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Short Report

Complete ^{1}H and ^{13}C NMR Assignments of Isojuripidine from *Solanum asterophorum* Mart.

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Isojuripidina, um alcalóide 3-aminoespirosolano foi isolado das partes aéreas de *Solanum asterophorum* Mart. A estrutura foi determinada usando uma combinação de técnicas de RMN homo (1D: RMN ^{1}H, RMN $^{13}C\{1H\}$, RMN^{13}C-DEPT135; 2D: COSY, ^{1}H-^{1}H-NOESY) e heteronuclear 2D (HSQC e HMBC) e espectro de massas de alta resolução. Foi feita também a atribuição inequívoca dos deslocamentos químicos dos átomos de carbono e hidrogênio dos derivados 3-*N*,6-*O*-diacetil-isojuripidina e 3-*N*-cinamoil-isojuripidina.

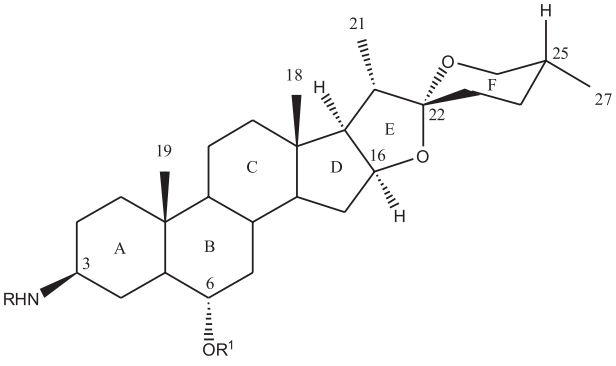
Isojuripidine was isolated from the aerial parts of *Solanum astherophorum* Mart. Its structure was determined using a combination of homo- (1D ^{1}H NMR, ^{13}C NMR-HBBD and ^{13}C NMR-DEPT) and heteronuclear 2D NMR techniques (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HSQC, HMBC), and HREIMS. The unambiguous assignments of ^{1}H and ^{13}C NMR data of derivatives 3-*N*,6-*O*-diacetyl-isojuripidine and 3-*N*-cinnamoyl-isojuripidine are described.

Keywords: Solanaceae, *Solanum asterophorum*, isojuripidine

Introduction

The genus *Solanum* is considered to be one of the largest and most complex among the Angiosperms. It is comprised of about 1000 species and approximately 3000 epithets are described.[1] The genus is well represented in Brazil and is widely distributed from north to south in diverse phytogeographic regions. Many of the species are endemic to the country. In the Northeast of Brazil, many *Solanum* species are widely used in popular medicine and are commonly known as *jurubeba,* the word originating from the Tupi-guarani, *yu'beba,* which refers to the presence of prickles on some of them.[2] *Solanum asterophorum* is known as *jurubeba-de-fogo* and its roots are popularly used in the treatment of liver diseases.[2]

As part of our chemical and pharmacological studies of Brazilian *Solanum*,[4-10] we report the first chemical investigation on *S. asterophorum*. From the methanol extract of *S. asterophorum* the steroidal alkaloid isojuripidine **1** was isolated. The spectroscopic data of **1** and of its derivative 3-*N*,6-*O*-diacetyl-isojuripidine **1a** and 3-*N*-cinnamoyl-isojuripidine **1b** (Figure 1) are described. The compound **1b** is a new derivative. The unambiguous assignments of ^{1}H and ^{13}C NMR data of isojuripidine **1** and derivatives **1a** and **1b** are reported for the first time and involved a combination of homo- (1D ^{1}H NMR, ^{13}C NMR-HBBD and ^{13}C NMR-DEPT) and heteronuclear 2D NMR techniques (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HSQC, HMBC), and HREIMS. Isojuripidine was isolated previously only from *Solanum paniculatum*[11] and no NMR data was published.



