

A New Sesquiterpene Hydroperoxide from the Aerial Parts of *Aster oharai*

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Phytochemical works on the aerial parts of *Aster oharai* (Compositae) led to the isolation of a new sesquiterpene hydroperoxide, 7 α -hydroperoxy-3,11-eudesmadiene (**2**) and seven known compounds, teucdiol B (**1**), α -spinasterol (**3**), oleanolic acid (**4**), α -spinasterol 3-O- β -D-glucopyranoside (**5**), methyl 3,5-di-O-caffeoyl quinate (**6**), 3,5-di-O-caffeoylquinic acid (**7**), 3,4-di-O-caffeoylquinic acid (**8**). The chemical structures of **1-8** were established by chemical and spectroscopic methods. Compound **2** showed cytotoxicity against cultured human tumor cell lines *in vitro*, SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), and HCT15 (colon) with ED₅₀ values ranging from 3.86-17.21 μ g/mL.

Key words: *Aster oharai*, Compositae, Sesquiterpene hydroperoxide, Quinic Acid, Cytotoxicity

INTRODUCTION

Aster oharai (Compositae), a perennial herb, is distributed at Ullung island in Korea. It has been used as a traditional medicine to treat asthma and diuresis (Kim, 1996). In our previous study on this plant, five new diterpenes were isolated from the *n*-hexane fraction of the MeOH extract of the aerial parts (Choi *et al.*, 2002). In a continuation of our study on this plant, we isolated further a new sesquiterpene hydroperoxide (**2**), a sesquiterpene (**1**), a triterpene (**4**), two sterols (**3** and **5**) and three caffeoylquinic acids (**6-8**) as minor constituents. This paper describes the isolation and structural characterization of these compounds and cytotoxic activities of a new sesquiterpene hydroperoxide.

MATERIALS AND METHODS

General experimental procedure

Mps: uncorr. Optical rotations: Jasco P-1020 Polarimeter. NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl₄, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography:

Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh), Licroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F₂₅₄ plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar[®]-A Si 60 (240 \times 10 mm).

Plant material

Aster oharai Nakai was collected at Ullung island, Gyeong-Sangbuk-Do, Korea in July 1999. A voucher specimen (SKK-99-002) was deposited in the College of Pharmacy at SungKyunkwan University.

Cytotoxicity test *in vitro*

Sulforhodamin B Bioassay (SRB) was used for cytotoxicity test. The activity of a compound was tested at several concentrations against five cultured human tumor cells *in vitro* (Skehan *et al.*, 1990); A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon).

Extraction, separation and purification of compounds

The half-dried and chopped aerial parts of *Aster oharai* (5 kg) were extracted with MeOH three times at room temperature for two weeks. The resultant MeOH extract (400 g) was subjected to successive solvent partitioning to give *n*-hexane (46 g), CH₂Cl₂ (5 g), EtOAc (12 g) and *n*-BuOH (20 g) soluble fractions. The *n*-hexane soluble frac-

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tion (46 g) was chromatographed over silica gel column using a gradient solvent system of *n*-hexane:EtOAc (10:1-0:1) to give six subfractions (H1-H6). The subfraction H2 (2 g) was chromatographed over silica gel eluting with *n*-hexane:EtOAc (3:1) to give three subfractions (H21~H23). The subfraction H21 (300 mg) was further subjected to Sephadex LH-20 (CH₂Cl₂:MeOH, 1:1) column chromatography and purified with silica gel Lobar[®]-A column (*n*-hexane:EtOAc=6:1) to afford **1** (12 mg). The subfraction H23 (300 mg) was further purified using a RP-18 Lobar[®]-A column (70% MeCN) to afford **2** (10 mg). The subfraction H4 (2.8 g) was chromatographed over silica gel column (CH₂Cl₂:MeOH = 25:1) to give four subfractions (H41-H44). The subfraction H41 (900 mg) was chromatographed on Sephadex LH-20 (CH₂Cl₂:MeOH = 1:1) and purified over silica gel Lobar[®]-A column (*n*-hexane:EtOAc = 2:1) to afford **3** (30 mg). The subfraction H43 (750 mg) was chromatographed on a RP-18 Lobar[®]-B column (80% MeCN) to give two subfractions (H431 and H432). The subfraction H431 (200 mg) was chromatographed on Sephadex LH-20 and silica gel Lobar[®]-A column (*n*-hexane:EtOAc = 1:1) to afford **4** (20 mg). The subfraction H44 (150 mg) was purified over silica gel (CH₂Cl₂:MeOH = 40:1) and Sephadex LH-20 (CH₂Cl₂:MeOH = 1:1) to afford **5** (15 mg).

