Flavanes from *Dracaena cambodiana*

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Two new flavanes, (2*S*)-4′,7-dihydroxy-6,8-dimethylflavane (**1**), and (2*S*)-5,7-dihydroxy-4′-methoxy-8-methylflavane (**2**), together with five known flavanes, (2*S*)-3′,7-dihydroxy-4′-methoxy-8-methylflavane (**3**), (2*R*)-4′,7-dihydroxy-8-methylflavane (**4**), (±)-3′,7-dihydroxy-4′-methoxyflavane (**5**), (±)-4′,7-dihydroxy-3′-methoxyflavane (**6**), and (2*S*)-4′,7-dihydroxyflavane (**7**), were isolated from the stem of *Dracaena cambodiana*. Their structures were determined by spectroscopic techniques (UV, IR, 1D and 2D NMR). Their antimicrobial activities were preliminarily examined by the filter paper disc agar diffusion method.

Key words: Dracaena cambodiana, Flavane, (2*S*)-4′,7-Dihydroxy-6,8-dimethylflavane, (2*S*)-5,7-Dihydroxy-4′-methoxy-8-methylflavane, Antimicrobial Activity

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 haemostatic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory, antioxidant, *etc.* activity [1]. *Dracaena cambodiana* Pierre ex Gagnep. (Agavaceae), known as one of the dragon's blood trees, is endemic to the Hainan island of 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 there are only four steroidal saponins which have been isolated from the fruits of *D. cambodiana* [4]. In an effort to search for new bioactive compounds from tropical medicinal plants in Hainan Province of China, the ethanol extract from the stem of *D. cambodiana* was found to show antimicrobial activities. Bioassay-guided fractionation of the ethanol extract led to the isolation of two new flavanes, (2*S*)-4′,7-dihydroxy-6,8-dimethylflavane (**1**) and (2*S*)-5,7-dihydroxy-4′-methoxy-8-methylflavane (**2**), together with five known flavanes **3** – **7** (Fig. 1). In this paper, we describe the isolation and structure elucidation of compounds **1** and **2**, as well as the antimicrobial activities of compounds **1** – **7**.

Results and Discussion

Compound **1**, obtained as colorless crystals, had a molecular formula $C_{17}H_{18}O_3$ based on its HRMS ((–)-ESI) (*m/z* = 269.1165; calcd. 269.1178 for $C_{17}H_{18}O_3$, $[M–H]^-$), which was supported by its ^{13}C NMR and DEPT data (Table 1). The ^{13}C NMR (DEPT) spectra of **1** showed the presence of twelve aromatic carbons (δ_C = 113 – 158), together with an oxygenated CH (δ_C = 78.7) and two CH_2 (δ_C = 31.4 and 25.8) groups, indicating a flavane skeleton. In addition, two methyl signals (δ_C = 16.2 and 8.9) were observed. In the ^1H NMR spectrum, an AA′BB′ spin system at δ_H = 7.25 (d, *J* = 8.5 Hz, 2H) and 6.79 (d, *J* = 8.5 Hz, 2H) indicated a 4′-OH substituent in ring B. In the HMBC spectrum (Fig. 2), the 1H singlet (δ_H = 6.61) showed correlations to the carbon at δ_C = 25.8 (C-4) and to a methyl group (δ_C = 16.2), which indicated that the aromatic proton in ring A belonged to C-5, and the methyl group (δ_C = 16.2) was attached to C-6. The HMBC correlation of the 3H singlet (δ_H = 2.13) of the methyl group (δ_C = 16.2) to the carbon at δ_C = 152.6 indicated that the C-7 position was substituted by a hydroxyl group, so that the other methyl group (δ_C = 8.9) was connected with C-8. The configuration at C-2 was proposed to be *S*, similar to compound **7** [5], based on the negative sign of its specific rotation. Thus, the structure of **1** was established as (2*S*)-4′,7-dihydroxy-6,8-dimethylflavane.

