyl quinic acid, (−) 3,5-dicaffeoyl-*muco*-quinic acid (2) and three known compounds, (−) 3,5-dicaffeoyl quinic acid (1), (−) 4,5-dicaffeoyl quinic acid (3), (−) 5-caffeoyl quinic acid (4). The structures were established by high resolution spectroscopic methods. The antiviral effects against HIV-1 integrase of the compounds was evaluated. (−) 3,5-Dicaffeoyl-*muco*-quinic acid (2) exhibited potent antiviral activity with an IC_{50} value of 7.0±1.3 μg/ml.

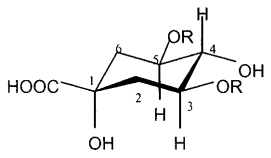
Keywords *Aster scaber*; Asteraceae; caffeoyl quinic acid; (−) 3,5-dicaffeoyl-*muco*-quinic acid; HIV-1 integrase

Aster scaber THUNB. (Asteraceae) is widespread and cultivated as a culinary vegetable in Korea.[1] *Aster* species has also been used in traditional Chinese medicine for treatment of bruises, snakebite, headache and dizziness.[2] A review of the literature of *A. scaber* shows that triterpene glycosides[3,4] and volatile compounds[5] were reported. In the course of our phytochemical studies on *Aster* species, we isolated a new caffeoyl quinic acid (**2**), together with three known caffeoyl quinic acids (**1**, **3**, **4**) from *A. scaber* cultivated in Korea. Quinic acid derivatives exhibited diverse biological activities, especially antiviral[6,7] and antihepatotoxic activities.[8] Most quinic acids are mono-, di-, or triesters of gallic, caffeic, and ferulic acids and are of several positional and stereochemical isomers.[9,10] Because of the structural similarities and complexities of quinic acid derivatives, the assignments of NMR spectra have not been unambiguous. Very recently, Pauli *et al.*[11] tried to assign the ^{1}H-NMR spectra of seven natural quinic acids through high resolution NMR techniques.

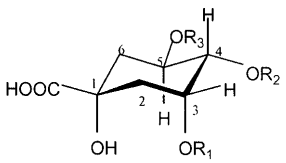
This paper deals with the isolation and structure determination of a new (**2**) and three known quinic acid derivatives (**1**, **3**, **4**), along with their inhibitory activity against human immunodeficiency virus type 1 (HIV-1) integrase.

Three known compounds, 3,5-dicaffeoyl quinic acid (**1**), 4,5-dicaffeoyl quinic acid (**3**) and 5-caffeoyl quinic acid (chlorogenic acid) (**4**) were characterized by comparing their physical and spectroscopic data with those reported in the literatures.[12,13]

Compound **2** was obtained as a yellowish gum ($[\alpha]_D$ −153.8°) and its molecular formula was determined to be $C_{25}H_{24}O_{12}$ by HRFABMS (*m/z* 517.1349, $[M+H]^+$). Its IR spectrum displayed absorption bands at 3300 and 1690 cm^{-1}, indicating the presence of hydroxy and ester groups. The ^{1}H-NMR spectrum of **2** showed very broad and distorted signals (see Experimental) and, therefore, their coupling constants were analyzed through DQF–^{1}H–^{1}H–COSY experiment. The ^{1}H-NMR spectrum showed signals by two methylenes at δ 1.96 (2H, m), 2.11 (1H, m) and 2.14 (1H, m), three oxygenated protons at δ 3.83 (1H, br s), 5.13 (1H, br s), and 5.19 (1H, br s), and five hydroxy protons at δ 5.30 (1H, br s), 9.18 (2H, br s) and 9.57 (2H, br s). The ^{13}C-NMR spectrum showed two methylene carbons at δ 35.9 and 37.0, four oxygenated carbons at δ 68.8, 71.8, 72.0 and 73.7, and a carbonyl carbon signal at δ 176.5. In addition, ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of two *trans*-caffeoyl groups.[10] The above spectral data were consistent with those of dicaffeoyl quinic acid derivatives.[12,13] The relationship of two caffeoyls and quinic acid was established by the analysis of ^{1}H–^{1}H COSY, HMQC and HMBC spectra. In HMBC spectrum, long-range connectivities were observed between H-3 and H-5 protons (δ 5.13 and 5.19) in quinic acid moiety and the caffeoyl ester carbons (δ 166.7 and 167.2). Thus, we speculated the structure of **2** to be 3,5-dicaffeoyl quinic acid; however, NMR data and HPLC retention time of **2** were quite different from those of known 3,5-dicaffeoyl quinic acid (**1**). The retention time of **2** in HPLC analysis was found to be greater than that of **1** (**2**: 35.7 min, **1**: 33.9 min) and when its ^{1}H-NMR spectrum of the quinic acid region was compared with those of **1**, H-3 and H-5 of **2** were shifted upfield, while H-4 was shifted downfield. Their coupling constants also showed differences (**2**: $J_{3,4}$=11 Hz and $J_{4,5}$=16 Hz; **1**: $J_{3,4}$=8 Hz and $J_{4,5}$=15 Hz). The large coupling constants between $J_{2ax,3}$ (14 Hz) and $J_{3,4}$ (11 Hz), and between $J_{4,5}$ (16 Hz) and $J_{5,6ax}$ (15 Hz) indicated the diaxial



