Two Bioactive Pentacyclic Triterpene Esters from the Root Bark of *Hibiscus syriacus*

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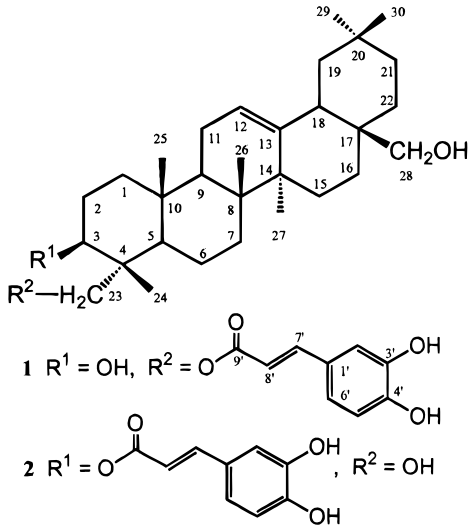
Two new triterpene caffeates have been isolated from the root bark of *Hibiscus syriacus*. Their structures were established through various spectral studies as 3β,23,28-trihydroxy-12-oleanene 23-caffeate (**1**) and 3β,23,28-trihydroxy-12-oleanene 3β-caffeate (**2**). Compounds **1** and **2** showed lipid peroxidation inhibitory activity and significant cytotoxicity against a panel of human cancer cell lines.

The genus *Hibiscus* belongs to the family Malvaceae and is widely distributed in eastern and southern Asia. The root bark of *H. syriacus* L. has been used as an antipyretic, anthelmintic, and antifungal agent in the Orient.[1,2] In our continuing investigation for biologically active substances from this plant,[3−5] we have isolated two pentacyclic triterpene caffeic acid esters from a $CHCl_3$-soluble extract of the root bark of *H. syriacus*. In this paper, we describe the isolation and structure elucidation of **1** and **2**.



The molecular formula of compound **1** was established as $C_{39}H_{56}O_6$ by HRFABMS (*m*/*z* 621.4149 $[M + H]^+$ −0.6 mmu). The IR spectrum of **1** suggested the presence of hydroxyl (3430 cm^{-1}) and α,β-unsaturated carbonyl (1690 cm^{-1}) groups. The ^1H NMR spectrum of **1** in CD_3OD exhibited signals due to three aromatic methine protons of an AMX type at δ 7.02, 6.94 and 6.79 and two trans-conjugated olefinic protons at δ 7.58 and 6.28 that were found to be identical with signals for authentic caffeic acid. The presence of caffeic acid was also supported by the UV maxima at 336, 300, and 225 nm. Additional signals in the ^1H NMR spectrum were typical for a triterpenoid. The 39 carbon signals observed in the ^{13}C NMR spectrum were characterized by a DEPT experiment, which suggested that **1** was a triterpene caffeic acid ester having an ester carbonyl, four sp^2 quaternary carbons, six sp^2 methines, two oxygenated methylenes, one oxygenated methine, six quaternary carbons, three methines, ten methylenes, and six tertiary methyls. The singlet signals for six methyls in the ^1H NMR spectrum and olefinic signals at δ 123.4 and 145.7 for C-12 and C-13, respectively, in the ^{13}C NMR spectrum indicated the presence of a Δ^{12}-oleanane skeleton in compound **1**.[6] From the presence of two hydroxymethyls and only six methyls, the general structure of **1** was suggested to be 23-hydroxyerythrodiol esterified with caffeic acid at the C-23 hydroxy function. Also, the ^{13}C NMR chemical shifts of all signals due to the triterpene portion of the molecule of **1** were in good agreement with those of erythrodiol,[7] except for C-2, C-3, and C-4. Further structural confirmation was performed by an HMBC NMR experiment,[8] which showed long-range correlations from the methylene protons at δ 4.04 and 4.13 to carbonyl carbon of caffeic acid at δ 169.0 (C-9′), as shown in Table 1. Therefore, **1** was assigned as 3β,23,28-trihydroxy-Δ^{12}-oleanene 23-caffeate. Complete ^1H and ^{13}C chemical shift assignments were made from the DQF−COSY, HMQC, and HMBC data.

Compound **2** was closely related to **1** in its physicochemical properties and NMR spectra, suggesting that it was also a triterpene caffeic acid ester. The molecular formula was determined to be $C_{39}H_{56}O_6$ by HRFABMS (*m*/*z* 621.4139 $[M + H]^+$ −1.6 mmu) and was the same as that of **1**. However, compound **2** differed from **1** in the ^1H and ^{13}C chemical shifts at H-3, H-23, C-2, C-3, and C-23. The signals of H-23, C-2, and C-23 in **2** were upfield from those of **1** while H-3 and C-3 were shifted downfield. In the HMBC spectrum, a long-range correlation from H-3 at δ 4.94 to ester carbonyl carbon of C-9′ at δ 169.2 was observed. From the above results, the structure of **2** was assigned as 3β,23,28-trihydroxy-12-oleanene 3β-caffeate.

