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Effect of indole alkaloids from roots of *Rauvolfia ligustrina* in the noradrenergic neurotransmission

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A R T I C L E I N F O

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A B S T R A C T

The new glucosyl sarpagan alkaloid designated as 21(*R**)-(*O*-*β*-glucosyl)-hydroxy-sarpagan-17-oic acid, along with eleven known alkaloids were isolated from a soluble alkaloidal fraction from the ethanol extract of *Rauvolfia ligustrina*. Their structures were elucidated by interpretation of spectroscopic data (1D and 2D NMR), HRESIMS experiment, GIAO ^{13}C NMR calculations, and comparison with literature data. All the isolated alkaloids were screened by their neuroinhibitory effects using the electrically stimulated mice vas deferens bioassay. Compounds **1**, **2** and **9** presented a potent inhibitory effect in the neurotransmission while **3** and **11** showed an acute neuroexcitatory effect. Compound **10** exhibited a very effective post-synaptic inhibitory activity.

1. Introduction

The pantropical genus *Rauvolfia* (Apocynaceae family) comprises 74 accepted species widely distributed throughout America, Africa and Asia continents [1]. Several *Rauvolfia* species have been used in traditional medicine for different purposes such as treatment of snakebite, malaria, gastrointestinal and central nervous system disorders [2–6]. Crude extracts and constituents from *Rauvolfia* species have shown pharmacological activities, such as anxiolytic [7], antihypertensive [8], anticancer [9] and antimicrobial [10].

The genus *Rauvolfia* is known as a prolific source of structurally diversified indole alkaloids, which are subdivided in different groups as sarpagan, yohimbine, heteroyohimbine, indolenine, oxindole, and anhydronium [11]. The most investigated species is *Rauvolfia serpentine*, worldwide known by its medicinal properties and as the reserpine producer, the first alkaloid indicated to the treatment of hypertension and schizophrenia in the 50's decade [2,12]. As part of our ongoing research for bioactive alkaloids [13–16] we have investigated the root extract of *R. ligustrina*, an annual shrub found in the northeast region of Brazil. Herein, a new glucosyl sarpagan alkaloid (**1**), together with eleven known indole alkaloids are described (see Fig.1). Additionally, their effects on the noradrenergic neurotransmission were also evaluated on the electrically stimulated mice vas deferens (biological tissue with a rich diversity of pharmacological presynaptic and postsynaptic receptors bioassay).

2. Experimental

2.1. General experimental procedures

Optical rotation was determined using a Jasco P-2000 digital polarimeter. 1D and 2D NMR spectra were obtained on a DRX-500 (Bruker) and DPX-300 (Bruker) operating at 500 and 300 MHz frequency for ^{1}H, respectively, and 125 and 75 MHz frequency for ^{13}C, respectively. High-resolution electrospray ionization mass spectra (HRESIMS) were acquired on Acquity UPLC-QTOF-ESI-MS (Waters). Chromatographic procedures were carried out using silica gel (40–63 μm, Merck), SPE cartridge C-18 (Phenomenex), Sephadex LH-20 (Pharmacia) and thin-layer chromatography (TLC) with pre-coated silica gel 60 F_{254} (Merck) using Dragendorff reagent to detect spots. HPLC (Shimadzu) analyses were performed with a system equipped with SPD-M20 diode array detector and semi-preparative C-18 column

