Journal of Chemistry, Vol. 46 (4), P. 503 - 508, 2008

ANTIFUNGAL CONSTITUENTS FROM THE STEMS OF *DRACAENA CAMBODIANA*

Received 6 September 2007, Revised 1 November 2007

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SUMMARY

The stems of Dracaena cambodiana have been investigated for antifungal constituents, resulting in the isolation of a new steroidal saponin dracagenin A (1) together with two known components 25S-namogenin B (2) and spriroconazole A (3). Their structures were elucidated on the basis of extensive spectroscopic analysis including 1D, 2D NMR and ESI MS. The isolated compounds were evaluated for their antifungal activities. 1 and 3 exhibited inhibitory activity against Aspergilus niger at the MICs of 50 μg/ml. No inhibition effects on F. oxysporum, S. cerevisiae and C. albicans have been found for the tested compounds. Interestingly, toxicity of 3 towards several cancer cell lines has been demonstrated. The IC_{50} of 3 against Hep-2, Lu, and RD are 2.002, 4.727, and 4.029 μg/ml, respectively.

Keywords: *Dracaenaceae, Dracaena cambodiana, Steroidal saponin, Dracagenin A.*

I - INTRODUCTION

Dracaena cambodiana (Dracaenaceae) is mostly distributed in the North of Vietnam. The plant has been found in ethnomedicine to treat menoschesis, ostealgia etc (Bich, 2006; Chi, 1997; Loi, 2001). Investigations on the chemical constituents of the genus revealed that the spirosterol and steroidal saponin are among the major components (Gonzalez, 2004; Mimaki, 1998). Interestingly, there has been no report on the phytochemical of *D. cambodiana*. On our continuing studies for antifungal agents, we have isolated a new steroidal saponin namely dracagenin A (**1**), one sprirosterol 25S-namogenin B (**2**) and one known steroidal saponin spriroconazole A (**3**). The antifungal activities of these compounds were also described.

II - MATERIALS AND METHODS

General experiment procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Chemical shifts are referenced to δ using tetramethylsilan (TMS) as an internal standard. The Electron Spray Ionization (ESI) mass spectrum was obtained using an AGILENT 1100 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silicagel 230 - 400 mesh (0.040-0.063 mm, Merck) or YMC RP-18 resins (30-50 μm, FuJisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates.

Plant material

The roots of *D. cambodiana* were collected in Hoabinh province, Vietnam in January 2006

and identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. An authentic sample was deposited at the Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and isolation

The dried roots of *D. cambodiana* (4 kg) were extracted with MeOH three times (7 days each time) and then concentrated under low pressure to obtain 150 g MeOH extract. The MeOH extract was suspended in water and partitioned with hexane, $CHCl_3$ and BuOH to obtain n-hexane (60 g), $CHCl_3$ (35 g), and BuOH (65 g) fractions. The $CHCl_3$ fraction was then chromatographed on silicagel column eluting with $CHCl_3$-MeOH gradient (from 10:1 to 1:1 v/v) to give fractions C1 (16 g), C2 (11 g), and C3 (7 g). C1 fraction was chromatographed on a silicagel column using $CHCl_3$-EtOAc (3:1) as eluent to give subfractions C3A (6.5 g), C3B (3.4 g), C3C (6.0 g). The C3C subfraction (6.0 g) was chromatographed on a silica gel column using a $CHCl_3$-MeOH (3:1 v/v) system as eluent yielded **2** (25 mg) as white powder. The BuOH fraction was chromatographed on silicagel column eluting with $CHCl_3$-MeOH gradient (from 10:1 to 1:1 v/v) to obtain fractions B1 (25 g), B2 (20 g), and B3 (12 g). B2 fraction showed the most active antifungal activity then selected for further isolation of bioactive compounds. The B2 fraction (20 g) was chromatographed on an YMC RP-18 column using a MeOH-H_2O (2:1 v/v) system as eluent yielded **1** (18.5 mg) and **3** (9 mg) as white amorphous powder.

