Bioactive Isoquinoline Alkaloids from *Corydalis saxicola*

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Key words
- *Corydalis saxicola* Bunting
- Fumariaceae
- isoquinoline alkaloids
- acetylcholinesterase

Abstract

Twelve isoquinoline alkaloids including two new nitro-containing tetrahydroprotoberberines, (−)-2,9-dihydroxyl-3,11-dimethoxy-1,10-dinitrotetrahydroprotoberberine (**1**) and (+)-4-nitroisoapocavidine (**2**), were isolated from the whole plant of *Corydalis saxicola* Bunting. The structures of the new compounds were established by spectroscopic analysis and chemical evidence. The inhibitory activity of these isolates against cholinesterase and canine parvovirus were evaluated. Compounds **1** and **1a**, (+)-1-nitroapocavidine (**5**), berberine (**8**), palmatine (**9**), dehydrocavidine (**10**), and sanguinarine (**11**) showed potent inhibitory activity against acetylcholinesterase with IC_{50} values of less than 10 µM, while only compound **1** possessed weak activity against canine parvovirus. Structure-activity studies demonstrated that the nitro substituents at ring A in the tetrahydroprotoberberines led to an increase in the anti-acetylcholinesterase activity.

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Introduction

Corydalis saxicola Bunting (Fumariaceae) is a perennial herb distributed mainly in southwest China [1]. As a traditional Chinese folk medicine, it is used for the treatment of hepatitis, diarrhea, stomachaches, and bleeding hemorrhoids [2]. The plant is rich in isoquinoline alkaloids with activities of anti-hepatitis B virus and DNA topoisomerase I inhibition [3–7]. In our continuing study to find bioactive alkaloids from medicinal plants [8–11], the chemical constituents of *C. saxicola* were investigated, which led to the isolation of 12 isoquinoline alkaloids including two new nitrotetrahydroprotoberberines: 2,9-dihydroxy-3,11-dimethoxy-1,10-dinitrotetrahydroprotoberberine (**1**) and 4-nitroisoapocavidine (**2**). Isoquinoline alkaloids from *Corydalis* were known for their antivirus and anti-acetylcholinesterase activities [6, 12]. Therefore, all of these isolates were evaluated for the activity against canine parvovirus (CPV), acetylcholinesterase (AChE), and butyrylcholinesterase (BuChE). The structural elucidation of the new compounds and bioassay results are reported.

Materials and Methods

General experimental procedures

Melting points were determined using an X-4 melting point apparatus (Yingyu Yuhua Apparatus Factory) and were not corrected. Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. CD spectra were measured in a Chirascan circular dichorism spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer with KBr disks. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as an internal standard. ESIMS and HRESI analyses were carried out on an API Qstar Pulsar 1 instrument. EIMS and HREIMS were carried out on a Waters Autospec Premier P776 mass spectrometer. Column chromatography was performed over silica gel G (80–100, 200–300, and 300–400 mesh), Sephadex LH-20 (40–70 µm; Amersham Pharmacia Biotech AB), HPLC (Agilent 1200, chromatography column: Zorbax SB-C_{18}, ϕ 9.4 × 250 mm, 5 µm), C-18 silica gel (40–75 µm; Fuji Silysia Chemical Ltd.), and MCI gel CHP 20P (polystyrene type, 75–150 µm; Mitsubishi Chem-

ical Corporation). TLC was conducted on precoated silica gel plates GF 254 (Qingdao).

Plant material

The whole plants of *C. saxicola* were collected from Jingxi County, Guangxi Zhuang Autonomous Region in June 2007. The plant was identified by Professor Chun-Lin Long (Kunming Institute of Botany, Chinese Academy of Sciences), and a voucher specimen (No. JX0701) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany.

Extraction and isolation

The whole plants of *C. saxicola* (1.5 kg) were extracted three times with MeOH (3 × 5 L) under reflux. The solvent was evaporated under reduced pressure to give a residue (379 g). The extract was separated by silica gel column chromatography (80 × 10 cm, No. 1007388, 80–100 mesh; Qingdao), eluted with $CHCl_3$–MeOH (30 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, and 1 : 1). Fractions were combined based on TLC analysis, and 6 fractions (A–F) were obtained. Fraction B (38 g) was subjected to C-18 reversed-phase (6 × 50 cm, LiChroprep RP-18; Merck) eluted with MeOH–H_2O (gradient from 1 : 20 to 95% MeOH) to afford 7 fractions (B_1–B_7). Fraction B_3 (5.4 g) was separated on silica gel CC (50 × 4 cm, No. 0803136, 200–300 mesh; Qingdao) eluted with $CHCl_3$-MeOH (20 : 1 to 0 : 1) to afford 6 fractions (B_{31}–B_{36}). The fraction B_{36} (468 mg) was applied to Sephadex LH-20 (200 cm, Sephadex LH-20) with MeOH and further purified by column chromatography (40 × 2 cm, No. 0100349, 300–400 mesh; Qingdao) eluted with $CHCl_3$: MeOH (5 : 1) to gain **1** (12.0 mg) and **10** (110 mg). The fraction B_{33} (676 mg) was purified on silica gel CC (40 × 2 cm, No. 0100349, 300–400 mesh; Qingdao) eluted with petroleum ether-Me_2CO (10 : 1 to 0 : 1) to afford 5 fractions (B_{331}–B_{335}), then the fraction B_{333} (50 mg) was purified by preparative TCL (petroleum ether-Me_2CO, 2 : 1) to gain **11** (5.0 mg) and **12** (6.0 mg).