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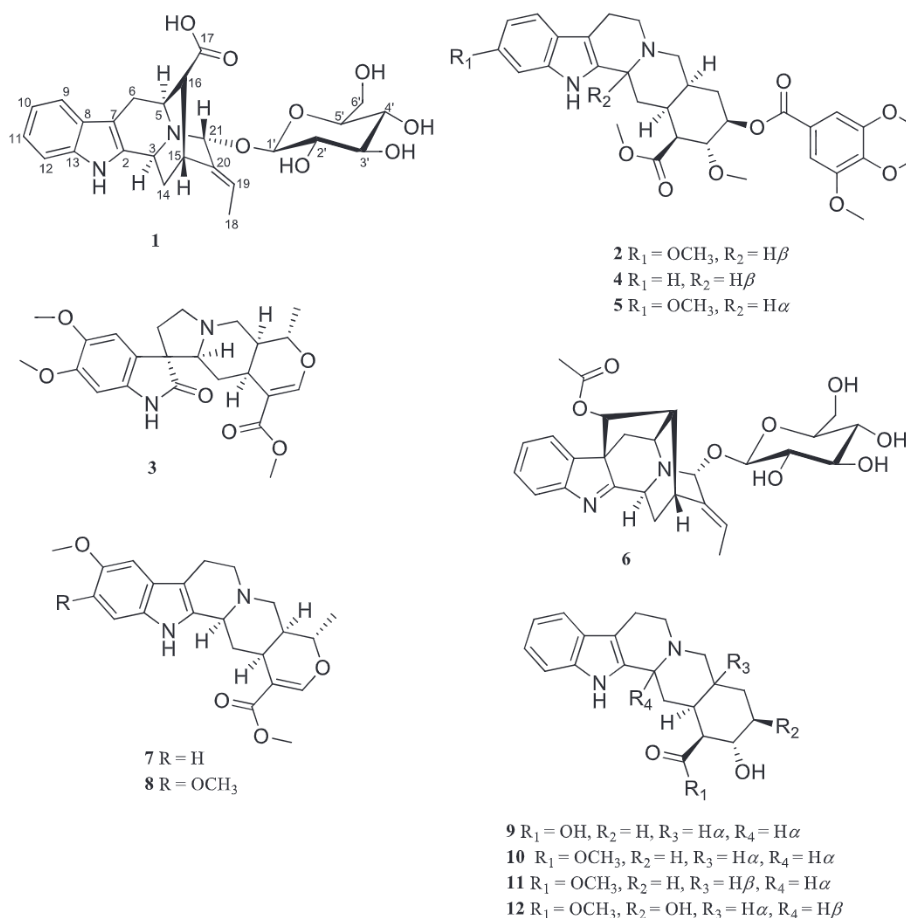

Fig. 1. Structures of alkaloids **1–12** isolated from roots of *R. ligustrina*.

(250 × 10 mm, 5 μm, Phenomenex) for isolation.

2.2. *Plant material*

Rauvolfia ligustrina was collected in September 2015 at Passa e Fica county, Rio Grande do Norte State – Brazil. The plant was authenticated by Dr. Maria Iracema B. Loiola. A voucher specimen (number 59.546) has been deposited in the Herbário Prisco Bezerra (EAC). SisGen: ACF36AA.

2.3. *Extraction and isolation*

Dried and powdered roots of *R. ligustrina* (1.7 Kg) were extracted with EtOH (2 × 8 L, 72 h each) at room temperature. The extract was concentrated under reduced pressure to yield a dark crude extract (123.0 g) which was suspended in 200 mL of 10% AcOH (pH 4) and successively partitioned with CH_2Cl_2 (3 × 100 mL) to give CH_2Cl_2-soluble fraction RD1 (19.2 g) and an aqueous fraction. The aqueous fraction was basified up to pH 10 with NH_4OH (25%, *v/v*) and extracted with CH_2Cl_2 (3 × 100 mL) to yield RD2 (6.8 g) after removal of the solvent.

Fraction RD1 (19.2 g) was subjected to a silica gel column chromatography eluted with CH_2Cl_2, CH_2Cl_2/EtOAc (3:1, 2:1, and 1:1), EtOAc, EtOAc/MeOH (8:2), and MeOH to afford seven fractions (RD1A – RD1G). Fraction RD1C (3.0 g) was washed with MeOH to afford compound **2** (523.6 mg), a yellowish precipitate, while the MeOH-soluble fraction was chromatographed on silica gel eluted with CH_2Cl_2, EtOAc and MeOH, pure or in binary mixtures to yield fourteen subfractions (RD1CA – RD1CN). Subfractions RD1CH (176.1 mg), RD1CJ (32.7 mg), and RD1CN (91.5 mg) were purified by semi-preparative HPLC using an isocratic solvent system [MeCN/H_2O (triethylamine 0.05%) 1:1] to afford compounds **3** (13.0 mg, t_R = 7.5 min), **4** (6.4 mg, t_R = 11.3 min), and **5** (9.4 mg, t_R = 15.4 min), respectively.

