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New Cytotoxic Tetrahydroprotoberberine-Aporphine Dimeric and Aporphine Alkaloids from *Corydalis turtschaninovii*

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Key words
- *Corydalis turtschaninovii*
- Papaveraceae
- tetrahydroprotoberberine-aporphine dimmer
- aporphine
- cytotoxicity

Abstract

Two new tetrahydroprotoberberine-aporphine dimeric alkaloids, corydaturtschines A (**1**) and B (**2**), and a new aporphine derivative, ethyl glausuccinate (**3**), together with 13 known protoberberine (**4**–**16**) and nine known aporphine alkaloids (**17**–**25**), were isolated from the tubers of *Corydalis turtschaninovii*. The structures of these new compounds were determined through spectral analyses, including extensive 2D-NMR data. The absolute configurations of the compounds were clarified by CD spectroscopic studies. The isolated compounds were tested for their cytotoxicity against four human cancer cell lines *in vitro* using a sulforhodamine B bioassay.

Introduction

Corydalis turtschaninovii Bess. (Papaveraceae) is a garden plant with blue flowers, native to Manchuria and Siberia [1]. *C. turtschaninovii* is a medicinally important species of *Corydalis* and its dried tubers, called yan-hu-suo, have been used as traditional Chinese medicine in the treatment of gastric and duodenal ulcer, cardiac arrhythmic disease, rheumatism, and dysmenorrhea [2], as well as memory dysfunction [3]. Previous pharmacological studies of *C. turtschaninovii* found that its extracts exhibited antiallergic [4] and antiamnesic activities [5]. The tuber contains several tertiary and quaternary alkaloids with pharmacologically important activity, such as corydaline, palmatine, coptisine, and columbamine [4–7]. In the course of our continuing search for biologically active compounds from Korean medicinal plant sources, we performed a phytochemical investigation of tubers of *C. turtschaninovii*. The purification of the $CHCl_3$-soluble fraction of its ethanol extract by repeated column chromatography led to the isolation of two new tetrahydroprotoberberine-aporphine dimeric alkaloids, corydaturtschines A (**1**) and B (**2**), and a new aporphine derivative, ethyl glausuccinate (**3**), together with 13 known protoberberine (**4**–**16**) and nine known aporphine alkaloids (**17**–**25**). The structures of these new compounds were determined through spectral analysis, including extensive 2D-NMR data. The absolute configurations of the compounds were determined by analysis of CD data. The isolated compounds were evaluated for their cytotoxicity against four human cancer cell lines *in vitro* using a sulforhodamine B bioassay. This paper describes the structural elucidation of the new compounds (**1**–**3**) and the cytotoxicity of isolated compounds (**1**–**25**).

Materials and Methods

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using a Shimadzu UV-1601 UV-visible spectrophotometer. CD spectra were measured on a JASCO J-715 spectropolarimeter. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ). FAB and HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and an Apollo Silica 5 µm column (250 × 10 mm) or Econosil RP-18 10 µm column (250 × 10 mm). Silica gel 60 (Merck; 70 ~ 230 mesh, and 230 ~ 400 mesh) and RP-C_{18} silica gel (Merck; 230–400 mesh) were used for column chromatography. TLC was performed using Merck

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precoated Silica gel F_{254} plates and RP-18 $F_{254\,s}$ plates. Spots were detected under UV light or by heating after spraying with 10% H_2SO_4 in EtOH (v/v). The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si gel 60 (240 × 10 mm) with an FMI QSY-0 pump (ISCO).

