D:C-Friedooleanane-Type Triterpenoids from *Lagenaria siceraria* and Their Cytotoxic Activity

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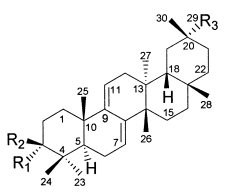
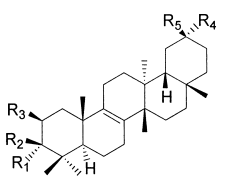
Four new D:C-friedooleanane-type triterpenes, 3β-*O*-(*E*)-feruloyl-D:C-friedooleana-7,9(11)-dien-29-ol (1), 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-ol (2), 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-oic acid (3), and methyl 2β,3β-dihydroxy-D:C-friedoolean-8-en-29-oate (6), together with five known triterpenes with the same skeleton, 3-epikarounidiol (4), 3-oxo-D:C-friedoolena-7,9(11)-dien-29-oic acid (5), bryonolol (7), bryononic acid (8), and 20-epibryonolic acid (9), were isolated from the methanol extract of the stems of *Lagenaria siceraria*. The structures of those compounds were elucidated using spectroscopic methods. Compounds 3 and 9 showed significant cytotoxic activity against the SK-Hep 1 cell line with IC_{50} values of 4.8 and 2.1 μg/ml, respectively. Based on these initially promising results, the two D:C-friedooleanane triterpenes merit further study as potential anticancer agents.

Key words *Lagenaria siceraria*; Cucurbitaceae; triterpene; friedooleanane; cytotoxic activity

Lagenaria siceraria (MOL.) STANDL. (bottle gourd), of the family Cucurbitaceae, is a climbing perennial plant widely cultivated as a vegetable crop in Taiwan and has been used as a folk medicine for detoxification. Previous investigations demonstrated that the crude extracts of fruits of *L. siceraria* possess antiinflammatory activity.[1)] There are also reports of the isolation of some flavonoids glycosides[2)] and cucurbitane-type triterpenes[3)] from the fruit of this plant. In our preliminary assay, the methanolic extract of the stems of *L. siceraria* exhibited cytotoxic activity against the SK-Hep-1 cell line (human hepatoma), which led us to investigate its bioactive principles. This resulted in the isolation of four new D:C-friedooleanane-type triterpenes, 3β-*O*-(*E*)-feruloyl-D:C-friedooleana-7,9(11)-dien-29-ol (**1**), 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-ol (**2**), 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-oic acid (**3**), and methyl 2β,3β-dihydroxy-D:C-friedoolean-8-en-29-oate (**6**), together with five known triterpenes with the same skeleton, 3-epikarounidiol (**4**),[4)] 3-oxo-D:C-friedoolena-7,9(11)-dien-29-oic acid (**5**),[4)] bryonolol (**7**),[5)] bryononic acid (**8**),[6)] and 20-epibryonolic acid (**9**).[7)] This paper deals with the extraction, purification, and structural elucidation of those constituents on basis of spectroscopic analysis, as well as the antineoplastic *in vitro* evaluation of these compounds.

