Two New Coumarins from *Herpetospermum caudigerum*

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The ethyl acetate extract from the seeds of *Herpetospermum caudigerum* was found to show protective effects on carbon tetrachloride (CCl₄) and thioacetamide (TAA)-induced acute hepatic injuries in mice. From the ethyl acetate extract, two new coumarins, herpetolide A (1) and herpetolide B (2), along with four known compounds, herpetone (3), dehydrodiconiferyl alcohol (4), 2,4-dihydroxypyrimidine (5) and stigmasterol (6) were isolated. The structures of the new coumarins were elucidated on the basis of chemical and physicochemical evidences. Herpetone exhibited protective effects on CCl₄-induced hepatocyte injury.

Key words *Herpetospermum caudigerum*; herpetolide A; herpetolide B; coumarin; herpetone

Herpetospermum caudigerum WALL (*H. pedunculatum* (SER.) BAILL.) (Cucurbitaceae) is distributed in southwest China, Nepal, and northeast India. The dried ripe seeds of *H. caudigerum* have been used for the treatment of liver diseases as a Tibetan medicinal herb in China.¹⁾ The ethyl acetate extract from the seeds of *H. caudigerum* was found to show protective effects on carbon tetrachloride (CCl₄) and thioacetamide (TAA)-induced acute hepatic injuries in mice.²⁾ From the ethyl acetate extract, a new sesqui-norlignan named herpetone was obtained during our study.³⁾ The continued study led to the isolation of two new coumarins named herpetolide A (1) and herpetolide B (2), along with herpetone (3), dehydrodiconiferyl alcohol (4),⁴⁾ 2,4-dihydroxypyrimidine (5)⁵⁾ and stigmasterol (6)⁶⁾ from the ethyl acetate extract. The structures of the new coumarins were determined on the basis of chemical and physicochemical evidences.

Compounds 1 was isolated as a white amorphous powder. Its molecular formula C₁₆H₁₄O₆ was established from the *quasi*-molecular ion peak at *m/z* 325.0682 [M+Na]⁺ in the HR-ESI-MS. The IR spectrum suggested the presence of hydroxyls (3384 cm⁻¹), a carbonyl group (1688 cm⁻¹), and benzene rings (1612, 1522, 1453 cm⁻¹). The presence of a 1,2,4,5-tetrasubstituted benzene ring and a 1,2,3,5-tetrasubstituted benzene ring were indicated from the ¹H-NMR spectrum (δ 8.02, 1H, s and δ 7.58, 1H, s; δ 7.05, 1H, d, *J*=2.6 Hz and δ 6.93, 1H, d, *J*=2.6 Hz), which was supported by the corresponding 12 downfield signals in the ¹³C-NMR. The ¹H- and ¹³C-NMR spectrum of 1 also suggested the presence of a hydroxymethyl group (δ _H 5.73, 1H, t, *J*=5.0 Hz, δ _H 4.89, 2H, d, *J*=5.0 Hz and δ _C 63.8), two methoxyl groups (δ _H 3.96 and 3.83, each 3H, s; δ _C 56.2 and 56.0) and a conjugated carbonyl group (δ _C 160.5). A detailed comparison of ¹H- and ¹³C-NMR data between 1 and reference data of similar compound reported by Tanahashi T. *et al.*⁷⁾ implied that 1 possessed the similar coumarin skeleton. The HMBC correlations between CH₂OH (δ 4.89) and C-4a (δ 111.1), C-5 (δ 140.1), C-6 (δ 115.0), along with the NOESY correlation between CH₂OH (δ 4.89) and H-1' (δ 8.02), H-6 (δ 6.93), indicated that the hydroxymethyl was located at C-5. Moreover, it was deduced that the hydroxy and two methoxyl groups were located at C-7, C-2' and C-3' respectively by the HMBC and NOESY experiments (Fig. 2). Consequently, compound 1 was elucidated as the structure showed in Fig. 1, and was trivially named as herpetolide A.

Its ¹H- and ¹³C-NMR spectra (Table 1) were completely assigned by detailed DEPT, HMQC, HMBC and NOSEY experiments.

Compound 2 was obtained as a white amorphous powder. Its molecular formula C₁₆H₁₂O₆ was established from the *quasi*-molecular ion peak at *m/z* 323.0523 [M+Na]⁺ in the HR-ESI-MS. The IR spectrum suggested the presence of hydroxyls (3356 cm⁻¹), carbonyl groups (1725, 1674 cm⁻¹), and benzene rings (1622, 1523, 1455 cm⁻¹). The ¹H- and ¹³C-NMR spectra of 2 were similar to those of 1, except for the difference of CHO signals in 2 (δ _H 10.51, 1H, s and δ _C 192.6) and CH₂OH signals in 1 (δ _H 5.73, 1H, t, *J*=5.0 Hz, δ _H 4.89, 2H, d, *J*=5.0 Hz and δ _C 63.8). The HMBC of 2 also revealed that the CHO was located at C-5. Thus, compound 2 was determined as the structure showed in Fig. 1, and was trivially named as herpetolide B. Its ¹H- and ¹³C-NMR spectra (Table 1) were assigned by detailed 2D-NMR experiments.

