

# ***In Vivo* Anti-Inflammatory Effect of a New Steroidal Saponin, Mannioside A, and Its Derivatives Isolated from *Dracaena mannii***

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## **INTRODUCTION**

The family Dracaenaceae (Agavaceae) with more than 480 species has a distribution in the tropic and subtropic dry climate regions throughout the world (Mimaki *et al.*, 1998). Many species of the genus *Dracaena* are used in African traditional medicine for the treatment of a variety of diseases. *Dracaena mannii* bark's decoction is used by the native people of the western highlands of Cameroon against abdominal pains. Furthermore, the mixture of its roots with "palm wine" is used in Cameroonian ethno-medicine for the treatment of male impotency (Noumi *et al.*, 1998). The occurrence of steroidal saponins in several Dracaenaceae plants is well documented (Mimaki *et al.*, 1999; Okunji *et al.*, 1991). Steroidal saponins are a class of natural products with strong biological and pharmacological effects. Some recently isolated steroidal saponins have been shown to possess antifungal, cytotoxic and antitumor effects (Zhang *et al.*, 2006; Sautour *et al.*, 2004; 2007) but only very few reports have been made regarding

their anti-inflammatory effect. In our ongoing search for new and/or potentially bioactive saponins from Cameroonian medicinal plants (Taponjougou *et al.*, 2002; 2003; 2005; Teponno *et al.*, 2006), the stem bark of *D. mannii* growing in the western highlands of Cameroon has been studied. In this study we found that the extracts of *D. mannii* exhibited significant *in vivo* anti-inflammatory activity. In the present paper we describe the isolation and structural elucidation of steroidal saponins from *D. mannii* and their structure-activity relationships on the *in vivo* anti-inflammatory test.

## **MATERIALS AND METHODS**

### **General procedure**

<sup>1</sup>H-NMR spectra were recorded in deuterated solvents (CD<sub>3</sub>OD and pyridine-d<sub>5</sub>) on a Bruker AMX-400 Spectrometer at 400 MHz while <sup>13</sup>C-NMR spectra were recorded in the same solvents and the same apparatus at 100 MHz. All chemical shifts (*d*) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (*J*) are in Hz. ESI-MS were taken on a LCQ Thermofiningan Apparatus. IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. Column chromatography

was performed using silica gel 60 Merck (0.040-0.063 mm) and sephadex LH-20. TLC was carried out on precoated Kieselgel 60 F<sub>254</sub> (Merck) plates which were first viewed with an ultraviolet lamp MULTIBAND UV- 254/365 nm for fluorescent spots and thereafter developed by spraying with 50% H<sub>2</sub>SO<sub>4</sub> and heating for 10 mins at 110°C. Solvents were distilled prior to use.

### Plants material

The stem bark of *Dracaena mannii* was collected in Dschang (West province of Cameroon) in March 2006 and authenticated by Dr G. Achoundong of the Cameroon National Herbarium, Yaoundé where specimens documenting the collection are deposited (Ref:12485/SRF/CAM).

### Extraction and isolation

The dried and pulverized stem bark of *Dracaena mannii* (2.5 Kg) was extracted three times (each time for 24 h) with 95% EtOH. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (252 g). Part of this extract (160 g) was suspended in water (300 mL) and extracted with EtOAc (26.3 g) and *n*-butanol (66.8 g), respectively. Part of the *n*-butanol extract (64.1 g)

was subjected to column chromatography on silica gel, with EtOAc-MeOH-H<sub>2</sub>O (85:15:5) as eluent, yielding four fractions (A-D). The fraction (B, 11.9 g) with steroid saponins was further subjected to repeated silica gel column chromatography (EtOAc-MeOH-H<sub>2</sub>O, 95:5:2) to afford compounds **3** (20 mg), **1** (900 mg), and **4** (9.57 g). Part of the EtOAc extract (24.4 g) was subjected to silica gel column chromatography and elution was performed with hexane-EtOAc increasing polarity to afford five main fractions (I-V). From fraction II (Hexane-EtOAc (8:2)) (4.00 g), compound **2** (152 mg) was obtained after further purification over silica gel column chromatography eluted with hexane-EtOAc (8:2) and recrystallisation in CH<sub>2</sub>Cl<sub>2</sub>.

