http://dx.doi.org/10.21577/0103-5053.20170105

J. Braz. Chem. Soc., Vol. 29, No. 1, 11-16, 2018.
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Article

Withanolides from Leaves of *Nicandra physalodes*

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Five new withanolides, designated as 15-oxo-nicaphysalin B, 6β,7α-dihydroxynicandrenone 10, 24α,25β-dihydroxy-nicandrenone-2 and a mixture of the epimers 17-(1α/1β-methylpropanone)-nicandrenone, in addition to six known others, were isolated from the leaf acetone extract from *Nicandra physalodes*. The complete structures of the five new withanolides, particularly their relative stereochemistries, were established by extensive spectroscopic analyses, including 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESIMS). The main isolated withanolides were evaluated for their antibacterial, antifungal and larvicidal properties, but, except nicandrenone, which showed marginal larvicidal activity, none of them was active.

Keywords: *Nicandra physalodes*, Solanaceae, withanolides

Introduction

According to the literature records,[1-3] the genus *Nicandra* (Solanaceae) consists of just three species: *N. physalodes* G, *N. john-tyleriana* S, and *N. yacheriana* S, all of them from South America. Despite the reports concerning to the identification of *N. john-tyleriana*[2] and *N. yacheriana*,[3] the site Plant List[4] just recognizes *N. physalodes* and *N. xsanderae*. All the other *Nicandra* species are considered as unresolved. Like several genera belonging to the Solanaceae family, plants of the *Nicandra* genus are a prolific source of withanolides (C_{28} steroidal lactones, ergostane-type). These secondary metabolites are widely known for their biological and pharmacological properties, such as cytotoxic, antitumor, immunosuppressive, antimicrobial, antifeedant, and anti-inflammatory.[5]

Nicandra physalodes has been the subject of several phytochemical and biological studies. From this species, several withanolides have been reported possessing either the normal or modified skeletons (ring-D aromatic).[6-12] *N. physalodes* is considered an ornamental plant possessing insect repellent properties. In fact, its main constituent named as nicandrenone showed antifeedant activity.[6-8,13]

In a continuing effort to isolate active compounds from the Brazilian Solanaceae, the acetone extract from *N. physalodes* was investigated. As results, five new withanolides (15-oxo-nicaphysalin B (**1**), 6β,7α-dihydroxynicandrenone 10 (**2**), 24α,25β-dihydroxy-nicandrenone-2 (**3**) and a mixture of the epimers 17-(1α/1β-methylpropanone)-nicandrenone (**4a/4b**)), in addition to nicandrenone-10 (**5**), nicandrenone (**6**), nicandrenone-7 (**7**),[10] nicandrenone-2 (**8**),[7] nicaphysalin B (**9**),[9] and 26*R*-withahisolide G (**10**),[12] were isolated. The isolated compounds were evaluated for their antibacterial and antifungal effects. Moreover, **2** and **6**, the main isolated compounds, were subjected to *Aedes aegypti* larvicidal assays.

Experimental

General experimental procedures

Optical rotations were measured with a Jasco P-2000 polarimeter, operating with a tungsten lamp at a wavelength of 589 nm at 20 °C. High resolution mass spectra were