Plant material

The tubers of *C. turtschaninovii* were purchased from Kyungdong herbal market, Seoul, Korea, in July 2007, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2007–7) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The tubers of *C. turtschaninovii* (10 kg) were extracted with 50% EtOH two times at room temperature. The ethanol extract (250 g) was suspended in distilled water (7.2 L) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, yielding 10, 30, 4, and 26 g, respectively. The $CHCl_3$ soluble fraction (28 g) was separated over a silica gel column (230–400 mesh, 600 g) eluted with $CHCl_3$-MeOH [15 : 1 (3.0 L), and 5 : 1 (3.0 L)] to afford seven fractions [Fr. A, 15 : 1, 1.0 L; Fr. B, 15 : 1, 1.0 L; Fr. C, 15 : 1, 1.0 L; Fr. D, 5 : 1, 0.5 L; Fr. E, 5 : 1, 0.5 L; Fr. F, 5 : 1, 1.0 L; and Fr. G, 5 : 1, 1.0 L]. Fr. A (3.5 g) and Fr. B (10.0 g) were mixed up and separated further over a silica gel column (230–400 mesh, 250 g, *n*-hexane/EtOAc = 3 : 1) to give seven subfractions [Fr. A1 – Fr. A7 (each 1.0 L)]. Fr. A1 (100 mg) was separated with a Lobar-A Si gel 60 (240 × 10 mm) column (*n*-hexane/EtOAc = 3 : 1) and further separated by preparative HPLC using a solvent of *n*-hexane/EtOAc (3 : 1) at a flow rate of 2.0 mL/min (Apollo Silica 5 µm column; 250 × 10 mm; 5 µ particle size; Shodex refractive index detector) to yield **5** (10 mg, t_R = 13.5 min). Fr. A2 (3.2 g) was separated over a Sephadex LH-20 column (150 g; Pharmacia Co.; CH_2Cl_2/MeOH = 1 : 1) and further separated by preparative reversed-phase HPLC using a solvent of 80% MeOH, over 30 min at a flow rate of 2.0 mL/min (Econosil RP-18 10 µ column; 250 × 10 mm; 10 µ particle size; Shodex refractive index detector) to yield **6** (12 mg, t_R = 12.5 min) and **7** (70 mg, t_R = 14.0 min) and by preparative HPLC, as described above, to obtain **8** (6 mg, *n*-hexane/$CHCl_3$/EtOAc = 5 : 3 : 5, t_R = 11.5 min) and **9** (6 mg, *n*-hexane/CH_2Cl_2/EtOAc = 3 : 1 : 1, t_R = 13.5 min). Fr. A3 (350 mg) was separated by preparative HPLC using a solvent of *n*-hexane/$CHCl_3$/EtOAc (6 : 3 : 5), as described above, to obtain **18** (13 mg, t_R = 12.5 min) and **10** (30 mg, t_R = 13.5 min). Fr. A4 (800 mg) was separated over a RP-C_{18} silica gel column (230–400 mesh, 10 g, 70% MeOH) and further separated by preparative HPLC using a solvent of $CHCl_3$/MeOH (50 : 1), as described above, to yield **11** (15 mg, t_R = 12.5 min), **12** (45 mg, t_R = 14.0 min), and **13** (8 mg, t_R = 15.0. min). Fr. A5 (350 mg), Fr. A6 (350 mg), and Fr. A7 (550 mg) were mixed up and separated over a RP-C_{18} silica gel column (230–400 mesh, 10 g, 80% MeCN) to give seven subfractions [Fr. A51 – Fr. A57 (each 1.0 L)]. Fr. A52 (130 mg) was separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 30 : 1) and further separated by preparative HPLC using a solvent of $CHCl_3$/MeOH (30 : 1), as described above, to yield **2** (6 mg, t_R = 11.5 min) and **19** (16 mg, t_R = 13.5 min). Fr. A53 (300 mg) was separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 10 : 1) to yield **21** (4 mg). Fr. A54 (50 mg) was separated by preparative HPLC using a solvent of $CHCl_3$/MeOH (50 : 1), as described above, to yield **1** (6 mg, t_R = 12.0 min) and **20** (8 mg, t_R = 12.5 min). Fr. A55 (500 mg) was separated over a silica gel column (230–400 mesh, 3.5 g, $CHCl_3$/MeOH = 50 : 1) to yield **24** (180 mg). Fr. A56 (150 mg) was separated by preparative HPLC using a solvent of *n*-hexane/CH_2Cl_2/EtOAc (2 : 1 : 1), as described above, to yield **4** (12 mg, t_R = 14.5 min) and **17** (32 mg, t_R = 16.0 min). Fr. C (1.3 g) was separated over a RP-C_{18} silica gel column (230–400 mesh, 10 g, 100% MeOH) to give three subfractions [Fr. C1 – Fr. C3 (each 1.0 L)]. Fr. C1 (120 mg) was separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 20 : 1) and further separated by preparative HPLC using a solvent of $CHCl_3$/MeOH (45 : 1), as described above, to yield **3** (25 mg, t_R = 12.0 min) and **23** (18 mg, t_R = 14.5 min). Fr. C2 (1 g) was separated over a silica gel column (5.5 g, $CHCl_3$/MeOH = 50 : 1) and further separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 200 : 1) to yield **22** (20 mg). Fr. D (2.5 g) was separated over a silica gel column (230–400 mesh, 10 g, $CHCl_3$/MeOH = 15 : 1, and 5 : 1) to give eight subfractions [Fr. D1 – Fr. D4 ($CHCl_3$/MeOH = 15 : 1, each 1.0 L), Fr. D5 – Fr. D8 ($CHCl_3$/MeOH = 5 : 1, each 1.0 L)]. Fr. D8 (250 mg) was further separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 20 : 1) to yield **16** (20 mg). Fr. E (2.3 g) was separated over a Sephadex LH-20 column (200 g; Pharmacia Co.; CH_2Cl_2/MeOH = 1 : 1) and further separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 10 : 1) to yield **15** (6 mg). Fr. F (3.0 g) was separated over a RP-C_{18} silica gel column (230–400 mesh, 10 g, 60% MeOH and 100% MeOH) to give eight subfractions [Fr. F1 – Fr. F7 (60% MeOH, each 1.0 L), Fr. F8 (100% MeOH, 2.0 L)]. Fr. F2 (350 mg) was separated by a Sephadex LH-20 column (200 g; Pharmacia Co.; CH_2Cl_2/MeOH = 1 : 1) and further separated by using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 20 : 1) to yield **25** (6 mg). Fr. G (5.3 g) was separated over a silica gel column (230–400 mesh, 15 g, $CHCl_3$/MeOH = 15 : 1, and 5 : 1) to give seven subfractions [Fr. G1 – Fr. G4 ($CHCl_3$/MeOH = 15 : 1, each 1.0 L), Fr. G5 – Fr. G7 ($CHCl_3$/MeOH = 5 : 1, each 1.0 L)]. Fr. G7 (150 mg) was further separated using a Lobar-A Si gel 60 (240 × 10 mm) column (CH_2Cl_2/MeOH = 20 : 1) to yield **14** (20 mg).

