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Cytotoxic Isoflavanones from *Uraria clarkei*

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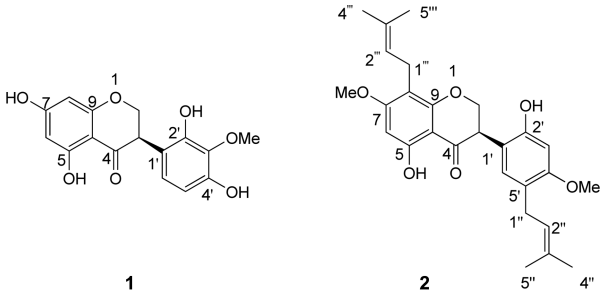
Two new isoflavanones, (3*R*) 5,7,3',4'-tetrahydroxy-2'-methoxyisoflavanone (**1**) and (3*R*) 5',8-di-(γ,γ-dimethylallyl)-2',5-dihydroxyl-4',7-dimethoxyl-isoflavanone (**2**), were isolated from *Uraria clarkei*, together with two known compounds dalbergioidin (**3**), 5,7-dihydroxy-2',4'-dimethoxyisoflavanone (**4**). The structures involving the absolute configuration of the new compounds were well elucidated by MS, IR, UV, CD, 1D and 2D NMR analyses. Cytotoxicity of the four compounds were assessed, results suggested that compound **2** possessed well cytotoxic activity, against the Hela, K562, and HL60 cell lines with IC_{50} values of 28.0, 40.6 and 35.1 μM, respectively.

Key Words : Isoflavanone, *Uraria clarkei*, Leguminosae, Cytotoxicity

Introduction

Uraria clarkei, belonging to the Leguminosae family, was mainly distributed in south-west of China, India, and Vietnam.[1] In China, the *U. clarkei* was folkly used for the treatment of postpartum hypogalactia, hemoptysis, and venomous snake bite.[2] During the course of our searching for antitumor active chemicals from natural source, the ethanol extracts of the *U. clarkei* was found to possess cytotoxic activity. To the best of our knowledge, there was no phytochemical investigation on this plant. With the aim of finding the constituents in operation, the *U. clarkei* was investigated and two new isoflavanones, named (3*R*) 5,7,3',4'-tetrahydroxy-2'-methoxyisoflavanone (**1**) and (3*R*) 5',8-di-(γ,γ-dimethylallyl)-2',5-dihydroxyl-4',7-dimethoxyl-isoflavanone (**2**) (Figure 1) were isolated from the petroleum ether extract, together with two known compounds dalbergioidin (**3**), 5,7-dihydroxy-2',4'-dimethoxyisoflavanone (**4**). Among them, compound **2** showed good activity when tested *in vitro* against Hela, K-562 and HL-60 cell lines using the microculture tetrazolium method (MTT) method. Herein we reported the isolation, structural elucidation and cytotoxicity of the above isolates.

Compound **1** was obtained as pale yellow powder. The HR-ESI-MS showed the quasi-molecular ion peak at *m/z* 319.0724 $[M+H]^+$ (calc. for $C_{16}H_{15}O_7^+$ 319.0743), in accordance with the molecular formula $C_{16}H_{14}O_7$. The IR spectrum displayed the absorptions at 3370, 1646, 1603, 1507, 1451 cm^{-1}, suggesting the existence of hydroxy, carbonyl and aromatic ring functions in the molecule, respectively. The ^{1}H-NMR spectrum revealed the presence of a pair of *ortho*-coupled aromatic protons at δ 6.57 (1H, *d*, *J* = 8.4 Hz), 6.49 (1H, *d*, *J* = 8.4 Hz), a pair of *meta*-coupled aromatic protons δ 5.92 (1H, *d*, *J* = 2.0 Hz), 5.90 (1H, *d*, *J* = 2.0 Hz), and one methoxyl signal at δ 3.80 (3H, *s*). The proton signals at δ 4.47 (1H, *t*, *J* = 11.2 Hz, H-2a), 4.37 (1H, *dd*, *J* = 11.2, 6.4 Hz, H-2b), 4.17 (1H, *dd*, *J* = 11.2, 6.4 Hz, H-3) in the ^{1}H-NMR spectrum (Table 1), combined with the carbon signals at δ 199.3 (C-4), 72.1 (C-2), 48.1 (C-3) in the ^{13}C-NMR (Table 1), implied compound **1** possessed an isoflavanone skeleton. Comparing the NMR data with those of secundiflorol H[3] and **4** suggested that there were two hydroxyl groups linked at the C-5 and C-7 in compound **1**. The main difference between compound **1** and secundiflorol H[3] was the replacement mode at ring B. The HMBC correlations (Figure 2) between H-3 (δ 4.17) and C-1' (δ 120.8), C-6' (δ 121.1), and C-2' (δ 148.2), δ 3.80 (3H, s, MeO) and C-3' (δ 148.2), H-5' (δ 6.57 (1H, *d*, *J* = 8.4 Hz)) and C-3' (δ 148.2), C-4' (δ 139.7), C-1' (δ 120.8), H-6' (δ 6.48 (1H, *d*, *J* = 8.4 Hz)) and C-3 (δ 48.1), C-2' (δ 147.8), C-4' (δ 139.7) established the substitution of 2',4'-dihydroxy-3'-methoxyl at ring B. To elaborated the absolute configuration of C-3, a CD curve was recorded. The positive Cotton effect at 345 nm due to the *n*-π* carbonyl transition and the equatorial position of ring B suggested the (3*R*) absolute configuration according to the literature[4] and was supported by the octant rule modified for the cyclic arylketones.[5] Thus, compound **1** was determined to be (3*R*) 5,7,2',4'-tetrahydroxyl-3'-methoxyl-isoflavanone.



