Cytotoxic Triterpenoids from *Berberis koreana*

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Abstract

A bioassay-guided fractionation and chemical investigation of the trunk of *Berberis koreana* led to the isolation and identification of three new triterpenoids, 2*α*,3*α*,19*α*-trihydroxy-urs-12-en-24-formyl-28-oic acid (**1**), 2*α*,3*β*,21*α*-trihydroxy-urs-12-en-28-oic acid (**2**), and 3*β*-acetyloxy-1-oxo-olean-12-en-28-oic acid (**3**), along with seven known triterpenoids (**4**–**10**). The structures of these new compounds were determined through spectral analysis, including extensive 2D-NMR data. The new compounds **1**–**3** showed significant cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 human tumor cell lines with IC_{50} values ranging from 7.17 to 48.73 µM.

Key words

Berberis koreana · Berberidaceae · triterpenes · cytotoxicity

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

The naturally occurring triterpenoids, especially oleanolic acid and ursolic acid, are well-known to have anticarcinogenic and antitumoral activity in experimental animals [1,2]. As a part of our ongoing search for cytotoxic constituents from natural Korean medicinal sources, MeOH extracts of the trunk of *Berberis koreana* (Berberidaceae), well-known as "Korean barberry", were selected for phytochemical investigation because its $CHCl_3$-soluble fraction showed cytotoxicity using a sulforhodamine B (SRB) bioassay in our screening procedures and cytotoxic triterpenoids were isolated from the active fraction in our previous study [3]. In the process of our continuing efforts to study this source [3,4], we further isolated three new triterpenoids (**1**–**3**) together with seven known ones (**4**–**10**) (**Fig. 1**) from the active $CHCl_3$-soluble fraction, using a bioassay-guided fractionation method and evaluated the cytotoxicities of all isolates (**1**–**10**).

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined to be $C_{30}H_{46}O_6$ by positive mode HR-ESI-MS data at *m/z* 525.3190 $[M + Na]^+$ (calcd. for $C_{30}H_{46}NaO_6$, 525.3192). The IR absorption bands at 3480 and 1710 cm^{-1} implied the presence of hydroxyl and carboxylic functionalities. The ^{13}C NMR spectrum of **1** (**Table 1**) showed a pair of NMR signals at δ_C 127.9 (C-12) and 139.0 (C-13), characteristic of the double bond of an ursane-12-en system and readily distinguished **1** from an isomeric olean-12-ene, in which the chemical shifts of the corresponding pair of signals could be around δ_C 123 and 145, respectively [5]. The ^1H and ^{13}C NMR data of **1** (**Table 1**) were similar to those of 2*α*,3*α*,19*α*-trihydroxy-urs-12-en-28-oic acid (**5**) isolated from the same extract, except for the signals corresponding to ring A [6]. The ^1H and ^{13}C NMR data at δ_H 9.52 (1H, s) and δ_C 208.1, respectively, were consistent with the presence of an aldehyde group [5]. Its position on ring A was deduced to be C-24 by comparison of ^{13}C NMR data for **1** and those for related triterpenes [5,7] and analysis of the HMBC experiment showing correlations of H-23 to C-3, C-4, C-5, and C-24, as well as of H-24 to C-3, C-4, C-5, and C-23 (**Fig. 2**). The relative configuration of **1** was determined to be identical to that of **5** by the interpretation of NOESY correlations. The NOESY correlations of the aldehyde group (δ_H 9.52) to H-2*β* (δ_H 3.90), H-3*β* (δ_H 3.66), and H-25 (δ_H 1.05) suggested that the aldehyde group had a 4*β*-configuration. Thus, compound **1** was assigned as 2*α*,3*α*,19*α*-trihydroxy-urs-12-en-24-formyl-28-oic acid. A survey of the literature revealed that the 4-epimer of **1** was reported from the roots of *Rubus ellipticus* var. *obcordatus* [8].