Isolated compounds

Corydaturtschine A (**1**): amorphous gum; $[\alpha]_D^{25}$: + 5.7 as shown in data of Corydaturtschine B (*c* 0.18, $CHCl_3$); IR (KBr): ν_{max} = 3364, 2945, 1648, 1510, 1460, 1279, 1056, 675 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 204 (4.17), 236 (3.52), 277 (4.11), 311 (4.21), 363 (4.38); CD (EtOH) λ_{max} ($\Delta\varepsilon$) 204 (− 29.8), 235 (− 4.0), 280 (+ 1.9) nm; FAB-MS: *m/z* = 661 $[M + H]^+$; HR-FAB-MS: *m/z* = 661.2557 $[M + H]^+$ (calcd. for $C_{39}H_{37}N_2O_8$: 661.2550). ^{1}H-NMR (CD_3OD, 500 MHz): see ● **Table 1**. ^{13}C-NMR (CD_3OD, 125 MHz): see ● **Table 2**.

Corydaturtschine B (**2**): amorphous gum; $[\alpha]_D^{25}$: + 14.5 (*c* 0.21, $CHCl_3$); IR (KBr): ν_{max} = 3386, 2945, 1647, 1511, 1457, 1276, 1028, 675 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 205 (4.23), 220 (4.10), 273 (4.23), 297 (4.51), 307 (3.78); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (+ 12.0), 236 (+ 18.4), 261 (− 8.1), 275 (− 16.4), 310 (+ 25.7) nm; FAB-MS: *m/z* = 648 $[M + H]^+$; HR-FAB-MS: *m/z* = 648.2841 $[M + H]^+$ (calcd. for $C_{39}H_{40}N_2O_7$: 648.2836). ^{1}H-NMR (CD_3OD, 500 MHz): see ● **Table 1**. ^{13}C-NMR (CD_3OD, 125 MHz): see ● **Table 2**.

Ethyl glausuccinate (**3**): amorphous gum; $[\alpha]_D^{25}$: + 45.7 (*c* 0.57, $CHCl_3$); IR (KBr): ν_{max} = 3388, 2945, 1667, 1517, 1461, 1254, 1030, 676 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 216 (3.75), 232 (2.75), 245 (3.12), 279 (4.42), 299 (4.80), 311 (4.27); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (+ 22.4), 238 (+ 12.4), 243 (+ 8.2), 263 (+ 4.3), 279

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Table 1 [1]H-NMR spectral data of compounds **1–3**[a].

H	1	2	3
3	7.22 s	6.77 s	6.60 s
4	7.79 d (5.5)	2.79 m	2.74 m
		3.26 m	3.23 m
5	8.92 d (5.5)	2.50 m	3.28 m
		3.66 m	3.38 m
6a		3.86 dd (13.5, 4.0)	3.64 br d (14.5)
7		2.80 t (13.5)	2.80 t (14.5)
		3.05 dd (13.5, 4.0)	3.06 dd (14.5, 4.0)
8	8.05 s	6.81 s	6.79 s
11	8.84 s	8.08 s	8.09 s
1′	6.84 s	7.51 s	[b]
4′	6.60 s	6.77 s	
5′	2.66 m	3.29 t (6.5)	
	3.11 m		
6′	2.56 td (11.0, 3.0)	5.25 t (6.5)	
	3.19 m		
8′	3.55 d (15.5)	10.34 s	
	4.24 d (15.5)		
11′	6.79 d (8.0)	8.01 d (9.0)	
12′	6.87 d (8.0)	7.73 d (9.0)	
13′	2.80 dd (15.5, 12.0)	8.74 s	
	3.24 dd (15.5, 3.5)		
14′	3.52 dd (12.0, 3.5)		
1-OCH_3	4.03 s	3.62 s	3.64 s
2-OCH_3	4.05 s	3.91 s	3.93 s
9-OCH_3			3.90 s
10-OCH_3	4.04 s	3.86 s	3.89 s
2′-OCH_3	3.88 s		
3′-OCH_3		3.96 s	
9′-OCH_3	3.87 s	4.12 s	
10′-OCH_3	3.86 s	4.24 s	

[a] [1]H-NMR run at 500 MHz in CD_3OD. Chemical shifts are given in δ values. Proton coupling constants (J) in Hz are given in parentheses; [b] $^{\delta}$H of 2′-3′ at 2.64 (2H, m, H-2′), 2.65 (2H, m, H-3′), and $^{\delta}$H of 5′-6′ at 4.11 (2H, q, J = 7.0 Hz, $\underline{CH_2}CH_3$), 1.23 (3H, t, J = 7.0 Hz, $CH_2\underline{CH_3}$), respectively

Table 2 [13]C-NMR spectral data of compounds **1–3**[a].

