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Article

Pentacyclic Triterpenes from Branches of *Maytenus robusta* and *in vitro* Cytotoxic Property Against 4T1 Cancer Cells

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Dois novos friedelanos, **1** e **2**, e cinco triterpenos pentacíclicos conhecidos foram isolados dos galhos de *Maytenus robusta*. Suas estruturas químicas foram identificadas como 3,16-dioxo-29-hidroxifriedelano (**1**), 3-oxo-16β,29-di-hidroxifriedelano (**2**), 3-oxofriedelano (**3**), 3β-friedelinol (**4**), 3,16-dioxofriedelano (**5**), 3-oxo-29-hidroxifriedelano (**6**) e 3,16-dioxo-12α-hidroxifriedelano (**7**). A estrutura e estereoquímica dos triterpenos **1** e **2** foram estabelecidas por infravermelho (IR), ressonância magnética nuclear (NMR) 1D/2D, espectrometria de massas de alta resolução com ionização química à pressão atmosférica (HR-APCIMS) e difração de raios X de pó. A atividade citotóxica *in vitro* dos triterpenos **1** a **6** foi avaliada frente a células de câncer de mama murino. Os triterpenos **1** e **2** apresentaram atividade citotóxica contra células 4T1 em baixa concentração.

Two new friedelane-type compounds **1** and **2** and five known pentacyclic triterpenes were isolated from branches of *Maytenus robusta*. Their structures were identified as 3,16-dioxo-29-hydroxyfriedelane (**1**), 3-oxo-16β,29-dihydroxyfriedelane (**2**), 3-oxofriedelane (**3**), 3β-friedelinol (**4**), 3,16-dioxofriedelane (**5**), 3-oxo-29-hydroxyfriedelane (**6**), and 3,16-dioxo-12α-hydroxyfriedelane (**7**). The structures and the stereochemistry of triterpenes **1** and **2** were established through infrared (IR), 1D/2D nuclear magnetic resonance (NMR), high-resolution atmospheric pressure chemical ionization mass spectrometry (HR-APCIMS) spectral data and powder X-ray diffraction. The *in vitro* cytotoxic property of triterpenes **1** to **6** on 4T1 murine breast cancer cells was evaluated. The triterpenes **1** and **2** showed cytotoxic activity against 4T1 cells at a lower concentration.

Keywords: *Maytenus robusta*, Celastraceae, pentacyclic triterpenes, *in vitro* 4T1 cells cytotoxicity

Introduction

Maytenus robusta is a terrestrial plant of Celastraceae family which includes 98 genera with approximately 1210 species.[1] In South America, different species of *Maytenus* are used in traditional medicine, mainly, for the treatment of gastric disorders.[2,3]

M. robusta is a rich source of pentacyclic triterpenes (PCTTs). Previous phytochemical studies of *M. robusta* leaves led to the isolation of 3β,11β-dihydroxyfriedelane, 3β-hydroxy-21β-*H*-hop-22(29)-ene, 3-oxo-21β-*H*-hop-22(29)-ene, 3-oxo-11β-hydroxyfriedelane, 3-oxofriedelane, 3β-hydroxyfriedelane and 3-oxo-29-hydroxyfriedelane.[4,5]

Potential antitumor properties have been attributed to several PCTTs in accordance with literature data. Maslinic acid demonstrated important activity against Caco-2 human colon-cancer cell line,[6] acetyl aleuritolic acid displayed

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cytotoxicity activity against central nervous system cancer cell lines,[7] and 3β,23-epoxy-friedelan-28-oic acid and 3-oxo-friedelan-28-oic acid showed antiproliferative effect in human leukemia HL-60 cells.[8] Semi-synthetic derivatives of oleanolic acid, denominated bardoxolone and bardoxolone methyl, were subjected to clinical trials, intravenously and orally administered as anti-cancer agents in patients with metastatic disease.[9] Furthermore, PCTTs of the lupane series and its semi-synthetic derivatives are known to present cytotoxicity activity against several cancer cells lines.[10-13]

The present study reports the isolation and structural elucidation of new triterpenes 3,16-dioxo-29-hydroxyfriedelane (**1**) and 3-oxo-16β,29-hydroxyfriedelane (**2**), along with five known friedelanes (**3** to **7**), from branches of *M. robusta* (Figure 1). Compounds **1** to **6** were subjected to *in vitro* cytotoxicity assays against murine breast cancer cells (4T1 tumor cells).

