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New cassane diterpenes from *Caesalpinia echinata*

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ABSTRACT

An investigation of the ethanolic extract from stems of *Caesalpinia echinata* Lam (Leguminosae-Caesalpinioideae) led to the isolation of five new cassane diterpenes along with known lambertianic acid. Their structures were determined based on spectroscopic methods. A preliminary study on leishmanicidal activity demonstrated that compounds **1, 2** and **6** were found to inhibit the growth of amastigote-*like* forms of *Leishmania amazonensis* without affecting mononuclear cells obtained from human peripheral blood.

1. Introduction

Caesalpinia echinata Lam. belongs to the Leguminosae-Caesalpinioideae family. It is a tropical tree that may reach up to 30 m in height and blooms yellow flowers from September until December. The species originally had a wide distribution in the Atlantic rainforest, ranging from Amazonas to São Paulo, especially in the coasts of Pernambuco and Rio de Janeiro [1]. Nowadays it is an endangered species [2] and it is found only in 7% of the original Atlantic Forest [3].

The kernel has astringent and tonic properties [1], but there are no pharmacological studies about *C. echinata*. Rezende et al. [4] analyzed the volatile constituents of *C. echinata* by GC-MS. *E*-β-ocimene was the major constituent of extracts obtained from flowers by a static cryogenic headspace while (*E*)-3-hexen-1-ol was the major constituent of extracts obtained from leaves by hydrodistillation in a Clevenger apparatus.

Previous investigations on species of the genus have displayed interesting biological activities such as antidiabetic [5], antiiflammatory [6], antiviral [7], antimalarial [8], antimicrobial [9] and antiplasmodal [10]. As part of an investigation of the chemical constituents of *Caesalpinia echinata* we report here the isolation and structure elucidation of five new cassane diterpenes **1**–**5,** and their preliminary evaluation of leishmanicidal activities.

2. Experimental procedures

2.1. General experimental procedures

TLC analyses were conducted on pre-coated silica gel G-60/F_{254} (0.25 mm, Merck) eluted with mixtures of $CHCl_3$/MeOH (65:50) and Hex/EtOAc (9:1). Spots were visualized after spraying the plates with vanillin–H_2SO_4. Preparative high speed co-current chromatography was carried out with a CCC-1000 model (High Speed Countercurrent Chromatograph, Pharma-Tech Research Corp) equipped by two pumps

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P-500 model (Pharmacia). Preparative circular chromatography (CCP) was carried out by Chromatotron 7924T model (Harrison Research). Semi-preparative HPLC purifications were carried out by a Shimadzu chromatograph, equipped with a LC6AD pump and a dual wavelength detector (SPD10A), using a Shim-pack® C_{18} column (5 μm, 250 × 20 mm i.d.) eluted with mixtures of MeCN/H_2O at a flow rate of 10 ml/min and detection at 210 nm and 254 nm. Electron impact mass spectrometry (EI-MS, 70 eV) was measured by Shimadzu QP5050A spectrometer, equipped with a direct insertion probe. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a Thermo Finnigan LCQ-Advantage spectrometer. ESI-Q/ToFMS analyses were performed using a Q-ToF MicroTM instrument (Micromass, Manchester, UK). Samples were diluted in methanol/Milli-Q1 water and introduced using a syringe pump with flow rates of 5–10 ml/min (electrospray). UV spectra (200–400 nm) were obtained by Shimadzu SPD M-10A VP Diode Array Detector. Infrared spectra were obtained by Shimadzu FTIR-8400 spectrometer, with the samples in KBr pellets. ^{1}H (400 MHz), ^{13}CNMR (100 MHz), DEPT-135, COSY, HMBC and NOESY experiments were carried out in a Bruker DRX 400 spectrometer. Chemical shifts were recorded in δ (ppm) using $CDCl_3$ as solvent. Complete assignments of the ^{1}H and ^{13}C chemical shifts of isolated compounds were solved by interaction using the Perch NMR Software (University of Kuopio, Finland). Conformational analyses were carried out by Perch's molecular modeling system (MMS). 3D molecular models were built with energetic optimization by Merck Molecular Force Field (MMFF94) based on superior force fields.

2.2. Plant material

Stems of *C. echinata* were collected at Fundação Zoo-Botânica, Belo Horizonte, Minas Gerais, Brazil, in March 2007. A voucher specimen (BHZB 6458) in complete form with flowers was deposited at the Herbarium of Fundação Zoo-Botânica of Belo Horizonte.