1 R^1=R=H
1a R^1=H, R=COCH=CHPh
1b R^1=R=Ac

Figure 1. Structure of isojuripidine **1** and its derivatives.

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Results and Discussion

The steroidal alkaloid isojuripidine **1** was isolated from the methanol extract of the aerial parts of *Solanum asterophorum* (Solanaceae). Compound **1** was obtained as an amorphous powder and gave a positive Dragendorff reagent test. The spectral data were in agreement of a (25*R*)-spirostan steroidal type skeleton, with the IR spectrum (KBr) showing absorptions at 3409 cm^{-1} (ν_{max} N-H and O-H), with a 25R-spirostan structure (supported by peaks at 982, 956, 920 and 899 cm^{-1}, intensity 920<899,)[12] and confirmed by ^{1}H NMR[13,14] and ^{13}C NMR.[15,16] As expected the ^{1}H NMR spectrum (Table 1) showed the signals of two methyl singlets, two methyl doublets, and a multiplet due to an oxymethine hydrogen. The presence of a β-amino and a carbinolic hydrogen, indicated an amino and hydroxyl group, respectively. The amino group would most probably be at C-3.

The ^{13}C NMR spectrum of **1** showed 27 signals. From these, the signals of C-16, C-22 and C-26 and one additional signal for a carbon-bearing nitrogen additionally supports the proposed aminospirosolane structure for **1**. EIMS fragments at *m/z* 115 and 139 corroborated this structure, while the peaks at *m/z* 56 and *m/z* 98 provided confirmation of the amino group at C-3,[17] and hydroxyl at C-6, respectively. HREIMS (IE, 70 eV) gave $[M]^+$ at 431.33979 corresponding to the molecular formula $C_{27}H_{45}NO_3$ (Calc. 431.33994), expected for **1**. Full assignment of ^{1}H and ^{13}C chemical shifts of **1** (Table 1) was achieved with the aid of ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY experiments, discussed below.

Only a few characteristic assignments can be made immediately from ^{1}H NMR, e.g. H-3, H-16, H-26$_{eq}$ and 26$_{ax}$ at δ_H 2.82, 4.23, 3.33 and 3.16, respectively. Comparative analysis of the ^{13}C NMR-HBBD and ^{13}C NMR-DEPT spectra was used to identify the number of signals attributed to three quaternary [$(C)_3$: all sp^3, ten methine [$(CH)_{10}$: all sp^3, including two oxygenated and one with amino group = $(CH)_7(O\text{-}CH)_2(HN\text{-}CH)$], ten methylene [$(CH_2)_{10}$: all sp^3 including one oxygenated = $(CH_2)_9(CH_2\text{-}O)$], and four methyl [$(CH_3)_4$] carbon atoms. Consequently, the expanded formula $(C)_3(CH)_7(O\text{-}CH)_2(N\text{-}CH)(CH_2)_9(CH_2\text{-}O)(CH_3)_4$=$C_{27}H_{42}NO_3$ was deduced, which after considering the presence of one hydroxyl and one amino group was established as $C_{27}H_{45}NO_3$ in accordance with the HREIMS. However, the complete assignments, especially in the most congested regions (^{1}H from δ_H 0.6 to δ_H 2.0, ^{13}C from δ_C 25 to 35) had to be made from 2D data.

The hydrogen assignment was divided into two spin systems. The first system includes the steroidal nucleus and ring E. The second system is the six-membered heterocyclic moiety at position 22 (Figure 1). For spin system 1, two starting points C$\underline{H}_3$-18 and H-6 were used on the COSY. The hydrogens on methyl-18 were used because steroidal compounds show a characteristic coupling between C$\underline{H}_3$-18 (δ_H 0.65) and C$\underline{H}_2$-12 (δ_H 1.63). This helps to penetrate into the heart of the convoluted region of the spectrum and to verify assignments made by the use of H-6 as the starting point. The carbinolic hydrogen H-6 (δ_H 3.14) was correlated with H-5 (δ_H 0.89) and H-5 with H-4 (δ_H 1.18). The assignment on H-4 leads to the assignment of ring A. The connection of H-6 to H-7 (δ_H 0.75) leads to continuous correlations which result in the assignment of rings B, C, D and E. The second spin system was assigned by using the two geminal hydrogens

