Artigo

TRACHYLOBANE AND KAURANE DITERPENES FROM *Croton floribundus* SPRENG.

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A new trachylobane diterpene *ent*-trachyloban-18,19-diol (**1**) was isolated from root bark of *Croton floribundus*, along with known diterpenes *ent*-trachyloban-19-oic acid (**2**), 15β-hydroxy-*ent*-trachyloban-19-oic acid (**3**), *ent*-trachyloban-19-ol (**4**), *ent*-kaur-16-en-19-oic acid (**5**), *ent*-kaur-16-ene-6α,19-diol (**6**) and *ent*-16α-hydroxykaur-11-en-19-oic acid (**7**). *ent*-trachyloban-18,19-diol (**1**) was submitted to derivatization reactions affording four new compounds (**8**-**11**). Cytotoxic activity of diterpenes **1**, **3**, **4**, **7**-**11** against three human cancer cell lines was evaluated. No compounds showed cytotoxic potential with IC_{50} values greater than 25 µg/mL. Compound **6** was evaluated against five human cancer cell lines, showing moderate effect against three cancer cell lines, MDA-MB-435, HCT-8 and HCT-116, with IC_{50} values of 14.32, 13.47 and 12.1 µg/mL, respectively.

Keywords: diterpenes; *Croton floribundus;* Euphorbiaceae.

INTRODUCTION

Trachylobane and kaurene are diterpenoids biosynthetically derived from the monocyclic membrane. Kaurenes have been reported in several families of higher plants, with kaurenoic acid and its natural derivatives found particularly in the Asteraceae and Euphorbiaceae families.[1] These compounds have been largely cited as cytotoxic, antitumoral, genotoxic, antiinflammatory, antibacterial, antifungal and moluscidal.[2-4] On the other hand, trachylobane is found in some species of Euphorbiaceae,[5] Annonaceae,[6] Asteraceae,[7] Lamiaceae[8] and Leguminosae.[9] Although the biological activities of trachylobane compounds are poorly investigated, they are cited to possess vasorelaxant and cytotoxic properties.[10]

As part of an investigative effort to find bioactive diterpenoids in native species from Northeastern Brazil flora, this study reports the phytochemical investigation of the root bark of *Croton floribundus* Spreng., a tree with ethnobotanical use in the treatment of syphilis and ulcers.[11] In this work, the new *ent*-trachyloban-18,19-diol (**1**) is reported, along with the known *ent*-trachyloban-19-oic acid (**2**),[12] *ent*-15β-hydroxytrachyloban-19-oic acid (**3**),[13] *ent*-trachyloban-19--ol (**4**),[12] *ent*-kaur-16-en-19-oic acid (**5**),[14] *ent*-kaur-16-en-6α,19-diol (**6**)[15] and *ent*-16α-hydroxykaur-11-en-19-oic acid (**7**).[16] *ent*-trachyloban-18,19-diol **1** was derivatized to the diacetate, dimethoxyl and diallyl derivatives **8**-**10**, respectively. In addition, an unusual oxidized product **11** was obtained by oxidation with PCC and chromic acid. The cytotoxicity of the compounds **1**, **3**, **4**, **6**-**11** against a small panel of cancer cell lines was evaluated (Figure 1).

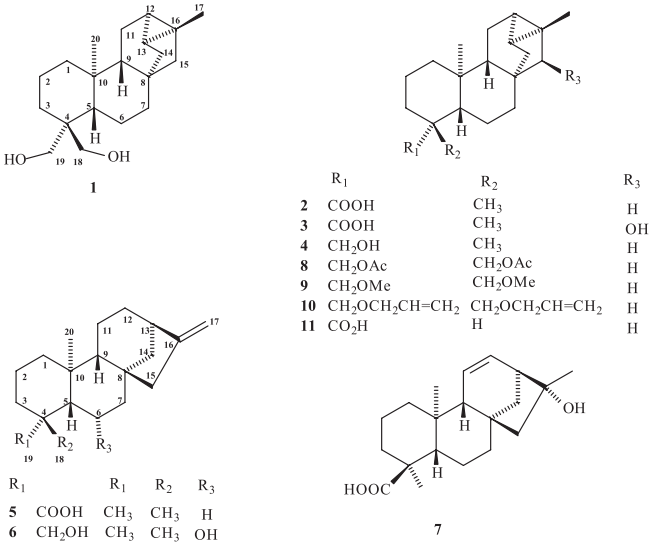

Figure 1. *Kaurene and trachylobane diterpenes isolated from C. floribundus (**1-7**) and chemical derivatives of compound **1** (**8-11**)*