No.	**1** (CD_3OD) δ_C	δ_H	**2** ($CDCl_3$) δ_C	δ_H
2	78.7 (d)	4.85 (dd, 10.0, 2.1)	77.1 (d)	4.95 (dd, 10.4, 2.2)
		2.15 (m, overlap)		2.17 (m)
3	31.4 (t)	1.34 (m)	29.4 (t)	1.93 (m)
		1.94 (m)		2.75 (ddd, 16.7, 8.9, 3.1)
4	25.8 (t)	2.64 (ddd, 14.8, 4.5, 4.0)	19.4 (t)	2.63 (ddd, 17.0, 11.2, 6.2)
5	128.6 (d)	6.61 (s)	154.0 (s)	–
6	117.8 (s)	–	91.0 (d)	6.05 (s)
7	152.6 (s)	–	155.9 (s)	–
8	113.3 (s)	–	103.2[a] (s)	–
4a	114.3 (s)	–	103.1[a] (s)	–
8a	152.9 (s)	–	152.5 (s)	–
1′	134.9 (s)	–	134.4 (s)	–
2′, 6′	128.2 (d)	7.25 (d, 8.5)	127.3 (d)	7.30 (d, 8.2)
3′, 5′	116.1 (d)	6.79 (d, 8.5)	115.2 (d)	6.80 (d, 8.2)
4′	157.9 (s)	–	155.0 (s)	–
6-Me	16.2 (q)	2.13 (s, overlap)	–	–
8-Me	8.9 (q)	2.07 (s)	7.6 (q)	2.06 (s)
OMe	–	–	56.4 (q)	3.76 (s)

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

[a] Possibility of exchange.

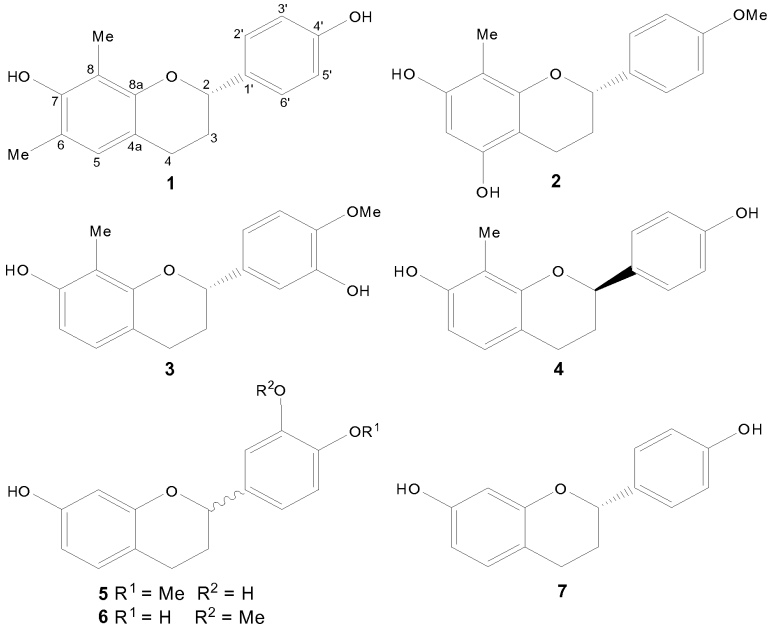

Fig. 1. Structures of compounds **1** – **7**.

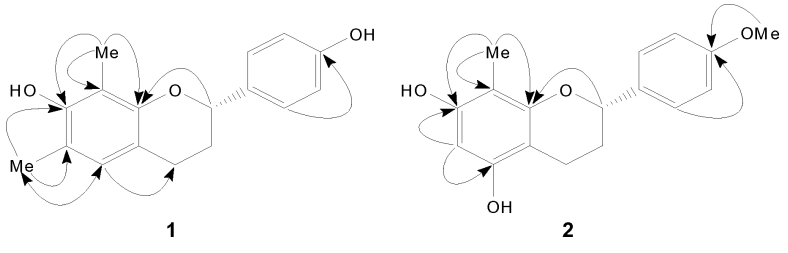

Fig. 2. Key HMBC correlations for **1** and **2** (H to C).

