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TWO NEW ALKALOIDS FROM THE SEEDS OF *CASSIA ALATA* AND THEIR BIOACTIVITIES

Pei-Song Yang,[1,2] Wei Zhang,[2] Xiao-Feng Shen,[2] Xin-Lin Wang,[2] Chao Li,[2] Xiao-Wei Gong,[2] Xu-Dong Zheng,[2] Dong-Lai Zhu,[2]* and Jia-Qiang Wang[2]*

[1] School of Chemical Science and Technology, Yunnan University, Kunming 650091, P.R. China; [2] Key Laboratory of Tobacco Chemistry of Yunnan Province, China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, P.R. China; E-mail: 16594939@qq.com, jszxtg_2015@163.com

Abstract – Two new alkaloids, 6-(hydroxymethyl)-3,9-dimethyl-7*H*-benzo[*de*]-quinolin-7-one (**1**) and 6-(hydroxymethyl)-8-methoxy-3,9-dimethyl-7*H*-benzo[*de*]-quinolin-7-one (**2**), together with four known alkaloids (**3**-**6**) were isolated from the seeds of *Cassia alata*. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1**-**6** were tested for their anti-tobacco mosaic virus (TMV) activities, and the results showed that compound **2** exhibited high anti-TMV activity with inhibition rates of 38.5%. This rate is higher than that of the positive control. The cytotoxicities of compounds **1**-**6** against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) were also tested. Compounds **1**-**6** showed weak inhibitory activities against some tested human tumor cell lines with IC_{50} values in the range of 2.5–7.5 μM.

Cassia alata (Linn.) is a native Fabaceae syn. Leguminosae family shrub of South America and can found widely in tropical regions. In Indonesia, Philippinnes and Thailand, this plant can be found all over the countries, sometimes cultivated for medicinal purposes.[1] In southern China, its roots, seeds, and leaves are used as herb medicines by Dai people, which has the effects of treating skin diseases, expel parasites, lowering blood pressure, relieving internal heat, and the like.[2,3] Previous phytochemical investigations on *C. alata* revealed that flavonoids[1,4-7] and alkaloids[1,7-9] had been isolated from this plant. Alkaloids from the plants of genus *Cassia* are known to have a variety of interesting biological profiles.[10-15] For the purpose of further utilizing *C. alata* and identifying more bioactive natural products from this plants, a

study on the seeds of *C. alata* was undertaken and lead to the isolation of two new (**1** and **2**) and four known (**3**-**6**) alkaloids. The structures of **1**-**6** were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. This paper deals with the isolation, structural elucidation, and their bioactivities of these compounds.

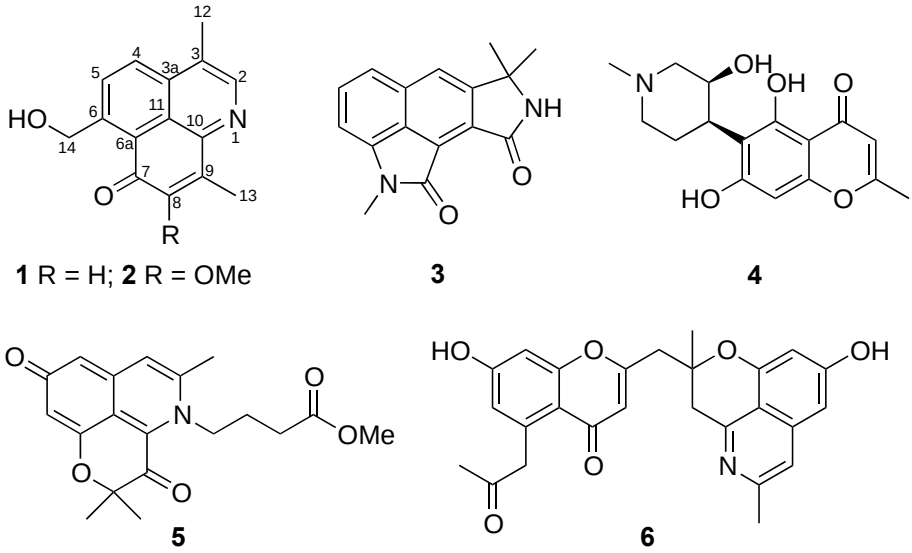

Figure 1. The structures of alkaloids from seeds of *C. alata*

A 70% aq. acetone extract prepared from the seeds of *C. alata* was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc. The EtOAc-soluble alkaloidal materials were subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new alkaloids, 6-(hydroxymethyl)-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one (**1**) and 6-(hydroxymethyl)-8-methoxy-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one (**2**), together with four known alkaloids (**3**-**6**). The structures of the compounds **1**-**6** were as shown in **Figure 1**, and the ^{1}H and ^{13}C NMR data of compounds **1** and **2** were listed in **Table 1**. The known compounds, cyclopiamide (**3**),[16] dysoline (**4**),[17] cassiarins H (**5**),[18] and cassiarin D (**6**),[19] were identified by the comparison of their spectroscopic data with literatures.