2.3. Extraction and isolation

Air-dried stems (55 g) of *C. echinata* were extracted with EtOH under ultrasonication at room temperature. The solvent was evaporated under reduced pressure to yield 1.6 g of crude EtOH extract. The crude extract (1.5 g) was purified by preparative high-speed co-current chromatography. The apparatus was equipped with three polytetrafluoroethylene preparative coils (total volume, 300 ml) and a 10 ml sample loop. With the rotor stopped, the coils were filled with the lower phase composition of a biphasic liquid system H_2O/MeOH/CH_2Cl_2 (4:6:5) at flow rate of 6 ml/min. The coils were then rotated at 1000 rpm and the upper phase was pumped in tail-to-head direction, at a flow rate of 4.5 ml/min. The extract (1.5 g) was dissolved in 10 ml of the biphasic solvent mixture and injected into the column. Lower phase was pumped at 3.0 ml/min and upper phase was pumped at 1.5 ml/min to give 22 fractions after TLC analysis. Fractions 17 and 18 (44 mg) were subjected to semi-preparative reversed-phase HPLC with MeCN:H_2O 40:60 → 60:40 in 40 min, 60:40 in 10 min, at a flow rate of 10 ml/min, to yield **1** (2 mg), **2** (2.5 mg) and **3** (6.5 mg). Fraction 19 (45 mg) was purified at the same conditions used before and yielded the compounds **4** (2.5 mg) and **5** (2 mg). Fraction 20 (300 mg) was subjected to semi-preparative circular chromatography on 1 mm plates of silica gel at a flow rate of 2–4 ml/min with hexane and ethyl acetate mixtures to furnish 23 subfractions. Compound **6** (4 mg) was obtained of subfractions 13–15 (18 mg) after column chromatography (silica gel) with Hex/EtOAc mixtures. Compound (**6**, Fig. 1): lambertianic acid, white powder; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.61 (s, 3 H, $\underline{CH_3}$-20), 1.00–1.06 (m, 1 H, H-1a), 1.07–1.09, (m, 1 H, H-3a), 1.24 (br s, 3 H, $\underline{CH_3}$-19), 1.31 (dd, J = 10.0 Hz, 5.0 Hz, 1 H, H-5), 1.47–1.56 (m, 1 H, H-2a), 1.54–1.62 (m, 1 H, H-9), 1.64–1.66 (m, 1 H, H-11a), 1.75 (td, J = 9.0, 9.0, 4.0 Hz, 1 H, H-11b), 1.79–1.86 (m, 1 H, H-1b),1.86–1.93 (m, 3 H, H-2b, H-6a and H-7a), 1.97 (dd, J = 11.0 Hz, 5.0 Hz, 1 H, H-6b), 2.15 (dd, J = 9.0, 4.0 Hz, 1 H, H-3b), 2.26 (dd, J = 14.0, 9.0 Hz, 1 H, H-12a), 2.43 (dd, J = 8.5, 3.0 Hz,1 H, H-7b), 2.56 (ddd, J = 14.0, 9.0, 4.0 Hz, 1 H, H-12b), 4.58 (m, 1 H, H-17a), 4.89 (m, 1 H, J = 1.9, 1.4 Hz, H-17b), 6.25 (m, 1 H, H-14), 7.19 (m, 1 H, H-16), 7.34 (m, 1 H, H-15), 10.57 (br s, 1 H, COO-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.9 (C-20), 19.91 (C-2), 23.60 (C-12), 24.29 (C-11), 26.09 (C-6), 28.99 (C-19), 38.03 (C-3), 38.71 (C-7), 39.07 (C-1), 40.39 (C-10), 44.13 (C-4), 55.27 (C-9), 56.24 (C-5), 106.53 (C-17), 110.97 (C-14), 125.47 (C-13), 138.75 (C-15) 142.69 (C-16) 147.84 (C-8), 182.08 (C-18); EI-MS *m/z* 316 ($[M]^+$), 279 (3), 223 (1.5), 167 (2.9), 149 (16), 139 (0.8), 104 (1), 88 (70), 70 (100), 61 (73).

2.4. Assay with Leishmania (Leishmania) amazonensis

Leishmanicidal activity was determined against amastigote-*like* forms that were obtained as previously described by Callahan et al. [11] and cell viability was determined using the methyl thiazolyl tetrazolium (MTT) assay described by Teixeira et al. [12].

2.5. Lymphocyte proliferation assay

Peripheral blood mononuclear cells (PBMCs) were prepared using the protocol previously described by Gazzinelli et al. [13] and the cell proliferation was determined by the MTT assay described by Jiang and Xu [14].

3. Results and discussion

As a part of an ongoing program devised for drug discovery from natural products, the EtOH extract from stems of *C. echinata* killed 94% of amastigotes-*like* forms of *Leishmania (Leishmania) amazonensis* and presented moderate inhibitory activity against human PBMC cells, stimulated with PHA, at 20 μg/ml (Table 3). The crude extract was fractionated by co-current HSCCC, preparative reversed phase HPLC and silica gel chromatography to give compounds **1**–**6** (Fig. 1).