Compound **2**, obtained as colorless crystals, had a molecular formula $C_{17}H_{18}O_4$ based on its HRMS ((–)-ESI) data (*m/z* = 285.1135; calcd. 285.1127 for $C_{17}H_{18}O_4$, $[M–H]^-$), which was supported by its ^{13}C NMR and DEPT data (Table 1). The ^1H NMR data of **2** were similar to those of **1** except for the appearance of a methoxy group (δ_H = 3.76) and the absence of a methyl group. The location of the methoxy group was deduced to be at C-4′ from MS data which showed an ion at *m/z* = 107 due to the cleavage of the C-2–C-1′ bond [6]. The location of the methyl group (δ_C = 7.6) was deduced to be at C-8 on the basis of HMBC correlations between the 3H singlet (δ_H = 2.06) of the methyl group to the carbons at δ_C = 103.2/103.1 (C-8), 155.9 (C-7) and 155.0 (C-8a) (Fig. 2). The aromatic proton (δ_H = 6.05) correlated with carbons at δ_C = 154.0 (C-5), 152.6 (C-7) and 103.2/103.1 (C-8/C-4a) indicating that it was assigned to C-6. The upfield shift of the aromatic protons observed at δ_H = 6.05 indicated that the C-5 and C-7 positions were oxygenated by hydroxyl groups [6]. The configuration at C-2 was tentatively assigned to be *S*, similar to compound **1**, on the basis of the negative sign of its specific rotation. CD spectra could not be obtained as yet due to technical problems. We take the assignment of the absolute stereochemistry on the basis of the specific rotation as sufficient for now, as both compounds **1** and **2** have only one chiral carbon atom each. Thus, the structure of **2** was established as (2*S*)-5,7-dihydroxy-4′-methoxy-8-methylflavane.

By comparing the ^1H and ^{13}C NMR data with those reported in the literature, compounds **3**–**7** were identified as (2*S*)-3′,7-dihydroxy-4′-methoxy-8-methylflavane (**3**) [7], (2*R*)-4′,7-dihydroxy-8-methylflavane (**4**) [8], (±)-3′,7-dihydroxy-4′-methoxyflavane (**5**) [9], (±)-4′,7-dihydroxy-3′-methoxyflavane (**6**) [9], (2*S*)-4′,7-dihydroxyflavane (**7**) [5].

This is the first phytochemical study on the stem of *D. cambodiana*. Antimicrobial tests demonstrated that most of the isolated compounds showed antibiotic activity against *Staphylococcus aureus* and *Candida albicans* (Table 2).

Table 2. Antimicrobial activities of **1**–**7** (expressed in mm diameter of the inhibition zone).

Compounds[a]	*Staphylococcus aureus*[b]	*Candida albicans*[c]
1	14.0	6.5
2	6.5	–
3	15.0	7.0
4	17.0	9.0
5	13.0	6.5
6	13.0	6.5
7	18.0	7.0

[a] Compounds: 50 μL of a solution with 10 mg μL^{-1} in MeOH of the compounds were each impregnated on filter paper discs of 6 mm size; [b] *Staphylococcus aureus*: the strains was cultured using Nutrient Agar (NA). 10 μL of a solution with 0.08 mg mL^{-1} kanamycin sulfate in water was used as control, whose diameter of inhibition zone was 31.0 mm; [c] *Candida albicans*: the strains was cultured using Yeast Extract Peptone Dextrose Medium (YPD). 10 μL of a solution with 12.8 mg mL^{-1} fluconazole in water was used as control, whose diameter of inhibition zone was 22.5 mm.

Experimental Section

General

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

Plant material

The stem of *D. cambodiana* was collected in Haikou, Hainan province, China (July 2007). The specimen was identified by Associate Professor 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* was deposited.