### Pennogenin-3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranoside (**1**)

White amorphous powder; ESI-MS: *m/z* 761 [M+Na]<sup>+</sup>, IR  $\nu_{\text{max}}^{\text{KBr}}$  (cm<sup>-1</sup>): 3325, 2950, 1733, 1456, 1081; <sup>1</sup>H-NMR (400 MHz, pyridine-d<sub>5</sub>):  $\delta$  0.55 (3H, d, *J* = 5.0 Hz, H-27), 0.77 (3H, s, H-19), 0.82 (3H, s, H-18), 1.08 (3H, d, *J* = 7.0 Hz, H-21), 1.57 (3H, d, *J* = 6.0 Hz, H-6''), 3.74 (1H, m, H-3), 4.76 (1H, d, *J* = 7.5 Hz, H-1'), 5.15 (1H, brs, H-6), 6.10 (1H, brs, H-1''), <sup>13</sup>C-NMR (100 MHz, pyridine-d<sub>5</sub>): Table I.

**Table I.** <sup>13</sup>C-NMR data (ppm, 125 MHz) of compounds **1-4** isolated from the *Dracaena mannii* stem bark

Carbon	1	2	3	4	Carbon	1	2	3	4
1	37.5	38.5	37.8	37.4	24	28.7	29.4	29.1	28.6
2	30.0	33.2	30.3	29.9	25	30.3	33.3	30.7	30.3
3	78.1	72.4	78.1	77.9	26	66.6	67.7	67.0	66.7
4	39.2	43.0	38.9	38.6	27	17.1	17.5	17.6	17.2
5	140.7	142.3	141.0	140.7	3-O-Gluc 1'	102.1		105.9	99.8
6	121.7	122.1	122.2	121.7	2'	75.6		75.9	78.2
7	32.2	32.3	32.4	31.7	3'	83.5		78.7	87.4
8	32.4	32.8	32.7	32.0	4'	69.8		71.8	70.5
9	50.0	51.4	50.5	50.1	5'	78.2		78.4	77.7
10	36.9	37.7	37.4	37.0	6'	62.4		62.9	62.2
11	20.9	21.7	21.2	20.8	3'-O-Rha 1''	102.8			102.5
12	31.9	32.5	32.1	31.9	2''	72.5			72.3
13	45.0	45.5	45.5	45.0	3''	72.6			72.5
14	53.0	53.9	53.3	52.9	4''	74.1			73.5
15	31.9	31.2	32.1	31.9	5''	69.6			69.8
16	89.9	90.6	90.3	89.9	6''	18.6			18.3
17	90.0	91.2	90.5	90.0	2'-O-Rha 1'''				103.7
18	17.1	17.4	17.5	17.0	2'''				72.4
19	19.3	18.8	19.7	19.3	3'''				72.7
20	44.7	45.8	45.1	44.7	4'''				73.7
21	9.6	9.0	10.1	9.6	5'''				69.8
22	109.7	110.9	110.2	109.7	6'''				18.5
23	32.4	32.0	32.7	32.3					
Solvent	C <sub>5</sub> D <sub>5</sub> N	CD <sub>3</sub> OD	C <sub>5</sub> D <sub>5</sub> N	C <sub>5</sub> D <sub>5</sub> N	Solvent	C <sub>5</sub> D <sub>5</sub> N	CD <sub>3</sub> OD	C <sub>5</sub> D <sub>5</sub> N	C <sub>5</sub> D <sub>5</sub> N

## Pennogenin (2)

White amorphous powder; ESI-MS:  $m/z$  883  $[2M+Na]^+$ , 413  $[M-H-H_2O]$ ; IR  $\nu_{\max}^{KBr}$  ( $cm^{-1}$ ): 3531, 2871, 1728, 1456, 1057;  $^1H$ -NMR (400 MHz,  $CD_3OD$ ):  $\delta$  0.80 (3H, d,  $J = 5.6$  Hz, H-27), 0.84 (3H, s, H-18), 0.89 (3H, d,  $J = 7.1$  Hz, H-21), 1.07 (3H, s, H-19), 3.26 (1H, m, H-3), 3.86 (1H, t,  $J = 7.8$ , H-16), 5.20 (1H, brs, H-6);  $^{13}C$ -NMR (100 MHz,  $CD_3OD$ ): Table I.

