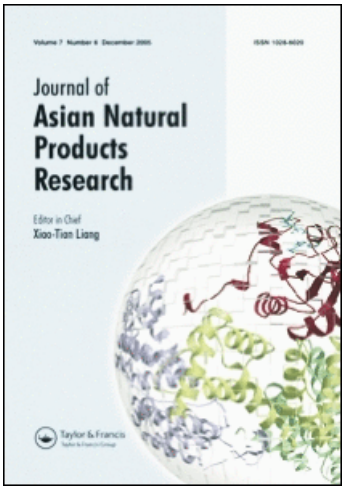


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# PREGNANE GLYCOSIDES FROM *DRACAENA COCHINCHINENSIS*

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Two new pregnane glycosides, named dracaenoside C and D, were isolated from the fresh stems of *Dracaena cochinchinensis*. Their structures were established as 3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (**1**) and 3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside (**2**) by means of 2D NMR spectral and chemical methods.

*Keywords*: Pregnane glycosides; Dracaenoside C; Dracaenoside D; *Dracaena cochinchinensis*; Agavaceae

## INTRODUCTION

Dragon's blood was used as a traditional Chinese medicine for more than 1500 years [1]. In China, the resin of *Dracaena cochinchinensis* S. C. Chen, named "longxuejie" was used as dragon's blood by local people for the treatment of diarrhea, diabetes, inflammation, bleeding, and as tonics. Chemical studies revealed that the resin contained a large amount of phenolic compounds, and some types of steroids, and aliphatic acids [2–6]. Steroidal saponins were also isolated from the fruits of *D. cochinchinensis* [7]. In a systematic chemical investigation on the resin and the original plants of dragon's blood we have reported two new C-22 steroidal lactone glycosides (dracaenoside A and B) from the fresh stems of this plant [8]. Further studies on the same plant led to the isolation of two new pregnane glycosides, named as dracaenoside C and D. Their structures were established as 3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (dracaenoside C, **1**) and 3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside (dracaenoside D, **2**) by means of 2D NMR spectral and chemical methods (Fig. 1).

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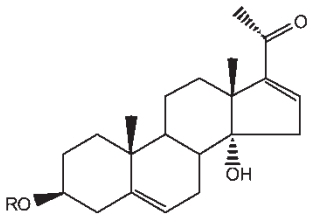


**1** α-L-rha*p* (1→2)[α-L-rha*p* (1→**4**)]-β-D-glc*p*

**2** α-L-rha*p* (1→2)[β-D-glc*p* (1→3)]-β-D-glc*p*

FIGURE 1 Structures of **1** and **2**.

## RESULTS AND DISCUSSION

Dracaenoside C was obtained as a white amorphous powder, $[\alpha]_D^{21}$ −41.90 in MeOH. Negative ion FABMS (a quasi-molecular peak at *m/z* 783 $[M-H]^+$) combined with $^{13}C$ NMR and DEPT indicated the molecular formula $C_{39}H_{60}O_{16}$. Acid hydrolysis with 1 M HCl in dioxane (v/v, 1:1) gave rhamnose and glucose in a ratio of about 2:1, which were further determined to be L-rhamnose and D-glucose by GC-MS analysis of a trimethylsilylated derivative of the acid hydrolysate (retention time for L-rhamnose of 7.17 min, D-glucose of 11.43 min), when compared with standard sugars. The $^{13}C$ and $^1H$ NMR spectra also exhibited the presence of two α-L-rhamnopyranosyl groups [anomeric protons at $\delta$ 5.84 (1H, brs) and $\delta$ 6.38 (1H, brs); secondary methyl groups at $\delta$ 1.61 (3H, d, $J = 6.04$ Hz) and 1.76 (3H, d, $J = 6.32$ Hz); anomeric carbons at $\delta$ 102.95 and 102.11] and a β-D-glucopyranosyl group [anomeric proton at $\delta$ 4.91 (1H, d, $J = 6.0$ Hz); anomeric carbon at $\delta$ 100.25]. Besides the sugar signals, 21 carbon peaks were observed in the $^{13}C$ NMR spectrum, which implied a $C_{21}H_{30}O_3$ composition for the aglycone. Preliminary analysis of the $^1H$ and $^{13}C$ NMR of the aglycone part led to the assignment of three tertiary methyl groups at $\delta$ 1.12, 2.15, 1.14 (each 3H, s), two olefinic protons at $\delta$ 5.27 (1H, brd) and $\delta$ 6.72 (1H, brs), indicating dracaenoside C to be a pregnane type glycoside. In the $^{13}C$ NMR spectrum, a ketone signal at $\delta$ 196.79 ascribed to C-20 was deduced by the downfield shift of a tertiary methyl group ($\delta$ 2.15), and its upfield shift revealed an olefinic group at C-16 (17). Another olefinic group was ascribed to C-5 by the downfield shift of the tertiary methyl groups (C-19) at $\delta$ 19.14. The assignment of the carbinol groups at C-3, C-14 was suggested by the resonance at $\delta$ 77.83, 82.78, by comparison with literature data [8]. Analysis of the HMBC spectrum with $^1H$-$^1H$ COSY and HMQC spectra led to the assignment of the aglycone carbons. The conformations of the ring junction, B/C *trans*, C/D *trans*, were ascertained by the ROESY correlations observed between H-8 and H-19, H-8 and H-11β, H-8 and H-18. The 3β orientation of the oxygen atom was confirmed by the ROESY observed between H-3 and H-1ax, H-3 and H-4eq (Fig. 2).