Experimental

All solvents used in this work were of analytical grade. All other chemicals were available commercially at a reagent grade and were used without further purification.

General experimental procedures

Optical rotations were measured on an ADP220 Bellinghan + Stanley Ltd polarimeter. Thermal analyses were carried out in a simultaneous thermogravimetric (TG) and differential thermal analyzer (DTA) Shimadzu DTG-60. The infrared (IR) spectra were recorded on a Perkin Elmer-Spectrum One (attenuated total reflectance, ATR) spectrometer. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 400.129 and 100.613 MHz, respectively, as well as correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) experiments were performed on a Bruker DRX400 *Avance* spectrometer, using $CDCl_3$ or $CDCl_3$ with two drops of pyridine-d_5 as solvent, with direct or inverse probes and a field gradient. The chemical shifts were registered in ppm (δ) using tetramethylsilane (TMS) as the internal standard. The coupling constants (J) were registered in hertz (Hz). High-resolution atmospheric pressure chemical ionization mass spectrometry (HR-APCIMS) spectra were acquired on a Shimadzu LCMS-IT-TOF system. The

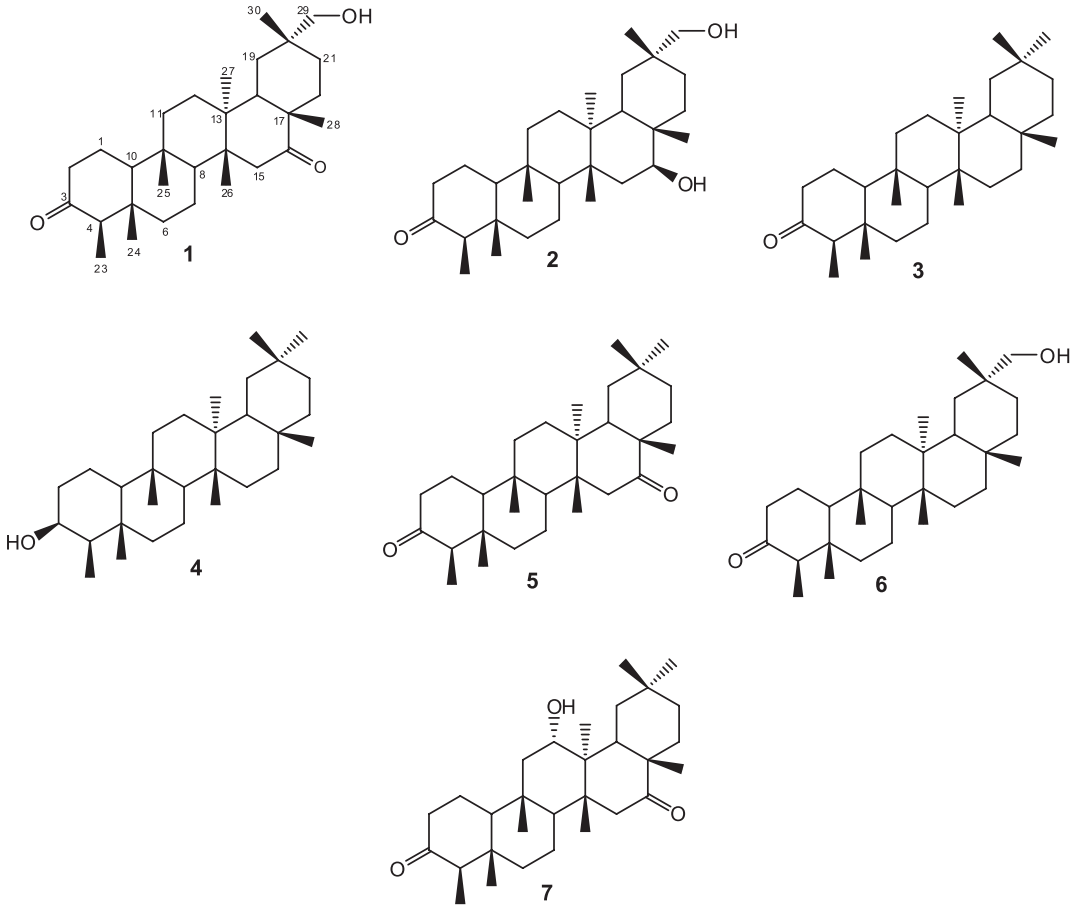

Figure 1. Chemical structures of triterpenes **1** to **7**.

