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

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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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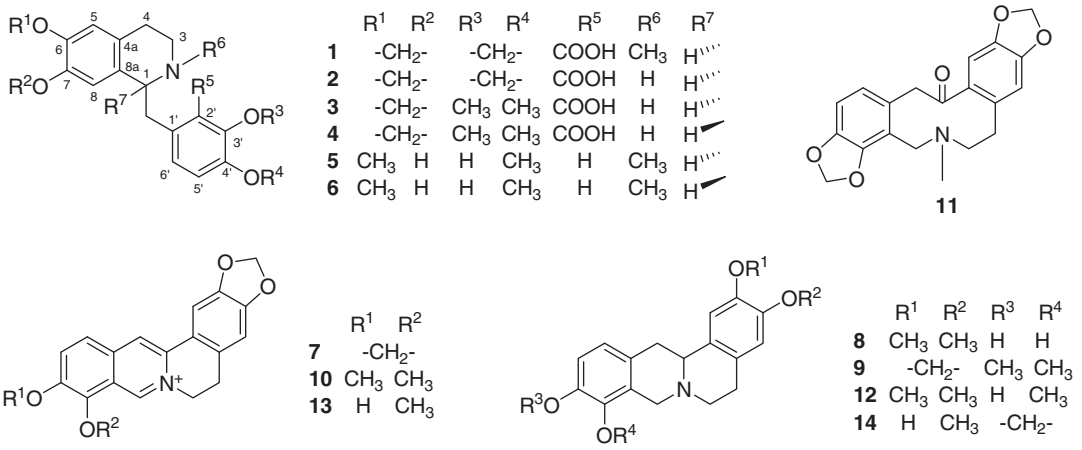

Figure 1. The structures of compounds **1–14** isolated from *C. ternata*.

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.

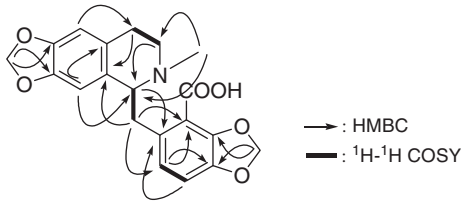

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

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