25*S*-Namogenin B (2): White powder, positive ESI-MS *m/z*: 447 $[M+H]^+$, 429 $[M+H-H_2O]^+$, 411 $[M+H-2H_2O]^+$; negative ESI-MS *m/z*: 445 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.46 (dd, *J* = 4.0, 11.5 Hz, H-1), 1.57/2.06 (m, H-2), 3.53 (m, H-3), 1.89/2.24 (m, H-4), 5.57 (d, *J* = 5.5 Hz, H-6), 1.94/2.08 (m, H-7), 1.97 (m, H-8), 1.59 (m, H-9), 1.60/2.11 (m, H-11), 1.42/1.75 (m, H-12), 1.61/1.95 (m, H-15), 4.60 (m, H-16), 2.29 (m, H-17), 0.94 (s, H-18), 1.07 (s, H-19), 2.10 (m, H-20), 1.01 (d, *J* = 7.0 Hz, H-21), 1.94/2.02 (m, H-23), 1.40/2.11 (m, H-24), 1.72 (m, H-25), 3.29 (d, *J* =2.5 Hz, H-26a), 3.93 (d, *J* = 11.0 Hz, H-26b), 0.90 (d, *J* = 7.0 Hz, H-27); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 77.7 (C-1), 42.0 (C-2), 67.9 (C-3), 42.6 (C-4), 137.6 (C-5), 125.5 (C-6), 26.2 (C-7), 35.7 (C-8), 43.7 (C-9), 43.2 (C-10), 22.6 (C-11), 32.0 (C-12), 44.0 (C-13), 87.6 (C-14), 39.4 (C-15), 81.9 (C-16), 58.8 (C-17), 20.0 (C-18), 13.0 (C-19), 42.2 (C-20), 14.5 (C-21), 110.0 (C-22), 26.1 (C-23), 25.8 (C-24), 27.1 (C-25), 65.1 (C-26), 16.1 (C-27).

Spriroconazole A (3): White powder, positive ESI-MS *m/z*: 907 $[M+Na]^+$; negative ESI-MS *m/z*: 883 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.11/1.90 (m, H-1), 1.31/1.91 (m, H-2), 3.66 (m, H-3), 2.29/2.47 (m, H-4), 5.40 (d, *J* = 5.0 Hz, H-6), 1.37/1.59 (m, H-7), 1.65 (m, H-8), 0.98 (m, H-9), 1.54/1.63 (m, H-11), 1.65/2.03 (m, H-12), 1.73 (m, H-14), 1.25/1.71 (m, H-15), 4.01 (dd, *J* = 7.0, 8.0 Hz, H-16), 0.85 (s, H-18), 1.07 (s, H-19), 2.11 (m, H-20), 0.94 (d, *J* = 7.0 Hz, H-21), 1.70/2.03 (m, H-23), 1.45/1.62 (m, H-24), 1.62 (m, H-25), 3.36/3.47 (m, H-26), 0.81 (d, *J* = 6.5 Hz, H-27), 4.53 (d, *J* = 7.0 Hz, H-1Glc), 3.58 (t, *J* = 9.0 Hz, H-3 Glc), 4.99 (d, *J* = 1.5 Hz, H-1 RhaI), 1.26 (d, *J* = 6.5 Hz, H-6 RhaI), 4.92 (d, *J* = 1.5 Hz, H-1 RhaII), 1.28 (d, *J* = 6.5 Hz, H-6 RhaII); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 38.6 (C-1), 30.8 (C-2), 79.1 (C-3), 39.3 (C-4), 141.9 (C-5), 122.9 (C-6), 32.8 (C-7), 33.3 (C-8), 51.5 (C-9), 38.0 (C-10), 21.7 (C-11), 33.2 (C-12), 45.8 (C-13), 53.1 (C-14), 32.1 (C-15), 90.6 (C-16), 91.3 (C-17), 17.5 (C-18), 19.8 (C-19), 45.5 (C-20), 9.1 (C-21), 111.0 (C-22), 32.5 (C-23), 29.4 (C-24), 31.3 (C-25), 67.7 (C-26), 17.5 (C-27), **Glc** (1) 100.2, (2) 97.4, (3) 88.4, (4) 70.5, (5) 77.5, (6) 62.6, **RhaI** (1) 102.8, (2) 72.3, (3) 72.5, (4) 73.6, (5) 70.1, (6) 17.8, **RhaII** (1) 103.9, (2) 72.2, (3) 72.4, (4) 73.8, (5) 70.9, (6) 18.1.

