Article

Journal of the Brazilian Chemical Society

https://dx.doi.org/10.21577/0103-5053.20240166

J. Braz. Chem. Soc. **2025**, *36*, 3, e-20240166, 1-8

Brazoide E, a New Alkaloid and Other Compounds from *Justicia wasshauseniana* (Acanthaceae)

Renata D. Fernandes,[a] Raimundo Braz-Filho,[a,b] Mário G. de Carvalho,[a] Denise M. Braz,[c] Luciano R. Suzart,[a] Tatiane N. Tejero,[a] Glauco F. Bauerfeldt[a] and Mariele R. S. Gonçalves[*,a]

[a]*Instituto de Química, Universidade Federal Rural do Rio de Janeiro (UFRRJ), 23890-000 Seropédica-RJ, Brazil*

[b]*Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), 28013-600 Campos dos Goytacazes-RJ, Brazil*

[c]*Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro (UFRRJ), 23890-000 Seropédica-RJ, Brazil*

A new alkaloid, named brazoide E (**6**), three known steroids (campesterol, β-sitosterol, stigmasterol), two glucosides (3-*O*-β-D-glucopyranosylsitosterol and 3-*O*-β-D-glucopyranosyl stigmasterol) and sucrose were isolated from the leaves and stems of *Justicia wasshauseniana*. Their structures were characterized using high-resolution electrospray ionization mass spectrometry (HRESIMS), nuclear magnetic resonance (1D and 2D NMR), and infrared (IR) spectroscopy. To reinforce the structural proposal, an acetylated derivative (**6a**) was obtained and its data compared to **6**. Moreover, circular dichroism spectroscopy (CD) analysis was performed to determine the absolute stereochemistry, time-dependent density functional theory (TD-DFT) at the wB97X-D/def2-TZVP level, adopting functionals M06-2X and wB97X-D and base aug-cc-pVDZ, was employed to confirm the CD analysis.

Keywords: Acanthaceae, *Justicia wasshauseniana*, alkaloid, steroids, glucosides

Introduction

Justicia L. is the largest genus of the Acanthaceae family, with approximately 600 species distributed globally, from tropical regions to temperate regions of North America.[1-3] The endemic species from Brazil, *Justicia wasshauseniana* Profice, is found in the Atlantic Forest in the states of Rio de Janeiro, Espírito Santo, and Bahia. Species of the genus *Justicia* share high morphological similarity, common names, and a variety of medical indications,[3,4] including antioxidant, anti-arthritic, anti-inflammatory, analgesic, anticancer, hepatoprotective, and larvicidal activities.[1] These medicinal properties have been traditionally explored in folk medicine, especially in the case of *J. gendarussa*, known as "*anador*" or "*anador grande*" and used to treat a wide range of health conditions, from fever to eye diseases.[5] Previous phytochemical studies with *J. gendarussa* reported the presence of triterpenoids, steroids, flavonoids, and alkaloids. Regarding the latter, four new molecules, named brazoides A-D were isolated from the leaf extract of this species.[5,6] Considering the relevance of this genus in terms of its popular use and the low knowledge about its chemical composition makes the study of its species relevant. Thus, this work describes the first phytochemical study of *Justicia wasshauseniana* about the isolation and structural elucidation of the new alkaloid named brazoide E (**6**), besides the identification of five steroids, including two glucosylates, and sucrose. Physical methods of organic analysis were used to define and identify the structures of the isolated compounds. Theoretical calculations based on density functional theory, adopting functionals M06-2X and wB97X-D and base aug-cc-pVDZ, were also performed.[7] Recently published works[8] support the choice of this level of theory for the prediction of UV-Vis spectra and circular dichroism spectroscopy (CD). Within this context we describe the

*e-mail: mariele.rondon@gmail.com
Editor handled this article: Hector Henrique F. Koolen (Associate)

phytochemical study of *J. wasshauseniana* including considerations on the stereochemistry of the new alkaloid identified in this work.

