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Dracaenogenins A and B, new spirostanols from the red resin of *Dracaena cochinchinensis*

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ABSTRACT

A 12(13→14)abeospirostanol dracaenogenin A (**1**) and a spirostanol dracaenogenin B (**2**) were isolated from Chinese dragon's blood, the red resin of *Dracaena cochinchinensis* (Agavaceae). Their structures were established as (14S,25R)12(13→14)abeospirosta-5,13(18)-diene-1β,3β,15α-triol (**1**) and (25R) spirost-5-ene-1β,3β,14α,15α-tetrol (**2**) by means of spectroscopic analysis, especially by 2D NMR spectra, and X-ray crystallographic analysis. Dracaenogenin A (**1**) is the first example of a 12(13→14)abeospirostane spirostanoid found in nature. Its biogenesis from ruscogenin (**3**) through namogenin (**4**) and **2** was tentatively proposed.

1. 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]. In China, the red resin of *Dracaena cochinchinensis* S. C. Chen (Agavaceae), called "longxuejie", was used as dragon's blood for promoting blood circulation and the treatment of inflammation, diarrhea, diabetes and bleeding. Chemical studies revealed that the resin contains many phenolic compounds, several steroids and aliphatic acids [2–6]. Steroidal saponins were isolated from the fruits of this plant [7]. Eighteen new steroidal glycosides including C_{22} steroidal lactone glycosides [8], pregnane glycosides [9] and steroidal saponins [10] were also isolated from the fresh stems of this plant by our group recently. As a systematic chemical investigation on "longxuejie", the Chinese dragon's blood, we have reported the isolation of 16 flavonoids from the red resin of *D. cochinchinensis* [11]. Further chemical study on Chinese dragon's blood led to the isolation of a novel 12(13→14)abeospirostanol dracaenogenin A (**1**) and a new spirostanol dracaenogenin B (**2**), together with three known sterols. This paper describes the structure determination of the new compounds by means of spectroscopic analysis, especially 2D NMR technique, and X-ray crystallographic analysis.

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2. Experimental

2.1. General methods

Melting points (mps, uncorrected) were determined by XRC-1 apparatus. Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. NMR spectra were measured in C_5D_5N and recorded on a Bruker AM-400 (for ^1H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR) instruments with TMS as internal standard; IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. FABMS (negative ion mode) spectra were recorded on a VG Auto Spec-300 spectrometer. Silica gel (200–300 mesh and 10–40 μm), RP-18 (40–63 μm) and Sephadex LH-20 (25–100 μm) were used for column chromatography.

2.2. Material

The red resin of *D. cochinchinensis* was purchased from Weihe Pharmaceutical Factory (Yunnan, China). A sample was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany. Identification of the extract was supported by an HPLC comparison with authentic sample.

2.3. Extraction and isolation

The red resin (1.0 Kg) of *D. cochinchinensis* was ground and successively extracted with $CHCl_3$, EtOAc, and MeOH. The $CHCl_3$ extract (90 g) was subjected to silica gel column chromatography, eluting with $CHCl_3$, $CHCl_3$/MeOH (20:1, 10:1, 10:2) and then MeOH, to give eight fractions (Fr. 1–8). Repeated column chromatography of Fr. 8 (4.5 g) on Sephadex LH-20, Rp-18, and silica gel followed with recrystallization in EtOH:H_2O yielded compounds **1** (33 mg), **2** (45 mg), daucosterol (65 mg), stigmast-5,22-diene 3-O-β-D-glucopyranoside (120 mg), and spirost- 5,25(27)-diene-1,3-diol 1-O-α-L-arabinopyranoside (76 mg).

2.3.1. *Dracaenogenin A* (1)

Colorless crystals, mp 145–146 °C. $[\alpha]_D = +0.0°$ ($c = 0.20$, MeOH). EIMS (positive ion mode): *m/z* (%) 444 $[M]^+$, 426 (24), 293 (80), 157 (97), 126 (100), 121 (92), 105 (98), 91 (88), 79 (52), 69 (72), 55 (71). HRESIMS (positive ion mode): *m/z* 467.2738 $[M(C_{27}H_{40}O_5)+Na]^+$ (Calc. 467.2773). IR (KBr) ν_{max}: 3431, 2934, 2873, 1628, 1459, 1369, 1280, 1074, 1043, 1015, 990, 915, 882. ^1H, and ^{13}C NMR: see Table 1.

