

Steroids from the Brazilian Zoanthids *Palythoa caribaeorum* and *Palythoa variabilis*

Francisco C. L. Pinto,^a José Gustavo L. Almeida,^a Edilberto R. Silveira,^a Arinice M. Costa,^b Larissa A. Guimarães,^b Diego V. Wilke,^b Letícia V. Costa-Lotuf,^c Maria da Conceição M. Torres^d and Otília Deusdênia L. Pessoa^{*a}

^aDepartamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará,
60021-970 Fortaleza-CE, Brazil

^bDepartamento de Fisiologia e Farmacologia, Universidade Federal do Ceará,
60430-270 Fortaleza-CE, Brazil

^cDepartamento de Farmacologia, Universidade de São Paulo,
05508-900 São Paulo-SP, Brazil

^dDepartamento de Química, Universidade Estadual da Paraíba,
58429-500 Campina Grande-PB, Brazil

Two unreported ergostane-type sterols 24(R)-7 α -hydroperoxy-ergost-5-en-3 β -ol and 6 β -carboxyl-24(R)-(8 \rightarrow 6)-abeo-ergostan-3 β ,5 β -diol, along with seven known ones were isolated from the hexane and alcohol extracts from the zoanthids *Palythoa caribaeorum* and *Palythoa variabilis*. The structures of the new compounds were determined using spectroscopic techniques, including 1D and 2D nuclear magnetic resonance (NMR), high-resolution electrospray ionization mass spectrometry (HRESIMS), and comparison with data previously published. 6 β -Carboxyl-24(R)-(8 \rightarrow 6)-abeo-ergostan-3 β ,5 β -diol showed moderate cytotoxicity against colon cancer cells (HCT-116) as previously described for others abeo-sterol derivatives.

Keywords: *Palythoa caribaeorum*, *Palythoa variabilis*, ergostane sterols, cytotoxic activity

Introduction

Zoanthids are cnidarians found in most marine environments, from temperate to tropical areas and from the intertidal zone to the deep sea below 5000 m, but are particularly common in tropical and subtropical seas.¹ The colonial zoanthid genus *Palythoa* (order Zoantharia) is represented by ca. 92 species.² It has been the subject of constant taxonomy investigations due to an incomplete understanding of their diversity.³ Many chemical and pharmacological studies have been conducted in order to investigate its biological potentialities. Several classes of compounds have been isolated from zoanthids, including ceramides,⁴ fatty acids,⁵ mycosporins,⁶ palytoxins,⁷ peptides,⁸ sterols,^{9,10} zoanthoxanthins,¹¹ prostaglandins,¹² lipidic α -amino acids,¹³ and pyrazines.¹⁴ Lipids and steroids represent the commonest classes of compounds isolated from *Palythoa* spp. (Figure 1). The most potent toxic non-

protein known, palytoxin, has been isolated from *Palythoa* species.¹⁵ The first natural lipid α -amino acids (C₃₀ and C₃₁) have been isolated from *Protospalythoa variabilis*, and exhibited cytotoxic effects against human cancer cell lines.¹³ *Protospalythoa* taxonomy previously assigned^{4,13} is under revision based on mitochondrial DNA analysis, indicating that *Protospalythoa* and *Palythoa* are indeed congeneric designations.¹⁶

The present investigation reports on the steroidal composition of *P. caribaeorum* and *P. variabilis*. Both species are abundant in shallow waters along the Brazilian Northeast coastline.

Experimental

General

Infrared (IR) spectra were obtained on a PerkinElmer FT-IR spectrum 1000 spectrometer. Optical rotations were measured on a PerkinElmer 341 digital polarimeter at 25 °C.

*e-mail: otilialoiola@gmail.com

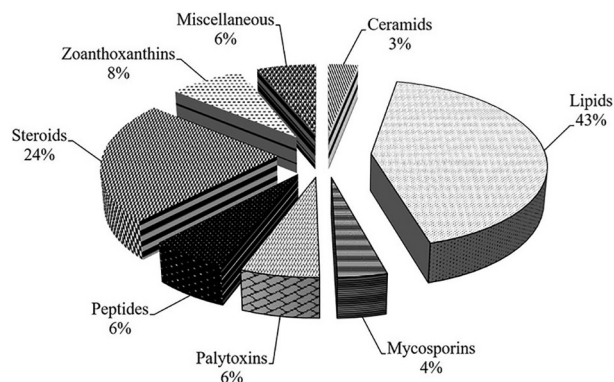


Figure 1. Percentage distribution by classes of the isolated, or identified, compounds from *Palythoa* species.