The EtOAc soluble fraction (12 g) was chromatographed over silica gel column using a solvent system of CHCl₃:EtOAc:MeOH (3:2:1) to give four subfractions (EA1-EA4). The second subfraction (5 g) was chromatographed over silica gel eluting with EtOAc:MeOH:H₂O (9:2:0.5) to give three subfractions (EA21-EA23). The subfraction EA21 (1 g) and EA22 (1.5 g) were purified over Sephadex LH-20 (MeOH) and RP-18 Lobar[®]-B column (50% MeOH) to afford **6** (150 mg) and **7** (200 mg), respectively. The subfraction EA3 (1 g) was chromatographed over silica gel eluting with EtOAc:MeOH:H₂O (9:2:0.5) to give three subfractions (EA31-EA33). The subfraction EA31 (0.3 g) was further purified with silica gel eluting with EtOAc:MeOH:H₂O (9:2:0.3) and Sephadex LH-20 (CH₂Cl₂:MeOH = 1:1) to afford **8** (15 mg).

Teucdiol B (1)

Colorless oil, EI-MS *m/z* (rel. int.): 238 (M⁺, 4), 220 (6), 205 (20), 203 (7), 197 (6), 191 (10), 187 (10), 177 (11), 163 (17), 149 (14), 145 (15), 135 (26); ¹H-NMR (500 MHz, CDCl₃): 0.95 (3H, s), 1.11 (3H, s), 1.81 (3H, brs), 2.22 (1H, dt, *J* = 12.0, 2.0 Hz, H-5), 5.01 (1H, t, *J* = 0.4 Hz), 5.09 (1H, brs); ¹³C-NMR (125 MHz, CDCl₃): 40.7 (C-1), 20.2 (C-2), 43.3 (C-3), 75.3 (C-4), 51.1 (C-5), 42.4 (C-6), 71.9 (C-7), 31.3 (C-8), 32.2 (C-9), 34.7 (C-10), 147.0 (C-11), 113.3 (C-12), 18.6 (C-13), 18.7 (C-14), 22.6 (C-15).

7 α -Hydroperoxy-3,11-eudesmadiene (2)

Colorless oil, [α]_D -6.3° (c 0.02, CHCl₃); IR: ν_{\max}^{neat} cm⁻¹ ν =

Table I. ¹H-NMR (δ in ppm, *J* in Hz), ¹³C-NMR (δ in ppm) data and selected HMBC, NOESY correlations of **2**

Position	¹ H (mult, <i>J</i> (Hz))	¹³ C	HMBC (¹ H \rightarrow ¹³ C)	NOESY
1	1.40 m (α)	37.4 t		H-5
	1.43 m (β)			H-15
2	2.01 m (α)	22.9 t		H-3
	2.10 m (β)			H-3
3	5.38 brs	121.4 d		H-14
4		134.5 s		
5	2.36 brd (α , 8.0)	40.7 d		H-1 (α), H-9 (α)
6	1.45 m (α)	28.9 t		H-12a
	2.18 brdt (β , 12.0, 2.0)			H-12a
7		85.9 s		
8	1.86 m (α)	27.5 t		H-12a
	1.80 m (β)			H-12a
9	1.50 m (α)	35.5 t	C-10	H-5
	1.24 m (β)			H-15
10		32.0 s		
11		148.4 s		
12	5.08 s (H-12a)	111.7 t	C-7, C-13	H-6, H-8
	5.02 t (1.5, H-12b)			C-7, C-13
13	1.86 m	18.9 q	C-7, C-11, C-12	H-12b
14	1.64 m	21.0 q	C-3, C-4, C-5	H-3
15	0.82 s	14.9 q	C-1, C-5, C-9, C-10	H-1 (β), H-9 (β)