Assignment of the sugar moieties was performed by the $^1H$-$^1H$ COSY and HMQC-TOCSY spectra combined with the HMBC spectrum. The respective linkage positions of the inner glucopyranosyl and the rhamnopyranosyl were at C-3 of the aglycone and C-2′ and C-4′ of the glucose moiety by observing the $^3J$ correlations of each anomeric proton signal with the corresponding carbon signal [$\delta_H$ 4.91 to $\delta_C$ 77.935 (C-3); $\delta_H$ 5.84 to $\delta_C$ 78.136 (C-2′), and $\delta_H$ 6.38 to $\delta_C$ 77.937 (C-4′), respectively].

All of the above results led to the full assignments of $^{13}C$ NMR data for dracaenoside C. The structure of this new glycoside was therefore elucidated as

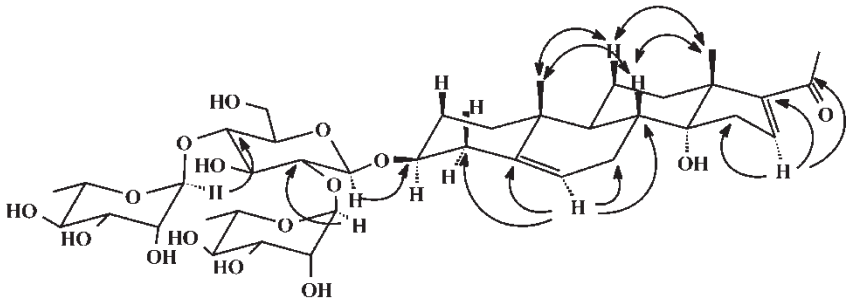

FIGURE 2 Selected HMBC and ROESY correlations of dracaenoside C ( → signal for HMBC; ← → signal for ROESY).



3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (**1**).

Dracaenoside D was obtained as a white amorphous powder, $[\alpha]_D^{21}$ −25.17 in MeOH. Negative ion HR-FABMS gave a quasi-molecular peak at *m/z* 799.3772 corresponding to a molecular formula of $C_{39}H_{60}O_{17}$. The $^{13}C$ and $^{1}H$ NMR spectra of dracaenoside D were closely related to those of dracaenoside C except for the sugar moieties, indicating that dracaenoside D is also a pregnane-type glycoside with an identical aglycone to that of dracaenoside C. The $^{13}C$ and $^{1}H$ NMR spectra showed the presence of a rhamnose and two glucose moieties in dracaenoside D, instead of the two rhamnose and a glucose in dracaenoside C. This was confirmed by acid hydrolysis of dracaenoside D which gave rhamnose and glucose in a ratio of 1:2. D-Glucose and L-rhamnose moieties were determined by GC-MS analysis, as for dracaenoside C. By comparison of the NMR data with those of dracaenoside C, the rhamnose moiety was deduced to be linked at the C-2′ position of the inner glucose and the terminal glucose moiety was deduced to be linked at C-3′ position of the inner glucose moiety by the significant downfield shift of C-3′ to 89.536. These assignments of the sugar moieties were consistent with the reference data [9]. Consequently, dracaenoside D was determined as 3β,14α-dihydroxypregna-5,16(17)-diene-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]-β-D-glucopyranoside (**2**).

Dracaenoside C and D are new naturally occurring C-21 pregnane glycosides.

