Two New Alkaloids from *Capparis himalayensis*

Yun-Qiu Li,[a] Shi-Lin Yang,[b] He-Ran Li,[a] and Li-Zhen Xu*,[a]

[a] *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing 100094, P. R. China: and* [b] *National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine; Nanchang 330006, P. R. China.*

Received July 17, 2007; accepted November 7, 2007; published online November 16, 2007

Two new alkaloids, Capparin A (1) and B (2), along with seven known compounds 6-methoxyindoline-2,3-dione (3), wogonin (4), oroxylin A (5), kaempferol (6), apigenin (7), quercetin (8) and luteolin (9), were isolated from the whole plant of *Capparis himalayensis*. Their structures have been established on the basis of spectral methods and the structure of 1 was confirmed by X-ray crystallographic analysis.

Key words *Capparis himalayensis*; Capparidaceae; alkaloid; X-ray

Capparis himalayensis belonging to Capparidaceae grows widely in Xinjiang Uygur Autonomous Region and Tibet of China. Its root barks, leaves and fruits are used for the treatment of rheumatism in traditional medicine.[1)] Previous investigations have led to the isolation of different chemical components showing various bioactivities from the *Capparis* genus[2—11)] while up to now, there is no report regarding chemical constituents of this species. In the present study, we attempted to find some bioactive components from *Capparis himalayensis* to support its development as a medicinal resource. This paper describes the isolation and structural elucidation of two new alkaloids 6-methoxyl-2′-(methylthio)-spiro[3*H*-indole-3,5′(4′*H*)thiazol]-2(1*H*)-one (**1**) and 6-methoxy-2-(methylthio)-1*H*-indole-3-carbaldehyde (**2**) from the ethanol extract of the plant along with seven known compounds. The known compounds were identified as 6-methoxyindoline-2,3-dione (**3**),[12)] wogonin (**4**),[13)] oroxylin A (**5**),[14)] kaempferol (**6**),[15,16)] apigenin (**7**),[15,16)] quercetin (**8**)[15,16)] and luteolin (**9**)[15)] by comparison of spectroscopic properties (MS, ^{1}H-NMR, ^{13}C-NMR, HMQC and HMBC). Among the known compounds, **3**, **4**, **5**, **7**, **9** were isolated from *Capparis* genus for the first time.

Results and Discussion

Compound **1** was obtained as yellow plates, and gave a positive reaction to dragendorff reagent. The molecular formula of **1** was determined to be $C_{12}H_{12}N_2O_2S_2$ by HR-EI-MS (*m*/*z* 280.0341 $[M]^+$). The IR spectrum displayed absorption bands for amide (3436 cm^{-1}), carbonyl (1715 cm^{-1}) and aromatic group (1615, 1505, 1463 cm^{-1}), respectively. The ^{1}H-NMR spectrum of **1** indicated the presence of a 1,2,4-trisubstituted benzene [δ_H 7.24 (d, J=8.4 Hz), 6.57 (dd, J=8.4, 1.8 Hz), 6.40 (d, J=1.8 Hz)] and a AB coupling system [δ_H 4.46, 4.36 (d, J=15 Hz)] corresponding to methylene (=N–CH_2–). In addition, the signal at δ_H 10.64 (s) showed the presence of an amidocyanogen. ^{1}H-NMR signals at δ_H 3.74 (s) and 2.50 (s) indicated the existence of a methoxyl and a methylthio, respectively (Table 1). The ^{13}C-NMR spectrum exhibited 12 carbon signals which revealed the existence of one carbonyl carbon atom (δ_C 177.5), one olefinic-quaternary carbon (>C=N–) (δ_C 161.8), and one methylene (δ_C 73.6), one quaternary (δ_C 64.2), one methoxyl (δ_C 55.3), one methylthio (δ_C 15.0), and one benzene ring (δ_C 160.6, 142.4, 125.1, 121.4, 107.6, 96.7) (Table 1). ^{1}H-, ^{13}C-NMR and EI-MS data strongly suggested the feature of spirobrassinin derivative.[17)] According to the molecular formula and the structure of 1,2,4-trisubstituted benzene indicated in ^{1}H-NMR spectrum, a methoxyl was suggested located at C-5 or C-6. In the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 1), the long-range coupling between H-12 (δ_H 3.74) and C-6 (δ_C 160.6); H-4 (δ_H 7.24) and C-3 (δ_C 64.2), C-6 (δ_C 160.6); H-5 (δ_H 6.57) and C-7 (δ_C 96.7), C-9 (δ_C 121.4); H-7 (δ_H 6.40) and C-5 (δ_C 107.6), C-9 (δ_C 121.4) indicated that the methoxyl was located at C-6. Thus **1** was identified as 6-methoxyl-2′-(methylthio)spiro[3*H*-indole-3,5′(4′*H*)thiazol]-2-one and all the ^{1}H and ^{13}C signals had been designed by 2D NMR. The relative structure of **1** was further confirmed by single crystal X-ray diffraction studies (Fig. 2). We named this compound Capparine A. In addition, single crystal X-ray analyses of **1** showed that it