Compound **2** was isolated as a white amorphous powder whose molecular formula was determined to be $C_{30}H_{48}O_5$ from the $[M + Na]^+$ peak at *m/z* 511.3402 (calcd. for $C_{30}H_{48}NaO_5$, 511.3399) in the positive-ion HR-ESI-MS. The ^{13}C NMR spectrum of **2** (**Table 1**) also displayed the characteristic signals for the double bond of an ursane-12-en system at δ_C 125.9 (C-12) and 137.8 (C-13) [5]. The ^1H and ^{13}C NMR data of **2** (**Table 1**) were similar to those of corosolic acid, except for the presence of an oxygenated methine at δ_H 3.85 (ddd, *J* = 3.5, 3.0, 2.5) in **2** [9]. Inspection of the ^1H and ^{13}C NMR data revealed that the chemical shifts of the ring E of **2** were nearly identical to those of methyl 3*β*,21*α*-dihydroxy-urs-12-en-28-oate [10]. This indicated that a hydroxyl group at C-21 of the ring E was present, which was confirmed by HMBC correlations from H-21 to C-17 and C-19 and from H-30 to C-21 (**Fig. 2**). The axial (*α*)-orientation of OH-21 was assigned by the observed coupling constants (ddd, *J* = 3.5, 3.0, 2.5) of H-21, which was indicative of an equatorial (*β*) proton coupled with three other protons [*J* = 3.5, 3.0, 2.5 for an equatorial (*β*) H-21; *J* = 10.5, 10.5, 5.5 for an axial (*α*) H-21] [10,11], and the relative configuration of **2** was identical to that of corosolic acid in the NOESY spectrum. Thus, compound **2** was determined to be 2*α*,3*β*,21*α*-trihydroxy-urs-12-en-28-oic acid.

Compound **3**, a white amorphous powder, had a molecular formula of $C_{32}H_{48}O_5$ as established by the $[M + Na]^+$ peak at *m/z* 535.3404 (calcd. for $C_{32}H_{48}NaO_5$, 535.3399) in the positive-ion HR-ESI-MS. The ^{13}C NMR spectrum of **3** (**Table 1**) exhibited a pair of NMR signals at δ_C 122.5 (C-12) and 143.4 (C-13), characteristic of the double bond of an olean-12-en system [5]. The ^1H and ^{13}C NMR data of **3** (**Table 1**) were similar to those of 3*β*-hydroxy-1-oxo-olean-12-en-28-oic acid, except for the signals corresponding to ring A with the presence of an acetyl group (δ_H 2.05; δ_C 171.1 and 21.1) [12]. The structure of ring A was confirmed by the interpretation of the HMBC spectrum (**Fig. 2**) in which correlations of H-3 to C-1 and C-5, H-23 to C-3, H-24 to C-3, and H-25 to C-1 and the correlation between H-3 (δ_H 4.58) and the acetyl group (δ_C 171.1) revealed the presence of a ketone at C-1 and an acetyloxy group at C-3. The coupling constants of H-3 (dd, $J_{2\alpha,3}$ = 6.0 Hz and $J_{2\beta,3}$ = 12.5 Hz) observed in the ^1H NMR spectrum indicated a *β*-orientation of OH-3, which was confirmed by NOESY correlations of H-3/H-23, H-3/H-5, H-3/H-2*α*, H-2*β*/H-25, and H-24/H-25. Thus, compound **3** was elucidated as 3*β*-acetyloxy-1-oxo-olean-12-en-28-oic acid.

The seven known triterpenoids were identified as 2*α*,3*α*,19*α*,23-tetrahydroxy-urs-12-en-28-oic acid (**4**) [6], 2*α*,3*α*,19*α*-trihydroxy-urs-12-en-28-oic acid (**5**) [6], annurcoic acid (**6**) [13], pomonic acid (**7**) [14], 2-oxopomolic acid (**8**) [15], dillenic acid A (**9**) [16], and alphitolic acid (**10**) [17] by comparison of their spectroscopic data with the reported data.

The cytotoxic activities of the isolates (**1**–**10**) were evaluated by determining their inhibitory effects on human tumor cell lines

