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Artigo

Brazoxide A, a New Tirucallane Triterpene from *Pilocarpus spicatus* subsp. *Aracatensis* Kaastra

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Brazoxido A, um Novo Triterpeno Tirucalano de *Pilocarpus spicatus* subsp. *Aracatensis* Kaastra

Resumo: Um novo triterpeno tirucalano, 3α,21α,21,23-diepoxi-tirucala-7,24-dieno, denominado brazoxido A, foi isolado das partes aéreas de *Pilocarpus spicatus* subsp. *Aracatensis* Kaastra. Sua estrutura e esteroquímica foi elucidada por métodos espectroscópicos, incluindo as técnicas de ressonância magnética nuclear (RMN) de 1D e 2D, espectrometria de massas de alta resolução com ionização por electrospray (AR-IES-EM), modelagem molecular e comparação com dados da literatura.

Palavras-chave: *Pilocarpus spicatus*; triterpeno tirucalano; brazoxido A.

Abstract

A new tirucallane triterpene, 3α,21α,21,23-diepoxy-tirucalla-7,24-diene, named brazoxide A, was isolated from aerial parts of *Pilocarpus spicatus* subsp. *Aracatensis* Kaastra. Its structure and stereochemistry was elucidated by spectroscopic methods, including 1D-, 2D-nuclear magnetic resonance (NMR), high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), molecular modeling techniques and comparison with literature data.

Keywords: *Pilocarpus spicatu*; tirucallane triterpene; brazoxide A.

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Brazoxide A, a New Tirucallane Triterpene from *Pilocarpus spicatus* subsp. *Aracatensis* Kaastra

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1. Introduction

Pilocarpus is one of the 150 genus that compose the Rutaceae family.[1] The species of this genus are distributed across much of the American continent.[2] There are seventeen species described to *Pilocarpus*, these fourteen can be found in Brazil where they are used in folk medicine for the treatment of several diseases.[3,4]

Different types of secondary metabolites have been reported to *Pilocarpus* species, such as alkaloids,[5,6] terpenes,[3,7,8] flavonoids,[7] coumarins,[9,10] hydrocarbons[11,12] and essential oil.[13-16]

In previous studies with *Pilocarpus spicatus*, was observed the presence of terpenes,[3] as well as the effect of the essential oil in developing nymphs of *Rhodnius prolixus*.[16] In this paper we describe the isolation and structural elucidation of a new triterpene tirucallano 3α,21α,21,23-diepoxy-tirucalla-7,24-diene, Brazoxide A (**1**), isolated from the aerial parts *Pilicarpus spicatus* subsp. *Aracatensis* Kaastra, species

known popularly as "Jaborandi da restinga".[16] We decided to study *P. spicatus* because it belongs to a genus rich in compounds with biological activity and also because there are few studies on this species.

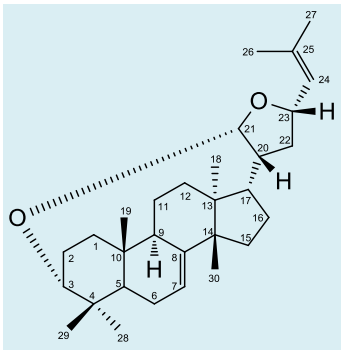

Figure 1. Tirucallane triterpene, brazoxide A, from aerial parts of *Pilocarpus spicatus*

2. Results and Discussion

The compound **1** was isolated in the form of white crystals. The high resolution mass spectrum (HS-ESI-MS) utilizing the ESI+ ionization mode showed the peak of the cationized molecule at *m/z* 439.3561 $[M + H]^+$, compatible with the molecular formula $C_{30}H_{46}O_2$ (calc. 438.3498). The IR spectrum showed absorptions in the region of 2960-2880 cm^{-1}, characteristic of C-H stretching of alkanes, absorptions between 1000-960 cm^{-1} of C-O stretching and absorption in 760 cm^{-1} characteristic of C-H sp^2 deformation of trisubstituted double bond.

In the ^1H NMR spectrum (Table 1) the presence of seven singlet of methyl protons was observed at δ_H 1.71, 1.69, 0.95 (signal for two methyl groups), 0.91, 0.89 e 0.76, these signals are characteristic of triterpene tirucallane skeleton. Also two olefinic protons were observed at δ_H 5.24 (sl, H-7) e 5.14 (d, J = 8.8 Hz, H-24), as well three oxymethines protons at in δ_H 5.10 (d, J = 2.4 Hz, H-21), 4.52 (m, H-23) e 3.52 (sl, H-3).

The DEPTQ NMR showed others chemical shifts for tirucallan triterpene in δ_C 118.41 (C-7) and 146.15 (C-8), which together with the signals at δ_C 124.43 (C-24), 99.66 (C-21), 75.62 (C-3), 72.94 (C-23), 51.89 (C-17), 50.87 (C-20), 49.94 (C-9) and 45.08 (C-5) suggesting a tetracyclic triterpene similar to 3-*epi*-flindissol.[17] The exceptions were observed in the signals δ_C 18.34, 75.62 and 99.66, attributed to the carbons C-2, C-3 and C-21, respectivelly.

