Bioorganic & Medicinal Chemistry Letters 20 (2010) 4487–4490

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Benzylisoquinoline alkaloids from the tubers of *Corydalis ternata* and their cytotoxicity

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ARTICLE INFO

Article history:
Received 22 April 2010
Revised 3 June 2010
Accepted 7 June 2010
Available online 12 June 2010

Keywords:
Corydalis ternate
Papaveraceae
Benzylisoquinoline alkaloids
Cytotoxicity

ABSTRACT

Chemical investigation of the tubers of *Corydalis ternata* resulted in the isolation and characterization of four new benzylisoquinoline alkaloids, *epi*-coryximine (**1**) and coryternatines A–C (**2–4**), along with 10 known alkaloids (**5–14**). Their structures were established on the basis of extensive spectroscopic data analyses and comparison with spectroscopic data reported. In addition, the cytotoxicities of the alkaloids (**1–14**) were evaluated by determining their inhibitory effects on several human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using the SRB assay. Compound **8** showed significant cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} = 8.34, 5.14, 7.87, and 2.86 μM, respectively). The four new compounds (**1–4**) exhibited selective cytotoxicity against the HCT-15 cell line.

Corydalis tuber has been used in the traditional Korean medicine for an analgesic, an antispasmodic, and a treatment of gastric ulcers.[1] Some components in *Corydalis* tuber have anticholinesterase, anti-amnesic, anti-inflammatory, antihypertensive, and analgesic effects.[2–4] It was also reported that *Corydalis* tuber depletes the levels of amygdaloid dopamine[5] and have neuroprotective effects in heat-stroke rats.[6] *Corydalis* tuber consists of the tubers of *Corydalis ternata* Nakai, *Corydalis turtschaninovii* Besser, and *Corydalis ambigua* Cham. & Schleht (Papaveraceae), and congeneric plants, although the actual species that compose this traditional medicine differ in each country.

C. ternata is the main species used for *Corydalis* tuber in Korea. The main chemical constituents of *C. ternata* are alkaloids, including berberine and coptisine.[1] Its well-documented component, protopine, decreases the glutamate level and increases the glutamate dehydrogenase (GDH) activity in the brains of rats.[7] As a part of our continuing search for cytotoxic constituents from Korean medicinal plants,[8–11] we investigated the MeOH extract of the tubers of *C. ternata* which showed considerable cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines in screening procedures. Although there have been several studies for cytotoxic constituents of congeneric plants,[12–15] the bioactive constituents of this plant have seldom been investigated. The MeOH extract of the tubers of *C. ternata* that were collected in Jinju, Korea, in May, 2009, was suspended in distilled H_2O. They were then partitioned with $CHCl_3$ after successive pretreatment with 1 N hydrochloric acid (HCl) and 1 N ammonium hydroxide (NH_4OH). Each fraction was subjected to various silica gel and reversed-phase column chromatography (Supplementary data); this yielded four new benzylisoquinoline alkaloids, *epi*-coryximine (**1**) and coryternatines A–C (**2–4**), along with ten known alkaloids (**5–14**) (Fig. 1). Herein, we describe the isolation and structure elucidation of the four new benzylisoquinoline alkaloids (**1–4**) and the cytotoxic activity of all isolated alkaloids (**1–14**) against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines.

Compound **1** was obtained as a colorless gum with $[\alpha]_D^{25}$ −60.2 (*c* 0.2, $CHCl_3$). The molecular formula of **1** was determined to be $C_{20}H_{19}NO_6$ by using positive mode HR-FABMS, which provided the molecular ion peak $[M+H]^+$ at *m/z* 370.1292 (calcd for $C_{20}H_{20}NO_6$, 370.1291), in conjunction with its ^{13}C NMR, which displayed 20 resonances. The UV spectrum of **1** exhibited absorption maxima at 205, 237, and 292 nm, suggesting the character of an isoquinoline alkaloid.[16] The IR spectrum of **1** showed a strong absorption at 1698 cm^{-1} and a wide absorption at 2925 cm^{-1}, indicating the presence of a carboxyl group. The ^1H NMR spectrum of **1** (Table 1) showed the following: two methylenedioxy groups at δ 5.97 and 6.02 (each 2H, s); two aromatic protons as singlet at δ 6.68 and 6.80; two coupled aromatic protons as doublet at δ 6.60 and 6.73; an *N*-methyl group at δ 2.67 (3H, s); four aliphatic protons as multiplet at δ 2.82–3.40; and three protons as an ABX system at δ 3.17 (1H, dd, *J* = 14.0, 6.5 Hz), 3.27 (1H, dd, *J* = 14.0, 6.5 Hz),