III - RESULTS AND DISCUSSION

Bioassay guided fractionation led to the isolation of three bioactive components. Compound **1** was obtained as white amorphous powder, The ESI MS (positive mode) showed the pseudomolecular $[M+Na]^+$ ion peak at *m/z* 1085 (corresponding to the molecular formula $C_{51}H_{82}O_{23}$). The ^{13}C-NMR spectrum of **1** showed

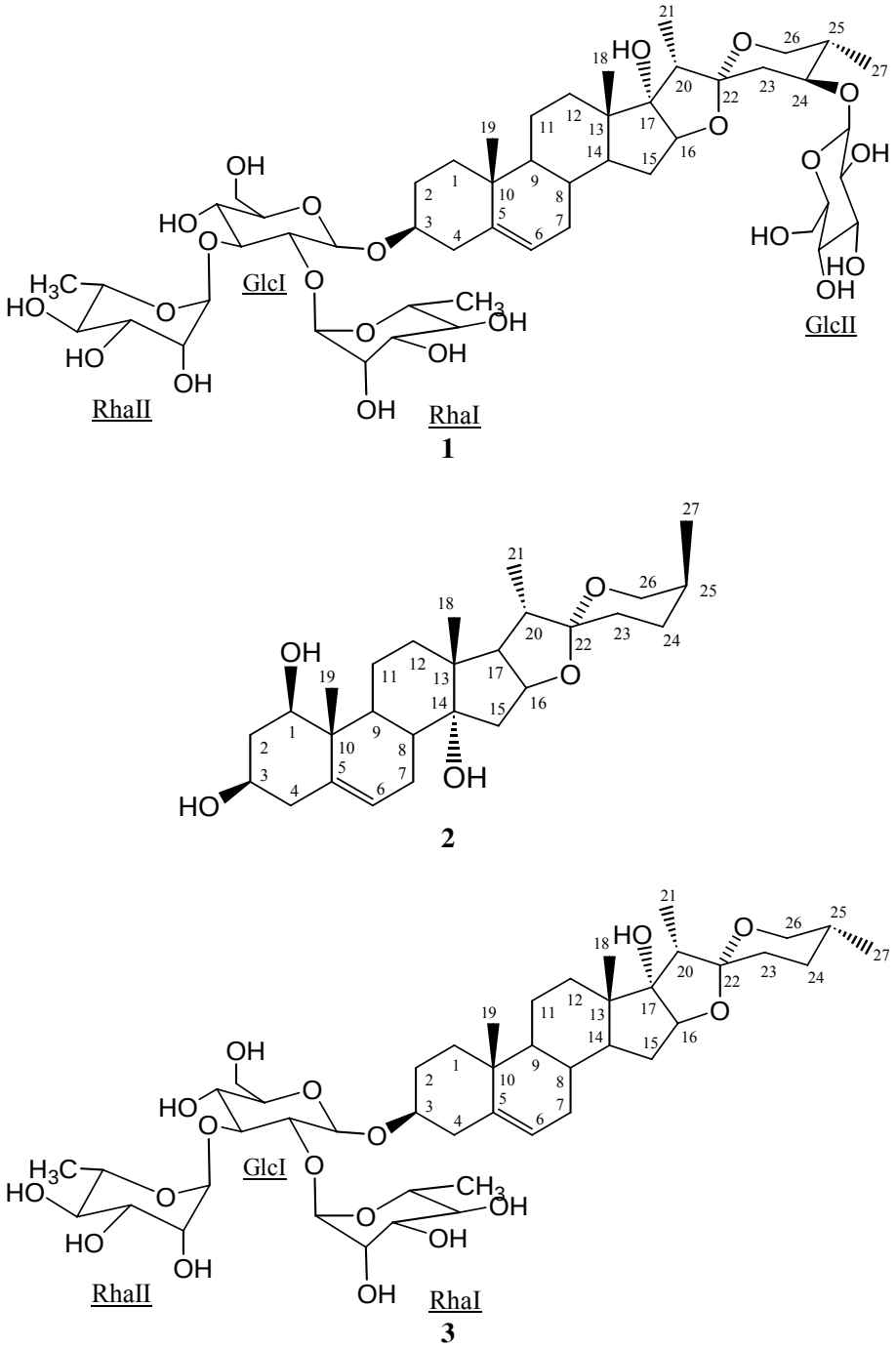

Figure 1: Structures of **1-3**

51 signals, among which 27 were assigned to the aglycone, the remaining 24 signals were indicative of the presence of four hexoses due to two D-glucoses and two L-rhamnoses. The structure of the aglycone moiety was recognized to be penogenin (3β,17α-dihydroxyspirost-5en) by ^{1}H- and ^{13}C-NMR spectral analysis using connectivities observed in COSY, HSQC, and

HMBC. The ^{1}H-NMR spectrum of **1** showed two three-proton singlet signals at δ 0.83 and 1.07 and two three-proton doublet signals at δ 0.91 (J = 7.5 Hz) and 1.00 (J = 6.5 Hz), which were characteristic of the spirostanol skeleton, as well as signals for four anomeric protons at δ 4.54 (d, J =7.5 Hz), 4.37 (d, J = 7.5 Hz), 5.00 (d, J = 1.5 Hz), 1.92 (d, J = 1.5 Hz). The salient features of this aglycone part were the ^{13}C-NMR signals at δ 141.9 (C-5) and 122.6 (C-6), characteristic of Δ^5-spirostene-type sapogenin. The proton coupling constant between H-25 and H-26$_{ax}$ (J = 11.5 Hz), and the ^{13}C-NMR shifts of the F-ring part (C-25/δ 38.5) gave an evidence for the C-25*R* configuration.