1 **2**

Figure 1. The structures of compounds **1** and **2**.

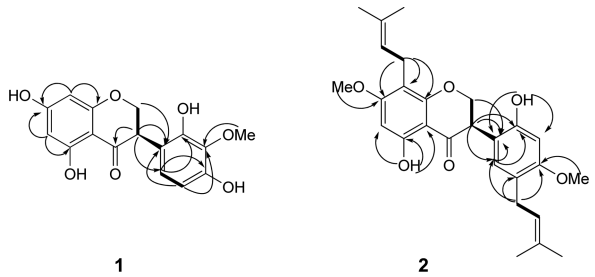
Compound **2** was obtained as pale yellow powder. The

Table 1. ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) data of compounds **1** and **2**. δ in ppm, *J* in Hz

position	**1**[a]		**2**[b]	
	δ_H	δ_C (mult.)	δ_H	δ_C (mult.)
2	4.47 (1H, dd, 11.2, 11.2) 4.37 (1H, dd, 11.2, 6.4)	72.1 (t)	4.85 (1H, dd, 11.6, 11.2) 4.69 (1H, dd, 11.6, 4.8)	69.5 (t)
3	4.17 (1H, dd, 11.2, 6.4)	48.1 (s)	3.93 (1H, dd,11.2, 4.8)	45.0 (s)
4	-	199.3 (s)	-	197.0 (s)
5	-	165.8 (s)	-	161.5 (s)
6	5.90 (d, 2.0)	97.2 (d)	6.04 (1H, s)	90.8 (d)
7	-	168.2 (s)	-	166.4 (s)
8	5.92 (d, 2.0)	96.1 (d)	-	110.3 (s)
9	-	165.2 (s)	-	160.9 (s)
10	-	103.8 (s)	-	101.6 (s)
1'	-	120.8 (s)	-	113.7 (s)
2'	-	148.2 (s)	-	154.2 (s)
3'	-	147.8 (s)	6.48 (1H, s)	101.0 (d)
4'	-	139.7 (s)	-	158.1 (s)
5'	6.57 (1H, d, 8.4)	111.9 (d)	-	122.8 (s)
6'	6.49 (1H, d, 8.4)	121.1 (d)	7.18 (1H, s)	127.6 (d)
1"	-	-	3.19 (2H, br d, 7.6)	27.9 (t)
2"	-	-	5.21-5.26 (1H, m)	122.8 (d)
3"	-	-	-	132.2 (s)
4"	-	-	1.68 (3H, br s)	25.8 (q)
5"	-	-	1.75 (3H, br s)	17.8 (q)
1"'	-	-	3.21 (2H, br d, 8.0)	21.00 (t)
2"'	-	-	5.12-5.16 (1H, m)	122.1 (d)
3"'	-	-	-	131.8 (s)
4"'	-	-	1.65 (3H, br s)	25.8 (q)
5"'	-	-	1.71 (3H, br s)	17.7 (q)
MeO-3'	3.80 (3H, s)	60.8 (q)	-	-
MeO-4'	-	-	3.76 (3H, s)	55.4 (q)
MeO-7	-	-	3.86 (3H, s)	55.9 (q)
OH-2'	-	-	7.70 (br s)	-
OH-5	-	-	11.73 (br s)	-

[a]measured in CD_3OD. [b]measured in $CDCl_3$.

