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# TETRAHYDROPROTOBERBERINE ALKALOIDS FROM *Corydalis saxicola*

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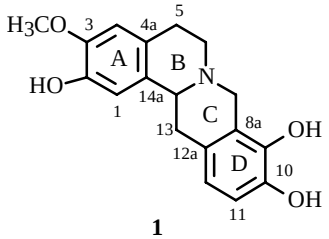
*Thirteen tetrahydroprotoberberines, 2,9,10-thrihydroxy-3-methoxytetrahydroprotoberberine (**1**), cavidine (**2**), thalictrifoline (**3**), mesotetrahydrocorysamine (**4**), stylopine (**5**), sinactine (**6**), apocavidine (**7**), cheilanthifoline (**8**), 13-β-hydroxystylopine (**9**), tetrahydropalmatine (**10**), tetrahydropalmatrubine (**11**), isocorypalmine (**12**), and scoulerine (**13**) have been isolated from the herb of* Corydalis. Saxicola *Bunting. Of these alkaloids, 2,9,10-thrihydroxy-3-methoxytetrahydroprotoberberine (**1**) was a new base. The alkaloids mesotetrahydrocorysamine (**4**), stylopine (**5**), sinactine (**6**), apocavidine (**7**), cheilanthifoline (**8**), 13-β-hydroxystylopine (**9**), tetrahydropalmatine (**10**), tetrahydropalmatrubine (**11**), isocorypalmine (**12**), and scoulerine (**13**), although previously known, were isolated for the first time from* Corydalis saxicola *Bunting.*

**Key words**: *Corydalis saxicola*, Papaveraceae, tetrahydroprotoberberine, 2,9,10-thrihydroxy-3-methoxy-tetrahydroprotoberberine.

*Corydalis saxicola* Bunting. (Papaveraceae) grows in south China and is an important component in various prescriptions in traditional Chinese medicine. It has been demonstrated to possess many pharmacological activities, including antibacterial, antiviral, and anticancer activities [1]. Clinically, *C. saxicola*. has been reported to have a potential effect of protecting hepatic tissues from hepatitis B virus and hepatitis A virus and can be used to treat hepatitis [2]. Moreover, *C. saxicola*. can also be used for alleviating fever, detoxification, and as a painkiller [3].

In our previous studies, a new nitro alkaloid was isolated from *C. saxicola* [4]. In this study, we investigated alkaloids from the herb of *C. saxicola*. Careful column chromatography of the chloroform fraction of the total alkaloids on silica gel yielded the following 13 alkaloids: 2,9,10-thrihydroxy-3-methoxytetrahydroprotoberberine (**1**), cavidine (**2**) [5], thalictrifoline (**3**) [5], mesotetrahydrocorysamine (**4**) [6], stylopine (**5**) [6], sinactine (**6**) [7], apocavidine (**7**) [8], cheilanthifoline (**8**) [9], 13$\beta$-hydroxystylopine (**9**) [10], tetrahydropalmatine (**10**) [11], tetrahydropalmatrubine (**11**) [12], isocorypalmine (**12**) [13], and scoulerine (**13**) [14]. Of the isolated bases, 2,9,10-thrihydroxy-3-methoxytetrahydro-protoberberine (**1**) was new. The alkaloids **4–13**, although previously known, were isolated for the first time from *C. saxicola*.

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Compound **1**: needle crystals, mp 232–234°C, UV ($\lambda_{max}$, MeOH): 280 nm. The ESI-MS afforded the positive ion at *m/z* 314 $[M+H]^+$, implying a molecular formula of $C_{18}H_{19}NO_4$, which was confirmed by the HRESI-MS ($[M+H]^+$ found 314.1025, calcd. 314.1023). The fragments with *m/z* 176 (7.5%) and 136 (100%) in the EI-MS suggested the substitution pattern of 9, 10-dihydroxy at ring D of tetrahydroprotoberberine.

The IR spectrum of **1** indicated the presence of phenolic hydroxy groups (3511 $cm^{-1}$). The $^1$H-NMR spectrum shows one methoxy at δ 3.87 (s), and four mutually coupling aliphatic protons at δ 2.51–3.32 (m, 4H, H-5, H-6). In addition, the chemical shifts of aliphatic protons of ring B and C were similar to those of scoulerine [14]. The aromatic region of the spectrum showed four protons: two at δ 6.71 and 6.42 (d, each 1H, J = 2 Hz), and the other two *ortho*-coupled protons at δ 6.52 and 6.64 (d, each 1H, J = 8 Hz), due to H-12 and H-11. The $^{13}$C-NMR spectrum gave eighteen carbon signals. A NOESY spectrum was run to establish the methoxy-substituted location. From the spectrum, the proton signal at δ 6.71 (s, 1H) was related to H-5 and H-OMe, which suggested the proton δ 6.71 (s, 1H) at C-4. So the 3-methoxy substituted pattern was determined. The structure of **1** was thus established as 2,9,10-thrihydroxy-3-methoxytetrahydro-protoberberine.