RESULTS AND DISCUSSION

Compound **1** was isolated as a colorless solid, with $[\alpha]_D^{20}$ -41° (*c* 0.1, $CHCl_3$), m.p. 148.2-149.3 °C. Its molecular formula $C_{20}H_{32}O_2$ was established based on its quasi-molecular ion at *m/z* 327.2349 $[M + Na]^+$ (calc for $C_{20}H_{32}O_2Na$ 327.2295), in the HRESIMS. The IR spectrum contained absorption bands of the hydroxyl group evidenced by the characteristic absorptions at 3332 and 1029 cm^{-1}.

The ^1H NMR spectrum displayed signals for two angular methyl groups at δ 0.90 (s, 3H-20) and 1.12 (s, 3H-17), and deshielding signals at δ 3.91 (d, *J* = 10.5 Hz, H-19a), 3.88 (d, *J* = 10.5 Hz, H-18a), 3.71 (d, *J* = 10.5 Hz, H-19b) and 3.33 (d, *J* = 10.5 Hz, H-18b), that

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were attributed to two oxymethylene groups attached to a quaternary carbon. In addition, the presence of the shielded doublets at δ 0.58 (d, *J* = 7.5 Hz, H-12) and 0.83 (dd, *J* = 7.5 and 2.4 Hz, H-13) of a tetrasubstituted cyclopropane ring suggested that **1** belongs to the trachylobane series.

Chemical shifts and comparative analysis of BB and DEPT-^{13}C NMR spectra revealed 20 lines in agreement with the suggested molecular formula. These data allowed clear deduction of the presence of two oxygen-bearing methylene carbons at δ 74.0 (C-18) and 65.0 (C-19), and 18 other sp^3 non-functionalized carbon signals (two methyls, eight methylenes, four methines and four quaternaries).

The unambiguous assignment of all carbons and hydrogens was possible by the HMQC spectrum analysis (Table 1). In particular, the presence of two magnetically equivalent carbons was evidenced by the correlations of the three hydrogens at δ 0.58 (H-12), 1.12 (H-17) and 1.60 (H-6) with a single signal at δ 20.7, and the correlations of hydrogens at δ 0.94 (H-5) and 1.14 (H-9) with the carbon at δ 53.5.

Table 1. ^{1}H and ^{13}C NMR (500 and 125 MHz) of **1** in $CDCl_3$, *J* in Hz, δ in ppm

C	HMQC		HMBC
	δ_H	δ_C	
1	1.52 (d, *J* = 11.2) 0.78 (td, *J* = 7.5 and 2.4)	39.0	2H-3, 3H-20
2	1.55 (d, *J* = 11.2) 1.35 (m)	17.3	-
3	2.00 (d, *J* = 11.2) 0.96 (m)	30.4	2H-1, H-18, H-19a
4	-	41.7	
5	0.94 (m)	53.5	2H-1, 2H-3, H-18, H-19, 3H-20
6	1.60 (m)	20.7	-
7	1.35 (m)	39.3	2H-15
8	-	40.6	2H-6, H-11, H-13
9	1.14 (m)	53.5	2H-1, 2H-11, H-12, 2H-15, 3H-20
10	-	38.2	-
11	1.88 (td, *J* = 13.0 and 2.4) 1.64 (ddd, *J* = 13.0; 7.5 and 2.4)	21.1	-
12	0.56 (d, *J* = 7.5)	20.7	H-13, 2H-14, 2H-15, 3H-17
13	0.80 (dd, *J* = 7.5 and 2.4)	24.4	2H-15, 3H-17
14	2.02 (m) 1.14 (m)	33.5	H-9; H-12, 2H-15
15	1.36 (d, *J* = 11.2) 1.23 (d, *J* = 11.2)	50.5	3H-17
16	-	22.6	2H-14
17	1,12 (s)	20.7	H-12, H 13, 2H-15
18	3.88 (d, *J* = 10.5) 3.33 (d, *J* = 10.5)	74.0	H-5, H-19
19	3.91 (d, *J* = 10.5) 3.71 (d, *J* = 10.5)	65.0	H-3, H-5, H-18
20	0.90 (s)	15.1	H-1b; H-5; H-9