Melting points, not corrected, were determined on a digital MQAPF-302 melting point apparatus (Microquímica), with a heating rate of 2 °C min⁻¹. ¹H (500 or 300 MHz) and ¹³C (125 or 75 MHz) nuclear magnetic resonance (NMR) spectra were performed either on a Bruker Avance DRX-500 or Avance DPX-300 spectrometer. High resolution electrospray ionization mass spectrometry (HRESIMS) was acquired using a liquid chromatography-mass spectrometry ion trap and time-of-flight (LCMS-IT-TOF, Shimadzu) spectrometer, consisting of an UFLC (ultra fast liquid chromatography) system coupled to an IT-TOF mass spectrometer equipped with electrospray ionization (ESI) source operating either in positive or negative mode. The mass spectra were recorded in the range of *m/z* 100-1000 Da, using a potential of 4.0 kV on the capillary, and nitrogen as the desolvation gas. The system was controlled by a LC-solution software, which was also used for data analysis. High-performance liquid chromatography (HPLC) analysis was carried out using an UFLC (Shimadzu) system equipped with a SPD-M20A diode array UV-Vis detector and a Phenomenex C₁₈ column, 5 μm (4.6 × 250 mm). The mobile phase consisted of H₂O-CH₃CN with a flow rate of 4.72 mL min⁻¹. For the column chromatography (CC) procedures, silica gel 60 [70-230 mesh (Vetec) or 230-400 mesh (Merck)], or Strata C₁₈-E cartridge (20 g/60 mL, 55 μm, 70 Å, Phenomenex) were used. Thin layer chromatography (TLC) was performed on pre-coated 0.2 mm silica gel aluminum sheets (silica gel 60 F₂₅₄, Merck), and the spots visualized by heating (ca. 100 °C) the plates sprayed with a vanillin/perchloric acid/EtOH solution.

Marine organisms

Both zoanths, *Palythoa caribaeorum* and *Palythoa variabilis*, were collected during low tide at Paracuru beach (3°24'0.22''S and 39°0'48.60''W), on the west coast of Ceará State, Brazil. Initially, the material was washed with

sea water and then stored under refrigeration. Specimens, *P. caribaeorum* (voucher No. 000976) and *P. variabilis* (voucher No. 000975), were authenticated by Dr Antonio Carlos Marques and deposited at the Museu de Zoologia, Universidade de São Paulo (MUZUSP-USP).

Extraction and isolation

The chemical investigation of *P. caribaeorum* and *P. variabilis* was conducted in two different ways. The first one was performed using the usual method, i.e., extraction with EtOH at room temperature and gravitational column chromatography over silica gel, while the second one was performed using extraction by sonication with MeOH, liquid-liquid partition, SPE (solid phase extraction) cartridges and HPLC chromatography.

P. caribaeorum (4.7 kg) and *P. variabilis* (3.4 kg), were cut in small pieces, washed with distilled water, dried at room temperature and extracted with *n*-hexane and then followed by EtOH 96% (3 times 6 L for each solvent), for 24 h. The *n*-hexane and EtOH extracts were filtered and evaporated under reduced pressure at 40 °C to yield, respectively, the crude extracts: 21.0 and 61.7 g from *P. caribaeorum*, and 19.8 and 23.3 g from *P. variabilis*.

The *P. caribaeorum* *n*-hexane extract (21.0 g) was initially fractionated over silica gel CC (108 g) using as the elution solvent *n*-hexane, *n*-hexane/EtOAc 4:1, 3:2, 2:3, 1:4, EtOAc and, finally, MeOH. The *n*-hexane/EtOAc 3:2 fraction (0.9 g) was subjected to silica gel CC by elution with *n*-hexane/EtOAc gradient (7:3 → 0:10) and then with MeOH to give 6 fractions (F1-F6), after TLC analysis. F6 (107.5 mg), a white amorphous powder, was washed with EtOAc to afford the pure compound **1** (31.5 mg).

The *P. variabilis* *n*-hexane extract (19.8 g) was fractionated over a silica gel CC by elution with *n*-hexane, *n*-hexane/CH₂Cl₂ 1:1, CH₂Cl₂, CH₂Cl₂/EtOAc 1:1, EtOAc, and, finally, MeOH. The CH₂Cl₂/EtOAc 1:1 fraction (4.3 g) was subjected to silica gel CC using a gradient of *n*-hexane/EtOAc (7:3 → 0:10) followed by MeOH to yield 6 sub-fractions (F1-F6), after TLC analysis. F4 (197.2 mg) was subjected to silica gel CC by elution with an isocratic solvent system consisting of *n*-hexane/EtOAc 7:3 to afford compound **2** (28.3 mg).

Additional samples of *P. caribaeorum* (2.0 kg) and *P. variabilis* (1.8 kg) were extracted with MeOH (3 × 1000 mL) under sonication for 15 minutes. The MeOH suspensions were filtered and concentrated under reduced pressure to approximately 1/3 of the total volume and then partitioned with *n*-hexane, CH₂Cl₂ and EtOAc (3 × 200 mL of each solvent), from which were obtained the corresponding materials: 3.0, 0.9 and 0.5 g from

P. caribaeorum and 11.2, 0.1 and 0.2 g from *P. variabilis*, after solvent evaporation under reduced pressure.

The EtOAc fraction (0.5 g), obtained from *P. caribaeorum*, was fractionated through a C₁₈ SPE cartridge using a gradient of MeOH/H₂O (2:8 → 10:0) as eluent. The H₂O/MeOH 6:4 (22.0 mg) and H₂O/MeOH 4:6 (14.0 mg) fractions were further purified by HPLC using a semi-preparative C₁₈ column, a solvent gradient of H₂O (0.1% TFA)/CH₃CN (9.5:0.5 → 1:9.0), at a flow rate of 4.7 mL min⁻¹, affording peaks with retention times (*t_R*) corresponding to 20-hydroxyecdysone¹⁷ (4.6 mg, *t_R* = 13.0 min), 3-*O*-acetyl-20-hydroxyecdysone¹⁷ (2.6 mg, *t_R* = 14.9 min) and 2-*O*-acetyl-20-hydroxyecdysone¹⁷ (1.4 mg, *t_R* = 15.6 min).

