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Two New Flavonoids from Dragon's Blood of *Dracaena cambodiana*

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Phytochemical investigation on dragon's blood of *Dracaena cambodiana* led to the discovery of two new flavonoid derivatives, cambodianin G (**1**) and cambodianin H (**2**). Their structures were elucidated on the basis of detailed spectroscopic analysis, including 1D and 2D NMR techniques and chemical methods. The two compounds were observed to exhibit antibacterial activities against *Staphylococcus aureus*, and compound **1** showed cytotoxicities against K562 and SGC-7901 cell lines.

Key Words : Dragon's blood, *Dracaena cambodiana*, Flavonoids, Cytotoxicity, Antibacterial activity

Introduction

Dragon's blood, known as a famous traditional medicine originated in the ancient Arabian area, has been used for the treatment of wounds, leucorrhea, fractures, diarrhea and piles as well as for intestinal and stomach ulcers for a long time.[1] Dragon's blood is the red resin excreted by a part of the genus *Dracaena* plants when encounter external injury or microbial invasion.[2] In China, *Dracaena cochinchinensis* (Lour.) and *Dracaena cambodiana* have been reported as the plants resource of dragon's blood.[3] Chemical studies revealed that the plants and dragon's blood of the genus *Dracaena* contains a wide diversity of flavonoids and steroids, which were reported to exhibit antibacterial, antioxidative, cytotoxic, antiestrogenic, thrombin inhibitory activities, and antiproliferative effects.[4-13] In this paper, two new flavonoids, cambodianin G (**1**) and cambodianin H (**2**) were obtained from dragon's blood of *D. cambodiana*. In addition, both compounds were assayed for their antibacterial activities against *Staphylococcus aureus*, as well as cytotoxicities against human chronic myelogenous leukemia cell line (K562) and human gastric cancer cell line (SGC-7901).

Experimental

General Experimental Procedures. Optical rotations were recorded using a Rudolph Autopol 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-500 spectrometer, using TMS as an internal standard. The HRESIMS spectra were measured with an API QSTAR Pulsar mass spectrometer. Column chromatography (CC) was performed over silica gel (Qingdao Marine Chemical Industry, Qingdao, China, 200-300 mesh, 10-40 μm), RP-18 (Fuji Silysia Chemical Ltd, United States, 20-45 μm), and Sephadex LH-20 (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed with silica gel GF254 (Marine Chemical Industry Factory, China) and RP-18 F254 (0.25 mm, Merck). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Materials. The dragon's blood of *D. cambodiana* was collected in Haikou, Hainan Province, China, in July, 2009, and a voucher specimen is deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

Extraction and Isolation. The air-dried and powered dragon's blood of *D. cambodiana* (3.0 kg) was extracted with 95% EtOH three times at room temperature. The ethanol extract was then filtered through absorbent gauze, and the filtrate was concentrated under reduced pressure to remove ethanol. The residue was suspended in H_2O and extracted with petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc fraction (500 g), which showed potent cytotoxic activity and antibacterial activity, was subjected to over silica gel CC (100 × 40 cm, 3.5 kg) eluted with increasing polarities of a mixture of chloroform and methanol (100:1–0:1, each 4 L) to yield 10 fractions (Fr.1–10). Fr.6 (55.6 g) was further separated on a silica gel CC (7.5 × 40 cm, 250 g) under reduced pressure, and eluted with a gradient solvent system of $CHCl_3$-MeOH (100:1–0:1) to yield ten fractions (Fr.6-1–10). Fr.6-6 (4.6 g) was subjected to vacuum liquid chromatography (VLC) over silica gel, using a step gradient elution of petroleum ether-ethyl acetate (1:0–0:1, v/v) followed with reverse phase RP-18 column chromatography eluting with MeOH-H_2O (2:1, v/v) to give compound **1** (3.0 mg). Fr.6-7 (5.6 g) was chromatographed on a silica gel column using a step gradient elution of petroleum ether-ethyl acetate (10:1–2:1, v/v), and followed by Sephadex LH-20 to yield compound **2** (5.0 mg).