Further evidence for a trachylobane skeleton was obtained by the long-range correlations in the HMBC spectrum. In this experiment, correlation signals for the hydrogen of cyclopropane moiety at δ 0.88 (H-13) with the carbons at δ 20.7 (C-12), 22.6 (C-16) and 40.6 (C-8) were observed, besides the correlation between the hydrogen at δ 0.56 (H-12) and the carbons at δ 53.5 (C-9), 33.5 (C-14), 20.7 (C-17) and 33.5 (C-14). In addition, the two oxymethylene groups at δ 3.91 and 3.71 (2H-19 and 2H-18, respectively) showed the same correlations with the carbons at δ 30.4 (C-3), 53.5 (C-5) and 41.7 (C-4), and confirmed the attachment of both groups to C-4 of the *trans*-decalin system.

On the basis of the levorotatory nature of **1**, along with the knowledge of the co-occurrence of *ent*-trachylobane diterpenoids in plants of the *Croton* genus, it was possible to suggest that compound **1** belongs to the *enantio* series. From the previously established evidence, compound **1** was determined to be the new *ent*-trachyloban-18,19-diol. Comparison of NMR data of compound **1** with other trachylobanes indicated a close structural similarity with *ent*-2α,18,19-traquilobantriol,[17] previously isolated from *Psiadia punctulata,* and that possesses one additional hydroxyl group at C-2.

Compounds **2**-**7** were identified as *ent*-trachyloban-19-oic acid (**2**), *ent*-15β-hydroxytrachyloban-19-oic acid (**3**), *ent*-trachyloban-19--ol (**4**), *ent*-kaur-16-en-19-oic acid (**5**), *ent*-kaur-16-en-6α,19-diol (**6**) and *ent*-16α-hydroxykaur-11-en-19-oic acid (**7**), by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was the major compound isolated from the roots of *C. floribundus.* Thus, considering the reported cytotoxic activity for kaurene and trachylobane diterpenoids, we analyzed the cytotoxic effects of the natural compounds **1**, **3**-**7**. In order to correlate cytotoxicity with chemical structure, four new semi-synthetic trachylobane derivatives **8**-**11** were prepared from **1**, and their cytotoxic activity also evaluated. *ent*-trachyloban-18,19-diol **1** was acetylated with acetic anhydride and pyridine to afford the diacetyl derivative **8**. Reaction with methyl iodide and KOH yielded the dimethoxyl **9**, while the reaction with allyl bromide and KOH yielded the diallyl **10**. In addition, oxidation with PCC yielded the unusual oxidized product **11**.

The ^{1}H NMR spectrum of compound **11** showed similar signals to those described for **1**. The only slight difference was the presence of one additional broad singlet at δ 2.46 (sl, H-4), and the disappearance of the two diastereotopic oxymethylene protons 2H-18 and 2H-19. The NMR ^{13}C spectrum of **11** showed only 19 spectral lines. The comparative analysis of the ^{13}C NMR data of **11** with those observed for compound **1** revealed a shielded signal attributable to one carboxyl group at δ 181.3 (C-19), in place of the signals of the oxymethylene groups at δ 65.0 and δ 74.0 and (C-18 and C-19) observed in **1**. These assignments suggested compound **11** is a nor-diterpene containing a carboxyl group at C-19. Although unexpected, the isolation of this compound could be explained by oxidation of the two oxymethylene groups in **1** to give a dicarboxyl acid precursor, followed by CO_2 loss. Indeed, the thermal instability of malonic acid derivatives toward decarboxylation is well-known, and has been proposed as being due to a possible concerted mechanism where proton transfer is concurrent with carbon-carbon bond fission.[18]

