

THREE NEW BENZAZEPINE ALKALOIDS FROM *THALICTRUM CIRRHOSUM* AND THEIR ANTI-ROTAVIRUS ACTIVITY

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Abstract – Three new benzazepine alkaloids, cirrhobenzazepines A-C (**1-3**), together with four known alkaloids (**4-7**) were isolated from the whole plants of *Thalictrum cirrhosum*. Their structures were elucidated by spectroscopic methods, including extensive ¹H, ¹³C, and 2D-NMR techniques. Compounds **1-7** were tested for their anti-rotavirus activity. The results revealed that compounds **1-7** exhibited potent anti-rotavirus activity with TI values in the range of 11.9-18.2, respectively.

Thalictrum plants are perennial herbs belonging to the family Ranunculaceae. This genus was established by Linnaeus in 1737, which has been studied continuously and systematically since then.^{1,2} *Thalictrum* includes more than 200 species around the world, mainly distributed in the North Temperate Zone, and about 69 species widely across China. Among them about 29 species are available for medicinal use.³ This genus is well known for its diverse pharmacological activities, including anti-tumor, anti-virus, anti-microbial, anti-tuberculosis, anti-inflammatory, anti-malarial activities, and the like.^{4,5}

Thalictrum cirrhosum Levl., is a perennial herb plant belong to *Thalictrum* genus. It is of 25 - 55 cm in height, grown in meadow slopes, alpine meadows and river banks at cold plateau in Yunnan province, P. R. China.² The whole plants of *T. cirrhosum* are used to treat some diseases, such as Jaundice hepatitis, digestive inflammation and allergy by Bai nationality people in Yunnan Province of China.^{6,7} Due to their versatile medicinal traditional uses, an increasing number of phytochemical studies have been carried out on this plant, and lot of bioactive natural products, especially some structural biodiversity alkaloids, have been reported.^{8,9}

The benzazepine ring system is an important structural motif in medicinal chemistry. There is a growing

interest in benzazepine derivatives due to their diversity in structural complexity and possess promising therapeutic perspectives.¹⁰⁻¹³ In order to increase pharmacodynamic value of *Thalictrum* plants, we now investigated the chemical constituents of the whole plant of *T. cirrhosum* collected in Dali Prefecture, Yunnan Province. As a result, three new benzazepine alkaloids (**1-3**), together with four known alkaloids (**4-7**) were isolated, and their structures were elucidated by spectral analysis. This paper describes the elucidation of the structures of these compounds and a preliminary evaluation of their anti-rotavirus activity.