2.3.2. *X-ray crystal structure analysis of* 1

Crystal data: $C_{27}H_{40}O_5.H_2O$, M = 444.61. Monoclinic system, space group: $P2_1$, $a = 11.473(1)$, $b = 7.933(1)$, $c = 13.948(1)$ Å, $\beta = 81.94(1)°$, $V = 1256.9(2)$ Å^3, $Z = 2$, $d = 1.222$ g cm^{-3}. Mo Kα radiation. A colorless crystal of dimensions 0.01 mm × 0.15 mm × 0.25 mm was used for X-ray measurements on a MAC DIP-2030 K diffractometer with a graphite monochromator. The maximum 36 value was set at 50.0°. The total number of independent reflections measured was 2815, of which 2228 were considered to be observed ($|F|^2 \geq 8\sigma|F|^2$).

Table 1 – ^1H and ^{13}C-NMR data of 1 (in C_5D_5N, δ in ppm and J in Hz)[a]

Number	δ_C	δ_H	ROESY[b]
1	77.8	3.76, dd, 3.8, 11.8	2α, 3, 9
2	43.0	2.57, m	1, 3
		2.25, m	4β, 19
3	68.5	3.94, m	1, 2α, 3, 4α
4	42.5	2.71	19
		2.69	1, 3, 6
5	140.1		
6	125.2	5.70, d, 4.6	4α, 7α, 7β
7	**34.0**	**2.53, m**	**7α, 8**
		2.15, m	**6, 9, 15, 16**
8	41.7	2.32, dd, 14.2, 5.6	7β, 11β, 18, 19
9	53.0	2.40, m	1α, 7β, 11β, 12β
10	44.9		
11	27.7	2.62, m	8, 12β, 18, 19
		1.93, m	9, 11α, 12α
12	34.0	2.73, m	11β, 18, 19, 21
		1.52, m	11α, 12β
13	159.1		
14	57.1		
15	79.9	4.54, d, 5.9	7α, 17, 26α, 14-OH
16	89.1	4.79, dd, 5.9, 9.7	7α, 17, 26α, 14-OH
17	52.9	2.99, dd, 2.9, 9.7	15, 16, 21, 14-OH
18	102.9	4.97, d, 2.6	17, 20
		5.11, d, 2.6	8
19	12.8	1.37, s	4β, 8
20	49.1	2.43, m	18, 21
21	17.3	1.01, d, 7.4	12α, 17, 20
22	109.6		
23	30.9	1.64, m	
		1.34, m	
24	28.9	1.55, m	
		1.50, m	
25	30.7	1.57, m	
26	67.7	3.45, dd, 4.0, 13.7	25, 27, 26β
		3.55, dd, 10.9, 13.7	15, 16, 27, 24α, 26β
27	17.2	0.57, d, 7.9	24α, 26α

[a] Data recorded on a Bruker AM-400 MHz spectrometer with reference to the solvents (δ_H 8.71/δ_C 149.9).
[b] Data recorded on a Bruker DRX-500MHz spectrometer.

The structure was solved by the directed method SHELX-86[6] and expanded using difference Fourier techniques, refined by the program and full-matrix least-squareds calculations. Hydrogen atoms were fixed at calculated positions. The final indices were $R_f = 0.087$, $Rw = 0.086(w = 1/\sigma|F|^2)$. Crystallographic data for the structure have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 242633). Copies of the data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.htm (or 12 Union Road,

Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

2.3.3. *Dracaenogenin B (2)*

Colorless crystals, mp. 163–164 °C. $[\alpha]_D = +13.7°$ (c = 0.2, MeOH). ESIMS (positive ion mode): *m/z* 485 $[M+Na]^+$, 463 $[M+H]^+$, HRESIMS (positive ion mode): *m/z* 463.3070 $[M(C_{27}H_{40}O_6)+H]^+$ (Calc. 463.3059). IR (KBr): ν_{max} 3373, 2963, 2937, 2874, 1631, 1461, 1377, 1356, 1069, 1039, 1014, 965, 917, 888 cm^{-1}. ^1H, and ^{13}C NMR: see Table 2.