The EtOAc fraction (1.0 mg mL⁻¹) from *P. variabilis* was subjected to HPLC using the conditions above mentioned. Following this procedure, and by comparison with the retention time, UV and MS the sterols 20-hydroxyecdysone, 3-*O*-acetyl-20-hydroxyecdysone and 2-*O*-acetyl-20-hydroxyecdysone were identified (Figure S19, Supplementary Information (SI)).

24(*R*)-7 α -Hydroperoxy-ergost-5-en-3 β -ol (**1**)

White amorphous powder; m.p. 127.1 °C; [α]_D²⁰ -34.0° (*c* 0.04, CHCl₃); IR (KBr) ν_{\max} 3425, 3297, 2936, 2875, 1461, 1376, 1058; ¹H and ¹³C NMR data, see Table 1; positive HRESIMS (*m/z* 433.3597 [M + H]⁺, calcd. for C₂₈H₄₉O₃, 433.3676).

6 β -Carboxyl-24(*R*)-(8 \rightarrow 6)-abeo-ergostan-3 β ,5 β -diol (**2**)

White amorphous powder; m.p. 182.5 °C; [α]_D²⁰ +28.0° (*c* 0.06, CHCl₃); IR (KBr) ν_{\max} 3492, 2948, 2928, 1711, 1152, 1081; ¹H and ¹³C NMR data, see Table 1; negative HRESIMS (*m/z* 447.3428 [M - H]⁻, calcd. for C₂₈H₄₇O₄, 447.3474).

Cytotoxic activity

The cytotoxic potential of **2** was assessed against a human colorectal tumor cell line (HCT-116), obtained from the Banco de Células do Rio de Janeiro (RJ, Brazil), using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium (MTT) reduction assay as described previously by Mosmann.¹⁸ Cells were seeded at 5 × 10⁴ cells mL⁻¹, treated with vehicle (0.4% DMSO), or with **2** at concentrations ranging from 0.007 to 110 μM and incubated for 24 or 72 h under 5% CO₂ atmosphere at 37 °C. Doxorubicin was used as positive control to confirm the proper sensitivity of the cell line. The inhibition concentration mean (IC₅₀) values were calculated along with their respective 95% confidence intervals, of at least

two experiments, by nonlinear regression using the software GraphPad Prism 6.0.

Cellular counting and viability

Cell viability was estimated on the basis of membrane integrity and evaluated by staining with propidium iodide (PI). HCT-116 cells were seeded in a 24-well plate (5 × 10⁴ cells mL⁻¹) and treated with **2** at 20, 40 or 80 μM for 24 h along with vehicle (0.4% DMSO) or 3.5 μM doxorubicin, as negative and positive controls, respectively. Cells were harvested with trypsin-EDTA 0.1% (Gibco, New York, USA), centrifuged and resuspended in a 5 μg mL⁻¹ PI solution (Sigma-Aldrich, Saint Louis, USA). After 5 min incubation in the dark, ten thousand events were acquired using the Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), on a gated region to exclude debris and doublets from the analysis. The experiments were repeated six times. The differences between negative control and experimental groups were determined by analysis of variance (ANOVA) followed by Dunnett's test using GraphPad Software 6.0 (Intuitive Software for Science, La Jolla, CA, USA). The minimal significance level was set at *p* < 0.05.

Results and Discussion

The chemical investigation of the extracts from the Brazilian zoanthids *P. caribaeorum* and *P. variabilis* allowed the isolation and characterization of two new sterols **1** and **2** (Figure 2), in addition to seven known sterols (Figure S1).

Compound **1**, an amorphous white powder, m.p. 127.1 °C; [α]_D²⁰ -34.0° (*c* 0.04, CHCl₃) had its molecular formula

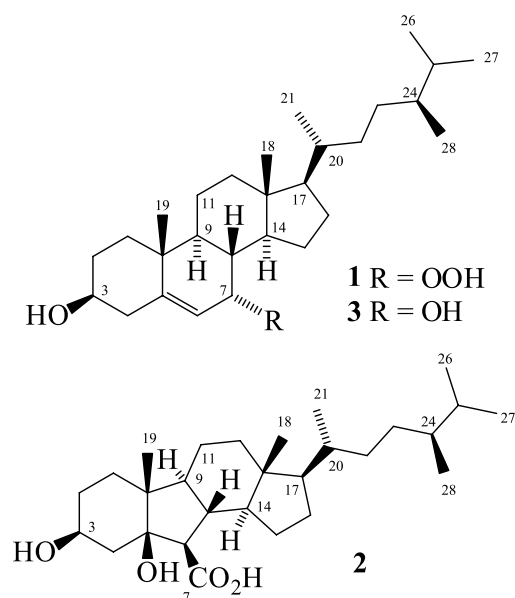


Figure 2. Chemical structures of the sterols **1-3**.