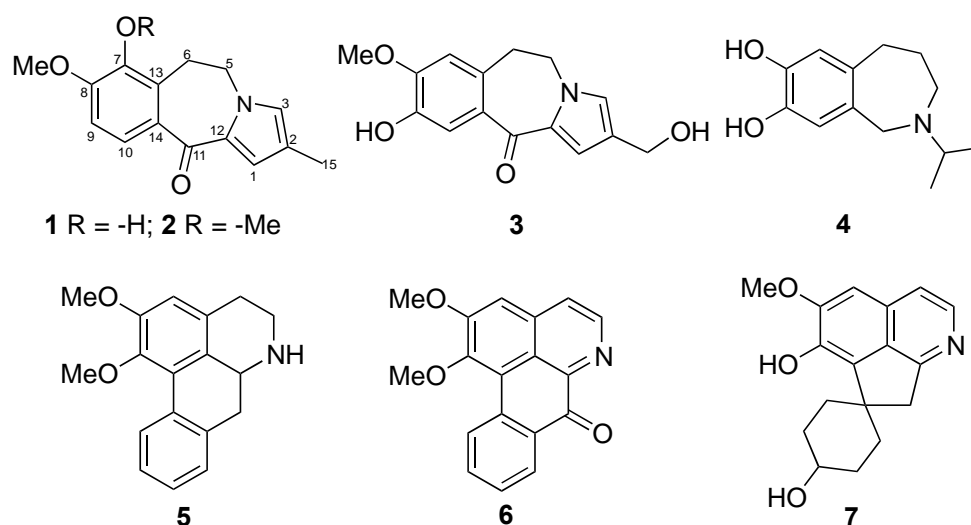


Figure 1. The alkaloids from *T.cultratum*

A 95% aq. ethanol extract prepared from whole plants of *T. cirrhosum* was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9.0 with saturated Na₂CO₃ aq. and extracted with EtOAc again. The EtOAc-soluble alkaloidal materials were subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford three new benzazepine alkaloids, cirrhobenzazepines A-C (**1-3**), together with four known alkaloids (**4-7**). The structures of the compounds **1-7** were shown in Figure 1, and the ¹H and ¹³C NMR data of **1-3** were listed in Table 1. The known compounds, compared with literature, were identified as scinamine D (**4**),¹⁴ nornuciferine (**5**),¹⁵ lysicamine (**6**),¹⁶ and prodensiflorin B (**7**).¹⁷

Compound **1** was obtained as yellow gum, and showed a positive response with Dragendorff's reagent (BiI₃·KI) on TLC. The molecular formula of **1**, C₁₅H₁₅NO₃, was deduced from HRESIMS (*m/z* 280.0958 [M+Na]⁺) and NMR spectral data (Table 1), representing nine degrees of unsaturation. The IR spectrum exhibited absorptions attributable to hydroxy (3412 cm⁻¹), carbonyl (1656 cm⁻¹), and aromatic (1606, 1560, and 1448 cm⁻¹) functionalities. The UV spectrum showed absorption maxima at 220, 250, 320 and

352 nm indicating the existence of a conjugated aromatic system. On the basis of the chemical shifts, HSQC, and HMBC, the 15 carbons and 15 protons resonances can attributed to one 1,2,3,4-tetrasubstituted benzene ring (C-7~C-10, C-13 and C-14, H-9 and H-10), one pyrrolo ring (C-1~C-3, C-12, N-4, H-1 and H-3),¹³ one *N*-connected -CH₂-CH₂- group (C-5 and C-6, H₂-5 and H₂-6),¹¹ one carbonyl group (C-11), one methyl group (C-15 and H₃-15), one methoxy group (δ_C 56.1 q, δ_H 3.79 s), and one phenolic hydroxy group (δ_H 10.89 s). By analysis of its HMBC correlations (Figure 2), the existence of the 1,2,3,4-tetrasubstituted benzene ring was supported by the HMBC correlations from H-9 to C-7, C-8, C-10, and C-14, from H-10 to C-8, C-9, C-13, and C-14, the existence of pyrrolo ring was supported by the HMBC correlations from H-1 to C-2, C-3, and C-12, from H-3 to C-1, C-2, C-12, the existence of *N*-connected -CH₂-CH₂- group was supported by the HMBC correlations from H₂-5 to C-6 and from H₂-6 to C-5.

Table 1. ¹H NMR and ¹³C NMR data (in CDCl₃) of compounds **1-3**

No.	Compound 1		Compound 2		Compound 3	
	δ_C (m)	δ_H (m, <i>J</i> , Hz)	δ_C (m)	δ_H (m, <i>J</i> , Hz)	δ_C (m)	δ_H (m, <i>J</i> , Hz)
1	110.4 d	6.76 s	110.2 d	6.77 s	110.9 d	6.83 s
2	123.6 s		123.3 s		124.9 s	
3	127.5 d	7.00 s	127.7 d	7.02 s	126.9 d	7.02 s
5	48.7 t	4.32 (d) 5.2	48.9 t	4.28 (d) 5.2	50.0 t	4.28 (d) 5.2
6	29.5 t	3.04 (d) 5.2	29.3 t	3.01 (d) 5.2	34.8 t	3.06 (d) 5.2
7	148.1 s		151.5 s		114.1 d	6.68 s
8	156.3 s		154.8 s		152.7 s	
9	115.2 d	7.09 (d) 8.2	114.7 d	7.19 (d) 8.2	148.5 s	
10	122.9 d	7.58 (d) 8.2	122.1 d	7.63 (d) 8.2	118.6 d	7.48 s
11	178.4 s		178.7 s		178.6 s	
12	133.3 s		133.1 s		133.7 s	
13	134.1 s		132.3 s		134.5 s	
14	121.4 s		120.3 s		126.1 s	
15	19.7 q	2.15 s	19.5 q	2.12 s	62.5 t	4.49 s
Ar-OH-7		10.89 s				
Ar-OH-9						10.86 s
-OMe-7			61.0 q	3.84 s		
-OMe-8	56.1 q	3.79 s	56.1 q	3.80 s	55.9 q	3.78 s