Table 1: NMR data of **1**

No.	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J, Hz)	HMBC (H to C)
1	38.6	1.13 m; 1.90 m	C-5
2	30.7	1.62 m; 1.91 m	
3	79.1	3.65 m	C-1 Glc
4	39.3	2.30 m; 2.47 m	C-2, C-5, C-6, C-10
5	141.9		
6	122.6	5.40 d (5.5)	C-8, C-10
7	32.8	1.37 m; 1.68 m	
8	33.3	1.65 m	
9	51.5	0.97 m	
10	38.0		
11	21.7	1.49 m; 1.63 m	
12	33.2	1.59 m; 2.03 m	
13	45.7		
14	53.9	1.74 m	
15	32.0	1.31 m; 2.05 m	C-15, C-17
16	90.9	3.99 t (6.5)	
17	91.8		
18	17.5	0.83 s	C-12, C-13, C-14, C-17
19	19.8	1.07 s	C-4, C-9
20	45.9	2.16 m	C-14, C-17, C-21, C-23
21	9.0	0.91 d (7.5)	C-17, C-20, C-22
22	113.8		
23	41.0	1.69 m; 2.22 m	C-24, C-25
24	82.0	3.62 m	
25	38.5	1.67 m	
26	65.8	3.38 m; 3.53 m	C-24, C-27
27	13.5	1.00 d (6.5)	C-24, C-26
GlcI (1 → C3)			
1	100.2	4.54 d (7.5)	C-3
2	79.4	3.47 m	
3	88.4	3.60 m	C-2 Glc, C-4, Glc
4	70.6	3.40 m	
5	77.5	3.29 m	
6	62.6	3.70 m; 3.88 m	
RhaI (1 → 2Glc)			
1	102.8	5.00 d (1.5)	C-2 Glc, C-3 Rha, C-5 Rha
2	72.3	3.64 m	

No.	δ_C[a,b]	δ_H[a,c] (*J*, Hz)	HMBC (H to C)
3	72.5	3.88 m	
4	73.6	3.43 m	
5	70.1	4.10 m	
6	17.8	1.26 d (6.0)	
RhaII (1 → 3Glc)			
1	103.9	4.92 d (1.5)	C-3 Glc, C-3 Rha', C-5 Rha'
2	72.2	3.86 m	
3	72.4	3.66 m	
4	73.8	3.43 m	
5	70.9	3.95 m	
6	18.1	1.28 d (6.0)	
GlcII (1 → C24)			
1	106.0	4.37 d (7.5)	C-24
2	75.5	3.17 m	
3	78.1	3.39 m	
4	71.7	3.33 m	C-5 Glc'
5	77.7	3.31 m	
6	62.8	3.70 m; 3.88 m	

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz, Chemical shift (δ) in ppm.

