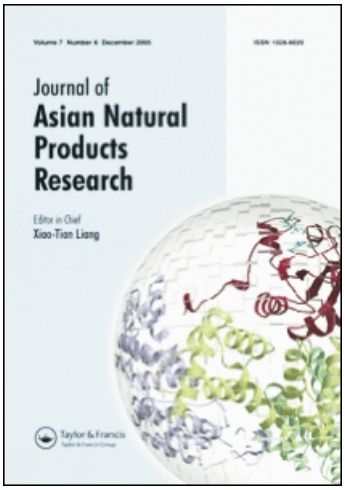

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Canthin-6-one alkaloids from *Picrasma quassioides* and their cytotoxic activity

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A new alkaloid, 4,5-dimethoxy-10-hydroxycanthin-6-one (**1**), was isolated from the stem of *Picrasma quassioides* Bennet (Simaroubaceae) together with four known canthin-6-one alkaloids, 8-hydroxycanthin-6-one (**2**), 4,5-dimethoxycanthin-6-one (**3**), 5-hydroxy-4-methoxycanthin-6-one (**4**), and 3-methylcanthin-5,6-dione (**5**). Their structures were elucidated on the basis of spectroscopic data. The cytotoxic activity of the canthin-6-one alkaloids was evaluated using human nasopharyngeal carcinoma (CNE2) and human liver cancer (Bel-7402) cell lines. Among these isolates, compounds **1**–**4** exhibited significant cytotoxic activity against CNE2 cell line.

Keywords: *Picrasma quassioides*; Simaroubaceae; canthin-6-one alkaloids; cytotoxic activity

1. Introduction

Picrasma quassioides Bennet (Simaroubaceae) is a small arbor mainly distributed in the tropical and subtropical zones. In Chinese traditional medicine, the stem has been used as a bitter stomachic, as well as an antimalarial, antitumor, and hypotensive agent. The present study deals with the isolation and structural elucidation of five alkaloids (**1**–**5**), including a new alkaloid (**1**) and an alkaloid (**2**) isolated from *Picrasma* genus for the first time together with the known canthin-6-one alkaloids (**3**–**5**) from the stems of this plant. The study by P.C. Kuo [1] showed that 9-methoxycanthin-6-one and canthin-6-one demonstrated significant cytotoxic activity against human lung cancer (A-549) and human breast cancer (MCF-7) cell lines. Another study by Fukamiya [2] showed that some canthin-6-one alkaloids, which have hydroxyl and(or) methoxyl substitutions at C-10 or C-11, have significant cytotoxic activities. To evaluate the cytotoxic activity of the canthin-6-one alkaloids, the cytotoxic properties of the isolated constituents have been studied. Among these isolates, compounds **1**–**4**, which have hydroxyl and (or) methoxyl, exhibited significant cytotoxic activity against CNE2 cell line.

2. Results and discussion

The 95% EtOH extract of the stems of *P. quassioides* was suspended in water and extracted with CH_2Cl_2, EtOAc and *n*-BuOH, successively. The CH_2Cl_2 and EtOAc extracts were then repeatedly subjected to silica gel, lipophilic Sephadex, and polyamide column chromatography to afford five compounds (**1**–**5**). Their structures (Figure 1) were identified as 4,5-dimethoxy-10-hydroxycanthin-6-one (**1**), 8-hydroxycanthin-6-one (**2**), 4,5-dimethoxycanthin-6-one (**3**), 5-hydroxy-4-methoxycanthin-6-one (**4**), and

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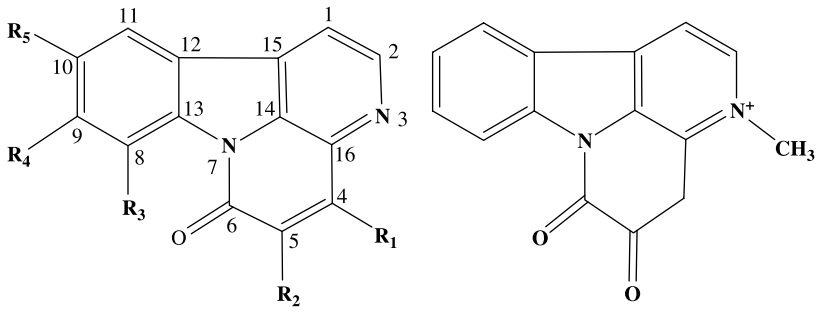

	R_1	R_2	R_3	R_4	R_5
1	OCH_3	OCH_3	H	H	OH
2	H	H	OH	H	H
3	OCH_3	OCH_3	H	H	H
4	OCH_3	OH	H	H	H
6	OH	OCH_3	H	H	OH
7	H	H	H	OH	H

5

Figure 1. The structures of compounds **1**–**7**.

