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Bioactivity-guided isolation of cytotoxic triterpenoids from the trunk of *Berberis koreana*

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ABSTRACT

A bioassay-guided fractionation and chemical investigation of the trunk of *Berberis koreana* resulted in the isolation and characterization of two new triterpenoids, 23-*trans*-*p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid (**1**), and 23-*cis*-*p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid (**2**), along with seven known triterpenoids (**3–9**). The structures of the new compounds were determined on the basis of spectroscopic analyses including 2D NMR. The cytotoxic activities of the triterpenes (**1–9**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using the SRB assay. Compounds **5** and **6** showed potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} (**5**): 4.37, 7.04, 9.72, and 5.83 μM, and IC_{50} (**6**): 5.57, 7.84, 13.29, and 5.61 μM, respectively).

Most of the *Berberis* species are evergreen and semi-evergreen shrubs or small trees distributed in the Northern Hemisphere, which have medical uses due to the presence of alkaloids with medicinal importance, principally 'berberine'.[1,2] *Berberis koreana* Palibin (Berberidaceae), well-known as 'Korean barberry', is an endemic species distributed throughout northern Korea. This medicinal plant has been used as a Korean traditional medicine for the treatment of various disorders such as gastroenteritis, sore throats, fever, and conjunctivitis.[3] Extracts from *B. koreana* were found to have neuroprotective,[4,5] cytotoxic, and antioxidant activities.[6] In spite of the various pharmacological uses of *B. koreana*, the chemical constituents of this plant except for alkaloids, including benzylisoqunoline, protoberberine derivatives, and pyrrole acids,[7–10] were little reported.

As part of our continuing search for bioactive constituents from Korean medicinal plants, we investigated a methanol extract of the trunk of *B. koreana* for cytotoxic constituents based on the fact that the extract showed considerable cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines in screening procedures. To identify the active ingredients responsible for cytotoxic activity, the MeOH extract of the trunk of *B. koreana* was fractionated by solvent (*n*-hexane, $CHCl_3$, *n*-BuOH), and then each fraction was evaluated for cytotoxicity against tumor cell lines using a sulforhodamine B (SRB) assay. It was found that the *n*-hexane-soluble and $CHCl_3$-soluble fractions showed cytotoxic activity against the tumor cell lines. We recently reported the isolation of biphenyl compounds and their cytotoxic activities from the active *n*-hexane-soluble fraction.[11] In our continuing study of this source, phytochemical investigation of the active $CHCl_3$-soluble fraction led to the isolation of two new triterpenoids, 23-*trans*-*p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid (**1**), and 23-*cis*-*p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid (**2**), along with seven known triterpenoids (**3–9**) (Fig. 1). This Letter describes the isolation and elucidation of the structures of the new triterpenoids (**1–2**), and the cytotoxic activity of **1–9** against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines.

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined to be $C_{39}H_{54}O_7$ by positive mode HR-FABMS data at *m/z* 657.3767 $[M+Na]^+$ (calcd for $C_{39}H_{54}NaO_7$, 657.3767). The UV spectrum exhibited absorption maxima at 224 and 311 nm, suggesting the presence of an aromatic ring in the molecule. The IR spectrum showed absorption bands for hydroxyl (3376 cm^{-1}), α,β-unsaturated carbonyl (1698 cm^{-1}), and aromatic (1606 and 1513 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) indicated the presence of six tertiary methyls at δ 0.80, 0.86, 0.90, 0.94, 0.98, 1.19 (each 3H, s), two hydroxymethine protons at δ 3.30 (1H, d, *J* = 3.0 Hz, H-3) and 3.91 (1H, m, H-2), a hydroxymethylene group at δ 3.66 and 4.10 (each 1H, d, *J* = 12.0 Hz, H-23), and an olefinic double bond at δ 5.26 (1H, t-like, *J* = 4.0 Hz, H-12). In addition, a 1,4-disubstituted aromatic ring at δ 6.83 (2H, d, *J* = 8.5 Hz, H-3′/5′) and 7.48 (2H, d, *J* = 8.5 Hz, H-2′/6′), and an olefinic double bond at δ 6.40 (1H, d, *J* = 16.0 Hz, H-8′) and 7.66 (1H, d, *J* = 16.0 Hz, H-7′) indicated the presence of a *trans*-*p*-coumaroyl group in **1** due to a characteristic *J* value of 16 Hz.[12] As expected, the ^{13}C NMR spectrum of **1**