3521, 3080, 1642, 1263, 885; ESIMS *m/z* (ret. Int.): 237 ([M+H]⁺, 8); HREIMS *m/z* 236.3300 (calcd for C₁₅H₂₄O₂, 236.3294); ¹H-NMR (500 MHz, CDCl₃): Table I; ¹³C-NMR (125 MHz, CDCl₃): Table I.

α -Spinasterol (3)

White needles, mp 168°C; EI-MS *m/z* (rel. int.): 412 (M⁺, 10), 394 (60), 379 (21), 351 (15), 282 (8), 271 (34), 253 (87), 239 (12), 229 (31), 213 (30), 201 (18), 187 (10), 173 (15), 159 (30), 147 (38), 133 (30), 105 (75), 95 (49), 81 (100), 67 (31), 55 (88); ¹H-NMR (CDCl₃, 500 MHz): δ 0.55 (3H, s, H-18), 0.79-0.82 (6H, m, H-27, H-29), 0.82 (3H, s, H-19), 0.84 (3H, d, *J* = 6.0 Hz, H-26), 1.02 (3H, d, *J* = 6.5 Hz, H-21), 3.60 (1H, m, H-3), 5.04 (1H, dd, *J* = 15.0, 8.5 Hz, H-23), 5.17 (1H, dd, *J* = 15.0, 8.5 Hz, H-22), 5.18 (1H, m, H-7); ¹³C-NMR (CDCl₃, 125 MHz): δ 37.18 (C-1), 31.52 (C-2), 71.05 (C-3), 39.04 (C-4), 40.30 (C-5), 29.66 (C-6), 117.47 (C-7), 139.57 (C-8), 49.49 (C-9), 34.24 (C-10), 21.57 (C-11), 39.50 (C-12), 43.30 (C-13), 55.15 (C-14), 23.02 (C-15), 28.49 (C-16), 55.95 (C-17), 12.05 (C-18), 13.03 (C-19), 40.79 (C-20), 21.06 (C-21), 138.15 (C-22), 129.48 (C-23), 51.26 (C-24), 31.87 (C-25), 21.37 (C-26), 19.00 (C-27), 25.38 (C-28), 12.22 (C-29).

Oleanolic acid (4)

White powder, mp 197°C; EI-MS m/z (rel. int.): 456 (M^+ , 6), 248 (100), 207 (30), 204 (32), 203 (72), 189 (30); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.74, 0.79, 0.89, 0.91, 0.92, 0.98 and 1.12 (each 3H, s), 2.83 (1H, bdd, $J = 14.0, 4.0$ Hz), 3.22 (1H, bdd, $J = 9.5, 4.0$ Hz) and 5.28 (each 1H, m); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 16.0 (C-25), 16.5 (C-24), 17.8 (C-26), 18.9 (C-6), 23.8 (C-30, 16, 11), 26.2 (C-27), 28.2 (C-2), 28.3 (C-15), 28.7 (C-23), 31.0 (C-20), 33.3 (C-29, 22, 7), 34.3 (C-21), 37.4 (C-10), 38.9 (C-1), 39.4 (C-4), 39.8 (C-8), 42.0 (C-18,14), 46.7 (C-19,17), 48.3 (C-9), 55.9 (C-5), 79.7 (C-3), 123.3 (C-12), 144.3 (C-13), 184.3 (C-28).

 α -Spinasterol-3-O- β -D-glucopyranoside (5)

White needles, mp 284°C; $^1\text{H-NMR}$ (500 MHz, pyridine- d_5): δ 0.58 (3H, s, H-18), 0.72 (3H, s, H-19), 0.85-0.89 (6H, m, H-27, H-29), 0.89 (3H, d, $J = 6.5$ Hz, H-26), 1.06 (3H, $J = 6.5$ Hz, H-21), 3.96-4.59 (1H, m, H-2~6), 5.04 (1H, d, $J = 7.5$ Hz, H-1), 5.06 (1H, m, H-23), 5.17 (2H, m, H-7, 22).