Experimental

Instrumentation and experimental procedure

The leaves and stems were ground in a Maqtron B-611 knife mill. The organic solvents of the extracts were removed using a Fisatom 550 rotary evaporator (São Paulo-SP, Brazil) at 40 °C under reduced pressure (300 mbar). 1D and 2D ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance II 400 MHz spectrometer (Bruker, Rheinstetten, Germany) equipped with a BB ^{1}H probe (400 MHz) and a Bruker Avance III 500 MHz spectrometer (Bruker, Rheinstetten, Germany) equipped with a BBO 500 MHz S2 5 mm z-gradient PLUS SP probe (500 MHz). Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), and dimethyl sulfoxide (D_3CSOCD_3) were used for the NMR experiments. High resolution mass spectra (HRESIMS) were obtained with a MICROTOF-Bruker Daltonics (Billerica, USA) spectrometer equipped with an electrospray ionization source. Low resolution mass spectra (EIMS) were obtained through electron ionization (70 eV) in a gas chromatograph with mass spectrometry (GC-MS) apparatus Shimadzu GCMS-QP2010 Plus (Mundelein, USA). The infrared spectra were obtained with KBr pastille in a Bruker VERTEX-70 (Billerica-USA) spectrometer with Fourier transform. The CD curve was recorded with a JASCO J-805 (São Paulo-SP, Brazil) spectrometer. Theoretical calculations were conducted with software ORCA (version 4.1.1)[9] to investigate the spectroscopic properties of stereoisomers of compound **6**. The density functional theory (DFT) calculations adopted the functionals M06-2X and wB97X-D and base aug-cc-pVDZ. Geometry and vibrational frequency optimization calculations for the global minima of each isomer were performed at the wB97X-D/def2-TZVP level. In all calculations, methanol solvent effects were considered with the polarizable continuum model CPCM (conductor-like polarizable continuum model). The energy differences (ΔE^0) reported in the present study included vibrational zero-point energy corrections.[10,11] Calculations of the vertically excited electronic states were performed at the TD-DFT, wB97X-D/def2-TZVP level for the lower energy conformations of the isomers of the new alkaloid. In all, 25 roots were requested in order to obtain the fine structure of the UV-Vis and CD spectra including the solvent effects with the CPCM method.[7,8]

Plant material harvesting

Leaves and stems of the species *Justicia wasshauseniana* Profice were collected in Parque Natural Municipal do Curió, Municipality of Paracambi, Rio de Janeiro state, Brazil (geographical location: 22°34'41.0"S and 43°42'34.0"W), in September 2009. The species was identified by Prof Denise Monte Braz and a voucher specimen was deposited in the Herbarium of the Department of Botany at UFRRJ, D. M. Braz 274 (RBR), The authorization to access the species through the number ACD32AD was granted by the Brazilian System for the Management of Genetic Heritage and Associated Traditional Knowledge, SisGen. After fifteen days of drying at room temperature and subsequent grinding in a knife mill, 453.4 g of stems and 657.4 g of leaves were obtained.

Extraction, isolation of the constituents, and derivatives

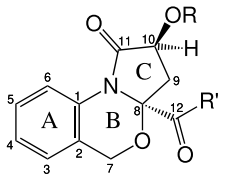
Stems (453 g) and leaves (657 g) were separately extracted with CH_2Cl_2 (5 L) and MeOH (4 L) by continuous maceration at room temperature, 72 h. The solvent was removed in a rotary evaporator at 40 °C under reduced pressure and the respective extracts were obtained: JwSD (*J. wasshauseniana* stems dichloromethane crude extract, 2.4 g); JwLD (*J. wasshauseniana* leaf dichloromethane crude extract, 20.6 g); JwSM (*J. wasshauseniana* stems methanol crude extract, 44.8 g) and JwLM (*J. wasshauseniana* leaf methanol crude extract, 55.1 g), the mass of JwLM was obtained after filtration to remove a precipitate that was identified as KCl. JwSD extract (2.2 g) was submitted to an open chromatographic column with a silica gel stationary phase (230-400 mesh) and eluted with a CH_2Cl_2/MeOH mixture in a gradient of increasing polarity, to obtain 58 fractions (JwSD-1-58). A white precipitate was obtained from the JwSD-20-31 subfraction (33.0 mg) soluble in dimethyl sulfoxide (DMSO), it was added acetone and filtered and the final material (30.0 mg, mp 290-292 °C) was obtained and characterized as a mixture of 3-*O*-β-D-glucopyranosylsitosterol (**4**) and 3-*O*-β-D-glucopyranosylstigmasterol (**5**). JwSM (26.3 g) was solubilized with MeOH/H_2O (8:2) and extracted with $CHCl_3$. The material obtained (JwSMC, 1.03 g) was subjected to chromatographic fractionation in an open silica gel column and eluted with CH_2Cl_2/MeOH in a gradient of increasing polarity from which 26 fractions were collected (JwSMC-1-26). The JwSMC-6-10 fractions were a solid material (30.0 mg) that was identified as a mixture of the steroidal saponins mentioned above.