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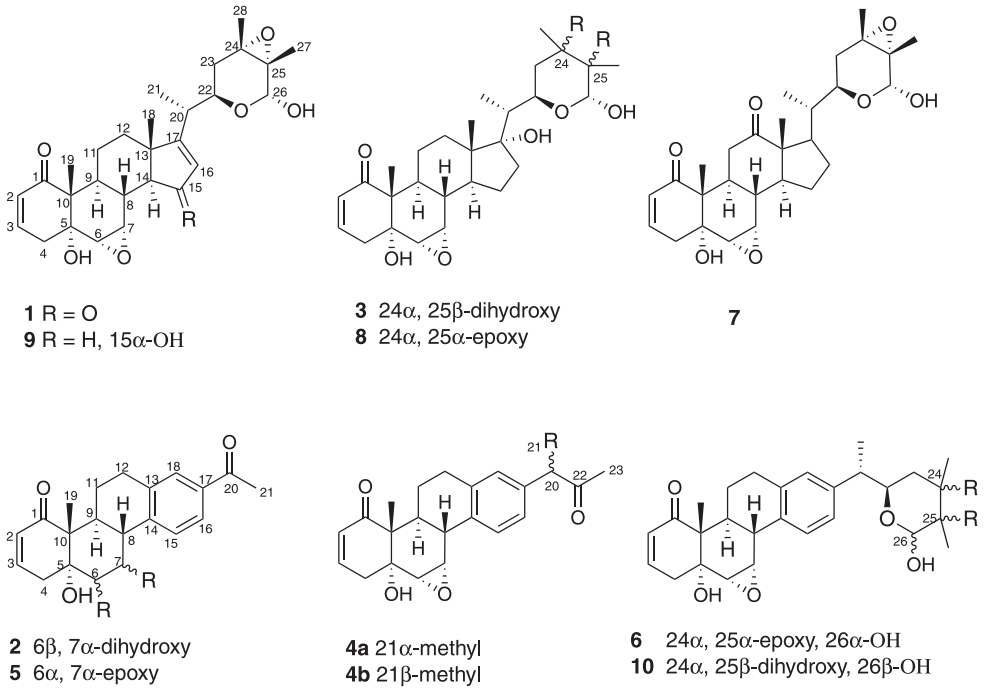

Figure 1. Structures of the withanolides (**1**-**10**) isolated from *Nicandra physalodes*.

recorded on a Waters Acquity UPLC system coupled with a quadrupole/time-of-flight (TOF) system (UPLC/Qtof MSE spectrometer) in the positive mode. The nuclear magnetic resonance (NMR) spectra were accomplished either on a Bruker Avance DRX-500 (resonance frequencies of 500 MHz for ^{1}H, and 125 MHz for ^{13}C) or DPX-300 (resonance frequencies of 300 MHz for ^{1}H, and 75 MHz for ^{13}C) spectrometers. The ^{1}H and ^{13}C chemical shifts were expressed in the δ scale and were referenced to TMS through the residual undeuterated solvent. High performance liquid chromatography (HPLC) was performed on a Shimadzu apparatus having a SPD-M20A UV-Vis detector, and a Phenomenex RP-C18 column, with a flow rate of 1 mL min^{-1} for the analytical column (4.6 × 250 mm, 5 μm) and 4 mL min^{-1} for the semi-preparative column (10 × 250 mm, 5 μm) using mixtures of MeCN, MeOH and H_2O as the solvents for the mobile phases. Column chromatography (CC) was performed with silica gel 60 (70-230 mesh) or 60 (230-400 mesh) for flash columns, Sephadex LH-20 (Pharmacia), or SPE C18 (Phenomenex) cartridges. Thin layer chromatography (TLC) was performed on precoated silica gel aluminum sheets (Merck) with fluorescent indicator (254 nm), and the spots were visualized by UV detection, and after spraying with a vanillin/perchloric acid solution, followed by heating at ca. 100 °C.

Plant material

The plant material was collected in 2015, in Guaraciaba do Norte (4° 06' 48.9'' S, 4° 51' 51.1'' W), Ceará State, Brazil. A voucher specimen (No. 58047) was deposited at Herbarium Prisco Bezerra (EAC) of the Universidade Federal do Ceará.

Extraction and isolation

Air-dried and powdered leaves of *N. physalodes* (1.2 kg) were macerated for 24 h with acetone (2 × 7.0 L). The resulting solution was evaporated under reduced pressure to yield 56.5 g of a crude extract, which was resuspended in MeOH/H_2O (6:4) and subjected to liquid-liquid partition with pure solvents of increasing polarity: *n*-hexane, CH_2Cl_2 and AcOEt, to yield three fractions, F_{hexane} (31.38 g), $F_{CH_2Cl_2}$ (7.30 g) and F_{AcOEt} (1.95 g), respectively. The $F_{CH_2Cl_2}$ fraction (7.30 g) was subjected to CC over Si gel 60 (70-230 mesh), by elution with CH_2Cl_2-AcOEt (9:1), CH_2Cl_2-AcOEt (7:3), CH_2Cl_2-AcOEt (1:1), CH_2Cl_2-AcOEt (3:7), EtOAc, EtOAc-MeOH (8:2), and MeOH yielding seven fractions: F1 (529.7 mg), F2 (3.08 g), F3 (682.3 mg), F4 (494.1 mg), F5 (861.1 mg), F6 (1.40 g) and F7 (157.0 mg).

