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Steroidal saponins from the roots of *Dracaena marginata* tam

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ABSTRACT

A phytochemical investigation on the roots of *Dracaena marginata* yielded nine steroidal saponins. Six of the saponins were isolated as three pairs of 22-hydroxy furostanol saponins and their 22-methoxy derivatives including two new minors identified as 26-O- β -D-glucopyranosyl-22 α -hydroxy-(25R)-furost-5-en-3 β , 26-diol-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranoside and its 22-methoxy derivative. The structures of the saponins were generally determined by 1D and 2D NMR data and acid hydrolysis. The cytotoxic activity of the saponin fraction and the isolates were tested against the two human tumor cell lines HepG2 and MCF7. The results revealed that the test isolates were weakly active against the used cell lines with IC_{50} values more than 30 μ g/ml. while the saponin mixture was moderately active against the cell line HepG2 with IC_{50} value equals to 13.4 μ g/ml.

Key words: *Dracaena marginata*; Agavaceae; steroidal saponin; cytotoxic activity.

INTRODUCTION

The members of *Agavaceae* family representing more than 580 species are distributed in the tropic and subtropic dry climate regions throughout the world. The genus *Dracaena* comprising about 60 species is distributed from the old world tropic regions to Canary Island. Many species of this genus are used in traditional medicine. In China, the red resin of *Dracaena cochinchinensis* known as Chinese dragon's blood, is used for promoting blood circulation and the treatment of traumatic and visceral hemorrhages [1]. *Dracaena Mannii* bark's decoction is used in Africa against abdominal pains while the mixture of its roots with "palm wine" is used for the treatment of male impotency [2]. Dragon's blood has been reported to have several medical applications as haemostatic, antidiarrhetic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory and antioxidant [3]. The antidiarrheogenic activity of *D. mannii* has been evaluated [4]. The occurrence of phenolic compounds and steroidal saponins in several *Dracaena* plants has been reported [3], [5], [6], [7], [8]. Steroidal saponins are class of natural compounds that proved to exhibit several biological and pharmacological properties [9-16].

Dracaena marginata tam. commonly known as Red edge Dragon Tree or Madagascar Dragon Tree, is a shrub grows to approximately 5m tall. It is one of the very best indoor ornamental plants. As a part of our interest in bioactive steroidal saponins [17], we describe in this report the isolation and identification of nine steroidal saponins including two new minor compounds from *D. Marginata*.

MATERIALS AND METHODS

General: ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Jeol α -400 FT-NMR spectrometer and chemical shifts are given as δ values with TMS as internal standard at 35°C in pyridine- d_5 . Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{\text{C-H}} = 145$ Hz) and HMBC (optimized for $^nJ_{\text{C-H}} = 8$ Hz) pulse sequences with a pulse field gradient. HPLC was performed on a Jasco system 800 instrument. GC analysis was carried out on Hitachi G-3000 gas chromatograph.

Plant material: Roots of *Dracaena marginata* were collected from El-Orman public garden, Giza, Egypt in November 2006. Plant identification was confirmed by Mrs. T. Labib, head specialist for plant identification in El-Orman public garden, Giza, Egypt. A voucher specimen was deposited in the Herbarium of NRC (CAIRC).

Extraction and Isolation: The air-dried roots of the *D. marginata* (1.0kg) were defatted with *n*-hexane then extracted twice with CHCl_3 followed by MeOH until exhaustion. The combined MeOH extract was evaporated under vacuum to dryness. The residue (45g) was suspended in H_2O and extracted with EtOAc (6x200 ml) then water saturated *n*-BuOH (6x200 ml). The *n*-BuOH extract (6.1g) was dissolved in H_2O (0.2%) and the H_2O solution was passed through a column chromatography packed with 500g Diaion HP-20 polymer gel (Mitsubishi). After washing the column with distilled water for several times, elution was carried out with 25%, 50%, 60%, 75% aqueous MeOH and finally with 100% MeOH. The collected fractions were examined by silica gel TLC (Merck) using solvent systems CHCl_3 -MeOH- H_2O (60:30:5) and *n*-BuOH-EtOH- NH_4OH (7:2:5) and visualized by spraying with 20% sulphuric acid in MeOH followed by heating at 110°C. Based on TLC analysis, similar fractions were then combined. Fractions eluted with 75% and 100% MeOH were found similar and contained saponin constituents. The combined saponin fraction (3.2 g) was applied on a column chromatography packed with 120 g PSQ 100B silica gel (Fuji silysia, Nagoya, Japan) and eluted with CHCl_3 -MeOH- H_2O with increasing polarity (70:27:3-58:35:7). A total of 44 fractions, 50 ml each, were collected. Similar fractions were combined after TLC analysis to yield 1-5 subfractions (A-E). The subfraction B (350mg) was subjected to repeated HPLC (column, Inertsil ODS- 30 mm x 50 cm; solvent, 20-36% CH_3CN in H_2O ; flow rate, 9.5ml/min.; detection, UV 205nm) to yield **1** and **2** (24 mg), **3** and **4** (15 mg), **5** and **6** (6.0 mg), **8** (9.0 mg), **9** (8.5 mg) and **10** (17 mg).

