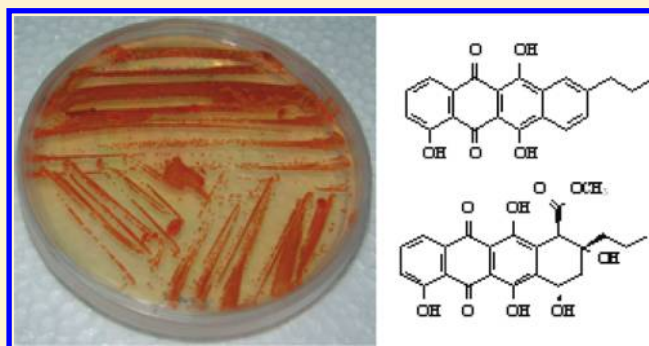


Anthracyclinones from *Micromonospora* sp.Thiciania da S. Sousa,[†] Paula C. Jimenez,[‡] Elthon G. Ferreira,[‡] Edilberto R. Silveira,[†]
Raimundo Braz-Filho,^{†,§} Otilia D. L. Pessoa,^{*,†} and Letícia V. Costa-Lotufo^{*,‡,§}[†]Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza, CE, 60.021-970, Brazil[‡]Instituto de Ciências do Mar, LABOMAR, Universidade Federal do Ceará, Fortaleza, CE, 60.165-081, Brazil[§]Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Fortaleza, CE, 60.430-270, Brazil

Supporting Information

ABSTRACT: Four new anthracyclinones, 4,6,11-trihydroxy-9-propyltetracene-5,12-dione (1), 1-methoxy-9-propyltetracene-6,11-dione (2), 7,8,9,10-tetrahydro-9-hydroxy-1-methoxy-9-propyltetracene-6,11-dione (3), and 10 β -carbomethoxy-7,8,9,10-tetrahydro-4,6,7 α ,9 α ,11-pentahydroxy-9-propyltetracene-5,12-dione (4), were isolated from a strain of *Micromonospora* sp. associated with the tunicate *Eudistoma vannamei*. All structures were established by 1D and 2D NMR (COSY, HSQC, HMBC, NOESY) and HRESIMS experiments. Compounds 1 and 4 were cytotoxic against the HCT-8 human colon adenocarcinoma cell line, with IC₅₀ values of 12.7 and 6.2 μ M, respectively, while compounds 2 and 3 were inactive.



The chemistry and pharmacology of marine microorganisms has been extensively investigated in the last five years with unprecedented results. The bacteria, particularly those from the order Actinomycetales, commonly known as actinomycetes have proven to be a prolific source of new and bioactive compounds.¹ The secondary metabolites produced by actinomycetes display a broad spectrum of biological activities including antibacterial, antifungal, antiprotozoal, anthelmintic, antiviral, insecticidal, cytotoxic, antioxidant, and anti-inflammatory.² Among the various genera within the actinomycetales, *Micromonospora* has been intensively investigated, leading to the isolation of several active secondary metabolites, such as anthraquinones,³ anthracyclines,⁴ alkaloids,⁵ and macrolides.⁶

As part of our efforts to obtain anticancer secondary metabolites from marine natural sources, we have investigated the extract of a *Micromonospora* sp. strain isolated from *Eudistoma vannamei*, a Brazilian endemic ascidian. *E. vannamei* is largely found along the coast of northeastern Brazil, especially the rocky beaches of Ceará State.⁷ The apoptosis-inducing activity found in extracts of *E. vannamei* were previously reported, while the chemical nature of these compounds remained unknown.^{7,8} Herein, we describe the isolation and structure elucidation of four new anthracyclinones (1–4). The cytotoxicity of these compounds was evaluated using the MTT assay against HCT-8 human adenocarcinoma cancer cells. The active EtOAc extract of *Micromonospora* sp. was fractionated using chromatographic methods (open silica gel column, Sephadex LH-20 and HPLC) to afford four anthracyclinones (1–4), Figure 1.