Table 2 – ^1H and ^{13}C-NMR data of 2 (in C_5D_5N, δ in ppm and *J* in Hz)[a]

Number	δ_C	δ_H	ROESY[b]
1	77.9	3.76, br d,11.8	2α,3,9
2	43.0	2.60 2.25, br d, 11.8	1, 2α, 2β, 3, 19
3	68.6	3.98, m	1, 2α, 3
4	42.6	2.72, m 2.70, m	19 3, 6
5	140.9		
6	126.6	5.82, d, 4.8	4α, 7α, 7β
7	30.8	2.85, t, 12.3 2.53	6, 7α, 8, 9, 15, 16
8	39.9	2.34 ddd, 12.0, 12.0, 5.0	7b, 18, 11β, 19
9	54.4	2.10 ddd, 12.3,12.0,4.8	1α, 7β, 11β, 12β
10	44.7		
11	30.7	2.74 1.69	8, 11α, 18, 19, 12β 12α, 9
12	28.1	1.62 1.46	11β, 18, 19 11α, 12β, 21
13	59.2		
14	79.5		
15	79.3	4.58, d, 3.8	7α, 17, 26α, 14-OH
16	90.5	4.74, dd, 6.3, 9.8	7α, 17, 26α, 14-OH
17	59.1	2.50, t, 10.4	15, 16, 21, 14-OH
18	23.0	1.35, s	
19	12.7	1.34, s	
20	42.4	2.53, m	18, 21
21	18.2	0.95, d, 7.4	12α, 17, 20
22	109.3		
23	29.2	1.40 1.37	
24	28.9	1.50 1.42	
25	30.4		
26	67.8	3.36, brd, 10.8 3.52, dd, 10.8, 9.6	25, 26β, 27 15, 16, 24α, 26β, 27
27	17.0	0.49, d, 6.43	

[a] Data recorded on a Bruker AM-400 MHz spectrometer with reference to the solvents (δ_H 8.71/δ_C 149.9).

[b] Data recorded on a Bruker DRX-500 MHz spectrometer.

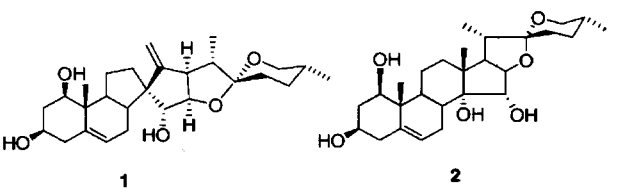


Fig. 1 – New spirostanols dracaenogenins A (1) and B (2) isolated from the red resin of *Dracaena cochinchinensis*.

3. Results and discussion

The $CHCl_3$ extract of Chinese dragon's blood, the red resin of *D. cochinchinensis*, was subjected to column chromatography on silica gel, Sephadex LH-20, and Rp-18 followed with recrystallization to give dracaenogenins A (1) and B (2) (Fig. 1), together with three known sterols, which were identified as daucosterol, stigmast-5,22-diene 3-O-β-D-glucopyranoside, and spirost-5,25(27)-diene-1,3-diol 1-O-α-L- arabinopyranoside, by 1D NMR and comparisons with authentic samples.

Compound **1** was obtained as colorless crystals, mp. 145–146 °C. Its molecular formula was assigned as $C_{27}H_{40}O_5$ on the basis of positive ion HRESIMS ($[M+Na]^+$, *m/z* 467.2738), indicating seven degrees of unsaturation. The ^{13}C NMR (including DEPT) data of **1** (Table 1) showed 27 carbon signals consisting of five quaternary carbons (including one oxygenated and two olefinic carbons), ten methines (including four oxygen-bearing and one olefinic carbons), nine methylenes (including one oxygen-bearing and one olefinic carbons), and three methyls, which clearly indicated a C_{27} sterol skeleton. The quaternary carbon signal at δ 109.6 (C-22) is characteristic for a spirostanol [12]. The ^1H NMR spectrum of **1** displayed an olefinic signal (δ 5.70, d, *J* = 4.6 Hz), an exomethylene signal [δ 4.97, 5.11 (each d, *J* = 2.6 Hz)], six proton signals attached to oxygenated carbons [δ 4.79 (dd, *J* = 9.7, 5.9 Hz), 4.54 (d, *J* = 5.9 Hz), 3.94 (m), 3.76 (dd, *J* = 11.8, 3.8 Hz), 3.55 (dd, *J* = 10.9, 13.7 Hz), 3.45 (dd, *J* = 4.0, 13.7 Hz)], and three methyl signals [δ 1.37 (s), 0.57 (d, *J* = 7.9 Hz), 1.01 (d, *J* = 7.4 Hz)]. Comparison of the ^{13}C NMR data of **1** with those of ruscogenin (**3**) [12], indicated that the signals arising from rings A–B and E–F were similar to each other, but the chemical shifts of rings C and D differed substantially. The additional oxygen-bearing carbon signal at δ 79.9 (d) was assigned to C-15 by detailed analysis of the 1D NMR, HMQC, and ^1H–^1H COSY spectra, which showed proton–proton correlations of H_{21}–H_{20}–H_{17}–H_{16}–H_{15}, and indicated that C-15 of **1** was hydroxylated. We conclude these observations that **1** has 1,3,15-trihydroxy substitution and 5-ene. However, unlike **3**, the ^1H NMR spectrum of **1** showed the presence of only one methyl singlet and two methyl doublets ascribed to the methyl groups attached at C-10, C-20, and C-25, in addition to an exomethylene group [δ 102.9 (t) and 159.1 (s)] in ^{13}C NMR spectrum. These observations suggested that the C-18 methyl group of the steroidal skeleton had become an exomethylene in the abeo-type skeleton. In the HMBC spectrum of **1** (Fig. 2), correlations of the exomethylene protons at δ 4.97 and 5.11 (H-18) with δ 57.1 (C-14), and 52.9 (C-17), and δ 4.54 (H-15) with δ 34.0 (C-12), confirmed that