20.6 g of JwLD were submitted to a selective filtration process in a separation funnel with silica gel (230-400 mesh)

as a filtering medium and eluted with hexane, CH_2Cl_2, ethyl acetate, and methanol. The CH_2Cl_2 fraction (JwLD-D) was chromatographed on silica gel (230-400 mesh) and eluted with a binary mixture of the CH_2Cl_2/MeOH solvents with a gradient of increasing polarity. From this column, 121 subfractions (JwLM-D-1-121) were collected and analyzed by analytical thin layer chromatography (TLC) and pooled according to their chromatographic profile. A white precipitate was observed in the pooled JwLM-D-11-19 and JwLM-D-20-30 subfractions and characterized as a steroid mixture (**1**, **2**, **3**, 65 mg). In the ethyl acetate fraction (JwLDAc), a precipitate soluble in DMSO was formed and a mixture of glucosides **4** and **5** was identified. 36.2 g of MeOH leaf extract (JwLM) were suspended in water and sequentially partitioned with chloroform, ethyl acetate and butanol solvents. The butanolic fraction (JwLM-B, 3.1 g) was chromatographed on silica gel and eluted with mixtures of hexane/ethyl acetate/methanol in gradients of increasing polarity (90:10:0 to 0:10:90). 29 subfractions (JwLM-B-1-29) were obtained, analyzed by TLC and pooled according to their chromatographic profile. The JwLM-B-1-2 (456.0 mg) and JwLM-B-3-7 (357.0 mg) subfractions were separately rechromatographed on Sephadex LH-20 and eluted with methanol. The purification of JwLM-B-1-2 resulted in 20 fractions, among which the grouped subfractions 2-4 were analyzed and identified as brazoide E (**6**, 80.0 mg). Part of these fractions (20.0 mg) were subjected to an acetylation reaction with acetic anhydride and pyridine, yielding the acetylated derivative **6a** (20.0 mg). The pooled JwLM-B-3-7 subfraction was rechromatographed, yielding 8 fractions, and the subfraction group of 3-5 (23.0 mg) containing an unidentified alkaloid. Analysis of fractions 6-8 from this last filtration led to the identification of sucrose (**7**).

Results and Discussion

Phytochemical investigation of the leaf (JwL) and stem (JwS) extracts of *Justicia wasshauseniana* led to the isolation of five steroids and a carbohydrate known in the literature and a new alkaloid (**6**, Figure 1). The steroidal saponins 3-*O*-β-D-glucopyranosylsitosterol (**4**) and 3-*O*-β-D-glucopyranosylstigmasterol (**5**), and sucrose (**7**), were identified by analysis of ^{1}H and ^{13}C NMR spectra and by comparison with spectral data described in the literature.[12] The same procedure was performed to identify the mixture of sitosterol (**1**), stigmasterol (**2**) and campesterol (**3**),[13] including additional GC-MS analysis and comparison with their mass spectra available at the database of the equipment (NIST08). In the identification of the new alkaloid, brazoide E (**6**) physical methods of organic analysis of the natural compound and acetylated derivative (**6a**) were used as described below.



6: R = H, R' = OH
6a: R = Ac, R' = OH

Figure 1. Structures of **6** and the acetylated derivative (**6a**).

Compound **6** was isolated as a methanol soluble brown amorphous solid. The ^{13}C distorsionless enhancement by polarization transfer including the detection of quaternary nuclei (DEPTQ) NMR spectrum revealed the presence of 11 carbon atoms (Table 1), in which five were methines (δ_C 118.7; 126.3; 124.2, 124.0 and 68.2), five quaternary carbons (δ_C 123.9; 132.9, C=C; δ_C 172.6 and 174.7, two C=O carbons; and δ_C 88.7, one sp^3 non-hydrogenated carbon connected to oxygen and nitrogen atoms), and two methylene, from which one is oxygenated [δ_C 64.1(CH_2O) and 40.3(CH_2)]. It was confirmed by ^{1}H NMR that compound **6** has hydrogens in an aromatic ring at

Table 1. NMR data obtained for compound **6** and its acetylated derivative (**6a**)

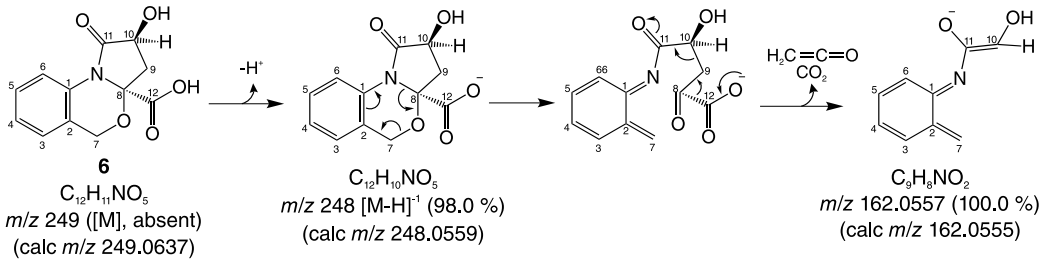
Position	**6**		**6a**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	132.9	–	131.8	–
2	123.9	–	122,6	–
3	124.2	7.11 (m)	124.4	7.06 (d, 8.2)
4	124.2	7.11 (m)	125.2	7.16 (t, 8.2)
5	126.8	7.26 (t, 8.2)	127.8	7.28 (t, 8.2)
6	118.7	8.35 (d, 8.2)	119.5	8.35 (d, (8.2)
7	64.1	5.26 (d,15.6, H-7a) 4.87 (d, 15.6, H7b)	64.8	5.28 (d, 15.6) 4.95 (d, 15.6)
8	88.7	–	87.0	–
9	40.3	2.89 (dd, 12.5, 8.3, H-9a) 2.07 (dd, 12.5, 9.6, H-9b)	37.8	3.12 (dd, 13.3, 8.5) 2.29 (dd, 13.3, 9.4)
10	68.2	4.57 (dd, 9.6, 8.3)	68.5	5.63 (t, 8.4)
11	174.7	–	167.3	–
12	172.6	–	171.8	–
2’	–			
3’	–			
$H_3C\underline{C}O_2$-10	–	–	170.3	–
$H_3\underline{C}CO$-10	–	–	20.7	2.19 (s)