Methyl 3,5-di-O-caffeoyl quinate (6)

Yellow gum, $[\alpha]_D -190.0^\circ$ (c 0.20, MeOH); FAB MS m/z : 531 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): δ 2.19 (brd, $J = 12.5$ Hz), 1.97 (brd, $J = 12.5$ Hz), 5.37 (brdd, $J = 9.5, 4.5$ Hz), 3.95 (dd, $J = 9.5, 3.0$ Hz), 5.29 (dt, $J = 7.0, 3.5$ Hz), 2.19 (brd, $J = 12.5$ Hz), 1.97 (brd, $J = 12.5$ Hz), 6.24/6.12 (d, $J = 16.0$ Hz), 7.48/7.41 (d, $J = 16.0$ Hz), 7.04/7.04 (d, $J = 2.5$ Hz), 6.77/6.76 (d, $J = 8.0$ Hz), 6.99/6.99 (d, $J = 8.0$ Hz), 3.59 (s); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD): δ 73.6 (C-1), 35.6 (C-2), 72.0 (C-3), 67.8 (C-4), 71.0 (C-5), 35.9 (C-6), 167.1 (C-1'), 166.4 (C-1''), 115.9 (C-2'), 115.8 (C-2''), 146.8 (C-3'), 146.7 (C-3''), 126.7 (C-4'), 126.5 (C-4''), 115.7 (C-5'), 114.8 (C-5''), 146.5 (C-6'), 146.1 (C-6''), 149.7 (C-7'), 149.4 (C-7''), 117.0 (C-8'), 116.9 (C-8''), 122.5 (C-9'), 122.3 (C-9''), 53.0 (OCH_3), 174.8 (CO).

3,5-Di-O-caffeoylquinic acid (7)

Yellow gum, $[\alpha]_D -220.4^\circ$ (c 0.20, MeOH); FAB MS m/z : 517 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): δ 2.13 (brd, $J = 12.5$ Hz), 1.86 (br m), 5.49 (dd, $J = 9.9, 4.5$ Hz), 3.39 (dd, $J = 9.9, 2.8$ Hz), 5.39 (brdd, $J = 6.5, 3.4$ Hz), 1.91 (br m), 6.25/6.22 (d, $J = 15.5$ Hz), 7.46/7.45 (d, $J = 15.5$ Hz), 7.06/7.06 (s), 6.76/6.75 (d, $J = 8.0$ Hz), 6.96/6.96 (d, $J = 8.0$ Hz); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD): δ 75.5 (C-1), 37.1 (C-2), 73.8 (C-3), 71.8 (C-4), 72.0 (C-5), 40.1 (C-6), 167.5 (C-1'), 167.3 (C-1''), 117.0 (C-2'), 116.9 (C-2''), 145.8 (C-3'), 145.5 (C-3''), 126.8 (C-4'), 126.7 (C-4''), 115.9 (C-5'), 115.7 (C-5''), 146.8 (C-6'), 146.8 (C-6''), 149.5 (C-7'), 149.4 (C-7''), 116.3 (C-8'), 116.3 (C-8''), 122.4 (C-9'), 122.0 (C-9''), 178.9 (COOH).