Initially, the *in vitro* anticancer activity of tested compounds was assessed against three human cancer cell lines (HCT-116; OVACR-8 and SF-295) by using MTT assay.[19] Based on data collected from three independent experiments, results showed that only *ent*-kaur-16-en-6α,19-diol (**6**) exhibited a moderate effect against the HCT-116 cell line with an IC_{50} value of 12.1 μg/mL (Table 2), while the other tested compounds showed IC_{50} greater than 25 μg/mL (data not shown). In a second set of experiments, compound **6** was tested against another three tumor cell lines (HL-60, HCT-8 and MDA-MB-435), showing moderate cytotoxic effects on MDA-MB-435 and HCT-8 cells with IC_{50} values of 14.3 and 13.5 μg/mL, respectively (Table 2). The cytotoxicity of kaurene diterpenoids has been widely discussed in the literature. Costa-Lotufo *et al.*[20] studied the effects of *ent*-kaur-16-en-19-oic acid (**5**) in developing sea urchin (*Lytechinus variegatus*) embryos on tumor cell growth. The results showed cytotoxic and anti-proliferative actions of *ent*-kaur-16-en-19-oic acid (**5**) against leukemic cells of 95.3%, and against both MCF-7 breast carcinoma and HCT-8 colon cancer cells of 47.5%. Cavalcanti *et al*.[3] demonstrated that kaurenoic acid also had moderate cytotoxicity against cancer cell lines, with

IC_{50} values ranging from 9.1 to 14.3 μg/mL, where its mechanism of action was apparently related to DNA interaction ultimately leading to the inhibition of topoisomerase I and apoptosis induction. Therefore, the structural similarity between *ent*-kaur-16-en-19-oic acid (**5**) and *ent*-kaur-16-en-6α,19-diol (**6**), justifies the moderate effect exhibited by the latter compound in this work.

Table 2. Cytotoxic activity expressed by IC_{50} in μg/mL of compound **6** for cancer cell lines[a]

	MTT IC_{50} μg/mL	
	ent-kaur-16-ene-6α, 19-diol (**6**)	Doxorubicin
OVCAR-8	> 25	0.26 (0.17-0.3)
SF-295	> 25	0.24 (0.2-0.27)
HCT-116	12.1 (9.1-16.1)	0.12 (0.09-0.17)
HL-60	> 25	0,02 (0,01-0,02)
MDA-MB-435	14.3 (12.8-16.0)	0.48 (0.34-0.66)
HCT-8	13.5 (10.2-17.8)	0.01 (0.01-0.02)

[a] Data are presented as IC_{50} values and 95% confidence intervals obtained by nonlinear regression for all cell lines from three independent experiments.

EXPERIMENTAL

General

IR spectra were recorded on a Perkin-Elmer FT-IR 1000 spectrometer (Waltham, USA), using a NaCl disc. The NMR spectra were acquired on a Bruker Avance DRX 500 spectrometer, equipped with an inverse detection probe head and z-gradient accessory working at 499.9 (^{1}H) and at 124.97 MHz (^{13}C), respectively. All pulse sequences were standard in the Bruker XWIN-NMR software, and all experiments conducted at room temperature. The samples, dissolved in $CDCl_3$ (0.6 mL), were transferred to 5 mm tubes. The ^{1}H and ^{13}C chemical shifts were expressed in the δ scale and referenced to TMS through the residual $CHDCl_3$ at δ 7.27 for proton and at δ 77.0 for carbon. High resolution mass spectra were recorded on an UltrOTOF-Q mass spectrometer (LC-IP-TOF model 225-07100-34, Shimadzu) either by positive or negative ionization modes of the ESI source. Column chromatography was performed over silica gel 60 (EMD, 70-230 mesh). TLC was performed on precoated silica gel aluminum sheets (Merck) and compounds were visualized by UV detection and by spraying with vanillin/perchloric acid/EtOH solution, followed by heating.

Plant material

C. floribundus was collected from Pacoti county (Ceará State, Northeast Brazil) and authenticated by E. P. Nunes of the Departamento de Biologia, Universidade Federal do Ceará. A voucher specimen (#39851) was deposited at the Herbarium Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brasil.

Extraction and isolation

Root bark (1.58 kg) from *C. floribundus* was dried, pulverized and extracted with hexane at room temperature. The solvent was removed under reduced pressure to give the crude hexane extract (97.06 g).