The fraction B_2 (400 mg) was separated by Sephadex LH-20 (200 cm, Sephadex LH-20) with MeOH to get 5 fractions (B_{21}–B_{25}). The fraction B_{23} (110 mg) was subjected to column chromatography (40 × 2 cm, No. 0100349, 300–400 mesh; Qingdao) eluted with $CHCl_3$-MeOH-diethylamine (40 : 2 : 1) to get 3 fractions (B_{231}–B_{234}), and the fraction B_{232} (58 mg) was chromatographed on a Sephadex LH-20 column (200 cm, Sephadex LH-20) to obtain **8** (15 mg) and **9** (17 mg).

The fraction C (4.0 g), which was subjected to column chromatography over MCI gel CHP 20P (80 × 10 cm, polystyrene type, 75–150 µm) was eluted with MeOH-H_2O (4 : 1 to 100% MeOH) to obtain 3 fractions (C_1–C_3). The fraction C_1 (3.2 g) was flashed on C_{18} silica gel (4 × 50 cm, LiChroprep RP-18; Merck) with MeOH-H_2O (gradient from 1 : 20 to 95% MeOH) to get 6 fractions (C_{11}–C_{16}). The fraction C_{15} (500 mg) was separated on silica gel CC (40 × 2 cm, No. 0100349, 300–400 mesh; Qingdao) eluted with $CHCl_3$-ethyl acetate (1 : 0 to 0 : 1) to obtain 5 fractions (C_{151}–C_{155}). The fraction C_{152} (106 mg) was purified by preparative TCL (petroleum ether-ethyl acetate, 2 : 1) to obtain a mixture and **5** (31 mg). The mixture was then submitted to Sephadex LH-20 (200 cm, Sephadex LH-20) with MeOH and preparative TCL (petroleum ether-ethyl acetate, 2 : 1) to gain **2** (11 mg). The fraction C_{151} (86 mg) was subjected to preparative TCL (petroleum ether-ethyl acetate, 2 : 1) to gain **7** (50 mg).

The fraction C_{14} (400 mg) was then submitted to Sephadex LH-20 (200 cm, Sephadex LH-20) with MeOH to obtain 4 fractions (C_{141}–C_{144}); then the fraction C_{142} (53 mg) was purified by preparative TCL ($CHCl_3$-MeOH, 6 : 1) to get **4** (15 mg). The fraction C_{143} (60 mg) was purified by preparative TCL ($CHCl_3$-MeOH, 1 : 1) to gain **3** (27 mg) and **6** (20 mg). The purity of compounds **1**–**12** was greater than 95% as determined by TLC and NMR.

(−)-2,9-Dihydroxy-3,11-dimethoxy-1,10-dinitrotetrahydroprotoberberine (**1**): yellow amorphous powder (MeOH); $[\alpha]_D^{14}$ − 586.3(*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 342 (3.82), 274 (3.73) nm; CD $\Delta\varepsilon$ (*c* 0.0020, MeOH) − 0.60 (240), − 1.52 (207); IR (KBr) ν_{max} 3432, 1629, 1517, 1327 cm^{-1}; ^1H and ^{13}C NMR, see ◉ **Table 1**; ESIMS *m*/*z* 418 $[M + H]^+$; HRESIMS *m*/*z* 418.1251 $[M + H]^+$ (calcd. for $C_{19}H_{20}N_3O_8$, 418.1250).

Preparation of 1a: K_2CO_3 (10.0 mg), Me_2CO (10 mL), and Me_2SO_4 (0.5 mL) were added to a solution of **1** (8.0 mg). The mixture was stirred for 12 h at 50 °C. Then, 25% $NH_3 \cdot H_2O$ (5 mL) was added and evaporated *in vacuo*. The residue was partitioned between H_2O (10 mL) and $CHCl_3$ (3 × 10 mL). The $CHCl_3$ layer was concentrated and purified by Sephadex LH-20 column chromatography (MeOH) to afford **1a** (7.9 mg): pink amorphous powder (MeOH); $[\alpha]_D^{23}$ − 112.1 (*c* 0.19, MeOH); CD (*c* 0.0020, MeOH) $\Delta\varepsilon$ −0.60 (240), − 1.52 (207); ^1H and ^{13}C NMR, see ◉ **Table 1**; ESIMS *m*/*z* 460 $[M]^+$.