Fraction RD2 (7.5 g) was chromatographed on Sephadex LH-20 eluted with MeOH to afford twelve fractions (RD2A – RD2L). Individual fractions RD2C (297.4 mg), RD2E (333.0 mg), RD2G (760.8 mg), RD2H (400.8 mg), and RD2J (350.4 mg) were fractionated on C-18 SPE cartridge eluted with MeOH/H_2O (6:4, 7:3, 8:2, and 100:0) to afford four main fractions each one. Fraction RD2C [eluted with MeOH/H_2O (6:4)] was purified by semi-preparative HPLC [isocratic MeCN/H_2O (trifluoroacetic acid 0.1%) 6:4] to yield **6** (15.3 mg, t_R = 6.5 min). Compound **9** (14.0 mg, t_R = 10.0 min) was isolated from fraction RD1E [eluted with MeOH/H_2O (6:4)] using the same HPLC mobile phase. The same HPLC method was used to isolate **7** (1.5 mg, t_R = 6.3 min) and **8** (18.6 mg, t_R = 9.4 min) from fraction RD1H [MeOH/H_2O (6:4)]. Fraction RD2G [MeOH/H_2O (7:3)] was purified by semi-preparative HPLC [isocratic MeCN/H_2O (trifluoroacetic acid 0.1%) 7:3] to yield **1** (4.1 mg, t_R = 5.9 min) and **10** (26.2 mg, t_R = 7.5 min). Compounds **12** (10.0 mg, t_R = 17.2 min) and **11** (30.5 mg, t_R = 19.4 min) were isolated from fraction RD2J [MeOH/H_2O (7:3)] using the above method.

2.3.1. *21(R*)-(O-β-glucosyl)-hydroxy-sarpagan-17-oic acid (1)*

Brown amorphous solid, $[\alpha]_D^{20}$ = + 9.0 (*c* = 0.1, MeOH); ^{1}H and ^{13}C NMR data (see Table 1); HRESIMS (positive): *m/z* 487.2094 $[M + H]^+$ (calc for $C_{25}H_{31}N_2O_8$, 487.2080, error 2.9 ppm).

2.4. *Noradrenergic neurotransmission study*

All the protocols described here were approved by the Ceará State University Committee in Ethics in Animal Research under protocol

Table 1
^1H and ^{13}C chemical shifts of alkaloid **1**.

Position	**1** (500 MHz, CD_3OD)	
	δ_H (multi, *J* in Hz)	δ_C
2		138.1[a]
3	4.80, d (7.2)	45.4
5	3.86, m	53.6
6a	2.74, d (15.8)	27.6
6b	3.15, dd (15.8, 4.6)	
7		104.8
8		129.1
9	7.39, d (7.9)	118.7
10	6.98, t (8.0)	120.0
11	7.06, t (7.9)	122.3
12	7.29, d (8.0)	112.1
13		138.5
14a	1.78, m	33.6
14b	2.16, t (11.7)	
15	3.33, m	31.3
16	2.37, d (7.2)	50.0
17		180.4[a]
18	1.71, d (6.8)	13.7
19	5.83, q (6.8)	126.5
20		136.0[a]
21	5.18, s	92.2
1'	4.67, d (7.8)	103.5
2'	3.31, m	78.1
3'	3.29, m	75.4
4'	3.28, m	71.7
5'	3.39, t (8.6)	78.5
6'a	3.79 dd (11.8, 1.9)	62.9
6'b	3.60 dd (11.8, 5.8)	

[a] ^{13}C Values determined by analysis of HMBC spectrum.