mass spectrometer was equipped with an atmospheric pressure chemical ionization (APCI) source operating in both the negative and positive modes. Detector voltage: 1.70 kV; interface temperature: 400 °C; CDL temperature: 250 °C; block heater temperature: 200 °C; nebulizing gas flow (N_2): 2.5 L min^{-1}. Analyses were carried out using manual injection. The samples were dissolved in $CHCl_3$ and then diluted with MeOH. Column chromatography (CC) processes were carried out using silica gel 60 (70-230 mesh). Thin layer chromatography (TLC) was carried out using precoated silica gel plates.

The powder X-ray diffraction (XRD) data were collected on a Shimadzu XRD-7000 under 40 kV, 30 mA, using Cu Kα (1.54056 Å) coupled with a polycapillary unit, graphite monochromator, scanned over an angular range of 3-35° (2θ) with a step size of 0.01° (2θ) and a time constant of 3 s $step^{-1}$. The sample holder was submitted to a spinning speed of 30 cycles *per* minute to reduce any preferred orientation and to minimize rugosity effects. The final matrix was averaged over 3 independent scans. The powder indexing tool used for peak identification, indexing and automatic space group determination was DICVOL91.[14]

Plant material

Branches of *M. robusta* were collected in June 2010 at Parque Estadual do Itacolomi, Ouro Preto City, Minas Gerais, Brazil. After botanical identification, a voucher specimen of *M. robusta* was deposited in the Herbário Professor José Badini, and identified by Dr Maria Cristina Teixeira Braga Messias, a botanist at the Departamento de Biodiversidade, Evolução e Meio Ambiente, Universidade Federal de Ouro Preto, under the code OUPR: 25559.

Extraction and Isolation

Branches of *M. robusta* were dried at room temperature until a constant weight was achieved and then powdered on a knife mill. A sample of the powder material (888.8 g) was submitted to exhaustive extraction with hexane, chloroform, ethyl acetate and methanol. During the recovery of hexane in a rotatory evaporator, a solid material (1.60 g) was precipitated and separated by filtration under reduced pressure. This solid was subjected to silica gel CC to obtain 3-oxofriedelane (**3**) (13.5 mg, hexane-$CHCl_3$ 2:8 v/v), 3β-friedelinol (**4**) (20.0 mg, $CHCl_3$), 3,16-dioxofriedelane (**5**) (494.4 mg, $CHCl_3$), 3-oxo-29-hydroxyfriedelane (**6**) (83.6 mg, $CHCl_3$), 3,16-dioxo-12α-hydroxyfriedelane (**7**) (17.6 mg, $CHCl_3$) and 3,16-dioxo-29-hydroxyfriedelane (**1**) (196.8 mg, $CHCl_3$-EtOAc 9:1 v/v). After complete removal of the solvent, the other portion of the hexane extract was obtained as viscous oil (3.67 g). This oil was submitted to silica gel CC to obtain an additional amount of 3-oxofriedelane (**3**) (7.5 mg, hexane-$CHCl_3$ 2:8 v/v), 3,16-dioxofriedelane (**5**) (131.5 mg, $CHCl_3$-EtOAc 9:1 v/v) and 3-oxo-29-hydroxyfriedelane (**6**) (34.0 mg, $CHCl_3$-EtOAc 9:1 v/v). The chloroform extract (14.0 g), obtained after extraction of branches with chloroform, was chromatographed on silica gel CC to obtain an additional amount of 3,16-dioxofriedelane (**5**) (50.9 mg, $CHCl_3$-EtOAc 8:2 v/v), 3-oxo-29-hydroxyfriedelane (**6**) (173.7 mg, $CHCl_3$-EtOAc 8:2 v/v) and 3-oxo-16β,29-hydroxyfriedelane (**2**) (213.1 mg, $CHCl_3$-EtOAc 7:3 v/v).