Compound **1** was isolated as a colorless amorphous solid. Its HR-EI-MS spectrum showed a molecular ion peak at *m/z* 616.4132, which corresponded to the molecular formula of $C_{40}H_{56}O_5$ and indicated 13 degrees of unsaturation. The IR spectrum displayed absorptions for hydroxyl (3403 cm^{-1}), conjugated ester (1703 cm^{-1}), conjugated double bond (1635, 1587, 817 cm^{-1}), and phenyl group (1587, 1514 cm^{-1}) functionalities. The ^{1}H-NMR spectrum of **1** (Table 1) showed the resonances of seven tertiary methyls [δ_H 0.83, 0.89, 0.90, 0.93, 0.97, 1.02, 1.06 (3H each, s)], two olefinic protons [δ_H 5.24 (1H, br s), 5.47 (1H, br s)], a hydroxymethyl group attached to a quaternary carbon [δ_H 3.21 (1H, d, J=10.4 Hz), 3.51 (1H, d, J=10.4 Hz)], and an oxomethine in proximity to an ester group [δ_H 4.62 (1H, dd, J=3.6, 11.2 Hz)]. In addition, the ^{1}H-NMR spectrum of **1** revealed the presence of an (*E*)-feruloyl moiety constituted by two coupled olefinic protons [δ_H 6.28 (1H, d, J=16.0 Hz) and 7.58 (1H, d, J=16.0 Hz)], a set of ABX aromatic proton signals [6.89 (1H, d, J=8.0 Hz), 7.02 (1H, d, J=1.6 Hz), and 7.05 (1H, dd, J=1.6, 8.0 Hz)], and a methoxy group [δ_H 3.92 (3H, s), which had a nuclear Overhauser effect (NOE) correlation with a signal at δ_H 7.02]. Forty carbon signals were observed in the ^{13}C-NMR spectrum of **1** (Table 2) and identified by the distortionless enhancement by polarization transfer (DEPT) experiments as seven sp^3 methyls, 10 sp^3 methylenes including one oxygenated carbon (δ_C 71.0), three sp^3 methines with one oxygenated carbon (δ_C 80.7), six quaternary sp^3 carbons, seven olefinic methines, five quaternary sp^2 carbons, one methoxy carbon (δ_C 55.9), and one conjugated carbonyl (δ_C 167.0). The UV absorption at λ_{max} 235 nm, together with the olefinic signals in the ^{1}H- and ^{13}C-NMR spectra of **1** (δ_H 5.24, 5.47; δ_C 114.6, 118.0, 142.1, 144.1) are consistent with a $\Delta^{7,9(11)}$-conjugated diene system,[8)] which led to the proposal that compound **1** is a D:C-friedooleana-7,9(11)-diene triterpene. The $\Delta^{7,9(11)}$-conjugated diene system was further supported by the heteronuclear multiple bond coherence (HMBC) correlations between H-6 (δ_H 2.22) and C-7 (δ_C 118.0), and between H-12 (δ_H 2.11) and C-11 (δ_C 114.6). In comparisons of the ^{1}H- and ^{13}C-NMR data with the known compound 3-epikarounidiol (**4**),[4)] their signals pattern were very similar to each other, except for an (*E*)-feruloyl substituent in **1** instead of a hydroxyl group in **4**. A downfield shift of H-3





	R_1	R_2	R_3
1	H	*O*-(*E*)-feruloyl	CH_2OH
2	H	*O*-(*E*)-coumaroyl	CH_2OH
3	H	*O*-(*E*)-coumaroyl	COOH
4	H	OH	CH_2OH
5	= O		COOH

	R_1	R_2	R_3	R_4	R_5
6	H	OH	OH	$COOCH_3$	CH_3
7	H	OH	H	CH_2OH	CH_3
8	= O		H	COOH	CH_3
9	H	OH	H	CH_3	COOH
10	H	OH	H	$COOCH_3$	CH_3

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Table 1. ^{1}H-NMR Data for **1**—**3**, **6** and **7** (400 MHz in $CDCl_3$)