## Pennogenin-3-O- $\beta$ -D-glucopyranoside (floribundasaponin A) (3)

White amorphous powder; ESI-MS:  $m/z$  615  $[M+Na]^+$ ; IR  $\nu_{\max}^{KBr}$  ( $cm^{-1}$ ): 3415, 2971, 1725, 1430, 1050;  $^1H$ -NMR (400 MHz, pyridine- $d_5$ ):  $\delta$  0.70 (3H, d,  $J = 5.7$  Hz, H-27), 0.98 (3H, s, H-18), 1.09 (3H, s, H-19), 1.26 (3H, d,  $J = 7.2$  Hz, H-21), 1.57 (3H, d,  $J = 6.0$  Hz, H-6''), 3.85 (1H, m, H-3), 4.90 (1H, d,  $J = 7.6$  Hz, H-1'), 5.28 (1H, brs, H-6);  $^{13}C$ -NMR (100 MHz, pyridine- $d_5$ ): Table I.

## Pennogenin-3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside (spiroconazole A) (4)

White amorphous powder; ESI-MS:  $m/z$  883  $[M - H]^-$ , 737  $[M - H - 146]$ , 591  $[M - H - 2 \times 146]$ ; IR  $\nu_{\max}^{KBr}$  ( $cm^{-1}$ ): 3435, 3006, 2918, 1660, 1437, 1406, 1029, 955, 706;  $^1H$ -NMR (400 MHz, pyridine- $d_5$ ):  $\delta$  0.55 (3H, d,  $J = 5.5$  Hz, H-27), 0.82 (3H, s, H-19), 0.94 (3H, s, H-18), 1.09 (3H, d,  $J = 7.5$  Hz, H-21), 1.50 (3H, d,  $J = 6.0$  Hz, H-6''), 1.61 (3H, d,  $J = 6.5$  Hz, H-6'''), 3.74 (1H, m, H-3), 4.90 (1H, d,  $J = 7.6$  Hz, H-1'), 5.18 (1H, brs, H-6), 5.77 (1H, brs, H-1'''), 5.87 (1H, brs, H-1'');  $^{13}C$ -NMR (100 MHz, pyridine- $d_5$ ): Table I.

## Acid hydrolysis of compound 1

Compound 1 was hydrolysed in 5%  $H_2SO_4$  under reflux for 3 h. After neutralization with  $NH_4OH$  followed by extraction with  $CHCl_3$ , the aqueous layer was evaporated in vacuo to give a crude sugar residue. The resulting residue was analysed by TLC (silica gel,  $CHCl_3$ -MeOH- $H_2O$  (64:40:8) in comparison with standard sugars. The spots of the product on TLC were identical to those of D-glucose ( $R_f$  0.32) and L-rhamnose ( $R_f$  0.51).

## Experimental animals

Wistar rats of either sex, aged between 12 and 16 weeks and weighing about 200 g obtained from the animal house of the Faculty of Science, University of Dschang, were used in these studies. The animals were housed in colony cages. They were exposed to daily 12 h light-dark cycle and have free access to standard laboratory diet and water. The ethic committee of the Cameroon Ministry of Scientific Research and Technology which has adopted the guidelines established by the European Union on Animal Care and Experimentation (CEE Council 86/609),