Compound 1: $^1\text{HNMR}$ (pyridine- d_5): δ 6.34 (1H, *brs*, Rha H-1), 5.32 (1H, *d*, $J=4.0$ Hz, H-6), 5.08 (1H, *d*, $J=7.5$ Hz, Glc II H-1), 4.93 (1H, *d*, $J=6.8$ Hz, Glc I H-1), 4.91 (1H, *m*, Rha H-5), 4.86 (1H, *brd*, $J=2.5$ Hz, Rha H-2), 4.79 (1H, *d*, $J=7.5$ Hz, Glc III H-1), 4.58 (1H, GlcII H-6), 4.54 (2H, Rha H-3 + GlcIII H-6), 4.45 (1H, *m*, H-16), 4.41 (1H, GlcI H-6), 4.38 (2H, GlcII H-6 $^+$ + Glc III H-6 $^+$), 4.28 (1H, Rha H-4), 4.25 (1H, GlcI H-6 $^+$), 4.22 (1H, GlcIII H-3), 4.19 (2H, GlcI H-2 + Glc III H-4), 4.16 (1H, Glc II H-3), 4.14 (1H, GlcI H-3), 4.09 (1H, GlcII H-4), 4.05 (1H, GlcI H-4), 4.03 (1H, GlcII H-5), 4.00 (1H, GlcIII H-2), 3.99 (1H, GlcII H-2), 3.93 (2H, H-26 + GlcIII H-5), 3.81 (1H, GlcI H-5), 3.63 (1H, *dd*, $J=11.0, 5.0$ Hz, H-26 $^+$), 1.74 (3H, *d*, $J=6.2$ Hz, Rha-Me), 1.32 (3H, *d*, $J=6.5$ Hz, Me-21), 1.06 (3H, *s*, Me-19), 0.99 (3H, *d*, $J=6.8$ Hz, Me-27), 0.90 (3H, *s*, Me-18); $^{13}\text{CNMR}$: see Table 1.

Compound 2: $^1\text{HNMR}$ (pyridine- d_5): δ 6.34 (1H, *brs*, Rha H-1), 5.35 (1H, *d*, $J=4.0$ Hz, H-6), 5.08 (1H, *d*, $J=7.5$ Hz, GlcII H-1), 4.93 (1H, *d*, $J=6.8$ Hz, GlcI H-1), 4.91 (1H, *m*, Rha H-5), 4.86 (1H, *brd*, $J=2.5$ Hz, Rha H-2), 4.83 (1H, *d*, $J=7.5$ Hz, GlcIII H-1), 4.58 (1H, GlcII H-6), 4.54 (2H, Rha H-3 + GlcIII H-6), 4.45 (1H, *m*, H-16), 4.41 (1H, GlcI H-6), 4.38 (2H, GlcII H-6 $^+$ + GlcIII H-6 $^+$), 4.28 (1H, Rha H-4), 4.25 (1H, GlcI H-6 $^+$), 4.22 (2H, GlcIII H-3 + Glc III H-4), 4.19 (1H, Glc I H-2), 4.16 (1H, GlcII H-3), 4.14 (1H, GlcI H-3), 4.09 (1H, GlcII H-4), 4.05 (1H, GlcI H-4), 4.03 (1H, GlcII H-5), 4.01 (1H, GlcIII, H-2), 4.00 (1H, GlcIII H-5), 3.99 (1H, GlcII H-2), 3.93 (1H, H-26), 3.81 (1H, GlcI H-5), 3.63 (1H, *dd*, $J=11.0, 5.0$ Hz, H-26 $^+$), 3.26 (*s*, OMe), 1.74 (3H, *d*, $J=6.2$ Hz, Rha-Me), 1.19 (3H, *d*, $J=6.5$ Hz, Me-21), 1.06 (3H, *s*, Me-19), 1.01 (3H, *d*, $J=6.8$ Hz, Me-27) 0.82 (3H, *s*, Me-18); $^{13}\text{CNMR}$: see Table 1.

Compound 3: $^1\text{HNMR}$ (pyridine- d_5): δ 6.34 (1H, *brs*, RhaI H-1), 5.80 (1H, *brs*, Rha II H-1), 5.31 (1H, *brd*, $J=4.0$ Hz, H-6), 4.92 (2H, GlcI H-1 + RhaI H-5), 4.80 (1H, *dd*, $J=3.0, 1.0$ Hz, RhaI H-2), 4.79 (1H, GlcIII H-1), 4.64 (1H, *dd*, $J=3.0, 1.0$ Hz, RhaII H-2), 4.59 (1H, *dd*, $J=9.0, 3.5$ Hz, RhaI H-3), 4.54 (1H, *dd*, $J=12.0, 2.0$ Hz, GlcIII H-6), 4.50 (1H, *dd*, $J=9.0, 3.0$ Hz, RhaII H-3), 4.33 (1H, *brd*, $J=12.0$ Hz, GlcIII H-6 $^+$), 4.07 (1H, *dd*, $J=12.0, 2.0$ Hz, GlcI H-6), 4.01 (1H, *dd*, $J=7.5, 7.5$ Hz GlcI H-2), 3.93 (2H, H-26 + GlcIII H-5), 3.63 (2H, H-26 $^+$ + GlcI H-5), 1.75 (3H, *d*, $J=6.5$ Hz, RhaI Me), 1.60 (3H, *d*, $J=6.5$ Hz, RhaII-Me), 1.32 (3H, *d*, $J=6.5$ Hz, Me-21), 1.04 (3H, *s*, Me-19), 0.99 (3H, *d*, $J=6.8$ Hz, Me-27), 0.90 (3H, *s*, Me-18); $^{13}\text{CNMR}$: see Table 1.