Compound 1 was isolated as a red powder. Its molecular formula, C₂₁H₁₆O₅, was deduced by HRESIMS spectrum from

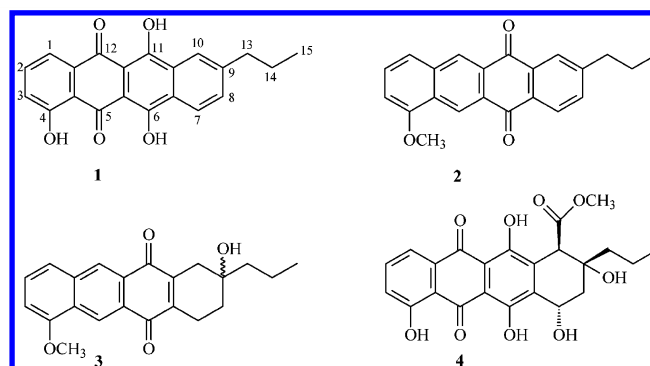


Figure 1. Structures of compounds 1–4 isolated from *Micromonospora* sp.

the quasimolecular ion peak [M + H]⁺. The IR spectrum displayed absorption bands at 3368, 1675, 1601, and 1589 cm⁻¹ for a hydroxyl, conjugated ketone, and aromatic ring, respectively. The ¹H NMR spectrum of 1 displayed signals for three chelated hydroxyl groups at δ 15.44 (s), 14.32 (s), and 12.47 (s) and aromatic protons at δ 8.40 (d, *J* = 8.3 Hz, H-7), 8.29 (s, H-10), and 7.64 (dd, *J* = 8.3, 1.4 Hz, H-8) corresponding to an AMX system. Also in the aromatic region were observed signals at δ 7.95 (H-1), 7.71 (H-2), and 7.28 (H-3). In addition, the ¹H NMR spectrum of 1 displayed signals for a propyl moiety (Table 1). The ¹³C NMR and DEPT spectra of 1 exhibited 21

Special Issue: Special Issue in Honor of Gordon M. Cragg

Received: September 30, 2011

Published: January 17, 2012

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 1–4^a

position	1		2		3		4	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	118.9	7.95, dd (8.1, 0.8)	122.1	7.65, d (7.6)	122.9	7.65, d (8.0)	119.9	7.85, d (8.0)
2	136.3	7.71, t (8.1)	130.1	7.60, t (7.6)	131.0	7.56, t (8.0)	137.6	7.70, t (8.0)
3	123.5	7.28, dd (8.1, 0.8)	107.3	6.99, d (7.6)VN	108.4	6.96, d (8.0)	125.0	7.30, d (8.0)
4	162.6		157.4		157.9		162.9	
4a	119.2		127.7		127.8		116.2	
5	185.5		124.6	9.27, s	123.6	9.30, s	191.1	
5a	106.5		129.3		129.4		111.7	
6	160.4		182.9		184.9		156.1	
6a	128.1		132.9		147.2		135.2	
7	125.4	8.40, d, (8.3)	127.9	8.32, d (8.0)	22.6	3.12, m 3.01, br d (18.7)	62.8	5.32, t (3.0)
8	132.8	7.64, dd (8.3, 1.7)	134.7	7.58, d (8.0)	33.2	2.03, m 1.65, m	35.2	2.29, d (3.0)
9	147.6		150.0		69.2		71.6	
10	124.5	8.29, s	127.3	8.19, br s	37.2	3.24, d (19.1) 2.78, d (19.1)	51.9	4.27, s
10a	131.0		134.6		145.4		137.4	
11	161.9		183.7		185.5		157.2	
11a	117.3		130.6		132.2		111.5	
12	183.6		129.0	8.77, s	128.6	8.96, s	186.3	
12a	134.4		136.4		136.8		133.6	
13	38.4	2.84, t (7.5)	38.4	2.79, t (7.5)	46.3	1.70, m	42.6	1.68, td (12.5, 3.7) 1.49, td (12.5, 4.0)
14	24.3	1.79, m	24.3	1.75, sext (7.5)	17.6	1.67, m	15.9	1.78, m 1.59, m
15	14.1	1.01, t (7.3)	13.9	1.00, t (7.5)	15.7	0.95, t (6.8)	14.8	1.00, t (7.2)
16							171.5	3.73, s
17							52.6	
4-OCH ₃			56.0	4.07, s	56.5	3.84, s		
4-OH	12.47, s						12.05, s	
6-OH	14.32, s						13.41, s	
11-OH	15.44, s						12.87, s	