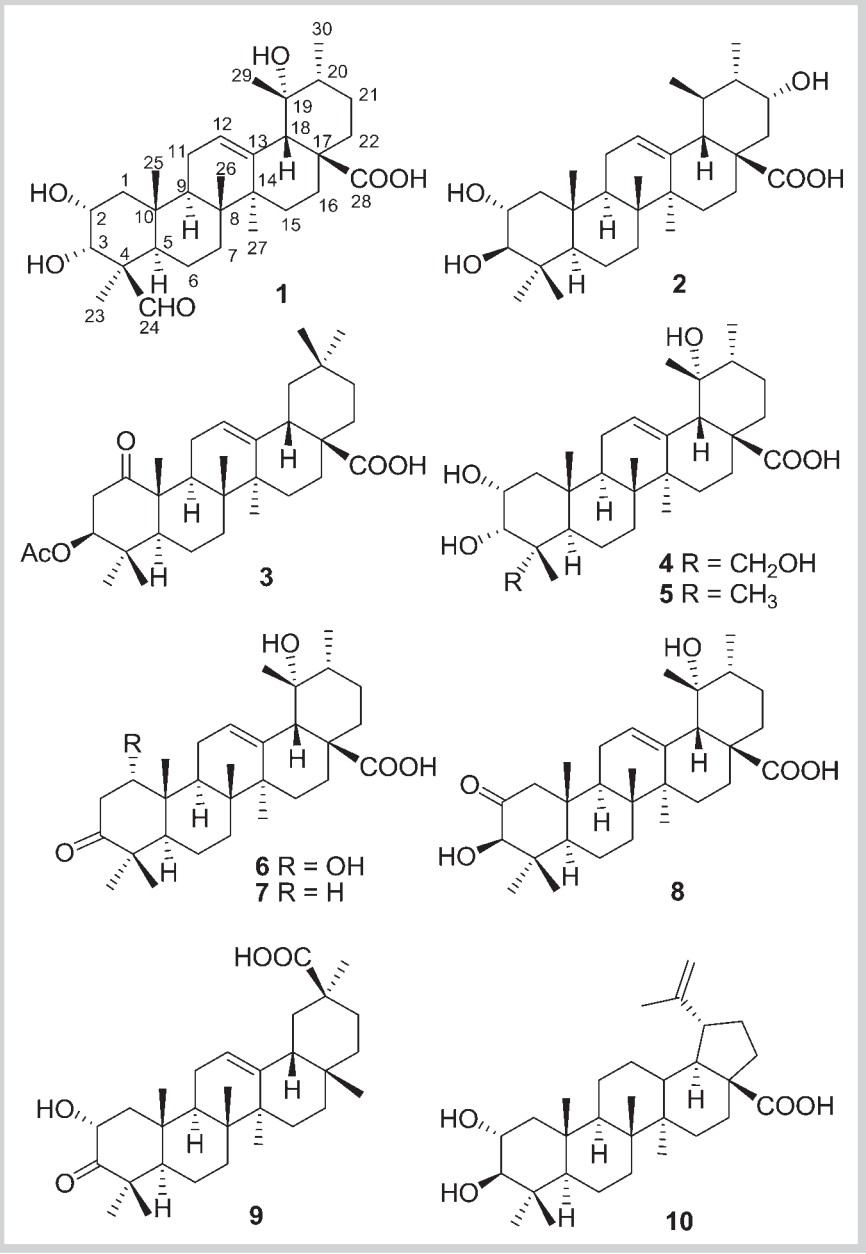

Fig. 1 Chemical structures of **1**–**10**.

(A549, SK-OV-3, SK-MEL-2, and HCT-15) using the SRB bioassay [18]. The results (**Table 2**) showed that all the tested triterpenoids (**1**–**10**) had consistent cytotoxicities against the A549 and SK-MEL-2 cell lines with IC_{50} values ranging from 7.17 to 90.67 µM. The new compounds **1**–**3** showed significant cytotoxicity against all tested tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) with IC_{50} values ranging from 7.17 to 48.73 µM. Of the known triterpenoids (**4**–**10**), compound **10** exhibited potent cytotoxicity against all of the cell lines tested with IC_{50} values of 8.07, 12.62, 3.76, and 9.92 µM, respectively, for the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines.

In conclusion, this study indicates that triterpenoids are the main active constituents responsible for the cytotoxic activity of the trunk of *B. koreana*. All the tested triterpenoids (**1**–**10**) showed cytotoxicity in various cancer cell lines, indicating that these compounds would be good bioactive molecules for the treatment of cancers.

Materials and Methods

The plant material was collected from Jeju Island, Korea, in December 2005. Samples of plant material were identified by one of the authors (K.R. Lee). A voucher specimen (SKKU 2005-10) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea. The air-dried and pulverized plant material (2.7 kg) was extracted with 80% aqueous MeOH two times (each 10 L × 4 h) under reflux. The resulting extract (220 g) was fractionated with *n*-hexane, $CHCl_3$, and *n*-BuOH, subsequently using H_2O. The active $CHCl_3$-soluble fraction (10 g) was applied to repeated column chromatography to purify the compounds **1**–**10** (Supporting Information).