Compound **1** was isolated as white amorphous powder and gave a molecular ion peak at *m/z* 495 $[M-H]^-$ in the negative ESI-MS spectrum and *m/z* 497.5985 $[M+H]^+$in the positive ESI-Q-TOF spectrum (calcd for $C_{29}H_{37}0_7$, 497.5999). The UV spectrum exhibited absorption bands at 210 nm and 279 nm, whereas the FT-IR (KBr) υ_{max} spectrum showed absorption bands at 3588, 2936, 2869, 1732, 1675, 1450, 1391, 1191 1144 and 950 cm^{-1}. The ^{1}H NMR spectrum of **1** (Table 1) showed resolved signals for one olefinic proton at δ 5.72 (1 H, br s) that was assigned for H-15 of α, β-unsaturated

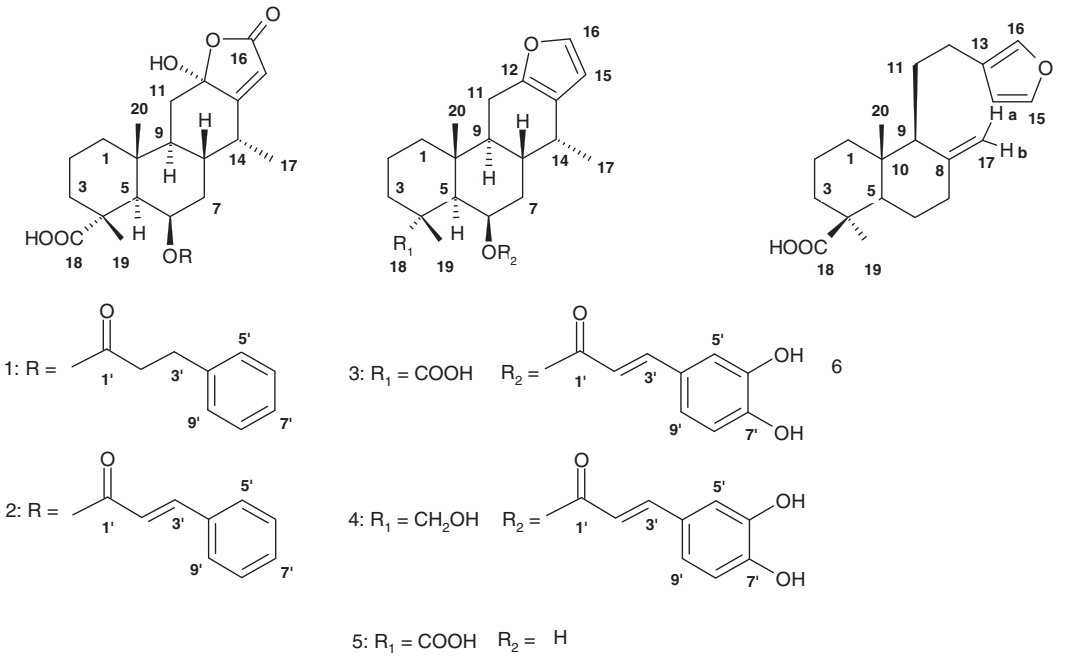

Fig. 1. Structure of compounds isolated from *Caesalpinia echinata*.

γ-lactone moiety, two tertiary methyl groups at δ 1.32 (s) and δ 1.08 (s), one secondary methyl group at δ 1.13 (d, $J=7.5$ Hz) and two α and β-carbonilic methylene protons at δ 2.96 and δ 2.64, respectively. One monosubstituted benzene ring was assigned by resonances at δ 7.18–7.26. Most of the methylene signals appeared as complex and overlapped multiplet and were assigned by HSQC, HMBC and COSY correlations (Table 1). The ^{13}C NMR and DEPT-135 spectra presented 29 carbon signals, eight quaternary including three carbonyl (δ 181.60, 172.26, 170.16), two olefinic carbons (δ 172.17 and δ 140.15), one carbon of a hemiketal (δ 105.24) and two others (C-4, δ 47.29 and C-10, δ 37.09). Also, these spectra showed the presence of seven methylene carbons, eleven methine carbons, comprising five aromatic carbons (δ 128.50 (2x), 128.23 (2x), 126.39), one olefinic carbon (δ 113.68), and five methine carbons (δ 72.09, 49.25, 45.09, 35.87, 35.81), besides two tertiary methyl groups (δ 18.35, 17.63) and one secondary methyl group (δ 12.94). The ^{13}C-NMR spectrum revealed all signals consistent with the structure of an α,β-butenolide hemiketal ring and hydrocinnamoyl ring moiety. The deshielded nature of the carbon at δ 72.09 and the proton attached to it at δ 5.13 (br d, $J=3.0$ Hz) suggested that the hydrocinnamoyl moiety is attached to C-6. Small vicinal coupling constants values between H-6 and H-5 ($^3J_{6eq,5ax}=3.0$ Hz) suggested an α-equatorial hydrogen attached to C-6. Besides, H-6 methine showed COSY and NOESY correlations to both vicinal proton signals at C-7. Other NOESY correlations observed were consistent with the configuration proposed for compound **1**. NOESY correlations between H-6 and H-5 at δ 1.96 (br d, $J=3.0$ Hz) and H-9 at δ 1.60 (m) suggested that protons were on the same face. A double doublet at δ 2.76 ($J=7.5$ and 3.0 Hz) was assigned to H-14 coupled with both CH_3-17 (δ 1.13 d, $J=7.5$ Hz) and an axial H-8 at δ 1.59 (m). H-14 proton was depicted as β-equatorial based on NOESY correlations and its small scalar coupling constant (3.0 Hz) with H-8. From these data, compound **1** was identified as 6β-O-2',3'-dihydrocinnamoyl-12-hydroxy-(13)15-en-16,12-olide-18-cassaneoic acid.