3,16-dioxo-29-hydroxyfriedelane (**1**)

White, amorphous powder; undergoes decomposition at 270 °C (TG bend); $[\alpha]_D^{20}$ –27 (c = 0.36, $CHCl_3$); IR ν_{max}/cm^{-1} 3470, 2934, 2844, 1718, 1671, 1446, 1392; NMR ($CDCl_3$ with two drops of pyridine-d_5) data of ^1H (400 MHz) and ^{13}C (100 MHz), see Table 1; HR-APCIMS (positive-ion mode, $[M+H-H_2O]^+$) calcd. 439.3576; found: 439.3526.

3-oxo-16β,29-hydroxyfriedelane (**2**)

White, amorphous powder; undergoes decomposition at 250 °C (TG bend); $[\alpha]_D^{20}$ –16 (c = 0.51, $CHCl_3$); IR ν_{max}/cm^{-1} 3390, 2918, 2850, 1712, 1450, 1388; NMR ($CDCl_3$ with two drops of pyridine-d_5) data of ^1H (400 MHz) and ^{13}C (100 MHz), see Table 2; HR-APCIMS (positive-ion mode, $[M+H-H_2O]^+$) calcd.: 441.3733; found: 441.3722.

MTT cytotoxicity assay

Dulbecco's modified eagle medium (DMEM) culture medium Gibco® was used to maintain the 4T1 murine breast cancer cells (obtained from American Type Culture Collection). MilliQ® water (Simplicity 185, Millipore, Bedford, USA) was used throughout the experiments. The 4T1 cells were employed in the *in vitro* cytotoxic evaluation. These cells were thawed, submitted to replication, and after having reached an adequate confluence, they were placed on three 96-well microtiter plates (500 *per* well), totaling 120,000 cells. Each sample was dissolved and diluted using DMSO and hot ethanol (approx. 55 °C) 1:9 solution, to reach a concentration ranging from 1×10^{-3} to 1×10^{-7} mol L^{-1}, in a volume of 100 μL. The cells were treated with the solution for 48 hours in DMEM supplemented with sodium bicarbonate (3.7 g L^{-1}), penicillin (5,000 units mL^{-1}), and 10% m/m sterile fetal bovine serum. The culture was maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Five replicates were used for an appropriate statistical evaluation. By means of [3-(4,5-dimethyl-2-thiazolyl-2,5-

Table 1. NMR spectral data[a] of 3,16-dioxo-29-hydroxyfriedelane (**1**)

Atom	δ_C	Type	δ_H	HMBC	COSY	NOESY
1	22.17	CH_2	1.89 β	3, 10	2	–
			1.62 α	–	2	10
2	41.34	CH_2	2.28	3, 10	1β	–
			2.38	3, 10	1α	–
3	212.20	C	–	–	–	–
4	57.91	CH	2.22 *ax* (α)	5, 23, 24	23	23, 6α, 10
5	41.96	C	–	–	–	–
6	40.82	CH_2	1.21 *ax* (α)	–	–	–
			1.70 *eq* (β)	5, 7, 8, 10	6α, 7α, 7β	23, 24
7	18.54	CH_2	1.40 *ax* (β)	–	–	24, 25, 26
			1.30 *eq* (α)			—
8	52.32	CH	1.40 *ax* (α)	–	–	–
9	37.51	C	–	–	–	–
10	58.99	CH	1.50 *ax* (α)	–	–	–
11	35.24	CH_2	1.19 *ax* (α)	–	–	–
			1.46 *eq* (β)			26
12	28.95	CH_2	1.38 (α, β)	–	–	–
13	38.96	C	–	–	–	–
14	40.59	C	–	–	–	–
15	50.17	CH_2	2.09 *ax* (α)	8, 13, 14, 16, 26	–	27
			2.40 *eq* (β)	13, 14, 16, 26	15	26, 28
16	218.38	C	–	–	–	–
17	45.68	C	–	–	–	–
18	43.24	CH	2.14 *ax* (β)	–	19α, 19β	12, 28, 30
19	30.14	CH_2	1.58 α	–	–	27
			1.30 β			–
20	32.68	C	–	–	–	–
21	27.04	CH_2	1.36	–	–	–
			1.68	17, 22	22	
22	31.14	CH_2	1.52	–	–	–
			1.91		21, 22	21
23	6.87	CH_3	0.89; d; *J* 6,8	3, 4, 5	–	–
24	14.56	CH_3	0.70; s	4, 5, 6, 10	–	–
25	17.25	CH_3	0,85; s	8, 9, 10, 11	–	–
26	20.27	CH_3	1.18; s	8, 13, 14, 15	–	25
27	15.93	CH_3	0.87; s	12, 13, 14, 18	–	–
28	27.44	CH_3	1.33; s	16, 17, 18, 22	–	–
29	74.06	CH_2	3.34; m	19, 20, 21, 30	–	19α, 19β, 21, 30
30	25.84	CH_3	1.13; s	19, 20, 21, 29	–	–