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2**

Position	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	10.64, s		12.11, s	
2		177.5		143.9
3		64.2		115.9
4	7.24, d (8.4)	125.1	7.87, d (9.0)	120.3
5	6.57, dd (8.4, 1.8)	107.6	6.83, dd (9.0, 2.4)	111.5
6		160.6		156.5
7	6.40, d (1.8)	96.7	6.90, d (2.4)	94.9
8		142.4		137.8
9		121.4		119.4
10		161.8	10.01, s	183.1
11	α 4.46, d (15.0); β 4.36, d (15.0)	73.6	3.80, s	55.2
12	3.74, s	55.3	2.66, s	16.7
13	2.50, s	15.0		

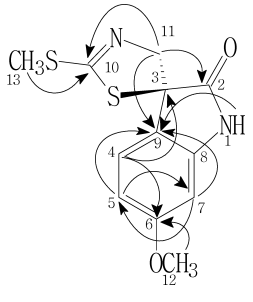

Fig. 1. Key HMBC Correlation of **1**

* To whom correspondence should be addressed. e-mail: xulizhen2002@hotmail.com

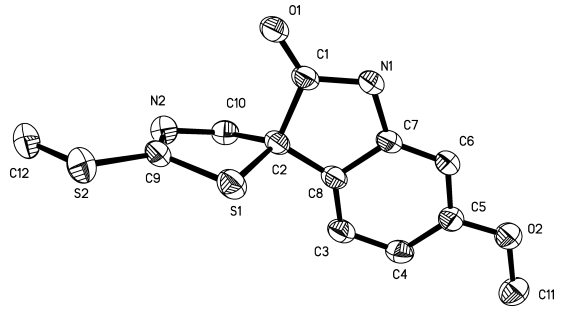

Fig. 2. The X-Ray Crystallographic Structure of **1**

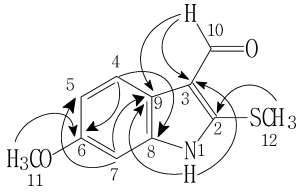

Fig. 3. Key HMBC Correlation of **2**

was racemic while the specific rotation suggested that levorotary enantiomer was a little bit more than dextrorotary enantiomer. One possible explanation for contradictory fact is that the sample used for specific rotary determination is obtained before single crystal formation, which contains more levorotatory enantiomer than dectrorotatory enantiomer. However, the single crystal grew for single crystal X-ray diffraction study was a result of the combination of levorotary enantiomer and dextrorotary in a 1 : 1 ratio. Since **1** was too little to resolute the anantiomers, we will proceed with the study in our continuous work.

Compound **2** was obtained as white amorphous powder. The molecular formula was determined to be $C_{11}H_{11}NO_2S$ by HR-EI-MS (*m*/*z* 221.0518 $[M]^+$). The IR spectrum displayed absorption bands for carbonyl (1618 cm^{-1}) and aromatic group (1581, 1515, 1453 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) indicated the presence of 1,2,4-trisubstituted benzene [δ_H 7.87 (d, J=9.0 Hz), 6.83 (dd, J=9.0, 2.4 Hz), 6.90 (d, J=2.4 Hz)], a methoxyl (δ_H 3.80, s), a methylthio (δ_H 2.66, s), an amidocyanogen (δ_H 12.11, br s) and an aldehyde group (δ_H 10.01, s). ^{13}C-NMR spectrum (Table 1) exhibited 11 carbon signals. In the HMBC spectrum (Fig. 3), the correlations between H-1 (δ_H 12.11) and C-3 (δ_C 115.9), C-9 (δ_C 119.4); H-4 (δ_H 7.87) and C-6 (δ_C 156.5), C-8 (δ_C 137.8); H-7 (δ_H 6.90) and C-9 (δ_C 119.4), C-5 (δ_C 111.5); H-11 (δ_H 3.80) and C-6 (δ_C 156.5) indicated the existence of 6-methyloxy-indole, the long-range coupling between H-10 (δ_H 10.01) and C-9 (δ_C 119.4), C-3 (δ_C 115.9) suggested an aldehyde group connected with C-3, the correlation between H-12 (δ_H 2.66) and C-2 (δ_C 143.9) indicated a methylthio connected with C-2. Furthermore, the NEOSY spectrum confirmed the methylthio and the methoxy connected with C-2 and C-6, respectively by correlations of H-1/H-12, H-11/H-5 and H-11/H-7 (Fig. 4). Thus compound **2** was identified as 6-methoxy-2-(methylthio)-1*H*-indole-3-carbaldehyde and all the ^{1}H and ^{13}C signals had been designed by 2D NMR. We named this compound Capparine B.