Position	1	2	3	6	7
1	1.63 m, 1.82 m	1.62 m, 1.82 m	1.59 m, 1.80 m	1.32 m, 2.15 m	1.19 m, 1.78 m
2	1.64 m, 1.76 m	1.63 m, 1.75 m	1.65 m, 1.84 m	4.10 dd (4.0, 6.8)	0.88 m, 1.70 m
3	4.62 dd (3.6, 11.2)	4.61 dd (4.0, 10.4)	4.58 dd (4.4, 11.6)	3.18 d (4.0)	3.21 dd (4.8, 11.6)
5	1.39 m	1.36 m	1.32 m	1.12 m	1.08 m
6	2.08 m, 2.22 m	2.01 m, 2.25 m	2.06 m, 2.20 m	1.47 m, 1.69 m	0.94 m, 1.68 m
7	5.47 br s	5.47 br s	5.41 br s	1.88 m, 2.07 m	1.94 m, 2.08 m
11	5.24 br s	5.22 br s	5.20 br s	1.94 m	1.84 m, 2.04 m
12	1.71 m, 2.11 m	1.71 m, 2.10 m	1.70 m, 2.16 m	1.23 m, 1.58 m	1.37 m, 1.50 m
15	1.36 m, 1.67 m	1.35 m, 1.66 m	1.27 m, 1.55 m	1.29 m, 1.48 m	1.49 m, 1.68 m
16	1.41 m, 1.69 m	1.42 m, 1.70 m	1.40 m, 1.70 m	1.32 m, 1.64 m	1.47 m, 1.54 m
18	1.58 m	1.58 m	1.53 m	1.48 m	1.55 m
19	1.35 m, 1.68 m	1.30 m, 1.70 m	1.72 m, 2.34 br d (16.0)	1.61 m, 2.35 d (15.6)	1.20 m, 1.58 m
21	1.24 m, 1.46 m	1.24 m, 1.46	1.43 m, 2.22 m	1.35 m, 2.16 m	1.28 m, 1.42m
22	0.88 m, 1.63 m	0.89 m, 1.65 m	0.89 m, 2.02 m	0.88 m, 2.06 m	0.92 m, 1.54 m
23	0.89 s	0.87 s	0.86 s	0.98 s	0.98 (s)
24	1.02 s	1.00 s	0.99 s	0.98 s	0.78 (s)
25	0.93 s	0.93 s	0.92 s	1.20 s	0.93 (s)
26	0.90 s	0.90 s	0.72 s	0.93 s	1.08 (s)
27	0.83 s	0.83 s	0.84 s	0.71 s	0.94 (s)
28	1.06 s	1.06 s	1.00 s	1.00 s	1.10 (s)
29	3.21 d (10.4), 3.51 d (10.4)	3.21 d (10.8), 3.51 d (10.8)			3.23 d (10.4), 3.40 d (10.4)
30	0.97 s	0.97 s	1.23 s	1.15 s	0.96 (s)
2′	7.02 d (1.6)	7.42 d (8.4)	7.42 d (8.8)		
3′		6.81 d (8.4)	6.82 d (8.8)		
5′	6.89 d (8.0)	6.81 d (8.4)	6.82 d (8.8)		
6′	7.05 dd (1.6, 8.0)	7.42 d (8.4)	7.42 d (8.8)		
7′	7.58 d (16.0)	7.59 d (16.0)	7.61 d (16.0)		
8′	6.28 d (16.0)	6.29 d (16.0)	6.29 d (16.0)		
OCH_3	3.92 s			3.59 s	

at δ_H 4.62 (1H, dd, J=3.6, 11.2 Hz) and the HMBC correlation between H-3 and C-9′ (δ_C 167.0) confirmed that the (*E*)-feruloyl group was attached to C-3. Hence, compound **1** was characterized as 3β-*O*-(*E*)-feruloyl-D:C-friedooleana-7,9(11)-dien-29-ol. Complete ^{1}H- and ^{13}C-NMR chemical shifts were established based on ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra.

Compound **2**, obtained as a colorless amorphous solid, gave a positive Liebermann–Burchard test. HR-EI-MS, ^{13}C-NMR, and DEPT spectra established the molecular formula of **2** as $C_{39}H_{54}O_4$, indicating 13 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl, conjugated ester, conjugated double bond, and phenyl group functionalities. The pattern of the ^{1}H-NMR spectrum (Table 1) of **2** closely resembled that of **1**, including seven tertiary methyls, two olefinic protons, an axial-oriented oxomethine proton, and a hydroxymethyl group attached to a quaternary carbon. The only difference was that the signals of the (*E*)-feruloyl moiety in **1** were replaced by a set of signals of an (*E*)-coumaroyl moiety, including the following resonances: δ_H 6.29 (1H, d, J=16.0 Hz), 6.81 (2H, d, J=8.4 Hz), 7.42 (2H, d, J=8.4 Hz), and 7.59 (1H, d, J=16.0 Hz). The (*E*)-coumaroyl moiety attached to C-3 was confirmed by the HMBC correlation between H-3 (δ_H 4.61) and C-9′ (δ_C 167.1). Thus compound **2** was elucidated as 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-ol.