molecular formula was determined to be $C_{27}H_{32}O_6$ by the positive HR-ESI-MS at *m/z* 453.2158 ($[M+H]^+$, calc. for $C_{27}H_{33}O_6$ 453.2242). Its IR spectrum displayed the presence of hydroxy (3360 cm^{-1}), carbonyl (1640 cm^{-1}), and aromatic ring (1601, 1507, 1453 cm^{-1}) functions. In the ^{1}H-NMR spectrum (Table 1), three singlet aromatic proton signals at δ 6.04 (1H, *s*, H-6), 6.48 (1H, *s*, H-3'), 7.18 (1H, *s*, H-6'), two γ,γ-dimethylallyl chains at δ 1.65 (3H, *br s*, H-4"'), 1.68 (3H, *br s*, H-4"), 1.71 (3H, *br s*, H-5"'), 1.75 (3H, *br s*, H-5"), 3.19 (2H, *br d*, *J* = 7.6 Hz, H-1"), 3.21 (2H, *br d*, *J* = 8.0 Hz, H-1"'), 5.12-5.16 (1H, *m*, H-2"'), 5.21-5.26 (1H, *m*, H-2") were exhibited, together with two methoxyl groups at δ 3.86 (3H, *s*), 3.76 (3H, *s*). Compound **2** was also deduced to be an isoflavanone by the characteristic signals at δ 4.85 (1H, *dd*, 11.6, 11.2 Hz, H-2a), 4.69 (1H, *dd*, 11.6, 4.8 Hz, H-2b), 3.93 (1H, *dd*, 11.2, 4.8 Hz, H-3) in the ^{1}H-NMR spectrum (Table 1), and the δ 197.0 (C-4), 69.5 (C-2), 45.0 (C-3) in the ^{13}C-NMR (Table 1). The HMBC spectrum (Fig. 2) showed the long range correlations of δ 3.86 (3H, s, MeO) with δ 166.4 (C-7), δ 3.76 (3H, s, MeO) with δ 158.1 (C-4'), δ 3.19 (2H, H-1") with δ 158.1 (C-4'), 122.8 (C-5'), and 127.6 (C-6'), δ 3.21 (2H, H-1"') with δ 166.4 (C-7), 110.3 (C-8), and 160.9 (C-9), as well as the δ 7.18 (1H, s, H-6') with δ 27.9 (C-1"), suggested the two methoxyl groups should be linked at C-7 and C-4', and the two γ,γ-dimethylallyl chains be linked at C-5' and C-8. The correlations between the two hydroxyls and the carbons in HMBC (Figure 2) allowed the assignments of 2',5-dihydroxyl substitution in compound **2**. The



1 **2**

Figure 2. Selected key HMBC (H→C) and COSY (H—H) correlations of compounds **1** and **2**.

Table 2. The cytotoxic activities of compounds **1**-**4**

Compounds	IC_{50} (μM)		
	Hela	K562	HL60
95% ethanol extract (μg/mL)	21.7 ± 4.3	24.3 ± 5.8	26.0 ± 6.1
1	71.4 ± 9.8	79.5 ± 11.7	90.9 ± 16.2
2	28.0 ± 6.6	40.6 ± 8.7	35.1 ± 6.1
3	113.5 ± 12.6	133.3 ± 15.7	128.1 ± 12.9
4	119.9 ± 13.6	105.7 ± 11.6	129.7 ± 10.7
Cis-platinum	7.3	9.5	11.5

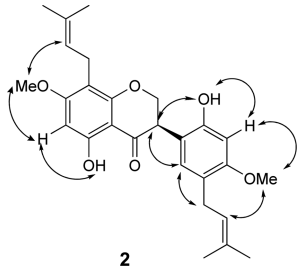


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Figure 3. Selected ROESY (H↔H) correlations of compound **2**.

above deduction were further verified by the ROESY correlations depicted in Figure 3. The positive Cotton effect at 346 nm for $n \sim \pi^*$ carbonyl transition in the CD established the (3*R*) absolute configuration.[3-5] Consequently, compound **2** was elucidated as (3*R*) 5,8-di-(γ,γ-dimethyl-allyl)-2',5-dihydroxyl-4',7-dimethoxyl-isoflavanone.

The known compounds dalbergioidin (**3**)[6], 5,7-dihydroxy-2',4'-dimethoxyisoflavanone (**4**),[7] isolated from this plant for the first time, were identified by comparison of their spectroscopic data with those literatures.

Compounds **1**-**4** were assayed for their cytotoxicity in Hela, K562 and HL60 cell lines. Results were summarized in Table 2. It was concluded that compound **2** possessed good activity against the tested Hela, K562, and HL60 cell lines, with IC_{50} values of 28.0, 40.6 and 35.1 μM, respectively. The other three compounds exhibited slight or moderate activities. *Cis*-platinum, an approved agent for the clinical *anti*-tumor treatment, was used as a positive control.