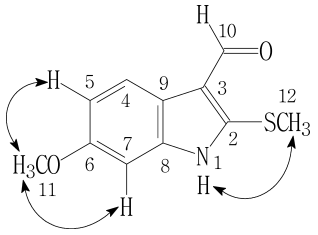

Fig. 4. Key NOESY Correlations of **2**

Experimental

General Experimenting Melting points were determined on Fisher–Johns and are uncorrected. UV spectra were taken on a Philips PYE Unican Pu 8800 Spectrophotometer. IR spectra were measured with a Nicolet 5700 of Thermo. NMR spectra were measured in DMSO-d_6 on a VARIAN INOVA 600 spectrometer with chemical shifts being represented in parts per million (ppm) and tetramethylsilane (TMS) as an internal standard. EI-MS and HR-EI-MS were measured on a Zabspec E spectrometer at an ionization voltage of 70 eV. The TLC and HPTLC employed precoated silica gel plates (Qingdao Haiyang Chem. Co., Ltd.). For the column chromatography, silica gel (Qingdao Haiyang) and Sephadex LH-20 (Pharmacia) were used. X-Ray crystallographic data collection for **1** was carried on a MAC DIP-2030K single crystal X-ray diffractometer.

Plant Material The plant of *Capparis himalayensis* was collected in Xinjiang Uygur Autonomous Region of China, in September, 2004 and identified by Doctor Guo-qiang Li. Voucher specimen (XC-04-0912) was deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation The air-dried plant material (18 kg) was powdered and extracted with 95% EtOH and 50% EtOH respectively. The alcohol extract were concentrated under reduced pressure and successively extracted with petroleum ether, $CHCl_3$, EtOAC and *n*-BuOH. The $CHCl_3$-soluble extract (60.8 g) was subjected to a silica gel column chromatography and eluted with petroleum ether/EtOAc (gradient, 1 : 0→0 : 1) to yield 26 fractions. Fr. 6-8 (3.7 g) were further separated with silica gel column using petroleum ether/EtOAc (gradient, 1 : 0→0 : 1) to yield 43 portions, and portion 27 was purified by Sephadex LH-20 to afford **4** (18 mg). Fr. 9-11 (1.5 g) were further separated with silica gel column using petroleum ether/EtOAc (gradient, 1 : 0→0 : 1) and Sephadex LH-20 to yield **2** (12 mg) and **5** (5 mg). Fr. 12-13 (5 g) were applied to silica gel column eluted with a gradient of petroleum ether/EtOAC and Sephadex LH-20 to give **1** (13 mg) and **3** (9 mg). EtOAC-soluble extract (116.3 g) was subjected to a silica gel column eluted with $CHCl_3$/MeOH (gradient, 1 : 0→0 : 1) to yield 28 fractions. Fr. 9 (4 g) was further separated with silica gel using petroleum ether/actone (gradient, 1 : 0→0 : 1) and Sephadex LH-20 to yield **6** (14 mg) and **7** (23 mg). Fr. 10-11 were separated with Sephadex LH-20 to yield **8** (25 mg) and **9** (10 mg) respectively.

