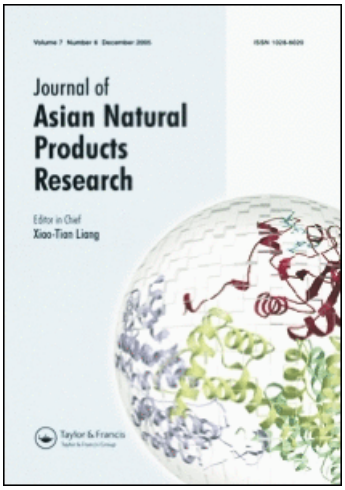

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A new cytotoxic homoisoflavonoid from *Dracaena cambodiana*

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A new homoisoflavonoid, named cambodianol (**1**), together with the two known flavanes, (2*S*)-7,3′-dihydroxy-4′-methoxy-8-methylflavane (**2**) and (2*R*)-7,4′-dihydroxy-8-methylflavane (**3**), were isolated from the stems of *Dracaena cambodiana*. Their structures were determined based on HR-ESI-MS and spectroscopic techniques (UV, IR, 1D-, and 2D-NMR). Compound **1** exhibited significant cytotoxic activities against K562 and SGC-7901 with the IC_{50} values of 1.4 and 2.9 μg/ml, respectively.

Keywords: *Dracaena cambodiana*; Agavaceae; homoisoflavonoid; cytotoxicity; cambodianol

1. Introduction

Dragon's blood is a deep red resin, which has been used as a famous traditional medicine since ancient times by many cultures. It has several therapeutic uses due to its hemostatic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory, antioxidant activities, etc. [1]. *Dracaena cambodiana* Pierre ex Gagnep (Agavaceae), known as one of the dragon's blood trees, is endemic to the Hainan Island in China [2]. Phytochemical studies on the plants of the genus *Dracaena* have previously led to the isolation of a number of phenolic compounds and a series of steroidal saponins [3], while only four steroidal saponins have been isolated from the fruits of *D. cambodiana* [4]. In an effort to search for new antitumor compounds from tropical medicinal plants in Hainan Province of China, the ethanol extract from the stems of *D. cambodiana* showed inhibitory activity against K562 and SGC-7901 cell lines. Bioassay-guided fractionation of the ethanol extract led to the isolation of a new homoisoflavonoid, named cambodianol (**1**), and the two known flavanes (2*S*)-7,3′-dihydroxy-4′-methoxy-8-methylflavane (**2**) and (2*R*)-7,4′-dihydroxy-8-methylflavane (**3**) (Figure 1). In this paper, we describe the isolation and identification of compounds **1**–**3**, as well as their cytotoxic activities against the K562 and SGC-7901 cell lines.

2. Results and discussion

Compound **1**, obtained as white amorphous powder, has a molecular formula $C_{18}H_{18}O_6$ based on its HR-ESI-MS at *m/z* 353.0994 $[M+Na]^+$, which was supported by the ^{13}C NMR and DEPT spectral data. A characteristic homoisoflavonoid ion fragment at *m/z* 121 revealed the presence of a methoxybenzyl group. The IR spectrum showed absorption bands for OH groups (3399 and 3269 cm^{-1}), aromatic ring (1620, 1509, and 1461 cm^{-1}), and a carbonyl group (1641 cm^{-1}). Four proton signals in the ^1H NMR spectrum at

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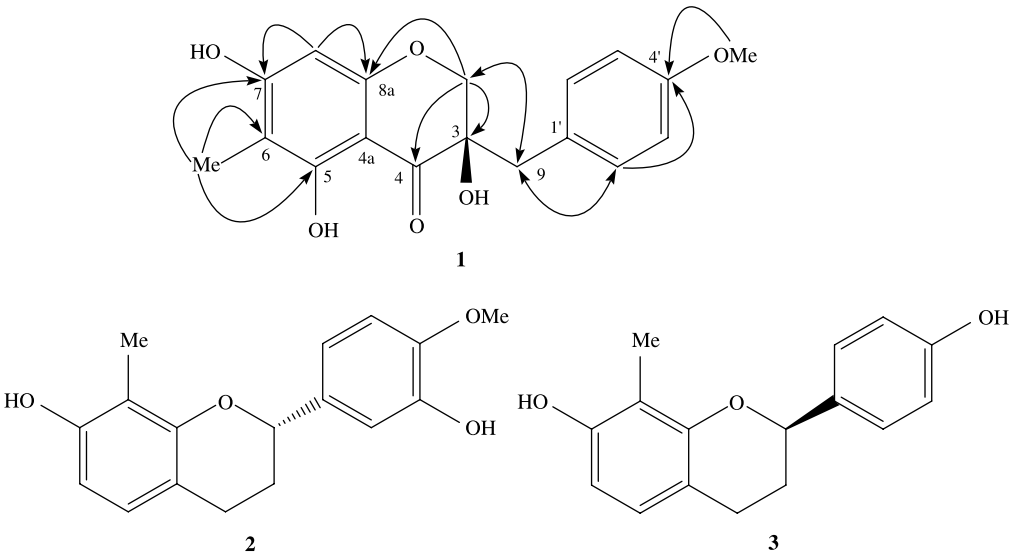