3,4-Di-O-caffeoylquinic acid (8)

Yellow powder, $[\alpha]_D -219.0^\circ$ (c 0.20, MeOH); FAB MS m/z : 517 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): δ 7.59 (1H, d, $J = 16.0$ Hz), 7.50 (1H, d, $J = 16.0$ Hz), 7.02 (1H, d, $J = 2.0$ Hz), 6.99 (1H, d, $J = 2.0$ Hz), 6.90 (1H, dd, $J = 8.0, 2.0$ Hz), 6.88 (1H, dd, $J = 8.0, 2.0$ Hz), 6.74 (1H, d, $J = 8.0$ Hz), 6.73 (1H, d, $J = 8.0$ Hz), 6.27 (1H, d, $J = 16.0$ Hz), 6.19 (1H, d, $J = 16.0$ Hz), 5.68 (1H, dt, $J = 10.0, 5.0$ Hz), 5.12 (1H, dd, $J = 10.0, 3.0$ Hz), 4.35 (1H, dt, $J = 3.0, 2.5$ Hz), 2.29 (1H, dd, $J = 14.0, 3.0$ Hz), 2.20 (2H, m), 2.02 (1H, dd, $J = 14.0, 6.0$ Hz); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD): δ 77.9 (C-1), 40.2 (C-2), 70.1 (C-3), 77.5 (C-4), 71.0 (C-5), 39.5 (C-6), 169.3 (C-1'), 169.4 (C-1''), 115.7 (C-2'), 115.7 (C-2''), 148.2 (C-3'), 148.4 (C-3''), 128.4 (C-4'), 128.4 (C-4''), 115.9 (C-5'), 115.8 (C-5''), 147.5 (C-6'), 147.5 (C-6''), 150.3 (C-7'), 150.3 (C-7''), 117.2 (C-8'), 117.4 (C-8''), 123.8 (C-9'), 123.8 (C-9''), 179.8 (COOH).

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil and its molecular formula was determined to be $\text{C}_{15}\text{H}_{26}\text{O}_2$ by EIMS (m/z 238, M^+). The IR spectrum of **1** exhibited strong absorption bands at 3550 and 3400 cm^{-1} , indicating the presence of hydroxyl groups. The $^1\text{H-NMR}$ spectrum showed the signals of two olefinic hydrogens at δ 5.01 (1H, t, $J = 0.5$ Hz, H-13a) and 5.09 (1H, brs, H-13b), and three methyl groups at δ 0.95 (3H, s, H-14), 1.11 (3H, s, H-15) and 1.81 (3H, s, H-12). The $^{13}\text{C-NMR}$ spectrum indicated the presence of a double bond (δ 113.3 and 147.0) and two oxygenated carbons (δ 71.9 and 75.3). The ^1H - and $^{13}\text{C-NMR}$ spectra of **1** were very similar to those of teucdiol A and B, a pair of isomers, which were isolated from *Teucrium heterophyllum* (Fraga *et al.*, 1993). The isomers were only differed in the disposition of the hydroxyl group at C-7. In the $^{13}\text{C-NMR}$ spectrum, the chemical shift of C-5 in teucdiol A (C-7 β hydroxy) was shifted upfield by 3ppm comparing with C-5 in teucdiol B (C-7 α hydroxy). The chemical shift (δ 51.1) of C-5 observed in compound **1** was same with the value for teudiol B (δ 51.1). Thus, the structure of **1** was determined as teucdiol B (Fraga *et al.*, 1993).

Compound **2** was obtained as a colorless oil with positive reaction on peroxide reagent (Lee, 1991). The molecular formula of **2** was assigned as $\text{C}_{15}\text{H}_{24}\text{O}_2$ based on $[\text{M}+\text{H}]^+$ at m/z 237 in electrospray ionization mass spectrum (ESI-MS) and HREIMS spectrum (M^+ , m/z 236.3300). The IR spectrum of **2** showed the presence of a hydroxyl group at 3521 cm^{-1} . The $^1\text{H-NMR}$ spectrum indicated signals due to three tertiary methyl groups at δ 0.82 (s), 1.64 (s), 1.86 (s), vinylic hydrogens of the isopropylidene group at δ 5.02 and 5.08. The $^{13}\text{C-NMR}$ spectrum showed 15 carbon signals, which included four