Compound **1** was isolated as a reddish gum. High-resolution ESIMS analysis gave a quasi-molecular ion at *m/z* 262.0838 $[M+Na]^+$, consistent with a molecular formula of $C_{15}H_{13}NO_2$, which indicated 10 degrees of unsaturation. The UV spectrum of **1** exhibited absorption bands at 210, 243, and 340 nm, highly suggesting the existence of aromatic chromophore. Strong absorption bands accounting for hydroxy (3410 cm^{-1}), carbonyl (1650 cm^{-1}), and aromatic groups (1610, 1567, and 1466 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H NMR spectrum of compound **1** showed four

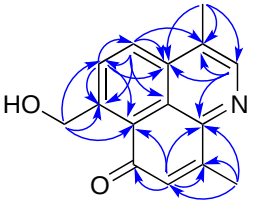

Figure 2. Key HMBC ( ) correlations of **1**

aromatic protons at δ_H 8.49 (1H, s, H-2), 8.08 (1H, d, J = 8.1 Hz, H-4), 7.69 (1H, d, J = 8.1 Hz, H-5) and 7.32 (1H, s, H-8), two methyl signals at δ_H 2.48 and 2.85 (each 3H, s, Me-12 and 13), a hydroxymethyl signal at δ_H 4.58 (2H, s, H_2-14). The ^{13}C NMR and DEPT spectra of compound **1** contained 15 carbon signals, including two methyl carbons (δ_C 13.5 and 16.3), a hydroxymethyl carbon (δ_C 62.4), four aromatic methine carbons (δ_C 141.4, 128.5, 131.9, and 132.3), seven aromatic quaternary carbons (δ_C 127.5, 130.9, 144.4, 125.5, 153.6, 146.6, and 119.6), and one quaternary carbonyl group (δ_C 183.7). All of the protons were assigned to the corresponding carbons by HSQC experiments. On the basis of the HMBC correlations (**Figure 2**) from H_2-14 to C-5, C-6, and C-6a, from H-5 to C-3a, C-6a, and C-14, and from H-4 to C-3, C-3a, C-11, and C-6, as well as the HMBC correlations from H_3-12 to C-2, C-3, and C-3a, and from H-2 to C-3, C-3a, C-10, and C-12, an isoquinoline moiety was established, with methyl groups attached to C-3 and hydroxymethyl to C-6.[20] HMBC correlations from H_3-13 to C-8, C-9, and C-10 suggested that the *β* carbon (C-9) of the *α,β*-unsaturated carbonyl unit was connected at C-10. The isoquinoline moiety and *α,β*-unsaturated carbonyl group accounted for 9 of the 10 sites of unsaturation, suggesting the presence of one remaining ring in the structure, and indicated that the carbonyl carbon (C-7) of the *α,β*-unsaturated carbonyl unit was connected to C-6a to form another ring. These were also supported by HMBC correlations of H-8 to C-6a and C-10. Thus, compound **1** was established as 6-(hydroxymethyl)-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one.

6-(Hydroxymethyl)-8-methoxy-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one (**2**) was also obtained as reddish gum and showed a quasi-molecular ion at *m/z* 292.0958 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 292.0950), corresponding to the molecular formula $C_{16}H_{15}NO_3$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. The chemical shift differences resulted from disappearance of an aromatic proton (δ_C 7.32, H-8) and appearance of a methoxy group (δ_C 52.7, δ_H 3.53) in **2**. These changes indicated that an aromatic proton in **1** was replaced by a methoxy group in **2**. The HMBC correlation between methoxy protons (δ_H 3.53) to C-8 suggested the methoxy group located at C-8. Accordingly, the structure of **2** was established.

Table 1. ^1H and ^{13}C-NMR spectral data of compound **1** (500/125 MHz, C_5D_5N)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	141.4 d		143.3 d	
3	127.5 s		127.5 s	
3a	130.9 s		130.3 s	
4	128.5 d	8.08 (d) 8.1	128.4 d	8.16 (d) 8.1
5	131.9 d	7.69 (d) 8.1	131.5 d	7.65 (d) 8.1
6	144.4 s		144.3 s	
6a	125.5 s		125.2 s	
7	183.7 s		181.5 s	
8	132.3 d	7.32 s	153.8 s	
9	153.6 s		121.1 s	
10	146.6 s		151.0 s	
11	119.6 s		120.5 s	
12	16.3 q	2.48 s	16.8 q	2.52 s
13	13.5 q	2.85 s	10.9 q	2.98 s
14	62.4 t	4.58 s	62.9 t	4.53 s
-OMe			52.7 q	3.53 s

Since certain of the isoquinoline alkaloids exhibit potential anti-TMV activities.[15,21] Compounds **1-6** were tested for their anti-tobacco mosaic virus (TMV) activities. The anti-TMV activities were tested by

half-leaf method, using ningnanmycin (a commercial product for plant disease in China, with inhibition rate of 31.2%) as a positive control.[22,23] The results showed that compounds **1** exhibited high anti-TMV activities with inhibition rates of 38.5% at the concentration of 20 μM. This rate is higher than that of the positive control. The other compounds also showed potential activities with inhibition rates in the range of 21.8%–26.2% at the concentration of 20 μM, respectively.