In order to seek the bioactive constituents for the treatment of liver diseases, effects of herpetone, a lignan compound of

Fig. 1. Structures of Herpetolide A, B and Herpetone from the Seeds of *H. caudigerum*

Fig. 2. Important HMBC and NOESY Correlations of Herpetolide A
HMBC: →; NOESY: ↔.

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Table 1. ^1H - and ^{13}C -NMR Data of **1** and **2** (600/150 MHz, $\text{DMSO}-d_6$, δ in ppm, J Hz)

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		160.5		160.0
3		113.6		113.8
4		128.9		126.5
4a		111.1		112.4
5		140.1		135.9
6	7.05, 1H, d (2.6)	115.0	7.31, 1H, d (2.6) ^{a)}	114.7
7		159.3		159.6
8	6.93, 1H, d (2.6)	101.4	7.29, 1H, d (2.6) ^{a)}	106.7
8a		152.8		152.9
1'	8.02, 1H, s	109.7	7.30, 1H, s	110.2
2'		154.3		154.2
3'		147.1		148.4
4'	7.58, 1H, s	114.6	7.61, 1H, s	114.2
—CH ₂ OH	5.73, 1H, t (5.0)	63.8		
	4.89, 2H, d (5.0)			
7-OCH ₃	3.83, 3H, s	56.0	3.91, 3H, s	56.3
2'-OCH ₃	3.96, 3H, s	56.2	3.95, 3H, s	56.6
—OH	9.98, 1H, br s		10.26, 1H, br s	
—CHO			10.51, 1H, s	192.6

a) Chemical shift may be exchanged.

Table 2. Effects of Herpetone on the Survival Rate of Rat Hepatocytes Treated with CCl_4

Group	Dose (mg/ml)	OD (mean $\pm$ S.D.)	Survival rate (%)
Medium		0.545 $\pm$ 0.06 $\blacktriangle\blacktriangle$	100
CCl_4 -treated		0.127 $\pm$ 0.02	23.3
Herpetone	0.5	0.257 $\pm$ 0.01 $\blacktriangle\blacktriangle$	47.1
	0.25	0.168 $\pm$ 0.04	30.8
	0.125	0.148 $\pm$ 0.01	27.1
	0.0625	0.142 $\pm$ 0.01	26.0

Compared with CCl_4 -treated, $\blacktriangle\blacktriangle p < 0.01$.

relatively high content isolated from the EtOAc extract of *H. caudigerum*, on the survival rate of CCl_4 -injured primary cultured rat hepatocytes were investigated (Table 2). The result showed that herpetone significantly improved the cell viability, indicating a protective effect on hepatocytes *in vitro*. In addition, Yuan *et al.* reported that herpetin, another lignan compound from this plant, showed significant inhibitory effects on HBV-DNA and the replication and expression of HBsAg and HBeAg.⁸⁾ These findings suggest that lignan constituents of this plant are worthy of further investigation.

Experimental

General Melting points were measured on an XRC-1 melting point apparatus and are uncorrected. UV and IR spectra were recorded on a UV-8500 spectrometer and a Nicolet 2000XV FT-IR spectrometer (KBr disk), respectively. Mass spectra were obtained on a VG-7070E mass spectrometer (EI-MS) and a Bruker Daltonics Bio TOF-Q mass spectrometer (HR-ESI-MS). NMR spectra were carried out on a Bruker Avance 600 spectrometer with TMS as the internal standard. Column chromatography was carried out on silica gel (200–300 mesh), Qingdao Haiyang Chemical Group Co., China). Semi-preparative HPLC was carried out using a Shimadzu 10A VP HPLC system (YWG-C₁₈ column, 250 $\times$ 10 mm, 10 μm).

Plant Material The seeds of *H. caudigerum* were purchased from Tibetan Shannan Herbal Company and identified by Prof. Minru Jia, College of Pharmacy, Chengdu University of Traditional Chinese Medicine (TCM). Voucher specimens (No. 2001-018a) have been deposited at the phytochemistry laboratory of Chengdu University of TCM.

Extraction and Isolation The dried and powdered seeds of *H. caudigerum* (5.3 kg) were refluxed with 95% ethanol (401 $\times$ 4 times, each 2 h). After removal of ethanol under reduced pressure, 370 g of residue was obtained. Mixed well with silica gel (1 kg), this residue was extracted in turns with petroleum ether, ethyl acetate, and 95% ethanol. After removal of solvents under reduced pressure, 90 g of petroleum ether extract, 30 g of ethyl acetate extract, and 45 g of ethanol extract were obtained.