$C_{28}H_{48}O_3$ deduced by HRESIMS analysis, by measurement of the protonated molecular ion peak $[M + H]^+$ at m/z 433.3597 (calcd. 433.3676), and by ^{13}C NMR-CPD (composite pulse decoupling) analysis. Comparing the ^{13}C NMR data of **1** with those of 7α -hydroxycampesterol (**3**),¹⁹ also isolated in this work (Table 1), a good match was observed, but with a significant difference for the corresponding chemical shift of the C-7 oxymethine carbon, δ_C 78.6 for **1** instead of 65.8 for **3** ($\Delta\delta_C$ 12.8 ppm). This is in agreement with a carbon bearing a hydroperoxy moiety ($-OOH$),²⁰ as supported by the molecular formula $C_{28}H_{48}O_3$. In fact, the HSQC (heteronuclear single quantum correlation) spectrum exhibited correlations for the protons at δ_H 4.15 (br s, 1H, H-7) and 3.62 (m, 1H, H-3) with the carbons at δ_C 78.6 (C-7) and 71.5 (C-3), respectively, in agreement with the chemical shifts for oxymethines bearing a peroxy and a hydroxyl groups, respectively.²⁰ The deshielding of H-7/C-7 (δ_H/δ_C 4.15/78.6), when compared with H-3/C-3 (δ_H/δ_C 3.62/71.5), is due to the β -effect of the additional oxygen of the hydroperoxyl group when compared with that of the corresponding hydroxyl group of **3**.¹⁹ According with the above data, and by analysis of the COSY (correlation spectroscopy) and HMBC (heteronuclear multiple bond correlation) spectra (Figure 3a), the structure of **1** was established. The NOESY (nuclear Overhauser effect spectroscopy) spectrum did not show any key correlations to prove the β -position for HO-3 and the α -position for HOO-7. However, comparison of the carbon chemical shifts of **1** with those reported to 7α -hydroperoxycholesterol (**1a**) and 7β -hydroperoxycholesterol (**1b**)²⁰ (Figure 3b), showed a very close similarity with the **1a**, particularly for the chemical shifts related to the carbocyclic chain (Figure 3c). Thus, the structure of compound **1** was defined as 7α -hydroperoxycampesterol.

Compound **2**, an amorphous white powder, had its $C_{28}H_{48}O_4$ molecular formula determined by HRESIMS, based on the deprotonated molecular ion peak $[M - H]^-$ at m/z 447.3498 (calcd. 447.3474), ^{13}C CPD and DEPT 135 NMR spectra. Its IR spectrum (KBr) exhibited absorption bands at 3.492 (ν_{OH}) and 1.711 (ν_{CO}) cm^{-1} indicating a carboxyl function. Comparison of 1H and ^{13}C NMR spectra of **2** with those of **1** (Table 1), revealed that these compounds share the same ergostane backbone, but with significant differences in the chemical shifts related to the A and B rings. In the ^{13}C CPD and DEPT NMR spectra, signals corresponding to oxymethine carbons at δ_C 67.3 (C-3) and 58.7 (C-6), and of a tertiary alcohol carbon at δ_C 82.9 (C-5), in addition to a carboxylic acid at δ_C 178.2 (C-7) were observed. The unequivocal position for the two hydroxyl groups, including the carboxyl acid moiety, was supported by the HMBC spectrum. Correlations were observed for the diastereotopic methylene protons at δ_H 1.64/1.62 (m, 2H, H-2) and 2.13/1.68 (m, 2H, H-4) with the oxymethine carbon at δ_C 67.3 (C-3), as well as the methyl signal at δ_H 0.96 (s, 3H, H-19) with the non-hydrogenated carbon at δ_C 82.9 (C-5) supporting the presence of two hydroxyl groups at C-3 and C-5, respectively. Likewise, correlations for the protons at δ_H 2.27 (H-6) and 2.09 (H-8) with the carbon at δ_C 178.2 (C-7) warrant the carboxyl group at C-6. These data, including COSY and HMBC analyses (Figure 4a) were in agreement with a rearranged five membered ring B of a *nor*-ergostane steroid.²¹ These kind of $C_6-C_5-C_6-C_5$ tetracyclic ergostane steroids are rare in nature. Few examples include similar sterols isolated from the sponges *Stelletta hiwasaensis*²² and *Svenzea zeai*.²³ The β -positions for both hydroxyl groups at C-3 and C-5, for the carboxyl acid moiety at C-6, and the side chain at C-17, including the *cis*-configuration for the fused A/B