Compounds **1** and **2** inhibited lipid peroxidation[9] with IC_{50} values of 2.3 and 1.1 μg/mL, respectively, with vitamin E (IC_{50} 1.6 μg/mL) used as a control. Also, the cytotoxicity of both compounds against several human tumor cell lines was examined (Table 2). Compound **1** was effective against the ACHN, SW620, HCT15, and SF539 cell lines, while **2** was active against the SW620 and HCT15 cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined on a MELTEMP II laboratory device and are uncorrected. Optical rotations were determined by using a Polartronic polarimeter. UV and IR spectra were recorded on a Shimadzu UV-260 spectrophotometer and a Laser Precision Analect RFX65S FT-IR spectrometer, respectively. NMR spectra were obtained using Varian UNITY 300 and UNITY 500

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Table 1. ^{1}H NMR and HMBC Spectral Data for Compound **1** in CD_3OD[a]

position	δ_H	HMBC (H→C)	position	δ_H	HMBC (H→C)
1	1.58[b]	C-3, 5, 10, 25	21	1.22[b]	C-20
	0.94[b]			1.13[b]	
2	1.65[b]		22	1.37[b]	
	1.54[b]	C-3, 10		1.25[b]	C-17, 18, 20
3	3.62 (dd, 11.0, 4.8)	C-2, 4, 23, 24	23	4.13 (d, 11.2)	C-3, 4, 5, 24, 9′
5	1.15[b]	C-3, 4, 9, 10, 24, 25		4.04 (d, 11.2)	C-3, 4, 5, 24, 9′
6	1.40[b]	C-5, 7, 10	24	0.71 (s)	C-3, 4, 5, 23
7	1.40[b]	C-5, 8	25	0.93 (s)	C-1, 9, 10
	1.22[b]	C-5, 8, 26	26	0.91 (s)	C-7, 8, 9, 14
9	1.56[b]	C-1, 8, 10, 11, 14, 25, 26	27	1.05 (s)	C-8, 14, 15
11	1.83[b]	C-8, 9, 12, 13	28	3.52 (d, 10.5)	C-22
12	5.17 (br t)	C-9, 11, 14, 18		3.10 (d, 10.5)	C-16, 17, 22
15	1.63[b]		29	0.89 (s)	C-19, 20, 21, 30
	0.88[b]		30	0.89 (s)	C-19, 20, 21, 29
16	1.77[b]	C-15, 17, 28	2′	7.02 (br s)	C-4′, 6′
	1.05[b]		5′	6.79 (d, 8.1)	C-1′, 3′, 4′
18	1.90[b]	C-12, 13, 14, 16, 17, 19, 28	6′	6.94 (br d, 8.1)	C-2′, 4′
19	1.67[b]	C-18, 20, 21, 29, 30	7′	7.58 (d, 15.9)	C-2′, 6′, 9′
	0.94[b]	C-13, 18, 20, 21	8′	6.28 (d, 15.9)	C-1′, 9′

[a] Proton resonance multiplicity and coupling constants (J in Hz) are given in parentheses. [b] The signals are either multiplets or overlapping with other peaks; assignments were performed by measuring the centre of crosspeaks in the COSY, HMQC, and HMBC spectra.

Table 2. ED_{50} Values (μg/mL) of Compounds **1** and **2** against Some Human Cancer Cell Lines

cell line	**1**	**2**	adriamycin
UACC62 (melanoma)	3.9	2.3	0.2
MCF7 (breast)	2.2	1.8	0.1
NCI-H23 (lung)	2.8	1.7	0.1
ACHN (renal)	1.2	2.1	0.3
UO-31 (renal)	2.0	1.7	0.0
PC-3 (prostate)	1.6	2.2	0.4
SW620 (colon)	1.1	1.0	0.1
HCT15 (colon)	0.8	1.3	0.6
SF539 (central nervous system)	1.4	2.0	0.2

NMR spectrometers in CD_3OD with TMS as an internal standard. Chemical shifts are given in δ from TMS. The 2D-NMR experiments were carried out on a Varian UNITY 500 NMR spectrometer operating at 500.05/112.5 MHz with $^nJ_{CH}$ = 8.3 Hz for HMBC. FABMS and HRFABMS were measured using glycerol as matrix and poly(ethylene glycol) as internal standard on a Kratos Concept-1S FAB mass spectrometer.

Plant Material. The root bark of *H. syriacus* was collected at Yusong, Chungnam Province, Korea, in October 1995, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejon, Korea. The voucher specimen is deposited in the Cell Function Regulator Research Unit Laboratory of KRIBB.

Extraction and Isolation. The dried root bark of *H. syriacus* (1.6 kg) was ground into a powder and extracted with MeOH at room temperature for 2 days. The MeOH extract was filtered and concentrated under reduced pressure. The liquid residue was washed with *n*-hexane and then partitioned between $CHCl_3$ and H_2O. Compounds **1** and **2** were obtained from the $CHCl_3$ layer by monitoring with lipid peroxidation inhibitory activity[9] in combination with analytical TLC. The $CHCl_3$ layer was concentrated in vacuo and the residue was chromatographed on a silica gel column eluted with *n*-hexane/EtOAc gradient mixtures. The active fractions eluted with *n*-hexane/EtOAc (2:1) to EtOAc were concentrated and then chromatographed over a silica gel column with $CHCl_3$/MeOH (100:1−5:1). The crude active constituents were applied to a Sephadex LH-20 column, eluting with MeOH, followed by an ODS column chromatographic separation with 70−90% aqueous MeOH to give compounds **1** (53 mg) and **2** (38 mg).

