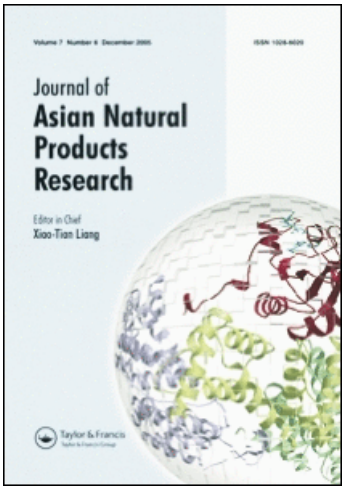

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C. -I. Chang[a]; Y. -H. Kuo[b]
[a] Graduate Institute of Biotechnology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan [b] Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

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Oleanane-type triterpenes from *Ludwigia octovalvis*

C.-I. CHANG† and Y.-H. KUO‡*

†Graduate Institute of Biotechnology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan
‡Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

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Two new oleanane-type triterpenes, (23*Z*)-feruloylhederagenin (**1**) and (23*E*)-feruloylhederagenin (**2**), together with two known oleanane-type triterpenes, β-amyrin acetate and β-amyrin palmitate, have been isolated from the whole plant of *Ludwigia octovalvis*. The structures of **1** and **2** were characterised on the basis of spectral evidence.

Keywords: *Ludwigia octovalvis*; Onagraceae; Triterpenes; (23*Z*)-Feruloylhederagenin; (23*E*)-Feruloylhederagenin

1. Introduction

Many aquatic plants have been used traditionally as folk medicines in Taiwan. One of these, *Ludwigia octovalvis* (Jacq.) P.H. Raven (Onagraceae), was used for the treatment of oedema, nephritis, and hypertension [1]. The crude extract of *L. octovalvis* has been reported possessing antidiabetic [2] and immunosuppressive [3] activities. Over 80 species of *Ludwigia* are present in the world, but few reports described their chemistry and biological activity. Only the other two species, *L. prostrate* [4,5] and *L. parviflora* [6], have been investigated for their chemical constituents, and four components have been reported. On the basis of our previous works towards the discovery of bioactive constituents from natural products, we continued our effort to search for the biological components from Taiwanese herbs. Recently, we had reported three new triterpenes which showed significant cytotoxicity against two human tumour cell lines, including oral epidermoid carcinoma KB and colorectal carcinoma HT29, with IC_{50} in the range 1.2–3.6 μM from the whole plant of *L. octovalvis* [7]. On the basis of these initially promising results, these new oleanane-type triterpenes merit further study as potential anticancer agents. In our continuing work on the same extract, we also isolated two new oleanane–type triterpenes (compounds **1** and **2**), together with two known triterpenes with the same skeleton, β-amyrin palmitate [8] and β-amyrin acetate [9].

*Corresponding author. Email: yhkuo@ntu.edu.tw

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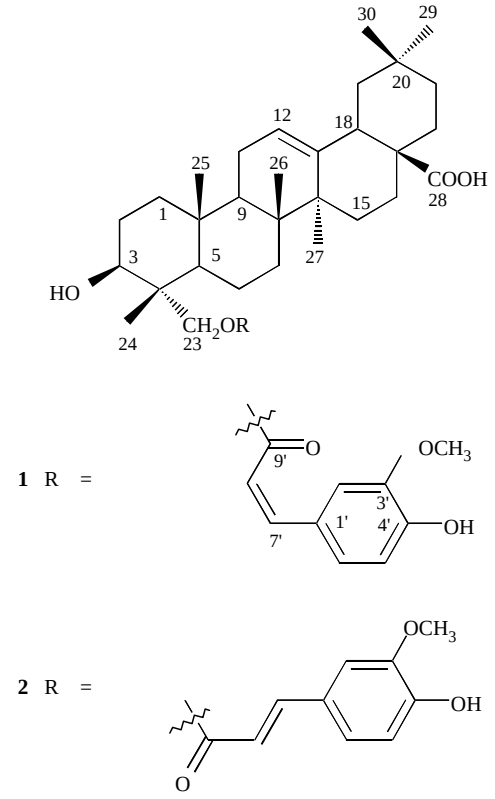

Figure 1. The structures of compounds **1** and **2**.

In this paper, we report the extraction, isolation, purification, and structural elucidation of two new *cis*- and *trans*-feruloyl esters of hederagenin (compounds **1** and **2**, figure 1).

2. Results and discussion

The MeOH extract of *Ludwigia octovalvis* was suspended in H_2O, and then partitioned sequentially using EtOAc and *n*-BuOH. Extensive column and high performance liquid chromatography of the EtOAc fraction furnished (23*Z*)-feruloylhederagenin (**1**) and (23*E*)-feruloylhederagenin (**2**), together with β-amyrin palmitate and β-amyrin acetate.