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doi:10.1016/j.bmcl.2010.06.035

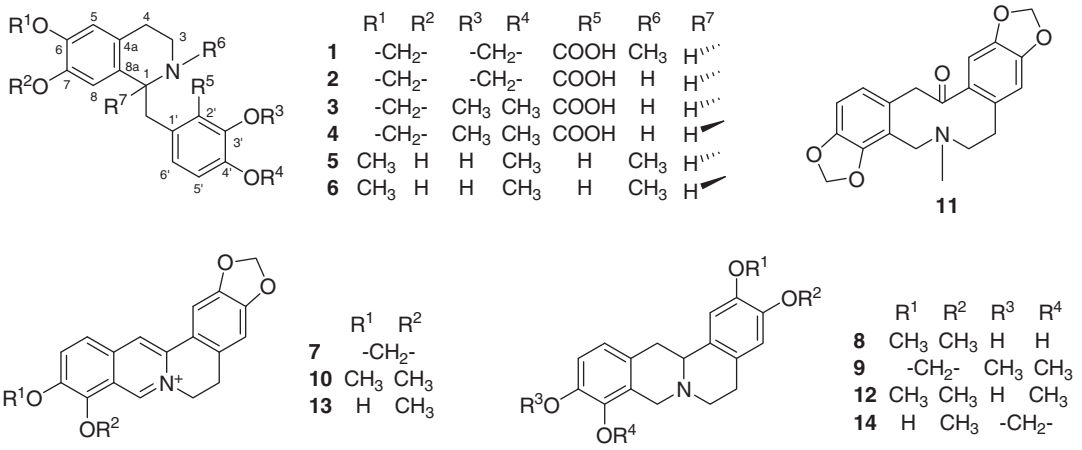

Figure 1. The structures of compounds **1–14** isolated from *C. ternata*.

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	4.77 (br t, 6.5)	67.1	4.57 (br t, 6.0)	56.1
3	3.40 (m)	49.8	3.06 (m)	40.8
			3.27 (m)	
4	2.82 (m)	24.2	3.01 (m)	28.8
	3.03 (m)		3.05 (m)	
4a		124.7		124.3
5	6.68 (s)	107.9	6.76 (s)	107.2[c]
6		147.8[a]		146.7[d]
7		147.5		146.4[d]
8	6.80 (s)	107.1	6.98 (s)	107.1[c]
8a		126.0[b]		127.3
9-CH_2–	3.17 (dd, 14.0, 6.5)	40.3	3.16 (dd, 15.0, 6.0)	42.2
	3.27 (dd, 14.0, 6.5)		3.40 (dd, 15.0, 6.0)	
1′		126.1[b]		127.0
2′		122.2		123.4
3′		146.2		145.6
4′		147.9[a]		148.7
5′	6.73 (d, 8.0)	108.7	6.75 (d, 8.0)	109.5
6′	6.60 (d, 8.0)	124.2	6.73 (d, 8.0)	124.1
O–CH_2–O	5.97 (s)	101.6	5.95 (s)	101.4
	6.02 (s)	101.7	5.96 (s)	101.6
COOH		170.9		171.1.
N-CH_3	2.67 (s)	42.4		

Assignments were based on 2D NMR methods, including HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants (in Hz) given in parentheses.
[a,b,c,d] May be interchanged.