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Male albino Swiss mice weighing 30–35 g were sacrificed by cervical displacement and 1 cm segment of the epididymal vas deferens (MVD) was rapidly excised and mounted into a 5 mL organ bath for isometric recordings of muscle contractility. The tissues were mounted vertically under 0.3 g passive tension and kept in a magnesium-free solution with the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 24, glucose 11, EDTA 0.027, ascorbic acid 0.1 (pH 7.4) gassed with 95% O_2 in 5% CO_2. The tissue was attached to a TRI210 force-displacement transducer (Panlab) connected to a Powerlab 8/30 data acquisition system (Powerlab) and the data was recorded and analyzed by using the Labchart 7.0 software.

2.4.1. Protocol 1

The tissue was driven by transmural electrical field stimulation (EFS) using double-ring platinum electrodes coupled to a Grass S88 stimulator (supramaximal; 0.5 ms; 0.1 Hz). After a 60 min equilibration period, test drugs were cumulatively added to the bath and the effect of each concentration (10^{-10} to 10^{-5} M) was observed over a 5 min interval. To check, for internal standard control, the neurogenic and noradrenergic nature of the effect, the tissues were incubated with 10^{-7} M tetrodotoxin (TTX) to block nerve conduction and show that we were indeed studying neuronal transmission (see Fig. 2A) and also with 10^{-7} M prazosin to show that the neurotransmitter involved is noradrenaline (see Fig. 2B).

2.4.2. Protocol 2

In order to check whether the inhibitory effects induced by the *Rauvolfia ligustrina* alkaloids in the neurotransmission were from presynaptic (neuronal) or postsynaptic (muscular) origin, we did the following protocol. The tissue was stimulated during 30 s with exogenous noradrenaline (10^{-5} M) to induce a phasic contraction as shown below (see Fig. 3). After two reproducible contractions, this stimulation was repeated in tissues previously incubated, for 5 min, with 10^{-7}, 10^{-6} or 10^{-5} M of the *R. ligustrina* alkaloids. The effect of noradrenaline in the presence of the different concentrations of the constituents was then recorded and measured.

2.5. Computational details

To elucidate the real structure of compound **1**, the two possible positions of the carboxylic group and hydrogen atom were drawn to prepare the input file and denominated as iso1 and iso2 isomers. Geometries of structures were optimized using the Density Functional Theory (DFT) method at mPW1PW91 functional [17] along with 6-31G(d,p) basis set [18] in Gaussian 09 package. Vibrational modes of the optimized geometries were calculated to determine whether the resulting geometries are true minima or transition states. In an attempt to reproduce the real medium of the researched molecule, the interaction between the two isomers and the solvent (methanol) were calculated at the same level of theory along with the Polarizable Continuum Model (PCM) [19,20] with the Integral Equation Formalism (IEF) [21].

The NMR isotropic shielding constants were determined from the optimized structures of iso1 and iso2 with mPW1PW91 functional [17] and 6-31G(d,p) basis set [18] level of theory based on the Gauge Independent Atomic Orbitals (GIAO) proposal [22–25], implemented in the Gaussian. The IEF-PCM solvation method was used with methanol as an implicit solvent to simulate the medium on the chemical shifts of the isomers. To correlate the theoretical calculated data with the experimental one, the theoretical isotropic shielding constants (σ_{calc}) of carbons were compared with the calculated isotropic shielding constants (σ_{TMS}) for the reference compound tetramethylsilane (TMS) as following: $\delta_{C(calc)} = \sigma_{C(TMS)} - \sigma_{calc}$ where the $\sigma_{C(TMS)} = 196.7367$ ppm was calculated at the same mPW1PW91/6-31G(d,p) level of theory. A supplemental analysis that correlates NMR chemical shifts and statistical analysis, named DP4+, allows the use of Quantum Chemical calculated NMR parameters combined with refined statistical data to elucidate the most likely structure among the isomers.