^{13}C data obtained by HSQC. ^{1}H NMR of compound **6** (D_3COD, 500 MHz and ^{13}C NMR at 125 MHz) and compound **6a** ($CDCl_3$).

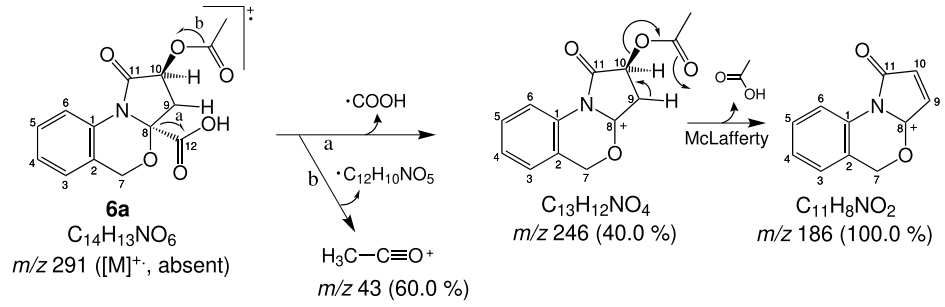
δ_H 7.11 (m, 2H), 7.26 (t, *J* 8.2 Hz) and 8.35 (d, *J* 8.2 Hz), ^{1}H-^{1}H correlated spectroscopy (COSY) correlations of H-6 (δ_H 8.35) with H-5 (δ_H 7.26) and H-4 (δ_H 7.11) with H-5, and H-3 (δ_H 7.11), confirmed the presence of a 1,2-disubstituted aromatic ring. These data allow us to propose the set of atoms: $C_2(C{=}O)_2(N{-}C{-}O)(CH)_4(OCH)(CH_2)_2$ (partial formula $C_{12}H_9NO_4$) which differs in 2 atoms of hydrogen and one oxygen from the molecular formula $C_{12}H_{11}NO_5$ that was deduced by HRESIMS in negative mode (*m/z* 248.0560 $[M-H]^-$), Scheme 1. This difference was justified by the presence of a hydroxy group, confirmed by the acetylation reaction yielding **6a** (Figure 1), and by the presence of a carboxyl group (Scheme 2).

The signals attached to oxygenated sp^3 carbons in an isolated AB system (CH_2O) with twinned coupling constant of 15.6 Hz corresponded to H-7a (δ_H 5.26) and H-7b (δ_H 4.87). The OCH–CH_2– group (ABX system) with of signals at δ_H 4.57 (dd, *J* 9.6 and 8.3 Hz, H-10) and two doublet signals at δ_H 2.89 (dd, *J* 12.5 and 8.3 Hz, H-9a) and δ_H 2.07 (dd, *J* 12.5 and 9.6 Hz, H-9b), were confirmed by ^{1}H-^{1}H-COSY. The heteronuclear single-quantum correlation (HSQC) spectroscopy contour map (Table 1) permitted the observation of the following correlations: δ_C/δ_H 118.7/8.35 (d, *J* 8.2 Hz), C-6; 126.8/7.26 (t, *J* 8.2 Hz), C-5; 124.2/7.11 (m, 2H), C-3 and C-4; 68.2/4.57 (dd, *J* 9.6 and 8.3 Hz), C-10; 64.1/5.26, 4.87 (d, *J* 15.6 and 16,6 Hz), C-7; 40.3/2.89 (dd, *J* 12.5 and 8.3 Hz) and 2.07 (dd, *J* 12.5 and 9.6 Hz), C-9 (Table 1). In the heteronuclear multiple bond correlation (HMBC) map, it was possible to observe the heteronuclear couplings of H-7 (δ_H 4.87 and 5.26) with C-2 (δ_C 124.2, $^2J_{HC}$), C-3 (δ_C 124.2, $^3J_{HC}$) and C-8 (δ_C 88.7, $^3J_{HC}$), indicating the direct bonding of C-7 with the aromatic ring and the oxygen atom attached to the C-8 carbon. The H-9 hydrogens (δ_H 2.07, 2.89) revealed cross-sectional correlation peaks with C-8 (δ_C 88.7, $^2J_{HC}$) and with the C-12 carboxylic carbon (δ_C 172.6, $^3J_{HC}$), and of H-10 (δ_H 4.57) with C-9 (δ_C 40.3, $^2J_{HC}$) and with C-11 (δ_C 174.7.$^3J_{HC}$). Comparison with literature data of synthetic compound 7-oxa-2-azatricyclo[7.4.0.02,6]trideca-1(9),10,12-trien-3-ones (Supplementary Information (SI) section)[14,15] and additional heteronuclear couplings revealed by HMBC is summarized in Figure 2. These interpretations allowed us to guarantee the unequivocal attributions of the chemical shifts (δ_C and δ_H) of compound **6**, including a comparison with 1D and 2D data from ^{1}H and ^{13}C NMR with the acetylated derivative **6a**, which confirmed the presence and the position of the hydroxyl group. Thus, the structure of the new alkaloid isolated from *Justicia wasshauseniana* was deduced as **6** (Figure 1). The results of extensive application of 1D and 2D (Figure 2) ^{1}H and ^{13}C NMR techniques were used for structural confirmation and to establish the δ_H and δ_C assignments of **6** and **6a** (Table 1). This structure of **6** is similar to the alkaloids named as brazoides C and D isolated from *J. gendarussa*[6] which contain the unit: *N*-(*o*-*O*-methylene)-phenyl amide, being named, however, as brazoide E.