2 (R= caffeoyl)



1 (R_1=R_3= caffeoyl, R_2=H)

3 (R_1= H, R_2=R_3= caffeoyl)

4 (R_1=R_2= H, R_3= caffeoyl)

caffeoyl :

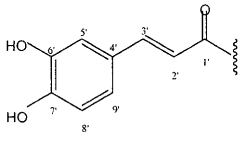


Fig. 1. Structures of Compounds **1**—**4**

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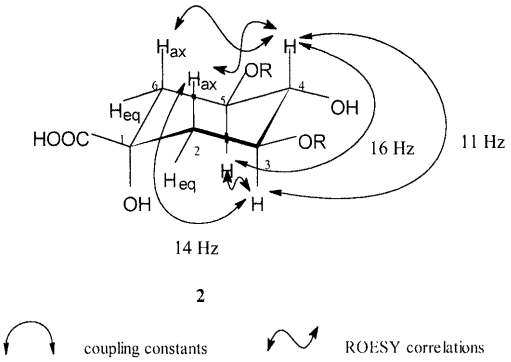

Fig. 2. Selected Coupling Constants and ROESY Correlations of **2**

Table 1. HIV-1 Integrase Inhibitory Activities of Compounds **1**—**4**

Compound	IC_{50} (μg/ml)[a]	Compound	IC_{50} (μg/ml)[a]
1	16.9±2.0	**3**	14.5±1.7
2	7.0±1.3	**4**	*ca.* 97.0

a) IC_{50} values with standard deviations are from at least three independent experiments.

relations of H-3 and H-4, and H-4 and H-5, respectively. Usually, the J values in the substituted cyclohexanes are expected to have over 10 Hz for axial–axial orientation.[14] Further, the ROESY spectrum exhibited the correlations of H-4, H-2_{ax} and H-6_{ax}, and of H-3 and H-5 (Fig. 2), implying that the configurations of H-3, H-4 and H-5 were axial, axial and axial, respectively. The configuration of OH group at C-1 was established to be axial by comparison of ^{1}H and ^{13}C chemical shifts values of the known 3-caffeoyl-muco-quinic acid, 3,5-dicaffeoylquinic acid and the other quinic acid derivatives.[8,10,11,13,15,16] Based on the evidences and comparison of NMR data of **1** and **2**, the structure of **2** was determined to be (−) 3,5-dicaffeoyl-*muco*-quinic acid. A *muco*-quinic acid derivative has been isolated from *Asimina triloba*.[10] Although the ^3J values and ROESY data are in agreement with the proposed chair conformation, comparatively large axial–equatorial $J_{5,6eq}$ (10 Hz) value indicates that the conformation could be deviated from a proposed chair form and seems to be consistent with a skewed or boat conformation.[10] Therefore, unambiguous determination of the absolute stereochemistry must be further studied, for example, by X-ray crystallography.