2α,3α,19α-Trihydroxy-urs-12-en-24-formyl-28-oic acid (**1**): white amorphous powder; m. p. 211–212 °C; $[\alpha]_D^{25}$ − 15.7 (*c* 0.20, MeOH); IR (KBr): ν_{max} = 3480, 2955, 1728, 1710, 1456, 1030, 670 cm^{-1}; ^1H

Table 1 ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1–3** in CD_3OD (δ in ppm)[a].

Position	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.76 (m); 2.02 (m)	40.5 t	1.03 (m); 2.06 (m)	46.2 t		213.1 s
2	3.90 (ddd, 10.5, 4.0, 2.0)	65.4 d	3.67 (ddd, 11.5, 10.0, 4.5)	68.2 d	2.83 (dd, 12.5, 12.5) 2.41 (dd, 12.5, 6.0)	41.8 t
3	3.66 (d, 2.0)	75.2 d	3.01 (d, 10.0)	83.1 d	4.58 (dd, 12.5, 6.0)	80.6 d
4		52.8 s		38.4 s		39.5 s
5	2.24 (m)	42.7 d	0.92 (m)	55.6 d	1.20 (m)	54.8 d
6	1.42 (m); 1.52 (m)	20.0 t	1.48 (m); 1.60 (m)	18.6 t	1.37 (m); 1.57 (m)	18.1 t
7	1.22 (m); 1.73 (m)	32.4 t	1.40 (m); 1.63 (m)	32.1 t	1.23 (m); 1.62 (m)	33.5 t
8		40.1 s		39.9 s		39.7 s
9	1.66 (m)	46.9 d	1.70 (m)	47.3 d	1.69 (m)	39.8 d
10		37.7 s		37.3 s		51.8 s
11	1.74 (m); 2.05 (m)	24.8 t	1.75 (m); 2.02 (m)	23.1 t	1.72 (m); 2.02 (m)	24.6 t
12	5.33 (br t, 2.5)	127.9 d	5.33 (br t, 3.5)	125.9 d	5.27 (br t, 3.0)	122.5 d
13		139.0 s		137.8 s		143.4 s
14		41.6 s		42.5 s		41.4 s
15	1.76 (m); 2.05 (m)	28.3 t	1.76 (m); 2.07 (m)	29.4 t	1.81 (m); 2.05 (m)	27.4 t
16	1.52 (m) 2.58 (dd, 16.5, 11.0)	25.4 t	1.53 (m); 2.35 (m)	27.5 t	1.54 (m); 2.05 (m)	23.3 t
17		48.6 s		48.3 s		46.2 s
18	2.53 (s)	53.9 d	2.22 (d, 10.5)	53.2 d	2.87 (dd, 15.5, 3.5)	41.3 d
19		72.3 s	1.66 (m)	22.1 d	1.12 (m); 1.68 (m)	46.7 t
20	1.38 (m)	41.9 d	1.70 (m)	42.9 d		31.7 s
21	1.22 (m); 1.73 (m)	26.0 t	3.85 (ddd, 3.5, 3.0, 2.5)	70.6 d	1.20 (m); 1.38 (m)	34.3 t
22	1.66 (m); 1.76 (m)	37.8 t	1.68 (dd, 14.0, 3.5) 1.98 (dd, 14.0, 3.0)	41.8 t	1.23 (m); 1.39 (m)	33.5 t
23	1.03 (s)	13.3 q	1.02 (s)	27.4 q	1.13 (s)	27.4 q
24	9.52 (s)	208.1 d	0.87 (s)	15.6 q	1.12 (s)	16.2 q
25	1.05 (s)	15.4 q	0.98 (s)	15.4 q	1.30 (s)	15.0 q
26	0.83 (s)	16.5 q	0.90 (s)	16.1 q	0.90 (s)	18.1 q
27	1.42 (s)	23.8 q	1.29 (s)	22.4 q	1.17 (s)	25.2 q
28		181.0 s		180.3 s		180.2 s
29	1.22 (s)	25.8 q	0.80 (d, 6.5)	17.0 q	0.91 (s)	32.1 q
30	0.95 (d, 6.5)	15.9 q	0.93 (d, 6.5)	17.1 q	0.95 (s)	23.3 q
OAc					2.05 (s)	21.3 q 171.1 s