Compound **2** was obtained as white amorphous powder and it showed a molecular ion $[M-H]^-$ at *m/z* 493 in the negative ESI-MS and *m/z* 495.5800 $[M+H]^+$in the positive ESI-Q-TOF (calcd for $C_{29}H_{35}0_7$, 495.5841). The UV spectrum exhibited absorption bands at 218 nm and 279 nm, whereas the F-TIR (KBr) υ_{max} spectrum showed absorption bands at 3459, 2939, 2869, 1690, 1440, 1400, 1209 and 1139 cm^{-1}. The ^{1}H and ^{13}C (Table 1) NMR spectroscopic data were closely related to those of **1** except at C-6, where the dihydrocinnamoyl ester was replaced by a cinnamoyl moiety (δ 6.38, d, $J=16.0$ Hz, H-2' and δ 7.66, d, $J=16.0$ Hz, H-3'). The configuration at C-6 was the same as in compound **1**, since the coupling constants of the associated protons were similar, and this was confirmed by NOESY experiments. Thus, compound **2** was established as 6β-O-cinnamoyl-12-hydroxy-(13)15-en-16,12-olide-18-cassaneoic acid.

Compound **3** was isolated as a white powder and the negative ESI-MS showed a molecular ion $[M-H]^-$ at *m/z* 493 and exhibited fragment ions at m/z 179, suggesting the presence of the caffeoyl ester moiety. It showed the quasimolecular ion at *m/z* 495.5820 $[M+H]^+$in the positive ESI-Q-TOF (calcd for $C_{29}H_{35}0_7$, 495.5841). The UV spectrum exhibited absorption bands at 220 nm, 309 nm and 327 nm, whereas the FT-IR (KBr) υ_{max} spectrum showed absorption bands at 3423, 2939, 2869, 1704, 1514, 1450, 1269 and 1157 cm^{-1}. A pair of doublets at δ 7.22 (d, $J=1.9$ Hz) and at δ 6.17 (d, $J=1.9$ Hz) in its ^{1}H NMR spectrum (Table 2) suggested the presence of a 2,3-disubstituted furan ring. The ^{1}H NMR spectrum revealed the presence of two terciary methyl groups at δ

Table 1
NMR spectroscopic data of the compounds **1** and **2** (^{1}H NMR, 400 MHz; ^{13}C NMR,100 MHz, $CDCl_3$; δ in ppm, multiplicities, *J* in Hz).