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) spectral data for isojuripidine **1** obtained by heteronuclear 2D shift-correlated HSQC and HMBC spectra, in DMSO-d_6. Chemical shifts (δ, ppm) and coupling constants (J in Hz, in parenthesis)[a]

	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
C				
10	35.7		3H-19	
13	40.1		H-12, 3H-18	
22	108.4		H-20	H-16, H-17, 3H-21
CH				
3	49.7	2.82	4$_{eq}$	
5	51.1	0.89		3H-19
6	67.0	3.14[b]	H-7$_{ax}$	
8	33.3	1.52[b]	H-7$_{ax}$	
9	53.0	0.60		H-1$_{ax}$, 3H-19, H-7$_{eq}$
14	55.4	1.09[b]	H-15$_{\beta}$	3H-18
16	80.1	4.23	H-15$_{\beta}$	H-14
17	61.8	1.61[b]		3H-21, 3H-18
20	41.1	1.75[b]	3H-21	
25	29.8	1.59[b]	3H-27	
CH_2				
1	36.4	1.61[b], H$_{eq}$; 0.92[b], H$_{ax}$		3H-19
2	25.7	1.69[b], H$_{eq}$; 1.46[b], H$_{ax}$		
4	27.0	2.12, H$_{ax}$; 1.18[b], H$_{eq}$		
7	41.7	1.85[b], H$_{eq}$; 0.75[b], H$_{ax}$		H-5
11	20.4	1.40[b], H$_{eq}$; 1.18[b], H$_{ax}$		
12	39.3	1.63[b], H$_{eq}$; 1.07[b], H$_{ax}$		
15	31.4	1.85[b], H$_{\alpha}$; 1.11[b], H$_{\beta}$		
23	30.9	1.57[b], H$_{eq}$; 1.47[b], H$_{ax}$		H-20
24	28.5	1.56[b], H$_{eq}$; 1.26[b], H$_{ax}$		3H-27
26	65.9	3.33, H$_{eq}$; 3.16[b], H$_{ax}$		3H-27
CH_3				
18	16.1	0.65 (s)		H-14, H-17$_{ax}$, H-12$_{ax}$
19	12.9	0.75 (s)		H-9
21	14.6	1.87 (d, 6.0 Hz)	H-20	H-17
27	17.1	0.70 (d, 6.4 Hz)		

[a] 2D homonuclear ^{1}H-^{1}H-COSY and heteronuclear HMBC spectra were also used for these assignments. Chemical shifts of hydrogen atoms obtained from 1D ^{1}H NMR spectra. Carbon signals corresponding to C, CH, CH_2 and CH_3 deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HSQC, HMBC and ^{1}H-^{1}H-COSY; [b] Superimposed by other signals.

H-26 as the starting point. The H-26 hydrogens absorb at δ_H 3.33 and 3.16 and were correlated to the other hydrogens on ring F and to methyl C$\underline{H}_3$-27 (δ_H 0.70).

The ^{1}H-^{13}C correlation was used interactively with COSY to differentiate the correlations between geminal and vicinal hydrogens. Subsequent to the completion of the hydrogen assignment, the assignments of the hydrogenated carbons were made from the one-bond ^{1}H-^{13}C correlations in a straightforward manner.