3-methylcanthin-5,6-dione (**5**) by analysis of both physical and spectroscopic data.

Compound **1**, yellow amorphous powder, showed a positive Dragendorff test, and its molecular formula was determined as $C_{16}H_{12}O_4N_2$ by HREIMS at *m/z* 296.0786 [M^+] and ^{1}H-, ^{13}C-NMR spectra. The UV spectrum with absorption maxima at 214 (4.42), 243 (4.32), 280 (4.26), 355 (4.01), 364 (3.99), and 370 (3.99) nm was similar to those of compounds **2**–**4**, which suggested that **1** has a canthin-6-one chromophore [3,6]. The ^{1}H-NMR spectrum of **1** showed a pair of *ortho*-coupled signals at δ 8.19 (1H, d, $J = 5.2$ Hz) and 8.76 (1H, d, $J = 5.2$ Hz), and ABX type signals at δ 7.15 (1H, dd, $J = 8.8$, 2.4 Hz), 7.63 (1H, d, $J = 2.4$ Hz), and 8.25 (1H, d, $J = 8.8$ Hz) corresponding to the three aromatic protons of the monosubstituted indole moiety. Comparison of MS and ^{1}H-NMR spectra of **1** with those of 4,5-dimethoxycanthin-6-one (**3**) [3] showed that **1** had a similar structure to **3**, except for the presence of an extra hydroxyl group in **1**.

The splitting pattern in the ^{1}H-NMR spectrum for the three aromatic protons of the indole part of **1** clearly revealed that the remaining hydroxyl group was located at either C-9 or C-10 position. By comparison with the reported ^{1}H- and ^{13}C-NMR assignments of 4,10-dihydroxy-5-methoxycanthin-6-one (**6**) [4] and 9-hydroxycanthin-6-one (**7**) [5] led us to favor the C-10 position; and this was confirmed by the HMBC correlations from H-8 to C-10 and C-12. From the above results, the remaining hydroxyl group was concluded to be located at C-10, and the structure of compound **1** was determined to be 4,5-dimethoxy-10-hydroxycanthin-6-one.

The known constituents (**2**–**5**) were identified by comparison of their spectral data (^{1}H and ^{13}C NMR and MS) with those reported in the literature [3,6,7].

The cytotoxic activities of the canthin-6-one alkaloids were tested against two human cancer cell lines. It was evident from Table 1 that canthin-6-one alkaloids reduced cell

Table 1. Cytotoxic activities of canthin-6-one alkaloids *in vitro* ($n = 4$, $p < 0.05$).

	IC_{50} (μg/ml)	
Test compound	CNE2	Bel-7402
1	11.6 ± 2.48	118.91 ± 67.42
2	13.43 ± 2.29	39.27 ± 9.72
3	9.86 ± 1.49	32.27 ± 9.74
4	7.96 ± 0.79	116.13 ± 47.56
5	23.72 ± 4.84	60.42 ± 17.51

growth in cultured CNE2 cell in a concentration-dependent manner and compounds **1**–**4**, which have hydroxyl and(or) methoxyl, demonstrated significant cytotoxic activity against CNE2 cell line, but the cytotoxic activities of the isolates against human liver cancer Bel-7402 cell line were not strong.

3. Experimental

3.1 General experimental procedures

Melting point was measured in a Buchi 540 instrument and is uncorrected. UV spectrum was obtained with a Shimadzu UV–visible recording spectrophotometer. IR spectra were recorded with a Nicolet Avatar 360 FT-IR spectrophotometer. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ using a Varian INOVA-400FT spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan LCQ-Advantage. For column chromatography, silica gel (Qingdao Marine Chemical Factory, Qingdao, China), polyamide (Taizhou Luqiao Sijia biochemistry plastics plant, Taizhou, China) and lipophilic Sephadex (Sigma, Stockholm, Sweden) were used.

3.2 Plant material

The stems of *P. quassioides* were collected in Guangdong province and identified by Prof. Jing-ping Li, Department of Pharmacognosy, School of Pharmaceutical Science, Central South University. A voucher specimen (060712) is kept in the Herbarium of School of Pharmaceutical Science, Central South University, China.