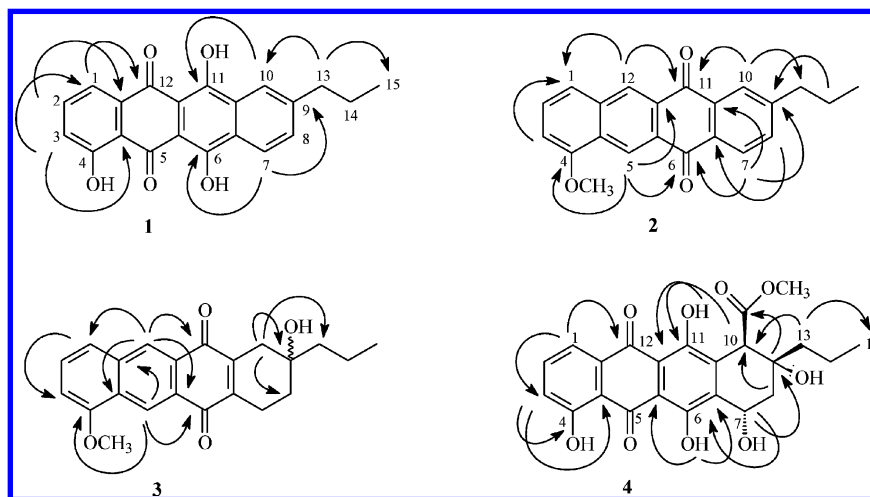
^aCDCl₃ (1, 2, and 4); C₅D₅N (3).

Figure 2. Important HMBC correlations for compounds 1–4.

carbon signals comprising one methyl, two methylenes, six sp^2 methines, and 12 non-hydrogen-bearing carbons, including two carbonyl (δ 183.6 and 188.5) and three oxygenated (δ 162.6, 160.4, and 161.9) carbons. The spectroscopic data are compatible with a trihydroxy-anthracyclinone bearing a propyl group. The isopropyl moiety at C-9 was determined by HMBC

correlations from H-10 (δ 8.29) to C-11 (δ 161.9) and C-13 (δ 38.4) and from H-8 (δ 7.64) to C-13. The key correlations from H-7 (δ 8.40) to C-6 (δ 160.4) and C-9 (δ 147.6), as well as from H-1 (δ 7.95) to C-12 (δ 183.6), were critical in determining the complete structure of 1 (Figure 2), which was established as 4,6,11-trihydroxy-9-propyltetracene-5,12-dione.