The crude hexane extract was coarsely chromatographed on a silica gel column by elution with hexane, CH_2Cl_2, EtOAc and MeOH to give 4 fractions. Chromatography of the EtOAc fraction (5.5 g) using hexane:EtOAc (70:30) as an isocratic eluting mixture afforded ten subfractions. Recrystallization of subfraction F-6 (940.5 mg) with hexane provided the compound *ent*-trachyloban-18,19-diol (**1**) (350.3 mg). The sub-F-2 (97.2 mg) was rechromatographed on a silica gel and eluted with a mixture of hexane/CH_2Cl_2 (90:10) to afford *ent*-trachyloban-19-oic acid (**2**) (20.2 mg). The compound *ent*-15β-hydroxytrachyloban-19-oic acid (**3**) (13.5 mg) was isolated from subfraction F-3 (335.2 mg) by repeated chromatography over silica gel (hexane-EtOAc 85:15). Chromatography of subfraction F-4 (427.0 mg) using hexane:EtOAc (90:10) as an isocratic eluting mixture afforded *ent*-kaur-16α-en-6α,19-diol (**6**) (32.0 mg). The subfraction F-5 (325.0 mg) was submitted to chromatography using hexane:EtOAc (90:10) as an isocratic eluting mixture to yield *ent*-16α-hydroxykaur-11-en-19-oic acid (**7**) (15.0 mg).

The dichloromethane fraction (5.0 g) was chromatographed over silica gel using a gradient system of hexane/CH_2Cl_2 affording 7 subfractions. The sub fraction F-3 (982.1 mg) was subjected to flash chromatography and eluted with hexane/CH_2Cl_2 (90:10) to give the compound *ent*-kaur-16-en-19-oic acid (**5**) (323.5 mg). The subfraction F-5 (923.3 mg) was subjected to CC and eluted using a mixture of hexane/CH_2Cl_2 with increasing polarity, yielding six fractions. Fraction 5 (923.3 mg) was submitted to semi-preparative HPLC using an isocratic hexane-isopropanol (99:1) system as the eluent, to obtain *ent*-trachyloban-19-ol (**4**) (510.0 mg).

*General procedure for preparation of derivative **8***

Compound **1** (30.4 mg, 0.1 mmol) was submitted to acetylation reaction using acetic anhydride (0.2 mmol) and pyridine (1 mL) with stirring for 3 h at room temperature. The reaction mixture was washed with $CuSO_4$ 5% solution and extracted with CH_2Cl_2 (3 x 20 mL). The CH_2Cl_2 phase was washed with water and dried with Na_2SO_4. Removal of the solvent yielded 28.3 mg (72.9 %) of compound **8**.

Compound **8**. Light yellow oil. $[\alpha]_D^{20}$: - 25 (*c* 0.1, $CHCl_3$). IR (cm^{-1}, NaCl disc) ν_{max}/cm^{-1}: 1737 (C=O), 1225 and 1032 (C-O). HR-ESIMS *m/z*: 411.2539 ([M+Na]$^+$, 411.2511, calc. for $C_{24}H_{36}O_4Na$). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 4.27 (1H, d, *J* = 11.2 Hz, H-19), 4.02 (1H, d, *J* = 11.2 Hz, H-19), 3.99 (1H, d, *J* = 11.2 Hz, H-18), 3.92 (1H, d, *J* = 11.2 Hz, H-18), 2.05 (3H, s, 18-OCOC$\underline{H}_3$), 2.03 (3H, s, 19-OCOC$\underline{H}_3$), 2.00 (1H, m, H-14), 1.89 (1H, td, *J* = 12.9, 2.5 Hz, H-11), 1.66 (1H, m, H-11), 1.62 (1H, m, H-3), 1.55 (1H, m, H-2), 1.52 (1H, m, H-1), 1.50 (1H, m, H-6), 1.41 (1H, m, H-2), 1.41 (1H, m, H-7), 1.36 (1H, m, H-15), 1.31 (1H, m, H-6), 1.28 (1H, d, *J* = 3.5 Hz, H-7), 1.23 (1H, m, H-15), 1.18 (1H, m, H-3), 1.17 (1H, m, H-5), 1.15 (1H, m, H-9), 1.12 (1H, m, H-14), 1.12 (3H, s, CH_3-17), 0.98 (3H, s, CH_3-20), 0.82 (1H, dd, *J* = 7.6, 2.3 Hz, H-13), 0.78 (1H, td, *J* = 12.0, 3.4 Hz, H-1), 0.57 (1H, d, *J* = 7.6 Hz, H-12). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$): 171.2 (18-O$\underline{C}$OCH$_3$), 171.2 (19-O$\underline{C}$OCH$_3$), 69.7 (C-18), 64.9 (C-19), 53.6 (C-9), 51.1 (C-5), 50.3 (C-15), 40.8 (C-8), 40.0 (C-4), 39,3 (C-7), 38.9 (C-1), 38.2 (C-10), 33.4 (C-14), 31,3 (C-3), 24.4 (C-13), 22.6 (C-16), 21.2 (18-OCO$\underline{C}$H$_3$), 21.1 (19-OCO$\underline{C}$H$_3$), 20.8 (C-6), 20.7 (C-12), 20.7 (C-17), 20.1 (C-11), 17.3 (C-2), 15,2 (C-20).