The cyclization was confirmated by IR and ^1H NMR experiments of the acetilated derivative, where no alteration was observed in the spectra. The cross peaks in the HMBC spectrum showed the correlation between the protons H-3 (δ_H 3.52) and C-21 (δ_C 99.66), corroborating with this information. Also the correlation observed in the NOESY between H-21 and H-3, showed the proximity of these nucleus, confirming the epoxidation in the compound **1**.

The conformational search generated only 2 conformers that after geometry optimization and vibrational frequencies analysis using DFT--B3LYP/6.311+G** have internal energies lower than - 3.4 10^{-6} kJ/mol (Figure 2). The mainly difference between the conformers is the distance between hydrogens at position C-3 and C21 around 3.03 Å.

Table 1. ^{1}H- and ^{13}C-NMR (400 and 100 MHz, resp.; $CDCl_3$), HMBC, COSY, and NOESY data for compound **1**, and ^{1}H- and ^{13}C-NMR (300 and 75 MHz, resp.; $CDCl_3$) for 3-*epi*-flindissol[17] (δ in ppm, *J* in Hz)

	^{1}H x ^{13}C HMQC		**^{1}H x ^{13}C HMBC**		**^{1}H x ^{1}H COSY**	**^{1}H x ^{1}H NOESY**	3-*epi*-flindissol	
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$			δ_C	δ_H
4	36.85	-	H-3; H-28; H-29				38.92	
8	146.15	-		H-30			145.45	
10	34.33	-	H-19				34.97	
13	44.15	-	H-18	H-30			43.70	
14	51.23	-	H-30	H-18			50.91	
25	137.54	-	H-26; H-27				136.84	
CH								
3	75.62	3.52 (sl)		H-1	H-2	H-21; H-23	79.16	3.24 (dd, 11.2, 3.9)
5	45.08	1.86		H-3; H-7; H-19; H-28; H-29			50.64	1.31
7	118.41	5.24 (sl)			H-6		118.13	5.27
9	49.94	2.49		H-7; H-19	H-11	H-5; H-18	48.75	2.23
17	51.89	2.30		H-21			50.54	1.79
20	50.87	1.77	H-17			H-23	50.13	2.20
21	99.66	5.10 (d, 2.4)		H-3; H-17	H-17		101.38	5.30 (d, 3.3)
23	72.94	4.52 (m)			H-22		73.93	4.81 (ddd, 10.4, 8.6, 4.6)
24	124.43	5.14 (d, 8.8)		H-26; H-27	H-23		124.80	5.14 (dm, 7.3, 1.2)
CH_2								
1	32.62	1.36, 1.65		H-3; H-19			37.12	1.13, 1.66
2	18.34	1.84, 1.66					27.60	1.64, 1.60
6	23.78	2.03, 1.91					23.92	2.14, 1.97
11	17.37	1.59, 1.50					17.51	1.54, 1.50
12	31.82	1.96, 1.76		H-18			31.65	1.74, 1.72
15	34.04	1.55, 1.47		H-30			33.78	1.54, 1.48
16	28.54	1.91, 1.25					27.35	1.88, 1.30
22	40.44	2.08, 1.19		H-24			39.61	2.12, 1.26
CH_3								

18	22.46	0.95 (s)		22.50	0.90 (s)
19	13.26	0.76 (s)	H-1	13.01	0.75 (s)
26	18.34	1.69 (s)		18.27	1.73 (s)
27	25.89	1.71 (s)		25.78	1,70 (s)
28	28.34	0.91 (s)	H-3; H-29	27.56	0.97 (s)
29	21.50	0.89 (s)	H-3	14.67	0.86 (s)
30	26.73	0.95 (s)		27.07	0.98 (s)