3. Results and discussion

The alkaloids (see Fig. 1) were isolated from an EtOH extract of *R. ligustrina* roots using chromatographic fractionation techniques (open silica gel column, Sephadex LH-20, C-18 SPE cartridge, and HPLC).

Compound **1** was isolated as brown amorphous powder, $[\alpha]_D^{20} = +9.0$ (*c* 0.1, MeOH). Its HRESIMS displayed a protonated molecule $[M + H]^+$ ion peak at *m/z* 487.2094 (calc for $C_{25}H_{31}N_2O_8$, 487.2080) indicating the molecular formula $C_{25}H_{30}N_2O_8$, which possesses 12 degrees of unsaturation. The ^1H NMR spectrum showed signals to four aromatic protons [δ_H 7.39 (d, $J = 7.9$ Hz, H-9), 7.29 (d, $J = 8.0$ Hz, H-12), 7.06 (t, $J = 7.9$ Hz, H-11), and 6.98 (t, $J = 8.0$ Hz, H-10)], one olefinic proton [δ_H 5.83 (q, $J = 6.8$ Hz, H-19)], as well as signals corresponding to five methine protons [δ_H 5.18 (s, H-21), 4.82 (d, $J = 7.2$ Hz, H-3), 3.86 (m, H-5), 3.33 (m, H-15), and 2.37 (d, $J = 7.2$ Hz, H-16)] being the three first bonded to nitrogenated carbons. Additionally, signals for two pair of diastereotopic methylene protons [δ_H 3.15 (dd, $J = 15.8$, 4.6 Hz, H-6a), 2.74 (d, $J = 15.8$ Hz, H-6b), 2.16 (t, $J = 11.7$ Hz, H-14a), and 1.78 (m, H-14b)] and a methyl group bonded to a sp^2 carbon [δ_H 1.71 (d, $J = 6.8$ Hz, Me-18)] were also observed. These data were suggestive of a sarpagan alkaloid skeleton, commonly found in *Rauvolfia* species [26]. A glucose moiety was evidenced by the proton signal at δ_H 4.67 (d, $J = 7.8$ Hz, H-1′) correlated with the signal at δ_C 103.5 (C-1′) of an anomeric carbon, suggesting a glucosyl sarpagan alkaloid for **1**.

The ^{13}C NMR, HSQC and HMBC spectra displayed signals to 25 carbons classified into one methyl, three methylenes, fifteen methines and six non-hydrogenated carbons, including a signal of a carboxyl acid at δ_C 180.4 (C-17) and five sp^2 hybridized carbons. In the HSQC spectrum, the signals at δ_C 92.2, 53.6 and 45.4, correlated with the signals at δ_H 5.18 (s, H-21), 3.86 (m, H-5) and 4.82 (d, $J = 7.2$ Hz, H-3),

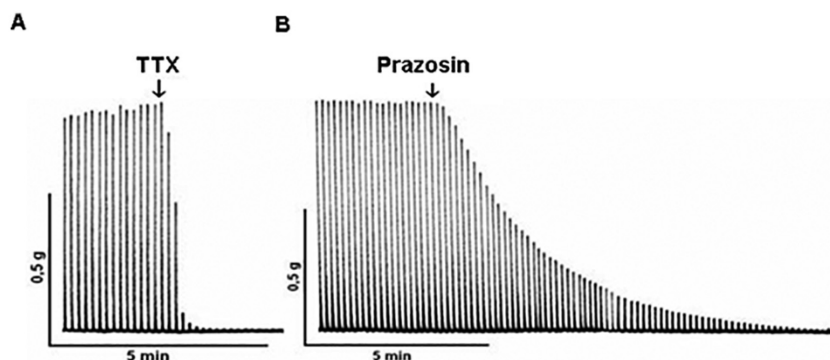

Fig. 2. Experiments showing the nature of the neurotransmission of the mice vas deferens (protocol 1) stimulated by electrical field (30 V; 1 ms; 0.5 Hz) once the twitches are completely inhibited by a neuronal sodium channel blocker, tetrodotoxin (TTX; 10^{-7} M) or by an alpha-adrenergic blocker (prazosin; 10^{-7} M).