The molecular formula of compound **3** was assigned as $C_{39}H_{52}O_2$ on the basis of HR-EI-MS ([M]$^+$ 600.3816), ^{13}C-NMR, and DEPT spectra, indicating the presence of 14 degrees of unsaturation. The IR spectrum showed absorption bands at 3354 cm^{-1} (hydroxyl), 1693 cm^{-1} (carbonyl), conjugated double bond (1630, 1588, 832 cm^{-1}), and phenyl group (1601, 1514 cm^{-1}) functionalities. The UV spectrum displayed two absorption maxima at λ_{max} 236 and 315 nm. The ^{1}H- and ^{13}C-NMR spectra of **3** (Tables 1, 2) exhibited seven tertiary methyls [δ_H 0.72, 0.84, 0.86, 0.92, 0.99, 1.00, 1.23 (3H each, s)], two olefinic protons [δ_H 5.20 (1H, br s), 5.41 (1H, br s)], an axial-oriented oxomethine proton [δ_H 4.58 (1H, dd, J=4.4, 11.6 Hz)], and a set of signals of (*E*)-coumaroyl [δ_H 6.29 (1H, d, J=16.0 Hz), 6.82 (2H, d, J=8.8 Hz), 7.42 (2H, d, J=8.8 Hz), 7.61 (1H, d, J=16.0 Hz); δ_C 116.1 (d), 116.4 (d), 127.6 (s), 130.2 (d), 144.4 (d), 157.8 (s), 167.6 (s)]. These spectroscopic characteristics closely resembled those of **2**. Unambiguous comparison of ^{1}H- and ^{13}C-NMR data between **2** and **3** revealed that the AB-type coupling proton signals of the hydroxymethylene of **2** were absent, instead, and an isolated carboxylic acid signal at δ_C 182.8 (C-29) was observed, indicating that **3** is an oxidized derivative of **2**. The carboxylic acid was assigned at C-29, which was supported by both HMBC correlations between H-19 (δ_H 2.34)/C-29 and H-30 (δ_H 1.23)/C-29 and NOE correlation between H-18 (δ_H 1.53)/H-30 (δ_H 1.23). Compound **3** was accordingly determined to be 3β-*O*-(*E*)-coumaroyl-D:C-friedooleana-7,9(11)-dien-29-oic acid.

Compound **6** was deduced to be a triterpenoid due to a positive Liebermann–Burchard test and was assigned the molecular formula of $C_{31}H_{50}O_4$ on the basis of the molecular ion peak at *m*/*z* 486.3705 in the HR-EI-MS, indicating the presence of seven degrees of unsaturation. The IR spectrum of **6** showed absorptions attributable to hydroxyl group (3369 cm^{-1}) and ester (1723 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 1) displayed seven tertiary methyls [δ_H 0.71, 0.93, 1.00, 1.15, 1.20 (3H each, s), 0.98 (3H×2, s)], two oxomethines [δ_H 3.18 (1H, d, J=4.0 Hz), 4.10 (1H,