Compound 4: $^1\text{HNMR}$ (pyridine- d_5): δ 6.34 (1H, *brs*, RhaI H-1), 5.80 (1H, *brs*, RhaII H-1), 5.33 (1H, *brd*, $J=4.0$

H_z, H-6), 4.92 (2H, GlcI H-1 + RhaI H-5), 4.83 (1H, *d*, *J*=7.5Hz, GlcII H-1), 4.80 (1H, *dd*, *J*=3.0, 1.0 Hz, RhaI H-2), 4.64 (1H, *dd*, *J*=3.0, 1.0 Hz, RhaII H-2), 4.59 (1H, *dd*, *J*=9.0, 3.0 Hz, RhaI H-3), 4.54 (1H, *dd*, *J*=12.0, 2.0 Hz, GlcIII H-6), 4.50 (1H, *dd*, *J*=9.0, 3.0 Hz, RhaII H-3), 4.33 (1H, *brd*, *J*=12.0 Hz, GlcIII H-6[^]), 4.07 (1H, *dd*, *J*=12.0, 2.0 Hz, GlcI H-6), 4.01 (1H, *dd*, *J*=7.5, 7.5Hz, GlcI H-2), 3.93 (2H, H-26 + GlcIII H-5), 3.63 (2H, H-26[^] + GlcI H-5), 1.75 (3H, *d*, *J*=6.5 Hz, RhaI-Me), 1.60 (3H, *d*, *J*=6.5Hz, RhaII-Me), 1.19 (3H, *d*, *J*=6.5 Hz, Me-21), 1.04 (3H, *s*, Me-19), 1.00 (3H, *d*, *J*=6.8 Hz, Me-27), 0.82 (3H, *s*, Me-18); ¹³CNMR : see Table 1.

Compound 5: ¹HNMR (pyridine- *d*₅): δ 6.35 (1H, *brs*, RhaI H-1), 5.86 (1H, *brs*, RhaII H-1), 5.32 (1H, H-6), 4.93 (1H, *d*, *J*=7.5 Hz, GlcI H-1), 4.79 (1H, *d*, *J*=7.5 Hz, GlcIII H-1), 4.67 (1H, *brs*, RhaI or RhaII, H-2), 4.58 (1H, *dd*, *J*=12.0, 2.7 Hz, GlcIII H-6), 4.36 (*dd*, *J*=12.0, 5.5 Hz GlcIII H-6[^]), 3.93 (2H, H-26+ GlcIII H-5), 3.63 (1H, *dd*, *J*=11.0, 5.0 Hz, H-26[^]), 3.70 (1H, *m*, GlcI H-5), 1.74 (3H, *d*, *J*=6.9Hz, RhaI, or RhaII-Me), 1.70 (3H, *d*, *J*=6.0 Hz, RhaI or RhaII-Me), 1.33 (3H, *d*, *J*=6.0 Hz, Me-21), 1.06 (3H, *s*, Me-19), 0.99 (3H, *d*, *J*=6.8 Hz, Me-27), 0.89 (3H, *s*, Me-18); ¹³CNMR : see Table 1.

Compound 6: ¹HNMR (pyridine- *d*₅): δ 6.35 (1H, *brs*, RhaI H-1), 5.86 (1H, *brs*, RhaII H-1), 5.32 (1H, H-6) 4.93 (1H, *d*, *J*=7.5 Hz GlcI H-1), 4.83 (1H, *d*, *J*=7.5 Hz, GlcIII H-1), 4.67 (1H, *brs*, RhaI or RhaII H-2), 4.58 (*dd*, *J*=12.0, 2.7 Hz, GlcIII H-6), 4.36 (1H, *dd*, *J*=12.0, 5.5 Hz GlcIII H-6[^]), 4.00 (GlcII H-5), 3.93 (1H, H-26), 3.70 (1H, *m*, GlcI H-5), 3.63 (1H, *dd*, *J*=11.0, 5.0 Hz, H-26[^]), 3.26 (*s*, OCH₃), 1.74 (3H, *d*, *J*=6.9 Hz, RhaI or RhaII-Me), 1.70 (3H, *d*, *J*=6.0 Hz, RhaI or RhaII-Me), 1.19 (3H, *d*, *J*=6.0 Hz, Me-21), 1.06 (3H, *s*, Me-19), 1.01 (3H, *d*, *J*=6.8 Hz, Me-27), 0.89 (3H, *s*, Me-18); ¹³CNMR : see Table 1.

