Three New Oleanane-Type Triterpenes from *Ludwigia octovalvis* with Cytotoxic Activity against Two Human Cancer Cell Lines

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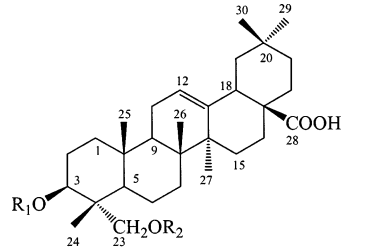
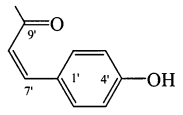
Three new oleanane-type triterpenes, (23*Z*)-coumaroylhederagenin (**1**), (23*E*)-coumaroylhederagenin (**2**), and (3*Z*)-coumaroylhederagenin (**3**), together with two known triterpene acids, oleanolic acid and ursolic acid, have been isolated from the whole plant of *Ludwigia octovalvis*, and their structures have been elucidated by spectroscopic methods. All three new triterpenes showed significant cytotoxicity against two human tumor cell lines, namely, oral epidermoid carcinoma KB and colorectal carcinoma HT29, and gave IC_{50} values in the range 1.2−3.6 μM.

Ludwigia octovalvis (Jacq.) P. H. Raven (Onagraceae), an aquatic plant, is widely distributed in wet areas of Taiwan. It is used as a traditional treatment for edema, nephritis, and hypertension. Previously, studies have shown that a crude extract of *Ludwigia octovalvis* possesses antidiabetic[1] and immunosuppressive[2] activities. *Ludwigia* is a very variable genus that contains over 80 species. Few reports have appeared in the literature on the chemistry and biological activity of this genus. Only two species, *L. prostrata*[3,4] and *L. parviflora*,[5] have been investigated for their chemical constituents, and four components, ellagic acid, gallic acid, orientin, and triethylchebulate, have been reported. In continuation of our previous work on the discovery of bioactive constituents from plants, we have elucidated the biological components from the Taiwanese herb *L. octovalvis*. We examined the methanolic extract of *L. octovalvis* and isolated three new oleanane-type triterpenes (compounds **1**−**3**), together with two known triterpene acids, oleanolic acid[6] and ursolic acid.[7] In this paper, we report the extraction, isolation, purification, and structural elucidation of three new *cis*- and *trans*-coumaroyl esters of hederagenin (compounds **1**−**3**), as well as the results of the cytotoxicity evaluation of these compounds.

Compound **1**, obtained as an amorphous powder, gave a positive Liebermann-Burchard test. The HREIMS displayed an ion peak at *m*/*z* 618.3932 $[M]^+$, consistent with the molecular formula $C_{39}H_{54}O_6$. The IR spectrum showed the presence of hydroxyl (3360 cm^{-1}), conjugated double bond (1623 and 700 cm^{-1}), conjugated ester (1700 cm^{-1}), and phenyl group (1595 and 1509 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) indicated the presence of six tertiary methyl singlets [δ 0.68, 0.71, 0.88, 0.90, 0.90, and 1.09 (3H each, s)], an olefinic proton characteristic of H-12 [δ 5.23 (br t)] of an oleanene skeleton,[6] a (*Z*)-coumaroyloxymethylene group attached to a quaternary carbon [δ 3.79 and 4.26 (1H each, d, J = 11.4 Hz), δ 5.81 and 6.88 (1H each, d, J = 12.6 Hz), 6.77 and 7.49 (2H each, d, J = 8.1 Hz)], an oxymethine proton [δ 3.36 (1H, t, J = 8.1 Hz)], and a typical H_β-18 proton of oleanolic acid [δ 2.78 (1H, dd, J = 11.5, 1.8 Hz)].[6] The ^{13}C NMR spectrum of **1** (Table 2) showed two olefinic carbon signals [δ 122.5 (d), 143.6 (s)], which was in good agreement with those of C-12 and C-13 of olean-12-ene derivatives,[8] and signals of a (*Z*)-coumaroyl moiety [δ 115.2 (d), 116.7 (d), 127.1 (s), 132.0 (d), 144.0 (d), 157.3 (s), 167.2 (s)].[9] From these spectral characteristics, compound **1** was considered as a hederagenin derivative with a (*Z*)-coumaroyl moiety. The HMBC spectrum of **1** showed a long-range correlation between H-23 (δ_H 3.79, 4.26) and C-9′ (δ_C 167.2), and several key NOESY correlations (H-23/H-3, H_α-6; H-3/H-5) suggested that the coumaroyl group was attached to C-23 and the hydroxyl group at C-3 was β-oriented. Hence, compound **1** was established as (23*Z*)-coumaroylhederagenin.