An aliquot of F1 (473.1 mg) was subjected to column chromatography on Sephadex LH-20, using methanol as eluent, resulting in the isolation of **5** (26.3 mg). The fraction F2 (3.08 g) was submitted by recrystallization using a mixture of hexane/AcOEt (1:1) with drops of MeOH, yielding compound **6** (2.8 g). Fraction F3 (682.3 mg) was washed with MeOH to give colorless crystals, compound **7** (13.9 mg). The residue from F3, soluble in MeOH, was

evaporated to give a white precipitate which was washed with hexane/AcOEt (1:1) with a few drops of MeOH, yielding compound **8** (56.2 mg).

The fraction F6 (1.40 g) was also submitted to a Sephadex LH-20 column eluting with MeOH to obtain 70 fractions, which after TLC analysis were pooled into eleven subfractions ($F6_A$-$F6_K$). $F6_C$ was subjected to successive Sephadex LH-20 columns, by elution with MeOH, and subsequent purification by chromatography on SPE C-18 cartridges using H_2O-MeOH (1:1) to MeOH 100%, yielding **9** (46.9 mg). The subfraction $F6_CA$ (63.1 mg) was chromatographed on SPE C-18 cartridges using H_2O/MeOH 30% to MeOH 100%, yielding 7 subfractions. The subfraction 4 (28.3 mg) was further purified by HPLC on a C-18 semi-preparative column using H_2O/MeCN (65:55 for 10 min), H_2O/MeCN (65:35 → 20:80 in 5 min) and H_2O/MeCN (20:80 for 10 min), at a flow rate of 4.2 mL min^{-1}, leading to the isolation of compound **1** (retention time, t_R 12.7 min, 2.9 mg).

Fraction F5 (861.1 mg) was applied to a Sephadex LH-20 column, by elution with MeOH, to yield 23 subfractions, which were grouped into 5 main subfractions ($F5_A$-$F5_E$), after TLC analysis. A precipitate was formed in $F5_D$, leading to the isolation of **2** (39.8 mg) as colorless crystals. Subfraction $F5_C$ (402.3 mg) was chromatographed on an SPE C-18 cartridge using mixtures of H_2O-MeCN (7:3) to MeCN-THF (9:1), resulting in 7 fractions ($F5_CA$-$F5_CG$). From fraction $F5_CB$ was obtained a precipitate, compound **3** (10.2 mg). The mother liquor of $F5_CB$ (201.4 mg) was submitted to CC over Si gel flash (230-400 mesh) by elution with CH_2Cl_2/MeOH (95:5 to 1:1) yielding 9 subfractions. Subfraction 4 (95.4 mg) was further purified by HPLC analyses on a C-18 semi-preparative column using H_2O/MeCN (70:30 → 55:45 in 20 min), flow rate of 3.5 mL min^{-1}, leading to compound **10** (t_R 14.6 min, 16.7 mg).

The F_{AcOEt} fraction (1.95 g) was submitted to CC over Si gel 60 (70-230 mesh), by elution with CH_2Cl_2-AcOEt (9:1), CH_2Cl_2-AcOEt (7:3), CH_2Cl_2-AcOEt (1:1), CH_2Cl_2-AcOEt (3:7), EtOAc, EtOAc-MeOH (8:2), and MeOH yielding 154 subfractions, which were grouped into 10 main fractions after TLC analysis: F1 (2.0 mg), F2 (14.3 mg), F3 (21.0 mg), F4 (46.8 mg), F5 (180.0 mg), F6 (543.6 g), F7 (360.0 mg), F8 (44.1 mg), F9 (510.0 mg) and F10 (133.9 mg). Subfraction F5 was applied to flash column over Si gel 60 (230-400 mesh) to give compound **4** (8.0 mg).