The proposed structure for brazoide E (**6**) allows us to suggest the stereoisomers (8*R*,10*R*) and (8*S*,10*S*), in addition to some conformational possibilities. Conformational analysis revealed a spectrum of five conformations for **6**, separated by at most 3.15 kcal mol^{-1}, at the M06-2x/aug-cc-pVDZ level (or 2.26 kcal mol^{-1}, at the



Scheme 1. Fragmentation proposed in order to justify the main peaks observed in the high-resolution mass spectrum (HRESIMS) of substance **6**.



Scheme 2. Fragmentation proposed in order to explain the main peaks observed in the low-resolution mass spectrum (EIMS) of the acetylated derivative (**6a**) of substance **6**.

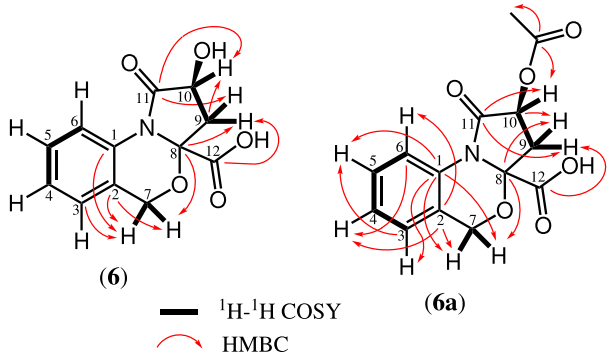
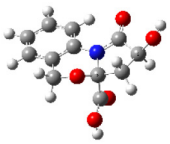


Figure 2. Selected HMBC correlation and ^{1}H-^{1}H COSY for compound **6** and acetylated derivative (**6a**).

wB97X-D level/aug-cc-pVDZ). All conformations come from rotations of O–H connected to C-10 and C-12 groups. The lowest energy conformation is distinguished by the interaction between the hydrogen of the OH group connected to carbon 10 and the carboxyl oxygen (C-11). The OH–O distance in this interaction is 2.57 Å and results in the formation of a 5-membered pseudoring. Excellent concordance is observed between the results obtained with the two functionals. The same configuration spectrum is expected for the (8*R*,10*R*) and (8*S*,10*S*) isomers, since they are mirror images. Table 2 presents the lowest energy geometries that form the spectrum of conformations of the (8*S*,10*S*) isomer of **6**, as well as the values of electronic energy, vibrational zero-point energy and relative energy obtained at levels CPCM(Methanol)/M06-2X/aug-cc-pVDZ and CPCM(Methanol)/wB97X-D/aug-cc-pVDZ, respectively.