*General procedure for preparation of derivative **9***

A total of 30.4 mg of compound **1** (0.1 mmol) was added to a stirred solution of KOH (44.8 mg, 0.8 mmol) in DMSO (3 mL), followed immediately by addition of methyl iodide (0.025 mL, 0.4 mmol). The mixture was stirred for 4 h at room temperature, after which the mixture was poured into water (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure, to give 19.5 mg (58.7%) of compound **9**.

Compound **9**. Light yellow oil. $[\alpha]_D^{20}$: - 41 (*c* 0.1, $CHCl_3$). IR (cm^{-1}, NaCl disc) ν_{max}/cm^{-1}: 1737 (C=O), 1225 and 1032 (C-O). HR-ESIMS *m/z* 355.2613 ($[M+Na]^+$, 355.2618, calc. for $C_{28}H_{36}O_2Na$). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 3.42 (1H, d, *J* = 9.4 Hz, H-19a), 3.30 (3H, s, 19-OC<u>H</u>$_3$), 3.30 (1H, m, H-18a), 3.27 (1H, m, H-19b), 3.26 (3H, s, 18-OC<u>H</u>$_3$), 3.15 (1H, d, *J* = 9.3 Hz, H-18b), 2.04 (1H, d, *J* = 11.7 Hz, H-14a), 1.88 (1H, td, *J* = 14.5, 11.6, 3.0 Hz, H-11a), 1.64 (1H, qd, *J* = 14.5, 7.9, 2.2 Hz, H-11b), 1.58 (2H, d, *J* = 14.5 Hz, H-3a), 1.54 (1H, t, *J* = 3.5 Hz, H-2a), 1.52 (2H, d, *J* = 4.1 Hz, H-6), 1.50 (1H, d, *J* = 3.1 Hz, H-1a), 1.37 (1H, m, H-15a), 1.36 (2H, d, *J* = 2.6 Hz, H-7), 1.33 (1H, m, H-2b), 1.25 (1H, s, H-5), 1.23 (1H, s, H-15b), 1.18 (1H, d, *J* = 11.6, 7.9 Hz, H-9), 1.14 (1H, m, H-14b), 1.12 (3H, s, H-17), 0.95 (3H, s, H-20), 0.80 (1H, dd, *J* = 7.7, 3.1 Hz, H-13), 0.77 (1H, td, *J* = 19.2, 13.0, 3.5 Hz, H-1b), 0.57 (1H, d, *J* = 7.7 Hz, H-12). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$): 78.5 (C-18), 74.2 (C-19), 59.3 (19-O<u>C</u>H$_3$), 59.3 (18-O<u>C</u>H$_3$), 53.2 (C-9), 50.2 (C-15), 49.5 (C-5), 41.5 (C-4), 40.7 (C-8), 39.1 (C-7), 38.9 (C-1), 38.0 (C-10), 33.3 (C-14), 30.9 (C-3), 24.2 (C-13), 22.4 (C-16), 20.6 (C-12), 20.6 (C-6), 20.5 (C-17), 19.9 (C-11), 17.6 (C-2), 15.0 (C-20).

*General procedure for preparation of derivative **10***

A total of 0.6 mmol (0.05 mL) of allyl bromide and 0.8 mmol (44.8 mg) KOH was added to a solution of 0.1 mmol (30.4 mg) of compound **1** dissolved in dry benzene. The system was stirred for 5 h under reflux. After completion of the reaction, the benzene was distilled under reduced pressure and the reaction material extracted with chloroform. The product was purified by column chromatography over silica gel using hexane/CH_2Cl_2 (1:1) as the eluent, to yield 8.7 mg of compound **10** (22.7%).