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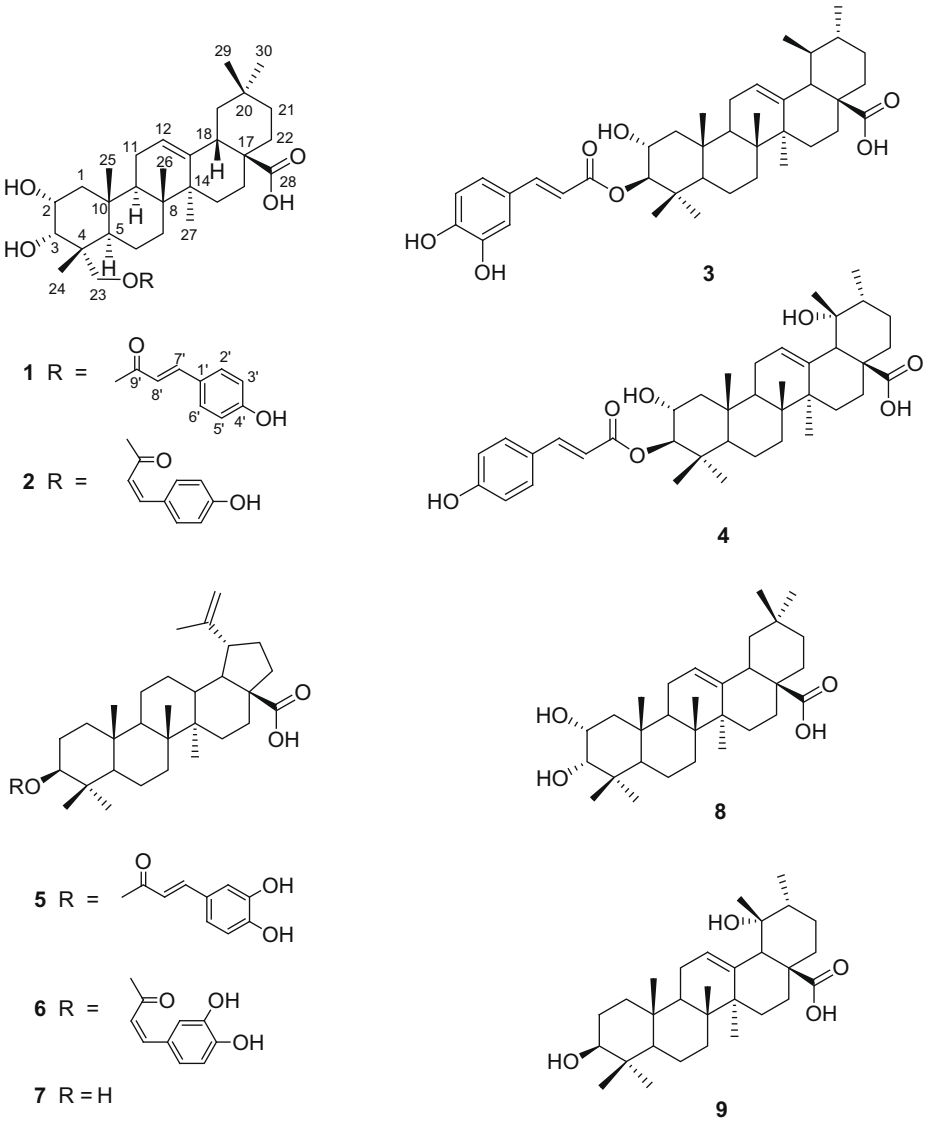