[a]$CDCl_3$, with two drops of pyridine-d_5, 100 or 400 MHz, δ in ppm, *J* in Hz.

diphenyl)-2*H*-tetrazolium bromide] MTT assay, metabolic feasibility assays were performed to evaluate the efficacy of the treatment employed. MTT was purchased from Sigma®. The results were plotted based on percentage of cell death *vs.* molar concentration of the sample, using the software Prism 6.0. A non-linear regression model (sigmoidal dose-response option) was used to determine the IC_{50} values. 4T1 cells treated with 2% Triton X-100 were used as positive damaged control 4T1. For negative control, the cells were treated with MTT stock solution added to 100 µL of medium alone. The experiments, in quintuplicate, were performed immediately. The IC_{50} (µM) were determined for compounds **1** (9.80), **2** (1.23), **3** (98.10), **4** (58.10), **5** (23.10) and **6** (11.00).

Table 2. NMR spectral data[a] of 3-oxo-16β,29-dihydroxyfriedelane (**2**)

Atom	δ_C	Type	δ_H	HMBC	COSY	NOESY
1	22.22	CH_2	1.59 β 1.84 α	–	– 1β	25 –
2	41.43	CH_2	2.33 β 2.23 α	–	–	– 1α , 1β
3	212.78	C		–	–	–
4	58.04	CH	2.18 α	–	23	23, 7*eq*
5	42.14	C	–	–	–	–
6	41.11	CH_2	1.16 *ax* (α) 1.61 *eq* (β)	–	– 6*ax*, 7*ax*	– 23, 24, 25
7	18.41	CH_2	1.36 *ax* (β) 1.46 *eq* (α)	–	–	25 –
8	53.32	CH	1.37 *ax* (α)	–	–	27
9	37.42	C	–	–	–	–
10	59.31	CH	1.43 *ax* (α)	–	–	–
11	35.61	CH_2	1.37 *ax* (α) 1.18 *eq* (β)	–	–	27 –
12	30.74	CH_2	1.25 *ax* (β) 1.61*eq* (α)	–	– 11*ax*	– 27
13	40.06	C	–	–	–	–
14	39.11	C	–	–	–	–
15	44.28	CH_2	1.48 (β) 1.86 (α)	–	– 15β	– –
16	74.40	CH	4.11 *eq* (α); t; *J* 8,8	15, 17, 18, 22, 28	15α, 15β	7*eq*, 15α, 22α, 27
17	36.45	C	–	–	–	–
18	44.09	CH	1.78 β	–	–	12*ax*, 21β, 26, 30
19	30.36	CH_2	1.63 β 1.33 α	–	–	12*ax*, 21β –
20	33.14	C	–	–	–	–
21	27.51	CH_2	1.40β 1.65α	–	–	–
22	36.45	CH_2	1.45α 1.76β	–	– 21β	– –
23	6.88	CH_3	0.88; d; *J* 6,6	3, 4, 5	–	–
24	14.60	CH_3	0.70; s	4, 5, 6, 10	–	–
25	18.06	CH_3	0.84; s	8, 9,10, 11	–	–
26	20.08	CH_3	1.11; s	8, 13, 14, 15	–	–
27	21.43	CH_3	1.02; s	12, 13, 14,18	–	–
28	25.44	CH_3	1.32; s	16, 17,18, 22	–	26
29	74.45	CH_2	3.36; m	19, 20, 21, 30	–	21α, 30
30	25.71	CH_3	1.13; s	19, 20, 21, 29	–	–

[a]$CDCl_3$, with two drops of pyridine-d_5, 100 or 400 MHz, δ in ppm, *J* in Hz.