Compound **10**. Light yellow oil. $[\alpha]_D^{20}$: - 43 (*c* 0.1, $CHCl_3$). IR (cm^{-1}, film) ν_{max}/cm^{-1}: 1093 (C-O), 1495 and 918 (=C-H).

HR-ESIMS *m/z*: 385.3101 ([M+H]+, 385.3026, calc. for $C_{26}H_{41}O_2$).^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 5.87 (1H, m, 18-OCH$_2$C<u>H</u>=CH$_2$), 5.87 (1H, m, 19-OCH$_2$C<u>H</u>=CH$_2$), 5.25 (2H, ddd, *J* = 17.2, 4.1, 1.8 Hz, 18-OCH$_2$CH=C<u>H</u>$_2$), 5.14 (2H, ddd, *J* = 10.4, 2.8, 1.5 Hz, 19-OCH$_2$CH=C<u>H</u>$_2$), 3.94 (2H, qt, *J* = 5.7, 1.5 Hz, 18-OC<u>H</u>$_2$CH=CH$_2$), 3.89 (2H, m, 19-OC<u>H</u>$_2$CH=CH$_2$), 3.49 (1H, d, *J* = 9.4 Hz, H-19), 3.36 (1H, d, *J* = 9.4 Hz, H-18a), 3.33 (1H, d, *J* = 9.4 Hz, H-19b), 3.26 (1H, d, *J* = 9.3 Hz, H-18b), 2.03 (1H, d, *J* = 11.7 Hz, H-14a), 1,88 (1H, td, *J* = 14.4, 11.3, 3.0 Hz, H-11a), 1.65 (1H, m, H-11b), 1.63 (1H, m, H-3a), 1.55 (1H, m, H-6), 1.50 (1H, m, H-2a), 1.49 (1H, dd, *J* = 13.0, 2.9 Hz, H-1a), 1,38 (1H, d, *J* = 2.9 Hz, H-2b), 1.36 (1H, m, H-15a), 1.36 (2H, m, H-7), 1.31 (1H, m, H-6), 1.30 (1H, m, H-3b), 1.29 (1H, m, H-5), 1.24 (1H, m, H-15b), 1.18 (1H, m, H-9), 1.29 (1H, m, H-14b), 1.12 (3H, s, H-17), 0.94 (3H, s, H-20), 0.80 (1H, dd, *J* = 7.6, 3.0 Hz, H-13), 0.77 (1H, td, *J* = 13.0, 3.7 Hz, H-1b), 0.57 (1H, d, *J* = 7.7 Hz, 1H, H-12). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$): 135.5 (18-OCH$_2$C<u>H</u>=CH$_2$), 135.4 (19-OCH$_2$C<u>H</u>=CH$_2$), 115.9 (18-OCH$_2$CH=C<u>H</u>$_2$), 115.9 (19-OCH$_2$CH=C<u>H</u>$_2$), 75.8 (C-18), 72.2 (18-OC<u>H</u>$_2$CH=CH$_2$), 72.1 (19-OC<u>H</u>$_2$CH=CH$_2$), 71.3 (C-19), 53.3 (C-19), 50.2 (C-15), 49.6 (C-5), 41,7 (C-4), 40.7 (C-8), 39.2 (C-7), 38.9 (C-1), 38.9 (C-10), 33.3 (C-14), 31.0 (C-3), 24.2 (C-13), 22.4 (C-16), 20.6 (C-6), 20.6 (C-17), 20.5 (C-12), 19.9 (C-11), 17.6 (C-2), 15.0 (C-20).

*General procedure for preparation of derivative **11***

Compound **1** (200.0 mg, 0.65 mmol) was added to a stirred suspension of PCC (433.0 mg, 2.0 mmol) and Si gel (500.0 mg) in CH_2Cl_2 (20 mL). After completion of the reaction (15 min), the mixture was diluted with 20 mL of ethyl ether and the supernatant passed through a Celite layer column (3.5 g). The solvent was removed under vacuum and the crude residue purified by column chromatography over silica flash by isocratic elution with hexane/CH_2Cl_2 (1:4) to yield compound **11** (10.2 mg, 5.4%).