dd, J=4.0, 6.8 Hz)], and one methoxy [δ_H 3.59 (3H, s)]. The ^{13}C-NMR spectrum of **6** (Table 2) revealed 31 carbon signals, which were assigned by DEPT experiments as seven sp^3 methyls, 10 sp^3 methylenes, four sp^3 methines with two oxygenated carbons, six quaternary sp^3 carbons, two quaternary olefinic carbons, one methoxy carbon, and one carbonyl. Based on the above characteristics and the ^{13}C-NMR chemical shifts of the tetrasubstituted double bond (δ_C 133.2, 134.7), compound **6** was considered to be a D:C-friedoolean-8-ene skeletal triterpene with a methyl ester and two hydroxyl substituents. By comparison of ^{1}H- and ^{13}C-NMR data with the known compound, 3-hydroxy-D:C-friedoolean-8-en-29-oic acid methyl ester,[9] the NMR spectra of **6** were very similar to those of **10**, except for the signals of the A ring part. The difference was the presence of an additional oxygenated methine [δ_H 4.10 (1H, dd, J=4.0, 6.8 Hz)], and the doublet of the doublet proton signal of H-3 in **10** was replaced by a broad doublet proton signal [δ_H 3.18 (1H, d, J=4.0 Hz)] in the ^{1}H-NMR spectrum. Two oxygenated methines located at C-2 and C-3, respectively, were confirmed by the HMBC correlations between H-5 (δ_H 1.12)/C-3 (δ_C 78.4) and H-2 (δ_H 4.10)/C-4 (δ_C 38.2). The NOE correlations between H-3 (δ_H 3.18) and H-5, together with the coupling constant of 4.0 Hz between H-2 (δ_H 4.10) and H-3, indicated that two hydroxyl groups were in a vicinal position and both in the β orientation. Therefore compound **6** was determined to be methyl 2β,3β-dihydroxy-D:C-friedoolean-8-en-29-oate.

Compound **7** was deduced to be a triterpenoid due to a positive Liebermann–Burchard test. The IR spectrum of **7** showed bands attributable to hydroxyl group (3330 cm^{-1}) functionality. The ^{1}H- and ^{13}C-NMR data (Tables 1, 2) indicated the presence of seven tertiary methyls [δ_H 0.78, 0.93, 0.94, 0.96, 0.98, 1.08, 1.10 (3H each, s)], an oxomethylene [δ_H 3.23 (1H, d, J=10.4 Hz), 3.40 (1H, d, J=10.4 Hz); δ_C 72.7], and an equatorial-orientated oxymethine [δ_H 3.21 (1H, dd, J=4.8, 11.6 Hz, H-3)]. In addition, the ^{13}C-NMR spectrum of **7** showed 30 carbon signals, containing two quaternary olefinic carbons signals (δ_C 133.4, 135.3) and its EI-MS spectrum displayed a molecular ion peak at m/z 442. Thus compound **7** was also considered to be a D:C-friedoolean-8-ene-type triterpene. The ^{1}H-NMR data of methyls of **7** were consistent with those of the known compound, bryonolol, which is the hydrolysis product of bryonolol diacetate, isolated from the seeds of *Trichosanthes kirilowii* by Akihisa *et al.*[4] In the paper, we reported the full assignments of ^{1}H- and ^{13}C-NMR chemical shift of **7** for the first time.

These D:C-friedooleane-type triterpenes were evaluated for their cytotoxic activity against human hepatoma SK-Hep 1 cells with etoposide as a positive control (IC_{50}=2.2 μg/ml). After 48 h of culture, compounds **3** and **9** exhibited significant growth inhibitory activity against the SK-Hep 1 cell line with IC_{50} of values 4.8 and 2.1 μg/ml, respectively. The other compounds were inactive with IC_{50} values of more than 10 μg/ml.

Table 2. ^{13}C-NMR Data for **1**—**3**, **6** and **7** (100 MHz in $CDCl_3$)