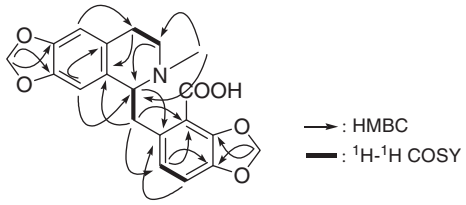

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

and 4.77 (1H, br t, J = 6.5 Hz). It indicated that alkaloid **1** possesses a benzylisoquinoline skeleton.[16,17] As expected, the ^{13}C NMR spectrum of **1** showed 20 carbon signals, classified as a methyl, 5 methylenes, 5 methines, and 9 quaternary carbon atoms by analysis of DEPT spectrum and showed a quaternary carbon at δ 170.9 for a carboxyl group, particularly. The full NMR assignments and connectivities of **1** were determined by using the ^{1}H–^{1}H COSY, HMQC, and HMBC spectroscopy data. The ^{1}H–^{1}H COSY spectra indicated the connectivity of partial structures written in bold lines (Fig. 2). In the HMBC experiment, long-range correlations were observed between the following protons and carbons: *N*-CH_3/C-1, C-3; H-5/C-4; H-8/C-1; H-1/C-1′; H-6′/C-2′; 9-CH_2/C-8a, C-2′. Meanwhile, the ^{1}H and ^{13}C NMR data of **1** were similar to those of coryximine,[16] except for the chemical shift and splitting pattern of H-1 [δ 4.77 (1H, br t, J = 6.5 Hz) in **1**; 4.05 (1H, dd, J = 6.1, 3.8 Hz) in coryximine]. These data suggest that alkaloid **1** is 1-epimer of coryximine.[16] The *S*-configuration of the chiral center at the benzylic position (C-1) of **1** was assigned on the basis of the CD spectrum of **1**, which showed the negative Cotton effect at 292 nm ([θ] = −2500).[18] Based on the above evidence, the structure of **1** was determined (Fig. 1) and named *epi*-coryximine. Although compound **1** is a stereoisomer of coryximine (1*R*-form), **1** (1*S*-form) was not yet reported.

Compound **2**, obtained as a colorless gum, has the molecular formula $C_{19}H_{17}NO_6$, as determined by the positive mode HR-FABMS data at *m/z* 356.1138 $[M+H]^+$ (calcd for $C_{19}H_{18}NO_6$, 356.1134). This compound showed UV maxima at 205, 236, and 292 nm and IR bands for a carboxyl group (2970 and 1741 cm^{-1}). The ^{1}H and ^{13}C NMR data of **2** were close to those of **1**. In particular, the ^{1}H NMR spectrum of **2** was almost identical to that of **1**, except the absence of the signal assignable to the *N*-methyl group (Table 1). Likewise, the ^{13}C NMR spectra of these compounds were very similar, except for the absence of a signal for the *N*-methyl group of **1** (δ 42.1). In addition, the signals assignable to C-1 (δ 56.1) and C-3 (δ 40.8) were present in the ^{13}C NMR spectrum of **2** instead of the corresponding signals for C-1 (δ 67.1) and C-3 (δ 49.8) of **1**, which also supported the absence of the *N*-methyl group in **2**.[19,20] The structure of **2** was confirmed by analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectroscopic data. Finally, the *S*-configuration of the chiral center at C-1 of **2** was determined on the basis of the CD spectrum, which showed the negative Cotton effect at 295 nm ([θ] = −3000).[18] Thus, the structure of **2** was established (Fig. 1) and named coryternatine A.

Compound **3** was obtained as a colorless gum with the molecular formula, $C_{20}H_{21}NO_6$, determined on the basis of the positive mode HR-FABMS data at *m/z* 372.1441 $[M+H]^+$ (calcd for $C_{20}H_{22}NO_6$, 372.1447). Compound **3** showed UV maxima at 206, 235, and 290 nm and IR bands for a carboxyl group (2970 and 1741 cm^{-1}). The ^{1}H and ^{13}C NMR data of **3** were similar to those of **2**. Particularly, the ^{1}H NMR spectrum of **3** was almost identical to that of **2**, except for the presence of the signals assignable to two methoxy groups in **3** (Table 2). This finding suggested that

Table 2
^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **3–4** in CD_3OD (δ in ppm)

Position	3		4	
	δ_H	δ_C	δ_H	δ_C
1	4.64 (br t, 5.5)	56.7	4.25 (dd, 6.5, 3.5)	56.2
3	3.04 (m)	41.7	2.94 (m)	40.7
	3.19 (m)		3.15 (m)	
4	3.02 (m)	28.3	2.88 (m)	27.5
4a		124.3		126.9
5	6.78 (s)	108.2	6.68 (s)	109.0
6		149.3		148.5
7		148.0		146.9
8	7.09 (s)	105.6	6.95 (s)	107.2
8a		124.9		129.7[a]
9-CH_2–	3.16 (m)	43.8	3.14 (m)	42.7
	3.38 (br d, 15.0)		3.33 (m)	
1′		130.7		129.8[a]
2′		125.2		125.8
3′		145.5		146.4
4′		151.4		151.5
5′	7.17 (d, 8.0)	113.6	6.93 (d, 8.0)	111.9
6′	7.11 (d, 8.0)	126.5	6.90 (d, 8.0)	126.4
O–CH_2–O	6.01 (s)	102.0	5.93 (s)	101.5
COOH		169.1		169.6
3′-OCH_3	3.89 (s)	59.8	3.77 (s)	59.8
4′-OCH_3	3.87 (s)	55.3	3.83 (s)	55.1

Assignments were based on 2D NMR methods, including HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants (in Hz) given in parentheses.
[a] May be interchanged.

one of the methylenedioxy functions of **2** was broken into two methoxy groups in **3**. As expected, the ^{13}C NMR spectrum of **3** showed two methoxy carbon signals (δ 59.8 and 55.3), the positions of which were confirmed to be at C-3′ and C-4′, respectively, from the HMBC correlations between the methoxy proton (δ 3.89) and C-3′ (δ 145.5) and between the methoxy proton (δ 3.87) and C-4′ (δ 151.4). On the basis of the CD spectrum showing the negative Cotton effect at 294 nm ($[\theta] = -1500$),[18] the structure of **3** was determined (Fig. 1) and named coryternatine B.