Figure 1. The structures of compounds **1**–**9** isolated from *B. koreana*.

showed 39 carbon signals, classified as 6 methyls, 10 methylenes, 12 methines, and 11 quaternary carbon atoms using DEPT spectrum analysis. The ^{1}H and ^{13}C NMR data suggested that **1** has an *O-trans-p*-coumaroyl group and an oleanane-type skeleton.[12,13] The framework of **1** was deduced from the presence of 6 tertiary methyls, a double bond, 2 hydroxymethines, and a hydroxymethylene, data of which were in agreement with the NMR spectral data of 2α,3α,23-trihydroxyolean-12-en-28-oic acid.[13] Thus, **1** could be a 2α,3α,23-trihydroxyolean-12-en-28-oic acid containing an *O-trans-p*-coumaroyl group. The full NMR assignments and connectivites of **1** were determined by HMQC, HMBC, and NOESY spectroscopic data analysis. The HMBC spectrum showed that H-23 at δ 4.10 and 3.66 (each 1H, d, J = 12.0 Hz) was correlated to C-3 (δ 80.2), C-5 (δ 43.1), and C-9′ (δ 168.2), indicating that the *O-trans-p*-coumaroyl group was located at C-23 (Fig. 2). This ester substituent at C-23 was also supported by the downfield shift observed for H-23 (C-23) in the ^{1}H and ^{13}C NMR spectrum, compared with those of 2α,3α,23-trihydroxyolean-12-en-28-oic acid.[13] Hydrolysis of **1** with 10% KOH in MeOH yielded 2α,3α,23-trihydroxyolean-12-en-28-oic acid[13] and *trans-p*-coumaric acid.[12] The relative configuration of H-2, H-3 and H-23 and other spatial information of **1** were further supported by NOESY experiments, wherein NOEs were observed between H-2 (δ 3.91) and H-24 (δ 0.86)/H-25 (δ 0.98), between H-3 (δ 3.30) and H-2 (δ 3.91)/H-24 (δ 0.86), and between H-23 (δ 4.10, 3.66) and H-5 (δ 0.92) (Fig. 3). Therefore, based on all the above evidence, the structure of **1** was assigned as 23-*trans-p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid.

Compound **2** was obtained as a white amorphous powder, with the molecular formula of $C_{39}H_{54}O_7$ as determined by positive mode HR-FABMS data at m/z 657.3766 $[M+Na]^+$. It showed UV maxima at 226 and 313 nm and IR bands for hydroxyl (3376 cm^{-1}), α,β-unsaturated carbonyl (1696 cm^{-1}), and aromatic (1605 and 1514 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR data of **2** were similar to those of **1**, except for the aromatic region (Table 1). The NMR data of **2** indicated a 1,4-disubstituted aromatic ring at δ 6.85 (2H, d, J = 8.5 Hz, H-3′/5′) and 7.63 (2H, d, J = 8.5 Hz, H-2′/6′)/δ 115.7 (C-3′/5′), 126.5 (C-1′), 133.8 (C-2′/6′), and 160.6 (C-4′), an olefinic double bond at δ 5.85 (1H, d, J = 13.0 Hz, H-8′) and 6.75 (1H, d, J = 13.0 Hz, H-7′)/δ 116.8 (C-8′) and 143.9 (C-7′), and a ester carbonyl carbon at δ 167.5 (C-9′). The coupling constant (13.0 Hz) of H-7′ and H-8′ suggested the presence of *cis*-olefinic protons.[12] Thus, it was presumed that compound **2** is a derivative of **1** containing an *O-cis-p*-coumaroyl group. In the HMBC spectrum, the

Table 1
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **1–2** in CD_3OD (δ in ppm)