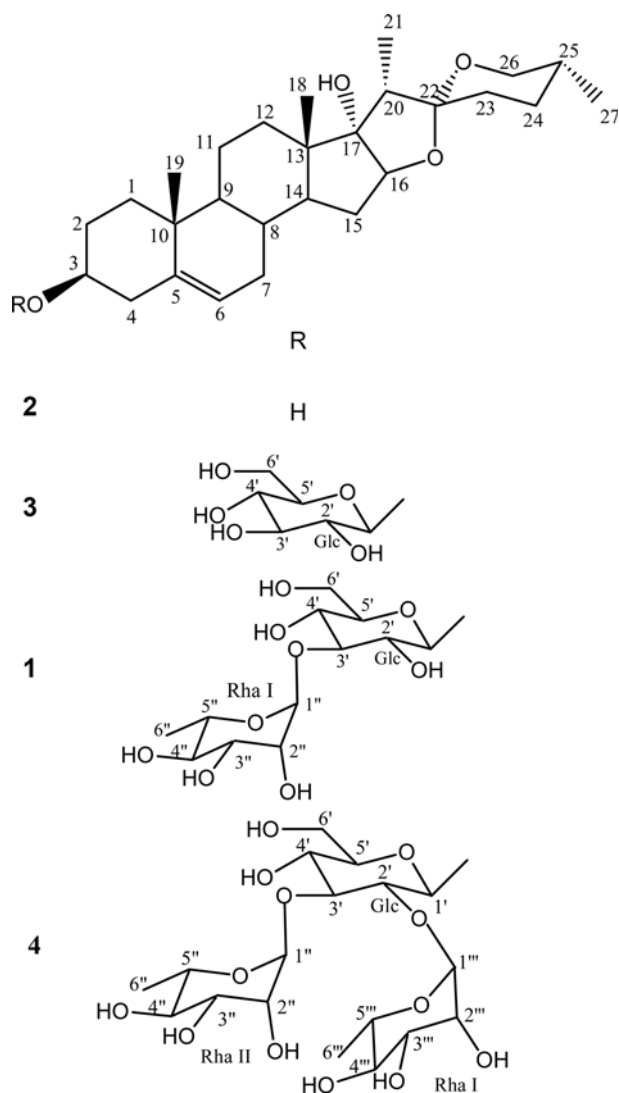


Fig. 1. Structures of the isolated compounds

approved all experimental procedures.

## Carrageenan-induced paw edema

The experiment was conducted as described by Winter *et al.* (1962). Animals were divided in 10 groups of 6 rats each. Groups 1 and 2 served as negative controls and received vehicles (distilled water, control<sub>1</sub> and the mixture of 2.5% DMSO and 2.5% tween 80, control<sub>2</sub>). Groups 3 and 4 (positive controls) received indomethacin (10 mg/kg) and aspirin (10 mg/kg) respectively. Groups 5 and 6 were treated respectively with the ethanol and n-butanol extracts at the dose of 600 mg/kg. Groups 7–10 received compounds 1 to 4 at the dose of 10 mg/kg. All the treatments were administered orally thirty minutes prior to the administration of 0.1 mL of carrageenan (1%, sub-plantarily). The volume of the paw was measured before treatments ( $V_0$ ) and 0.5, 1, 2, 3, 4, 5 and 6 h after carrageenan injection ( $V_t$ ) with the aid of a Ugo Basil Plethys-

mometer (7510). The edema was expressed as an increase in the volume of paw, and the percentage of inhibition for each rat and group was obtained as follow (Lanhers *et al.*, 1991):

$$\text{Percentage of inhibition} = \frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{exxay}}}{(V_t - V_0)_{\text{control}}} \times 100$$

## RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. Its ESIMS (positive mode) showed the pseudomolecular  $[M+Na]^+$  ion peak at  $m/z$  761 corresponding to the molecular formula  $C_{39}H_{62}O_{13}$ . The glycoside nature of **3** was shown by strong IR absorptions at 3325 and 1051  $cm^{-1}$  characteristic of hydroxyl groups and glycosidic linkages respectively (Dong *et al.*, 2001). The  $^{13}C$ -NMR spectrum showed 39 signals (Table I) among which 27 were assigned to the aglycone; the remaining 12 signals were indicative of the presence of two hexoses. The salient features of this spectrum were the presence of three signals at  $\delta$  140.7 (C-5), 121.7 (C-6) and 109.7 (C-22) characteristic of  $\Delta^5$ -spirostene type sapogenin (Agrawal *et al.* 1985). The structure of the aglycone moiety was recognized to be

