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A BIOACTIVE ALKALOID FROM THE FLOWERS OF *TROLLIUS CHINENSIS*

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Abstract –A new compound, named as trolline was isolated from the ethanol extract of the flowers of *Trollius chinensis* Bunge. The chemical structure of this compound was elucidated by NMR, MS, IR, UV spectra and single-crystal X-Ray analysis. The *in vitro* antibacterial and antiviral experiments revealed that trolline exhibited appreciable antibacterial activity against respiratory bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Trolline was also demonstrated to have moderate antiviral activity against influenza virus A and B.

INTRODUCTION

Trollius chinensis Bunge (Ranunculaceae) is a perennial herb widely distributed in northern China. Its flowers have been used to treat respiratory infections, pharyngitis, tonsillitis, and bronchitis by natives from ancient time.[1] Previous phytochemical reports revealed the existence of flavonoids, phenolic acids, and the constituents of volatile oil in the dried flowers.[2,3] Former pharmacological studies suggested that the bioactive constituents of this herb are flavonoids and phenolics.[4,5] The present paper described a new

alkaloid named as trolline and its antibacterial and antiviral activities. This is the first report on bioactive alkaloid from this plant.

RESULTS AND DISCUSSION

Compound (**1**) was obtained as a colorless plate crystal (MeOH). Its molecular formula was assigned as $C_{12}H_{13}NO_3$ by HR-ESI-MS spectrum. Its ^{13}C NMR and DEPT spectra showed four methylenes, three methines and five quaternary carbons. The ^1H NMR spectrum showed two singlet signals at δ 6.31 (1H, s, H-10) and 6.32 (1H, s, H-7), which suggested there was a benzene ring with two *para*-protons and four substituents in this compound (Figure 1). The ^1H-^1H COSY and HMQC spectra of **1** displayed two units existed in the structure. One of them (unit I) was composed of two methylenes [δ_H 2.75 (1H, m, H-5), 3.79 (1H, m, H-5), 1.43 (1H, m, H-6), 2.40 (1H, m, H-6); δ_C 36.7 (C-5), 27.3 (C-6)]. The other unit (unit II) comprised two methylenes [δ_H 2.36 (2H, m, H-1), 2.25 (1H, m, H-2), 2.06 (1H, m, H-2); δ_C 27.4 (C-1), 31.4 (C-2)] and one methine [δ_H 4.40 (1H, t, J=8.0 Hz, H-10b); δ_C 55.7(C-10b)]. Unit I jointed the benzene ring at C-6a (δ 123.8), because H-6 had HMBC correlation with C-7 (δ 115.5) and H-7 [δ 6.32 (1H, s)] had correlation with C-6. Unit II connected benzene ring at C-10a (δ 128.5), because HMBC correlations between H-10 [δ 6.31(1H, s)] and C-10b as well as between H-10b and C-10 (δ 111.8) were observed. C-10b connected C-5 through a nitrogen atom (N-4), which was supported by the downfield chemical shifts of C-10b and C-5 and the HMBC correlation between H-5 and C-10b. C-2 jointed the nitrogen atom through a carbonyl group [C-3 (δ 172.3)], because both H-5 and H-1 had correlations with C-3. The other two substituents at C-8 (δ 144.1) and C-9 (δ 144.3) of the benzene ring should be hydroxyl groups as deduced from the downfield chemical shifts of those carbons and the molecular formula of this compound.

The configuration of C-10b was determined by comparing the optical rotation of **1** with that of its analog, named (10b*S*)1,5,6,10b-tetrahydro-*2H*-pyrrolo[2,1-*a*]isoquinolin-3-one.[6] The latter is a synthesized compound having a skeleton the same as that of **1**. The only difference in the structures of these two compounds is that **1** has two hydroxyl groups. The optical rotation of **1** is $[\alpha]_D^{20}$ -197 º, which is close to that of (10b*S*)1,5,6,10b-tetrahydro-*2H*-pyrrolo[2,1-*a*]isoquinolin-3-one ($[\alpha]_D^{23}$ -223.6º (*c* 4.0, MeOH)), thus, the configuration of C-10b in **1** should be *S* form. On the basis of above evidence, the stereo-chemical structure of **1** was determined as (10b*S*)8,9-dihydroxy-1,5,6,10b-tetrahydro-*2H*-pyrrolo-[2,1-*a*]isoquinolin-3-one.

The result of a single-crystal X-Ray analysis (Figure 2) of **1** supported the structure elucidated based on the NMR spectral evidence.