1 R_1 = H, R_2 =



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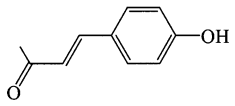
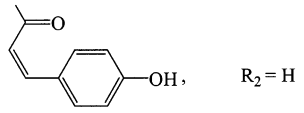


3 R_1 =



R_2 = H

Compound **2** was isolated as an amorphous powder and showed a molecular ion at *m*/*z* 618.3926, analyzing for $C_{39}H_{54}O_6$, and was seen to be an isomer of compound **1**. It also gave a positive Liebermann-Burchard test and showed IR absorption bands for hydroxyl (3370 cm^{-1}), conjugated double bond (1610 and 960 cm^{-1}), conjugated ester (1658 cm^{-1}), and phenyl (1590, 1580, and 1510 cm^{-1}) groups. The pattern of the proton signals (Table 1) was similar to those

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Table 1. ^{1}H NMR Data for **1**−**3** (500 MHz in $CDCl_3$)

no.	1	2	3
1	0.90 m, 1.58 m	0.96 m, 1.60 m	1.12 m, 1.61 m
2	1.10 m, 1.61 m	1.08 m, 1.67 m	1.22 m, 1.28 m
3	3.36 br t (8.1)	3.45 br t (7.8)	4.90 dd (4.5, 12.3)
5	0.92 m	1.08 m	1.33 m
6	1.33 m, 1.40 m	1.45 m, 1.53 m	1.30 m, 1.50 m
7	1.21 m, 1.31 m	1.30 m, 1.46 m	1.23 m, 1.50 m
9	1.50 m	0.94 m	1.63 m
11	1.85 m	1.89 m	1.88 m
12	5.23 br t (1.8)	5.26 br t (1.9)	5.26 br t (2.0)
15	1.05 m, 1.69 m	1.08 m, 1.71 m	1.08 m, 1.68 m
16	1.60 m, 1.96 m	1.60 m, 1.97 m	1.60 m, 1.97 m
18	2.78 dd (11.5, 1.8)	2.79 dd (11.6, 1.9)	2.79 dd (12.0, 2.0)
19	1.13 m, 1.59 m	1.13 m, 1.59 m	1.12 m, 1.60 m
21	1.20 m, 1.35 m	1.18 m, 1.33 m	1.19 m, 1.33 m
22	1.55 m, 1.75 m	1.55 m, 1.77 m	1.55 m, 1.73 m
23	3.79 d (11.4), 4.26 d (11.4)	3.86 d (11.7), 4.36 d (11.7)	2.94 d (12.3), 3.34 d (12.3)
24	0.71 s	0.80 s	0.59 s
25	0.90 s	0.94 s	0.90 s
26	0.68 s	0.73 s	0.73 s
27	1.09 s	1.10 s	1.03 s
29	0.88 s	0.88 s	0.86 s
30	0.90 s	0.90 s	0.88 s
2′, 6′	7.49 d (8.1)	7.39 d (8.4)	7.59 d (8.7)
3′, 5′	6.77 d (8.1)	6.82 d (8.4)	6.78 d (8.7)
7′	6.88 d (12.6)	7.60 d (15.9)	6.86 d (12.9)
8′	5.81 d (12.6)	6.27 d (15.9)	5.79 d (12.9)

Table 2. ^{13}C NMR Data for **1**−**3** (125 MHz in $CDCl_3$)

no.	1	2	3	no.	1	2	3
1	37.9	38.1	38.0	20	30.7	30.7	30.7
2	25.7	25.8	29.7	21	33.7	33.8	33.8
3	73.6	72.9	74.6	22	32.4	32.4	32.4
4	41.9	42.3	42.4	23	68.3	67.4	64.2
5	48.4	48.4	46.7	24	12.1	12.0	12.8
6	18.2	18.2	17.7	25	15.8	15.9	16.0
7	32.2	32.4	32.2	26	17.3	17.1	17.1
8	39.2	39.2	39.3	27	25.9	25.9	26.0
9	47.6	47.8	47.5	28	183.6	182.8	182.4
10	36.9	36.9	36.8	29	33.1	33.0	30.0
11	23.3	23.3	23.4	30	23.6	23.5	23.6
12	122.5	122.6	122.4	1′	127.1	126.9	127.3
13	143.6	143.5	143.8	2′, 6′	132.0	130.1	132.4
14	40.9	41.5	41.7	3′, 5′	115.2	115.9	115.0
15	27.6	27.6	27.7	4′	157.3	158.1	157.0
16	22.8	22.9	23.0	7′	144.0	145.2	144.3
17	45.8	46.5	45.9	8′	116.7	114.9	117.0
18	41.4	41.0	40.9	9′	167.2	167.8	167.5
19	46.5	45.8	46.5				