Cambodianin G (1): Red amorphous powder (MeOH); $[\alpha]_D^{27}$ –60 (*c* = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.81), 281 (1.98) nm; IR (KBr) 3015, 2934, 1740, 1646,

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **2** (δ in ppm , *J* in Hz)

No.	**1**[a]		**2**[b]	
	δ_C	δ_H	δ_C	δ_H
2	83.1 (d)	4.70 (d, 8.0)	83.2 (d)	4.64 (d, 8.1)
3	69.1 (d)	4.03 (m)	68.4 (d)	4.01 (m)
4	33.8 (t)	2.90 (m), 2.74 (m)	29.6 (t)	3.02 (m), 2.70 (m)
4a	113.2 (s)	–	108.3 (s)	–
5	127.7 (d)	6.62 (s)	155.1 (s)	–
6	115.4 (s)	–	108.5 (s)	–
7	153.3 (s)	–	153.3 (s)	–
8	113.0 (s)	–	106.9 (s)	–
8a	152.0 (s)	–	152.3 (s)	–
1'	131.7 (s)	–	131.0 (s)	–
2'	105.4 (d)	6.70 (s)	105.9 (d)	6.75 (s)
3'	149.1 (s)	–	148.5 (s)	–
4'	136.3 (s)	–	136.7 (s)	–
5'	149.1 (s)	–	148.5 (s)	–
6'	105.4 (d)	6.70 (s)	105.9 (d)	6.75 (s)
1"	134.7 (s)	–	134.0 (s)	–
2"	128.1 (d)	7.24 (d, 8.3)	127.9 (d)	7.29 (d, 8.5)
3"	116.1 (d)	6.79 (d, 8.3)	116.0 (d)	6.84 (d, 8.5)
4"	157.9 (s)	–	157.9 (s)	–
5"	116.1 (d)	6.79 (d, 8.3)	116.0 (d)	6.84 (d, 8.5)
6"	128.1 (d)	7.24 (d, 8.3)	127.9 (d)	7.29 (d, 8.5)
7"	78.7 (d)	4.95 (m)	78.1 (d)	4.96 (m)
8"	30.7 (t)	2.14 (m), 1.95 (m)	30.4 (t)	2.18 (m), 1.90 (m)
9"	25.7 (t)	2.86 (m), 2.67 (m)	20.6 (t)	2.80 (m)
5-OCH_3	–	–	59.9 (q)	3.71 (s)
3'-OCH_3	56.8 (q)	3.83 (s)	56.6 (q)	3.82 (s)
5'-OCH_3	56.8 (q)	3.83 (s)	56.6 (q)	3.82 (s)
8-CH_3	8.6 (q)	2.05 (s)	8.4 (q)	1.98 (s)

[a]Measured in CD_3OD. [b]Measured in CD_3COCD_3.

1462, 1378, 1235, 1124, 576 cm^{-1}; HRESIMS *m/z* 487.1723 $[M+Na]^+$ (calcd. for $C_{27}H_{28}O_7Na$, 487.1727); ^{1}H and ^{13}C NMR see Table 1.

Cambodianin H (2): Red amorphous powder (MeOH); $[\alpha]_D^{27}$ −120 (*c* = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.30), 276 (2.01) nm; IR (KBr) 3028, 2938, 1742, 1648, 1460, 1389, 1218, 1120, 670 cm^{-1}; HRESIMS *m/z* 517.1828 $[M+Na]^+$ (calcd. for $C_{28}H_{30}O_8Na$, 517.1833); ^{1}H and ^{13}C NMR see Table 1.

Antibacterial Activity: Two new compounds were tested for *in vitro* antibacterial activity against *Staphylococcus aureus* (SA, obtained from National Institutes for Food and Drug Control) by the filter paper disc agar diffusion method.[14] Twenty-five μL of the tested compounds (20 μg/μL) and control (kanamycin sulfate, 0.64 μg/μL) were impregnated on sterile filter paper discs of 6 mm size respectively, and then, aseptically applied to the surface of the agar plates. The plates were incubated at room temperature for 24 h. Then the diameters of the observed zones of inhibition surrounding each disc including the 6 mm disc diameter were measured and the activities are expressed in mm diameter of the inhibition zone. Experiments were done in triplicate and the results are mean values.