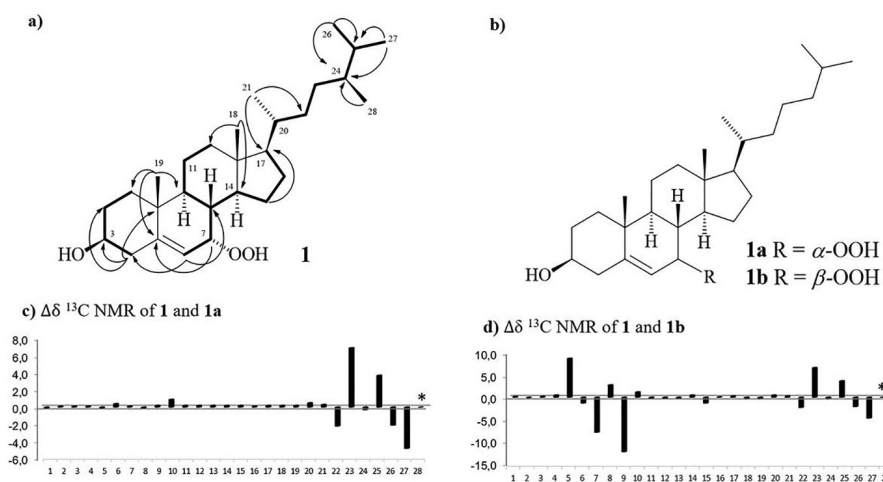


Figure 3. (a) Key 1H - 1H COSY (H—H) and HMBC (H→C) correlations for **1**; (b) structures of 7α -hydroperoxycholesterol (**1a**) and 7β -hydroperoxycholesterol (**1b**); (c) ^{13}C NMR comparison for **1** and **1a**, and (d) **1** and **1b**.²⁰ *Me-28 does not exist in cholestane sterols.

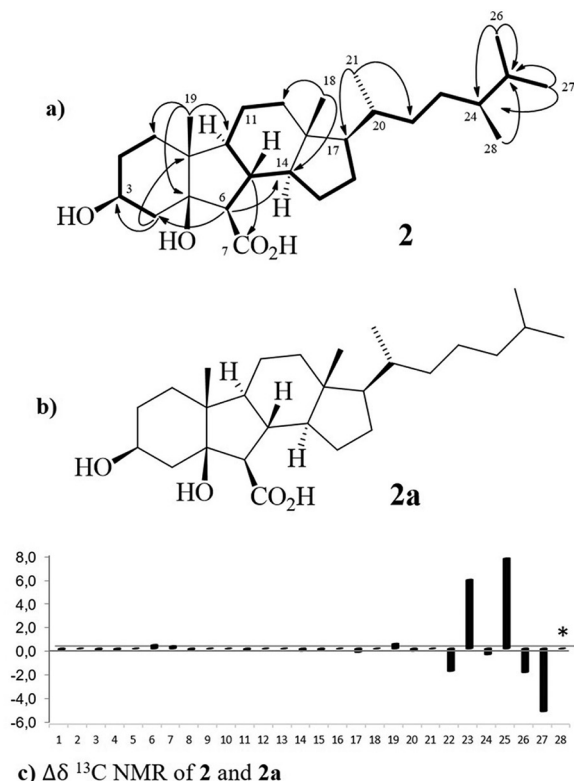


Figure 4. (a) Key ^1H - ^1H COSY (H—H) and HMBC (H→C) for compound **2**; (b) structure of *B-nor*-ergostan-3 β -5 β -diol-6 β -carboxyl acid (**2a**); (c) ^{13}C NMR comparison between **2** and **2a**.²¹ *Me-28 does not exist in cholestane sterols.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for **1** and **2** in CDCl_3 .

	1		2	
	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)
1	37.0	1.80 (m), 1.12 (m)	28.5	1.85 (m), 1.28 (m)
2	31.4	1.86 (m), 1.59 (m)	28.2	1.64 (m), 1.62 (m)
3	71.5	3.62 (m)	67.3	4.06 (s)
4	42.3	2.42 (m), 2.32 (t, 11.8)	43.7	2.13 (m), 1.68 (m)
5	148.8	—	82.9	—
6	120.3	5.73 (d, 3.2)	58.7	2.27 (d, 9.7)
7	78.6	4.15 (br s)	178.2	—
8	37.3	1.60 (m)	42.7	2.09 (m)
9	43.7	1.30 (m)	51.4	1.16 (m)
10	37.6	—	45.5	—
11	21.1	1.50 (m)	21.6	1.71 (m), 1.68 (m)
12	39.2	2.00 (m), 1.20 (m)	39.8	2.03 (m), 1.11 (m)
13	42.5	—	44.8	—
14	49.2	1.42 (m)	56.4	1.16 (m)
15	24.6	1.88 (m), 1.12 (m)	24.2	1.16 (m), 1.51 (m)
16	28.4	1.90 (m), 1.30 (m)	28.4	1.40 (m), 1.36 (m)
17	55.9	1.20 (m)	55.6	1.11 (m)
18	11.5	0.66 (s)	12.7	0.69 (s)
19	18.4	0.99 (s)	19.3	0.96 (s)
20	36.3	1.38 (m)	36.2	1.36 (m)
21	19.1	0.91 (d, 6.6)	17.7	0.92 (d, 6.7)
22	33.9	1.32 (m), 0.98 (m)	33.9	1.38 (m), 0.95 (m)
23	30.6	1.40 (m), 0.99 (m)	30.7	1.36 (m), 0.95 (m)
24	39.2	1.20 (m)	39.2	1.18 (m)
25	31.7	1.58 (m)	31.6	1.55 (m)
26	20.7	0.86 (d, 6.6)	20.7	0.84 (d, 6.7)
27	17.8	0.79 (d, 6.6)	17.7	0.78 (d, 6.7)
28	15.6	0.78 (d, 6.6)	15.6	0.77 (d, 6.7)