The ethyl acetate extract (30 g) was subjected to silica gel column chromatography (ϕ 6 $\times$ L 50 cm) and eluted with petroleum ether/ethyl acetate (6:1, 3:1, 1:1, 1:2) to give fractions A–D (A, 1.8 g; B, 1.2 g; C, 0.6 g; D, 2.0 g). Compound **1** (12 mg) was obtained by crystallization of fraction A from petroleum ether/ethyl acetate, while the remnant was then subjected to silica gel column (ϕ 3 $\times$ L 30 cm) and eluted with petroleum ether/acetone to yield compound **2** (9 mg). Compound **3** (31 mg) was obtained by crystallization of fraction B from petroleum ether/ethyl acetate (3:1). Compound **4** (5 mg) was obtained by HPLC (C-18 column, $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 65:35) from fraction C. The fraction D was subjected to silica gel column chromatography (ϕ 3 $\times$ L 30 cm) and eluted with petroleum ether/acetone (2:1 to 1:1) to give fractions D1, D2. Compound **5** (20 mg) was obtained by crystallization of fraction D1 from petroleum ether/acetone, and compound **6** (15 mg) was obtained by crystallization of fraction D2 from petroleum ether/acetone.

Herpetolide A (1): White amorphous powder. mp 95–96.5 $^\circ\text{C}$. ^1H - and ^{13}C -NMR data see Table 1. IR (KBr) cm^{-1} : 3384, 1688, 1612, 1522, 1453, 1308, 1155. UV λ_{max} (CH_3OH) nm (log ϵ): 260 (3.64), 303 (3.18), 335 (2.88). HR-ESI-MS m/z : 325.0682 (Calcd for $\text{C}_{16}\text{H}_{14}\text{O}_6\text{Na}$: $[\text{M}+\text{Na}]^+$, 325.0683). MS m/z : 302 (M^+), 285, 84.

Herpetolide B (2): White amorphous powder. mp 105–107 $^\circ\text{C}$. ^1H - and ^{13}C -NMR data see Table 1. IR (KBr) cm^{-1} : 3356, 1725, 1674, 1622, 1523, 1455, 1315, 1159. UV λ_{max} (CH_3OH) nm (log ϵ): 247 (3.84), 304 (3.30), 346 (3.22). HR-ESI-MS m/z : 323.0523 (Calcd for $\text{C}_{16}\text{H}_{12}\text{O}_6\text{Na}$: $[\text{M}+\text{Na}]^+$, 323.0527). MS m/z : 300 (M^+), 272, 257, 113.

Protective Effects of Herpetone on Primary Cultured Rat Hepatocytes Treated with CCl_4 Hepatocytes were obtained from rat liver by means of collagenase perfusion method.⁹⁾ Cell suspensions were seeded into 96-well-plates ($\geq 4 \times 10^4$ cells/well) and incubated at 37 $^\circ\text{C}$ in a humidified atmosphere of 5% CO_2 for 48 h. CCl_4 was then added to the culture medium at a final concentration of 8 mmol/l to induce hepatocyte injury. After 24 h, herpetone of various concentrations were added to the culture medium and the cells were further cultured for 48 h followed by the addition of 50 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) into each well. The cells were incubated under the same conditions for additional 4 h. Then, the culture medium was removed. After the addition of 150 μl of DMSO, the optical densities at 570 nm were measured and the survival rates of hepatocytes were calculated [survival rate = OD (sample group)/OD (medium group) $\times$ 100%].

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References

- 1) "The Drug Standard of Ministry of Health of P. R. China (Tibetan Medicines)," Vol. 9, ed. by State Pharmacopoeia Committee of P. R. China, 1995, p. 641.
- 2) Zhang M., "Ph. D. Dissertation of Chengdu University of Traditional Chinese Medicine," Chengdu University of Traditional Chinese Medicine, Chengdu, 2003, pp. 46–51.
- 3) Zhang M., Dong X. P., Deng Y., Wang H., Li X. N., Song Q., *Acta Pharm. Sinica*, **41**, 659–661 (2006).
- 4) Salama O., Chaudhuri P. K., Sticher O., *Phytochemistry*, **20**, 2603–2604 (1981).
- 5) Ellis P. D., Dunlap R. B., Pollard A. L., Seidman K., Cardin A. D., *J. Am. Chem. Soc.*, **95**, 4398–4403 (1973).
- 6) Zhang X. Q., Qi J., Ye W. C., Zhao S. X., *J. Chin. Pharm. Univ.*, **35**, 404–405 (2004).
- 7) Tanahashi T., Kuroishi M., Kuwahara A., Nagakura N., Hamada N., *Chem. Pharm. Bull.*, **45**, 1183–1185 (1997).
- 8) Yuan H. L., Liu Y., Zhao Y. L., Xiao X. H., *J. Chin. Pharm. Sci.*, **14**, 140–143 (2005).
- 9) Tan H. L., Yang M. H., Wang Y. G., Ma Z. H., Gao Y., *Chin. J. Appl. Physiol.*, **22**, 509–512 (2006).