Compound 8: ¹HNMR (pyridine-*d*₅): δ 6.29 (1H, *d*, *J*=1.5 Hz, Rha H-1), 5.59 (1H, *d*, *J*=5.5 Hz, H-6), 5.32 (1H, *brs*, H-27), 5.04 (1H, *brs*, H-27[^]), 4.97 (1H, *d*, *J*=7.3 Hz, Xyl H-1), 4.90 (1H, *d*, *J*=7.3 Hz, GlcH-1), 4.73 (1H, *d*, *J*=7.3 Hz, Ara H-1), 4.11 (1H, *dd*, *J*=9.0, 4.0 Hz, Rha H-3), 4.05 (1H, *dd*, *J*=9.0, 9.0 Hz, Rha H-4), 3.24 (3H, *s*, OMe), 1.72 (3H, *d*, *J*=6.2 Hz, Rha-Me), 1.41 (3H, *s*, Me-19), 1.12 (3H, *d*, *J*=6.7 Hz, Me-21), 0.83 (3H, *s*, Me-18); ¹³CNMR : see Table 2.

Compound 9: ¹HNMR (pyridine- *d*₅): δ 6.43 (1H, *d*, *J*=1.5 Hz, Rha H-1), 5.75 (1H, *dd*, *J*=9.5, 9.5 Hz, Rha H-4), 5.62 (1H, *d*, *J*=6.0 Hz, H-6), 5.23 (1H, *s*, H-27), 5.14 (1H, *d*, *J*=7.8 Hz, Fuc H-1), 5.07 (1H, *s*, H-27[^]), 4.93 (1H, *d*, *J*=7.5 Hz, Xyl H-1), 4.89 (1H, *dq*, *J*=9.7, 6.1 Hz, Rha H-5), 4.82 (1H, *d*, *J*=12.0 Hz, H-26), 4.71 (1H, *brd*, *J*=3.3 Hz, Rha H-2), 4.68 (1H, *d*, *J*=7.5 Hz, Ara H-1), 3.99 (1H, *d*, *J*=12.0 Hz, H-26[^]), 2.0 (3H, *s*, Ac), 1.47 (3H, *d*, *J*=6.5 Hz, Fuc-Me), 1.42 (3H, *d*, *J*=6.0 Hz, Rha-Me), 1.37 (3H, *s*, Me-19), 1.06 (3H, *d*, *J*=7.0 Hz, Me-21), 0.95 (3H, *s*, Me-18); ¹³CNMR : see Table 2.

Compound 10: ¹HNMR (pyridine- *d*₅): δ 6.50 (1H, *d*, *J*=1.5 Hz, Rha H-1), 6.07 (1H, *dd*, *J*=3.5, 1.5 Hz, Rha H-2), 5.91 (1H, *dd*, *J*=10.2, 3.5 Hz, Rha H-3), 5.63 (1H, H-6), 5.61 (1H, *dd*, *J*=10.2, 10.2 Hz, Rha H-4), 5.23 (1H, *d*, *J*=1.2 Hz, H-27), 5.14 (1H, *d*, *J*=8.0 Hz, Fuc H-1), 5.08 (1H, *s*, H-27[^]), 4.99 (1H, *dq*, *J*=10.2, 6.0 Hz, Rha H-5), 4.83 (1H, *d*, *J*=11.0 Hz, H-26), 4.79 (1H, *d*, *J*=7.7 Hz, Xyl H-1), 4.63 (1H, *d*, *J*= 7.7 Hz, Ara H-1), 4.53 (1H, *dd*, *J*=8.0, 8.0 Hz, Ara H-2), 4.00 (1H, *d*, *J*=11.0 Hz, H-26[^]), 2.12, 2.02 and 1.87 (each 3H, *s*, Ac), 1.47 (3H, *d*, *J*=6.3 Hz, Fuc-Me) 1.43 (3H, *d*, *J*=6.2 Hz, Rha-Me), 1.35 (3H, *s*, Me-19), 1.07 (1H, *d*, *J*=7.0 Hz, Me-21), 0.96 (3H, *s*, Me-18); ¹³CNMR : see Table 2.

General method for acid hydrolysis: Each saponin (2mg) in dioxane (50 µl) and 2M HCl (50 µl) was heated at 100°C for 1.0 h. The dioxane was evaporated and the residue was diluted with distilled water and extracted with CHCl₃. The H₂O layer was passed through an Amberlite IRA-60E column (6x60mm) and the eluate obtained was then concentrated. The sugar components in the residue were detected by GC analysis after being converted to their thiazolidine derivatives as previously described [18],[19].

Assay method for cytotoxic activity

Cell culture: HepG2 (hepatocellular carcinoma) and MCF7 (breast carcinoma) human tumor cell lines were cultured in 95% humidity and 5% CO₂ at 37°C. The cell lines were maintained in RPMI-1640 supplemented with 10% foetal bovine serum.

Cytotoxicity test: The acid phosphatase assay was used to assess cytotoxicity according to the described method [20]. In 96 well plates, 5000 cells were seeded per well and left to attach overnight then treated with test samples for three days. For each plate, a substrate solution was prepared where 20 mg tablet of PNPP (*p*-nitro phenyl phosphate,

Sigma; cat. No. N2765) was dissolved in 10 ml buffer solution (0.1 M sodium acetate, 0.1 % triton X-100, PH=5). Cell monolayers were washed twice with 250 μ l PBS (Phosphate buffer saline). PNPP substrate solution (100 μ l) was added to each well, and then plates were incubated for 4 hours at 37°C. 1N sodium hydroxide solution (10 μ l) was added to each well and absorbance was measured directly at wavelength 405 nm. All samples were tested in triplicates and 1 μ l DMSO was used as negative control while 0.5 μ M adriamycin was used as positive control. The isolates were tested at 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 μ g/ml, while the saponin fraction was tested at 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μ g/ml. Percent cytotoxicity is calculated by the formula:

$[1-(D/S)] \times 100$, where D and S denote the optical density of test sample and solvent respectively.