C	1	2	3
1	149.6 s	145.1 s	145.3 s
1a	120.1 s	127.0 s	127.1 s
1b	121.7 s	127.2 s	121.5 s
2	156.7 s	152.7 s	153.5 s
3	106.0 d	110.1 d	110.3 d
3a	135.4 s	129.4 s	129.5 s
4	123.3 d	27.3 t	27.5 t
5	145.6 d	53.3 t	53.2 t
6a	145.0 s	56.0 d	56.2 d
7	181.4 s	35.5 t	32.2 t
7a	126.0 s	127.9 s	127.5 s
8	109.9 d	111.3 d	111.1 d
9	150.2 s	147.5 s	148.8 s
10	153.8 s	149.0 s	148.2 s
11	110.2 d	111.8 d	111.8 d
11a	129.1 s	122.8 s	123.9 s
1′	110.6 d	108.6 d	176.4 s
2′	143.9 s	150.5 s	29.5 t
3′	149.3 s	152.3 s	29.5 t
4′	110.9 d	111.1 d	172.7 s
4′a	127.0 s	129.6 s	
5′	29.1 t	27.3 t	60.9 t ($\underline{C}H_2CH_3$)
6′	51.6 t	56.2 t	14.3 q ($CH_2\underline{C}H_3$)
8	54.0 t	146.3 d	
8′a	127.1 s	122.2 s	
9′	145.0 s	151.3 s	
10′	150.9 s	145.2 s	
11′	111.3 d	123.4 d	
12′	123.9 d	126.2 d	
12′a	128.2 s	133.7 s	
13′	36.2 t	120.1 d	
14′	59.2 d	137.4 s	
14′a	129.0 s	119.1 s	
1-OCH_3	60.1 q	60.6 q	60.5 q
2-OCH_3	55.9 q	56.0 q	56.1 q
9-OCH_3			56.1 q
10-OCH_3	56.7 q	56.4 q	56.2 q
2′-OCH_3	60.6 q		
3′-OCH_3		57.2 q	
9′-OCH_3	56.3 q	57.0 q	
10′-OCH_3	56.2 q	62.0 q	

[a] [13]C-NMR run at 125 MHz in CD_3OD. Chemical shifts are given in δ values

(−12.8), 299 (+15.7) nm; FAB-MS: m/z = 492 $[M + Na]^+$; HR-FAB-MS: m/z = 492.1995 $[M + Na]^+$ (calcd. for $C_{26}H_{31}NNaO_7$: 492.1998). [1]H-NMR (CD_3OD, 500 MHz): see ▶ **Table 1**. [13]C-NMR (CD_3OD, 125 MHz): see ▶ **Table 2**.

Isolation of methyl glausuccinate (3a)

The tubers of *C. turtschaninovii* (5 g) were extracted with 80% MeOH two times at room temperature and fractionated. The isolation of **3a** progressed as described above for the isolation of **3**, except that EtOH was not used. This methanolic extract yielded methyl ester of glausuccine (**3a**; 1.3 mg).

Methyl glausuccinate (**3a**): amorphous gum; FAB-MS: m/z = 456 $[M + H]^+$; [1]H-NMR (CD_3OD, 500 MHz): δ 8.09 (1H, s, H-11), 6.79 (1H, s, H-8), 6.60 (1H, s, H-3), 3.93 (3H, s, 2-$OC\underline{H_3}$), 3.90 (3H, s, 9-$OC\underline{H_3}$), 3.89 (3H, s, 10-$OC\underline{H_3}$), 3.68 (3H, s, 4′-$OC\underline{H_3}$), 3.66 (3H, s, 1-$OC\underline{H_3}$), 3.64 (1H, br d, J = 14.0 Hz, H-6a), 3.36 (1H, m, H-5a), 3.30 (1H, m, H-5b), 3.22 (1H, m, H-4a), 3.05 (1H, dd, J = 14.0, 4.0 Hz, H-7a), 2.79 (1H, t, J = 14.0 Hz, H-7b), 2.74 (1H, m, H-4b), 2.68–2.60 (4H, m, H-2′, H-3′).

Hydrolysis of ethyl glausuccinate (3)

Compound (**3**; 5 mg) was hydrolyzed with 10% EtOH/KOH (2 mL) at 40 °C for 1 h. The mixture was acidified with 1 N HCl, then H_2O (5 mL) was added, and the product was extracted twice with $CHCl_3$. The combined $CHCl_3$ layer was evaporated under reduced pressure to give the $CHCl_3$ extract (2.2 mg). This was purified over a silica gel Waters Sep-Pak Vac 6cc [$CHCl_3$/MeOH 50 : 1] to give glausuccine (**3b**; 1.1 mg).

Glausuccine (**3b**): amorphous gum; FAB-MS: m/z = 442 $[M + H]^+$; HR-FAB-MS: m/z = 442.1881 $[M + H]^+$ (calcd. for $C_{24}H_{28}NO_7$: 442.1866); [1]H-NMR (CD_3OD, 500 MHz): δ 8.09 (1H, s, H-11), 6.78 (1H, s, H-8), 6.59 (1H, s, H-3), 3.92 (3H, s, 2-$OC\underline{H_3}$), 3.90 (3H, s, 9-$OC\underline{H_3}$), 3.88 (3H, s, 10-$OC\underline{H_3}$), 3.65 (3H, s, 1-$OC\underline{H_3}$), 3.61 (1H, br d, J = 14.0 Hz, H-6a), 3.36 (1H, m, H-5a), 3.28 (1H, m, H-5b), 3.20 (1H, m, H-4a), 3.03 (1H, dd, J = 14.0, 4.0 Hz, H-7a), 2.76 (1H, t, J = 14.0 Hz, H-7b), 2.69 (1H, m, H-4b), 2.61–2.55 (4H, m, H-2′, H-3′).

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Cytotoxicity assay

A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines [8]. The cell lines (National Cancer Institute, Bethesda, MD, USA) used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (purity ≥ 98%; Sigma) was used as a positive control. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analysis (purity ≥ 95%).

Results and Discussion

An ethanol extract of tubers of *C. turtschaninovii* was partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Repeated chromatographic purification of the $CHCl_3$-soluble fraction afforded three new alkaloids (**1**–**3**), together with thirteen known protoberberine (**4**–**16**) and nine known aporphine alkaloids (**17**–**25**). The three new alkaloids (**1**–**3**) were two new tetrahydroprotoberberine-aporphine dimers, rarely reported from natural sources, and a new aporphine alkaloid containing succinic acid ethyl ester group.