Compound **11**. Colorless solid. $[\alpha]_D^{20}$: - 25 (*c* 0.1, $CHCl_3$). IR (cm^{-1}, NaCl disc) ν_{max}/cm^{-1}: 3400-2700 (O-H) and 1687 (C=O). HR-ESIMS *m/z*: 287.2089 ($[M-H]^-$, 287.2011, calc. for $C_{19}H_{27}O_2$). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$): 2.46 (1H, s, H-4), 2.11 (1H, d, *J* = 12.7 Hz, H-3a), 2.05 (1H, d, *J* = 11.7 Hz, H-14a), 1.91 (1H, m, H-11a), 1.90 (1H, m, H-6a), 1.86 (1H, m, H-2a), 1.66 (1H, ddd, *J* = 14.5; 7.6, 2.0 Hz, H-11b),1.56 (1H, d, *J* = 12.9 Hz, H-1a), 1.48 (1H, d, *J* = 11.7 Hz, H-14b), 1.44 (1H, m, H-6b), 1.40 (1H, m, H-2b), 1.40 (1H, m, H-5), 1.40 (2H, m, H-7), 1.40 (1H, m, H-15a), 1.31 (1H, m, H-3b), 1.24 (1H, m, H-15b), 1.13 (3H, s, CH_3-17), 1.09 (1H, d, *J* = 2.0 Hz, H-9), 0.90 (3H, s, CH_3-20), 0.82 (1H, dd, *J* = 7.6, 3.0 Hz, H-13), 0.77 (1H, td, *J* = 13.2, 3.8 Hz, H-1b), 0.58 (1H, d, *J* = 7.6 Hz, H-12). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$): 181.3 (C-19), 52.3 (C-9), 50.3 (C-15), 49.4 (C-5), 42.7 (C-4), 40.9 (C-8), 39.1 (C-1), 38.8 (C-7), 38.6 (C-10), 33.1 (C-14), 28.3 (C-3), 26.6 (C-6), 24.3 (C-13), 22.4 (C-16), 20.5 (C-12), 20.5 (C-17), 19.5 (C-11), 18.0 (C-2), 12.4 (C-20).

CYTOTOXIC ACTIVITY AGAINST TUMOR CELL LINES

Cell line and cell culture

The tumor cell lines used in this work were HCT-8 (colorectal adenocarcinoma), HCT-116 (colorectal adenocarcinoma), MDA-MB 435 (melanoma); OVCAR-8 (ovarian carcinoma); HL-60 (leukaemia) and SF-295 (glioma), kindly provided by the National Cancer Institute (Bethesda, MD, USA). All cancer cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin at 37 °C with 5% CO_2.

MTT assay

The cytotoxicity of compounds **1**, **3**, **4**, **7**-**11** was tested against three tumor cell lines, while compound **6** was tested against six tumor cell lines using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H--tetrazolium bromide (MTT) (Sigma Aldrich Co., St. Louis, MO/USA) reduction assay.[16] For all experiments, cells were plated in 96-well plates (10^5 cells/well for adherent cells or 3×10^5 cells/well for suspended cells in 100 µL of medium). The tested compounds (0.05-25 µg/mL) dissolved in DMSO were added to each well (using the HTS – high-throughput screening - biomek 3000 device - Beckman Coulter Inc., Fullerton, California, USA) and incubated for 72 h. Doxorubicin was used as the positive control (0.009-5 µg/mL). Control groups received the same amount of DMSO. After 69 h of incubation, the supernatant was replaced by fresh medium containing MTT (0.5 mg/mL). Three hours later, the MTT formazan product was dissolved in 150 µL of DMSO, and absorbance measured at 595 nm (DTX 880 Multimode Detector, Beckman Coulter Inc., Fullerton, CA, USA).

CONCLUSIONS

C. floribundus proved a prolific source of diterpenes, corroborating previously reported results for the *Croton* species. In this work, the cytotoxic activity of kaurene and trachylobanes isolated from the roots, besides the semi-synthetic trachylobane derivatives of new *ent*-trachyloban-18,19-diol (**1**), was evaluated. However, based on data collected from three independent experiments, results showed that only *ent*-kaur-16-ene-6α,19-diol (**6**) exhibited a moderate effect against the three cancer cell lines, MDA-MB-435, HCT-8 and HCT-116, with IC_{50} value of 14.32, 13.47 and 12.1 µg/mL, respectively. The other tested compounds showed IC_{50}> 25 µg/mL.

SUPPLEMENTARY MATERIAL

Available at http://quimicanova.sbq.org.br, in pdf file with free access.

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