[a] Assignments were based on 2D NMR including HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses

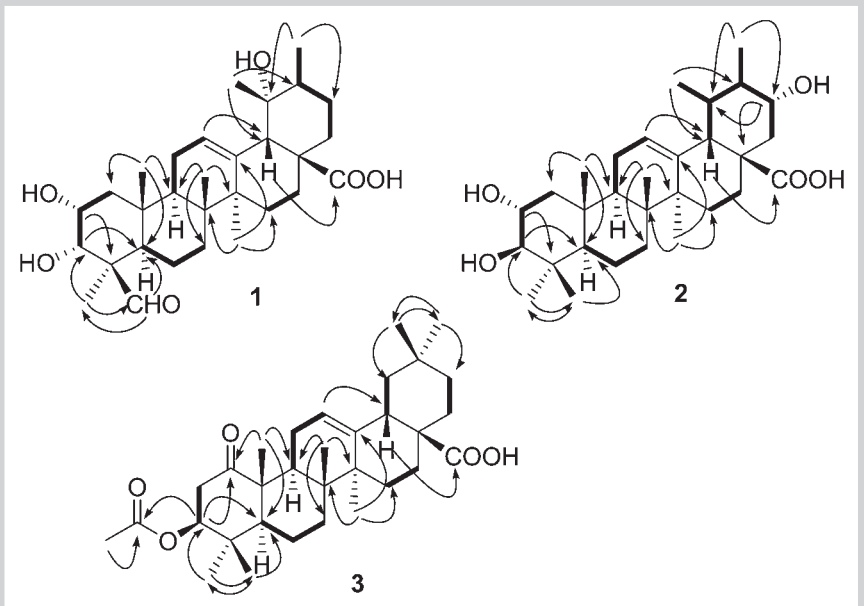

Fig. 2 Key ^{1}H-^{1}H COSY (bold) and HMBCs (→) of **1–3**.

Table 2 Cytotoxicity of compounds **1**–**10** against four cultured human tumor cell lines using the SRB bioassay *in vitro*.

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	36.37	28.06	12.46	48.73
2	7.17	7.48	8.76	8.02
3	14.65	19.46	10.38	27.80
4	90.67	> 100.0	33.52	> 100.0
5	42.71	58.43	26.87	72.37
6	27.35	50.64	35.19	60.43
7	80.17	> 100.0	41.84	> 100.0
8	37.22	36.07	30.51	57.36
9	13.42	35.26	13.08	22.61
10	8.07	12.62	3.76	9.92
Doxorubicin[b]	0.01	0.01	0.01	0.18

[a] IC_{50} value of compounds against each tumor cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*. [b] Doxorubicin as a positive control

(500 MHz) and ^{13}C (125 MHz) NMR data, see **Table 1**; ESI-MS: $m/z = 525$ $[M + Na]^+$; HRESIMS: $m/z = 525.3190$ $[M + Na]^+$ (calcd. for $C_{30}H_{46}NaO_6$, 525.3192).

2α,3β,21α-Trihydroxy-urs-12-en-28-oic acid (**2**): white amorphous powder; m.p. 208–209 °C; $[\alpha]_D^{25} + 36.4$ (*c* 0.15, MeOH); IR (KBr): $\nu_{max} = 3478$, 2955, 1705, 1454, 1032, 665 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see **Table 1**; ESI-MS: $m/z = 511$ $[M + Na]^+$; HRESIMS: $m/z = 511.3402$ $[M + Na]^+$ (calcd. for $C_{30}H_{48}NaO_5$, 511.3399).

3β-Acetyloxy-1-oxo-olean-12-en-28-oic acid (**3**): white amorphous powder; m.p. 252–253 °C; $[\alpha]_D^{25} - 24.5$ (*c* 0.12, MeOH); IR (KBr): $\nu_{max} = 3480$, 2955, 1707, 1698, 1455, 1031, 675 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see **Table 1**; ESI-MS: $m/z = 535$ $[M + Na]^+$; HRESIMS: $m/z = 535.3404$ $[M + Na]^+$ (calcd. for $C_{32}H_{48}NaO_5$, 535.3399).

A detailed description of the bioassays is available as Supporting Information. The positive control, doxorubicin (purity ≥ 98%), was purchased from Sigma. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analyses (purity ≥ 95%).

Supporting information

The general experimental procedures, the isolation details, and bioassay protocol are available as Supporting Information.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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