LC₅₀ values were determined by SPSS computer program (SPSS for windows, statistical analysis software package / version 9 / 1989 SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

The defatted methanolic root extract of *D. marginata* was partitioned between water and n-butanol. The n-butanol soluble phase was subjected to a porous polymer gel Diaion HP-20 column chromatography. The eluted saponin fraction, was chromatographed on silica gel followed by repeated HPLC to afford nine steroidal saponins **1-6** and **8-10** including two new ones **5** and **6**. The furostanol saponins **1-6** were isolated as three pairs of 22- hydroxy furostanol saponins and their 22-methoxy derivatives. Their structures were determined from the NMR spectra of each pair with almost identical NMR signals except those around C-22 (Tables 1 and 2).

The furostanol structure of **1** was evidenced from spectral analysis. The ¹H and ¹³CNMR spectra showed the presence of four methyl signals [δ_{H} 0.99 (3H, *d*, *J*=6.8 Hz), δ_{C} 17.5 (Me-27); δ_{H} 1.32 (3H, *d*, *J*=6.5 Hz), δ_{C} 16.5 (Me-21); δ_{H} 0.90 (3H, *s*), δ_{C} 16.4 (Me-18); δ_{H} 1.06 (3H, *s*), δ_{C} 19.4 (Me-19)] as well as a quaternary carbon signal at δ 110.7 attributable to C-22. The glycosidic nature of **1** was indicated by presence of four anomeric signals [δ_{H} 6.34 (1H, *brs*), δ_{C} 102.2; δ_{H} 5.08 (1H, *d*, *J*=7.5 Hz), δ_{C} 104.6; δ_{H} 4.93 (1H, *d*, *J*=6.8 Hz), δ_{C} 100.1; δ_{H} 4.79 (1H, *d*, *J*=7.5 Hz), δ_{C} 104.9]. The full aglycone structure was established as 22 α -hydroxy-(25 R)-furost-5-en-3 β , 26 diol based on 1D and 2D (¹H-¹H COSY, HMQC, HMBC) NMR spectra. The C-25R configuration was established based on the difference of δ values of the geminal CH₂-26 ($\Delta\delta_{\text{H}}$ 0.3 ppm) [21]. The spectra also allowed to identify the sugar units as 2,3 disubstituted β -glucopyranose and three terminal sugars comprising two β -glucopyranoses and one α -rhamnopyranose. The anomeric centers of the three glucopyranoses were each determined to have β -configuration based on large ³*J*_{H-1,H-2} values. The δ values of C-3 and C-5 signals of the rhamnopyranose indicated α configuration. Acid hydrolysis of **1** yielded the sugars D-glucose and L-rhamnose. The positions of the sugar units were confirmed by the long range HMBC correlations. Finally compound **1** was identified as 26-*O*- β -D-glucopyranosyl-22 α -hydroxy-(25R)-furost-5-ene-3 β -26-diol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside, identical to protogracillen [22].

Compound **2** is a furostanol glycoside, showed in the ¹HNMR spectrum, a methoxyl signal at δ 3.26 (δ_{C} 47.3) as well as two signals due to Me-21 and Me-27 at δ 1.19 (*d*, *J*= 6.5 Hz) and 1.01 (*d*, *J*=6.8 Hz), respectively. The carbon signals due to C-22 and C-23 were located in the ¹³CNMR spectrum at δ 112.7 and 30.9, respectively. Therefore, **2** was elucidated as 26-*O*- β -D-glucopyranosyl-22 α -methoxy-(25R)-furost-5-en-3 β , 26-diol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-glucopyranoside, identical to methyl protogracillin [22].

Compound **3** is another furostanol glycoside with an aglycone moiety identical to that of **1** from NMR data. The ¹³C and ¹HNMR data revealed that the 3-*O*-sugar chain of **3** contained two terminal α -L-rhamnopyranose units and an inner β -D-glucopyranose unit. According to the glycosylation shift effect, the two terminal α -L-rhamnopyranoses were attached to the hydroxyls groups at C-2 and C-4 of the inner β -D-glucopyranose unit. From these results **3** was concluded to be 26-*O*- β -D-glucopyranosyl-22 α -hydroxy-(25R)-furost-5-en-3 β , 26-*O*-diol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranoside, identical to protodioscin [23].

Applying the same approach used in determining the structure of **2**, saponin **4** was concluded to have the structure of 26-*O*- β -D-glucopyranosyl-22 α -methoxy-(25R)-furost-5-en-3 β , 26-diol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranoside, identical to the methylprotodioscin [22,24].