The quaternary carbons were assigned by using long-range ^{1}H-^{13}C correlations obtained from the HMBC experiment, which primarily reveals connectivity between hydrogens and carbons separated by two and three bonds. The angular methyl hydrogen correlations are particularly useful in rendering this information. The quaternary carbons C-10 (δ_C 35.7), C-13 (δ_C 40.1), and C-22 (δ_C 108.4) showed correlations to methyl hydrogen C$\underline{H}_3$-19 (δ_H 0.75), C$\underline{H}_3$-18 (δ_H 0.65), and C$\underline{H}_3$-21 (δ_H 1.87), respectively. In addition C-22 exhibited three bond correlations to H-16 (δ_H 4.23), H-17 (δ_H 1.61), and two bond correlation to H-20 (δ_H 1.75). This signal (H-20) also showed a three bond correlation to C-13.

The NOESY spectrum of **1** was used to confirm the *trans* junctions of the A/B and B/C rings and their corresponding conformations. The α-axial orientation of H-5 was confirmed by a strong NOE observed between H-5 (δ_H 0.89) and the hydrogens assigned to H-2_{eq} (δ_H 1.69), H-3_{ax} (δ_H 2.82), H-4_{ax} (δ_H 2.12), H-4_{eq} (δ_H 1.18), and H-9 (δ_H 0.60). The axial orientation of H-6 was confirmed by the strong correlation with H-7_{eq} (δ_H 1.85), H-8 (δ_H 1.52), H-11_{ax} (δ_H 1.18), and C$\underline{H}_3$-19 (δ_H 0.75). The dipolar interaction was observed between CH_3-27_{eq} (δ_H 0.70), with H-23_{ax} (δ_H 1.47), and 26_{ax} (δ_H 3.16), indicating spatial proximity of these hydrogen atoms. These data were used to confirm the configuration *25R*-3β-amino-5-22α-*O*-spirostan-6α-ol. This proposal is in agreement with the specific rotation $[\alpha]_D$=-50 (literature $[\alpha]_D$=-47).[11]

The acetylation with acetic anhydride and acylation with cinnamoyl chloride furnished diacetylisojuripidine **1a** and the new *N*-cinnamoylisojuripidine **1b** respectively**.** The last compound was chemoselectively formed. The structures were confirmed by ^{1}H and ^{13}C NMR assignments (Tables 2). As expected, H-6 was shifted from δ_H 3.14 to 4.50 in **1a**. This is the first report of ^{13} C NMR data assignments for the isojuripidine **1** (*25R*-3β-amino-5-22α-*O*-spirostan-6α-ol) and the derivatives **1a** (*25R*-3β-acetanamide-5α-22α-*O*-spirostan-6α-acetate) and **1b** (*25R*-3β-*N*-cinnamoyl-5α-22α-*O*-spirostan-6α-ol).

The 3-aminospirostane alkaloid (jurubidine-type) form a relatively small group of *Solanum* alkaloids.[18] Only few reports of this type of compound are described on the literature[19] juripidine[20] itself and isojuripidine,[11] besides soladunalinidine[21] and few others aza-ketal isomers are solely representative members.

Table 2. ^{13}C (200 MHz) and ^{1}H NMR (50.3 MHz) chemical shifts* of the derivatives of isojuripidine from *S. asterophorum*

	^{13}C		^{1}H	
	1a	**1b**	**1a**	**1b**
OCO-Me	171.1	-	2.14 s	
NCO-Me	169.3	-	1.92 s	
1’	-	165.9		
1”	-	134.7		
10	36.5	36.2		
13	40.5	40.3		
22	109.2	109.3		
CH				
3	49.1	48.8	3.55 m	3.69 m
5	48.2	52.0		
6	72.1	68.8	4.50 m	3.30 m
8	33.7	33.7		
9	53.5	53.5		
14	55.8	55.7		
16	80.6	80.7	4.35 m	4.33 m
17	62.0	61.8		
20	41.6	42.0		
25	30.3	30.0		
2’	-	120.7		6.34 d (15.8)
3’	-	140.4		7.51 d (15.8)
2”,6”	-	127.5		7.44 m
3”,5”	-	128.6		7.29 m
4”	-	129.8		7.28 m
CH_2				
1	37.4	37.4		
2	28.5	27.9		
4	29.7	28.9		
7	39.6	41.3		
11	23.0	20.6		
12	37.7	39.4		
15	31.6	31.3		
23	31.2	31.1		
24	28.7	28.4		
26	66.8	66.6	3.33 m, 3.84 m	3.34 m, 3.41 m
CH_3				
18	16.4	16.2	0.72	0.69
19	13.2	13.0	0.83	0.76
21	14.4	14.1	0.93 d (8.8)	0.89 d (6.6)
27	17.1	18.8	0.75 d (6.0)	0.73 d (6.4)
OCO-C$\underline{H}_3$	21.3	-		
NCO-C$\underline{H}_3$	23.7	-		