(+)-4-Nitroisoapocavidine (**2**): yellow powder ($CHCl_3$); m.p. 290–293 °C; $[\alpha]_D^{25}$ + 82.7 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 424 (3.05), 343 (3.49), 279 (3.78), 240 (3.93) nm; CD $\Delta\varepsilon$ (*c* 0.0021, MeOH) + 0.23 (207); IR (KBr) ν_{max} 3430, 1628, 1534, 1510, 1467, 1358, 1292 cm^{-1}; ^1H and ^{13}C NMR, see ◉ **Table 1**; ESIMS *m*/*z* 385 $[M + H]^+$; EIMS *m*/*z* 384 $[M]^+$ (45), 369 (8), 354 (7), 223 (5), 162 (100); HREIMS *m*/*z* 384.1322 $[M]^+$ (calcd. for $C_{20}H_{20}N_2O_6$, 384.1321).

General procedure for preparation of hydrochlorides

A drop of concentrated hydrochloric acid was added to a solution of the alkaloids (1.0 mg) in MeOH (0.5 mL). The mixture was evaporated under reduced pressure to yield the hydrochlorides of compounds **1**–**7**.

Bioassays

Microplate assay for AChE and BuChE: The AChE inhibitory activities of samples were measured quantitatively by the improved Ellman's method with tacrine (Sigma, purity 98%) as the positive control (IC_{50} = 0.19 µM) [13, 14]. Inhibitory activity of compounds against BuChE was performed by the improved Ellman's method using tetraisopropylpyrophosphoramide (Sigma, purity 98%) as the positive control (IC_{50} = 1.35 µM) [13, 15].

Cell culture and cytotoxicity assays: The embryonic feline kidney F81 cell line (Cell Bank of the Chinese Academy of Sciences, Shanghai) was used for growing canine parvovirus, which was isolated by the authors in Yunnan Agricultural University. The cells were grown in monolayer in a 5% carbon dioxide and 95% atmosphere at 37 °C, with minimal essential medium (MEM; HyClone) containing 10% fetal bovine serum (HyClone). Cytotoxicity assays were performed by the WST-8 method [16, 17], using Cell Counting Kit-8 (CCK-8; Dojindo) according to the supplier recommendations. Briefly, cells were incubated in a 96-well microculture plate (Corning) in the absence or presence of twofold serial dilutions of compounds **1**–**12** and ribavirin (Sigma). After 4 days of culture, 10 µL of CCK-8 solution was added, and the cells were incubated for 1 hour. The number of surviving cells was measured with a Bio-Tek ELx 800.

ELISA microplate reader could show the detection wavelength of 450 nm (L_1) and the reference wavelength of 650 nm (L_2). The 50% cytotoxic concentration (CC_{50}) was obtained by nonlinear

Table 1 ^{1}HNMR data for compounds **1**, **1a**, and **2** (δ in ppm, J in Hz).