In addition to four degrees of unsaturations for benzene ring, three degrees of unsaturations for pyrrolo ring, and one degree of unsaturation for carbonyl group, the still one ring was needed to meet the requisition of nine degrees of unsaturation. The spectral data of **1** were similar to these of known compound, 5,6-dihydro-8,9-dihydroxy-11*H*-pyrrolo[2,1-*b*][3]benzazepin-11-one.¹³ The chemical shift differences only resulted from the substituents group variation. Two hydroxy group in the known

compound were replaced by a hydroxy, a methoxy, and a methyl in **1**. In addition, the HMBC correlations (Figure 2) from H₂-6 to C-7, C-13, C-14, from H₂-5 to C-3, C-12, from H-3 to C-5, C-12, from H-1 and H-10 to C-11 also suggested that a seven numbered benzazepine ring was formed between the benzene, *N*-connected -CH₂-CH₂-, carbonyl group, and pyrrolo ring, and **1** was confirmed as a tricyclic benzazepine alkaloid.

Since the skeleton of compound **1** was determined, the positions of substituents (methoxy, phenolic hydroxy, and methyl group) also can be determined by further analysis of its HMBC data (Figure 2). The HMBC correlations from H₃-15 to C-1, C-2, C-3, from H-1 and H-3 to C-15 indicated that the methyl group located at C-2. The HMBC correlation from the methoxy protons (δ_{H} 3.79) to C-8 indicated that the methoxy groups located at C-8. In addition, the HMBC correlations from the phenolic hydroxy proton (δ_{H} 10.89) to C-7, C-8, C-13 indicated that the phenolic hydroxy group was located at C-7. From the above analysis, the structure of compound **1** was elucidated, and gave the trivial name of cirrhobenzazepine A.

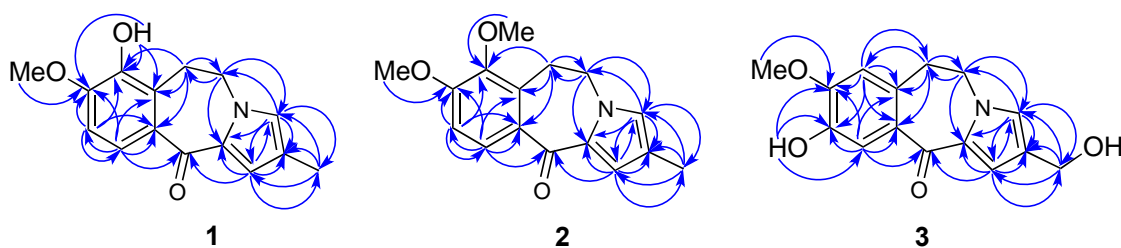



Figure 2. Key HMBC() correlations of compounds **1-3**

Cirrhobenzazepine B (**2**) was obtained as a pale yellow gum and showed a quasi-molecular ion at m/z 294.1112 $[\text{M}+\text{Na}]^+$ in the HRESIMS (calcd m/z 294.1106), corresponding to the molecular formula $\text{C}_{16}\text{H}_{17}\text{NO}_3$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from the down-field shift of C-7 from δ_{C} 148.1 ppm to δ_{C} 151.5 ppm, and the disappearance of a phenolic hydroxy resonance and appearance of a methoxy resonance in **2**. These changes indicated that the phenolic hydroxy group at C-7 in **1** was converted into a methoxy group (δ_{C} 61.0 q, δ_{H} 3.84 s) in **2**. The HMBC correlation from this methoxy proton (δ_{H} 3.84) to C-7 also supported this deduction. In addition, the positions of the other methoxy group and methyl can also be determination by further analysis of its HMBC correlations (Figure 2). The structure of **2** was therefore defined.

