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Two New Macrocyclic Alkaloids from *Albizia inopinata* †

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SUMMARY. Two new macrocyclic spermine alkaloids were isolated as a mixture from the leaves of *Albizia inopinata*. These alkaloids are being reported here for the first time and were given the trivial names felipealbizine A and felipealbizine B. They were identified on the basis of spectroscopic techniques, including 2D NMR experiments, as well as by comparison with related compounds from previous reports. Preliminary studies indicated that the compounds shown a possible pharmacological depressor activity on the central nervous system.

RESUMEN. "Dos nuevos alcaloides macrocíclicos de *Albizia inopinata*". De las hojas de *Albizia inopinata* fue aislada una mezcla de dos nuevos alcaloides macrocíclicos del tipo espermina, denominados felipealbizina A y felipealbizina B. Los nuevos alcaloides fueron identificados en base a técnicas espectroscópicas, incluyendo RMN 2D y comparación con compuestos anteriormente publicados. Estudios farmacológicos preliminares indicaron que estos compuestos muestran una posible actividad depresora en el sistema nervioso central.

INTRODUCTION

The family Leguminosae is widely distributed around the world, mainly in tropical, subtropical and temperate zones, where it is represented by trees of small to medium size. The family is formed by several genera, including *Albizia*. There are aproximately 150 species of this genus, yet to be chemically studied. In Brazil, the species *A. inopinata* is found sparsely distributed around the country, and is especially abundant in the Northeast. Its use in folk medicine is not known, but it is very used as ornamental tree in streets and squares [1]. There are references to the use of the bark of *Albizia julibrissin* for the treatment of insomnia [2] and the bark of *Albizia lebbek* for the treatment of asthma in India [3]. There is also a report of the use of *Albizia zygia* on psychiatric disorders in Nigeria [4]. Studies with seeds of *Albizia amara* [5] suggested that this genus is rich in several classes of natural products such as saponins, lignans, steroids, triterpenes, and in some species, macrocyclic-type alkaloids. The absence of any reference to *Albizia inopinata* on Chemical Abstracts and on the NAPRALERT database led us to investigate the occurrence of similar alkaloids in this species, as well as the biological activity of these constituents.

MATERIAL AND METHODS

Plant Material

The leaves of *Albizia inopinata* (Harms) G.P. Lewis were collected near the city of Santa Rita, Paraíba, Brazil, in April 1997 and were identified by the botanist Maria de Fátima Agra. A voucher specimen (Agra e Gois 3599) is kept at the herbarium Prof. Lauro Pires Xavier, João Pessoa, Paraíba, Brazil.

KEY WORDS: *Albizia inopinata*, Leguminosae, Macrocyclic alkaloid, Felipealbizine A, Felipealbizine B.
PALABRAS CLAVE: Albizia inopinata, Leguminosae, Alcaloide macrocíclico, Felipealbizina A, Felipealbizina B

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Extraction and Purification

The leaves were dried at 40 °C and grounded in a Harley-type mill. Approximately 1 kg of the dried material was exhaustively percolated with 70% aqueous ethanol and the crude extract was concentrated under reduced pressure to afford 205 g of residue. This material was dissolved in 1000 ml of aqueous acetic acid (4%), stirred and the residue filtered through Celite. The filtrate was extracted with chloroform (3 x 400 ml) and the aqueous phase was alkalinised with NH_4OH. This alkaline phase was submitted to a new extraction with chloroform (5 x 400 ml) and the resultant chloroform extract dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude total alkaloidal fraction (9.8 g). This fraction was subjected to preparative thin layer chromatography over silica gel eluting with chloroform-methanol-cyclohexan-diethylamine (5:5:8:2). The material was developed three times in this solvent system, yielding a mixture of alkaloids (0.104 g) .The above solvent system was adapted from published data [6] on analytical TLC. A sample of the material was submitted to spectroscopic (UV, IR, ^1H and ^{13}C NMR, EIMS and FABMS) analysis.

Animals

The experiments were conducted on male Swiss Albino mice (25-30 g) and on male Wistar rats (200-300 g). The animals were kept at constant room temperature (25 ± 2 °C) and submitted to 12 h light/12 h (6:00 - 18:00 h) dark cycle with free access to food and water.

Spontaneous mobility test

Locomotor activity was measured in a plastic activity cage (size 40 x 40 cm). Mice were divided into 4 groups of 10 each. Saline 0.9%, amphetamine (3 mg/kg), haloperidol (5 mg/kg) or FLA (10 mg/kg) were i.p. injected. The readings were taken immediately after the drug administration (basal) and at 60, 120, 180 and 240 min later [7,8].