Position	1		1a		2	
	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]	δ_H[e]	δ_C[f]
1		140.0 (qC)		144.9 (qC)	6.77 (1H, s)	110.0 (CH)
2		139.7 (qC)		140.7 (qC)		148.8 (qC)
3		149.0 (qC)		154.8 (qC)		142.0 (qC)
4	6.93 (1H, s)	114.4 (CH)	7.45 (1H, s)	116.9 (CH)		137.5 (qC)
4a		127.2 (qC)		127.8 (qC)		119.1 (qC)
5	3.01 (1H, m)	29.8 (CH_2)	3.60 (2H, m)	23.9 (CH_2)	3.89 (1H, m)	22.3 (CH_2)
	2.88 (1H, ddd, 16.3, 5.3, 5.3)				2.85 (1H, m)	
6	3.13 (1H, m)	48.2 (CH_2)	4.10 (2H, m)	51.0 (CH_2)	3.95 (1H, m)	51.2 (CH_2)
	2.69 (1H, ddd, 11.4, 7.5, 5.3)				3.14 (1H, m)	
8	4.16 (1H, d, 16.5)	53.8 (CH_2)	5.42 (1H, d, 16.4)	61.0 (CH_2)	4.90 (1H, d, 15.5)	52.0 (CH_2)
	3.78 (1H, d, 16.5)		5.15 (1H, d, 16.4)		4.02 (1H, d, 15.5)	
8a		122.9 (qC)		119.8 (qC)		108.4 (qC)
9		150.3 (qC)		150.9 (qC)		143.7 (qC)
10		140.4 (qC)		143.2 (qC)		146.4 (qC)
11		146.2 (qC)		151.6 (qC)	6.76 (1H, d, 8.0)	109.0 (CH)
12	7.67 (1H, s)	108.1 (CH)	7.90 (1H, s)	111.1 (CH)	6.70 (1H, d, 8.0)	121.6 (CH)
12a		126.2 (qC)		122.9 (qC)		130.2 (qC)
13	3.19 (1H, dd, 18.0, 3.6)	32.2 (CH_2)	4.00 (1H, m)	33.1 (CH_2)	3.64 (1H, m)	36.1 (CH)
	2.98 (1H, dd, 18.0, 11.4)		3.56 (1H, m)			
14	3.90 (1H, dd, 11.4, 3.6)	55.4 (CH)	5.01 (1H, dd, 11.3, 5.6)	61.3 (CH)	4.66 (1H, br s)	63.6 (CH)
14a		122.3 (qC)		116.0 (qC)		120.9 (qC)
2-OMe			3.94 (3H, s)	62.5 (CH_3)	3.89 (3H, s)	56.8 (CH_3)
3-OMe	3.92 (3H, s)	56.8 (CH_3)[g]	4.01 (3H, s)	57.2 (CH_3)		
7-NMe			3.52 (3H, s)	50.9 (CH_3)		
9-OMe			4.04 (3H, s)	61.7 (CH_3)		
11-OMe	3.93 (3H, s)	57.0 (CH_3)[g]	4.02 (3H, s)	56.9 (CH_3)		
13-Me					1.28 (3H, d, 6.8)	17.9 (CH_3)
OCH_2O					5.97 (1H, d, 1.5)	102.1 (CH_2)
					5.93 (1H, d, 1.5)	

[a] Measured in CD_3OD-DMSO-d_6 (6 : 1) at 500 MHz. [b] In CD_3OD-DMSO-d_6 (6 : 1) at 100 MHz. [c] In acetone-d_6 at 500 MHz. [d] In acetone-d_6 at 100 MHz. [e] In $CDCl_3$-CD_3OD (6 : 1) at 400 MHz. [f] In $CDCl_3$-CD_3OD (6 : 1) at 100 MHz. [g] Data under the same entry are interchangeable

regression analysis of logistic curves (the value of $L_1 - L_2$ to different concentrations of compounds).

Protection assay for CPV-infected F81 cells: By the determination of cell viability, the ability of the compounds to protect CPV-infected F81 cells from death was evaluated [18, 19] using the WST-8 method as described above. Tissue culture medium infective dose ($TCID_{50}$) of 300 viral particles with twofold serial dilutions of the compounds were added to each test well, and the plates were reincubated for 4 days to allow development of a cytopathologic effect (CPE) if any. The number of surviving cells was measured. The 50% effective concentration (EC_{50}) and therapeutic index (TI) were calculated. Ribavirin (Sigma, purity 99.5%) was used as a positive control (EC_{50} = 121.72 μM; TI = 10.5) (**Fig. 1**).

Supporting information

The optical rotation values and CD data of compounds **1**–**7** as well as 1D NMR and 2D NMR spectra of compounds **1**, **1a**, and **2** are available as Supporting Information.