Bioassay of Cytotoxic Activity: These compounds were evaluated for their cytotoxic activity against K562, and SGC-7901 cancer cell line by MTT assay. The logarithmic phase cells (90 μL) were seeded onto 96-well plates at the concentration of 5×10^4 cell/mL. After 24 h, different concentrations of sample (0.10, 0.40, 1.60, 6.25, 25, 100 μg/mL), dissolved in DMSO, was added at 10 μL per well respectively and each concentration had 3 replicate wells. Control cells were treated with DMSO alone and positive controls with Paclitaxel. The cells were carried out and observed by XS-212 Biological microscope after incubation for 72 h. MTT was dissolved at 5 mg/mL in PBS and used essentially as previously described.[15] Briefly, 15 μL of MTT solution were added to each well and the cells were further incubated at 37 °C for 4 h. Then the supernatant was removed and 100 μL DMSO was added into each well. In the end, the absorbance (A value) at wavelength of 490 nm was measured with a MK3 Microtitre plate Reader. Data were expressed as mean absorbance value (OD) of triplicate samples + standard error. Percentage-specific cytotoxicity (%) was calculated as follows: cell inhibition (%) = (1 – average O.D. of wells/ average O.D. of control wells) × 100%. IC_{50} values were calculated as the concentration (μg/mL) of samples causing 50% inhibition of cell viability.

Results and Discussion

Compound **1** was obtained as red amorphous powder and had a molecular formula $C_{27}H_{28}O_7$ based on its HRESIMS (*m/z* = 487.1723, calcd. 487.1727 for $C_{27}H_{28}O_7$, $[M+Na]^+$), indicating 14 degrees of unsaturation. This formula can also be validated through its ^{13}C NMR and DEPT spectra. In the ^{1}H NMR spectrum, four protons at δ_H 4.70 (1H, d, *J* = 8.0 Hz, H-2), δ_H 4.03 (1H, m, H-3), δ_H 2.74 (1H, m, H-4α), and δ_H 2.90 (1H, m, H-4β) indicated a typical flavan-3-ol moiety, which was confirmed by the ^{1}H-^{1}H COSY corrections between H-3 (δ_H 4.03) to H-2 (δ_H 4.70) and H-4 (δ_H 2.74, 2.90). A singlet at δ_H 6.70 (2H, s, H-2', 6') and two methoxyl groups [δ_H 3.83 (6H, s, 3', 5'-OCH_3)] indicated the existence of a 4-hydroxy-3,5-dimethoxyphenyl ring, which was determined to be connected with C-2 by HMBC corrections from H-2 (δ_H 4.70) to C-2', 6' (δ_C 105.4). Five protons at δ_H 4.95 (1H, m, H-7"), δ_H 1.95 (1H, m, H-8"α), δ_H 2.14 (1H, m, H-8"β), δ_H 2.67 (1H, m, H-9"α), and δ_H 2.86 (1H, m, H-9"β) exhibited the presence of a typical flavan moiety, which was confirmed by the ^{1}H-^{1}H COSY corrections between H-8" (δ_H 1.95, 2.14) to H-9" (δ_H 2.67, 2.86). A pair of A_2X_2 aromatic protons at δ_H 7.24 (2H, d, *J* = 8.3 Hz, H-2", 6") and δ_H 6.79 (2H, d, *J* = 8.3 Hz, H-3", 5") indicated the existence of a 4-hydroxyphenyl ring, which was determined to be connected with C-7" by HMBC corrections from H-7" (δ_H 4.95) to C-2", 6" (δ_C 128.1). The ^{13}C and ^{1}H NMR spectra data of **1** (Table 1) were very similar to those of daphnotin B,[16] except for appearance of an additional methylene at δ_C 30.7 (C-8") instead of an oxygenated methine, together with appearance of an additional methyl group (δ_C 8.6, δ_H 2.05),