Antimicrobial assay

The antimicrobial activity of all compounds was evaluated against four bacteria: *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12128, *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 11303, while the antifungal activity was performed with two fungi: *Candida albicans* ATCC 90028 and *Candida tropicalis* ATCC 750. Before the assays procedures, each bacterial and fungi species was grown in tryptic soy agar (TSA, Himedia, USA) and yeasts in Sabouraud dextrose agar (SDA, Himedia, USA) for 24 h at 37 °C. The bacterial and fungi cells were inoculated in tryptic soy broth and Sabouraud dextrose broth (TSB, SDB, Himedia, USA) and incubated for 18 h at 37 °C under constant agitation. Subsequently, cell's concentration of each microorganism was adjusted to 1×10^6 cell mL^{-1} using spectrophotometry and calibration curves previously determined for each bacterium. For yeasts, the cell culture was adjusted using a Neubauer chamber. The minimal inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) were determined by the broth microdilution method according with the guidelines from the National Committee for Clinical Laboratory Standards (NCCLS), M7-A6[14] and M27-A2,[15] with some modifications. Briefly, different concentrations of the compounds (7.8 to 500 μg mL^{-1}) were prepared in a medium (with 4% of dimethyl sulfoxide) and aliquots of 100 μL of each compound were mixed with the microbial suspensions (100 μL) in the 96-well polystyrene plates. The plates were incubated at 37 °C during 24 h under constant agitation. The MIC value was established as the lowest concentration of compound able to inhibit the visible growth of the microorganism. MMC, considered the lowest concentration that completely inhibited microbial growth in the plates, was determined by transferring 10 μL from each well without visible growth into agar plates.

Larvicidal bioassay

Compounds **2** and **6** (12.5 to 500 μg mL^{-1}) were placed in a beaker (50 mL) and dissolved in DMSO/H_2O 1.5% (20 mL). Fifty instar III larvae of *Aedes aegypti* were delivered to each beaker. After 24 h at room temperature, the number of dead larvae was counted and the lethal percentage calculated. A control using DMSO/H_2O 1.5% was carried out in parallel. For each sample, 3 independent experiments were run.[16]

15-Oxo-nicaphysalin B (**1**)

White amorphous solid; $[\alpha]_D^{20}$ +43.9 (*c* 0.1, MeOH); ^1H (500 MHz, pyridine-d_5) and ^{13}C (75 MHz, pyridine-d_5) NMR data, see Table 1; HRESIMS (high resolution electrospray ionization mass spectrometry) (positive mode) *m/z*, calcd. for $C_{28}H_{37}O_7$ $[M + H]^+$: 485.2539, found: 485.2527.

6β,7α-Dihydroxynicandrenone 10 (**2**)

Colorless crystal; $[\alpha]_D^{20}$ −105.0 (*c* 0.1, MeOH); ^{1}H (500 MHz, pyridine-d_5) and ^{13}C (75 MHz, pyridine-d_5) NMR data, see Table 1; HRESIMS (positive mode) *m/z*, calcd. for $C_{21}H_{25}O_5$ $[M + H]^+$: 357.1702, found: 357.1703.

24α,25β-Dihydroxy-nicandrenone-2 (**3**)

Colorless crystal; $[\alpha]_D^{20}$ −4.76 (*c* 0.1 $CHCl_3$); ^{1}H (500 MHz, pyridine-d_5) and ^{13}C (75 MHz, pyridine-d_5) NMR data, see Table 1; HRESIMS (positive mode) *m/z*, calcd. for $C_{28}H_{43}O_8$ $[M + H]^+$: 487.2696, found: 487.2695.

17-(1α/1β-Methylpropanone)-nicandrenone-10 (**4a**,**4b**)

White amorphous solid; ^{1}H (500 MHz, pyridine-d_5) and ^{13}C (75 MHz, pyridine-d_5) NMR data, see Table 1; HRESIMS (positive mode) *m/z*, calcd. for $C_{23}H_{27}O_4$ $[M + H]^+$: 367.1909, found: 367.1916.