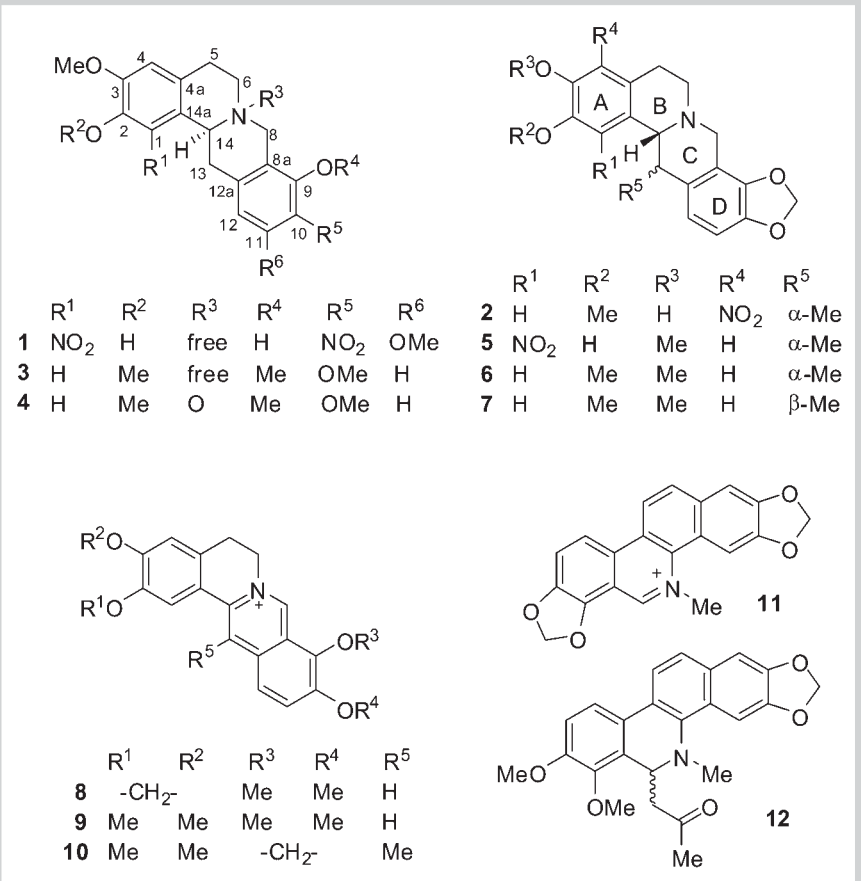
Results and Discussion

The molecular formula $C_{19}H_{19}N_3O_8$ of **1** was determined from the HRESIMS ([M + H]$^+$ 418.1251, calcd. 418.1250). The IR spectrum of **1** indicated the presence of hydroxy (3432 cm^{-1}), aromatic (1629 cm^{-1}), and nitro (1327 and 1517 cm^{-1}) functional groups. NMR data of **1** (**Table 1**) showed the presence of two pentasubstituted phenyl rings [δ_H 7.67 (1H, s, H-12), 6.93 (1H, s, H-4); δ_C 150.3 (qC), 149.0 (qC), 146.2 (qC), 140.4 (qC), 140.0 (qC), 139.7 (qC), 127.2 (qC), 126.2 (qC), 122.9 (qC), 122.3 (qC), 114.4 (CH), 108.1 (CH)]. The ^{1}H NMR spectrum also showed signals for two aryl methoxy groups, three aliphatic protons (H_2-13 and H-14) as an AMX system, and two aliphatic protons of the same methylene (H_2-8) as an AX system, together indicating that **1** may be a tetrahydroprotoberberine [20].

Using the ^{1}H–^{1}H COSY and HMBC results (**Fig. 2**), the carbon skeleton of tetrahydroprotoberberine **1** was established. The ROESY spectrum showed the correlations of H-4/3-OMe and H-12/11-OMe, helping to establish that the two methoxy groups were located at C-3 and C-11, respectively.

The remaining four substituent groups in the two benzene rings were deduced as two hydroxy groups and two nitro groups according to the molecular formula of **1**. In order to determine their locations, a derivative (**1a**) of **1** was prepared.

The ESI-MS afforded the positive ion of **1a** at m/z 460 [M]$^+$, which implied that three more *O*- or *N*-methyl groups were linked to **1** through the reaction. The ^{1}H-NMR spectrum (**Table 1**) of **1a** indicated four *O*-methyl groups (δ_H 3.94, 4.01, 4.02, and 4.04) and one *N*-methyl group (δ_H 3.52). The 3-OMe and 11-OMe groups were located on **1a** by the ROESY correlations (**Fig. 2**) of H-4/3-OMe and H-12/11-OMe, respectively, while 2-OMe and 9-OMe groups were determined by the HMBC correlations (**Fig. 2**) from H-4 and 2-OMe to C-2, and H_2-8 and 9-OMe to C-9, respectively. Thus, the remaining two nitro groups must be



	R^1	R^2	R^3	R^4	R^5	R^6
1	NO_2	H	free	H	NO_2	OMe
3	H	Me	free	Me	OMe	H
4	H	Me	O	Me	OMe	H

	R^1	R^2	R^3	R^4	R^5
2	H	Me	H	NO_2	α-Me
5	NO_2	H	Me	H	α-Me
6	H	Me	Me	H	α-Me
7	H	Me	Me	H	β-Me

	R^1	R^2	R^3	R^4	R^5
8	$-CH_2-$		Me	Me	H
9	Me	Me	Me	Me	H
10	Me	Me	$-CH_2-$		Me

Fig. 1 Structures of compounds **1**–**7**.