The anti-HIV-1 integrase activities of compounds **1**—**4** were investigated.[17] As shown in Table 1, compound **2** showed most the potent inhibitory activity against HIV-1 integrase (IC_{50} value of 7.0±1.3 μg/ml).

Experimental

NMR: in DMSO-d_6, Bruker AMX 500 and Varian UNITY INOVA 500 spectrometers. IR: in Nujol, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Prep. HPLC: JAI LC-908 model with refractive index detector, UV detector and Econosil C18 10u column (250 mm×22 mm). *Anal.* HPLC: Gilson 306 dual pump, 811C dynamic mixer, 112 UV/VIS detector (280 nm) and Microsorb-MV column (5 mm×200 mm, 5 μm), eluent,[15] CH_3CN and 5% acetic acid (1 : 49, v/v) for 10 min, the linear gradient from 1 : 49 to 3 : 7 for 40 min, and 3 : 7 for 10 min; flow rate 1.0 ml/min. Column chromatography: silica gel (Merck, 70—230 mesh and 230—400 mesh). TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm).

Extraction and Isolation The *Aster scaber* cultivated was purchased in ChukRyung Mt., Kyungi-Do, Korea in September 1997. A voucher specimen (SKK-98-001) is deposited in the College of Pharmacy at SungKyunKwan University. The dried and chopped aerial parts of *A. scaber* (2.1 kg) were extracted with MeOH two times at room temperature and once at 50 °C for 5 h. The resultant MeOH extract (200 g) was separated by successive solvent partitioning, gave methylene chloride (CH_2Cl_2, 28 g), ethylacetate (EtOAc, 17 g) and *n*-butanol (BuOH, 33 g) soluble fractions. The EtOAc soluble fraction (17 g) was chromatographed on a silica gel column using EtOAc : MeOH : H_2O (9 : 2 : 0.5) to give six subfractions (E1—E6). The subfraction E4 was chromatographed on Sephadex LH-20 to give a mixture, which was separated with HPLC (35% MeOH) to afford yellowish gum **1** (95 mg) and **2** (75 mg). The subfraction E5 was chromatographed on Sephadex LH-20 and purified with HPLC (35% MeOH) to give yellowish gum **3** (98 mg).

The BuOH soluble fraction (33 g) was chromatographed on Diaion HP-20 column using H_2O and MeOH to give two subfractions (B1 and B2). The subfraction B1 was chromatographed on Sephadex LH-20 (MeOH) to give a mixture. The mixture was purified with HPLC (35% MeOH) to afford yellowish gum **4** (230 mg).

(−) 3,5-Dicaffeoyl quinic acid (**1**): Yellowish gum. $[\alpha]_D$ −183.4° (c= 0.51, MeOH). FAB-MS *m/z* (rel. int.): 517 ($[M+H]^+$, 100). ^{1}H-NMR (500 MHz, DMSO-d_6): δ: 1.86 (1H, br m, H-2_{eq}), 1.91 (2H, br m, H-6), 2.13 (1H, br d, J=12.5 Hz, H-2_{ax}), 3.73 (1H, br d, J=9.3 Hz, H-4), 5.21 (1H, br s, H-3), 5.30 (1H, br dd, J=15.5, 8.9 Hz, H-5), 6.25—6.22 (each 1H, d, J= 15.5 Hz, H-2′), 6.76—6.75 (each 1H, d, J=8.0 Hz, H-8′), 6.96 (2H, d, J= 8.0 Hz, H-9′), 7.06 (2H, s, H-5′), 7.46—7.45 (each 1H, d, J=15.5 Hz, H-3′). Coupling constants in DQF-^{1}H-^{1}H-COSY (Hz): $^2J_{2ax,2eq}$=15, $^2J_{6ax,6eq}$=14, $^3J_{2ax,3}$=9, $^3J_{2eq,3}$=6, $^3J_{3,4}$=8, $^3J_{4,5}$=15, $^3J_{5,6ax}$=17, $^3J_{5,6eq}$=8. ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 37.1 (C-2), 40.1 (C-6), 71.8 (C-4), 72.0 (C-5), 73.8 (C-3), 75.5 (C-1), 115.9—115.7 (C-5′), 116.3—116.3 (C-8′), 117.0—116.9 (C-2′), 122.4—122.0 (C-9′), 126.8—126.7 (C-4′), 145.8—145.5 (C-3′), 146.8—146.8 (C-6′), 149.5—149.4 (C-7′), 167.5—167.3 (C-1′), 178.9 (COOH).