*Spectra were recorded in $CDCl_3$ and the chemical shifts are expressed on the δ scale with TMS as internal standard (values in parenthesis are coupling constants in Hz).

Experimental

The NMR spectra of compound **1** were run on a Bruker Advance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) in DMSO-d_6. The compounds **1a** and **1b** were taken on a

Bruker AC 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) in $CDCl_3$. FT-IR spectra were obtained on a Bomen-Michelson spectrophotometer using KBr. High-resolution mass spectra were obtained by electron impact on a VG Autospec spectrometer.

Plant material

The aerial parts of *Solanum asterophorum* were colleted in Brazil, Paraiba, municipality of Areia, in 2003. Voucher specimens (Agra 1744) are deposited at the Herbarium Prof. Lauro Pires Xavier (JPB), Universidade Federal da Paraiba. The powdered aerial parts of *S. asterophorum* (396.0 g) were extracted with MeOH in a Soxhlet apparatus. The extract was concentrated under vacuum in a rotaevaporator. The crude residue (37.5 g), after standing in the refrigerator, furnished a white precipitate that was separated from the extract and recristallyzed from methanol to yield 205.0 mg of **1**, mp 200 °C (Lit.[11] 204-5 °C); $[\alpha]^{20}_{D}$ -50.0 (Lit.[11] -47.0); IR (KBr) ν_{max}/cm^{-1}: 3409, 2951, 2929, 2873, 2850, 1617, 1449, 1385, 1242, 1176, 1154, 1055, 982, 956, 920, 899 and 866; HREIMS Calc. for $C_{27}H_{45}NO_3$ 431.33994; Found 431.33979.

*Synthesis of **1a**.*

A mixture of 90.0 mg of isojuripidine **1** with 1 mL of pyridine and 1 mL acetic anhydride was stirred at room temperature for 24 h. After TLC inspection, the reaction was added to crushed ice with the aid of ethanol. The solids were collected on a Buchner and recrystallized from methanol, yielding 75.1 mg (70 %) of **1b** as white crystals. IR (KBr) ν_{max}/cm^{-1}: 3416, 2950, 2935, 2872, 1753, 1639, 1452, 1239, 1042, 982, 900 and 865.

*Synthesis of **1b**[22]*

To a mixture of **1** (23.0 mg) in 10 mL of dichloromethane with 1 mL of triethylamine at room temperature was added a solution of cinnamoyl chloride (10.0 mg, 1.12 mmol) in 1 mL of dichloromethane. The resulting mixture was stirred for 24 hours at room temperature, the solvents removed by vacuum and the product crystallized in methanol, giving the *N*-cinnamoyl derivative **1a** as colourless crystals (14.7 mg, 52 % yield), with mp 327-330 °C; IR (KBr) ν_{max}/cm^{-1}: 3409, 2949, 2927, 2851, 1651, 1620, 1451, 1240, 1176, 1052, 900 and 788; MS (70 eV) 544 (4%, M^+ - OH), 415 (2%), 387 (2%), 256 (13%), 131 (7%), 77 (5%) and 69 (100%).

Supplementary Information

All spectra obtained for the assignments are given as PDF file for consulting, free of charge, at http://jbcs.sbq.org.br next to the article PDF link.

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