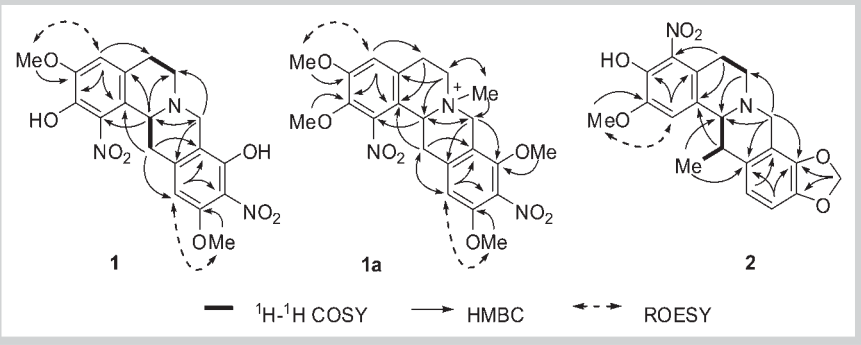


Fig. 2 Key 2D NMR correlations of **1**, **1a**, and **2**.

linked to C-1 and C-10, respectively. Therefore, the planar structure of **1** was elucidated as 2,9-dihydroxy-3,11-dimethoxy-1,10-dinitrotetrahydroprotoberberine.

The absolute configuration of **1** was determined as 14*S* according to the strong negative Cotton effect at 207 nm ($\Delta\varepsilon$ · –1.52) in the CD spectrum of **1** hydrochloride [21].

Compound **2** was obtained as yellow powder, which has the molecular formula of $C_{20}H_{20}N_2O_6$ according to its HREIMS at *m*/*z* 384.1322 $[M]^+$ (calcd. 384.1321). The fragments with *m*/*z* 223 (5%) and 162 (100%) in the EIMS suggested the substitution pattern of the 13-methyl-9,10-methylenedioxy moiety at rings C and D [3]. The ^{1}H-NMR spectrum displayed one *O*-methyl group at δ 3.89 (s), one methyl group at δ 1.28 (d), and one methylenedioxy group at δ 5.97 (d) and 5.93 (d), respectively. Comparison of NMR data of compound **2** with those of known (+)-1-nitroapocavidine (**5**) [3] and isoapocavidine [22,23] revealed that compound **2** is very similar to the two known alkaloids except for the substitution pattern in ring A. The presence of ROESY correlations (**Fig. 2**) between H-1 and 2-OMe implied that the nitro group should be located at C-4 in **2** rather than at C-1 in **5**. In the 13-methyl tetrahydroprotoberberines with a *cis* configuration between H-13 and H-14, the carbon chemical shift of 13-Me is about 18 ppm rather than 22 ppm in those with a *trans* configuration [22,24,25]. Therefore, the relative configuration of **2** was confirmed as 4-nitroisoapocavidine. The absolute configuration of **2** was deduced as 13*S*, 14*R* by comparison of its CD spectrum with that of known (+)-cavidine (**6**) (see Supporting Information) [26,27].

Ringdahl et al. have studied the CD spectra of a series of 14*S*-tetrahydroprotoberberines and suggested that the intense 206–210 nm negative Cotton effects correlate directly with the absolute configuration of 14*S* [21]. However, 14*R*-tetrahydroprotober-

Table 2 AChE inhibitory activity of alkaloids from *Corydalis saxicola*.

Compound	IC_{50} (μM)
1	8.77 ± 0.20
1a	3.34 ± 0.69
2	53.30 ± 0.50
3	43.20 ± 1.20
4	64.40 ± 1.60
5	1.70 ± 0.31
6	> 200
7	14.50 ± 0.20
8	1.87 ± 0.48
9	2.20 ± 0.46
10	9.92 ± 0.23
11	1.93 ± 0.01
12	58.70 ± 1.34
Tacrine	0.19 ± 0.02

berines were not reported in the literature. We found that 14*R* compounds (**2** and **5**–**7**) showed positive Cotton effects at 206–210 nm, whose intensity was changed according to the configuration of C-13. In the case of the *cis* configuration between H-13 and H-14 (**2**, **5**, and **6**), the Cotton effects are much weaker than that in the *trans* one (**7**) (see Supporting Information).

To the best of our knowledge, compounds **1** and **2** are among the very few examples of the natural tetrahydroprotoberberine alkaloids with a nitro group.

The known alkaloids (−)-tetrahydropalmatine (**3**) [21,28], (−)-corynoxidine (**4**) [29], (+)-1-nitroapocavidine (**5**) [3], (+)-cavidine (**6**) [26,27], (+)-thalictrifoline (**7**) [26,27,30], berberine (**8**) [31,32], palmatine (**9**) [33], dehydrocavidine (**10**) [34], sanguinarine (**11**) [31], and 8-acetonyldihydrochelerythrine (**12**) [35] were determined by comparison of their spectral data with those in the literature.