Compound **4** was obtained as a colorless gum. The molecular formula of **4** was deduced from the positive mode HR-FABMS and found to be the same as that of **3**. The ^{1}H and ^{13}C NMR data of **4** were very similar to those of **3**, except for the chemical shift and splitting pattern of H-1 [δ 4.25 (1H, dd, J = 6.5, 3.5 Hz) in **4**; 4.64 (1H, br t, J = 5.5 Hz) in **3**]. Analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC correlations led to the establishment of the same planar structure for both compounds. Comparison of the CD spectrum of **3** ($[\theta]_{294} = -1500$) and **4** ($[\theta]_{296} = +5300$)[18] showed that the absolute configuration at C-1 of **4** ($[\alpha]_D^{25}$ +122.5) was *R*-form. Thus, compound **4** was found to be an 1-epimer of **3**, and it was named coryternatine C.

Ten known compounds were also isolated and identified as (*S*)-reticuline (**5**),[21] (*R*)-reticuline (**6**),[21] coptisine (**7**),[22] demethylcorydalmine (**8**),[23] tetrahydroberberine (**9**),[24] berberine (**10**),[22] protopine (**11**),[25] corydalmine (**12**),[26] thalifendine (**13**),[27] and cheilanthifoline (**14**)[17,28] by comparisons with previously published data. To the best of our knowledge, compounds **5**, **6**, **8**, **9**, **12**, **13**, and **14** were isolated for the first time from this plant.

Compounds **1–14** were evaluated for cytotoxicity against the A549 (non–small cell lung carcinoma), SK-OV-3 (malignant ascites from ovary), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma) human tumor cell lines by using the SRB assay.[29] The results of these assays (Table 3) showed that compound **8** exhibited significant cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} = 8.34, 5.14, 7.87, and 2.86 μM, respectively). Protoberberine-type alkaloids (**7**–**10** and **12**–**14**) also showed cytotoxicity against the four human tumor cell lines, yet the protopine-type alkaloid (**11**) was inactive (Table 3). Additionally, the benzylisoquinoline-type alkaloids (**1–6**), including the four new compounds, were selectively cytotoxic against the HCT-15 cell line. These bioactivity data could provide valuable information for future synthetic and pharmacologic studies to identify cytotoxic compounds that are more potent and selective against cancer cells.

Table 3
Cytotoxicity of compounds **1–14** against four cultured human cancer cell lines in the SRB assay

Compound	IC_{50}[a] (μM)			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	>30.0	>30.0	>30.0	27.75
2	>30.0	>30.0	>30.0	23.29
3	>30.0	>30.0	>30.0	24.58
4	>30.0	>30.0	>30.0	27.63
5	>30.0	>30.0	>30.0	29.44
6	>30.0	>30.0	>30.0	26.83
7	23.96	>30.0	>30.0	29.07
8	8.34	5.14	7.87	2.86
9	>30.0	>30.0	27.94	>30.0
10	6.27	16.44	13.76	16.59
11	>30.0	>30.0	>30.0	>30.0
12	>30.0	28.54	27.20	26.35
13	28.26	>30.0	25.67	>30.0
14	20.63	27.41	22.24	29.84
Doxorubicin[b]	0.021	0.003	0.012	0.038

[a] 50% inhibitory concentration; the concentration of the compound that caused a 50% inhibition of cell growth.
[b] Doxorubicin as positive control.

In conclusion, this study indicates that alkaloids are cytotoxic components of the tubers of *C. ternata*. Moreover, four new benzylisoquinoline alkaloids, *epi*-coryximine (**1**) and coryternatines A–C (**2–4**), with selective cytotoxicity against the HCT-15 cell line were isolated from this source. The active alkaloids may possess therapeutic potential against diverse tumor types.

Acknowledgments

This study was supported by a grant (09112KFDA890) from the Korean Food & Drug Administration in 2009. We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their assistance with the NMR spectroscopic and mass spectrometric measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.035.

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