Results and Discussion

Compound **1** was isolated as white solid powder, and its molecular formula, $C_{30}H_{48}O_3$, was established by HR-APCIMS (*m/z*: 439.3526 $[M+H-H_2O]^+$, calcd. 439.3576). In the IR spectrum of **1** two intense absorptions bands, at 1718 and 1671 cm^{-1}, and a band at 3470 cm^{-1} were observed, which were attributed to carbonyl groups and hydroxyl group, respectively.[15]

The ^{13}C NMR spectral data of **1** were closely related to those of 3-oxo-29-hydroxyfriedelane and 3,16-dioxofriedelane reported by Mahato and Kundu.[16] This information enables to identify **1** as a friedelane derivative with one hydroxyl bonded to C-29 and two oxo

groups bonded to C-3 and C-16. In the ^{1}H NMR spectrum of **1**, six singlet signals (δ_H 0.70, 0.85, 0.87, 1.13, 1.18 and 1.33) and a doublet (δ_H 0.89, *J* 6.8 Hz) were observed, corresponding to seven methyl groups. In the ^{1}H NMR spectrum there was also detected a signal at δ_H 3.34 (m, 2H). In the HSQC contour map this signal was correlated with the carbon signal of C-29 (δ_C 74.06). Correlations between the signal of H-29 (δ_H 3.34) with the signals of C-19 (δ_C 30.14), C-20 (δ_C 32.68), C-21 (δ_C 27.04) and C-30 (δ_C 25.84) were observed in the HMBC contour map. In this map, the doublet at δ_H 0.89, attributed to the hydrogen attached to C-23, showed a correlation with the signals at δ_C 41.96 (C-5), δ_C 57.91 (C-4) and δ_C 212.20 (C-3). These NMR data confirmed the presence of an oxo group attached to C-3. In the HMBC contour map, they were also identified correlations between the signal (δ_H 1.33) of hydrogen attached to C-28 with the signals at δ_C 45.68 (C-17), δ_C 43.24 (C-18), δ_C 31.14 (C-22) and δ_C 218.38 (C-16). These NMR data confirmed the location of a second oxo group attached to C-16. A boat-like conformation of ring D and E was established mainly based on the correlation between H-18 and H-30 observed in the NOESY contour map. By detailed analysis of the HSQC, HMBC and NOESY contour maps, it was possible to establish all chemical shift assignments of compound **1** (Table 1). The stereochemistry of triterpene **1** is in agreement with the powder X-ray diffractometry results (Figure 2) and it is possible to establish that, in solid state, the ring A of triterpene **1** is in an intermediate state between boat and chair-like conformation.

Compound **2** was isolated as a white solid powder, and its molecular formula, $C_{30}H_{50}O_3$, was established by HR-APCIMS (*m/z*: 441.3722 $[M+H-H_2O]^+$, calcd. 441.3733). In the IR spectrum of **2** an absorption band at 3390 cm^{-1}, characteristic of hydroxyl group, and at 1712 cm^{-1}, corresponding to a carbonyl group, were observed.[15]

The ^{13}C NMR spectral data of **2** were closely related to those reported for celasdin A by Kuo and Kuo.[17] This information confirms triterpene **2** as a friedelane derivative with an oxo group bonded to C-3 and two hydroxyl groups bonded to C-16 and C-29. In the ^{1}H NMR spectrum, the six singlet signals (δ_H 0.70, 0.84, 1.02, 1.11, 1.13 and 1.32) and a doublet (δ_H 0.88, *J* 6.6 Hz) observed were attributed to seven methyl groups. In this spectrum, signals at δ_H 3.36 (m, 2H) and at δ_H 4.11 (t, 1H, *J* 8.8 Hz) were also detected. In the ^{13}C NMR spectra, signals at δ_C 74.40, 74.45 and at 212.78 were visualized and associated with two carbinolic groups and one carbonyl group respectively. By the analysis of HSQC contour map, the correlation between the signal at δ_H 3.36 with the signal of C-29

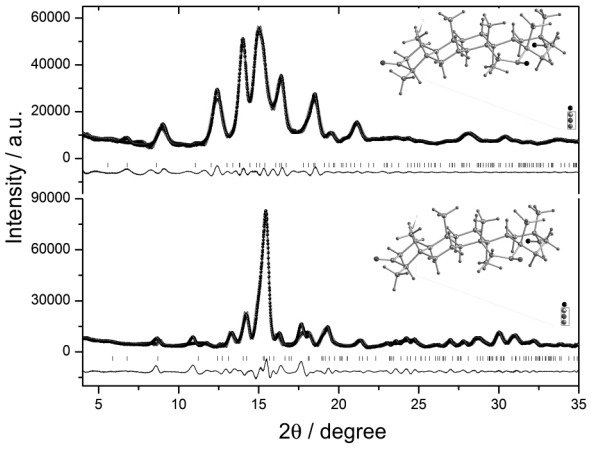

Figure 2. Rietveld fitted X-ray diffraction for compound **1** (above) and compound **2** (below). The chemical structures derived from the fittings are presented as insets on the diffractogram.