The inhibitory activity of all compounds against AChE, BuChE, and canine parvovirus were evaluated. The results showed that compounds **1** and **1a**, (+)-1-nitroapocavidine (**5**), berberine (**8**), palmatine (**9**), dehydrocavidine (**10**), and sanguinarine (**11**) owned potent inhibitory activity against acetylcholinesterase with IC_{50} values less than 10 μM (**Table 2**). Structure-activity relationships indicated that (i) all of tested berberines (**8**–**10**) were active (IC_{50} < 10 μM). Other berberines such as jatrorrhizine, dehydrocorydaline, pseudocolumbamine, coptisine, and pseudopalmatine possessed similar potency [20,36]. However, phenolic hydroxy groups (dehydrocorydalmine and dehydrocorytenchine) could reduce the activity [20]; (ii) nitro substitutions at ring A, especially at C-1, in the tetrahedroprotoberberines (**2**, **5**, and **6**) could increase the activity.

All of these compounds were inactive against BuChE (IC_{50} > 200 μM), while compound **1** showed weak protection activity of CPV-infected F81 cells from death (EC_{50} = 182.06 μM; SI = 2.2).

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Conflict of Interest

There are no conflicts of interest among the authors.

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References

1 Flora of Guangxi. Nanning: Guangxi Science and Technology Press; 1993: 410
2 Zhonghua Bencao, Vol. 3. Shanghai: Shanghai Science and Technology Press; 1999: 638–640
3 *Li HL, Zhang WD, Zhang W, Zhan C, Liu RH.* A new nitro alkaloid from *Corydalis saxicola* bunting. Chin Chem Lett 2005; 16: 367–368
4 *Wu YR, Ma YB, Zhao YX, Yao SY, Zhou J, Zhou Y, Chen JJ.* Two new quaternary alkaloids and anti-hepatitis B virus active constituents from *Corydalis saxicol*. Planta Med 2007; 73: 787–791
5 *Li HL, Zhang WD, Han T, Zhang C, Liu RH, Chen HS.* Tetrahydroprotoberberine alkaloids from *Corydalis saxicola*. Chem Nat Comp 2007; 43: 173–175
6 *Li HL, Han T, Liu RH, Zhang C, Chen HS, Zhang WD.* Alkaloids from *Corydalis saxicola* and their anti-hepatitis B virus activity. Chem Biodivers 2008; 5: 777–783
7 *Cheng XX, Wang DM, Jiang L, Yang DP.* DNA topoisomerase I inhibitory alkaloids from *Corydalis saxicola*. Chem Biodivers 2008; 5: 1335–1344
8 *Wang YH, Long CL, Yang FM, Wang X, Sun QY, Wang HS, Shi YN, Tang GH.* Pyrrolidinoindoline alkaloids from *Selaginella moellendorfii*. J Nat Prod 2009; 72: 1151–1154
9 *Zhao FW, Sun QY, Yang FM, Hu GW, Luo JF, Tang GH, Wang YH, Long CL.* A new type of lycopodium alkaloid, lycoposerramine-A, from *Lycopodium serratum* thunb. Org Lett 2010; 12: 3922–3925
10 *Zhao FW, Luo M, Wang YH, Li ML, Tang GH, Long CL.* A piperidine alkaloid and limonoids from *Arisaema decipiens*, a traditional antitumor herb used by the dong people. Arch Pharm Res 2010; 33: 1735–1739
11 *Tang GH, Chen DM, Qiu BY, Sheng L, Wang YH, Hu GW, Zhao FW, Ma LJ, Wang H, Huang QQ, Xu JJ, Long CL, Li J.* Cytotoxic amide alkaloids from *Piper boehmeriaefolium*. J Nat Prod 2011; 74: 45–49
12 *Hung TM, Na M, Dat NT, Ngoc TM, Youn U, Kim HJ, Min BS, Lee J, Bae K.* Cholinesterase inhibitory and anti-amnesic activity of alkaloids from *Corydalis turtschaninovii*. J Ethnopharmacol 2008; 119: 74–80
13 *Ellman GL, Courtney KD, Andres V, Featherstone RM.* A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88–95
14 *Sun QY, Yang FM.* Comparative study on microassays for screening acetylcholinesterase inhibitors. Chin Pharmacol Bull 2008; 24: 1387–1392
15 *Yang FM, Sun QY.* Study on microassay for screening butyrylcholinesterase inhibitors. Chin Pharmacol Bull 2009; 25: 690–692
16 *Ishiyama M, Shiga M, Sasamoto K, Mizoguchi M, He PG.* A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. Chem Pharm Bull 1993; 41: 1118–1122
17 *Tominaga H, Ishiyama M, Ohseto F, Sasamoto K, Hamamoto T, Suzuki K, Watanabe M.* A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Anal Commun 1999; 36: 47–50
18 *Heldt CL, Hernandez R, Mudiganti U, Gurgel PV, Brown DT, Carbonell RG.* A colorimetric assay for viral agents that produce cytopathic effects. J Virol Methods 2006; 135: 56–65
19 *Mu LZ, Zhang YL, Zhang MJ, Li L, Zhang Y, Liu SC.* Antiviral activities of pinon shell polysaccharide on CDV and CPV *in vitro*. Chin J Vet Sci 2009; 29: 1111–1114
20 *Tsai SF, Lee SS.* Characterization of acetylcholinesterase inhibitory constituents from *Annona glabra* assisted by HPLC microfractionation. J Nat Prod 2010; 73: 1632–1635
21 *Ringdahl B, Chan RPK, Craig JC, Manske RHF.* Proterberine alkaloids: chiroptical properties and absolute configuration. J Nat Prod 1981; 44: 75–79