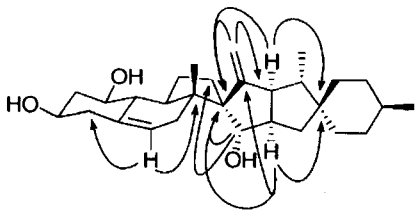
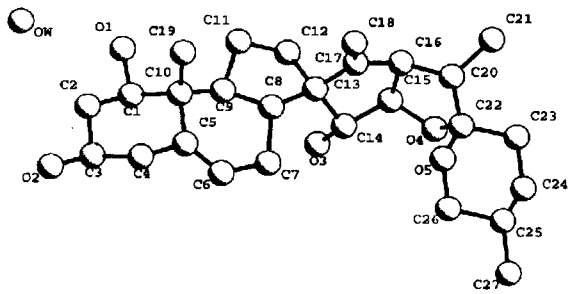


Fig. 2 – Important HMBC correlations of dracaenogenin A (1).



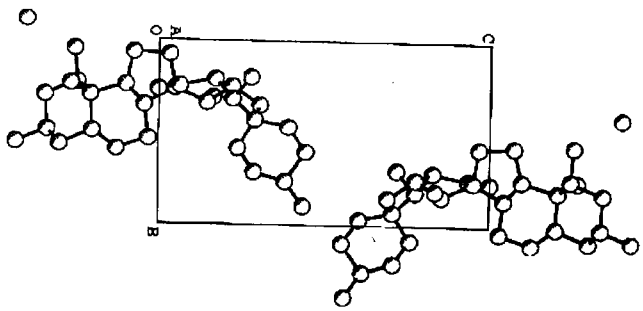


Fig. 3 – X-Ray crystallographic structure of 1.

the C-ring of **1** was cleaved between C-12 and C-13 to form the 12(13→14)abeospirostanol structure.

The similarity of the NMR data for the F-ring with those of **3** and the IR spectral evidence (band at 1369 cm^{-1} due to symmetric deformation of the C-25 equatorial Me-group, and the greater intensity of the band at 882 cm^{-1} than that at 917 cm^{-1}) revealed the C-25 of **1** to have R configuration [13]. Furthermore, the single crystal X-ray diffraction confirmed the novel *seco*-ring-C skeleton of **1** and established the S configuration at C-14 (Fig. 3). Therefore, the structure of dracaenogenin A (**1**) was established as (14S,25R)12(13→14)abeospirost-5,13(18)-dien-1β,3β,15α-triol.

Compound **2**, was shown to have a molecular formula of $C_{27}H_{42}O_6$ by positive HRESIMS (found: 463.3070 $[M+H]^+$, calcd: 463.3059) combined with ^{13}C NMR and DEPT, indicating six degrees of unsaturation. The ^1H NMR spectrum of **2** displayed one olefinic proton [δ 5.82 (d, J = 4.80 Hz)], six protons attached to oxygenated carbons [δ 4.74 (dd, J = 9.84, 6.30 Hz), 4.58 (1H, brs), 3.98 (m), 3.76 (brd, J = 11.84 Hz), 3.52 (t, J = 10.86 Hz), 3.36 (brd, J = 9.56 Hz)], two singlet methyls [δ 1.34, 1.35 (each s)], and two doublet methyls [δ 0.49 (d, J = 6.43 Hz), 0.95 (d, J = 7.35 Hz)]. Observed in the ^{13}C NMR (including DEPT) spectra of **2** were five quaternary carbons including two oxygenated and one olefinic carbons, 10 methines including four oxygen-bearing carbons and one olefinic carbons, eight methylenes including an oxygen-bearing one, and four methyls, which clearly indicated a C_{27} sterol skeleton. The characteristic quaternary carbon signal at δ 109.75 (C-22) indicated **2** to be a spirosterol [12]. The similarity of NMR spectral signals of **2** with those of **3** led to the assignment of 1,3-dihydroxy substitution and 5-ene in **2**. However, unlike **3**, compound **2** showed two more hydroxyl substitutions. In addition, the signals ascribed to ring-D in **3** were significantly changed in **2**. The signal ascribed to C-16 (δ 82.0) in **3** was shifted significantly downfield to δ 90.5 in **2** [12]. Moreover, instead of the methine and methylene in **3**, an oxy-bearing quartenary carbon and an oxygenated methine were tentatively ascribed to C-14 and C-15 for **2**. These NMR features led to assignment of two hydroxyl substitutions on C-14 and C-15, which was further supported by analysis of 2D NMR including ^1H–^1H COSY, HMBC and HMQC spectral data. The 25R configuration was deduced by comparison of the NMR data with those of **3**, combined with IR spectral evidence (band at 888 cm^- stronger than band at 917 cm^-) [13].