Compounds **5** and **6** obtained as minor compounds were 22-hydroxy furostanol saponin and its 22-methoxy derivative, respectively, from the positive color reaction in Ehrlich test and NMR analysis. The ¹H NMR spectrum of **5** and **6** showed one set of signals with regard to Me-19 at δ 1.06 (*s*) and Me-18 at δ 0.89 (*s*), two signals due to Me-27 at δ 0.99 (*d*, *J* = 6.8 Hz) and 1.01 (*d*, *J* = 6.8 Hz) together with two signals for Me-21 at δ 1.33 (*d*, *J* = 6.0 Hz) and 1.19 (*d*, *J* = 6.0 Hz) as well as an olefinic proton signal at δ 5.32. Further, a methyl signal at δ 3.26 (*s*, OCH₃) was also observed for **6**. In the sugar area of the spectrum, the three anomeric proton signals at δ 4.93 (*d*, *J* = 7.5 Hz), 6.35 (*brs*) and 5.86 (*brs*) together with two methyl signals at δ 1.74 (*d*, *J* = 6.9 Hz) and 1.70 (*d*, *J* = 6.0 Hz) were assigned to a glucose and two rhamnose units after acid hydrolysis which afforded D-glucose and L-rhamnose. The two anomeric proton doublets at δ 4.79 (*d*, *J* = 7.5 Hz) and 4.83 (*d*, *J* = 7.5 Hz) were assigned to the glucose units at aglycone C-26 (δ 75.3) for **5** and **6**, respectively. The ¹³C NMR spectrum of **5** and **6** exhibited signals due to the aglycone moiety almost identical to those of **1** and **2** as well as **3** and **4**, respectively. The C-25 configuration was deduced to be (R) based on the difference of δ values for H₂-26 (Δδ_H 0.3 ppm) [21]. The ¹³C NMR assignments of the sugar moieties of **5** and **6** were achieved by referring to literature data of methyl glycosides [25]. The assignments indicated the presence of a 3,4-disubstituted β-glucopyranose unit and three terminal sugar comprising one β-glucopyranose attached to aglycone C-26 and two rhamnopyranose units. The β-configuration of the glucose units were proven by the large ³J_{H-1,H-2} values (7.5 Hz). The α-configuration of each anomeric centre of the rhamnose units was established from the δ values of C-3 and C-5. From the above findings, it was concluded that **5** and **6** possess identical sugar chain linked to aglycone C-3 and identified as α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside. The marked difference between the NMR data due to this moiety and the corresponding data of **3** and **4** as well as the data of spiroconazole **A 7** having two terminal rhamnoses attached to inner glucose at C-2 and C-3 [26], confirmed the structure of the trisaccharide moiety for **5** and **6**. Thus **5** was assigned the structure of 26-*O*-β-D-glucopyranosyl-22α-hydroxy-(25R)-furost-5-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside and **6** was its 22-methoxy derivative.

Compound **8** was shown to be 22-methoxy furostanol saponin from ¹H and ¹³C NMR spectra [δ 3.24 (3H, *s*); δ_C 112.4 and 47.3]. The ¹H NMR spectrum also showed three methyl proton signals at δ 0.83 (*s*, Me-18), 1.12 (*d*, *J* = 6.7 Hz, Me-21) and 1.41 (*s*, Me-19), exomethylene protons at δ 5.32 (*s*) and 5.04 (*s*). Further, the spectrum revealed the presence of four sugar units assigned by ¹³C NMR analysis and after acid hydrolysis to be 2,3-disubstituted α-L-arabinopyranose unit and three terminal units comprising a β-D-glucopyranose, a β-D-xylopyranose and an α-L-rhamnopyranose. From detailed NMR analysis, the structure of **8** was shown to be 26-*O*-β-D-glucopyranosyl-22-methoxy-furost-5,25(27)-diene-1β,3β,22,26-tetrol-1-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside [27], [28]. This is the first reported occurrence of compound **8** in genus *Dracaena*. Compound **9** was shown to be spirostanol saponin from the ¹H NMR spectrum which displayed proton signals arising from the aglycone moiety at δ 0.95, 1.37, (each *s*), 1.06 (*d*, *J* = 7.0 Hz), exomethylene protons at δ 5.23 and 5.07 and acetyl group at δ 2.0. The full structure of **9** was shown from detailed ¹H and ¹³C NMR analysis and after acid hydrolysis to be (23S, 24S)-spirost-5,25(27)-diene-1β,3β,23,24-tetrol-1-*O*-[(4-acetyl-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranosidyl)-24-*O*-β-D-fucopyranoside] [29]. Compound **9** is reported here for the first time from genus *Dracaena*.

Saponin **10** showed NMR spectra similar to that of **9** except the presence of three acetyl groups [δ 1.87, 2.02, 2.12; δ_C 170.3, 170.4, 170.5] substituted the three hydroxyl groups of the terminal rhamnose unit. Therefore the structure of **10** was shown to be (23S, 24S)-spirost-5,25(27)-diene-1β,3β,23,24-tetrol-1-*O*-[(2,3,4-*O*-triacetyl-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside]-24-*O*-β-D-fucopyranoside [8], [29].

The saponin mixture and the isolates 1-6 and 8-10 were tested *in vitro* for their cytotoxic activities against the cell lines HepG2 and MCF7. The results (Table 3) showed weak activities towards the used cell lines with IC₅₀ values more than 30 μg/ml except the saponin mixture which gave moderate activity against the cell line HepG2 with IC₅₀ value equal to 13.4 μg/ml.