Compound **2** was obtained as an orange powder. Its molecular formula, $C_{22}H_{18}O_3$, was deduced by HRESIMS spectrum from the quasimolecular ion peak $[M + H]^+$. Its IR spectrum displayed an intense absorption band at 1675 for a conjugated ketone, as well as skeletal bands at 1617 and 1589 cm^{-1} . The 1H NMR spectrum of **2** displayed two deshielded singlets at δ 9.27 (H-5) and 8.77 (H-12) for protons *peri* to the carbonyls, signals for two AMX systems, one at δ 7.60 (H-2), 6.99 (H-3), and 7.65 (H-1) and a second at δ 8.32 (H-7), 7.58 (H-8), and 8.19 (H-10). Additionally, the 1H NMR spectrum showed signals for a methoxyl and a propyl group (Table 1). The ^{13}C NMR and DEPT spectra of **2** displayed 22 carbon signals comprising two methyls (one of them a methoxyl), two methylenes, eight sp^2 methines, and 10 non-hydrogen-bearing carbons, including two carbonyls (δ 183.7 and 182.9). These data showed that **2** is also an anthracyclinone derivative, however with a different arrangement of carbonyl carbons than **1**. The observed long-range correlations of H-10 (δ 8.19) with C-11 (δ 183.7) and C-13 (δ 38.4), of H-7 (δ 8.32) with C-6 (δ 182.9) and C-9 (δ 150.0), and of H-14 (δ 1.75) with C-9 indicated the position of the isopropyl group at C-9, as well as the carbonyl groups at C-6 and C-11 (C ring) (Figure 2). On the basis of these data, the structure of **2** was established as 4-methoxy-9-propyltetracene-6,11-dione.

Compound **3** was isolated as a yellow powder. Its molecular formula, $C_{22}H_{22}O_4$, was determined by HRESIMS spectrum. The IR spectrum exhibited absorption bands at 3400, 1660, and 1615 cm^{-1} for hydroxyl, conjugated ketone, and carbon-carbon double bonds, respectively. The 1H NMR spectrum of **3** exhibited two isolated signals at δ 9.30 (s) and 8.96 (s) in the aromatic region, as well as signals at δ 7.65 (H-1), 7.56 (H-2), and 6.96 (H-3). Additionally, the 1H NMR spectrum showed signals for a methyl group and eight methylene protons (Table 1). The ^{13}C NMR and DEPT spectra of **3** exhibited 22 carbon signals comprising one methyl, one methoxyl, five methylenes, five sp^2 methines, and 10 non-hydrogen-bearing carbons, including two carbonyls (δ 185.5 and 184.9) and one oxygenated sp^3 (δ 69.2) carbon. The spectroscopic data were compatible with a methoxylated anthracyclinone derivative bearing a propyl group. The HMBC experiment showed correlations of the protons at δ 9.30 (H-5) and 8.96 (H-12) with the carbonyls at δ 184.9 (C-6) and 185.5 (C-11), respectively, and for the diastereotopic methylene protons at δ 3.24/2.78 (2H-10) with the carbons at δ 69.2 (C-9), 33.2 (C-8), and 46.3 (C-13). Other important HMBC correlations are depicted in Figure 2. On the basis of the data, the structure of compound **3** was established as the new 7,8,9,10-tetrahydro-9-hydroxy-4-methoxy-9-propyltetracene-6,11-dione. It was not possible to unambiguously determine the stereochemistry of the single stereocenter of **3**, but on the basis of models from literature the α -positioning of the hydroxyl at C-9 was suggested.⁹

Compound **4** was also isolated as a yellow powder. Its molecular formula, $C_{23}H_{22}O_8$, was deduced by HRESIMS. Its IR spectrum showed an absorption band at 3400 cm^{-1} corresponding to a hydroxyl group and bands at 1729 and 1606 cm^{-1} corresponding to an ester and conjugated ketone, respectively. The 1H NMR spectrum of **4** exhibited signals at δ 7.85 (H-1), 7.70 (H-2), and 7.30 (H-3), which were shown to be vicinal by coupling constants. In addition, the 1H NMR spectrum exhibited signals for an oxymethine proton at δ 5.32 (H-7) and a benzylic methine α -positioned to a carboxyl at δ 4.27 (H-10), along with signals for a methoxyl and a propyl moiety (Table 1). The ^{13}C NMR and DEPT spectra of **4**