rings was inferred by direct comparison of the chemical shifts described to *B-nor*-ergostan-3 β -5 β -diol-6 β -carboxyl acid (**2a**),²⁴ which showed a good match from C-1 through C-21 (Figure 4b). On the other hand, the observed NOE displayed for H-6 at δ_{H} 2.27 (d, J 9.7 Hz) with H-9 and H-14 both at δ_{H} 1.16 (m), was an additional support for a β -orientation of the carboxylic acid group.²⁴ Thus, the structure of **2** was established as 6 β -carboxyl-24(*R*)-(8 \rightarrow 6)-*abeo*-ergostan-3 β ,5 β -diol.

In addition to the new compounds **1** and **2**, seven known sterols have also been isolated: 7 α -hydroxycampesterol (**3**),¹⁹ 5 α ,8 α -*epi*-dioxycampesterol (**4**),²⁵ campesterol (**5**),²⁶ 24(*R*)-ergost-7-en-3 β ,5 α ,6 β -triol (**6**),²⁷ 20-hydroxyecdysone (**7**),¹⁷ 2-*O*-acetyl-20-hydroxyecdysone (**8**)¹⁷ and 3-*O*-acetyl-20-hydroxyecdysone (**9**)¹⁷ (Figure S1). Additionally, GC-MS analysis of the hexane fraction (see SI), allowed the identification of the following sterols: gorgosterol (m/z 426), 7 α -hydroxycampesterol (m/z 416), campesterol (m/z 400), crinosterol (m/z 398), cholest-4-en-3-one (m/z 398), cholesta-3,5-dien-7-one (m/z 396), cholesterol (m/z 386) and cholesta-5,22-dien-3-ol (m/z 384)¹⁰ (Table S2 and Figure S20).

Orostanal, an *abeo*-sterol isolated from a marine sponge, and its derivatives, are known to have moderated cytotoxic effects.²⁸ The scanty literature attributes the cytotoxic activity of these class of compounds to apoptosis

induction.^{21,28} The cytotoxic activity of **2** was assessed by the MTT assay against a human colorectal tumor cell line (HCT-116). Compound **2** showed inhibition concentration mean (IC_{50}) values of 50 and 4 μM after 24 and 72 h treatment, respectively. Flow cytometry was performed after 24 h to assess the cellular concentration and viability as well (Figure 5). Compound **2** reduced cell number and increased non-viable cells at the higher concentrations. Compound **1** was not tested because it decomposed previously to the assay.

Conclusions

In this work, we described the chemical investigation of sterols from *P. caribaeorum* and *P. variabilis*. Nine sterol compounds were isolated, two of which being new. A series of cholestane and ergostane sterols were identified by GC-MS analysis. Compound **2**, an uncommon $C_6-C_5-C_6-C_5$ tetracyclic *abeo*-ergostan sterol, showed moderate cytotoxicity against the human colorectal tumor (HCT-116) cell line.