## EXPERIMENTAL

**General**. NMR spectra were operated on a Bruker DRX-500 spectrometer at 500 MHz for $^1$H-NMR and 125 MHz for $^{13}$C-NMR. Chemical shifts are expressed in δ values with reference to TMS as internal standard, and coupling constants (J) are given in Hz.; EI-MS was recorded on a Varian MAT-212 mass spectrometer and HRESI on a Q-TOF micro mass spectrometer; Melting point was measured on a RY-2 melting point apparatus and are uncorrected; IR was recorded on a Bruker Vector 22 spectrometer with KBr pellet; Column chromatography was performed on silica gel (200–300 mesh, Yantai, P. R. China), silica gel H (10–40 μm, Yantai, P. R. China), and Sephadex LH-20 (Pharmacia); TLC analysis was run on HSGF254 precoated silica gel plates (10–40 μm, Yantai, P. R. China).

**Plant Material**. The herb of *C. saxicola* was collected in Jinchengjiang, Guangxi Province, P.R. China, in July of 2003, and authenticated by Prof. Hanchen Zheng, Department of Pharmacognosy, Second Military Medical University. The voucher specimens (collection No. 188) are deposited at the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, China.

**Extraction and Isolation**. The dried and powdered herb of *C. saxicola* (10 kg) was extracted with 200 L methanol by infiltration. The solvent was evaporated under vacuum to afford 937 g crude extract, which was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate and aqua-saturated *n*-butanol successively. The chloroform partition (50 g) was subject to chromatography on a silica gel column (5.0×45 cm) eluting with a gradient mixtures of $CHCl_3$ and MeOH (0 to 100% MeOH) to yield 15 fractions, YB1-15. Fraction YB-14 (2.7 g), upon standing overnight, gave powder and **1** (24.0 mg) was obtained by recrystallization in methanol. Fraction YB-1 (0.9 g) was chromatographed on a silica gel column (2.0×25 cm) eluting with 10% MeOH in $CHCl_3$, giving **2** (11.0 mg), 3 (7.0 mg), and **4** (12.0 mg). Fraction YB-3 (0.5 g) was purified in a silica gel column (2.0×25 cm) to afford compounds **5** (32.0 mg) and **6** (21.0 mg) ($CHCl_3$–MeOH 15:1). Compounds **7** (14.0 mg), **8** (12.0 mg), and **9** (28.0 mg) were obtained from fraction YB-7 (silica gel column chromatography (2.0×25 cm), $CHCl_3$–MeOH 12:1). Fraction YB-10 (1.4 g) was separated on a silica column (2.0×25 cm) ($CHCl_3$–MeOH 10:1~1:1) to give 4 fractions. The second fraction afforded a colorless powder after concentration, which was purified by recrystallization in methanol to give compound **10** (26.0 mg). Compound **11** (35.0 mg) were obtained from the fourth part by further chromatography on a silica gel column (2.0×25 cm). Fraction YB-14 (0.7 g) was chromatographed on a silica gel column (2.0×25 cm), eluting with a gradient of MeOH in $CHCl_3$, (10–30%), yielding 12 (14.0 mg) and **13** (17.0 mg).

**Compound 1**: 2,9,10-thrihydroxy-3-methoxytetrahydro-protoberberine, needle crystals, mp 182–184°C, UV ($\lambda_{max}$, MeOH): 281 nm; ESI-MS: *m/z* 314 $[M+H]^+$.

$^1$H NMR (δ, ppm, $CDCl_3$, J/Hz): 6.42(1H, d, J = 2, H-1), 6.71 (1H, d, J = 2, H-4), 2.51–3.32 (4H, m, H-5 and H-6), 3.63, 4.03 (each 1, d, J = 15, H-8), 6.52 (1H, d, J = 8, H-11), 6.44 (1H, d, J = 8, H-12), 2.83 (1H, dd, J = 15, 2, H-13), 3.24 (1H, dd, J =15, 7, H-13), 4.29 (1H, dd, J = 7.2, H-14), 3.87 (3H, s, H-$OCH_3$).

$^{13}$C NMR (δ, DMSO, $CDCl_3$): 107.6 (C-1), 146.5 (C-2), 141.2 (C-3), 114.9 (C-4), 129.8 (C-4a), 30.4 (C-5), 50.1 (C-6), 53.8 (C-8), 116.5 (C-8a), 145.2 (C-9), 144.3 (C-10),113.5 (C-11), 121.1 (C-12), 135.8 (C-12a), 135.6 (C-13), 61.2 (C-14), 129.1 (C-14a), 56.4 ($OCH_3$-3).

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