exhibited 23 carbon signals, revealing a similar structure to compounds **1–3**, with additional signals for a carbomethoxy moiety and an additional sp^3 oxymethine carbon. The HMBC experiment showed correlation of the signal at δ 7.85 (H-1) with the carbonyl carbon at δ 186.3 (C-12). A hydroxyl group was placed at C-7 with correlations of the oxymethine proton at δ 5.32 (H-7) with the carbons at δ 156.1 (C-6), 137.4 (C-10a), and 71.6 (C-9). The carbomethoxy group was located at C-10 through correlations of the signal at δ 4.27 (H-10) with the carbons at δ 157.2 (C-11), 42.5 (C-13) and the carboxyl carbon at δ 171.5 (C-16) (Figure 2). The relative stereochemistry of the stereocenters of **4** (D ring) was deduced by the NOESY experiment, coupling constant values, and comparison with literature data for similar compounds.^{4,10} The NOE correlations of H-10 (δ 4.27) with H-13a (δ 1.68) and H-14a (δ 1.78) suggested that both carbomethoxy and propyl groups were β -positioned. The configuration of C-7 was determined on the basis of the multiplicities and coupling constant values displayed for H-7 and the homotopic methylene protons 2H-8. On the basis of the data above it was inferred that the D ring adopts a skew-type conformation, in which the oxymethine proton H-7 is β in order to hold the same dihedral angles (about 60°), and justifying the small *J* value and the multiplicity of H-7 (t) and 2H-8 (d). The structure of **4** was established as 10 β -carbomethoxy-7,8,9,10-tetrahydro-4,6,7 α ,9 α ,11-pentahydroxy-9-propyltetracene-5,12-dione, an analogue of ϵ -rhodomycinone, whose difference is the presence of a propyl group instead of an ethyl group at C-9.¹⁰

Anthracyclines comprise a group of aromatic glycosidic microbial polyketides common in Actinomycetes, typically from *Streptomyces* spp.,¹¹ but also from the genus *Micromonospora*.^{3,4,12–14} They are a diverse group of molecules, which differ in the number and position of hydroxyl or methoxyl groups in the anthraquinone nuclei, in the substitution pattern and stereochemistry of the D ring, and in the complexity of the glycoside moieties.¹¹ The anthracyclines are among the most studied natural products due to their importance in cancer therapeutics.^{11,15} The molecular mechanism of action of anthracyclines includes DNA damage mediated by topoisomerase II or by reactive oxygen species.¹⁵ The aglycones of the anthracyclines, named anthracyclinones, common in Actinomycetes,⁹ have been also isolated from the genus *Micromonospora*.¹⁶

The cytotoxic activity of compounds **1–4** was evaluated against HCT-8 human adenocarcinoma cells (Table 2).

Table 2. Cytotoxicity of Compounds 1–4 on HCT-8 Cells, Evaluated by the MTT Assay after 72h Exposure^a

	1	2	3	4	doxorubicin
IC ₅₀	12.7	>70	>70	6.2	0.1
95% CI	9.9–19.1			5.0–7.6	0.06–0.13

^a IC₅₀ (μ M) values and 95% CI were obtained by nonlinear regression using the GraphPad program (Intuitive Software for Science, San Diego, CA, USA).

Interestingly, the 5,12-anthracyclinones **1** and **4** displayed moderate cytotoxicity, with IC₅₀ values of 12.7 and 6.2 μ M, respectively, while 6,11-anthracyclinones derivatives **2** and **3** were considered inactive. In general the anthracyclines are 80- to 100-fold more active than their respective aglycones.^{17,18} Indeed the anthracycline doxorubicin, used as positive control, was 141 and 69 times more active than **1** and **4**, respectively. The synthesis and isolation of new enantiomerically pure