Compound **1**: Pale yellow plates crystal ($CHCl_3$–CH_3OH), mp 170—173 °C; $[\alpha]_D^{25}$ −9.88° (c=0.16, MeOH); UV λ_{max} (MeOH) nm (log ε): 224 (3.34), 198 (2.86); HR-EI-MS *m*/*z*: 280.0341 $[M^+]$ (Calcd for $C_{12}H_{12}N_2O_2S_2$, 280.0340); EI-MS *m*/*z* (%): 280 (M^+, 73), 233 (22), 207 (100),175 (51), 162 (16); IR (cm^{-1}): 3436, 3169, 3057, 2926, 2835, 1715, 1587, 1505, 1463, 1344, 1281, 1159; ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) see Table 1.

Single-Crystal X-Ray Structure Determination Crystals of **1** was crystallized from $CHCl_3$–CH_3OH (1 : 1). Crystal data: $C_{12}H_{12}N_2O_2S_2$, MW=280.38, triclinic, space group *P*-1, a=5.750 (1) Å, b=10.189 (2) Å, c=11.988 (2) Å, α=106.96°, β=96.97°, γ=102.59°, V=642.6 (3) Å^3, Z=2, D_{calc}=1.449 g/cm^3, T=283 K, Crystal size: 0.05×0.30×0.60 mm. Single-crystal analysis of **1** was made on a MAC DIP-2030K diffractometer with monochromated Mo$K\alpha$ radiation (50 kV, 60 mA). A total of 1647 unique reflections were recoded, of which 1545 were considered observed on the basis $|F|^2 \geq 2\sigma|F|^2$. The structure was solved by direct methods with the use of the SHELX-97 program, and all hydrogen atoms were located from a difference Fourier map. Final *R*-factors were R=0.0700 and R_w=0.1756.

Compound **2**: White amorphous powder ($CHCl_3$–MeOH), mp 202—204 °C (dec); UV λ_{max} (MeOH) nm (log ε): 333 (2.83), 284 (2.97), 247 (3.17), 223 (3.17), 192 (3.29); HR-EI-MS *m*/*z*: 221.0518 $[M^+]$ (Calcd for $C_{11}H_{11}NO_2S$, 221.0511); EI-MS *m*/*z* (%): 221 (M^+, 100), 206 (68), 178 (18); IR (cm^{-1}): 3092, 2949, 1618, 1581, 1515, 1453, 1349, 1146, 1092, 828,

726; ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) see Table 1.

Acknowledgments We would like to express our gratitude to Professor Y. Lu (Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China) for the X-ray crystallography.

References

1) Jiangsu College of New Medicine. "A Dictionary of the Traditional Chinese Medicines," People's Hygiene Publisher, Beijing, 1986, p. 843.
2) Schraudolf H., *Phytochemistry*, **28**, 259—260 (1989).
3) Dekker T. G., Fourie T. G., Matthee E., Snyckers F. O., *Phytochemistry*, **26**, 1845—1846 (1987).
4) Ahmad V. U., Arif S., Amber A. R., Fizza K., *Liebigs Ann. Chem.*, **1987**, 161—162 (1987).
5) Calis I., Kuruuzum A., Ruedi P., *Phytochemistry*, **50**, 1205—1208 (1999).
6) McLean W. F. H., Blunden G., Jewers K., *Biochem. Syst. Ecol.*, **24**, 427—434 (1996).
7) Ahmad V. U., Ismail N., Amber A. R., *Phytochemistry*, **28**, 2493—2495 (1989).
8) Ahmad V. U., Ismail N., Arif S., Amber A. R., *J. Nat. Prod.*, **55**, 1509—1512 (1992).
9) Pelotto J. P., Del P. M., Maria A., *Biochem. Syst. Ecol.*, **26**, 577—580 (1998).
10) Sharaf M., El-Ansari M. A., Saleh N. A. M., *Fitoterapia*, **71**, 46—49 (2000).
11) Sharaf M., El-Ansari M. A., Saleh N. A. M., *Biochem. Syst. Ecol.*, **25**, 161—166 (1997).
12) Neelu K., Kristin J., *J. Med. Chem.*, **50**, 21—39 (2007).
13) Shen X. L., Zeng H. F., Chen Z., *J. Chin. Pharm.*, **37**, 14—17 (2003).
14) Wang H. Y., Xiao L. H., Liu L., *Journal of Shenyang Pharmaceutical University*, **20**, 339—341 (2003).
15) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahedron*, **34**, 1389—1397 (1978).
16) Shen C. C., Chang Y. S., Ho L. K., *Phytochemistry*, **34**, 843—845 (1993).
17) Takasugi M., Monde K., Katsui N., Shirata. A., *Chem. Lett.*, **1987**, 1631—1632 (1987).