Compound **1** was obtained as an optically active amorphous gum, and was positive for Dragendorff's reagent. The molecular formula was determined to be $C_{39}H_{36}N_2O_8$ from the $[M + H]^+$ peak at *m*/*z* 661.2557 (calcd. for $C_{39}H_{37}N_2O_8$: 661.2550) in the HR-FAB-MS. The relatively weak molecular ion in the FAB-MS suggested a dimeric alkaloid with one diphenyl ether linkage [9]. The IR spectrum indicated that **1** possessed hydroxyl (3364 cm^{-1}), carbonyl (1648 cm^{-1}), and aromatic (1510 cm^{-1}) groups. The UV spectrum showed an absorption band at λ_{max} 311 nm, suggesting an oxoaporphine component [10]. The molecular formula required 23 degrees of unsaturation, of which this oxoaporphine unit provided 13, with the remaining ten from a tetrahydroprotoberberine unit. This dimeric alkaloid was also supported by the UV spectra with the absence of a prominent absorption band at λ_{max} 282–289 nm, characteristic of tetrahydroprotoberberines [11, 12], which could be hidden under or combined with the oxoaporphine absorption. The overall UV spectrum was consistent with that of an aporphine-tetrahydroprotoberberine dimer [13]. The dimeric nature of the alkaloid was suggested by the ^{1}H-NMR spectral data (**Table 1**) that showed the presence of six sharp singlets representing aromatic methoxy groups (δ 3.86, 3.87, 3.88, 4.03, 4.04, 4.05). The aromatic protons were observed as singlets at δ 6.60, 6.84, 7.22, 8.05, and 8.84, the last being characteristic of the H-11 proton in an aporphine [11, 12], and as doublets at δ 6.79, 6.87, 7.79, and 8.92. These data, combined with the knowledge that 2,3,9,10-oxygenation is common in the protoberberine series, strongly suggested that **1** was a dimeric form that contained a 1,2,9,10-tetraoxygenated oxoaporphine linked to a 2,3,9,10-tetraoxygenated tetrahydroprotoberberine through a diaryl ether bridge in each monomer [10–12]. The ^{1}H- and ^{13}C-NMR spectra of **1** showed several features observed for 1,2,9,10-tetraoxygenated oxoaporphine. Three aromatic singlets (δ 7.22, 8.05, and 8.84), two aromatic doublets (δ 7.79 and 8.92) in the ^{1}H-NMR spectrum, and a carbonyl carbon (δ 181.4) as well as four oxygenated aromatic carbon signals (δ 149.6, 150.2, 153.8, and 156.7) in the ^{13}C-NMR spectrum were similar to those of oxoglaucine (**20**), which was isolated from this plant [14]. The structure of the monomer, 1,2,9,10-tetraoxygenated oxoaporphine, was ultimately elucidated by the interpretation of the ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY NMR data. The COSY allowed for proton signals at δ 7.22, 8.05, and 8.84, defining the different spin systems, and showed a correlation between H-4 (δ 7.79) and H-5 (δ 8.92). These resonances were then correlated to their respective carbons by the HSQC experiment. The skeleton of the oxoaporphine portion of **1** was assembled on the basis of the HMBC and NOESY correlations. The remaining NMR data of **1** displayed 17 carbon signals and 13 proton signals assignable to the tetrahydroprotoberberine portion, except for three methoxy signals. The ^{1}H- and ^{13}C-NMR spectra clearly showed four aromatic protons at δ 6.60 (s), 6.79 (d, *J* = 8.0 Hz), 6.84 (s), and 6.87 (d, *J* = 8.0 Hz), and one methine (δ 59.2), four methylenes (δ 29.1, 36.2, 51.6, and 54.0), and oxygenated aromatic carbons (δ 143.9, 145.0, 149.3, and 150.9), which were very similar to those of (−)-corypalmine (**11**) isolated from this plant [15]. This was also confirmed by 2D NMR data; HMBC and NOESY correlations consistent with the 2,3,9,10-tetraoxygenated tetrahydroprotoberberine portion. Finally, the NOESY experiment confirmed the position of the substituent on the aporphine-tetrahydroprotoberberine framework (**Fig. 1**). NOESY correlations were observed from the H-1′, H-4′, and 2′-*O*-methyl resonances in the protoberberine subunit to the H-8 and 10-*O*-methyl resonances in the oxoaporphine portion of the structure, which established the structure of **1** as protoberberine, corypalmine linked at C-3′ to the oxoaporphine, oxoglaucine via the C-9 phenolic hydroxyl group. The absolute configuration of **1** was determined from its CD spectrum by comparison with those of the (−)-tetrahydropalmatine [16, 17], because the diphenyl ether linkage of **1** would not prevent the two components from behaving as individual monomers conformationally [9]. The CD spectrum showed three Cotton effect maxima at 204, 235, and 280 nm (two negative and one positive), indicating that **1** has an *S*-configuration at C-14′ [16, 17]. Thus, compound **1** was characterized as shown in the structural drawings and named as corydaturtschine A.