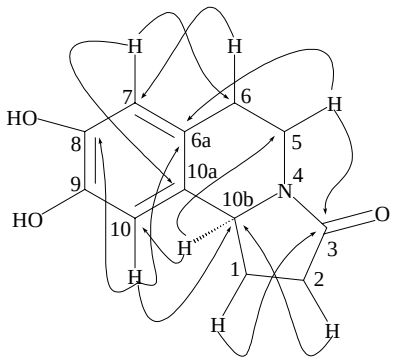

Figure 1 Selective HMBC correlations for **1**

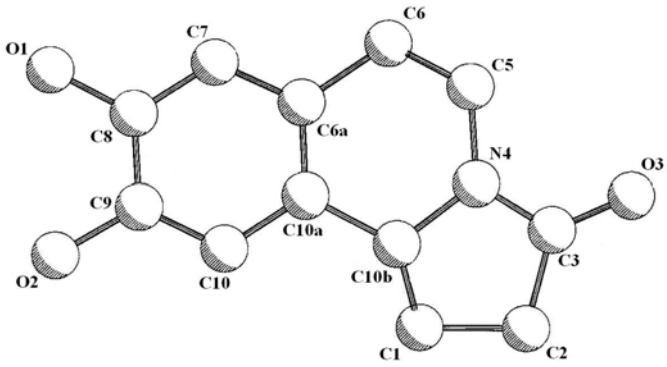

Figure 2 Structure and solid-state conformation of **1**

In *in vitro* antibacterial experiment, compound (**1)** showed appreciable antibacterial activity against both Gram-positive and Gram-negative bacteria, with MIC values of 32, 128 and 128 mg/L against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*, respectively (Table 1). Compound (**1)** also showed moderate antiviral activity against influenza virus A with an IC_{50} of 56.8 μg/mL (Table 2).

The present results suggest that this alkaloid may play a role in the anti-infectious effect of the herbal drug.

Table 1 *In vitro* antibacterial activities of **1**

Name of bacteria	MIC (mg/L)
Staphylococcus aureus	32
Klebsiella pneumoniae	128
Streptococcus pneumoniae	128
Streptococcus pyogenes	256
Pseudomonas aeruginosa	>256
Haemophilius influenzae	>256

Table 2 *In vitro* inhibitory effects of **1** against influenza virus A and B

Sample	CC_{50} (μg/mL)	virus A		virus B	
		IC_{50} (μg/mL)	SI	IC_{50} (μg/mL)	SI
1	273.03	56.8	4.81	-	-
RBV	>2000	2.2	>909.1	31.4	>63.7

EXPERIMENTAL

General Method

Melting point was measured on an XT-4A apparatus without correction. UV spectrum was recorded on a Varian Cary Eclipse 300 spectrophotometer using MeOH as the solvent. IR spectrum (film) was determined on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer. NMR spectra were obtained on a Bruker DRX 500 NMR spectrometer with the solvent as internal standard. HR-ESI-MS spectrum was measured on a Bruker APEX Ⅱ mass spectrometer. Single-crystal X-Ray analysis was performed on a Rigaku R-Axis Rapid IP Image Plate diffractometer with the Mo K*a* radiation and a graphite

monochromator. Silica gel (200-300 mesh) used in column chromatography was provided by Tsingtao Marine Chemistry Co. Ltd. Polyamide (30-60 and 200 mesh) was produced by Wuxi Electronic Teaching Apparatus Co. Ltd in Jiangsu Province, China.

Plant Material

The experimental material was obtained in October, 2000, from Anguo Chinese crude drug market in Hebei Province of China, and authenticated by Prof. Shao-Qing Cai as the flowers of *T. chinensis*. The voucher specimen (No. 2594) was deposited in the herbarium of Pharmacognosy, School of Pharmaceutical Sciences, Peking University.

Extraction and Isolation

The dried flowers of *T. chinensis* (8 kg) were extracted under reflux with 95% ethanol for 3 times (2 h/time). After being concentrated *in vacuo*, the ethanol crude extract (1.6 kg) was redissolved in water, and partitioned successively with petroleum ether and ethyl acetate (EtOAc). The EtOAc-soluble part (205 g) was separated by polyamide (30-60 mesh) column chromatography, eluted with H_2O and gradient ethanol. Eight major fractions (Fr. A- Fr. H) were obtained from the concentrated eluates. Fr. A (17.5 g) was subjected to silica gel chromatography, using gradient $CHCl_3$-MeOH from 30:1 to 3:1 as eluent to give 4 fractions (Fr. A-1-Fr. A-4). Compound (**1)** (120 mg) was crystallized from the Fr. A-2 (1.5 g) by using MeOH.