Compound **1**, obtained as an amorphous powder, gave a positive Liebermann–Burchard test for an unsaturated triterpenoid. The HREI-MS of ion peak at *m*/*z* 648.4069 $[M]^+$, consistent with the molecular formula $C_{40}H_{56}O_7$. The EI-MS spectrum exhibited characteristic fragmentation of ferulic acid (*m*/*z* 177) and the Δ^{12}-triterpene skeleton (*m*/*z* 203, 248), and by cleavage of the ester bond, the ions corresponding to oleanolic acid (*m*/*z* 454) and phenolic acid moieties [10,11]. The IR spectrum showed the presence of hydroxyl (3350 cm^{-1}), conjugated double bond (1620 and 700 cm^{-1}), conjugated ester (1691 cm^{-1}), and phenyl group (1595 and 1510 cm^{-1}) functionalities. The ^{1}H-NMR spectrum of **1** (table 1) indicated the presence of six tertiary methyl singlets [δ 0.72, 0.75, 0.88, 0.91, 0.91, and 1.05 (3H each, s)], an olefinic proton characteristic of H-12 [δ 5.28 (br t)] of an oleanene skeleton

Table 1. ^{1}H- and ^{13}C-NMR data for **1** and **2** (500 MHz in $CDCl_3$).

	1		***2***	
No.	δ_C	δ_H	δ_C	δ_H
1	38.2 t	0.91 m, 1.57 m	38.2 t	0.94 m, 1.60 m
2	25.9 t	1.10 m, 1.61 m	25.8 t	1.10 m, 1.60 m
3	72.7 d	3.35 br t (8.0)	72.7 d	3.44 br t (8.1)
4	41.6 s		42.4 s	
5	48.1 d	0.95 m	48.3 d	1.07 m
6	18.2 t	1.33 m, 1.41 m	18.1 t	1.38 m, 1.50 m
7	32.2 t	1.22 m, 1.30 m	32.2 t	1.31 m, 1.45 m
8	39.2 s		39.3 s	
9	47.7 d	1.50 m	47.8 d	1.58 m
10	36.9 s		37.0 s	
11	23.4 t	1.88 m	23.4 t	1.87 m
12	122.6 d	5.28 br t (1.8)	122.6 d	5.26 br t (1.8)
13	143.5 s		143.5 s	
14	40.9 s		41.5 s	
15	27.6 t	1.06 m, 1.65 m	27.6 t	1.08 m, 1.68 m
16	22.9 t	1.61 m, 1.92 m	22.9 t	1.60 m, 1.92 m
17	46.4 s		46.4 s	
18	41.4 d	2.80 dd (11.4, 1.8)	41.1 d	2.80 dd (11.4, 1.8)
19	45.8 t	1.14 m, 1.58 m	45.8 t	1.13 m, 1.59 m
20	30.7 s		30.7 s	
21	33.8 t	1.20 m, 1.33 m	33.8 t	1.19 m, 1.32 m
22	32.4 t	1.55 m, 1.72 m	32.4 t	1.56 m, 1.73 m
23	67.2 t	3.82 d (11.4)	67.3 t	3.88 d (12.2)
		4.25 d (11.4)		4.37 d (12.2)
24	12.0 q	0.75 s	12.0 q	0.80 s
25	15.8 q	0.91 s	15.9 q	0.95 s
26	17.1 q	0.72 s	17.1 q	0.72 s
27	25.9 q	1.05 s	25.9 q	1.11 s
28	182.7 s		182.7 s	
29	33.1 q	0.88 s	33.1 q	0.86 s
30	23.5 q	0.91 s	23.5 q	0.91 s
1′	126.2 s		126.3 s	
2′	112.5 d	7.76 d (1.6)	109.4 d	7.01 d (1.5)
3′	145.8 s		146.4 s	
4′	146.4 s		148.1 s	
5′	113.8 d	6.86 d (8.0)	114.8 d	6.91 d (8.2)
6′	125.8 d	7.03 dd (8.0, 1.6)	123.2 d	7.06 dd (8.2, 1.5)
7′	144.1 d	6.83 d (12.5)	145.4 d	7.61 d (16.0)
8′	116.0 d	5.81 d (12.5)	115.1 d	6.28 d (16.0)
9′	167.7 s		167.7 s	
$-OCH_3$	56.0 q	3.90 s	56.0 q	3.92 s

[12], a (*Z*)-feruloyloxymethylene group attached to a quaternary carbon [δ 3.82 and 4.25 (1H each d, $J = 11.4$ Hz), 5.80 (1H, s, $-$OH, disappeared on D_2O exchange), 5.81 and 6.83 (1H each, d, $J = 12.5$ Hz), 7.03 (1H, dd, $J = 8.0$, 1.6 Hz), 7.76 (1H, d, $J = 1.6$ Hz), and 6.86 (1H, d, $J = 8.0$ Hz), 3.90 (3H, s)], an oxymethine proton [δ 3.35 (1H, t, $J = 8.0$ Hz)], and a typical H_β-18 proton of oleanolic acid [δ 2.80 (1H, dd, $J = 11.4$, 1.8 Hz)] [12]. The methoxy group [δ 3.90 (3H, s)] of (*Z*)-feruloyl moiety showed a correlation with a signal at δ 7.76 (1H, d, $J = 1.6$ Hz) in the NOESY spectrum. The ^{13}C NMR spectrum of **1** (table 1) show two olefinic carbon signals [δ 122.6(d), 143.5(s)] which was in good agreement with those of C-12 and C-13 of olean-12-ene derivatives [13] and signals of (*Z*)-feruloyl moiety [δ 112.5 (d), 113.8 (d), 116.0 (d), 125.8 (d), 126.2 (s), 144.1 (d), 145.8 (s), 146.4 (s), 167.7 (s)] [14]. From these characteristic spectral data, compound **1** was considered as a hederagenin derivative