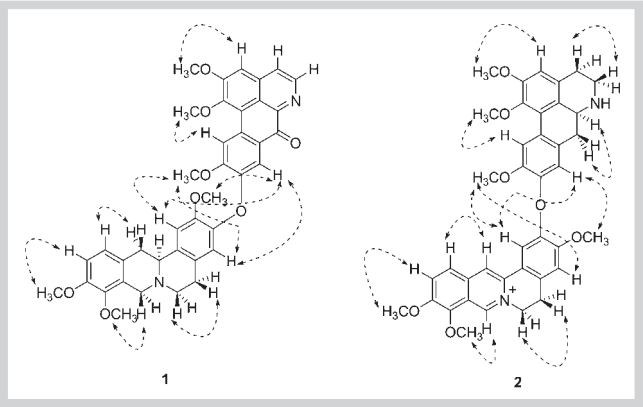

Fig. 1 Key NOESY correlations of **1** and **2**.

Compound **2** was obtained as an optically active amorphous gum and was positive for Dragendorff's reagent. The FAB-MS showed a weak molecular ion peak $[M + H]^+$ at *m*/*z* 648 corresponding to the molecular formula $C_{39}H_{39}N_2O_7$, which was confirmed by the $[M + H]^+$ peak at *m*/*z* 648.2841 (calcd. for $C_{39}H_{40}N_2O_7$: 648.2836) in the HR-FAB-MS. The relatively weak molecular ion in the FAB-MS and the formula support a dimeric alkaloid with one diphenyl ether group, and the UV absorptions at 220, 273, and 307 nm indicated an aporphine unit [11, 12]. This unit would provide 10 of the 22 degrees of unsaturation required by the for-

mula, with the remaining twelve from a tetrahydroprotoberberine. The ^{13}C-NMR spectrum with 27 aromatic carbons between 152.7 and 110.1 ppm were in accord with the proposed dimer. The ^{1}H-NMR spectra (**Table 1**) of **2** revealed six *O*-methyls and nine aromatic protons, of which the upfield shifted *O*-methyl at δ 3.62 was characteristic of the *O*-methyl attached at C-1 in an aporphinoid, and the aromatic singlet protons at δ 6.77, 6.81, and 8.08 were assigned to the aporphine unit, being similar to those of norglaucine (**19**) isolated from this plant [18,19]. The structure of the aporphine portion of **2** was established on the basis of the 2D NMR data, including ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY. The ^{1}H-NMR data of **2** showed six additional aromatic protons at δ 6.77 (s), 7.51 (s), 7.73 (d, J = 9.0 Hz), 8.01 (d, J = 9.0 Hz), 8.74 (s), and 10.34 (s), the last being characteristic of the H-8 proton in a palmatine [20], and two methylene signals at δ 3.29 (t, J = 6.5 Hz) and 5.25 (t, J = 6.5 Hz). The ^{13}C-NMR spectrum showed 17 additional carbon signals composed of two methylenes (δ 27.3 and 56.2) and oxygenated aromatic carbons (δ 145.2, 150.5, 151.3, and 152.3), except for three methoxy signals. These NMR data were similar to those of columbamine (**14**) isolated from this plant [20,21]. This tetrahydroprotoberberine portion was also established by the HMBC and NOESY correlations. Finally, the aromatic substitution position of the oxygen-linked aporphine-tetrahydroprotoberberine dimer was confirmed by the NOESY experiment (**Fig. 1**). NOESY correlations were observed from the H-1′ and 3′-*O*-methyl signals in the protoberberine subunit to the H-8 and 10-*O*-methyl signals in the aporphine portion of **2**, which indicated the connection on the aporphine-tetrahydroprotoberberine framework of **2**. The positive Cotton effect curve of high intensity at 236 nm indicated the *S*-configuration for C-6a [22,23]. This is also supported by the nearly identical appearance of its CD spectrum with that of the (+)-(*S*)-oconovine analogue of the aporphine portion of **2** [9]. Thus, the structure of **2** was identified as shown in the structural drawings and named corydaturtschine B.

Compound **3**, obtained as an amorphous gum, was optically active ($[\alpha]_D^{25}$: +45.7) and positive for Dragendorff's reagent. In its HR-FAB-MS spectrum, the molecular ion $[M + Na]^+$ was observed at *m/z* 492.1995 $[M + Na]^+$ (calcd. for $C_{26}H_{31}NNaO_7$: 492.1998), suggesting the molecular formula $C_{26}H_{31}NO_7$. The IR spectrum indicated that **3** possessed hydroxyl (3388 cm^{-1}), amide carbonyl (1667 cm^{-1}), and aromatic (1517 cm^{-1}) groups. The UV spectrum showed absorption bands at λ_{max} 216, 279, and 311 nm, which were characteristic of an aporphinoid [11,12]. The 1D-NMR (^{1}H and ^{13}C) of **3** indicated the presence of six methylenes, four methines, eleven quaternary carbons, one methyl, and four methoxy groups. The ^{1}H-^{1}H COSY spectrum of **3** showed four sets of correlations as follows: between CH_2-4 (δ 2.74 and 3.23) and CH_2-5 (δ 3.28 and 3.38), between CH-6a (δ 3.64) and CH_2-7 (δ 2.80 and 3.06), between CH_2-2′ (δ 2.64) and CH_2-3′ (δ 2.65), and between -$\underline{CH_2}CH_3$ (δ 4.11) and -$CH_2\underline{CH_3}$ (δ 1.23). In the HMBC spectrum, correlations of H-11 with C-7a (δ 127.5) and C-9 (δ 148.8) and of H-8 with C-10 (δ 148.2) and C-11a (δ 123.9) were observed. Cross-peaks of H-3 with C-1 (δ 145.3) and C-1b (δ 121.5) were also shown. These data suggested the presence of a 1,2,9,10-tetrasubstituted aporphine unit. On the other hand, the CH_2-5 (δ 3.28 and 3.38) and CH-6a (δ 3.64) signals linked to a nitrogen atom were correlated to C-3a (δ 129.5) and C-6a (δ 56.2), and C-1a (δ 127.1), C-3a (δ 129.5), C-5 (δ 53.2) and C-7a (δ 127.5), respectively. Methoxy groups (δ 3.64, 3.93, 3.90, and 3.89) were bonded to C-1 (δ 145.3), C-2 (δ 153.5), C-9 (δ 148.8), and C-10 (148.2), respectively, as determined by their HMBC correlations (**Fig. 2**). This