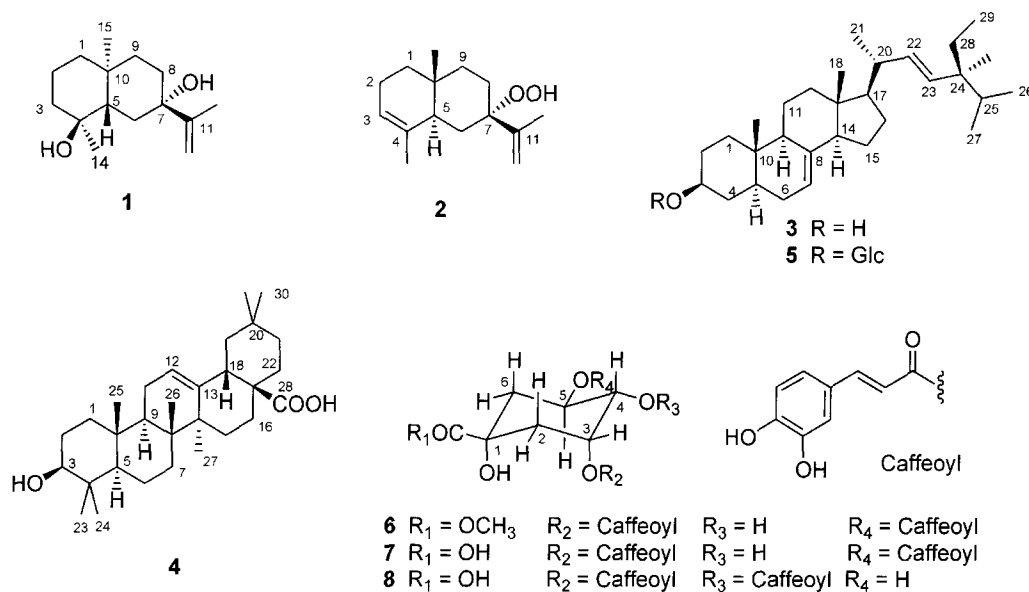


Fig. 1. Structures of compounds 1-8

olefinic carbons (δ 111.7, 121.3, 134.5, 148.4), three methyl carbons (δ 14.9, 18.9, 20.9) and an oxygenated carbon (δ 85.9). These data implies that the compound **1** was presumed to be a eudesmane type sesquiterpene with a tertiary hydroperoxide group. The hydroperoxide group was assigned at C-7, considering the downfield shift of the carbons at C-11 (δ 148.4) and C-12 (δ 111.7) (Bohlmann *et al.*, 1975). These data suggested that **2** is 7-hydroperoxy-3,11-eudesmadiene. The chemical shift of β -methyl group at C-10 in eudesmane sesquiterpenes was shifted upfield by 5-10 ppm comparing with α -methyl group in ^{13}C -NMR spectrum (Oliveira *et al.*, 2000; Fraga *et al.*, 1993). The C-15 (δ 14.9) of **2** was shifted upfield by 7 ppm comparing with α -methyl group at C-10, therefore C-15 of **2** should be β -orientation. The absence of correlation between H-5 and H-15 in NOESY spectrum indicated their *trans*-configuration, therefore, H-5 should be α -orientation (axial). The chemical shift of C-11 in eudesmane sesquiterpenes with C-7 α hydroxy group shifted upfield by 5 ppm comparing with C-7 β hydroxy group and C-12 at C-7 α hydroxy group shifted downfield by 5ppm comparing with C-7 β hydroxy group in the ^{13}C -NMR spectrum (Oliveira *et al.*, 2000; Fraga *et al.*, 1995). The downfield shift of H-5a (δ 2.36, brd) in the ^1H -NMR spectrum may be ascribed to the same plane of the 7-hydroperoxide group, therefore, the stereochemistry of hydroperoxide was to be α -orientation (axial) (Lu *et al.*, 1993). The structure of **2** was determined as 7 α -hydroperoxy-3,11-eudesmadiene. The correlations in the HMBC and NOESY spectra were in full support of the proposed structure (Table I). Compound **2** was first isolated from natural sources.

Compounds **3** (α -spinasterol, Goad, 1997), **4** (oleanolic acid, Mahato *et al.*, 1994; Ahmad *et al.*, 1994) and **5** (α -

spinasterol 3-O- β -D-glucopyranoside, Iida *et al.*, 1980) were characterized by comparing their physical and spectroscopic data with those of reported literatures.