Table 1. ¹³CNMR data of compounds 1-6 in pyridine-d₅

C	1	2	3	4	5	6	7 ²
1	37.6	37.6	37.6	37.6	37.5	37.5	
2	30.2	30.2	30.2	30.2	30.2	30.2	
3	77.8	77.8	78.2	78.2	77.9	77.9	
4	38.8	38.8	39.1	39.1	39.3	39.3	
5	140.9	140.9	140.9	140.9	140.9	140.9	
6	121.9	121.9	121.8	121.8	121.8	121.8	
7	32.4	32.2	32.2	32.2	32.3	32.3	
8	31.7	31.7	31.8	31.8	31.7	31.7	
9	50.4	50.4	50.5	50.4	50.4	50.4	
10	37.2	37.2	37.2	37.2	37.2	37.2	
11	21.1	21.1	21.2	21.1	21.2	21.2	
12	40.0	39.8	40.0	39.8	40.0	40.0	
13	40.9	40.9	40.9	40.9	40.9	40.9	
14	56.6	56.6	56.6	56.6	56.7	56.7	
15	32.5 ^a	32.4 ^a	32.4 ^a	32.4 ^a	32.5 ^a	32.5 ^a	
16	81.2	81.4	81.1	81.4	81.2	81.4	
17	63.9	64.2	63.9	64.2	63.9	64.2	
18	16.4	16.3	16.4	16.3	16.4	16.3	
19	19.4	19.4	19.4	19.4	19.4	19.4	
20	40.7	40.5	40.7	40.5	40.8	40.5	
21	16.5	16.3	16.5	16.3	16.5	16.3	
22	110.7	112.7	110.7	112.7	110.7	112.7	
23	37.2	30.9	37.2	30.8	37.1	30.9	
24	28.4	28.3	28.4	28.3	28.4	28.3	
25	34.3	34.3	34.3	34.3	34.3	34.3	
26	75.3	75.3	75.2	75.2	75.3	75.3	
27	17.5	17.2	17.5	17.2	17.5	17.3	
-O-CH ₃		47.3		47.3		47.3	
3- O- sugar							
3-O-GlcI							
1	100.1	100.1	100.4	100.4	104.6	104.6	99.8
2	77.1	77.1	78.9	78.9	71.9	71.9	78.2
3	89.5	89.5	77.9	77.9	89.6	89.6	87.4
4	69.7	69.7	78.2	78.2	78.6	78.6	70.5
5	77.9	77.9	76.9	76.8	76.8	76.8	77.7
6	62.5 ^b	62.5 ^b	61.4	61.4	61.6	61.6	62.2
2-O-Rha							
1	102.2	102.2	102.0	102.0	102.5	102.5	103.7
2	72.5	72.5	72.5	72.5	72.5 ^b	72.5 ^b	72.4
3	72.8	72.8	72.9	72.9	72.9	72.9	72.7
4	74.2	74.2	74.2	74.2	74.2 ^c	74.2 ^c	73.7
5	69.6	69.6	69.5	69.5	69.5	69.5	69.8
6	18.7	18.7	18.5	18.5	18.5	18.5	18.5
3-O-GlcII							
1	104.6	104.6					
2	75.0	75.0					
3	78.7 ^c	78.7 ^c					
4	71.6	71.6					
5	78.6 ^c	78.6 ^c					
6	62.6 ^b	62.6 ^b					
4-O-RhaII							
1			103.0	103.0	102.7	102.7	102.5
2			72.5	72.5	72.6 ^b	72.6 ^b	72.3
3			72.8	72.8	72.9	72.9	72.5
4			73.9	73.9	74.0 ^c	74.0 ^c	73.5
5			70.5	70.5	70.4	70.4	69.8
6			18.6	18.6	18.7	18.7	18.3
26-O-GlcIII							
1	104.9	105.0	105.0	105.0	105.1	105.1	
2	75.3	75.3	75.3	75.3	75.3	75.3	
3	78.7 ^c	78.6 ^c	78.6 ^b	78.6 ^b	78.5 ^d	78.5 ^d	
4	71.9	71.9	71.9	71.9	71.9	71.9	
5	78.5 ^c	78.5 ^c	78.5 ^b	78.5 ^b	78.6 ^d	78.6 ^d	
6	62.6	62.9	63.0	62.9	62.9	62.9	

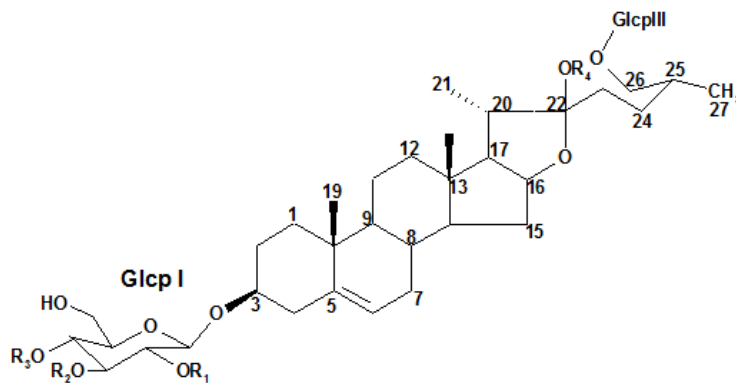
Assignments with the same superscript may be reversed in each column.
Data taken from reference [26].