Extraction and isolation

The dried and crushed stem (13.3 kg) of *D. cambodiana* was extracted three times with 95 % EtOH at r. t. The extract was evaporated under reduced pressure to dryness and then partitioned in succession between H_2O and petroleum ether, EtOAc, and then *n*-BuOH. The EtOAc fraction (150.0 g), which showed inhibitory activity towards *Staphylococcus aureus* and *Candida albicans*, was chromatographed on a silica gel column using a step gradient elution of $CHCl_3$-MeOH (100 : 1 – 0 : 100, v/v) to afford 10 fractions. Fraction 2 was submitted to repeated column chromatography on silica gel and Sephadex LH-20, and finally yielded compounds **1** (4.5 mg), **3** (26.3 mg), **4** (161.8 mg), **5** (10.2 mg), and **6** (9.2 mg). Fraction 3 was submitted to repeated column chromatography on silica gel and Sephadex LH-20, and finally yielded compounds **2** (19.0 mg) and **7** (50.3 mg).

*(2S)-4′,7-Dihydroxy-6,8-dimethylflavane (**1**)*

Colorless crystals. – M. p. 148 – 151 °C. – $[\alpha]_D^{27} = -31.0$ (c = 0.58, MeOH). – UV (MeOH): λ (log ε_{max}) = 209 (0.65), 284 (0.20) nm. – IR (KBr): ν = 3423, 2927, 2858, 1617, 1565, 1480, 1463, 1375, 1103 cm^{-1}. – HRMS ((–)-ESI): *m/z* = 269.1165 (calcd. 269.1177 for $C_{17}H_{18}O_3$, $[M–H]^-$). – ^1H and ^{13}C NMR: see Table 1.

*(2S)-5,7-Dihydroxy-4′-methoxy-8-methylflavane (**2**)*

Colorless crystals. – M. p. 163 – 165 °C. – $[\alpha]_D^{27} = -14.0$ (c = 0.67, MeOH). – UV (MeOH): λ (log ε_{max}) = 237 (0.85), 285 (0.40) nm. – IR (KBr): ν = 3382, 2933, 2869, 1612, 1513, 1454, 1340, 1231, 1205, 1174, 1111, 1033 cm^{-1}. – HRMS ((–)-ESI): *m/z* = 285.1135 (calcd. 285.1127 for $C_{17}H_{18}O_4$, $[M–H]^-$). – ESI-MS: *m/z* = 285 $[M–H]^-$, 107. – ^1H and ^{13}C NMR: see Table 1.

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

Colorless oil. – $[\alpha]_D^{27} = -20.0$ (c = 0.52, MeOH).

*(2R)-4′,7-Dihydroxy-8-methylflavane (**4**)*

Colorless crystals. – M. p. 132 – 135 °C. – $[\alpha]_D^{27} = +15.0$ (c = 0.41, MeOH).

*(±)-3′,7-Dihydroxy-4′-methoxyflavane (**5**)*

Colorless crystals. – M. p. 158 – 159 °C. – $[\alpha]_D^{27} = 0$ (c = 0.72, MeOH).

*(±)-4′,7-Dihydroxy-3′-methoxyflavane (**6**)*

Amorphous solid. – $[\alpha]_D^{27} = 0$ (c = 0.43, MeOH).

*(2S)-4′,7-Dihydroxyflavane (**7**)*

Colorless crystals. – M. p. 195 – 196 °C. – $[\alpha]_D^{27} = -34.5$ (c = 0.74, MeOH).

Antimicrobial activity

All compounds were tested for *in vitro* antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* strains (obtained from Hainan Provincial Institute for Drug Control, China) by the filter paper disc agar diffusion method [10]. Compounds and control were each impregnated on sterile filter paper discs of 6 mm size, and aseptically applied to the surface of the agar plates. The plates were incubated at r. t. 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 expressed in mm diameter of the inhibition zone. Experiments were done in triplicate, and the results are mean values. The results are reported in Table 2.

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