The connection of the sugars part to C-3 of the aglycone was confirmed by the correlation of an anomeric proton (δ_H 4.54) and C-3 position (δ_C 79.1) in the HMBC spectrum. This carbohydrate consisted of one disubstituted glucose and two terminal rhamnose moieties. The linkage positions of the sugars were determined by HMBC spectrum. Accordingly, the anomeric proton signal observed in ^{1}H-NMR spectrum at δ_H 5.00 correlated with C-2 (δ_C 79.4) of glucose, the anomeric proton signal observed in ^{1}H-NMR spectrum at δ_H 4.92 correlated with C-3 (δ 88.3) of glucose. Furthermore, the presence of two upfield signals of methyl protons at δ 1.26 (d, J = 1.5 Hz), and 1.28 (d, J = 1.5 Hz) in its ^{1}H-NMR spectrum confirmed that the two deoxyhexopyranose units were rhamnose moieties. The rhamnopyranosyl residues were shown to be terminal units as suggested by the absence of any glysosydation shift for their carbon resonances. The 2,3 distribution of the glucopyranosyl moiety was shown by downfield chemical shifts at δ 79.4 (C-2 GlcI) and 88.3 (C-3 GlcI) in the ^{13}C-NMR spectrum. The NMR data were in good agreement with literature data (Tepono, 2001; Yokosuka, 2002).

The remaining sugar moiety was also determined by extensive spectroscopic studies. The strong downfield shift signal at C-24 (δ 82.0) in ^{13}C-NMR spectrum was indicative of the presence of one sugar moiety at this position. The correlation observed in HMBC spectrum between the anomeric proton (δ_H 4.37 (d, *J* = 7.5 Hz)) and C-24 (δ_C 82.0) confirmed that one glucose unit connected to C-24 of the F-ring. By above evidence and comparison with published data (Mimaki, 2001), **1** was identified as 24-*O*-β-D-glucopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopysanosyl-(1→3)]-*β*-D-glucopyranosylpennogenin which were named as dracagenin A. This is the first report of this compound from the nature.

Compound **2** was obtained as white powder. Inspecting the NMR data of **2** revealed that **2** was a spirostane-type sterol. The NMR data of **2** were similar to those of the aglycone of **1** except for the appearance of two hydroxyl group at C-1 (δ_H 3.46 (dd, J = 4.0, 11.5 Hz)/ δ_C 77.7) and C-14 (δ_C 87.6) and the upfield shifts of the signals of F-ring from C-23 to C-27. This evidence suggested to the 25*S* configuration of **2**. The 13C-NMR spectrum of **2** showed the upfield

shift of the oxygenated signal (δ 58.8) assigned to C-17 in **1**. Therefore, C-17 was considered as a methine group. The spectroscopic data of **2** were compared with those of 25*S*-namogenin B and found to match (Tran, 2001). By carried out the same methods on structure elucidation using spectroscopic evidence, compound **3** were determine to be spriroconazole A, which were isolated from *D. manii*, *D. arborea*, and *Dioscorea bulbifera* L.var *sativa* (Tepono, 2001).

The isolated compounds were evaluated for their antifungal activities. **1** and **3** exhibited inhibitory activity against *Aspergilus niger* at the MICs of 50 μg/ml. No inhibition effects on *F. oxysporum*, *S. cerevisiae* and *C. albicans* have been found for the tested compounds. **2** was inactive against all tested fungi. Interestingly, toxicity of **3** towards several cancer cell lines has been demonstrated. The IC_{50} of **3** against Hep-2, Lu, and RD are 2.002, 4.727, and 4.029 μg/ml, respectively. Thus, **1** and **3** should be considered to be promising antifungal agents from *D. cambodiana*.

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