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1	2.02 (m), 1.10 (m)	42.6	2.02 (m), 1.12 (m)	42.8
2	3.91 (m)	67.2	3.94 (m)	67.3
3	3.30 (d, 3.0)	80.2	3.32 (d, 3.0)	80.3
4		42.8		42.4
5	0.92 (m)	43.1	0.92 (m)	43.2
6	1.62 (m), 1.44 (m)	19.3	1.62 (m), 1.47 (m)	19.5
7	1.71 (m), 1.53 (m)	33.7	1.73 (m), 1.54 (m)	33.4
8		39.5		39.8
9	1.66 (m)	47.7	1.66 (m)	48.0
10		39.3		39.4
11	1.98 (m), 1.81 (m)	24.1	1.99 (m), 1.80 (m)	24.5
12	5.26 (t-like, 4.0)	123.6	5.27 (t-like, 4.0)	123.3
13		145.4		145.1
14		41.2		41.2
15	1.78 (m)	28.9	1.75 (m)	28.6
16	1.99 (m), 1.59 (m)	24.1	2.01 (m), 1.57 (m)	24.7
17		47.4		47.8
18	2.86 (dd, 15.5, 3.5)	41.6	2.85 (dd, 15.5, 3.5)	41.8
19	1.66 (m), 1.12 (m)	47.6	1.67 (m), 1.10 (m)	47.6
20		31.7		31.5
21	1.38 (m), 1.19 (m)	35.0	1.37 (m), 1.21 (m)	35.7
22	1.35 (m), 1.23 (m)	34.0	1.34 (m), 1.23 (m)	34.3
23	4.10 (d, 12.0) 3.66 (d, 12.0)	73.1	4.12 (d, 12.0) 3.70 (d, 12.0)	73.3
24	0.86 (s)	17.9	0.84 (s)	17.9
25	0.98 (s)	16.7	1.00 (s)	16.4
26	0.80 (s)	17.0	0.82 (s)	17.2
27	1.19 (s)	26.6	1.19 (s)	26.5
28		182.0		181.6
29	0.90 (s)	33.9	0.92 (s)	33.3
30	0.94 (s)	22.5	0.95 (s)	22.2
1′		125.7		126.5
2′/6′	7.48 (d, 8.5)	130.4	7.63 (d, 8.5)	133.8
3′/5′	6.83 (d, 8.5)	116.9	6.85 (d, 8.5)	115.7
4′		161.2		160.6
7′	7.66 (d, 16.0)	144.9	6.75 (d, 13.0)	143.9
8′	6.40 (d, 16.0)	115.7	5.85 (d, 13.0)	116.8
9′		168.2		167.5

Assignments were based on 2D NMR including HMQC, HMBC, and NOESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in hertz in parentheses.

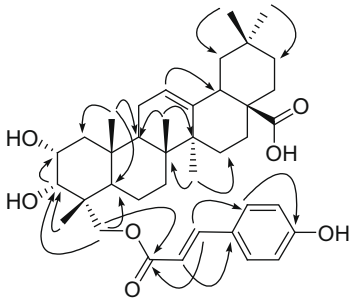

Figure 2. Key HMBC correlations of compound **1**.

proton signals at δ 4.12 and 3.70 (each 1H, d, J = 12.0 Hz, H-23) showed correlations with C-3 (δ 80.3), C-5 (δ 43.2), and C-9′ (δ 167.5), indicating that the *O-cis-p*-coumaroyl group was located at C-23. Moreover, the cross peaks in the NOESY spectrum of **2** indicated that the corresponding substituents in **2** have the same orientations as those in **1**. Thus, based on all the above evidence, the structure of **2** was determined to be 23-*cis-p*-coumaroyloxy-2α,3α-dihydroxyolean-12-en-28-oic acid.

Seven known compounds were isolated and identified as 3β-*trans*-caffeoyloxy-2α-hydroxyurs-12-en-28-oic acid (**3**),[14] 3-*O*-*trans-p*-coumaroyltormentic acid. (**4**),[15] betulinic acid 3β-*trans*-caffeate (**5**),[16] betulinic acid 3β-*cis*-caffeate (**6**),[16] betulinic acid (**7**),[17] 3-epimaslinic acid (**8**),[13] and pomolic acid (**9**)[18] by comparisons with previously published data. To the best of our knowledge, this is the first report on chemical analysis of triterpenoids from *B. koreana*.