## EXPERIMENTAL

### General Experimental Procedures

NMR spectra were run on Bruker AM-400 (400 MHz for $^{1}H$ NMR and 100 MHz for $^{13}C$ NMR) and DRX-500 (500 MHz for 2D NMR) instruments with TMS as internal standard; IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. FAB-MS spectra were recorded on a VG Auto Spec-300 spectrometer. UV spectra were obtained on a Shimadzu double-beam 210A spectrophotometer. GC-MS was run on Fisons MD-800 GC/MS instruments using a 30QC2/AC-5 fused silica capillary column (30 m × 0.25 mm) under the following condition: filament current: 4.2 A; column temperature: 180°C/260°C, programmed increase of 5°C $min^{-1}$; carrier gas: He; head pressure: 12 psi; EI-MS: 70 eV; ion source temperature: 250°C. Silica gel (200–300 mesh and 10–40 μm), RP-18 (40–63 μm) and Sephadex LH-20 were used for column chromatography.

## Plant Material

The fresh stems of *D. cochinchinensis* were collected at Xishuangbanna, Yunnan, China. A voucher specimen is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in the west China, Kunming Institute of Botany, Chinese Academy of Sciences.

## Extraction and Isolation

The fresh stems (25.0 kg) were chipped and extracted with MeOH at 60°C. After removing of the MeOH under reduced pressure, the viscous concentrate was partitioned between $H_2O$ and n-BuOH. Column chromatography of the n-BuOH layer on silica gel, eluting with a gradient mixture of $CHCl_3$–MeOH–$H_2O$, and finally with MeOH, gave five fractions (I–V). Fraction III was chromatographed over ODS silica gel, eluting with MeOH–$H_2O$, and silica gel with $CHCl_3$–MeOH–$H_2O$ to give dracaenoside C (30 mg) and dracaenoside D (16 mg).

*Dracaenoside C*. (**1**) $C_{39}H_{60}O_{16}$, is an amorphous white powder, $[\alpha]_D^{21}$ −41.90 (MeOH, *c* 0.2); Negative ion FAB-MS *m/z* 783$[M-H]^+$; UV (MeOH) $\lambda_{max}$ (nm): (log $\varepsilon$): 237; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3433 (br, OH), 2933 (CH), 1652 (C═O), 1374, 1041, 887; $^{13}C$ NMR: see Table I; $^1H$ NMR see Table II.

*Acid hydrolysis of dracaenoside C*. A solution of dracaenoside C (5 mg) in 1 M HCl (dioxane–$H_2O$, 1:1, 1 ml) was heated at 100°C for 2 h. After removing the solution under reduced pressure, the residue was suspended with $H_2O$ and extracted with chloroform 3 times. The monosaccharide fraction was dried and dissolved in pyridine (5 ml). Then trimethylchlorosilane (0.5 ml) was added and the mixture was kept at room temperature for 30 min. After drying under reduced pressure, the reaction mixture was extracted with ether (0.5 ml), and the ether layer was analyzed by GC-MS. The retention time of L-rhamnose and D-glucose were 7.17 and 11.43 min, respectively.

*Dracaenoside D*. $C_{39}H_{60}O_{17}$, is an amorphous white powder, $[\alpha]_D^{21}$ −25.17 (MeOH, *c* 0.4). Negative ion FAB-MS: *m/z* 799 $[M-H]^+$; 637 ($M^+$–162–H). Negative ion

TABLE I $^{13}C$ NMR spectral data of dracaenoside C (**1**) and D (**2**) (100 MHz, in pyridine-$d_5$)

| | *C* | *D* | | *C* | *D* |
|---|---|---|---|---|---|
| 1 | 37.61 | 37.60 | | Glc (inner) | Glc (inner) |
| 2 | 30.24 | 30.12 | 1′ | 100.25 | 99.88 |
| 3 | 77.94 | 78.53 | 2′ | 78.14 | 77.91 |
| 4 | 39.10 | 38.80 | 3′ | 76.96 | 89.54 |
| 5 | 140.89 | 140.82 | 4′ | 77.94 | 69.59 |
| 6 | 122.37 | 122.41 | 5′ | 78.00 | 78.74 |
| 7 | 26.79 | 26.79 | 6′ | 61.29 | 62.45 |
| 8 | 34.13 | 34.13 | | Rha | Rha |
| 9 | 43.69 | 43.65 | 1″ | 102.11 | 102.28 |
| 10 | 37.68 | 37.60 | 2″ | 72.59 | 72.53 |
| 11 | 19.87 | 19.84 | 3″ | 72.79 | 72.84 |
| 12 | 26.79 | 26.79 | 4″ | 74.17 | 74.15 |
| 13 | 52.40 | 52.39 | 5″ | 69.61 | 69.59 |
| 14 | 82.78 | 82.76 | 6″ | 18.72 | 18.76 |
| 15 | 41.79 | 41.77 | | Rha | Glc |
| 16 | 143.22 | 143.16 | 1‴ | 102.95 | 104.58 |
| 17 | 153.52 | 153.53 | 2‴ | 72.59 | 74.99 |
| 18 | 20.51 | 20.49 | 3‴ | 72.91 | 77.14 |
| 19 | 19.14 | 19.12 | 4‴ | 73.97 | 71.52 |
| 20 | 196.79 | 196.76 | 5‴ | 70.46 | 77.67 |
| 21 | 27.13 | 27.12 | 6‴ | 18.57 | 62.45 |