Cirrhobenzazepine C (**3**) was also obtained as yellow gum with a molecular formula as $\text{C}_{15}\text{H}_{15}\text{NO}_4$, according to the ion peak of m/z 296.0894 ($[\text{M}+\text{Na}]^+$) in the HRESIMS. The UV and IR spectra of **3** were similar to those of **1**. Comparison of the NMR spectra of these two compounds revealed that the obvious chemical shift differences resulted from the proton signals on benzene ring. A pair of doublets at δ_{H} 7.09

and 7.58 (d, 8.2) in **1** were replaced by two singlets at δ_{H} 6.68 and 7.48 in **3**. In addition, a methyl resonance in **1** was also replaced by a hydroxymethyl resonance in **3**. The HMBC correlation from the methoxy protons (δ_{H} 3.78) to C-8 indicated that the methoxy group located at C-8. The HMBC correlations from the phenolic hydroxy proton (δ_{H} 10.86) to C-8, C-9, C-10 indicated that the phenolic hydroxy group was located at C-9. Finally, The HMBC correlations from H₃-15 to C-1, C-2, C-3, from H-1 and H-2 to C-15 indicated that the hydroxymethyl group located at C-2. From the above analysis, the structure of compound **3** was elucidated.

Since certain of the alkaloids from *Thalictrum* genus exhibit potential anti-viral activity,¹⁸⁻²⁰ compounds

1-7 were tested for their anti-rotavirus activity.

Their ability to prevent the cytopathic effects of rotavirus in MA104 cells was tested according to our previous literatures,^{21,22} and their effects were measured in parallel with the determination of antiviral activity using ribavirin as positive control. The results (Table 2) revealed that compounds **1-7** exhibited potent anti-rotavirus activity with therapeutic index (TI) value in the range of 11.9-18.2.

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained using a Shimadzu UV-1900 spectrophotometer. A Bio-Rad FTS185 spectrophotometer was used for scanning IR spectra. ¹H, ¹³C, and 2D-NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. ESIMS and HRESIMS analyses were measured on Agilent 1290 UPLC/6540 Q-TOF mass spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. Semi-preparative HPLC was performed on an Agilent 1260 preparative liquid chromatograph with Zorbax PrepHT GF (2.12 mm \times 25 cm) or Venusil MP C₁₈ (2.0 mm \times 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40 - 63 μm , Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75 - 150 μm , Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5% H₂SO₄ in ethanol and heating.

Plant Material. The whole plants of *Thalictrum cirrhosum* (Levl.) were collected in Heqing country,

Table 2. Anti-rotavirus activity of compounds **1-7**

No.	CC ₅₀ ($\mu\text{g/mL}$)	EC ₅₀ ($\mu\text{g/mL}$)	TI (CC ₅₀ /EC ₅₀)
1	212.7	14.8	14.4
2	238.4	15.2	15.7
3	254.8	14	18.2
4	132.9	12.4	10.7
5	185.6	14.1	13.2
6	202.8	17.0	11.9
7	218.5	15.7	13.9
Ribavirin	278.4	13.6	20.5

CC₅₀: mean (50%) value of cytotoxic concentration; EC₅₀: mean (50%) value of effective concentration; TI: therapeutic index, CC₅₀/EC₅₀.

Dali prefecture of Yunnan Province, People's Republic of China, in September 2017. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-17-09-96) has been deposited in Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, P. R. China.