1						**2**			
Position	δH (mult. *J*, Hz)	δC DEPT	COSY	HMBC	NOESY	δH (mult. *J*, Hz)	δC DEPT	COSY	HMBC
1eq(β)	1.78 *m*	41.03	1α, 2α	3, 9	1α, 11α	1.84 *m*	41.09	1α, 2α	3
1ax(α)	1.18 *ddd* (12.8, 12.8, 5.0)		1β		1β	1.25 *m*		1β	5, 10
2ax(β)	1.61 *m*	17.97		5, 10	19β	1.66 *m* (2H)	17.96	1α, 1β, 3α, 3β	19
2eq(α)	1.58 *m*		1β						
3eq(β)	1.76 *dd* (12.8, 3.0)	39.00	2α	5, 20	1β	1.79 *m*	39.05	2β	19
3ax(α)	1.69 *dd* (12.8, 5.0)		2β	1		1.70 *m*		1β, 3β	1, 4, 5
4		47.29		5, 19			47.41		5, 19
5ax(α)	1.96 *br d* (3.0)	49.25	6α	2, 4, 10, 18, 20	6α, 3α	2.10 *br d* (1.8)	49.41	6α, 7α	2, 4, 10
6eq(α)	5.13 *br d* (3.0)	72.09	5α, 7α		5α, 7α, 9α, 19β	5.30 *td* (3.0, 1.8, 1.8)	72.12	5α, 7α, 7β	
7	1.61 *m* (2H)	34.95	6α	8, 17	6α	1.86 *m*	35.17	6α, 7α, 9α	
						1.73 *m*		6α, 7β	8
8ax(β)	1.59 *m*	35.81	9α, 14β	17	14β	1.96 *ddd* (10.0, 6.0, 3.0)	36.15	14β, 7α, 9α	17
9ax(α)	1.60 *m*	45.09	8β, 11α	5	6α	1.75 *m*	45.18	8β,11β, 11α	20
10		37.09		5			37.25		5, 20
11ax(β)	1.37 *d* (13.0)	37.57	11α	20	8β, 11β	1.49 *d* (13.0)	37.68	9α, 11α	20
11eq(α)	2.39 *dd* (13.0, 3.0)		11β		1β, 11α	2.47 *dd* (13.0, 3.0)		9α, 11β	8, 20
12		105.24		15			105.31		15
13		172.17		17			172.31		17
14β	2.76 *dd* (7.5, 3.0)	35.87	8β, 17α	17	17α	2.94 *dd* (7.0, 5.0)	35.96	9α, 17	17
15	5.72, *br s*	113.68		12, 16	14β	5.72 *br s*	113.72		12, 16
16		170.16		15			170.25		15
17α	1.13. *d* (7.5, 3H)	12.94	14β	8, 13, 14, 16	14β	1.19 *d* (7.0, 3H)	12.96	14β	8, 13, 14
18		181.60		19			182.20		19
19β	1.32 *s* (3H)	18.35	5α	3, 5, 18	20	1.39 *s* (3H)	18.38	3β	3, 4, 10, 18
20β	1.08 *s* (3H)	17.63	5α	1, 5, 9, 10	19	1.26 *s* (3H)	18.02	11β	1, 5, 9, 10
1'		172.26					166.27		3'
2'	2.64 *td* (2× 7.5, 3.0, 2H)	36.30			3'	6.38 *d* (16.0, 2H)	118.30	3'	4'
3'	2.96 *td* (2× 7.5, 3.0, 2H)	30.85	5′, 9′	5', 9'	2'	7.66 *d* (16.0, 2H)	145.28	2'	1', 5', 9'
4'		140.15		3', 6', 8'			134.18		2'
5'	7.19 *m*	128.23	3′, 7′	7', 9'	6'	7.52 (*m*)	128.15	6'	3', 8'
6'	7.27 *m*	128.50	8′	4', 8'	5', 7'	7.39 (*m*)	128.94	5'	5', 9'
7'	7.18 *m*	126.39	5′, 9′	5', 9'	6', 8'	7.40 (*m*)	130.53	5',9'	5', 6', 8', 9'
8'	7.26 *m*	128.50	6′	4', 6'	9', 7'	7.39 (*m*)	128.94	7'	5', 9'
9'	7.19 *m*	128.23	5′, 7′	5', 7'	8'	7.52 (*m*)	128.15	8'	3', 6'

1.47 and 1.37 and one secondary methyl group at δ 0.97 (d, *J* = 7.0 Hz), five aliphatic methylenes (δ 1.79 and 1.23, 1.69 and 1.60, 1.86 and 1.73, 1.96 and 1.73, 2.65 and 2.52) and five aliphatic methines (δ 5.40, 2.57, 2.21, 2.09, 1.72). A caffeoyl ester ring moiety was confirmed by signals of aromatic protons found at δ 6.88–7.11, and two coupled doublets at δ 7.55 (*J* = 15.8 Hz) and at δ 6.17 (*J* = 15.8 Hz) assigned for olefinic protons. Moreover, the ^{13}C NMR and DEPT-135 of **3** (Table 2) showed a total of 29 carbon signals, nine quaternary represented by two carbonyl (δ 181.06, 166.66) and five olefinic carbons that includes two furan carbons (δ 149.44 and 122.01) and three aromatic carbons (δ 148.34, 145.80 and 126.33). These spectra showed the presence of five methylene carbons (δ 41.39, 18.22, 39.00, 35.87, 21.44), twelve methine carbons, comprising three aromatic carbons (δ 121.34, 115.12 and 113.99), two furan olefinic carbons (δ 140.16 and 109.26), two olefinic carbons in caffeoyl ester moiety (δ 144.80 and 115.29) and five others (C-5, δ 49.72, C-6, δ 72.14, C-8, δ 31.19, C-9, δ 45.58, C-14, δ 30.87). In addition, ^{13}C-NMR spectrum of **3** revealed the signals of two tertiary methyl groups (δ 17.99, 17.72) and one secondary methyl group (δ 17.44). The configuration of compound **3** was determined by analysis of coupling constants and NOESY data. The small coupling constant between H-5 at δ 2.21 (br d, *J* = 2.0 Hz) and H-6 at δ 5.40 (br d, *J* = 2.0 Hz) indicated that H-6 is at α-equatorial position. In the NOESY spectrum H-6 had cross-peaks with H-5 and H-5 with H-9, confirmed that H-5 was axial and that the C-6 was equatorial. These observations allowed for **3** to be proposed as being 6β-*O*-6',7' dihydroxycinnamoyl-18-vouacapaneoic acid.