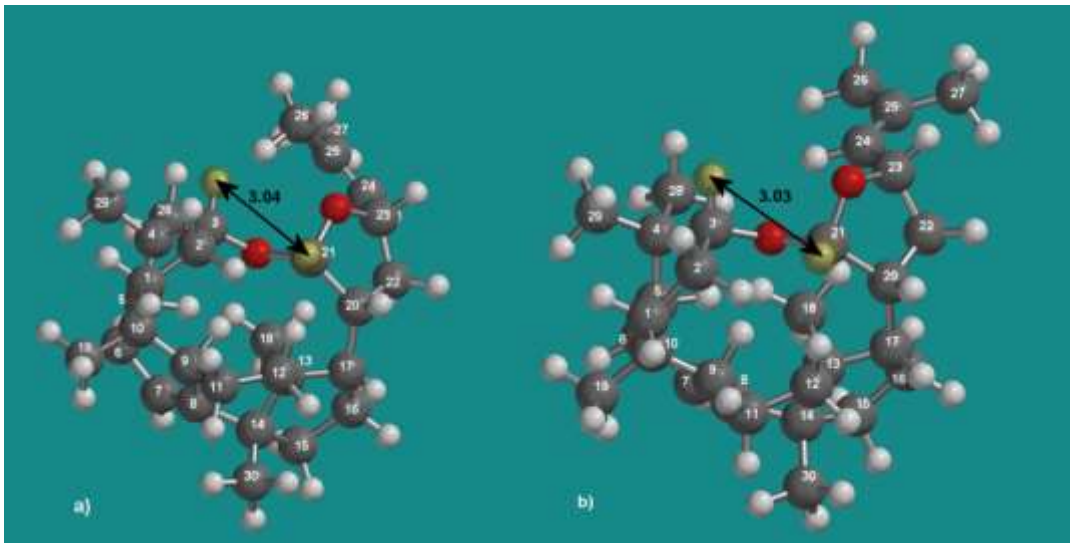

Figure 2. The black arrows show the distance in angstroms between the hydrogens H-3 and H-21: a) 3.04 Å and b) 3.03 Å. Lowest energy geometry of conformers: a) -3,468,111.12 kJ/mol and b) -3,468,119.71 calculated by DFT-B3LYP/6.311+G**

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on Shimadzu IRPrestige-21 spectrophotometer. One-dimensional (^{1}H and ^{13}C) and two-dimensional (gHMQC, gHMBC, gCOSY and gNOESY) NMR experiments were performed on a VARIAN spectrometer (MR-400) operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). $CDCl_3$ was used as the solvent with TMS as an internal standard. HR-ESI-MS was obtained using a MicroTOF-Q system from Brüker. Conventional chromatographic methods were used for column chromatography (CC) (silica gel 60, Merck, 0.04 – 0.063 mm). Medium pressure liquid chromatography (MPLC) was performed using the Buchi system of binary gradient flash separation, in which the chromatograph was equipped with two pump modules (C-601 and C-605), controller module (C-615), Knauer UV detector and columns packed with silica gel (Merck, 0.063-0.20 mm). Silica gel TLC plates PF_{254} 7749 (Merck) revealed with iodine or under UV light (254/366 nm) were used to monitor chromatographic purification procedures.

3.2. Extraction and isolation

The aerial parts of *Pilocarpus spicatus* were collected in the municipality of Matureia, Paraíba State, Brazil, in June 2011 and identified by Prof. Dr. Maria de Fátima Agra, Universidade Federal da Paraíba. A dried specimen is deposited in the Herbário Professor Lauro Pires Xavier of UFPB under N°. 7428.

The plant material (4.5 Kg), dried and pulverized, was extracted with 95% EtOH at room temperature. The extract obtained was concentrated in a rotatory evaporator under reduced pressure at 40 °C, yielding 474.5 g ethanolic extract. A portion (100.0 g) was suspended in MeOH:H_2O (7:3) and partitioned with hexane, CH_2Cl_2 and EtOAc to obtain the hexane (4.7 g), dichloromethane (6.2 g) and ethyl acetate (4.6 g) extracts. The hexane extract (4.0 g) was submitted to MPLC, with the column packed with silica gel 60 (0.063-0.200 mm), utilizing a flow rate of 35 mL min^{-1} and mobile phase of the solvents hexane, EtOAc and MeOH, pure or in binary mixtures, in increasing order of polarity, yielding 48 fractions. The fractions were analyzed by analytical TLC and combined according to their chromatographic profiles. Fractions 34-36 (221.5 mg) were submitted to CC utilizing silica gel 60 (0.04-0.063 mm) and the eluents hexane, EtOAc and MeOH, resulting in 18 fractions of 15 mL each, which were analyzed by analytical TLC, and the subfraction 3 yielded compound **1** (15.3 mg).

3.3. Molecular modeling

We draw the structure of the compound **1** using Marvin 6.0.1, 2012, ChemAxon (http://www.chemaxon.com). The software Standardizer, JChem 6.0.0, 2013, ChemAxon (http://www.chemaxon.com) was used to canonize structure, add hydrogens, and clean the molecular graph in three dimensions and finally saved in .sdf format. The process uses a divide-and-conquer approach. The structure split into small fragments is organized into a build tree using their original connectivity information. Conformers generated for the initial structure (represented by the root node in the build tree) are also optimized. The building process uses a proprietary extended version of the Dreiding force field.[18]

All geometry optimization and conformational search were performed using Spartan for Windows 10.0 software.[19] The geometry of the chemical structure of the compound was initially optimized using MMFF force field and,[20] afterwards, we performed a new geometry optimisation based on the semi-empirical method AM1 (Austin Model 1).[21] We selected the systematic search method, analyzing until 10000 conformers and selecting until 10 conformers of lowest minimum energy using AM1. The dihedrals were evaluated by rotation in accordance with the standard (default) conditions of the program. The conformers of lowest minimum energy were selected and performed geometry optimization and vibrational mode calculation using DFT (density functional theory) at B3LYP/6-31G* level.[22,23]

4. Conclusions

The analysis of spectral data and comparison with literature data allowed to elucidate the compound **1** as the tirucallane triterpene, 3α,21α,21,23-diepoxy-tirucalla-7,24-diene, named trivially as brazoxide A, a new, unusual natural product, since this is the first report of cyclization of this triterpenes via connections at C-3-O-C-21.

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