Extraction and Isolation. The air-dried and powdered whole plants of *T. cirrhosum* (3.2 kg) were extracted with 95% aq. EtOH, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na₂CO₃ aq. and extracted with EtOAc again. The EtOAc-soluble alkaloidal materials (38.5 g) were applied to silica gel (200-300 mesh) column chromatography, eluting with CHCl₃/MeOH gradient system (10:0, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. Further separation of fraction B (9:1, 6.22 g) by silica gel column chromatography, eluted with CHCl₃/Me₂CO (9:1-2:1), yielded mixtures B1–B7. Sub-fraction B1 (9:1, 1.46 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (68% MeOH/H₂O, flow rate 20 mL/min) to give **5** (12.2 mg) and **6** (16.8 mg). Sub-fraction B2 (8:2, 1.15 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (52% MeOH/H₂O, flow rate 20 mL/min) to give **1** (16.0 mg), **2** (15.5 mg) and **7** (14.3 mg). Sub-fraction B3 (7:3, 1.24 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (42% MeOH/H₂O, flow rate 20 mL/min) to give, **3** (17.2 mg) and **4** (18.5 mg).

Anti-rotavirus assay. The human rotavirus Wa group was used to infect the cell culture MA104 *in vitro*, the 50% cytotoxicity concentration (CC₅₀) and half maximal effective concentration (EC₅₀) were evaluated, and the ribavirin was used as positive control.^{21,22} MA-104 cells (1×10⁵ cells *per* well) were grown in 96-well plates for 48 h. The media were removed and replaced by new media containing serial dilutions of compounds under test. After incubation for 72 h, the media were discarded, and 5 μL of MTT solution was added to each well. Plates were then incubated at 37 °C for 4 h. The solution was removed, and 100 μL of 0.04 mol/L HCl-isopropanol were added to each well to dissolve formazan crystals. Using a microplate reader, the absorbance of each well was measured at 540 nm. After subtracting the background absorbance at 655 nm, the 50% CC₅₀ of each compound was estimated by regression analysis.

In the mixed treatment assay, each compound was mixed with a 0.01 multiplicity of infection (MOI) of the rotaviruses at various concentrations (1-160 μg/mL) and incubated at 4 °C for 1 h. The mixtures were inoculated in triplicates onto near confluent MA-104 cell monolayers (1×10⁵ cells *per* well) for 1 h with occasional rocking. The solution was removed and the cells replaced with eagles minimum essential medium (EMEM) containing 1 μg/mL trypsin. The cells were incubated for 72 h at 37 °C under 5% CO₂

atmosphere until the cells in the control showed complete viral cytopathic effect (CPE) by light microscopy. EC₅₀ was estimated by regression analysis.

Cirrhobenzazepine A (1): C₁₅H₁₅NO₃, obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 220 (4.33), 250 (3.68), 320 (3.82), 352 (3.67) nm; IR (KBr) ν_{max} 3412, 3055, 2947, 1656, 1606, 1560, 1448, 1267, 1179, 1146, 1065, 1027, 896, 793 cm⁻¹; ¹H NMR and ¹³C NMR data (in CDCl₃, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 280 [M+Na]⁺; HRESIMS *m/z* 280.0958 [M+Na]⁺ (calcd for C₁₅H₁₅NNaO₃, 280.0950).

Cirrhobenzazepine B (2): C₁₆H₁₇NO₃, obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 220 (4.19), 253 (3.57), 325 (3.76), 355 (3.62) nm; IR (KBr) ν_{max} 3062, 2944, 1658, 1602, 1557, 1442, 1263, 1176, 1072, 922, 853 cm⁻¹; ¹H NMR and ¹³C NMR data (in CDCl₃, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 294 [M+Na]⁺; HRESIMS *m/z* 294.1112 [M+Na]⁺ (calcd for C₁₆H₁₇NNaO₃, 294.1106).

Cirrhobenzazepine C (3): C₁₅H₁₅NO₄, obtained as yellow gum; UV (MeOH) λ_{max} (log ε) 218 (4.21), 252 (3.69), 324 (3.70), 355 (3.62) nm; IR (KBr) ν_{max} 3428, 3032, 2940, 1658, 1608, 1542, 1455, 1276, 1162, 1060, 976, 843 cm⁻¹; ¹H NMR and ¹³C NMR data (in CDCl₃, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 296 [M+Na]⁺; HRESIMS *m/z* 296.0894 [M+Na]⁺ (calcd for C₁₅H₁₅NNaO₄, 296.0899).

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