anthracyclinone moieties is a key strategy for developing new anthracyclines with improved therapeutic efficacy and reduced cardiotoxicity.¹⁹ Further studies are in progress to obtain the anthracyclines from the isolated anthracyclinones and also to characterize their mechanism of cytotoxic action.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points (mp) were measured on a Marconi N480D apparatus. Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. IR spectra were obtained on a Perkin-Elmer FT-IR spectrum 1000 spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were acquired using a LCMS-IT-TOF (Shimadzu) spectrometer. ¹H (500 MHz) and ¹³C (125 MHz) NMR spectra were performed on a Bruker DRX-500 spectrometer. HPLC analysis was carried out using a UFLC (Shimadzu) system equipped with a SPD-M20A diode array UV-vis detector and a Phenomenex C₁₈ column, 5 μm (4.6 × 250 mm). Column chromatography was carried out on silica gel 60 (70–230 mesh, Vetec or 230–400 mesh, Merck), Sephadex LH-20, or SPE C₁₈ cartridges (Strata C18-E, 20 g/60 mL, 55 μm, 70 Å) from Phenomenex. TLC was performed on precoated silica gel aluminum sheets (Kieselgel 60 F₂₅₄, 0.20 mm, Merck), and compounds were visualized by heating (~100 °C) the plates sprayed with a vanillin/perchloric acid/EtOH solution.

Microorganism. *Eudistoma vannamei*, an endemic tunicate from the northeastern coast of Brazil, was manually collected at Taíba Beach, Ceará (03°30'21.23' S; 038°53'40.16' W) and sprayed with 70% EtOH for superficial decontamination. The sample was washed in autoclaved seawater and kept frozen at –20 °C for 4 weeks. The sample was processed further under sterile conditions: crushed colonies of the tunicate were spread on various agar-media plates and left at 28 °C. Four weeks later, an orange-brown spore-forming colony was separated from GYM media (malt extract, glucose, and yeast extract from Difco). This strain was identified as *Micromonospora* sp. based on 16S rRNA gene sequencing and BLAST analysis. The nucleotide sequence was submitted to NCBI GenBank and is available under the accession number JN797618. *Micromonospora* sp. was then grown in 36 replicates of 2.0 L Erlenmeyer flasks containing 500 mL of A1 broth (10 g of starch, 2 g of peptone, and 4 g of yeast extract from Difco in 1 L of seawater 75%) under 200 rpm agitation during 10 days at room temperature and then extracted with EtOAc.

Extraction and Isolation. The EtOAc extract (18 L) was dried over Na₂SO₄ and concentrated over reduced pressure to give 1.6 g of the crude extract. This material was fractionated over silica gel by elution with *n*-hexane, 1:1 *n*-hexane/CH₂Cl₂, CH₂Cl₂, 1:1 CH₂Cl₂/EtOAc, EtOAc, 1:1 EtOAc/MeOH, and MeOH to yield seven fractions (F-1 to F-7). F-2 (143.3 mg) was subjected to a silica gel column using a gradient of *n*-hexane/CH₂Cl₂ (8:2, 7:3, 1:1), CH₂Cl₂, and MeOH to yield 43 fractions of approximately 8 mL each. Compound 1 (4 mg) was isolated from F-17/20, obtained by elution with *n*-hexane/CH₂Cl₂ (7:3). F-26/30 (48.6 mg, obtained by elution with *n*-hexane/CH₂Cl₂, 1:1) was applied to HPLC analysis using a RP-C₁₈ column (250 mm × 10 mm i.d. × 5 μm) in the isocratic mode using 95:5 methanol/water as eluent (v/v) and a flow rate of 4.5 mL min^{–1} with an injection volume ("loop") of 200 μL to yield compound 2 (5.9 mg). Compound 3 (6.5 mg) was isolated from F-3 (160.4 mg) by HPLC using a RP-C₁₈ column (250 × 10 mm i.d. × 5 μm) in the isocratic mode, with acetonitrile/water, 8:2 (v/v), and a flow rate of 4.7 mL min^{–1} with an injection volume ("loop") of 200 μL. F-4 (203 mg) was subjected to silica gel flash column chromatography using an isocratic system of *n*-hexane/EtOAc (9:1) to provide 23 fractions of approximately 3 mL each. Compound 4 (2.1 mg) was isolated from F-16/20.