TABLE II $^{1}$H NMR spectral data of dracaenoside C (**1**) and D (**2**) (400 MHz, in pyridine-$d_5$, $J$ in Hz)

| | *C* | *D* |
|---|---|---|
| 1 | 1ax,1.00, m; 1eq,1.70, m | 1ax, 0.98, m; 1eq, 1.66, m |
| 2 | 2ax,1.82, m; 2eq, 2.03, m | 2eq, 2.03, brd; 2ax, 1.76, m |
| 3 | 3.83, m | 3.90, m |
| 4 | 4eq2.80, dd, $J$ = 3.6, 9.6 Hz; 4ax, 2.74, dd, $J$ = 11.0, 11.8 Hz | 4eq, 2.80, m; 4ax, 2.76, m |
| 6 | 5.27, brd, $J$ = 4.0 Hz | 5.45, brd, $J$ = 2.8 Hz |
| 7 | 1.89, m; 2.55, m | 1.85, m; 2.55, m |
| 8 | 1.98, m | 1.91, m |
| 9 | 2.00, m | 1.93, m |
| 11 | 1.62, m | 1.62, m |
| 12 | 12ax, 2.59, m; 12eq, 2.45, m | 12eq, 2.45, m; 12ax, 2.59, m |
| 15 | 15ax, 2.54, brd, $J$ = 14.84 Hz; 15eq, 2.51, brd, $J$ = 14.84 Hz | 15ax, 2.54, brd, $J$ = 15.0 Hz; 15eq, 2.51, brd, $J$ = 15.0 Hz |
| 16 | 6.72, brs | 6.71, brs |
| 18 | 1.14, s | 1.14, s |
| 19 | 1.12, s | 1.13, s |
| 21 | 2.15, s | 2.15, s |
| | Glc (inner) | Glc (inner) |
| 1′ | 4.96, d, $J$ = 6.4 Hz | 4.90, d, $J$ = 6.4 Hz |
| 2′ | 4.38, m | |
| 3′ | 4.19, m | |
| 4′ | 4.20, m | |
| 5′ | 3.62, brs | |
| 6′ | 4.06, dd, $J$ = 12.4, 3 Hz | |
| | Rha | Rha |
| 1″ | 6.38, brs | 6.38, brs |
| 2″ | 4.83, brs | 4.82, brs |
| 3″ | 4.63, dd, $J$ = 9.30, 3.2 Hz | 4.57, dd, $J$ = 9.32, 3 Hz |
| 4″ | 4.35, m | 4.32, m |
| 5″ | 4.91, m | 4.82, m |
| 6″ | 1.76, d, $J$ = 6.16 Hz | 1.76, $J$ = 6.16 Hz |
| | Rha | Glc |
| 1‴ | 5.84, brs | 5.08, d, $J$ = 7.46 Hz |
| 2‴ | 4.67, brs | |
| 3‴ | 4.53, dd, $J$ = 9.32, 3 Hz | |
| 4‴ | 4.32, m | |
| 5‴ | 4.90, m | |
| 6‴ | 1.61, d, $J$ = 6.04 Hz | |

HR-FABMS: *m/z* 799.3772 ($[M-H]^+$; calcd. 799.3752). UV (MeOH) $\lambda_{max}$ (nm) (log $\varepsilon$): 238. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3430 (br, OH), 2932, 1652 (C═O), 1373, 1048, 888. $^{13}$C NMR: see Table I; $^{1}$H NMR: see Table II.

*Acid hydrolysis of dracaenoside D.* Dracaenoside D (5 mg) was subjected to acid hydrolysis as described for dracaenoside C to give a mixture of monosaccharides. The monosaccharides were identified as D-glucose and L-rhamnose by direct TLC comparison with authentic samples and GC-MS analysis of the corresponding trimethylsilylated derivatives. The retention times of L-rhamnose and D-glucose were 7.17 and 11.43 min, respectively.

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