pennogenin ( $3\beta,17\alpha$ -dihydroxyspirost-5-ene) by  $^1H$ - and  $^{13}C$ -NMR analysis (Table I) using connectivities observed in  $^1H$ - $^1H$  COSY, HMQC and HMBC spectra and was in full agreement with the literature data (Mahato *et al.*, 1981; Hufford *et al.*, 1988). TLC of the mild acid hydrolyzed **1** revealed the presence of glucose and rhamnose. Two anomeric carbon atoms detected at  $\delta$  102.9 (C-1'') and 102.2 (C-1') in the  $^{13}C$ -NMR spectrum, were shown to be attached to proton signals at  $\delta$  6.10 (1H, brs) and 4.76 (1H, d,  $J = 7.5$  Hz), respectively, in the HSQC experiment. The ring proton of each monosaccharide residue was assigned starting from the readily identifiable anomeric proton using  $^1H$ - $^1H$  COSY spectrum. Analysis of 2D experiments ( $^1H$ - $^1H$  COSY, HSQC and HMBC) followed by comparison of  $^1H$ - and  $^{13}C$ -NMR data of the sugar moiety with those reported in the literature (Dong *et al.*, 2001; Hufford *et al.*, 1988; Teponno *et al.*, 2006) revealed the presence of an inner glucopyranosyl residue and a terminal rhamnose (Table I). The coupling constants observed for the two anomeric proton signals at 4.76 (1H, d,  $J = 7.5$  Hz) and 6.10 (1H, brs) suggested that the linkages of the glucose and rhamnose were of the  $\beta$  and  $\alpha$  forms, respectively. The sequence of the sugar chains were obtained by HMBC experiment. Cross peak correlation observed in the HMBC spectrum between the anomeric proton at  $\delta_H$

**Table II.** Effects of aspirin, indomethacin, extracts and pennogenin derivatives from *Dracaena mannii* on rat hind paw edema induced by carrageenan

Group	Dose (mg/kg)	$\Delta V$ (mL) (Percentage inhibition)						
		0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control <sub>1</sub>	-	0.46 ± 0.12	0.81 ± 0.16	1.27 ± 0.23	1.90 ± 0.23	2.06 ± 0.21	1.99 ± 0.29	1.80 ± 0.22
Aspirin	10	0.83 ± 0.10 (31.76%)	1.07 ± 0.11 (41.43%)	1.29 ± 0.13 (49.51%)	1.69 ± 0.12 (40.25%)	1.97 ± 0.16 (32.32%)	2.04 ± 0.11 (30.89%)	1.90 ± 0.12 (30.38%)
Indométhacin	10	0.79 ± 0.08 (20%)	1.10 ± 0.08 (21.43%)	0.98 ± 0.06 (52.43%)	0.93 ± 0.09 <sup>c</sup> (60.99%)	0.98 ± 0.10 <sup>c</sup> (62.36%)	1.32 ± 0.11 (49.03%)	1.58 ± 0.04 <sup>c</sup> (32.91%)
Control <sub>2</sub>	-	0.84 ± 0.14	1.39 ± 0.19	2.05 ± 0.13	2.41 ± 0.12	2.62 ± 0.07	2.58 ± 0.10	2.36 ± 0.11
Ethanol extract	600	0.59 ± 0.09 (54.76%)	0.55 ± 0.09 <sup>y</sup> (74.10%)	0.71 ± 0.09 <sup>y</sup> (70.73%)	1.29 ± 0.09 <sup>y</sup> (53.94%)	1.08 ± 0.06 <sup>y</sup> (58.78%)	1.20 ± 0.13 <sup>y</sup> (62.01%)	1.03 ± 0.10 <sup>â</sup> (56.35%)
n-butanol extract	600	0.38 ± 0.02 (29.76%)	0.36 ± 0.05 <sup>y</sup> (60.43%)	0.60 ± 0.08 <sup>y</sup> (65.36%)	1.11 ± 0.09 <sup>y</sup> (46.47%)	1.08 ± 0.13 <sup>y</sup> (58.78%)	0.98 ± 0.12 <sup>y</sup> (53.49%)	1.03 ± 0.06 <sup>â</sup> (56.35%)
<b>1</b>	10	0.38 ± 0.13 (54.76%)	0.27 ± 0.10 <sup>y</sup> (80.57%)	0.74 ± 0.07 <sup>y</sup> (63.90%)	1.17 ± 0.02 <sup>y</sup> (51.45%)	1.12 ± 0.06 <sup>y</sup> (57.25%)	1.11 ± 0.08 <sup>y</sup> (56.98%)	0.85 ± 0.04 <sup>â</sup> (63.98%)
<b>2</b>	10	1.09 ± 0.14 (-29.76%)	0.97 ± 0.14 (30.21%)	1.79 ± 0.09 (12.68%)	2.17 ± 0.10 (9.96%)	2.19 ± 0.16 (16.41%)	2.12 ± 0.06 (17.83%)	2.04 ± 0.10 (13.56%)
<b>3</b>	10	0.42 ± 0.12 (50%)	0.40 ± 0.10 <sup>y</sup> (71.22%)	0.40 ± 0.10 <sup>y</sup> (54.15%)	1.40 ± 0.09 <sup>y</sup> (41.91%)	1.59 ± 0.22 <sup>y</sup> (39.31%)	1.45 ± 0.21 <sup>y</sup> (43.80%)	1.21 ± 0.14 <sup>y</sup> (53.99%)
<b>4</b>	10	0.44 ± 0.10 (47.62%)	0.47 ± 0.07 <sup>y</sup> (66.19%)	1.20 ± 0.17 <sup>â</sup> (41.46%)	1.85 ± 0.20 (23.24%)	1.76 ± 0.13 <sup>α</sup> (32.82%)	1.70 ± 0.04 <sup>β</sup> (34.11%)	1.57 ± 0.09 <sup>β</sup> (33.47%)