(10b*S*)8,9-dihydroxy-1,5,6,10b-tetrahydro-2*H*-pyrrolo[2,1-*a*]isoquinolin-3-one (1): mp 165-167 °C, colorless plate crystal (MeOH); UV λ_{max} (MeOH): 289, 255 nm; IR (KBr) ν_{max}: 3003, 2931, 1677, 1592, 1424, 1268, 1024, 762 cm^{-1}; $[\alpha]^{20}_D$ -197 ° (*c*=0.8, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHZ) δ: 2.36 (2H, m, H-1), 2.25 (1H, m, H-2), 2.06 (1H, m, H-2), 2.75 (1H, m, H-5), 3.79 (1H, m, H-5), 1.43 (1H, m, H-6), 2.40 (1H, m, H-6), 6.32 (1H, s, H-7), 6.31 (1H, s, H-10), 4.40 (1H, t, *J*=8.0 Hz, H-10b); ^{13}C NMR (DMSO-d_6, 125 MHZ) δ: 27.4 (C-1), 31.4 (C-2), 172.3 (C-3), 36.7 (C-5), 27.3 (C-6), 123.8 (C-6a), 115.5 (C-7), 144.1 (C-8), 144.3 (C-9), 111.8 (C-10), 128.5 (C-10a), 55.7 (C-10b); HR-ESI-MS (positive) *m/z* 220.0969 $[M+1]^+$ (calcd for $C_{12}H_{13}NO_3$, 219.0895).

Crystallographic Analysis of 1

Transparent colorless plate crystal was grown from MeOH and a crystal with the size of 0.65 × 0.20 × 0.10 mm was used for X-Ray diffraction work. All reflection data were collected on Rigaku R-Axis Rapid IP Image Plate diffractometer with the Mo K*a* radiation using a graphite monochromator at the temperature of 23 ± 1°C, the voltage of 50 kV, and the current of 300 mA. The distance between the crystal and the image plate was 127 mm. A total of 1760 unique reflections were collected using the ω scan from 0 to 220° at the speed of 8°/min with oscillational angle of 5°, $2\theta_{max}$ = 55.0°. 1570 reflections with $|F|^2 \geq 2\sigma|F|^2$ were considered as observed and were used for structure determination. Empirical

formula, $C_{12}H_{13}O_3N$; M_r = 219.24, crystal system, orthorhombic; space group, *P*cab; a = 9.256 (1) Å, b = 13.256 (1) Å, c = 16.578 (1) Å, V = 2034.2 (1) Å^3, Z = 8, D_c = 1.432 g/cm^3. The structure was solved by direct method (SHELXS 86). All of the non-hydrogen atoms were refined by the block least-square matrix. R_F = 0.043, R_w = 0.049 (w = $1/\sigma|F|^2$).

Antibacterial experiment *in vitro*

All bacteria were obtained from the Institute of Clinical Pharmacology of Peking University. These included *Klebsiella pneumoniae* 02-63, *Pseudomonas aeruginosa* 02-123, *Haemophilius influenzae* 02-102, *Staphylococcus aureus* 01-159, *Streptococcus pneumoniae* 02-19, and *Streptococcus pyogenes* M1371. The *in vitro* antibacterial activity against a variety of Gram-positive and Gram-negative organisms was determined by standard agar dilution method, according to NCCLS (National Committee for Clinical Laboratory Standard). 2 μL of cultures of test strains in the concentration of 5×10^5 CFU/mL were inoculated on Mueller Hinton agar containing different concentrations of the test compound. The MIC values (minimum inhibitory concentration at which the microbes failed to grow into a visible spot) were determined after incubation at 37 °C for 16-18 h.

Antiviral Effect *in vitro*

Madin-Darby canine kidney (MDCK) cells were obtained from the Institute of Virology, Chinese Academy of Preventive Medicine (Beijing, China), and grown as specified in Eagle's Minimum Essential Medicine with 10% heat inactivated fetal bovine serum (FBS) plus antibiotics (penicillin, 200 u/mL; streptomycin, 200 u/mL). Influenza viruses A (JF 190-15) and B (JF 197-13) were both from the Institute of Virology, Chinese Academy of Preventive Medicine. MDCK cell monolayers at about 85% confluence were infected with Influenza virus A or B at a multiplicity of infection (MOI) of 0.05 PFU per cell. After 2 h of adsorption at 37 °C, the cells were washed twice with phosphate-buffered saline (PBS) and incubated at 37 °C in the maintenance medium (MEM plus 0.1% BSA and 0.005% trypsin) with or without test compound or ribavirin (RBV), which had been diluted in a series of concentration. Cells were harvested when the cytopathic effect (CPE) was visually evident in viral control well (without compounds), usually 36 to 48 h after inoculation. CPE was observed and recorded under the microscope. The drug concentration required for 50% virus inhibition (IC_{50}) was calculated by using linear regression analysis of a half-log dilution series covering the effective concentrations of the tested drug. Cytotoxicity evaluation of compounds was conducted in parallel with the antiviral assays by CPE method. The selective index (SI) was calculated from the ratio of CC_{50}/IC_{50}.

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