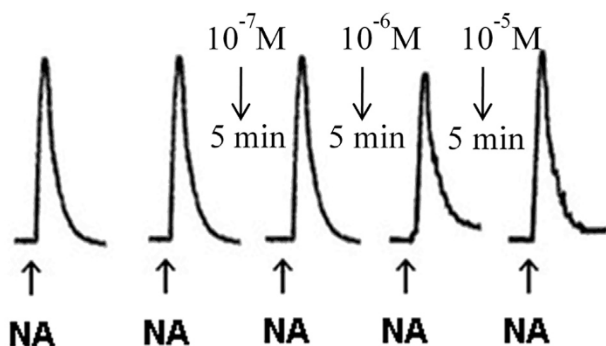

Fig. 3. Representation of the protocol 2 for testing the previously incubated for 5 min *Rauvolfia ligustrina* alkaloids (10^{-7} to 10^{-5} M) in contractions induced by the exogenous noradrenaline (NA; 10^{-5} M) addition in mouse vas deferens (MVD).

respectively, were inferred to the nitrogenated carbons of the sarpagan aglycone. The unequivocal position of the carboxyl acid group was determined based on the HMBC correlations of the methine proton at δ_H 2.37 (d, J = 7.2 Hz, H-16) with the carbon signals at δ_C 180.4 (C-17), 136.0 (C-20), 33.6 (C-14), and 27.6 (C-6). Similarly, the position of the glucosyl moiety was defined based on the cross peak between the methine proton (δ_H 4.67, H-1′) of the anomeric carbon with the downfield nitrogenated carbon at δ_C 92.2 (C-21). Comparison of the above NMR data with those of the sarpagan-type alkaloid rauverine C, previously isolated of *Rauvolfia verticilata* [27], were very similar. The only difference was the absence of the glucose moiety and the carboxyl group, whose C-17 is a sp^3 carbon bonded to two methoxy groups.

The relative configuration of the sarpagan core was deduced based on comparison literature data from analogous compounds and by NOESY spectrum (see Fig. 4) and supported by the literature [28,29].

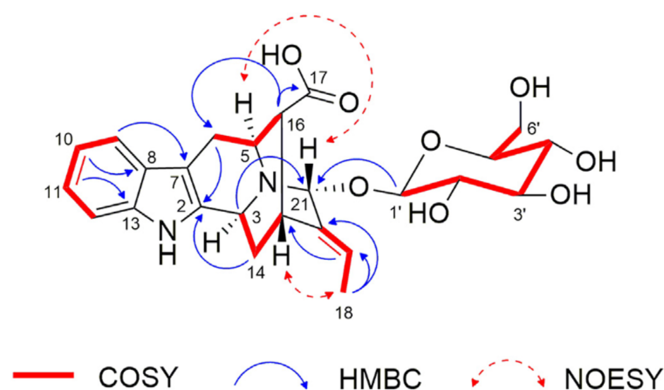

Fig. 4. Key COSY, HMBC, and NOESY correlations of alkaloid **1**.

The NOESY spectrum displayed spatial interactions for H-5 (δ_H 3.86) with H-21 (δ_H 5.18) indicating that *O*-glucose was *α*-oriented, while the dipolar interactions between the Me-18 (δ_H 1.71) and H-15 (δ_H 3.86) supported de *E*-configuration for the double bond.