Compound **4** showed the molecular ion $[M\text{-}H]^-$ at *m/z* 480 in negative ESI-MS spectrum and *m/z* 479.5805 $[M-H]^-$ in the negative ESI-Q-TOF (calcd for $C_{29}H_{37}0_6$, 479.5845). The UV spectrum exhibited absorption bands at 218 nm, 309 nm and 327 nm, whereas the FT-IR (KBr) υ_{max} spectrum showed absorption bands at 3441, 2929, 2855, 1695, 1518, 1446, 1382, 1273 and 1177 cm^{-1}. The ^{1}H and ^{13}C NMR spectral data (Table 2) of **4** revealed the same cassane-type skeleton as **3** which contains a furan and a 6',7'-dihydroxy-*trans*-cinnamoyl moieties. The major difference was the replacement of the acid carboxilic group by a hydroxymethylene group at C-18. The ^{1}H-NMR spectrum presented two coupled doublets at δ 3.64 (*J* = 11.0 Hz) and at δ 3.18 (*J* = 11.0 Hz), and the ^{13}C-NMR spectrum showed an oxygenated carbon signal at δ 71.6 corresponding to hydroxymethylene group. The hydroxymethyl proton H-18A at δ 3.18 showed HMBC correlation with carbon signal of methyl group (C-19), and H-19 and H-20 showed HMBC correlations with C-18. Thus, the structure of compound **4** was determined as 6*β*-*O*-cinnamoyl-18-vouacapaneol.

Compound **5** exhibited molecular peak $[M+H]^+$ at *m/z* 333 by positive ESI-MS and *m/z* 331.4198 $[M-H]^-$ in the negative ESI-Q-TOF (calcd for $C_{20}H_{27}0_6$, 331.4259). The UV spectrum

Table 2
NMR spectroscopic data of the compounds **3**–**5** (^{1}H NMR, 400 MHz; ^{13}C NMR,100 MHz, $CDCl_3$; δ in ppm, multiplicities, *J* in Hz).