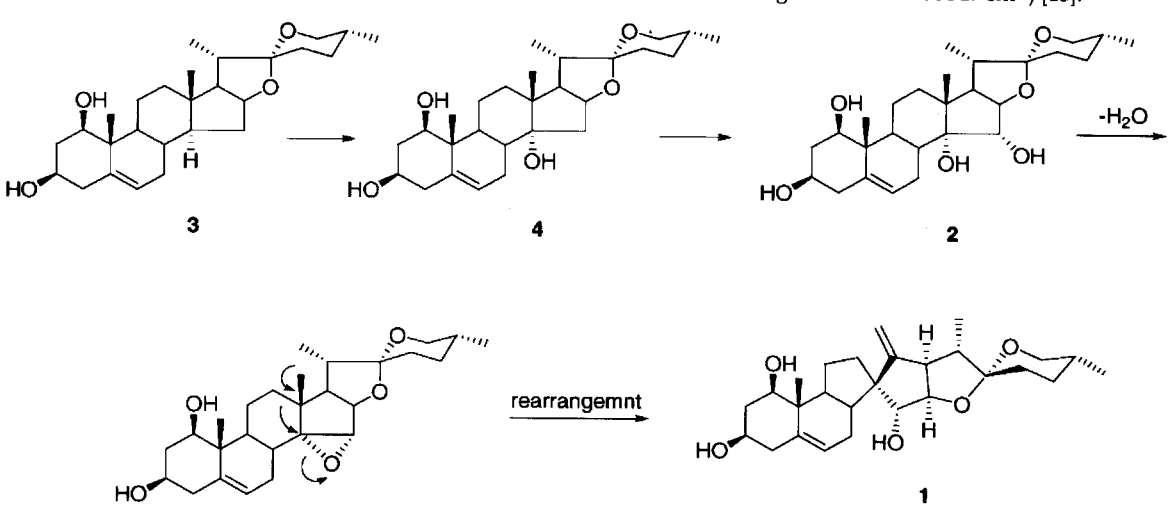


Fig. 4 – Postulated biogenesis of 1.

The relative stereochemistry of **2** was determined by a ROESY experiment (Table 2). The C-15 hydroxyl group was assigned to be in the α-orientation due to the presence of a ROESY correlation between H-15 and H-20, as well as a broad singlet of H-15. Accordingly, **2** was elucidated to be (25R) spirost-5-ene-1β,3β,14α,15α-tetrol, and was named dracaenogenin B.

The C_{27} steroidal glycosides are widely distributed in the plant kingdom [14]. Ruscogenin and its glycosides occur mainly in Liliaceae and some genus of Agavaceae (*Dracaena*, *Sanseveria*, *Nolina*, etc.). Up to now, many steroid sapogenins with multi-hydroxyl substitution, such as namogenin were obtained from the genus *Dracaena* [15,16]. Dracaenogenin A (**1**) represents the first example with a novel 12(13→14)abeospirostane skeleton in nature. Generally, the 1,3-dihydroxy-5-ene spirostane skeleton is relatively stable. Endomycorrhiza may play an important role in the biogenesis of dracaenogenin A while the red resin (Chinese Dragon's blood) formed from the plant. As shown in Fig. 4, a plausible biosynthetic origin for the skeleton of compound **1** is proposed, in which the carbon skeleton of **1** is biosynthesized from ruscogenin (**3**) [16], through namogenin (**4**) [15] and dracaenogenin B (**2**).

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