Unfortunately, C-16 configuration could not be determined from NOESY spectrum, thus, theoretical calculations methods were performed to correctly assign **1** as isomer 16*R* (iso1) or 16*S* (iso2). For these two isomers, the ^{13}C isotropic magnetic shielding ($\delta_{C\ calc}$) values were calculated using GIAO method with mPW1PW91/6-31G(d,p) level of theory. The theoretical ^{13}C isotropic magnetic shielding values predicted for iso1 and iso2 are reported in Table 2 in comparison to the experimental ^{13}C chemical shifts (δ_{exp}). The results obtained (see Fig. 5) indicated that the correlation coefficients (R^2) between the calculated and experimental data from linear regression analysis were 0.9743 (see Fig. 5a) and 0.9741 (see Fig. 5b) for iso1 and iso2, respectively, suggesting iso1 is the favorable isomer.

To verify this assumption, DP4+ probability analysis was applied [30,31], to distinguish between isomers iso1 and iso2. As shown in

Table 2

Calculated ^{13}C nuclear magnetic shielding ($\delta_{C\ calc}$) using GIAO method with mPW1PW91/6-31G(d,p) level of theory and ^{13}C NMR experimental data ($\delta_{C\ exp}$) for iso1 and iso2.

Position	$\delta_{C\ exp}$	$\delta_{C\ calc}$ 16*R* (iso1)	Δδ (iso1)	$\delta_{C\ calc}$ 16*S* (iso2)	Δδ (iso2)
2	138.1	141.9	−3.8	141.3	−3.2
3	45.4	64.0	−18.6	62.9	−17.5
5	53.6	63.8	−10.2	65.8	−12.2
6	27.6	33.6	−6.0	29.7	−2.1
7	104.8	113.8	−9.0	112.4	−7.6
8	129.1	124.0	5.1	123.9	5.2
9	118.7	113.6	5.1	113.6	5.1
10	120.0	116.3	3.7	116.3	3.7
11	122.3	115.7	6.6	115.8	6.5
12	112.1	108.3	3.8	108.3	3.8
13	138.5	130.0	8.5	130.0	8.5
14	33.6	31.9	1.7	29.7	3.9
15	31.3	44.3	−13.0	42.0	−10.7
16	50.0	54.1	−4.1	55.6	−5.6
17	180.4	169.9	10.5	170.8	9.6
18	13.7	15.6	−1.9	15.4	−1.7
19	126.5	120.8	5.7	119.3	7.2
20	136.0	139.7	−3.7	142.5	−6.5
21	92.2	93.7	−1.5	93.4	−1.2
1’	103.5	104.4	−0.9	104.1	−0.6
2’	78.1	68.1	10.0	68.0	10.1
3’	75.4	73.2	2.2	73.1	2.3
4’	71.7	71.6	0.1	71.7	0
5’	78.5	68.4	10.1	68.5	10.0
6’	62.9	61.5	1.4	61.6	1.3
uDP4+		81.02%		18.98%	
sDP4+		98.22%		1.78%	
DP4+		99.58%		0.42%	