The absolute configuration of the alkaloid was determined with the aid of CD analysis, ((300 µL, MeOH) $[\theta]_{250}$ = –33.000 and $[\theta]_{220}$ = + 62.700), Figure 3a. To define the stereoisomer that corresponds to this cotton effect (CE), theoretical calculations were made to simulate the circular dichroism spectra for the (8*R*, 10*R*) and (8*S*, 10*S*) isomers, which are presented in Figure 3b. In this, the red lines delimit the experimentally observed region in the theoretical spectrum. As expected, the profiles observed in the CD spectrum follow the absorption maxima of the UV spectrum. The spectrum of the (8*S*, 10*S*) isomer agrees with the experimental spectrum

Table 2. Stationary points obtained at the CPCM(Methanol)/X/aug-cc-pVDZ and (X = M06-2X and wB97X-D) levels as possible conformations of the (8*S*,10*S*) isomer of **6**

Geometry	Functional	Energy / hartree	Vibrational energy from point zero / hartree	ΔE^0 / (kcal mol^{-1})
	M06-2X wB97X-D	–894.626017 –894.654656	0.224081 0.223895	0.18 0.13
	M06-2X wB97X-D	–894.6216452 –894.6513457	0.224435 0.223982	3.15 2.26
	M06-2X wB97X-D	–894.624060 –894.652797	0.223842 0.224121	1.26 1.46
	M06-2X wB97X-D	–894.624716 –894.653566	0.224096 0.224409	1.01 1.13
	M06-2X wB97X-D	–894.626150 –894.654877	0.223920 0.223912	0.00

ΔE^0: energy difference between different conformations, adjusted to the zero-point energy.

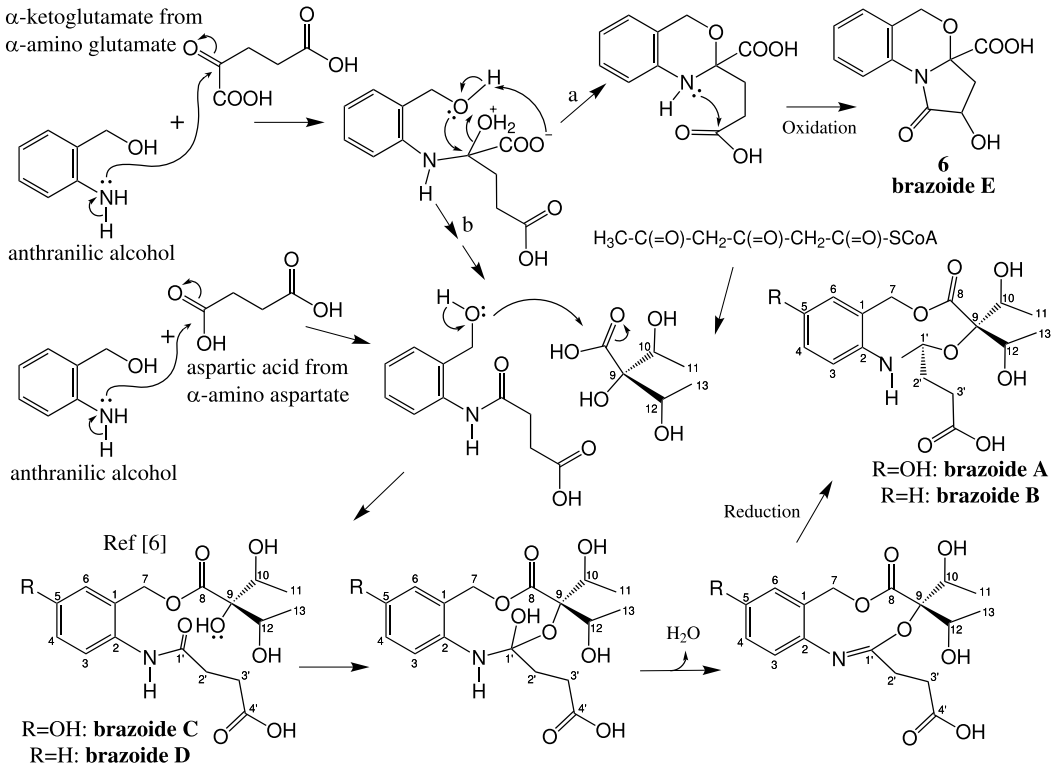
obtained for **6**, (Figure 3a): from 190 nm onwards, an increase in intensity is observed, forming a band with a positive sign and peak at 196 nm; the second peak shows a negative sign with a peak at 225 nm. The theoretical spectrum obtained for the isomer (8*R*,10*R*) shows an inverted profile, with a negative signal peak at 196 nm and a positive signal peak at 225 nm. The predicted spectrum at CPCM(Methanol)/TDDFT-wB97X-D/def2-PVTZ level (Figure 3b) is compared satisfactorily in terms of profile and peak intensity with the experimental spectrum, in the 200 to 350 nm range (Figure 3a). However, differences are expected between the wavelength values referring to the maximum of the absorption bands in the experimental and theoretical UV spectra. Even so, a shift of only 25 nm was obtained as a difference between the theoretical and experimental absorption maxima, with the theoretical results being shifted to lower wavelengths. Thus, theoretical calculations corroborate the interpretation of experimental circular dichroism spectra based on empirical projection rules, confirming the stereochemistry (8*S*,10*S*) in **6**.