3.3 Extraction and isolation

The dried stem of *P. quassioides* was extracted with 95% EtOH. After removal of the solvent by evaporation, the residue (280 g) was extracted with CH_2Cl_2, EtOAc, and *n*-BuOH, successively. The CH_2Cl_2 extract (66 g) was subjected to a column of silica gel and eluted with CH_2Cl_2 : MeOH (100:0, 98:2, 95:5, 9:1, 4:1, 1:1, and 0:100) to afford seven fractions. Fraction 3 (10 g) was subjected to a column of silica gel and eluted with CH_2Cl_2 : MeOH (98:2, 95:5, and 90:10) to afford fractions 13–15 (270 mg). Then, fractions 13–15 was subjected to a column of polyamide and eluted with CH_2Cl_2 : MeOH (98:2 and 95:5) to afford fractions 6–13 (57 mg). At the end, fractions 6–13 was subjected to a column of lipophilic Sephadex and eluted with CH_2Cl_2 : MeOH (98:2) to afford compounds **1** (18 mg) and **2** (10 mg). Fraction 1 (9 g) was subjected to a column of silica gel and eluted with PE : EtOAc (20:1, 10:1, 5:1, 3:1, 1:1, and 0:1) to afford compound **3** (45 mg). Fraction 2 (8.3 g) was subjected to a column of silica gel and eluted with CH_2Cl_2:MeOH (100:0, 98:2, 95:5, and 0:100) to afford compound **4** (2.1 g). Fraction

Table 2. ^{1}H-NMR spectral data of compounds **1**, **6**, and **7** in DMSO-d_6.

Position	1	6	7
H-1	8.19 (d, 5.2)	8.21 (d, 5.2)	8.14 (d, 5.0)
H-2	8.76 (d, 5.2)	8.78 (d, 5.2)	8.76 (d, 5.0)
H-4			8.10 (d, 10)
H-5			6.96 (d, 10)
H-8	8.25 (d, 8.8)	8.29 (d, 8.8)	8.00 (d, 2.5)
H-9	7.15 (dd, 8.8, 2.4)	7.17 (dd, 8.8, 2.4)	
H-10			7.00 (dd, 9, 2.5)
H-11	7.63 (d, 2.4)	7.65 (d, 2.5)	8.17 (d, 9)
OMe-4	4.34 (s)		
OMe-5	3.95 (s)	3.89 (s)	

Table 3. ^{13}C-NMR spectral data of compounds **1**, **6**, and **7** in DMSO-d_6.

Position	1	6	7
C-1	117	116.3	116
C-2	145.4	144.2	146
C-4	153	151.7	140
C-5	141.5	135.6	128
C-6	157.6	157	159
C-8	117.3	116.4	103
C-9	119.1	118.4	160.5
C-10	156	155	114
C-11	109.6	108.8	124.7
C-12	126.4	125.5	115.6
C-13	132.2	131.9	140.5
C-14	130	129.1	129.9
C-15	128.8	128.3	131.7
C-16	133.6	131.9	135
OMe-4	62.1		
OMe-5	61.3	59.7	

4 (4.2 g) was subjected to a column of silica gel and eluted with CH_2Cl_2 : MeOH (98:2, 95:5, and 90:10) to afford compound **5** (500 mg).

*3.3.1 Compound **1***

Yellow amorphous powder (MeOH), mp 227–229°C. IR (KBr) ν_{max} cm^{-1}: 3414, 3147, 2926, 1663, 1591, 1574, 1463, 1441, 1386, 1209, 1141, and 1085. UV (MeOH) λ_{max} nm (log ε): 214 (4.42), 243 (4.32), 280 (4.26), 355 (4.01), 364 (3.99), and 370 (3.99). For ^{1}H and ^{13}C NMR spectral data see Tables 2 and 3, respectively. HREIMS: *m/z* 296.0786 $[M]^+$ (calculated for $C_{16}H_{12}O_4N_2$, 296.0792).

3.4 Cytotoxic studies

CNE2 and Bel-7402 cell lines were obtained from XiangYa Central Experiment Laboratory and were cultured at 37°C with 5% CO_2, using RPMI-1640 media containing 10% new-born calf serum. MTT assay was used to study the cytotoxic properties of the evaluated samples. The assay was performed *in vitro* using the method described in cell culture [8]. The cells (5×10^3) were seeded in each well plate. After incubation for 4 h, the test compound with different concentrations (in μg/ml) were added to respective wells and after further 48 h, 20 μl of MTT (5 mg/ml stock solution, Sigma) was added to each well. The medium was discarded after 4 h and the formazan blue, which formed in the cells, were dissolved with 100 μl of DMSO. The rate of color production was measured at 490 nm in a spectrophotometer (ELx 800_{NB} Universal Microplate supported by BIO-TEK Instruments Inc., Winooski, VT, USA). All experiments were conducted under the standard laboratory illumination. The percentage of inhibition of cell viability was computed with reference to the MTT reduction in control (without test compound). The experimental measurements were made in four replicates each and the average value was taken as percentage inhibition. The IC_{50} values were calculated based on the bliss method.

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