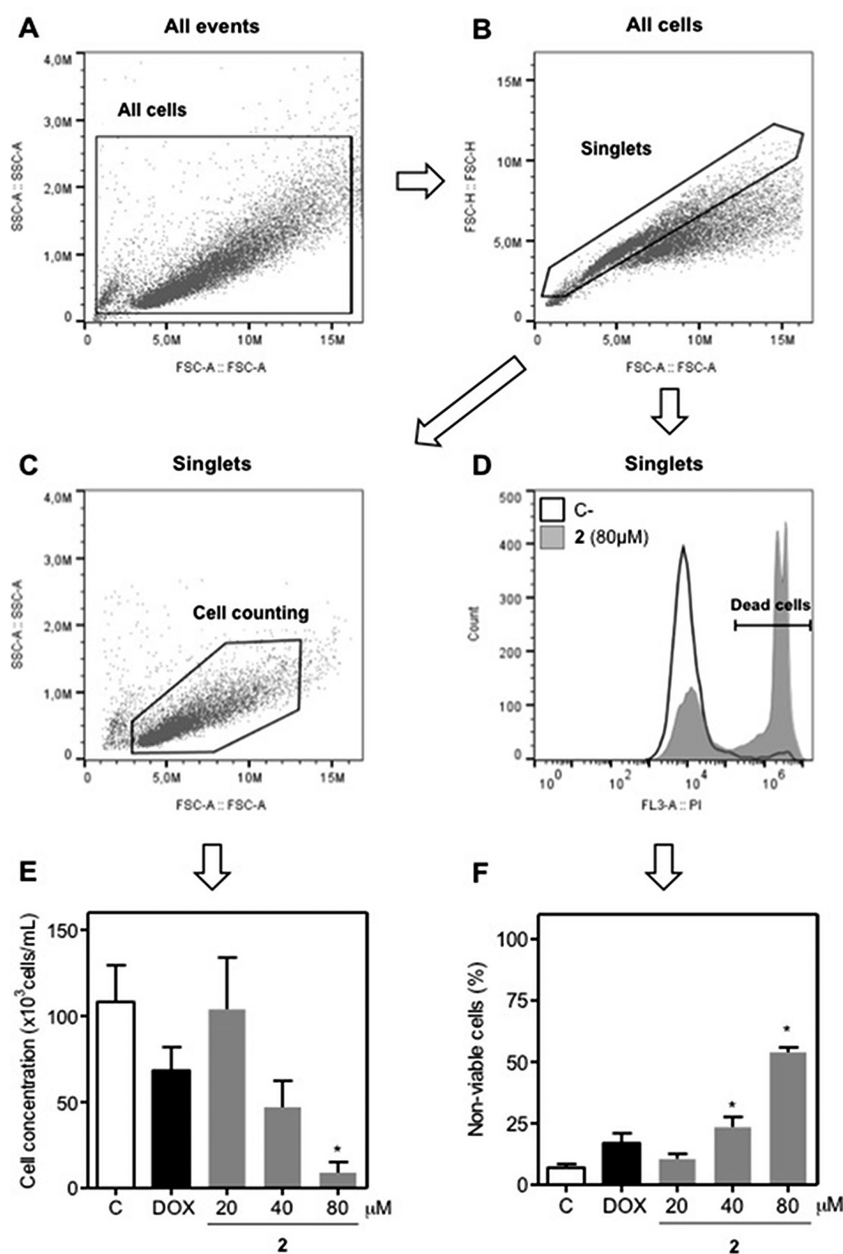


Figure 5. Treatment of cancer colon cells HCT-116 with **2** decreases cell counting and causes cell death. (A) to (D) depict gating strategies to analyze cell number (C) and dead cells stained with propidium iodide (D) among the single cells of each sample. (E) and (F) depict cell counting and percentage of dead cells, respectively. HCT-116 cells treated with DMSO at 0.4% (C), doxorubicin at 3.5 μM (DOX) or 6 β -carboxyl-24(*R*)-(8 \rightarrow 6)-*abeo*-ergostan-3 β ,5 β -diol (**2**) at 20, 40 or 80 μM during 24 h. Ten thousand events were acquired in the singlets gate region from each sample. Data obtained from 6 experiments performed in triplicates by flow cytometric analysis. * $p < 0.05$ compared to negative control by ANOVA followed by Dunnet's test.

Supplementary Information

Experimental details of the isolation of the known compounds and supplementary information (Figures S1-S20 and Tables S1-S2) are available free of charge at <http://jbcs.s bq.org.br> as a PDF file.

Acknowledgments

The authors thank the Governmental Brazilian Agencies CNPq, FUNCAP and CAPES for financial support and fellowships.