Table 2. ¹³CNMR data of compounds 8-10 in pyridine-d₅

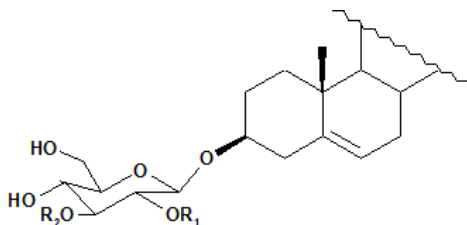
C	8	9	10
1	83.7	83.9	84.0
2	37.4	37.5	37.7
3	68.3	68.1	68.0
4	43.9	44.0	43.9
5	139.6	139.5	139.4
6	124.8	124.9	125.0
7	32.1	32.0	32.0
8	33.2	33.0	33.0
9	50.5	50.4	50.3
10	43.0	42.9	42.9
11	24.1	24.0	24.0
12	40.4	40.5	40.5
13	40.6	40.8	40.8
14	56.8	56.7	56.7
15	32.1	32.4	32.4
16	81.3	82.1	82.2
17	63.9	61.5	61.5
18	16.9	16.8	16.8
19	15.1	14.7	15.0
20	40.7	37.5	37.5
21	16.3	15.0	14.8
22	112.4	111.8	111.8
23	31.8	70.3	70.4
24	28.4	83.0	83.0
25	147.3	144.0	144.0
26	72.1	61.5	61.5
27	110.7	113.7	113.7
OCH ₃	47.3		
3- O- sugar			
Ara			
1	100.4	100.7	100.3
2	74.2	72.9	72.6
3	84.4	85.1	85.1
4	69.6	69.9	69.9
5	67.1	67.2	67.2
Rha			
1	101.8	100.9	97.7
2	72.5	72.3	70.6
3	72.6	70.0	70.2
4	74.0	76.5	72.0
5	69.6	66.6	66.4
6	19.1	18.5	18.2
Xyl			
1	106.4	106.7	106.6
2	74.6	74.6	74.6
3	78.6	78.5	78.4
4	71.0	70.9	71.0
5	66.9	67.3	67.5
Glc		Fuc	Fuc
1	104.0	106.3	106.3
2	75.2	73.1	73.1
3	78.5	75.4	75.4
4	71.7	72.9	72.9
5	78.6	71.6	71.6
6		17.3	17.3
Ac		170.7	170.5
		21.0	170.4
			170.3
			20.7
			20.7
			20.8

**Table 3. The cytotoxicities of the saponin fraction and isolates against the tumor cell lines
Cell lines, IC₅₀ (mg/ml)**

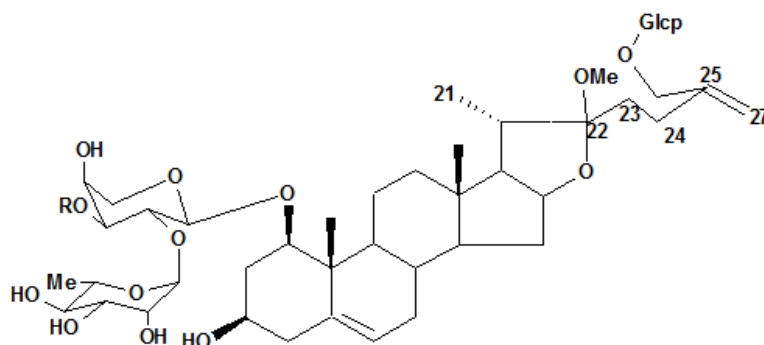
Compound	HepG2	MCF7
Saponin fraction	13.4	35.0
1,2	> 50	> 50
2,4	29.8	> 50
5,6	> 50	> 50
8	> 50	> 50
9	> 50	> 50
10	> 50	> 50
Adriamycin	6.9	2.5



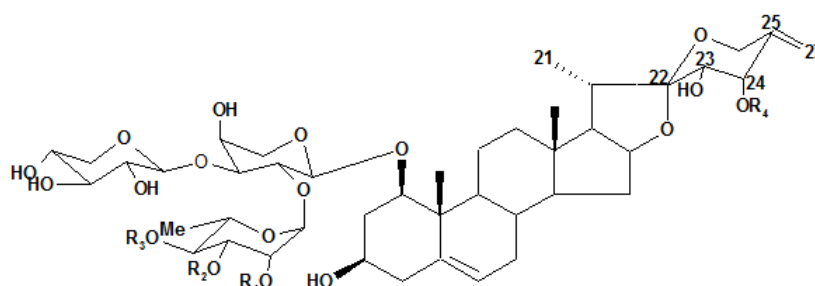
	R ₁	R ₂	R ₃	R ₄
1	Rhap	Glcp II	H	H
2	Rhap	Glcp II	H	CH ₃
3	Rhap I	H	Rhap II	H
4	Rhap I	H	Rhap II	CH ₃
5	H	Rhap I	Rhap II	H
6	H	Rhap I	Rhap II	CH ₃



	R ₁	R ₂
7	Rhap I	Rhap II



	R
8	Xylp



	R ₁	R ₂	R ₃	R ₄
9	H	H	Ac	Fucp
10	Ac	Ac	Ac	Fucp

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