3						**4**				**5**			
Position	δ (mult. *J*, z)	δC DEPT	COSY	HMBC	NOESY	δ (mult. *J*, z)	δC DEPT	COSY	HMBC	δ (mult. *J*, z)	δC DEPT	COSY	HMBC
1eq(β)	1.79 *d* (12.5)	41.39	1α	3, 10, 20	1α	1.76 *br d* (13.0)	41.72	3β	3, 20	1.72 *m*	41.44	5α, 20β	9, 10
1ax(α)	1.23 *dd* (12.5, 4.0)		2β		1β	1.11 *dt* (13.0, 13.0, 3.0)		2β, 3a, 20	2	1.18 *ddd* (12.5, 12.5, 4.0)		1β, 2β	
2ax(β)	1.69 *m*	18.22	3β, 5α	5, 19	11β	1.72 *m*	18.17	1β, 2α	1	1.59 *m*	18.06	2α	
2eq(α)	1.60 *dd* (13.0, 3.0)		3β		2β	1.55 *dd* (14.0, 4.0)		1β, 2β		1.69 *m*		2β	
3eq(β)	1.86 *dd* (13.0, 4.0)	39.00	3α	1, 5, 19	19	1.69 *m*	37.08	2α, 3α	5, 19	1.69 *m*	40.40		19
3ax(α)	1.73 *m*		3β		5α	1.22 *m*		2β, 3β					
4		47.23		5, 19			38.31		5, 19		47.98		5, 7
5ax(α)	2.21 *br d* (2.0)	49.72	7β, 3α	2, 4, 9, 10, 18, 19	7α, 9α	1.75 *m*	48.40	6α	4, 9, 20	1.85 *br s*	50.73	6α	1
6eq(α)	5.40 *br d* (2.0)	72.14	5α, 7α, 7β	8, 10	5α, 7α, 7β	5.60 *ddd* (4.0, 3.0, 3.0)	69.73	5α, 7α, 9α		4.15 *m*	70.18	5α, 7β, 7α	
7(β)	1.96 *td* (14.0, 3.0, 3.0)	35.87	7β, 8β		14	2.34 *m*	36.46	9α		1.80 *ddd* (13.0, 13.0, 4.0)	38.74		6α, 7α
7(α)	1.73 *m*		7β, 8β	5, 8	6α	1.88 *ddd* (14.0, 3.0, 3.0)		8β	5, 8	1.69 *m*		7β	
8ax(β)	2.09 *m*	31.19	7α, 7β	6, 11	11β, 14β, 20	2.10 *m*	31.12	7α, 9α	7, 11	2.18 *m*	30.70	7α, 7β, 9α	
9ax(α)	1.72 *m*	45.58	11α, 11β	5, 11, 20	6α, 11α, 17	1.64 *m*	45.67	6β, 7β, 11β, 14β	20	1.62 *m*	46.22	8β, 11β	1, 10, 20
10		37.26		1, 5, 6, 11, 20			37.78		5, 20		37.25		5, 20
11ax(β)	2.52 *dd* (17.0, 10.0)	21.44	9α	8, 9, 10, 12, 13, 20	1β	2.52 *m*	21.80		8	2.48 *dd* (16.0, 10.0)	21.71	11β	12
11eq(α)	2.65 *dd* (17.0, 6.5)		9α		9α, 1α	2.65 *ddd* (15.0, 15.0, 7.0)		7α, 9α, 11β		2.61 *dd* (16.0, 7.0)		9α, 11α	
12		149.44		11, 15, 16			149.45		11		149.35		11
13		122.01		11, 15, 16, 17			122.55		11		122.27		15
14β	2.57 *dd* (7.0, 5.0)	30.87	17	12, 13, 17	7β, 8β, 17	2.57 *m*	31.14	8β, 17	12, 17	2.61 *m*	31.26	17α	13
15	6.17 *d* (1.9)	109.26	16	12, 13, 16	16	6.18 *d* (1.8)	109.49	16	16	6.19 *d* (2.0)	109.52	16	15
16	7.22 *d* (1.9)	140.16	15	15	15	7.23 *d* (1.8)	140.44	15	15	7.23 *d* (2.0)	140.45	15	13
17α	0.97 *d* (7.0)	17.44	14β, 8β, 9α	12, 13, 14	9α, 14β, 15,	0.92 *d* (7.0, 3H)	17.60	14	8, 13, 14	0.98 *d* (7.0, 3H)	18.06	14β	8
18B		181.06		3, 4, 5, 19		3.64 *d* (11.0)	71.60	18A			183.59		19
18A						3.18 *d* (11.0)		18B	19				
19β	1.47 *s* (3H)	17.99	3β, 20	1, 3, 4, 5, 18	3β, 6α	0.96 *s* (3H)	19.56		3, 4, 5, 18	1.62 *s* (3H)	18.87		3, 5, 18
20β	1.37 *s* (3H)	17.72	19	1, 5, 9, 10	1β, 2β, 8β, 2'	1.34 *s* (3H)	18.07	1α	1, 5, 9, 10, 18	1.27 *s* (3H)	17.84		1, 5, 9, 10
1'		166.66		2', 3', 5', 9'			167.18		3'				
2'	6.17 *d* (15.8)	115.29	3'	3', 5', 9'	5', 9', 20	6.22 *d* (16.0)	116.62	3'	4'				
3'	7.55 *d* (15.8)	144.80	2'	5', 7', 9'	5', 9', 20	7.54 *d* (16.0)	145.24	2'	1',5'				
4'		126.33		2', 8'			127.70		2', 8'				
5'	7.11 *d* (1.9)	113.99	9'	3', 9'	2', 3'	7.07 *d* (2.0)	114.30	8', 9'	3', 7', 9'				
6'		145.80		5', 8'			146.19		4, 8'				
7'		148.34		5', 9'			148.77		5'				
8'	6.88 *d* (8.0)	115.12	9'	4', 6'	9'	6.86 *d* (8.5)	115.52	5'	4', 6'				
9'	6.92 *d* (8.0, 1.9)	121.34	8'	3', 5'	2', 3'	6.99 *dd* (8.5, 2.0)	121.64	5'	4', 5'				

exhibited absorption bands at 220 nm and 279 nm, whereas the FT-IR (KBr) υ_{max} spectrum showed absorption bands at 3441, 2930, 2871, 1695, 1459, 1395, 1199 and 1172 cm^{-1}. The ^{1}H NMR spectrum (Table 2) exhibited signals of two tertiary methyl groups at δ 1.62 (s) and δ 1.27 (s), and a secondary methyl group at δ 0.98 (d, $J = 7.0$ Hz). In the low field region of the spectrum, two protons of a 1,2-disubstituted furan resonated at δ 7.23 (d, $J = 2.0$ Hz, H-16) and at δ 6.19 (d, $J = 2.0$ Hz, H-15). Analysis of the ^{13}C NMR and DEPT-135 spectra of **5** revealed signals of one carbonyl at δ 183.59, four carbons of the furan ring (δ 149.35, 140.45, 122.27, 109.52) and one oxygenated carbon at δ 70.18. One oxymethine proton at δ 4.15 (m) was assigned for H-6 that showed correlations with H-5 and H-7 in the COSY experiment. The configurations of compound **5** were the same as in compound **3** and **4** and it was identified as 6β-hydroxi-18-vouacapaneoic acid.