Figure 1. The structures of compounds **1**–**3** and key HMBC correlations of **1** (H to C).

δ 4.06 (1H, d, $J = 11.2$ Hz, H_a-2), 3.99 (1H, d, $J = 11.2$ Hz, H_b-2), 2.98 (1H, d, $J = 14.0$ Hz, H_a-9), and 2.92 (1H, d, $J = 14.0$ Hz, H_b-9), and four carbon signals in the ^{13}C NMR spectrum at δ 73.6 (t, C-2), 74.1 (s, C-3), 200.6 (s, C-4), and 41.6 (t, C-9) indicated the presence of 3-hydroxy-3-benzyl-4-chroma-none [5]. An AA′BB′ spin system at δ 7.20 (d, $J = 8.6$ Hz, 2H) and 6.85 (d, $J = 8.6$ Hz, 2H) in the ^{1}H NMR spectrum indicated the C-4′ in ring B was oxygenated. In addition, an aromatic proton (δ 6.06, s, 1H), a methyl signal (δ 1.98, s, 3H), and a methoxyl signal (δ 3.77, s, 3H) were observed in the ^{1}H NMR spectrum. Their locations were deduced by HMBC experiment (Figure 1). The methoxyl was connected with C-4′ on the basis of HMBC correlations between the 3H singlet (δ 3.77) and the carbon at δ 160.7 (C-4′). The other three aromatic carbons with oxygen function were observed at δ 163.6, 166.5, and 162.5 in the ^{13}C NMR spectrum, which indicated that ring A was a phloro-glucinol ring [6]. In the HMBC spectrum (Figure 1), both the aromatic proton (δ 6.06, s, 1H) and the two protons of C-2 showed correlations with the carbon at δ 162.5 (C-8a), which indicated that the aromatic proton in ring A belonged to C-8; therefore, the methyl group (δ 1.98, s, 3H) was attached to C-6. The configuration at C-3 was proposed to be *R*, similar to that of dracol, a homoisoflavan analog isolated from *D. draco* [7], based on the negative sign of its specific rotation. Thus, the structure of **1** was established as (3*R*)-3,5,7-trihydroxy-6-methyl-3-(4′-meth-oxybenzyl)-4-chroma-none, named cambo-dianol.

Along with the new homoisoflavonoid, two known flavanes, which were isolated from this plant for the first time, were identified as (2*S*)-7,3′-dihydroxy-4′-methoxy-8-methylflavane (**2**) [8,9] and (2*R*)-7,4′-dihydroxy-8-methylflavane (**3**) [10] by com-paring their 1D-NMR spectral data and optical rotation with those reported in the literature.

The cytotoxic activities of compounds **1**–**3** were evaluated against K562 and SGC-7901 cell lines by MTT method (Table 1). The new compound **1** exhibited significant cytotoxicity against the two cell lines with the IC_{50} values of 1.4 and 2.9 μg/ml, respectively. Compounds **2** and **3** showed moderate cytotoxic activities against the two cell lines.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of compound **1** (in acetone-d_6).

Position	δ_C	δ_H (J in Hz)	HMBC (H to C)
2	73.6 t	4.06 (d 11.2) 3.99 (d 11.2)	C-3, C-4, C-9, C-8a
3	74.1 s		
4	200.6 s		
5	163.6 s		
6	106.2 s		
7	166.5 s		
8	96.2 d	6.06 s	C-6, C-7, C-8a, C-4a
9	41.6 t	2.98 (d 14.0) 2.92 (d 14.0)	C-2, C-3, C-4, C-1′, C-2′, C-6′
4a	101.9 s		
8a	162.5 s		
6-Me	8.1 q	1.98 s	C-5, C-6, C-7
1′	128.9 s		
2′	133.6 d	7.20 (d 8.6)	C-9, C-4′, C-6′
3′	115.2 d	6.85 (d 8.6)	C-1′, C-4′, C-5′
4′	160.7 s		
5′	115.2 d	6.85 (d 8.6)	C-1′, C-3′, C-4′
6′	133.6 d	7.20 (d 8.6)	C-9, C-2′, C-4′
4′-OMe	56.4 q	3.77 s	C-4′
3-OH		4.75 s	C-3
5-OH		12.01 s	C-5, C-6, C-4a