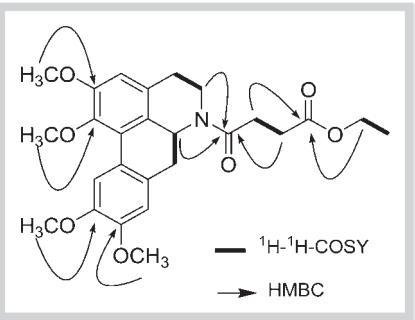

Fig. 2 Key HMBC and ^{1}H-^{1}H-COSY correlations of **3**.

evidence confirmed **3** as an aporphine skeleton. Moreover, the methylene CH_2-2′ (δ 2.64) and -$\underline{CH_2}CH_3$ (δ 4.11) showed a cross-peak with C-4′ (δ 172.7) and the methylene CH_2-3′ (δ 2.65) showed a cross-peak with C-1′ (δ 176.4) (**Fig. 2**), suggesting that **3** contained an additional succinic acid ethyl ester group. This unit was attached at a nitrogen atom of **3**, based on the HMBC correlation between H_2-5 (δ 3.28 and 3.38), H-6a (δ 3.64) and C-1′ (δ 176.4). Detailed analysis of the HMBC correlations permitted assignment of the planar structure of **3** as shown in the structural drawings. The absolute stereochemistry of **3** was determined by its CD spectrum and optical rotation value ($[\alpha]_D^{25}$: +45.7). The strong positive specific rotation indicated the *S*-configuration for C-6a [24,25], which was also supported by the positive Cotton effect curve of high intensity at 238 nm [22,23]. This aporphine alkaloid was named glausuccine.

The ethyl glausuccinate (**3**) could be an artifact during isolation because EtOH was used to extract the tubers of *C. turtschaninovii*. To identify whether the ethyl glausuccinate (**3**) was a genuine natural compound or an isolation artifact, tubers of *C. turtschaninovii* were extracted with 80% MeOH. In this case, methyl glausuccinate (**3a**) was isolated from the methanolic extract, and there was no detectable trace of the ethyl ester form (**3**), indicating that the free carboxylic acid, glausuccine (**3b**), must be a genuine natural product. To confirm this, tubers of *C. turtschaninovii* were extracted on cold (3–5 °C) using chloroform for 3 days. The extract contained glausuccine (**3b**) which was verified by a confirmatory TLC along with the produced **3b** after the hydrolysis of ethyl glausuccinate (**3**).

The known compounds, yuanhunine (**4**) [4], (−)-tetrahydrocoptisine (**5**) [26], isocorybulbine (**6**) [27], (+)-corydaline (**7**) [4], (+)-nandinine (**8**) [28], (+)-corybulbine (**9**) [4], (*S*)-(−)-canadine (**10**) [4], (−)-corypalmine (**11**) [15,29], (−)-tetrahydropalmatine (**12**) [4], (−)-isocorypalmine (**13**) [27], columbamine (**14**) [20, 21], dehydrocorydaline (**15**) [30], pseudodehydrocorydaline (**16**) [5], didehydroglaucine (**17**) [31], dehydrolirioferine (**18**) [32], (+)-norglaucine (**19**) [18,19], oxoglaucine (**20**) [14,19], (+)-laurotetanine (**21**) [33], 7-hydroxydehydroglaucine (**22**) [34], 7-methyldehydroglaucine (**23**) [35], (+)-glaucine (**24**) [36], and (+)-*N*-methyllaurotetanine (**25**) [33] were identified by comparison of physical and spectroscopic data (UV, IR, ^{1}H- and ^{13}C-NMR, and MS data) with literature values (**Fig. 3**).

The cytotoxicity of the isolates (**1**–**25**) against A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cell lines was evaluated using the SRB assay *in vitro*. The majority of the isolated alkaloids exhibited cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cells (**Table 3**), although several compounds (**4, 10**, and **14**) showed little cytotoxicity (IC_{50} > 100 µM). In particular, new compounds (**1**–**3**) showed good cytotoxicity, with IC_{50} values ranging from 8.75 µM to 36.89 µM. The results (**Table 3**) re-