Results and Discussion

Compound **1** was isolated as a white amorphous powder. Its HRESIMS showed a protonated molecular ion $[M + H]^+$ at *m/z* 485.2527 (calcd. *m/z* 485.2539) indicating the molecular formula $C_{28}H_{36}O_7$, 11 degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Table 1) showed typical signals of a withanolide with an A-type nucleus (group I),[5] particularly similar to those reported for nicaphysalin B, also isolated in this study, already reported for *N. physalodes*.[9] In fact, comparing the ^{1}H and ^{13}C resonances of both compounds, the most significant difference was the presence of an additional conjugated α,β-unsaturated carbonyl signal at δ_C 207.1 (C-15) for **1** instead the signal at δ_C 77.0 (Table S1, Supplementary Information), related to an oxymethine carbon, of nicaphysalin B (**9**). The signals at δ_C 207.1 (C-15), 188.8 (C-17) and 130.2 (C-16) indicated an α,β-conjugated ketone, therefore, the C_{15}-oxidized form of nicaphysalin B. In the heteronuclear multiple bond correlation (HMBC) spectrum, the correlations for the olefinic proton at δ_H 6.02 (d, *J* 1.9 Hz, H-16) with the carbon signals at δ_C 207.1 and 188.8, and for both methyl protons at δ_H 1.19 (s, Me-18) and 0.99 (d, *J* 6.3 Hz, Me-21) with the non-hydrogenated olefinic carbon at δ_C 188.8, supported the structure suggested. Based on these findings, the complete structure of **1**, including its relative stereochemistry, was defined as 15-oxo-nicaphysalin B.

Compound **2** was isolated as colorless crystals. Its HRESIMS displayed a protonated molecular ion peak at *m/z* 357.1703 (calcd. *m/z* 357.1702) indicating the molecular formula $C_{21}H_{24}O_5$, which comprises 10 degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **2** were similar to those of nicandrenone 10 (nic-10) (**5**), a withanolide possessing a D-aromatic ring, previously isolated from *N. physalodes*.[10] The deshielding observed for the proton signals at δ_H 4.39 (t, *J* 4.1 Hz, H-6) and 5.21 (br s, H-7), as well as the carbon signals at δ_C 75.9 (C-6) and 73.8 (C-7) in relation to those of nic-10 [δ_H 3.33 (H-6) and 4.08 (H-7) / δ_C 57.4 (C-6) and 54.6 (C-7)] (Table S1, Supplementary Information) supported the opening of the epoxy to generate two hydroxylated carbinolic carbons. The relative stereochemistry of C-6 and C-7 stereocenters was established based on nuclear Overhauser spectrum (NOESY), that also allowed to differ between the diastereotopic protons H-4β (δ_H 3.60) and H-4α (δ_H 2.56). The spatial interaction of the signals at δ_H 1.69 (Me-19) / 3.60 (H-4β), and δ_H 2.56 (H-4α) / 4.39 (H-6) allowed to assign a β-position for HO-6, while, and consequently, the HO-7 was α-positioned. Thus, compound **2** was characterized as 6β,7α-dihydroxynicandrenone 10.

Compound **3** was isolated as colorless crystals. Its molecular formula $C_{28}H_{42}O_8$, with 8 degrees of unsaturation, was determined through the protonated ion peak at *m/z* 487.2695 (calcd. *m/z* 487.2696) in the HRESIMS. Likewise, as seen for **1** and **2**, the ^{1}H and ^{13}C NMR data of **3** was compatible with a withanolide nucleus similar to those of **1** and **8** (nic-2) (Table S1, Supplementary Information), another withanolide previously isolated from *N. physalodes*.[7] However, its tertiary oxygenated carbons C-24 and C-25 (δ_C 74.5 and 74.4) were more deshielded than the correspondent carbons of compound **1** (δ_C 63.8 and 62.6) and compound **8** (δ_C 63.5 and 63.0) (Table S1, Supplementary Information), indicating the opening epoxide of the lactol moiety. The relative stereochemistry of **3** was assigned based on ^{1}H and ^{13}C chemical shifts reported for nicandrenone-2 (**8**),[7] and correlations displayed in the NOESY spectrum, which exhibited spatial interactions for the well-established α-positioned H-22 and Me-21, as well as for H-22 and Me-27. From these data, the Me-28 was β-positioned. Thus, the final structure of **3** was defined as the 24α,25β-dihydroxy-nicandrenone-2.