(δ_H 74.45) was observed. Correlations among the signal of H-29 (δ_H 3.36) with the signals of C-19 (δ_C 30.36), C-20 (δ_C 33.14), C-21 (δ_C 27.51) and C-30 (δ_C 25.71) were observed in HMBC contour map. In this map, correlations between the signal of H-16 at δ_H 4.11 with the signals of C-15 (δ_C 44.28), C-17 (δ_C 36.45), C-18 (δ_C 44.09), C-22 (δ_C 36.45) and C-28 (δ_C 25.44) were also visualized. In the NOESY contour map, correlations of H-16 (δ_H 4.11) with the signal of H-27 (δ_H 1.02) and H-7 (*eq*, δ_H 1.46) were detected. These correlations enable to confirm that H-16 has α-configuration and the hydroxyl group linked to C-16, has a β-orientation. In the HSQC contour map, the correlation between hydrogen signal at δ_H 0.88 (d, *J* 6.6 Hz, H-23) with the carbon signal of C-23 at δ_C 6.88 was also verified. In the HMBC contour map, correlations of H-23 with the signals of C-3 (δ_C 212.78), C-4 (δ_C 58.04) and C-5 (δ_C 42.14) were detected. These correlations enabled to confirm C-3 as carbonyl group. The analysis of the NOESY contour map enables to establish that rings D and E of triterpene **2** have a boat-like conformation. The conformation of these rings was confirmed mainly by the correlation between H-18 and H-30. Furthermore, correlations between H-16 and H-28 and among H-29 and H-27 were not observed in the NOESY contour map. The complete chemical shift assignment of triterpene **2**, in Table 2, was established by detailed analysis of HSQC, HMBC, COSY and NOESY contour maps. The spatial position of atoms, obtained by Rietveld refinement for compound **2,** is consistent with the information obtained after analysis of the 1D/2D NMR spectra. It is possible to determine that, in solid state, the hydroxyl attached to C-16 is in beta position in plan A. In low energy reaching, in solid state, ring A is in an intermediate state between boat and chair-like conformation.

Compounds **3** to **7** were isolated as white, amorphous solid materials. The chemical structures of these compounds were confirmed by comparison on thin-layer chromatography (TLC) with authentic samples and through the similarity with ^{13}C NMR data previously published.[16,18,19]

The final structure solution of triterpenes **1** and **2** (Figure 2) was obtained by the Rietveld refinement using the lowest energy profile fitting option. This technique has been recently employed to analyze the chemical structure of 3-oxofriedalane.[20] The peaks were searched and fitted with a modified Pseudo-Voigt peak profile, performing both modified Pawley and Rietveld refinement to optimize powder diffraction parameters and crystal structure so that the best possible agreement between simulated and experimental powder patterns was achieved. Initially, the pattern was processed for background contribution; peaks were searched and manually inspected, when necessary, to prevent background fluctuations from being considered reflections. The molecule was sketched using ChemSketch®.[21] The hydrogen atoms were introduced based on the NMR spectra and the molecule allowed to have its energy minimized by the Monte Carlo method before using it as a model. The crystal structure solution was first obtained without any preferred orientation correction and close-contact penalty. The results were compared with the fit using Marck-Dollese preferred orientation and close-contact penalty algorithm. The result that combined the lowest final energy obtained by Monte Carlo optimization was used as the solution. The corresponding structures were deposited at the CCDC under the numbers 974658 for unit cell parameters: a = 11.5986, b = 22.6631 and c = 5.3911 for space group P212121 (**1**) and 974657 for unit cell parameters: a = 11.7751, b = 23.9910 and c = 5.4078 for space group P212121 (**2**).