References

- Irei, Y.; Nozawa, Y.; Reime, J. D.; *Zool. Stud.* **2011**, *50*, 426.
- Reimer, J.; *Palythoa lamouroux*, 1816, 2014. Available at World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=205785>, accessed in December 2016.
- Hibino, Y.; Todd, P. A.; Yang, S.; Benayahu, Y.; Reimer, J. D.; *Hydrobiologia* **2013**, *733*, 31.
- Almeida, J. G. L.; Maia, A. I. V.; Wilke, D. V.; Silveira, E. R.; Braz-Filho, R.; La Clair, J. J.; Costa-Lotufo, L. V.; Pessoa, O. D. L.; *Mar. Drugs* **2012**, *10*, 2846.
- Imbs, A. B.; *Biochem. Syst. Ecol.* **2014**, *54*, 213; Carballeira, N. M.; Reyes, M.; *J. Nat. Prod.* **1995**, *58*, 1689; Miralles, J.; Diop, M.; Ferrel; Kornprobst, J. M.; *Comp. Biochem. Physiol.* **1989**, *94B*, 91.
- Hirata, Y.; Uemura, D.; Ueda, K.; Takano, S.; *Pure Appl. Chem.* **1979**, *51*, 1875; Ueda, K.; Takano, S.; Uemura, D.; Hirata, Y.; *Tetrahedron Lett.* **1978b**, *49*, 4909; Ito, S.; Hirata, Y.; *Tetrahedron Lett.* **1977**, *28*, 2429.
- Riobó, P.; Franco, J. M.; *Toxicon* **2011**, *57*, 368.
- Lazcano-Pérez, F.; Vivas, O.; Román-González, S. A.; Rodríguez-Bustamante, E.; Castro, H.; Arenas, I.; García, D. E.; Sánchez-Puig, N.; Arreguín-Espinosa, R.; *Toxicon* **2014**, *82*, 112; Pettit, G.; Fujii, O.; Hasler, J. A.; Schmidt, J. A. M.; Michel, C.; *J. Nat. Prod.* **1982**, *45*, 263; Pettit, G.; Fujii, O.; Hasler, J. A.; Schmidt, J. A. M.; *J. Nat. Prod.* **1982**, *45*, 272.
- Elbagory, A. M.; Meyer, M.; Ali, A.-H. A. M.; Ameer, F.; Parker-Nance, S.; Benito, M. T.; Doyagüez, E. G.; Jimeno, M. L.; Hussein, A. A.; *Steroids* **2015**, *101*, 110; Shigemori, H.; Sato, Y.; Kagata, T.; Kobayashi, J.; *J. Nat. Prod.* **1999**, *62*, 372; Fedorov, S. N.; Stonik, V. A.; Elyakov, G. B.; *Khim. Prirodn. Soedin.* **1988**, *4*, 603.
- Diop, M.; Leung-Tack, D.; Braekman, J. C.; Kornprobst, J. M.; *Biochem. Syst. Ecol.* **1986**, *14*, 151; Miralles, J.; Diop, M.; Ferre, L.; Kornprobst, J. M.; *Comp. Biochem. Physiol.* **1988**, *8*, 209; Kelecom, A.; Salé-Cava, A. M.; *Comp. Biochem. Physiol.* **1982**, *72*, 677.
- Cariello, L.; Crescenz, S.; Zanetti, L.; *Comp. Biochem. Physiol.* **1978**, *63B*, 77.
- Han, C.; Qi, J.; Shi, X.; Sakagami, Y.; Shibata, T.; Uchida, K.; Ojika, M.; *Biosci. Biotechnol. Biochem.* **2006**, *70*, 706.
- Wilke, D. V.; Jimenez, P. C.; Pessoa, C.; Moraes, M. O.; Araujo, R. M.; Silva, W. M. B.; Silveira, E. R.; Pessoa, O. D. L.; Braz-Filho, R.; Lopes, N. P.; Costa-Lotufo, L. V.; *J. Braz. Chem. Soc.* **2009**, *20*, 1455; Wilke, D. V.; Jimenez, P. C.; Araújo, R. M.; Silva, W. M. B.; Pessoa, O. D. L.; Silveira, E. R.; Pessoa, C. O.; Moraes, M. O.; Skwarczynski, M.; Simerska, P.; Toth, I.; Costa-Lotufo, L. V.; *Bioorg. Med. Chem.* **2010**, *18*, 7997.
- Uemura, D.; Toya, Y.; Watanabe, I.; Hirata, Y.; *Chem. Soc. Jpn.* **1979**, 1481.
- Moore, R. E.; Sheuer, P. J.; *Science* **1971**, *172*, 495.
- Reimer, J. D.; Ono, S.; Takishita, K.; Tsukahara, J.; Maruyama, T.; *Zool. Sci.* **2006**, *23*, 87.
- Cachet, N.; Genta-Jouve, G.; Ivanisevic, J.; Chevaldonne, P.; Sinniger, F.; Culioli, G.; Pérez, T.; Thomas, O. P.; *Sci. Rep.* **2015**, *5*, 1; Calcagno, M. P.; Camps, F.; Cool, J.; Melé, E.; *Eur. J. Entomol.* **1995**, *92*, 278.
- Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.
- Kobayashi, M.; Krishna, M. M.; Haribabub, B. B.; Anjaneyulu, V.; *Chem. Pharm. Bull.* **1993**, *41*, 87.
- Sung, P.-J.; Lin, M.-R.; Chen, J.-J.; Lin, S.-F.; Wu, Y.-C.; Hwang, T.-L.; Fang, L.-S.; *Chem. Pharm. Bull.* **2007**, *55*, 666.
- Lin, W.-H.; Fang, J.-M.; Cheng, Y.-S.; *Phytochemistry* **1998**, *48*, 1392.
- Miyamoto, T.; Kodama, K.; Aramaki, Y.; Higuchi, R.; Soest, R. W. M. V.; *Tetrahedron Lett.* **2001**, *42*, 6349.
- Wei, X.; Rodriguez, A. D.; Wang, Y.; Franzblau, S. G.; *Tetrahedron Lett.* **2007**, *48*, 8851.
- Schenast, J. C.; Witter, D. P.; Grant, E.; Boldt, G. E.; Offer, J.; Wentworth, J. P.; *Angew. Chem. Int. Ed.* **2009**, *48*, 9469.
- Ioannou, E.; Abdel-Razika, A. F.; Zervouc, M.; Christfidisa, D.; Alexi, X.; Vagiassa, C.; Alxis, M. N.; Roussis, V.; *Steroids* **2009**, *74*, 73.
- Zhang, X.; Cambrail, A.; Miesch, M.; Roussi, S.; Raul, F.; Marchioni, E.; *J. Agric. Food Chem.* **2006**, *54*, 1196.
- Madaio, A.; Piccialli, V.; Sica, D.; *J. Nat. Prod.* **1989**, *52*, 952.
- Cui, J.; Qi, B.; Gan, C.; Liu, Z.; Huang, H.; Lin, Q.; Zhao, D.; Huang, Y.; *Mar. Drugs* **2015**, *13*, 2488.

Submitted: August 26, 2016

Published online: December 15, 2016