of compound **1**, including six tertiary methyl singlets, an olefinic proton characteristic of H-12 [δ 5.26 (br t)] of an oleanane skeleton,[6] an oxymethine proton [δ 3.45 (1H, br t, J = 7.8 Hz, H-3)], and a typical H_β-18 proton of oleanolic acid [δ 2.79 (1H, dd, J = 11.6. 1.9 Hz)]. The only difference is a (*E*)-coumaroyl group in **2** instead of a (*Z*)-coumaroyl group in **1**. The coumaroyl moiety was connected at C-23 due to the chemical shifts of the H-23 protons being similar to that of **1**. The HMBC spectrum of **2** showed a mutual correlation between H-23 (δ_H 3.86, 4.36) and C-9′ (δ_C 167.8) and together with the key NOESY correlations (H-23/H-3, H_α-6; H-3/H-5) further proved the coumaroyl group was attached to C-23 and the hydroxyl group at C-3 was β-oriented. In addition, NMR signals similar to those of compound **1** were observed (Table 2); thus, compound **2** was established as (23*E*)-coumaroylhederagenin.

The molecular formula of **3** was assigned as $C_{39}H_{54}O_6$, based on HREIMS, the same as **1** and **2**. It contained a (*Z*)-coumaroyl moiety due to IR absorption bands at 3380 (−OH), 1703 (conjugated ester), 1620 (conjugated double bond), 1595 and 1510 cm^{-1} (phenyl group) and ^{1}H NMR signals (Table 1) at δ 5.79 and 6.86 (1H each, d, J = 12.9 Hz) and δ 6.78 and 7.59 (2H each, d, J = 8.7 Hz). The ^{13}C NMR spectrum (Table 2) exhibited 39 carbon signals for six methyls, two olefinic carbons, one carbonyl, two oxygenated carbons (CH and CH_2), 10 methylenes, three methines, six quaternary carbons, and nine carbons from a coumaroyl unit. By comparison of the ^{1}H and ^{13}C NMR data of **3** with **2** and **1**, compound **3** was assigned as a hederagenin derivative with an extra (*Z*)-coumaroyl moiety. The (*Z*)-coumaroyl unit was linked at C-3 and caused a downfield shift of H-3 to δ 4.90 (dd, J = 12.3, 4.5 Hz). On the basis of the above evidence, compound **3** was assigned as (3*Z*)-coumaroylhederagenin. Compounds **1**−**3** all exhibited characteristic mass fragmentation peaks of oleanolic acid at *m*/*z* 248, 203, 133, and 119,[10] and a coumaroyl moiety fragmentation peak at *m*/z 147 was observed in their mass spectra.

Table 3. Cytotoxicity of Compounds **1**−**3**

cell line	cell type	growth inhibition constant (IC_{50}) [μM] 1	2	3	VP-16[a]
KB	oral epidermoid carcinoma	1.6 ± 0.10	1.3 ± 0.05	1.2 ± 0.01	1.1 ± 0.02
HT29	colorectal carcinoma	3.6 ± 0.08	2.4 ± 0.08	2.1 ± 0.04	2.3 ± 0.08

[a] Positive control substance.

The three new oleanane-type triterpenes, (23*Z*)-coumaroylhederagenin (**1**), (23*E*)-coumaroylhederagenin (**2**), and (3*Z*)-coumaroylhederagenin (**3**), were evaluated for their cytotoxic activity against human oral epidermoid carcinoma KB cells and colorectal carcinoma HT29 cells. After 72 h of treatment, all three exhibited IC_{50} ranges from 1.2 to 3.6 μM (Table 3). The potencies of these oleanane-type triterpenes for three cell lines were similar to the clinically used anticancer drug etoposide (VP-16, IC_{50} 1.1−2.3 μM) (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-180 digital spectropolarimeter. UV spectra were measured in MeOH on a Shimadzu UV-1601PC spectrophotometer. The IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. The NMR spectra were recorded in $CDCl_3$ at room temperature on a Bruker DMX-500 SB spectrometer, and the solvent resonance was used as internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EIMS and HREIMS were recorded on a Finnigan TSQ-700 and a JEOL SX-102A spectrometer, respectively. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). HPLC was performed using a Lichrosorb Si 60 (10 μm) column (250 × 10 mm).