Position	**1**	**2**	**3**	**6**	**7**
1	35.2 t	35.2 t	35.3 t	40.7 t	35.0 t
2	24.3 t	24.3 t	24.5 t	71.4 d	27.9 t
3	80.7 d	80.7 d	81.2 d	78.4 d	79.0 d
4	38.1 s	38.1 s	38.3 s	38.2 s	38.8 s
5	47.9 d	47.9 d	48.3 d	50.4 d	50.7 d
6	23.7 t	23.7 t	23.9 t	18.8 t	19.2 t
7	118.0 d	118.0 d	118.0 d	27.4 t	27.4 t
8	142.1 s	142.1 s	141.9 s	133.2 s	135.3 s
9	144.1 s	144.1 s	144.3 s	134.7 s	133.4 s
10	36.2 s	36.2 s	36.4 s	36.9 s	37.5 s
11	114.6 d	114.6 d	114.6 d	20.7 t	20.8 t
12	39.2 t	39.2 t	39.1 t	30.3 t	30.7 t
13	39.9 s	39.9 s	40.7 s	37.1 s	37.6 s
14	37.3 s	37.3 s	37.7 s	41.8 s	40.6 s
15	27.4 t	27.4 t	27.2 t	24.9 t	26.6 t
16	36.7 t	36.7 t	37.2 t	37.0 t	36.3 t
17	31.6 s	31.6 s	31.6 s	30.9 s	31.1 s
18	44.4 d	44.4 d	45.4 d	44.7 d	43.0 d
19	27.1 t	27.1 t	30.7 t	30.7 t	28.6 t
20	32.7 s	32.7 s	40.4 s	40.4 s	33.1 s
21	29.8 t	29.8 t	29.7 t	29.9 q	28.9 t
22	34.2 t	34.2 t	32.8 t	34.4 t	37.4 t
23	27.6 q	27.6 q	27.9 q	29.6 q	28.0 q
24	16.6 q	16.6 q	16.7 q	17.0 q	15.6 q
25	20.7 q	20.7 q	20.8 q	21.6 q	19.8 q
26	22.0 q	22.0 q	19.2 q	22.0 q	25.8 q
27	19.1 q	19.1 q	20.5 q	17.1 q	18.0 q
28	31.0 q	31.0 q	31.2 q	31.2 q	31.3 q
29	71.0 t	71.0 t	182.8 s	179.3 s	72.7 t
30	29.9 q	29.9 q	33.3 q	32.9 q	27.7 q
1′	127.1 s	127.5 s	127.6 s		
2′	109.2 d	129.9 d	130.2 d		
3′	146.7 s	115.8 d	116.1 d		
4′	147.8 s	157.3 s	157.8 s		
5′	114.6 d	115.8 d	116.1 d		
6′	123.0 d	129.9 d	130.2 d		
7′	144.4 d	143.9 d	144.4 d		
8′	116.2 d	116.4 d	116.4 d		
9′	167.0 s	167.1 s	167.6 s		
OCH_3	55.9 q			51.5 q	

Experimental

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. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ at room temperature on a Varian Mercury plus 400 NMR spectrometer, and the solvent resonance was used as an internal shift reference (TMS as standard). The 2D NMR spectra were recorded using standard pulse sequences. EI-MS and HR-EI-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 (Merck). Column chromatography was performed on silica gel (230—400 mesh ASTM, Merck). HPLC was performed using a Lichrosorb silica gel 60 (5μm) column (250×10 mm).

Plant Material The stems of *L. siceraria* were collected in Ping-Tung, Taiwan, in July 2005. The plant material was identified by Prof. Sheng-Zehn Yang, Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen was deposited in the Herbarium of that institution.