Table 2. TMV Infection inhibition activities of compounds **1**-**6**

Compounds	Inhibition rate (%)	IC_{50} (μM)	Compounds	Inhibition rate (%)	IC_{50} (μM)
1	38.5 ± 3.4	29.8	**5**	25.4 ± 3.5	63.4
2	26.2 ± 3.0	60.5	**6**	21.8 ± 2.8	76.3
3	22.3 ± 2.8	74.6	ningnanmycin	31.2 ± 3.2	36.8
4	23.3 ± 3.1	68.7			

All results are expressed as mean ± SD; n = 3 for all groups.

Since certain of the isoquinoline alkaloids exhibit potential cytotoxicity.[24-26] The cytotoxicities of compounds **1-6** were also tested using a previously reported procedure.[27,28] The cytotoxic abilities against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) by MTT-assay were summarized in **Table 3**. The results revealed that compounds **1-6** showed moderate-to-weak inhibitory activities against some tested human tumor cell lines with IC_{50} values in the range of 2.5–7.5 μM.

Table 3. Cytotoxic activity of compounds **1-6**

Compounds	Cell lines and IC_{50} (μM)				
	NB4	A549	SHSY5Y	PC3	MCF7
1	2.5	3.8	4.2	>10	6.4
2	3.6	>10	4.8	3.9	3.0
3	>10	5.2	>10	6.1	7.5
4	3.3	4.8	>10	6.1	>10
5	4.0	5.8	>10	8.4	6.5
6	5.3	5.8	6.2	>10	7.3
Taxol	0.03	0.02	0.05	0.05	0.05

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, human prostate cancer cell; MCF7, human breast adenocarcinoma cell.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard, and the chemical shifts (δ) were expressed in ppm. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant Material. The seeds of *C. alata* were collected in Zhaifang village, Dehong Prefecture of Yunnan Province, People's Republic of China, in September 2014. The identification of plant material was verified by Prof. Ning Yuan. A voucher specimen (Ynni-14-09-48) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered seeds of *C. alata* (2.6 kg) were extracted with 70% aq. acetone, and the extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 9 with saturated Na_2CO_3 aq. and extracted with EtOAc. The EtOAc-soluble alkaloidal materials (28.6 g) were applied to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$/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, 5.22 g) by silica gel column chromatography, eluted with $CHCl_3$/Me_2CO (9:1-2:1), yielded mixtures B1–B7. Fraction B1 (9:1, 0.26 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (65% MeOH/H_2O, flow rate 12 mL/min) to give **6** (12.2 mg). Fraction B2 (8:2, 0.58 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (52% MeOH/H_2O, flow rate 12 mL/min) to give **1** (10.5 mg), **2** (12.2 mg), **3** (5.2 mg), and **5** (13.8 mg). Fraction B4 (6:4, 0.65 g) was subjected to silica gel column chromatography using petroleum ether/acetone, and then semi-preparative HPLC (38% MeOH/H_2O, flow rate 12 mL/min) to give **4** (13.6 mg).

Anti-TMV Assays. The anti-TMV activity was tested using the half-leaf method,[20,21] and ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as positive control.

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control).[22,23]

6-(Hydroxymethyl)-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one (1): reddish gum, UV λ_{max} (log ε) (nm): 210 (4.26), 243 (3.85), 340 (3.86); IR (KBr) ν_{max} (cm^{-1}): 3410, 3186, 2952, 1650, 1610, 1567, 1466, 1381, 1319, 1263, 1134, 1035, 862, 728 cm^{-1}; ESI-MS *m/z*: 262 $[M+Na]^+$, HR-ESI-MS *m/z*: 262.0838 $[M+Na]^+$ (calcd 262.0844, $C_{15}H_{13}NNaO_2$).

6-(Hydroxymethyl)-8-methoxy-3,9-dimethyl-7*H*-benzo[*de*]quinolin-7-one (2): reddish gum, UV λ_{max} (log ε) (nm): 210 (4.30), 249 (3.89), 345 (3.82); IR (KBr) ν_{max} (cm^{-1}): 3414, 3180, 2950, 1655, 1610, 1562, 1460, 1382, 1323, 1258, 1137, 1041, 897, 749 cm^{-1}; ESI-MS *m/z*: 292 $[M+Na]^+$, HR-ESI-MS *m/z*: 292.0958 $[M+Na]^+$ (calcd 292.0950, $C_{16}H_{15}NNaO_3$).

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