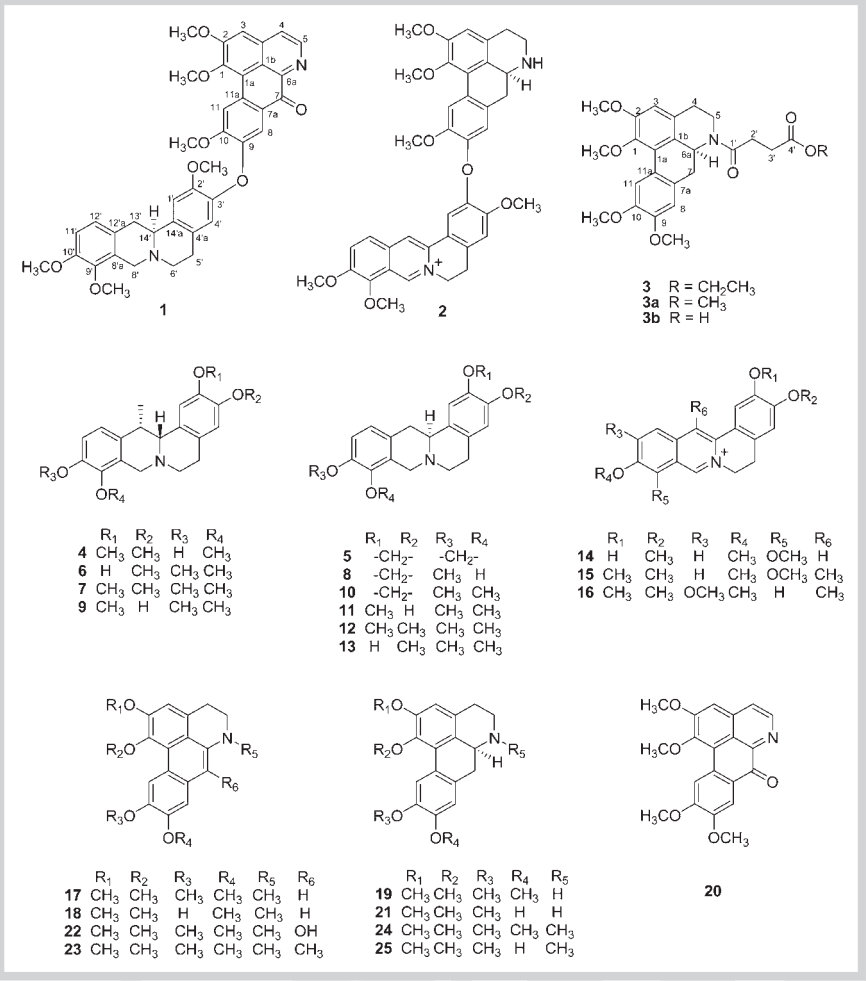

Fig. 3 Chemical structures of compounds **1**–**25**.

vealed that the aporphine-type seems to have better cytotoxicity than the tetrahydroprotoberberine-type compared to activities of compounds **4**–**16** and **17**–**25**.

Acknowledgements

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Table 3 Cytotoxicity of compounds **1–25** 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
1	12.9 ± 0.8[b]	8.75 ± 0.05	12.28 ± 0.19	14.21 ± 1.18
2	35.13 ± 5.58	30.28 ± 5.72	10.08 ± 0.84	29.31 ± 3.69
3	36.89 ± 0.20	26.34 ± 0.27	33.84 ± 0.08	30.67 ± 3.07
4	> 100.0	60.63 ± 3.53	53.36 ± 3.23	51.20 ± 4.53
5	93.75 ± 2.72	80.10 ± 0.18	87.02 ± 5.38	88.75 ± 2.22
6	40.06 ± 0.49	22.43 ± 1.19	29.61 ± 3.09	15.55 ± 1.04
7	43.90 ± 2.42	40.11 ± 5.89	31.33 ± 3.44	39.84 ± 0.90
8	41.33 ± 3.57	33.90 ± 2.19	23.67 ± 4.04	33.96 ± 1.71
9	65.54 ± 3.39	40.48 ± 0.33	42.07 ± 1.28	38.22 ± 2.83
10	> 100.0	75.86 ± 7.83	60.89 ± 7.42	86.37 ± 2.31
11	66.34 ± 3.54	41.57 ± 0.97	35.63 ± 0.61	44.52 ± 2.52
12	46.78 ± 0.87	48.17 ± 1.54	34.31 ± 1.21	65.75 ± 1.53
13	67.32 ± 6.09	47.37 ± 2.19	47.66 ± 5.66	67.32 ± 3.50
14	> 100.0	> 100.0	42.91 ± 2.92	> 100.0
15	55.57 ± 4.75	48.82 ± 1.20	28.76 ± 2.63	60.06 ± 0.77
16	95.18 ± 1.84	84.44 ± 2.97	36.15 ± 0.64	97.84 ± 0.44
17	4.51 ± 0.24	4.53 ± 0.13	4.21 ± 0.16	4.76 ± 0.07
18	11.05 ± 0.23	10.10 ± 0.18	5.23 ± 0.38	10.83 ± 0.27
19	39.4 ± 1.4	19.38 ± 1.87	32.41 ± 2.43	21.97 ± 1.51
20	12.21 ± 0.97	7.19 ± 0.68	15.40 ± 3.23	13.10 ± 1.64
21	30.03 ± 5.24	26.19 ± 1.31	26.64 ± 6.13	18.60 ± 2.37
22	15.94 ± 0.45	12.29 ± 0.47	5.99 ± 0.31	15.15 ± 0.77
23	31.67 ± 1.25	15.90 ± 0.70	20.86 ± 1.47	27.70 ± 1.74
24	26.76 ± 3.82	21.57 ± 1.01	20.39 ± 1.45	18.63 ± 4.15
25	12.61 ± 0.81	13.64 ± 1.18	11.21 ± 0.75	15.06 ± 1.18
Doxorubicin[c]	0.026 ± 0.005	0.067 ± 0.003	0.006 ± 0.001	0.013 ± 0.017

[a] IC_{50} value of compounds against cancer cell lines, defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*;
[b] Data are expressed as mean ± SEM of three independent experiments; [c] Doxorubicin as positive control

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