with a (*Z*)-feruloyl moiety. The HMBC spectrum of **1** showed a long range correlation between H-23 (δ_H 3.82, 4.25) and C-9′ (δ_C 167.7) and several key NOESY correlations (H-23/H-3, H_α-6; H-3/H-5) suggested that the feruloyl group attached to C-23 and the hydroxyl group at C-3 was in β-oriented. Hence, compound **1** was established as (23*Z*)-feruloylhederagenin.

Compound **2** was isolated as an amorphous powder and showed a molecular ion at *m/z* 648.4067, analysing for $C_{40}H_{56}O_7$, an isomer of compound **1**. It also gave positive Liebermann–Burchard test and showed IR absorption bands to hydroxyl (3360 cm^{-1}), conjugated double bond (1610, 960 cm^{-1}), conjugated ester (1690 cm^{-1}), and phenyl group (1590, 1580, and 1509 cm^{-1}). The pattern of the proton signals (table 1) was similar to those of compound **1**, including six tertiary methyl singlets, an olefinic proton characteristic of H-12 [δ 5.26 (br t)] of oleanane skeleton [12], an oxymethine proton [δ 3.44 (1H, t, $J = 8.1$ Hz, H-3)], and a typical H_β-18 proton of oleanolic acid [δ 2.80 (1H, dd, $J = 11.4$, 1.8 Hz)] [12]. The following data, δ 7.01 (1H, d, $J = 1.5$ Hz), 6.91 (1H, d, $J = 8.2$ Hz), 7.06 (1H, dd, $J = 8.2$, 1.5 Hz), 5.01 (1H, s. –OH, exchangeable), 6.28, 7.61 (each 1H, d, $J = 16.0$ Hz), and 3.92 (3H, s, NOESY correlation with δ 7.01), confirmed the presence of (*E*)-feruloyl functionality. The only difference is a (*E*)-feruloyl group in **2** instead of a (*Z*)-feruloyl group in **1**. The feruloyl moiety connected on C-23 due to the chemical shift of H-23 protons are similar to **1**. The HMBC spectrum of **2** showed mutual correlation between H-23 (δ_H 3.88, 4.37) and C-9′ (δ_C 167.7) together with the key NOESY correlations (H-23/H-3, H_α-6; H-3/H-5) further proved a feruloyl group attached to C-23 and a hydroxyl group at C-3 was β-oriented, in addition to similar NMR signals (table 1) to compound **1**; thus, compound **2** was established as (23*E*)-feruloylhederagenin.

3. Experimental

3.1 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. EI-MS and HREI-MS 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).

3.2 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.

3.3 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 8 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)), fr. 8 (6000 ml, EtOAc). Fr. 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 8 frs (each 700 ml), fr. 4A–fr. 4H. HPLC of fr. 4F on a Merck Lichrosorb Si 60 column (10 μm, 250 × 10 mm) with *n*-hexane/EtOAc (6:4) as eluent, 2 ml/min, afforded (23*Z*)-feruloylhederagenin (**1**) (2 mg) and (23*E*)-feruloylhederagenin (**2**) (3 mg), retention time: 23, 25 min, respectively. Fr. 3 gave β-amyrin palmitate (11 mg) and β-amyrin acetate (8 mg).

3.3.1 (23*Z*)-Feruloylhederagenin (1). Amorphous white powder; $[\alpha]_D^{25}$ +10.8 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (4.51), 231 (4.01), 304 (4.30) nm; IR (KBr) ν_{max} 3350, 1691, 1620, 1595, 1510, 700 cm^{-1}; ^1H NMR and ^{13}C NMR data, see table 1; EI-MS *m/z* 648 (M^+, 4), 602 (4), 454(6), 436 (7), 248 (100), 203 (97), 177(72), 147 (17), 133 (32), 119(28); HREI-MS *m/z* 648.4069 $[M]^+$ (calcd for $C_{40}H_{56}O_7$ 648.4028).

3.3.2 (23*E*)-Feruloylhederagenin (2). Amorphous white powder; $[\alpha]_D^{25}$ +7.6. (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.40), 219 (4.02), 306 (4.52) nm; IR (KBr) ν_{max} 3360, 1690, 1610, 1590, 1580, 1509, 960 cm^{-1}; ^1H NMR and ^{13}C NMR data, see table 1; EI-MS *m/z* 648 (M^+, 2), 602 (3), 454 (5), 436 (8), 248 (100), 203 (96), 147 (15), 133 (30), 119 (30); HREI-MS *m/z* 648.4067 $[M]^+$ (calcd for $C_{40}H_{56}O_7$ 648.4028).

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