and a singlet aromatic proton δ_H 6.62 (1H, s) in **1**. In the HMBC spectrum, corrections from the singlet aromatic proton H-5 (δ_H 6.62) to C-4 (δ_C 33.8), C-9" (δ_C 25.7), and C-7 (153.3), and from the methyl singal at δ_H 2.03 to C-7 (δ_C 153.3), C-8 (δ_C 113.0), and C-8a (δ_C 152.0) indicated that the methyl group was located at C-8 and the singlet aromatic proton was assigned to C-5. The relative configuration of **1** was obtained through an analysis of coupling constants and the nuclear overhauser effect spectroscopy (NOESY) spectrum. H-2 and H-3 were determined to be α-, β-oriented, respectively, based on the coupling constant ($^3J_{H\text{-}2,\ H\text{-}3}$ = 8.0 Hz). Furthermore, comparison of the NOESY spectrum of **1** with those of daphnotins A, B[16], a pair of configuration isomers, showed that configuration of H-7" was β-oriented according to no correlations from the methoxyl groups of aromatic ring at C-2 to the protons of aromatic ring at C-7", as same as daphnotin B. Thus, compound **1** was deduced, and named cambodianin G.

The molecular formula of **2** was assigned to be $C_{28}H_{30}O_8$ on the basis of its positive HRESI-MS ($[M+Na]^+$, *m/z* 517.1828, calcd. 517.1833). The ^1H and ^{13}C NMR spectra of **2** (Table 1) were closely related to those of **1**, except for the appearance of an additional methoxyl group (δ_C 59.9) in **2**. The HMBC corrections of the two methylene signals of H-9" (δ_H 2.80) and H-4 (δ_H 2.70, 3.02) with C-5 (δ_C 155.1), and the methoxyl signal (δ_H 3.71) with C-5 (δ_C 155.1) indicated that the methoxyl group (δ_C 59.9) was located at C-5. The same configuration at H-2, 3, and 7" of **2** as **1** was determined by its coupling constant ($^3J_{H\text{-}2,\ H\text{-}3}$ = 8.1 Hz), and the NOESY spectrum. On the above evidence, compound **2** was identified and named as cambodianin H.

Antibacterial assay exhibited that compounds **1** and **2** possessed inhibitory effects on SA. The diameters of inhibition zones were 9.91 and 8.89 mm, respectively, and the inhibition zones of positive control (kanamycin sulfate) was 19.51 mm. Compounds **1** and **2** were evaluated for their cytotoxic activity against K562, and SGC-7901 by MTT method. The results indicated that compound **1** showed cytotoxicity against K562 and SGC-7901 cell line with the IC_{50} value of 9.5 and 16.2 μg/mL, respectively. The positive control (Paclitaxel) showed cytotoxicities against the two human cancer cell lines with the IC_{50} value of 5.1 and 1.6 μg/mL, respectively.

Previous phytochemical studies on plants and dragon's blood of genus *Dracaena* have led to the isolation of a series of flavonoids (chalcones, dihydrochalcones, flavanes, homoisoflavanes, flavones and homoisoflavones), which were the main active constituents. Both **1** and **2**, which were rarely

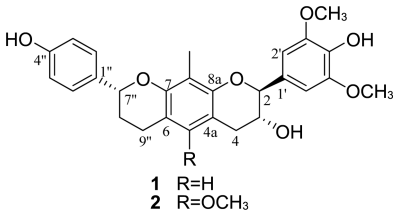

Figure 1. Structures of compounds **1** and **2**.

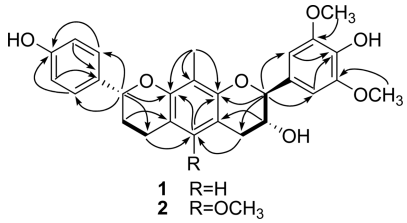

Figure 2. The key HMBC (→) and ^1H–^1H COSY (—) correlations of **1** and **2**.

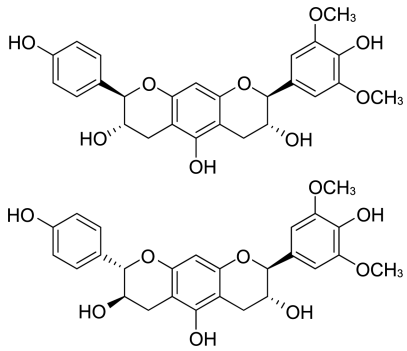

Figure 3. Structures of daphnotin A and B.

encountered in flavan-3-ol skeleton, were identified in genus *Dracaena* for the first time. Biological activities of such compounds haven't been reported. Therefore, more experiments are needed to discovery their wide spectrum of biological activities.

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