Effect of FLA on ptosis

Several neuroleptics administration in rats causes symptoms such as ptosis and catalepsy [9]. For this experiment three groups of ten rats were used. While one group received saline (0.9%), the others were given haloperidol (HAL) 5 mg/kg, i.p. or FLA at 10 mg/kg, i.p. The degree of ptosis was noted on a scale ranging from 0 to 3, where 0 = no effect, 1 = mild effect, 2 = moderate effect and 3 = intense effect. The degree of ptosis was measured at 15, 30, 60 and 90 min after the injection. The degree of ptosis was compared between control and treated groups of rats.

Active Avoidance Test

The animals were trained in an active-avoidance paradigm in a 25 x 25 x 40 cm shuttle-box with a buzzer located at the midline on the lid of the box and a floor consisting of stainless steel grid bars. The box was divided into two equal compartments by a wood panel with an 8x7 cm hole in the middle. A sound (conditioned stimulus) was presented during 20 s. The unconditioned stimulus was a 0.4 mA scrambled shock for a maximum of 40 s or until the rat escaped to the other compartment of the cage. FLA was administered at doses of 5, 10 and 20 mg/kg and only those animals which in earlier assays had shown avoidance responses in at least 80% of trials were used [10].

Statistical analysis

The data obtained were evaluated by one-way analysis of variance (ANOVA). Further pos-hoc comparisons were made by Student's t-test, except in the evaluations of ptosis for which the Kruskal-Wallis test was employed. The results were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Recently, several macrocyclic alkaloids from *Albizia* species were isolated using HPLC and DNA-affinity chromatography [5,6,11], as well as conventional chromatographic techniques [12-14]. However, in most circunstances this type of compounds is identified as a mixture of 2 or 3 substances, depending on the particular species studied [6,13].

The material isolated showed a strong positive reaction to Dragengroff reagent. The IR spectra showed absorption at 3300 and 1647 cm^{-1} (amide). The absence of resonance in the region of δ_C 40-45 in ^{13}C NMR spectra as well as the absence of resonance between δ_H 2.30-2.10 in the ^1H NMR spectra suggest that the isolate is devoid of *N*-methyl groups in the macrocyclic ring of budmunchiamine-type alkaloids. A peak at *m/z* 255 observed in the EIMS confirmed the presence of this characteristic fragment [9]. The ^1H NMR spectra showed a broad singlet at δ_H 1.19 (CH_2) and a triplet at δ_H 0.90 (CH_3) suggestive of an aliphatic side chain. Two superimposed multiplets at δH 3.37-3.20 and a broad singlet at

δ_H 8.34 are indicative of a $CONHCH_2$ moiety and a signal at δ_H 2.32 - 2.90 can be assigned to the hydrogens H-3 and H-4 respectively (Table 1). The signal at δ_H 1.70 is assigned to H-7 and the one at δ_H 1.64 (H-11, H-12, and H-16) are in agreement with the published values for budmunchiamine alkaloids [13,14] (Table 1). The ^{13}C NMR spectra revealed some signals occurring in duplicate, for example the ones atributed to the carboxylic function (δ_C 172.70 and 172.78) and those of the methyl groups of the aliphatic side chain (δ_C 14.09 and 14.30).

C	^1H x ^{13}C-HMQC-$^1J_{CH}$ Felipealbizine A and B		^1H x ^{13}C-HMBC-$^nJ_{CH}$ Felipealbizine A and B	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
2	172.78/172.70	-	H-3	
		2.32 (d, J=14.1)		
3	40.65	2.14 (dd, J=14.1, 8.2)		
4	55.61/55.27	2.90	H-3	H-6
6	46.80/46.87	2.65	H-7	
7	28.55	1.70		
8	47.71	2.70		H-6
10	46.87/46.80	2.60	H-11	H-12
11	26.65 - 26.52	1.64	H-10, H-12	
12	26.65 - 26.52	1.64		
13	45.76/45.62	2.65		
15	48.23/48.17	2.65 (m)		
16	26.65/26.52	1.64 (m)		
17	37.48/37.36	3.37 (m) /3.20 (m)	H-16	H-15
HN-1	-	8.34 (sl)		

Table 1. NMR data of the macrocyclic ring of feli-pealbizine A (**1**) and B (**2**) from *Albizia inopinata*.