Experimental

General Experiment Procedures. Optical rotations were determined on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-210A spectrophotometer. CD curves were measured on Jasco J-720 spectrometer. IR Spectra were obtained on a Bio-Rad FTS-135 spectrometer. 1D and 2D NMR spectra were tested by Bruker AM-400 NMR, with TMS as internal standard. Ms were recorded on VG Auto Spec-3000 spectrometer. Column chromatography (CC): Silica gel (SiO_2; 200-300 mesh; Qingdao Meigao Chemical Company, Qingdao, China), Sephadex LH-20 (20-150 μm; Pharmacia Fine Chemical Co. Ltd., Sweden).

Planta Material. The aerial part of *Uraria clarkei* were collected in Simao, Yunnan province, P.R. China, in August 2009, and identified by Dr. Jingmei Lu from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (2009-08-03) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities.

Extraction and Isolation. The dried and powdered aerial part of *U. clarkei* (8 kg) was extracted with 95% EtOH (70 L) under reflux for three times. The extract was concentrated *in vacuo*, and then partitioned between water, petroleum ether, EtOAc and *n*-BuOH, respectively, to provide a petroleum ether fraction (100 g), EtOAc (80 g) and *n*-BuOH fraction (200 g). The petroleum ether fraction was fractionated by silica gel column chromatography (CC) with gradient elution (petroleum ether/EtOAc 30:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1 (v/v)) to yield 7 fractions (Frs. 1-7). Fr. 5 (5 g) was subjected to a silica gel CC (200 g) and eluted with $CHCl_3$/MeOH (98:2, 95:5) to afford 4 fractions (Frs. 5a-5d). Fr. 5b (500 mg) was further purified by silica gel CC (80 g, $CHCl_3$/Me_2CO, 80:20) to give compound **1** (116 mg). Fr. 5c (360 mg) was performed on a silica gel CC (50 g, $CHCl_3$/Me_2CO, 70:30), followed by a sephadex LH-20 CC ($CHCl_3$/MeOH 1:1) to obtain compound **3** (120 mg). Fr.4 (7 g) was repeated subjected to CC (SiO_2, $CHCl_3$/MeOH 98:2, 95:5), further separated by sephadex LH-20 to yield compounds **2** (138 mg) and **4** (175 mg).

Compound 1: Pale yellow powder. $[\alpha]_D^{20.5}$ –38.0 (c = 0.359, MeOH). UV: λ_{max}^{MeOH} (log ε) 289 (2.93). CD (c = 0.103, MeOH) λ_{max} (nm; Δε): 255 (+0.491), 345 (+0.210). IR (KBr) cm^{-1}: 3370, 1646, 1603, 1507, 1451, 1263, 1171, 1050. ^{1}H- and ^{13}C-NMR data, see Table 1. ESI-MS (pos.): 319 ($[M+H]^+$). HR-ESI-MS (pos.): 319.0724 ($[M+H]^+$, $C_{16}H_{15}O_7^+$; calc. 319.0743).

Compound 2: Pale yellow powder. $[\alpha]_D^{21.8}$ = –27.3 (c = 0.225, MeOH). UV: λ_{max}^{MeOH} (log ε) 291 (2.96). CD (c = 0.101, MeOH) λ_{max} (nm; Ää): 258.5 (+0.435), 346 (+0.059). IR(KBr) cm^{-1}: 3360, 1640, 1601, 1507, 1453, 1274, 1108, 1010. ^{1}H- and ^{13}C-NMR data, see Table 1. ESI-MS (pos.): 453 ($[M+H]^+$). HR-ESI-MS: 453.2158 ($[M+H]^+$, $C_{27}H_{33}O_6^+$; calc. 453.2242).

Cytotoxicitic Assay. Cytotoxic activities were evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method using the Hela, K562 and HL60 cell lines. Briefly, the cell suspensions (200 mL) at a density of 5×10^4 cells mL^{-1} were plated in 96 well microtiter plates and incubated for 24 h at 37 ºC in a humidified incubator at 5% CO_2. The tested compound solution (2 mL in DMSO) at different concentrations was added to each well and further incubated for 72 h under the same conditions. Then, 20 mL of the MTT solution was added to each well and incubated for 4 h. The old medium (150 mL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 540 nm. Dose-response curves were generated and the IC_{50} values were defined as the concentration of compound required to inhibit cell proliferation by 50%. *Cis*-platinum, an approved agent for the

treatment of many tumors, was used as the positive control.

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