Compound 1: mp 140−150 °C; $[\alpha]_D$ +15° (*c* 1.0, MeOH); UV λ_{max} (MeOH) (log ϵ) 225 (4.44), 300 (4.12), 336 (4.18) nm; IR (KBr) ν_{max} 3435, 2930, 1707, 1691, 1603, 1464, 1446, 1385, 1263, 1169, 1033, 1005 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR (CD_3OD, 125 MHz) δ 169.0 (C-9′), 149.7 (C-4′), 146.9 (C-7′), 146.8 (C-3′), 145.7 (C-13), 127.6 (C-1′), 123.4 (C-12), 123.0 (C-6′), 116.5 (C-5′), 115.0 (C-2′, 8′), 72.7 (C-3), 69.7 (C-28), 66.5 (C-23), 49.4 (C-9), 49.0 (C-5), 47.7 (C-19), 43.8 (C-18), 43.2 (C-4), 42.8 (C-14), 41.0 (C-8), 39.8 (C-1), 38.1 (C-17), 37.8 (C-10), 35.2 (C-21), 33.7 (C-29), 33.4 (C-7), 32.2 (C-22), 31.8 (C-20), 27.4 (C-2), 26.5 (C-15), 26.4 (C-27), 24.6 (C-11), 24.0 (C-30), 22.8 (C-16), 19.2 (C-6), 17.3 (C-26), 16.4 (C-25), 12.7 (C-24); FABMS *m/z* 621 (M + H)$^+$, 643 (M + Na)$^+$; HRFABMS *m/z* 621.4149 (M + H)$^+$ ($C_{39}H_{57}O_6$ requires 621.4155).

Compound 2: mp 140−145 °C; $[\alpha]_D$ +52° (*c* 1.0, MeOH); UV λ max (MeOH) (log ϵ) 224 (4.21), 300 (4.05), 330 (4.14) nm; IR (KBr) ν max 3433, 2926, 1705, 1687, 1604, 1464, 1446, 1385, 1270, 1178, 1004 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.51 (1H, d, J = 15.6 Hz, H-7′), 7.02 (1H, d, J = 1.2 Hz, H-2′), 6.77 (1H, d, J = 8.1 Hz, H-5′), 6.93 (1H, dd, J = 8.1, 1.2 Hz, H-6′), 6.23 (1H, d, J = 15.6 Hz, H-8′), 5.20 (1H, br t, H-12), 4.95 (1H, dd, J = 10.5, 6.0 Hz, H-3), 3.53 (1H, d, J = 10.8 Hz, H-28), 3.35 (1H, d, J = 11.7 Hz, H-23), 3.15 (1H, d, J = 11.7 Hz, H-23), 3.11 (1H, d, J = 10.8 Hz, H-28), 1.22 (3H, s, Me-27), 1.06 (3H, s, Me-25), 1.01 (3H, s, Me-26), 0.89 (6H, s, Me-29, 30), 0.82 (3H, s, Me-24); ^{13}C NMR (CD_3OD, 125 MHz) δ 169.2 (C-9′), 149.6 (C-4′), 146.9 (C-7′), 146.9 (C-3′), 145.8 (C-13), 127.7 (C-1′), 123.4 (C-12), 122.9 (C-6′), 116.5 (C-5′), 115.6 (C-8′), 115.1 (C-2′), 75.8 (C-3), 69.8 (C-28), 64.7 (C-23), 49.0 (C-9), 47.9 (C-19), 47.8 (C-5), 43.8 (C-18), 43.0 (C-4, 14), 41.0 (C-8), 39.2 (C-1), 38.1 (C-10, 17), 35.3 (C-21), 33.8 (C-29), 33.1 (C-7), 32.3 (C-22), 31.8 (C-20), 26.6 (C-15, 27), 24.7 (C-11), 24.2 (C-2), 24.0 (C-30), 22.9 (C-16), 18.8 (C-6), 17.4 (C-26), 16.5 (C-25), 13.9 (C-24); FABMS *m/z* 621 (M + H)$^+$; HRFABMS *m/z* 621.4139 (M + H)$^+$ ($C_{39}H_{57}O_6$ requires 621.4155).

Antioxidative Activity. Antioxidative activities of **1** and **2** were evaluated as inhibitory activity against lipid peroxidation in rat liver microsomes according to a previously described method,[9] with minor modification. The reaction was initiated by the addition of 100 μM $FeSO_4 \cdot 7H_2O$ to a mixture of ascorbic acid (0.2 mM) and the microsomal suspension (0.5 μg protein/mL). Lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm.

Cytotoxicity. Cytotoxic activity against a small panel of human tumor cell lines was estimated according to NCI protocols.[10]

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