The theoretical UV spectrum of **6** is shown in Figure 3c. Bandwidths were increased for better comparison with the experimental spectrum. At this resolution, four peaks are observed, with the maximum at 145, 165, 189 and 223 nm. The peak at 189 covers 3 electronic excitations: 189 nm and 185 nm, which refer to the excitation n $\rightarrow \pi^*$ (HOMO−1 $\rightarrow$ LUMO+1 and HOMO−1 $\rightarrow$ LUMO, respectively) and 191 nm, the lowest intensity peak, which refers to excitation $\pi \rightarrow \pi^*$ (HOMO $\rightarrow$ LUMO+2). The peak at 223 covers two electronic excitations of predominant characteristic $\pi \rightarrow \pi^*$ at 236 nm (HOMO $\rightarrow$ LUMO+1), 222 nm (HOMO $\rightarrow$ LUMO) and two electronic excitations of characteristic n $\rightarrow \pi^*$ at 224 nm (HOMO−2 $\rightarrow$ LUMO) and 214 nm (HOMO−3 $\rightarrow$ LUMO+2). The frontier orbitals related to these electronic transitions are shown in the Supplementary Information section, in which the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are orbitals 64 and 65, respectively, as long as HOMO-3, HOMO-2 and HOMO-1 are orbitals 61, 62 and 63 and LUMO+1 and LUMO+2 are 66 and 67.

Finally, a biosynthetic proposal for the new alkaloid and for those known brazoides was summarized in Scheme 3.

Conclusions

The present investigation was the first chemical study of the species *Justicia wasshauseniana* Profice (Acanthaceae) which led to the isolation and structural determination of a new alkaloid: (8*S*,10*S*)*N*-(phenyl-*o*-methylene-*O*-8)-10-hydroxy-8-carboxylic acid γ-lactam (**6**, brazoide E). Theoretical calculations allowed us to define the stereochemistry of the new alkaloid. The steroidal saponins, 3-*O*-β-D-glucopyranosylsitosterol (**4**)



Scheme 3. Biosyntetic route proposal for the biological production of brazoides A, B, C, D[6] and E.

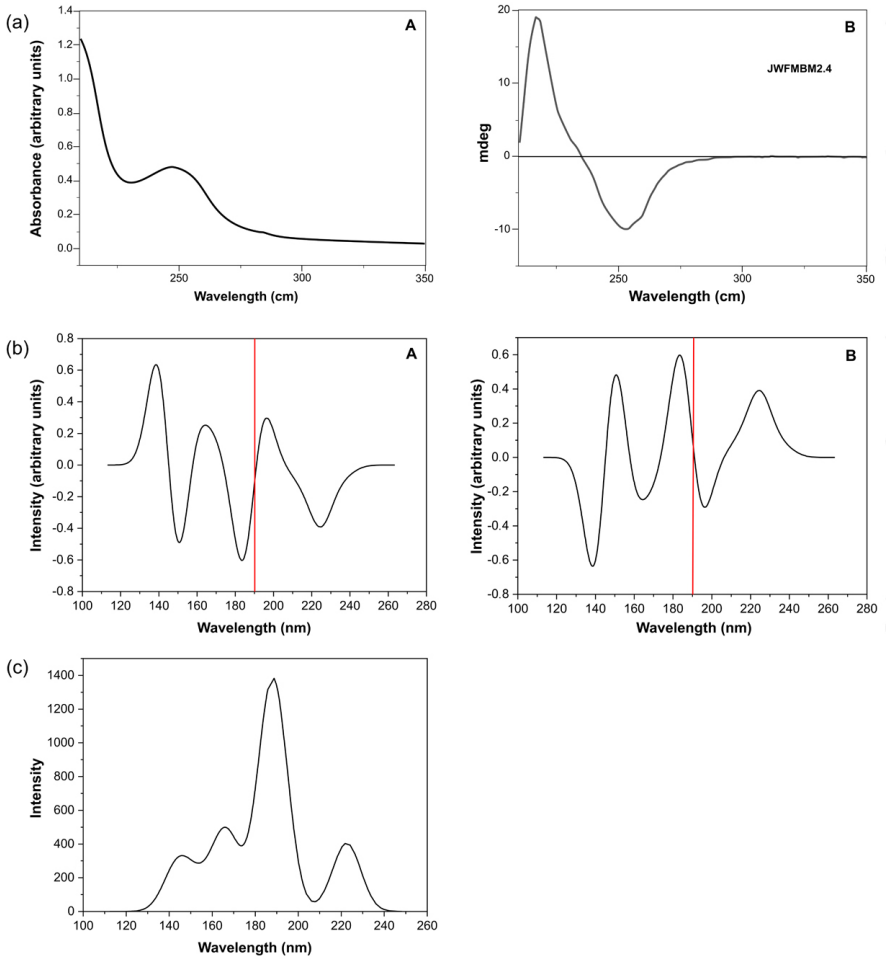


Figure 3. Representation of the experimental and theoretical spectroscopies of **6**; (a) experimental UV (A) and CD (B) spectra (methanol) of compound **6**; (b) simulated CD spectra for the CPCM/TDDFT level **6** isomers wB97X D/ def2 PVTZ: (A) (8*S*,10*S*); (B) (8*R*,10*R*); (c) theoretical UV spectrum of **6**, obtained at the CPCM/TDDFT wB97X D/def2-PVTZ level.