(−) 3,5-Dicaffeoyl-*muco*-quinic acid (**2**): Yellowish gum. $[\alpha]_D$ −153.8° (c=0.78, MeOH). UV λ_{max} (EtOH) nm (log ε): 328 (4.52), 300sh (4.40), 244 (4.28), 219 (4.43). IR (nujol) cm^{-1} : 3300, 1690, 1600. HR-FAB-MS *m/z*: 517.1349 ($[M+H]^+$, Calcd for $C_{25}H_{25}O_{12}$: 517.1346). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.96 (2H, m, H-2_{eq}, H-6_{eq}), 2.11 (1H, m, H-2_{ax}), 2.14 (1H, m, H-6_{ax}), 3.83 (1H, br s, H-4), 5.13 (1H, br s, H-3), 5.19 (1H, br s, H-5), 5.30 (1H, br s, 4-OH), 6.23—6.14 (each 1H, d, J=16.0 Hz, H-2′), 6.77—6.73 (each 1H, d, J=8.0 Hz, H-8′), 6.99—6.98 (each 1H, br d, J= 8.0 Hz, H-9′), 7.04 (2H, d, J=2.5 Hz, H-5′), 7.46—7.43 (each 1H, d, J= 16.0 Hz, H-3′), 9.18 (2H, br s, caffeoyl OH), 9.57 (2H, br s, caffeoyl OH). Coupling constants in DQF-^{1}H-^{1}H-COSY (Hz): $^2J_{2ax,2eq}$=13, $^2J_{6ax,6eq}$=12, $^3J_{2ax,3}$=14, $^3J_{3,4}$=11, $^3J_{4,5}$=16, $^3J_{5,6ax}$=15, $^3J_{5,6eq}$=10. ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 35.9 (t, C-2), 37.0 (t, C-6), 68.8 (d, C-4), 71.8 (d, C-5), 72.0 (d, C-3), 73.7 (s, C-1), 115.3—115.3 (d, C-5′), 116.0—115.9 (d, C-8′), 117.0—116.9 (d, C-2′), 122.5—122.2 (d, C-9′), 126.8—126.7 (s, C-4′), 146.2—145.8 (d, C-3′), 146.7—146.7 (s, C-6′), 149.5—149.4 (s, C-7′), 167.2—166.7 (s, C-1′), 176.5 (s, COOH).

(−) 4,5-Dicaffeoyl quinic acid (**3**): Yellowish gum. $[\alpha]_D$ −257.8° (c= 0.46, MeOH). FAB-MS, ^{1}H- and ^{13}C-NMR data were in good agreement with those in previous papers.[12,13,16]

(−) 5-Caffeoyl quinic acid (**4**): Yellowish gum. $[\alpha]_D$ −24.8° (c=0.49, MeOH). FAB-MS, ^{1}H- and ^{13}C-NMR data were in good agreement with those in previous papers.[12,13,16]

Bioassay of HIV-1 Integrase Inhibitory Activity[17] A standard reaction assay of endonucleolytic activity was carried out in the presence of potential inhibitor containing 0.1 pmol of duplex oligonucleotide substrate and 15 pmol of HIV-1 integrase. Inhibitors of drugs were dissolved in 100% DMSO and added to the reaction mixture; the final mixture was 5% DMSO. Reaction products were visualized by autoradiography of the wet gel. IC_{50} values were calculated by scanning bands on Kodak-5 film (Image Master VDS, Pharmacia Biotech., Piscataway, NJ, U.S.A.).

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