Extraction and Isolation Air-dried pieces of the stems of *L. siceraria* (19.4 kg) were extracted three times with methanol (90 l) at room temperature (7 d each). The MeOH extract was evaporated *in vacuo* to give a black residue, which was suspended in H_2O (3 l), and then partitioned sequentially using EtOAc and *n*-BuOH (3 l×3). The EtOAc fraction (195 g) was chromatographed over silica gel, using mixtures of *n*-hexane and EtOAc of increasing polarity as eluents. Twenty-two fractions were collected: fr. 1 [4000 ml, *n*-hexane], fr. 2 [3000 ml, *n*-hexane–EtOAc (49 : 1)], fr. 3 [3000 ml, *n*-hexane–EtOAc (45 : 5)], fr. 4 [4000 ml, *n*-hexane–EtOAc (40 : 10)], fr. 5 [4000 ml, *n*-hexane–EtOAc (37 : 13)], fr. 6 [3000 ml, *n*-hexane–EtOAc (35 : 15)], fr. 7 [3000 ml, *n*-hexane–EtOAc (33 : 17)], fr. 8 [4000 ml, *n*-hexane–EtOAc (30 : 20)], fr. 9 [3000 ml, *n*-hexane–EtOAc (28 : 22)], fr. 10 [3000 ml, *n*-hexane–EtOAc (25 : 25)], fr. 11 [3000 ml, *n*-hexane–EtOAc (24 : 26)], fr. 12 [4000 ml, *n*-hexane–EtOAc (22 : 28)], fr. 13 [3000 ml, *n*-hexane–EtOAc (20 : 30)], fr. 14 [3000 ml, *n*-hexane–EtOAc (17 : 33)], fr. 15 [4000 ml, *n*-hexane–EtOAc (15 : 35)], fr. 16 [4000 ml, *n*-hexane–EtOAc

(12 : 38)], fr. 17 [3000 ml, *n*-hexane–EtOAc (10 : 40)], fr. 18 [3000 ml, *n*-hexane–EtOAc (8 : 42)], fr. 19 [4000 ml, *n*-hexane–EtOAc (5 : 45)], fr. 20 [3000 ml, *n*-hexane–EtOAc (3 : 47)], fr. 21 [3000 ml, *n*-hexane–EtOAc (1 : 49)], and fr. 22 (7000 ml, EtOAc). Fraction 16 was further chromatographed on a silica gel column (3×45 cm) eluted with CH_2Cl_2–EtOAc (100 : 1) to yield seven fractions (each 700 ml), fr. 16A—G. HPLC of fraction 16C eluted with *n*-hexane–EtOAc (7 : 3) 2 ml/min afforded **8** (6.2 mg, t_R=18.5 min) and **5** (10.1 mg, t_R=23.4 min), respectively. HPLC of fraction 16G eluted with CH_2Cl_2–acetone (20 : 1) 2 ml/min afforded **4** (1.1 mg, t_R= 15.2 min). Fraction 17 was further purified on a silica gel column (3×45 cm) eluted with CH_2Cl_2–acetone (100 : 1). Eight fractions (each 700 ml) were obtained: fr. 17A—H. HPLC of fraction 17F eluted with *n*-hexane–EtOAc (4 : 1) 2 ml/min afforded **6** (10.5 mg, t_R=18.6 min) and **2** (1.3 mg, t_R=22.4 min), respectively. HPLC of fraction 17H eluted with *n*-hexane–EtOAc (1 : 1) 2 ml/min afforded **1** (2.5 mg, t_R=16.4 min) and **7** (1.0 mg, t_R=25.0 min), respectively. Fraction 19 was further chromatographed on a silica gel column (3×45 cm) eluted with CH_2Cl_2–acetone (100 : 1) to generate six fractions (each 600 ml), fr. 19A—F. HPLC of fraction 19E eluted with *n*-hexane–EtOAc (7 : 3) 2 ml/min afforded **3** (7.5 mg, t_R=16.7 min). Fraction 20 was further chromatographed on a silica gel column (3×45 cm) eluted with CH_2Cl_2–acetone (100 : 1) to generate six fractions (each 600 ml), fr. 20A—F. HPLC of fraction 20C eluted with *n*-hexane–EtOAc (7 : 3) 2 ml/min afforded **9** (120 mg, t_R=19.3 min).

3β-*O*-(*E*)-Feruloyl-D:C-friedooleana-7,9(11)-dien-29-ol (**1**): Colorless amorphous solid, $[\alpha]_D^{20}$ −30.1° (*c*=0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 218 (4.30), 235 (4.41), 335 (4.20); IR (KBr) cm^{-1}: 3403, 1703, 1635, 1587, 1514, 1455, 1377, 1270, 1168, 1031, 987, 817; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 616 $[M]^+$ (5), 598 (1), 551 (2), 407 (3), 368 (6), 353 (3), 313 (4), 256 (10), 227 (11), 213 (15), 185 (17), 129 (34), 97 (45), 83 (57), 69 (69), 55 (100); HR-EI-MS *m/z* 616.4132 (Calcd for $C_{40}H_{56}O_5$, 616.4130).