Plant Material. The whole plant of *Ludwigia octovalvis* was collected in Ping-Tung, Taiwan, in July 2002. The plant material was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University. A voucher specimen (No. 174841) has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. Air-dried pieces of the whole plant of *L. octovalvis* (5 kg) were extracted three times with methanol (30 L) at room temperature (7 days each time). The MeOH extract was evaporated in vacuo to leave a black residue, which was suspended in H_2O (2 L) and then partitioned sequentially using EtOAc and *n*-BuOH (1 L × 3). The EtOAc fraction (86 g) was chromatographed on Si gel using *n*-hexane and EtOAc of increasing polarity as eluent to obtain eight fractions: fr. 1 [3000 mL, *n*-hexane/EtOAc (19:1)], fr. 2

[4000 mL, *n*-hexane/EtOAc (9:1)], fr. 3 [3000 mL, *n*-hexane/EtOAc (8:2)], fr. 4 [40000 mL, *n*-hexane/EtOAc (7:3)], fr. 5 [3000 mL, *n*-hexane/EtOAc (5:5)], fr. 6 [3000 mL, *n*-hexane/EtOAc (4:6)], fr. 7 [(3000 mL, *n*-hexane/EtOAc (2:8)), and fr. 8 (6000 mL, EtOAc). Fraction 5 was further chromatographed on a Si gel column (5 × 45 cm, Merck 230−400 mesh) eluted with CH_2Cl_2/EtOAc (8:1) to obtain eight fractions (each 700 mL): fr. 4A−4H. HPLC of fr. 4D on a Merck Lichrosorb Si 60 column (10 μm, 250 × 10 mn) with *n*-hexane/EtOAc (6:4) as eluent, 2 mL/min, afforded (23*Z*)-coumaroylhederagenin (**1**) (9 mg) and (23*E*)-coumaroylhederagenin (**2**) (12 mg), with retention times of 18.0 and 21.0 min, respectively. HPLC of fr. 4E on a Merck Lichrosorb Si 60 column (10 μm, 250 × 10 mn) as eluent, 3 mL/min, afforded (3*Z*)-coumaroylhederagenin (**3**) (12 mg), retention time 22.0 min. Fraction 5E gave oleanonic acid[6] (8 mg) and ursolic acid[7] (6 mg).

(23*Z*)-Coumaroylhederagenin (1): amorphous white powder; $[\alpha]^{25}_D$ +14.6° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3360, 1700, 1623, 1595, 1509, 700 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 209 (4.51), 227 (4.01), 312 (4.30) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 618 (M^+, 1), 600 (2), 436 (5), 248 (100), 203 (93), 133 (24), 119(24), 147 (58); HREIMS *m*/*z* $[M]^+$ 618.3932 (calcd for $C_{39}H_{54}O_6$, 618.3922).

(23*E*)-Coumaroylhederagenin (2): amorphous white powder; $[\alpha]^{25}_D$ +6.8 ° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3370, 1688, 1610, 1590, 1580, 1510, 960 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 206 (4.40), 230 (4.02), 314 (4.52) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 618 (M^+, 1), 600 (3), 436 (4), 248 (98), 203 (100), 147 (55), 133 (35), 119 (35); HREIMS *m*/*z* $[M]^+$ 618.3926 (calcd for $C_{39}H_{54}O_6$, 618.3922).

(3*Z*)-Coumaroylhederagenin (3): amorphous white powder; $[\alpha]^{25}_D$ +9.6° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3380, 1703, 1620, 1595, 1510, 705 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 209 (4.30), 226 (4.01), 311 (4.27) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 618 (M^+, 2), 600 (3), 436 (5), 248 (100), 203 (97), 147 (57), 133 (26), 119 (24); HREIMS *m*/*z* $[M]^+$ 618.3930 (calcd for $C_{39}H_{54}O_6$, 618.3922).

Cytotoxicity Assay. Human oral epidermoid carcinoma KB cells and colorectal carcinoma HT-29 cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cells in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. The cells were exposed to various concentrations of the test drugs for 72 h. The methylene blue dye assay was used to evaluate the effects of the test drugs on cell growth, as described previously.[11] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically in a comparison with the control.

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