Compound **4** was obtained as a white amorphous solid. Its molecular formula was assigned as $C_{23}H_{26}O_4$, with 11 degrees of unsaturation, based on the protonated ion peak $[M + H]^+$ at *m/z* 367.1916 (calcd. *m/z* 367.1909) in the HRESIMS. The ^{1}H and ^{13}C NMR data of **4** were also similar to those reported for nicandrenone-10 (nic-10, **5**) (Table S1, Supplementary Information), but its heteronuclear single quantum correlation (HSQC) spectrum presented signals at δ_C/δ_H 53.7/3.80 (q, *J* 6.9 Hz, 1H), and 18.1/1.46 and 1.45 (d, *J* 6.9 Hz, 2 × 3H), that were associated to an additional methine (CH-20) and two methyl groups (2 × Me-21), respectively. In the HMBC spectrum, the correlation of the

Table 1. ^{1}H (500 MHz) and ^{13}C (75 MHz) NMR data for compounds **1**-**4**, in pyridine-d_5 (δ in ppm, J in Hz)

Position	**1**		**2**		**3**[a]		**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	203.7	–	204.9	–	204.4	–	204.0	–
2	129.1	6.00, dd (12.4, 2.6)	129.0	6.16, dd (10.0, 2.5)	129.5	6.03, dd (10.0, 1.6)	129.4	6.05, dd (10.0, 2.4)
3	141.5	6.55, ddd (12.4, 4.8, 2.6)	142.4	6.70, ddd (10.0, 5.1, 3.0)	141.0	6.58, ddd (10.0, 4.9, 1.9)	141.5	6.61, ddd (10.0, 4.9, 2.0)
4	38.1	2.65, d (18.1) 2.53, dd (18.1, 4.8)	36.5	3.60, d (19.0) 2.56, dd (19.0, 5.1)	38.2	2.64, d (16.5) 2.54, dd (16.5, 4.8)	38.4	2.74, d (18.8) 2.64, dd (18.8, 5.0)
5	74.4	–	79.5	–	74.0	–	74.0	–
6	55.3	3.04, d (4.2)	75.9	4.39, t (4.1)	55.9	3.27, t (2.3)	57.6	3.30, d (3.7)
7	57.5	3.61, d (3.5)	73.8	5.21, br s	56.8	3.12, d (3.7)	55.0	4.06, t (3.2)
8	27.3	2.33, br s	40.4	3.79, d (12.2)	36.0	2.03, m	39.9	3.06, d (11.4)
9	35.1	2.33, br s	33.5	3.25, td (12.2, 1.5)	35.8	1.69, m	32.7	2.45, td (15.2, 3.2)
10	54.5	–	54.1	–	52.1	–	52.7	–
11	22.8	2.25, m 1.62, m	26.3	3.07, dt (12.0, 2.2) 1.56, qd (12.0, 4.0)	31.3	2.74, dd (13.2, 5.1) 1.23 dd (13.2, 6.6)	25.0	3.12, m 1.55, m
12	33.3	1.50, m	31.7	3.18, td (16.0, 5.1) 2.90, dt (16.0, 2.8)	33.1	1.32, m	30.2	2.77, m
13	48.1	–	145.2	–	49.6	–	138.3	–
14	56.2	2.33, br s	140.2	–	45.5	1.36, m	139.6	–
15	207.1	–	127.5	7.68, d (8.1)	22.6	1.46, m 1.34, m	126.4	7.65, d (7.9)
16	130.2	6.02, d (1.9)	126.0	7.83, dd (8.1, 1.6)	22.8	3.13, m 1.55, m	125.84 (**4a**) 125.78 (**4b**)	7.16, d (7.9)
17	188.8	–	135.9[b]	–	86.8	–	138.7	–
18	14.5	1.19, s	130.1	7.79, d (1.6)	15.2	1.23, s	128.97 (**4a**) 128.91 (**4b**)	7.02, s
19	25.9	1.20, s	16.1	1.69, s	18.5	1.03, s	14.6	1.22, s
20	37.4	2.53, dd (18.1, 7.2)	197.9	–	37.7	2.06, m	53.7	3.80, q (6.9)
21	17.7	0.99, d (6.3)	26.9	2.52, s	16.6	1.05, d (6.9)	18.1	1.46, d (6.9) (**4a**) 1.45, d (6.9) (**4b**)
22	69.2	4.20, t (9.2)			70.9	4.06, t (4.6)	208.7	–
23	35.4	1.97, d (12.0) 1.64, d (12.0)			33.5	2.03, m 1.77, m	28.8	2.09, s
24	63.8	–			74.5	–		
25	62.6	–			74.4	–		
26	92.8	5.40, d (6.1)			93.7	5.19, s		
27	17.6	1.49, s			18.7	1.62, s		
28	18.9	1.36, s			24.0	1.40, s		
OH-5	–	5.73, s	–	6.65, s	–	6.35, s	–	6.22, s
OH-6			–	7.52, d (5.0)				
OH-7			–	7.70, s				
OH-26	–	7.93, d (8.8)						