Each value represents the mean ± SEM of 6 animals; <sup>α</sup>P < 0.001 statistically significant compared to negative control<sub>1</sub>, <sup>α</sup>P < 0.05; <sup>β</sup>P < 0.01; <sup>γ</sup>P < 0.0001. Statistically significant compared to negative control<sub>2</sub>.

4.76 (H-1') and the carbon atom at  $\delta_C$  78.1 (C-3) showed that the glucopyranosyl moiety is linked at C-3 of the aglycone. The cross peak correlation observed between the signal of the anomeric proton at  $\delta_H$  6.10 (H-1'') and the carbon atom of the glucopyranosyl moiety at  $\delta_C$  83.5 (C-3') suggested the location of the rhamnopyranosyl unit at C-3'. The common D-configuration for glucose and L-configuration for rhamnose were assumed to be those of the most commonly encountered analogues in the plant kingdom. Thus, the structure of compound **1** was elucidated as pennogenin-3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranoside, a new natural secondary metabolite to which we assigned the trivial name mannoside A.

Compound **2** was isolated as white amorphous powder. It was identified as pennogenin by comparison of the ESI-MS,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data with those previously reported in the literature (McAnuff *et al.*, 2005). Compounds **3** and **4** were also obtained as white amorphous powders. They were respectively identified as pennogenin-3-O- $\beta$ -D-glucopyranoside (floribundasaponin A) and pennogenin-3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside (spiroconazole A) by comparison of their ESIMS,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data with those previously reported in the literature (Mahato *et al.*, 1981; Teponno *et al.*, 2006).

The sub-plantar injection of the carrageenan produced an inflammatory edema which increased gradually, reaching its maximum at the 4<sup>th</sup> hour after injection of the phlogistic agent (Table II). All the extracts presented significant ( $P < 0.001$ ) anti-inflammatory effects with maximum activity observed within 2 h. The ethanol and the n-butanol extracts exhibited an inhibitory percentage of 74 and 65%, respectively. The tested compounds showed, one hour after carrageenan injection, a maximum inhibitory activity of 80.57%, 71.22%, 66.19%, and 30.21% for compounds **1**, **3**, **4** and **2**, respectively. Although reduced, the significant activity was maintained during all the experimental period for compounds **1**, **3** and **4**. The indomethacin produced an anti-inflammatory activity which increased gradually to reach a maximum of inhibition (62.36%,  $P < 0.001$ ) at the 4<sup>th</sup> hour.

Additional information could be deduced from the structure-activity relationship of the tested pennogenin glycosides. Globally all the pennogenin glycosides (**1**, **3** and **4**) were more potent than pennogenin (**2**). Attachment of D-glucose to pennogenin was observed to significantly improve (from 30.21% to 71.22% edema reduction) the anti-inflammatory activity (compound **3**). One sugar moiety (rhamnose) substitution to C-3 of D-glucose in compound **1** led to a little higher activity (from 71.22% to 80.57% edema reduction) than that of compound **3** whereas, in compound **4**, two rhamnose moieties attached at both sites of C-2 and C-3 of D-glucose in compound **3** slightly

reduced the activity (from 71.22% to 66.19% edema reduction).

Although further studies are needed to ascertain the biomedical and molecular mechanisms underlying the anti-inflammatory activity of these compounds, the present study has documented the anti-inflammatory properties of *Dracaena mannii* stem bark. Furthermore, it has been demonstrated that pennogenin glycosides play a significant role in the overall anti-inflammatory response induced by this plant.

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