3. Experimental

3.1 General experimental procedures

Melting points were obtained on a Beijing Taike X-5 stage apparatus and are uncorrected. Optical rotation was recorded using a Rudolph Autopol III polarimeter. The UV spectra were measured on a Beckman DU800 spectrometer. The IR spectra were obtained on a Nicolet 380 FT-IR instrument, as KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS spectra were measured with an API QSTAR Pulsar mass spectrometer. Column chromatography was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Plant material

The stems of *D. cambodiana* were collected in Haikou, Hainan Province, China (July 2007). The specimen was identified by Associate Prof. Zheng-Fu Dai of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 20070701) of *D. cambodiana* is deposited.

3.3 Extraction and isolation

The dried and crushed stems of *D. cambodiana* (13.3 kg) were extracted thrice with 95% EtOH at room temperature. The extract was evaporated under reduced pressure to dryness and then partitioned in succession between H_2O and petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction (150.0 g), which showed cytotoxic activities against K562 and SGC-7901 cells, was separated into 10 fractions on a silica gel column using step gradient elution of $CHCl_3$–MeOH (100:1–0:1, v/v). The bioactive fraction 2 (18.0 g) was subjected to chromatography on silica gel column with petroleum ether–acetone (7:1, v/v) as eluent, and yielded 10 subfractions. Subfraction 7 (2.2 g), subfraction 9 (1.9 g), and subfraction 10 (2.3 g) were subjected

to repeated column chromatography on silica gel with $CHCl_3$–MeOH (50:1–30:1, v/v) as eluent and Sephadex LH-20 with EtOH as eluent, yielding compounds **1** (120.2 mg), **2** (26.3 mg), and **3** (161.8 mg), respectively.

*3.3.1 (3R)-3,5,7-trihydroxy-6-methyl-3-(4′-methoxybenzyl)-4-chromanone (**1**)*

$C_{18}H_{18}O_6$, white amorphous powder; mp 168–170°C; $[\alpha]_D^{27}$ − 24.0 ($c = 0.15$, $CHCl_3$); UV (MeOH) λ_{max} (nm) (log ε): 298 (1.75); IR (KBr) ν_{max} (cm^{-1}): 3399, 3269, 2934, 2911, 1641, 1620, 1509, 1461, 1439, 1299, 1239, 1168, 1118, 1101, and 1070; ^1H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS *m/z*: 353.0994 $[M+Na]^+$ (calcd for $C_{18}H_{18}O_6$ Na, 353.1001).

*3.3.2 (2S)-7,3′-dihydroxy-4′-methoxy-8-methylflavane (**2**)*

$C_{17}H_{18}O_4$, colorless oil; $[\alpha]_D^{27}$ − 20.0 ($c = 0.52$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 286 (0.37); IR (KBr) ν_{max} (cm^{-1}): 3423, 2923, 2851, 1613, 1518, 1460, 1270, 1210, and 1088.

*3.3.3 (2R)-7,4′-dihydroxy-8-methylflavane (**3**)*

$C_{16}H_{16}O_3$, colorless crystal; mp 132–135°C; $[\alpha]_D^{27}$ + 15.0 ($c = 0.41$, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 285 (0.18); IR (KBr) ν_{max} (cm^{-1}): 3373, 2922, 2872, 1614, 1602, 1520, 1495, 1451, 1431, 1341, 1226, and 1080.

3.4 Cell cultures and in vitro cytotoxicity assay

The human myeloid leukemia cell line (K562) and human gastric cell line (SGC-7901) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology. Cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate at 37°C, 5% CO_2. The MTT assay was performed according to the method described previously in the literature [11]. The IC_{50} values are listed in Table 2.

Table 2. *In vitro* cytotoxic activity of compounds **1**–**3** against two cell lines (IC_{50}, μg/ml).

	Cell lines	
Compounds	K562	SGC-7901
1	1.4	2.9
2	5.0	49.5
3	14.9	25.3
Mitomycin C[a]	7.1	8.8

[a] Mitomycin C was used as a positive control.

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