3β-*O*-(*E*)-Coumaroyl-D:C-friedooleana-7,9(11)-dien-29-ol (**2**): Colorless amorphous solid, $[\alpha]_D^{20}$ −37.7° (*c*=0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 222 (4.02), 236 (4.36), 317 (4.20); IR (KBr) cm^{-1}: 3378, 1698, 1685, 1625, 1606, 1590, 1514, 1455, 1372, 1270, 1168, 1012, 987, 837, 739, 525; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 586 $[M]^+$ (3), 551 (5), 523 (5), 423 (3), 407 (4), 368 (18), 256 (14), 236 (15), 213 (17), 185 (19), 129 (35), 97 (55), 83 (68), 69 (85), 55 (100); HR-EI-MS *m/z* 586.3982 (Calcd for $C_{39}H_{54}O_4$, 586.4024).

3β-*O*-(*E*)-Coumaroyl-D:C-friedooleana-7,9(11)-dien-29-oic acid (**3**): Yellow oil, $[\alpha]_D^{20}$ −32.0° (*c*=0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm: 212 (3.80), 236 (4.41), 315 (4.40); IR (KBr) cm^{-1}: 3354, 1693, 1630, 1601, 1588, 1514, 1455, 1367, 1265, 1207, 1158, 992, 832, 744, 525; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 600 $[M]^+$ (53), 554 (4), 523 (3), 421 (16), 345 (60), 253 (13), 147 (100), 107 (35), 91 (43), 69 (63), 55 (84); HR-EI-MS *m/z* 600.3816 (Calcd for $C_{39}H_{52}O_2$, 600.3816).

Methyl 2β,3β-dihydroxy-D:C-friedoolean-8-en-29-oate (**6**): Colorless solid, $[\alpha]_D^{20}$ +22.9° (*c*=0.3, $CHCl_3$); IR (KBr) cm^{-1}: 3369, 1723, 1455, 1372, 1202, 1148, 1060, 1026, 982, 812, 730, 695, 515; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 486 $[M]^+$ (68), 471 (76), 453 (53), 435 (17), 427 (20), 409 (18), 393 (36), 275 (77), 257 (78), 249 (100), 245 (65), 223 (17), 189 (45); HR-EI-MS *m/z* 486.3705 (Calcd for $C_{31}H_{50}O_4$, 486.3711).

Bryonolol (**7**): Colorless solid; IR (KBr) cm^{-1}: 3330, 1455, 1372, 1026, 734; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 442 $[M]^+$ (2), 424 (4), 406 (17), 391 (29), 337 (8), 241 (49), 229 (68), 189 (34), 147 (33), 119 (49), 95 (61), 81 (63), 69 (71), 55 (100).

Assay of Cytotoxicity The cytotoxicity of compounds **1**—**9** was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method based on the procedure reported in the literature.[10] SK-Hep 1 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine 2 mM, 1% penicillin/streptomycin (penicillin 10000 U/ml and streptomycin 10 mg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C. A volume of SK-Hep 1 cells 100 μl at a density of 1×10^5 cells/ml was incubated under the same conditions for 24 h in a 96-well flat-bottomed microplate. Test samples dissolved in DMSO were added to the medium and incubated for 48 h. Subsequently, the wells were incubated with MTT (100 μl/well concentrated at 5 mg/ml) at 37 °C for 4 h. After removing the supernatant, 200 μl of DMSO was added to redissolve the formazan crystals. The absorbance of the resulting formazan was read using an enzyme-linked immunosorbent assay plate reader at 550 nm. The results were assayed in triplicate experiments. The ratio of cell viability (%) was calculated using the following formula: [(experimental absorbance−background absorbance)/(control absorbance−background absorbance)]×100. The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control.

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