Even using few micromoles, all tested samples (triterpenes **1** to **6**) presented a relevant *in vitro* cytotoxicity activity against 4T1 cells (Figure 3). In a study developed by Lu *et al.*,[22] 4T1 cells were treated with doxorubicin, one of the most effective antitumor drugs, and the observed result was an IC_{50} of 3.27 μM. In the present work, using the same cell line, triterpene **2** induced an IC_{50} of 1.23 μM. This value is 2.64 times smaller than the IC_{50} induced by doxorubicin in 4T1 cells. These results suggest compound **2** as a potential antitumor agent. Compound **2**, which presented the highest cytotoxic activity, has an oxo group at carbon C-3 and two hydroxyl groups bonded to C-16 and C-29 respectively. The cytotoxic property of triterpene **1** was slightly lower than compound **2**. The difference between them was associated with C-16, which is connected to a hydroxyl group in triterpene **2** and to an oxo group in compound **1**. Compounds **2** and **6** differ only by the substitution of the hydroxyl group attached to C-16 for one hydrogen. Compound **6** is about ten times less active against 4T1 cells than triterpene **2** that has hydroxyl group attached to C-16. The cytotoxic activity of the triterpenes subjected to assays againt 4T1 cells seems to be also related to C-29. Compound **5** differs from triterpene **1** by presenting a hydrogen atom instead of a hydroxyl group attached to C-29 and is about ten times less active against 4T1 cells than triterpene **1**. Triterpene **3** differs from compound **2** by presenting only hydrogen atoms bonded to C-16 and C-29. This configuration resulted in a reduction of the cytotoxic activity of triterpene **3** about 100 times. Compound **4** differs from compound **3** by presenting a hydroxyl group bonded to C-3 instead of oxo group and **4** was about two times more potent than **3**.

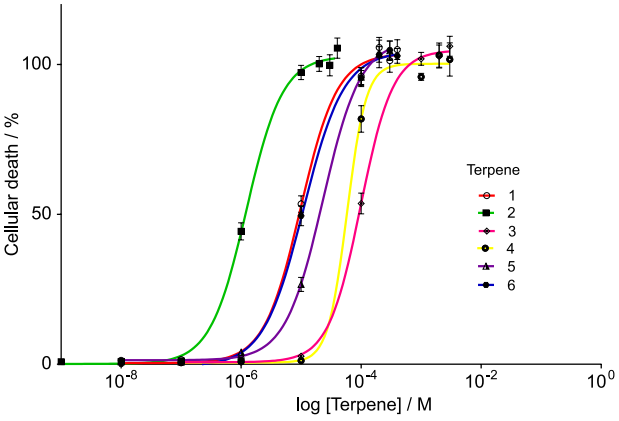

Figure 3. Representative curves of surviving 4T1 cells *vs.* the log of compound molar concentration. The IC_{50} value was evaluated through non-linear regression (n = 5).

According to the results obtained for compounds **1** to **6**, it is possible to establish that the presence of hydroxyl groups bonded to C-16 and C-29 may contribute in the increase of cytotoxicity of friedelane-type triterpenes against 4T1 cells. Moreover, hydroxyl group bonded to C-3 also contributed to increase the cytotoxicity. However, more detailed studies can reveal if this behavior is exclusive of hydroxyl group or if other organic groups can also act the same way. Detailed studies regarding the structure-activity relationship, including 3D-QSAR models, can help to evaluate the role of these groups in the cytotoxicity activity, since the mechanism of antitumor activity of friedelane-type triterpenes was not found in the literature. The results obtained in this work along with more detailed studies of the mechanisms of cell death (necrosis, apoptosis) may contribute to a better assessment of the potential of PCTTs in the treatment of cancer.

Conclusions

Seven friedelane-type triterpenes were isolated from hexane and chloroform extracts of the branches of *M. robusta*. The 1D/2D NMR spectral data and crystalline powder X-ray diffraction data of 3,16-dioxo-29-hydroxyfriedelane (**1**) and 3-oxo-16β,29-hydroxyfriedelane (**2**) are herein described for the first time. These two triterpenes presented a promising cytotoxic activity against 4T1 cells. Further studies will certainly contribute to the evaluation of the activity of these pentacyclic triterpenes against other tumor cells.

Supplementary Information

Supplementary data (Figures S1-S27) are available free of charge at http://jbcs.sbq.org.br as PDF file. Crystallographic data (excluding structure factors) for the structures in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 974658 and 974657. Copies of the data can be obtained, free of charge, in www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk.

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