1,6,11-Trihydroxy-9-propyltetracene-5,12-dione (1): red resin; IR (KBr) ν_{\max} 3400, 2919, 2853, 1594, 1453, 1383, 1219, 1028, 772 cm^{–1}; ¹H (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Tables 1 and 2; positive HRESIMS *m/z* 349.1063 (calcd) [M + H]⁺ (calcd for C₂₁H₁₆O₅, 349.1076).

1-Methoxy-9-propyltetracene-6,11-dione (2): orange powder; mp 192.3–192.7 °C; IR (KBr) ν_{\max} 3368, 2956, 2922, 1675, 1601, 1294, 1271, 970, 772, 748 cm^{–1}; ¹H (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Tables 1 and 2; positive HRESIMS *m/z* 331.1345 (calcd) [M + H]⁺ (calcd for C₂₂H₁₈O₃, 331.1334).

7,8,9,10-Tetrahydro-9-hydroxy-1-methoxy-9-propyltetracene-6,11-dione (3): yellow resin; [α]_D²⁰ –65 (c 0.02, CHCl₃); IR (KBr) ν_{\max} 3400, 2952, 2921, 2849, 1660, 1615, 1385, 1290, 1068, 770, 746 cm^{–1}; ¹H (500 MHz, Py-d₃) and ¹³C NMR (125 MHz, Py-d₃) data, see Tables 1 and 2; positive HRESIMS *m/z* 351.1575 (calcd) [M + H]⁺ (calcd for C₂₂H₂₂O₄, 351.1596).

10β-Carbomethoxy-7,8,9,10-tetrahydro-4,6,7α,9α,11-pentahydroxy-9-propyltetracene-5,12-dione (4): yellow powder; mp 173.5–173.8 °C; [α]_D²⁵ –61.5 (c 0.013, CHCl₃); IR (KBr) ν_{\max} 3400, 2952, 2922, 2849, 1739, 1729, 1606, 1460, 1277, 1022, 771 cm^{–1}; ¹H (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Tables 1 and 2; negative HRESIMS *m/z* 441.1170 (calcd) [M – H][–] (calcd for C₂₃H₂₂O₉, 441.1185).

Cytotoxicity Evaluation: MTT Assay. Cytotoxic activity was evaluated against the HCT-8 human cancer adenocarcinoma cell line obtained from Children's Mercy Hospital, Kansas City, MO, USA. Cells were grown in RPMI-1640 medium supplemented with 2 mM glutamine, 10% fetal calf serum, 100 μg/mL streptomycin, and 100 U/mL penicillin and housed at 37 °C with a 5% CO₂ atmosphere. The cell cultures were regularly split to keep them in a logarithmic phase of growth.

The compounds were tested at concentrations ranging from 0.01 to 25 μg/mL during 72 h, and the effect on cell proliferation was evaluated *in vitro* using the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay, as described by Mosmann.²⁰ IC₅₀ (the concentration that inhibits growth by 50%) values were calculated, along with the respective 95% CI (confidence interval), by nonlinear regression using the software GraphPad Prism 5.0.

ASSOCIATED CONTENT

Supporting Information

IR, HRESIMS, and ¹H and ¹³C NMR spectra of compounds 1–4 are available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*(O.D.L.P.) Tel: +55-85-33669441. Fax: +55-85-33669782. E-mail: opessoa@ufc.br. (L.V.C.-L.) Tel: + 55-85-33668255. Fax: +55-85-33668333. E-mail: costalotufog@gmail.com.

Notes

[#]Pesquisador Emérito Visitante, FAPERJ/UENF/UFRRJ.

ACKNOWLEDGMENTS

The authors thank the National Agencies CNPq, FUNCAP, CAPES, and PRONEX for financial support.

DEDICATION

Dedicated to Dr. Gordon M. Cragg, formerly Chief, Natural Products Branch, National Cancer Institute, Frederick, Maryland, for his pioneering work on the development of natural product anticancer agents.