Compound **6** exhibited molecular peak at *m/z* 316 ($[M]^+$) by EI-MS suggesting the molecular formula $C_{20}H_{28}O_3$. All the spectroscopic data observed for compound **6** were identical to those described for lambertianic acid [15].

Compounds **1** and **2** can be considered as derivatives from vinhaticoic acid, a furanocassane-type diterpene isolated from *Dipteryx lacunifera* Ducke (Leguminosae-Papilionoideae), a species that occurs in Brazil [16]. Previous studies on species of this genus reported the same skeleton with a methine on C-5 [17–21]. Compounds**1**–**5** are cassane diterpenes with *trans*-cinnamoyl or *trans*-hydrocinnamoyl groups as side chain at C-6. The relative configuration of compounds **1**–**5** was defined by NOESY results and built with energy-minimized conformation (Fig. 2). All structures adopt *trans*-junction of the three hexagonal rings and the *trans*-cinnamoyl ester groups were placed on opposite face of COOH groups.

Table 3
Results of the biological assays of the isolated compounds.

Compounds*	% Leishmanicidal activity[a]	%Inhibition of PBMC proliferation[b]
1	56 ± 13	114 ± 8
2	69 ± 8	112 ± 37
3	26 ± 19	124 ± 13
4	43 ± 21	–
5	27 ± 0	122 ± 12
6	62 ± 9	120 ± 28
EtOH extract	94 ± 6	60 ± 5
AMB**	70 ± 10	–
DMSO***	6 ± 2	100

*Extract and compounds were tested at 20 μg/ml. **Amphotericin B at 0.2 μg/ml was used as positive drug control. ***DMSO was tested at 0.1% v/v. (–) not tested.
[a] Amastigote-*like* forms of *Leishmania (Leishmania) amazonensis*.
[b] Human peripheral blood mononuclear cells, stimulated with PHA.

Compounds **1**, **2** and **6** were tested against *Leishmania (Leishmania) amazonensis* (Table 3) and neither of them is more active than the crude extract, but they are not toxic to human peripheral blood mononuclear cells in vitro at a concentration of 20 μg/ml. In this paper, we reported for the first time, the isolation and elucidation of furan and hemiketal cassane-type diterpenes and also the evaluation of their leishmanicidal activity.

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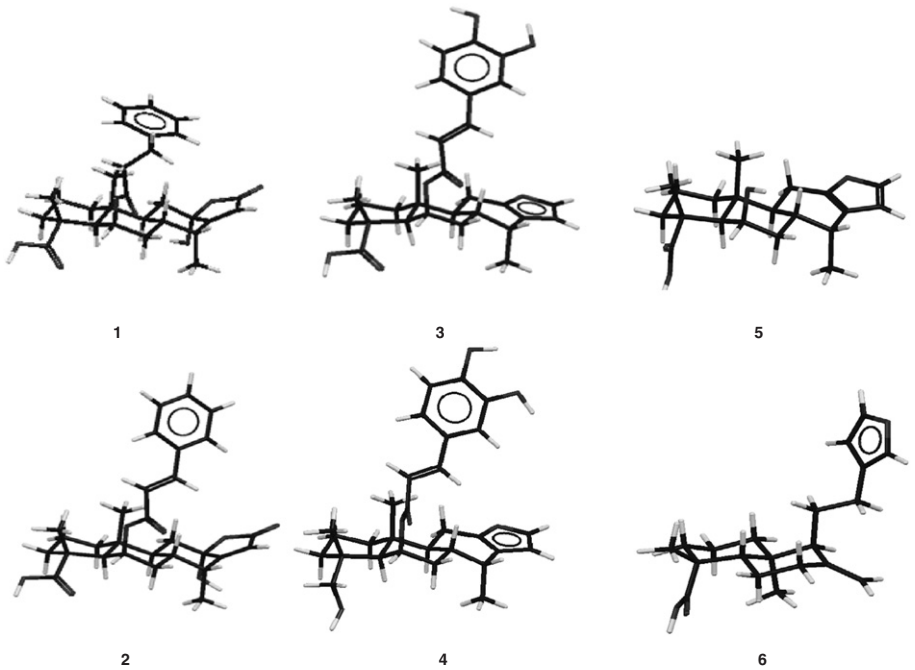

Fig. 2. Conformational analyses of compounds **1**–**6** by PERCH NMR software.

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