Compound **6** was obtained as yellowish gum ($[\alpha]_D$ -190.0°) and its molecular formula was determined to be $\text{C}_{26}\text{H}_{27}\text{O}_{12}$ by FABMS (m/z 531, $[\text{M}+\text{H}]^+$). The ^1H -NMR spectrum showed signals by two *trans*-caffeoyl groups [δ 7.48 & 7.41 (1H each, d, J = 16.0 Hz), 7.04 & 7.04 (1H each, d, J = 2.5 Hz), 6.99 & 6.99 (1H each, d, J = 8.0 Hz), 6.77 & 6.76 (1H each, d, J = 8.0 Hz) and 6.24 & 6.12 (1H each, d, J = 16.0 Hz)] and three oxygenated protons [δ 3.95 (1H, dd, J = 9.5, 3.0 Hz), 5.29 (1H, dt, J = 7.0, 3.5 Hz), and 5.37 (1H, brdd, J = 9.5, 4.5 Hz)]. The ^{13}C -NMR spectrum showed two methylene carbons at δ 35.6 and 35.9, four oxygenated carbons at δ 67.8, 71.0, 72.0 and 73.6, and a carbonyl carbon signal at δ 174.8. The above observed ^1H - and ^{13}C -NMR spectral data were typical in dicaffeoyl quinic acids. The position of two caffeoyls on quinic acid was established by the downfield shift of the proton signals at δ 5.29 (H-5) and δ 5.37 (H-3) and carbon signals at δ 71.0 (H-5) and (δ 72.0 (H-3) spectra. Thus, the structure of compound **6** was determined as methyl 3,5-dicaffeoyl quinate. The NMR spectral and physical data of compound **6** were in good agreement with those reported in the literature (Lin *et al.*, 1999).

Compound **7** was obtained as yellowish gum ($[\alpha]_D$ -220.4°) and its molecular formula was determined to be $\text{C}_{25}\text{H}_{24}\text{O}_{12}$ by FABMS (m/z 517, $[\text{M}+\text{H}]^+$) and ^1H , ^{13}C -NMR spectral data. Its IR spectrum displayed absorption bands at 3300 and 1690 cm^{-1} , indicating the presence of hydroxy and ester groups. ^1H and ^{13}C -NMR spectra of **7** were similar to those of **6**. The major difference between the two compounds was the absence of methoxy signal (δ 53.0) and

the presence of acid group (δ 178.9, COOH) in **7**. Thus, the structure of compound **7** was determined as 3,5-dicaffeoyl quinic acid. The NMR spectral and physical data of compound **7** were in good agreement with those reported in the literature (Basnet *et al.*, 1996).

Compound **8** was obtained as yellowish gum ($[\alpha]_D^{25}$ -219.0°) and its molecular formula was determined to be C₂₅H₂₃O₁₂ by FABMS (m/z 517, [M+H]⁺). ¹H and ¹³C-NMR spectra of **8** indicated the presence of two *trans*-caffeoyl groups and were also similar to those of **7**. The major differences were the chemical shift of H-4 and H-5 at the quinic acid moiety in **8**. The H-4 of **8** was shifted downfield by 1.73 ppm and the H-5 shifted upfield by 1.04 ppm relative to those of **7**, also, the H-3 and H-4 was shifted downfield by about 1.6 ppm relative those of free quinic acid (Iwahashi *et al.*, 1985). Thus, the structure of compound **8** was determined as 3,4-dicaffeoyl quinic acid. The NMR spectral and physical data of compound **8** were in good agreement with those reported in the literature (Basnet *et al.*, 1996).

The cytotoxicities of the compound **2** was tested by SRB (Sulforhodamin B) bioassay against five cultured human tumor cells. Compound **2** exhibited moderate cytotoxicity, with ED₅₀ values of 15.16, 17.21 and 3.86 µg/mL against SK-OV-3, SK-MEL-2 and HCT15, respectively, meanwhile, no activities against A549 and XF498.

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