REFERENCES

- (1) Jensen, P. R.; Mincer, T. J.; Williams, P. G.; Fenical, W. *Antonie van Leeuwenhoek* **2005**, *87*, 43–48.
- (2) Kekuda, T. R. P.; Shobha, K. S.; Onkarappa, R. J. *Pharm. Res.* **2010**, *3*, 250–256.
- (3) Igarashi, Y.; Yanase, S.; Sugimoto, K.; Enomoto, M.; Miyanaga, S.; Trujillo, M. E.; Saiki, I.; Kuwahara, S. *J. Nat. Prod.* **2011**, *74*, 862–865.

- (4) Yang, S.-W.; Chan, T.-M.; Terracciano, R. P.; Loeberberg, D.; Chen, G.; Patel, M.; Gullo, V.; Pramanik, B.; Chu, M. *J. Antibiot.* **2004**, *57*, 601–604.
- (5) Charan, R. D.; Schlingmann, G.; Janso, J.; Bernan, V.; Feng, X.; Carter, G. T. *J. Nat. Prod.* **2004**, *67*, 1431–1433.
- (6) Gaertner, A.; Ohlendorf, B.; Schulz, D.; Zinecker, H.; Wiese, J.; Imhoff, J. F. K. *Mar. Drugs* **2011**, *9*, 98–108.
- (7) Jimenez, P. C.; Fortier, S. C.; Lotufo, T. M. C.; Pessoa, C.; Moraes, M. E. A.; Moraes, M. O.; Costa-Lotufo, L. V. *J. Exp. Mar. Biol. Ecol.* **2003**, *287*, 93–101.
- (8) Jimenez, P. C.; Wilke, D. V.; Takeara, R.; Lotufo, T. M. C.; Pessoa, C.; Moraes, M. O.; Lopes, N. P.; Costa-Lotufo, L. V. *Comp. Biochem. Physiol.* **2008**, *151*, 391–398.
- (9) Clark, B.; Capon, R. J.; Stewart, M.; Lacey, E.; Tennant, S.; Gill, J. H. *J. Nat. Prod.* **2004**, *67*, 1729–1731.
- (10) Johdo, O.; Ishikura, T.; Takeuchi, A. *J. Antibiot.* **1991**, *44*, 1110–1120.
- (11) Laatsch, H.; Fotso, S. *Top. Curr. Chem.* **2008**, *282*, 3–74.
- (12) Igarashi, Y.; Trujillo, M. E.; Martínez-Molina, E.; Yanase, S.; Miyanaga, S.; Obata, T.; Sakurai, H.; Saiki, I.; Fujita, T.; Furumai, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3702–3705.
- (13) He, H.; Ding, W. D.; Bernan, V. S.; Richardson, A. D.; Ireland, C. M.; Greenstein, M.; Ellestad, G. A.; Carter, G. T. *J. Am. Chem. Soc.* **2001**, *123*, 5362–5363.
- (14) Nair, M. G.; Mishra, S. K.; Putnam, A. R.; Pandey, R. C. *J. Antibiot.* **1992**, *45*, 1738–1745.
- (15) Beretta, G. L.; Zunino, F. *Top. Curr. Chem.* **2008**, *283*, 1–19.
- (16) Ströck, K.; Zeeck, A.; Antal, N.; Fiedler, H.-P. *J. Antibiot.* **2005**, *58*, 103–110.
- (17) Dessypris, E. N.; Brenner, D. E.; Hande, K. R. *Cancer Treat. Rep.* **1986**, *70*, 487–490.
- (18) Dessypris, E. N.; Brenner, D. E.; Baer, M. R.; Hande, K. R. *Cancer Res.* **1988**, *48*, 503–506.
- (19) Acmatowicz, O.; Szechner, B. *Top. Curr. Chem.* **2008**, *282*, 143–186.
- (20) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.