[a]Spectra taken at 300 MHz; [b]overlapped.

methine proton at δ_H 3.80 (H-20) with the carbons at δ_C 125.84a/125.78b (C-16) and 208.7 (C-22), and both 1.46 and 1.45 (2 × Me-21) also with C-22 supported the butanone moiety directly connected at the benzene ring through the α-carbon. These data are also in agreement with an epimeric mixture at C-20. Thus, the complete structure of **4** was assigned as a mixture of 17-(1α-methylpropanone)-nicandrenone-10 (**4a**) and 17-(1β-methylpropanone)-nicandrenone (**4b**).

The isolated compounds **1**-**10** were evaluated for their antibacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermis*) and antifungal (*Candida albicans* and *Candida tropicalis*) activities. Unfortunately, all tested compounds were inactive against both bacterial and fungal strains. In addition, **2** and **6**, the main isolated compounds, were assayed against the third stage larvae of *Aedes aegypti*, but only **6** was active, showing a marginal activity with an IC_{50} 256.2 ppm.

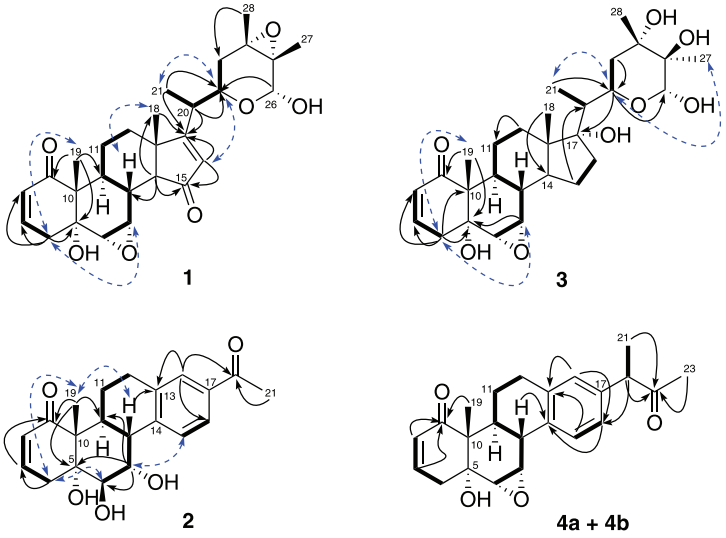

Figure 2. Key COSY (H—H) and HMBC (H ⟶C) correlations of **1**-**4a/4b**, and NOESY (H ⇠⇢H) correlations of **1**-**3**.

Conclusions

The chemical investigation of *N. physalodes* afforded five new withanolide derivatives (**1**-**3** and **4a/4b**) and six known ones (**5**-**10**). Since previous reports described the repellant properties for *N. physalodes*, our expectative was that some of the isolated compounds could display some antibacterial, antifungal or larvicidal effects, but, unfortunately, except for nicandrenone (**6**) which showed weakly larvicidal activity, all were inactive.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.org.br as a PDF file.

Acknowledgments

The authors are grateful to CNPq, CAPES, PRONEX, FUNCAP for the fellowships and financial support.

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Submitted: November 28, 2016
Published online: June 8, 2017