and 3-*O*-β-D-glucopyranosylstigmasterol (**5**) were found in different extracts of the plant. The other compounds, a mixture of steroids and sucrose, were identified. The result of this study contributes to expanding knowledge about the chemical composition of *Justicia* species, well-known due to popular uses with many pharmacological activities.

Supplementary Information

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

Acknowledgments

The authors would like to thank the research funding institutions CNPq, CAPES and FAPERJ for financial support and for study and research grants to Prof Darí Cesarin-Sobrinho for the circular dichroism essay, and to Dr Ari Miranda da Silva (UFRJ, IPPN, Analytical Central, RJ) and Carolina Passos Cunha (Embrapa Agroindustria de Alimentos, Laboratorio de Cromatografia Líquida, Guaratiba, RJ) for calculating the high-resolution mass spectra (HRESIMS).

Author Contributions

Renata D. Fernandes and Luciano R. Suzart were responsible for the execution of the chemical experimentation, isolation and derivatization of the described substances; Raimundo Braz Filho and Mário G. de Carvalho for the characterization and elucidation of the molecules, as well as for the writing and revision of the manuscript; Denise M. Braz for botanical identification; Tatiane Nicola Tejero and Glauco Favilla Bauerfeldt for computational analyses and simulations, as well as for contributing to the manuscript production; Mariele R. S. Gonçalves for manuscript revision and writing editing.

References

1. Corrêa, G. M.; Alcântara, A. F. C.; *Braz. J. Pharmacog.* **2012**, *22*, 220. [Crossref]

2. Wasshausen, D. C.; *Brittonia* **2002**, *54*, 286. [Crossref]
3. Braz, D. M.; *Phytotaxa* **2015**, *236*, 184. [Crossref]
4. Chagas, E. C. O.; Costa-Lima, J. L.; *Flora e Funga do Brasil*; Jardim Botânico do Rio de Janeiro, Brazil, https://floradobrasil.jbrj.gov.br/FB106949, accessed in July 2024.
5. Sonal, P.; Maitreyi, Z.; *Asian J. Tradit. Med.* **2011**, *6*, 61. [Link] accessed in July 2024
6. Souza, L. G. S.; Almeida, M. C. S.; Lemos, T. L. G.; Ribeiro, P. R. V.; Canuto, K. M.; Braz-Filho, R.; Del Cistia, C. N.; Sant'Anna, C. M. R.; Barretog, F. S.; de Moraes, M. O.; *J. Braz. Chem. Soc.* **2017**, *28*, 1281. [Crossref]
7. Komjáti, B.; Urai, A.; Hosztafi, S.; Kökösi, J.; Kováts, B.; Nagy, J.; Horváth, P.; *Spectrochim. Acta, Part A* **2016**, *155*, 95. [Crossref]
8. Wang, Z.-Q.; Huang, C.; Huang, J.; Han, H.-Y.; Li, G.-Y.; Wang, J.-H.; Sun, T.-M.; *RSC Adv.* **2014**, *4*, 14373. [Crossref]
9. Neese, F.; *ORCA*, 4.1.1; Max Planck Institute for Coal Research; Mülheim an der Ruhr, Germany, 2019.
10. Sammaritano, M. A.; Vera, M. G.; Cometto, P. M.; Tejero, T. N.; Bauerfeldt, G. F.; Mellouki, A.; *Chem. Phys. Lett.* **2020**, *755*, 137757. [Crossref]
11. Tejero, T. N.; Bauerfeldt, G. F.; *Arkivoc* **2020**, *part ii*, 20. [Crossref]
12. Velandia, J. R.; de Carvalho, M. G.; Braz-Filho, R.; Werle, A. A.; *Phytochem. Anal.* **2002**, *13*, 283. [Crossref]
13. Jain, P. S.; Bari, S. B.; *Asian J. Plant Sci.* **2010**, *9*, 163. [Crossref]
14. Jha, A.; Naidu, A. B.; Abdelkhalik, A. M.; *Org. Biomol. Chem.* **2013**, *11*, 7559. [Crossref]
15. Zhou, Y.; Zhai, Y.; Ji, X.; Liu, G.; Feng, E.; Ye, D.; Zhao, L.; Jiang, H.; Liu, H.; *Adv. Synth. Catal.* **2010**, *252*, 373. [Crossref]

Submitted: December 6, 2023
Published online: August 19, 2024