22 *Rücker G, Breitmaier E, Zhang GL, Mayer R.* Alkaloids from *Dactylicapron torulosa*. Phytochemistry 1994; 36: 519–523
23 *Halbsguth C, Meissner O, Häberlein HH.* 3-O-Methylquercetin more selectively inhibits phosphodiesterase subtype 3. Planta Med 2003; 69: 305–309
24 *Iwasa K, Cushman M.* Characterization of an unusual tetrahydroprotoberberine conformer by carbon-13 nuclear magnetic resonance spectroscopy. J Org Chem 1982; 47: 545–552
25 *Takao N, Iwasa K, Kamigauchi M, Sugiura M.* Studies on the alkaloids of papaveraceous plants. XXIX. Conformational analysis of tetrahydroprotoberberines by Carbon-13 magnetic resonance spectroscopy. Chem Pharm Bull 1977; 25: 1426–1435
26 *Hughes DW, Holland HL, Maclean DB.* ^{13}C Magnetic resonance spectra of some isoquinoline alkaloids and related model compounds. Can J Chem 1976; 54: 2252–2260
27 *Iwasa K, Gupta YP, Cushman M.* Absolute configuration of the cis-and trans-13-methyl tetrahydroprotoberberines, total synthesis of (+)-thalicticavidine, (+)-canadine, (±)-, (−)-, and (+)-thalictrifoline, and (±)-, (−)-, and (+)-cavidine. J Org Chem 1981; 46: 4744–4750
28 *Blanchfield JT, Sands DPA, Kennard CHL, Byriel KA, Kitching W.* Characterisation of alkaloids from some Australian *Stephania* (Menispermaceae) species. Phytochemistry 2003; 63: 711–720
29 *Kim DK, Lee KT, Baek NI, Kim SH, Park HW, Lim JP, Shin TY, Eom DO, Yang JH, Eun JS.* Acetylcholinesterase inhibitors from the aerial parts of *Corydalis speciosa.* Arch Pharmacol Res 2004; 27: 1127–1131
30 *Iwasa K, Gupta YP, Cushman M.* The absolute configurations of (+)-thalictrifoline and (+)-corydalic acid methyl ester total synthesis of (+)-thalictrifoline. Tetrahedron Lett 1981; 22: 2333–2336
31 *Blasko G, Cordell GA, Bhamarapravati S, Beecher CWW.* Carbon-13 NMR assignments of berberine and sanguinarine. Heterocycles 1988; 27: 911–916
32 *Janssen RHAM, Lousberg RJJC, Wijkens P, Kruk C, Theuns HG.* Assignment of ^{1}H and ^{13}C NMR resonances of some isoquinoline alkaloids. Phytochemistry 1989; 28: 2833–2839
33 *Hussain RA, Kim J, Beecher CWW, Kinghorn AD.* Unambiguous carbon-13 NMR assignments of some biologically active protoberberine alkaloids. Heterocycles 1989; 29: 2257–2260
34 *Bhakuni DS, Chaturvedi R.* The alkaloids of *Corydalis meifolia* [J]. J Nat Prod 1983; 46: 466–470
35 *Martínez-Martínez FJ, Padilla-Martínez II, Hernández-Carlos B, Pérez-Gutiérrez RM, García-Báez EV.* X-ray diffraction and total ^{1}H and ^{13}C NMR assignment of (RS)-5,6-dihydro-7,8dimethoxy-5-methyl-6-(2-oxopropyl)-(2,3-methylenedioxyphenyl)-[c]-phenanthridine ((RS)-6-acetonyldihydrochelerythrine). J Chem Crystallogr 2002; 32: 63–68
36 *Xiao HT, Peng J, Liang Y, Yang J, Bai X, Hao XY, Yang FM, Sun QY.* Acetylcholinesterase inhibitors from *Corydalis yanhusuo.* Nat Prod Res, advance online publication 8 July 2011; doi: 10.1080/14786410802496911