C	^1H x ^{13}C-HMQC-$^1J_{CH}$		^1H x ^{13}C-HMBC-$^nJ_{CH}$		Methyl linoleate	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	C	δ_C
1'	34.08	1.60 -1.30		H-3a	5	29.60
2'	28.95	1.40 - 1.00	H-3'	H-4'	6	29.20
3'	29.35	1.40 - 1.00			7	29.20
4'	27.23	1.90	H-5'	H-6'	8	27.20
5'	130.75	5.40 - 5.10			9	130.20
6'	127.32	5.40 - 5.10			10	127.80
7'	25.56	2.70	H-6', 8'	H-5', H-9'	11	25.60
8'	128.72	5.40 - 5.10			12	128.30
9'	128.33	5.40 - 5.10			13	128.30
10'	25.56	2.70	H-9', 11'	H-8', H-12'	14	25.60
11'	127.10	5.40 - 5.10			15	127.20
12'	132.06	5.40 - 5.10	H-13'	H-10', H-14'	16	131.90
13'	20.59	2.05	H-14'		17	20.60
14'	14.30	0.90 (t, J=7.5)			18	14.30

Table 2. NMR data of the side chain of felipealbizine A (**1**) and methyl linoleate.

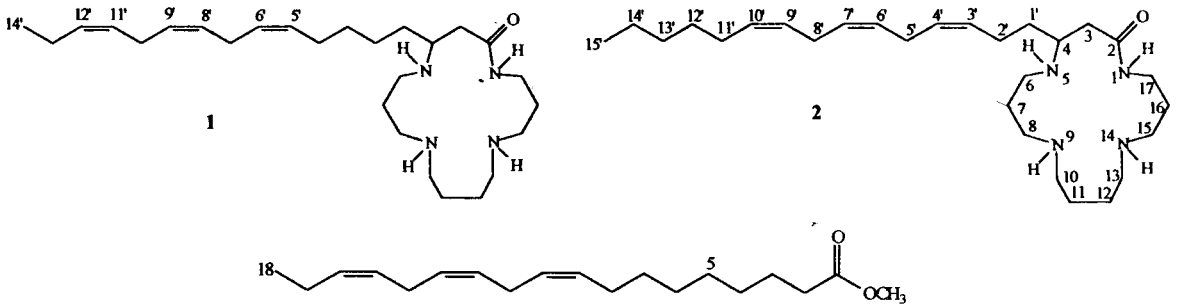
C	^{1}H x ^{13}C-HMQC-$^1J_{CH}$		^{1}H x ^{13}C-HMBC-$^nJ_{CH}$	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
1'	34.18	1.60 - 1.30		
2'	23.75	2.10	H-3'	H-4'
3'	131.86	5.40 - 5.10		
4'	129.75	5.40 - 5.10		
5'	25.62	2.70		
6'	129.45	5.40 - 5.10		
7'	128.82	5.40 - 5.10		
8'	25.62	2.70		
9'	129.08	5.40 - 5.10		
10'	130.26	5.40 - 5.10		
11'	27.26	1.90		
12'	29.84	1.40 - 1.00		
13'	31.53	1.40 - 1.00	H-12', H-14'	H-11', H-15'
14'	22.59	1.40 - 1.00		
15'	14.09	0.83 (t)		

Table 3. NMR data of the side chain of felipealbizine B (**2**).

The ^{13}C NMR spectra showed 12 olefinic signals (δ 127.10 to 132.06), which were assigned to the sp2 carbons of the side chain. These data together with the peaks at *m/z* 446 and 460 in the FABMS referent to the side chain fragments led us to identify both alkaloids.

These compounds have unsaturated side chains, what makes them unique. They were given the trivial names of Felipealbizine A (**1**) and Felipealbizine B (**2**) as a homage to Felipe de Albizzi, a Florentian noble of the XVIII century from whose gardens the first *Albizia* species was described [15].

The structure of the fragment relative to **1**'s side chain (low abundance) was confirmed by comparison with data published for the linolenic acid methyl ester. **1** and **2** differ from each other by the position of the double bonds occurring in the side chain. **2** also has one additional methylene carbon compared to **1**.



Methyl linoleate

In preliminary studies using rats or mice, it was observed that the mixture containing the alkaloids (FLA) induced a marked ptosis and reduction of spontaneous movement. The alkaloids (FLA, 10 mg/kg, ip) were able to decrease the conditioned avoidance response in as much as 44% in rats compared with the control group ($p < 0.05$). These results taken together indicate a possible CNS depressing activity. Further investigation is under way to delineate the pharmacological profile of these compounds.

The mixture of **1** and **2** is a viscous oil with the following spectroscopic features: IR: cm^{-1}: 3420, 3300 (NH), 2928, 1647, (CO), 1454, 1375, 1062, 750; MS: M+ 460 (6%), 446 (2%), and *m/z* 255 (2%); ^{1}H NMR and 13C NMR: see Tables 1-3.

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