Compounds **1–9** were evaluated for cytotoxicity against the A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma) human tumor cell lines using the SRB assay in vitro.[19] The results (Table 2) showed that all the tested triterpenes (**1–9**) exhibited consistent cytotoxicity against four human tumor cell lines with IC_{50} values ranging from 4.37 μM to 41.73 μM. It was found that compounds **5** and **6** showed potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} (**5**): 4.37, 7.04, 9.72, and 5.83 μM, and IC_{50} (**6**): 5.57, 7.84, 13.29, and 5.61 μM, respectively). Compounds **1** and **2** exhibited selective inhibitory activity against the HCT-15 cell line (IC_{50} (**1**): 8.57 and IC_{50} (**2**): 9.74), in particular. In the structure activity relationship (SAR), it appears that the caffeoyl or coumaroyl function in the triterpene skeleton improves cytotoxicity, as compounds **1/2** and **5/6** with the above function were more active than compounds of their core structure, **8** and **7**, respectively. The caffeoyl moiety seems to increase more inhibitory activity than coumaroyl moiety, since compounds **3**, **5**, and **6** were more active than compounds **1**, **2**, and **4**. The coumaroyl moiety seems to have a good effect on cytotoxicity selectively against the HCT-15 cell line, due to activities of compounds **1**, **2**, and **4**. The cytotoxicity may also be more

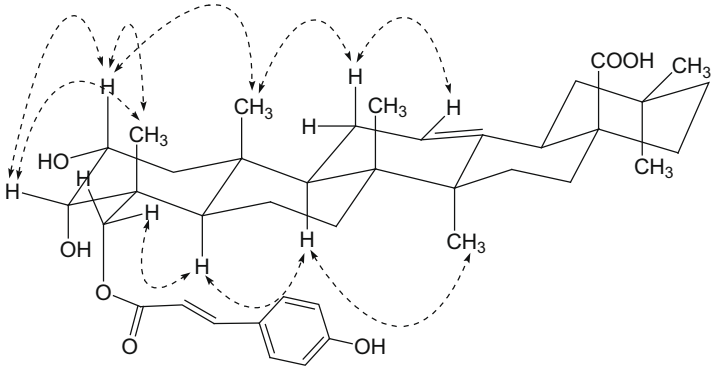

Figure 3. Key NOESY correlations of compound **1**.

Table 2
Cytotoxicity of compounds **1**–**9** against four cultured human cancer cell lines using the SRB assay in vitro

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
$CHCl_3$ extract	15.97	9.87	22.20	19.52
1	14.64	33.92	19.51	8.57
2	15.14	36.27	21.47	9.74
3	9.43	14.52	12.17	7.88
4	20.68	41.03	31.15	11.02
5	4.37	7.04	9.72	5.83
6	5.57	7.84	13.29	5.61
7	10.82	17.68	17.44	14.25
8	22.10	41.73	19.52	12.53
9	17.22	13.95	19.16	13.72
Doxorubicin[b]	0.16	0.38	0.04	0.82

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

positively influenced by *trans*-type than *cis*-type of the above function, compared to activities of compounds **1/2** and **5/6**, respectively. This SAR study could provide valuable data for future synthetic and pharmacological studies with the aim of obtaining cytotoxic compounds that are more potent and selective toward cancer cells.

In summary, we focused our investigation on cytotoxic principles from the trunk of *B. koreana* and found two new triterpenes (**1–2**) with selective cytotoxic activity against the HCT-15 cell line and seven cytotoxic known tritepenes (**3–9**) against human tumor cell lines. Thus, it is possible to demonstrate that isolated triterpenoids might possess beneficial therapeutic potential against various tumors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.156.

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