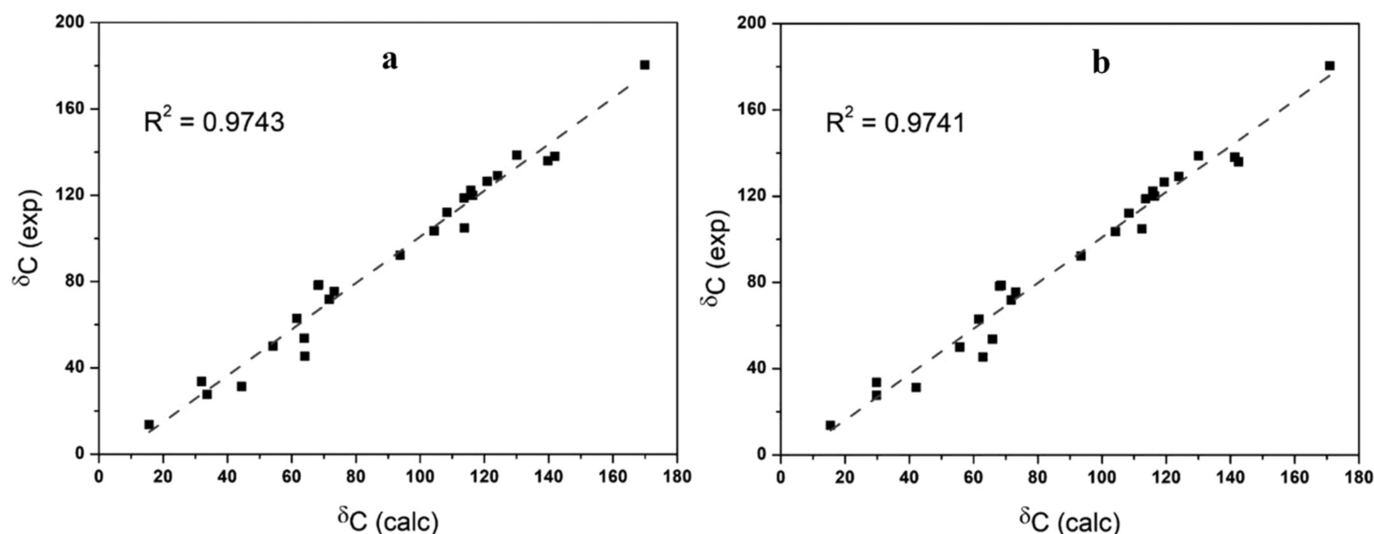

Fig. 5. The correlation between the experimental chemical shift ($\delta_{C\ exp}$) versus the calculated magnetic isotropic shielding ($\delta_{C\ calc}$) using GIAO method with mPW1pw91/6-311G(d,p) level of theory for (a) iso1 and (b) iso2.

Table 2, the unscaled DP4+ (uDP4+), scaled DP4+ (sDP4+) and DP4+ data of the carbons also suggested that the real identity of the conformer is iso1. Fig. S29 and Fig. S30 showed the optimized structure of the conformers iso1 and iso2, respectively, using the Density Functional Theory (DFT) method with mPW1PW91/6-31G(d,p) level of theory. The iso1 and iso2 conformers showed Gibbs free energies (G) of −1,054,909.95 kcal mol^{-1} (G_1) and − 1,054,906.25 kcal mol^{-1} (G_2), respectively, with an energy difference ($\Delta G = G_1 - G_2$) of −3.7004 kcal mol^{-1}, which gives greater stability to the iso 1 conformer. Accordingly, the structure of **1** was established as the new glycosylated sarpagan alkaloid 21(*R**)-(*O*-*β*-glucosyl)-hydroxy-sarpagan-17-oic acid.

To the best of our knowledge, sarpagan glucosyl alkaloids were only reported to *Rauvolfia* leading us to suggest these compounds as possible chemomarker to the genus [32–34].

A plausible biogenetic pathway for alkaloid **1** was suggested based on the biosynthesis of monoterpenoid indole alkaloids sarpagine (via vellosimine) and ajmaline (via 16-*epi*-vellosimine) from *Rauvolvia* [26,35] As well-established the first step consist of condensation via Pictet-Spengler reaction between tryptamine and secologanin to afford strictosidine which is converted via several steps into 16-*epi*-vellosimine that epimerize in vellosimine. In the sequence, hydroxylation followed by glycosylation could afford compound **1** as summarized in Fig. 6.

In addition to the new glycosylated sarpagan alkaloid (**1**) the known indole alkaloids reserpine (**2**) [32], isocarapanaubine (**3**) [36], deserpidine (**4**) [37], isoreserpine (**5**) [38], raucaffricine (**6**) [39], aricine (**7**) [40], isoreserpiline (**8**) [41], yohimbic acid (**9**) [42], *α*-yohimbine (**10**) [43], corynanthine (**11**) [43], and 18*β*-hydroxy-3-epi-*α*-yohimbine (**12**) [44] were also isolated.

Alkaloids **1–12** (see Fig. 1) were evaluated for their neurogenic contraction inhibition. According to protocol 1, compounds 21(*R**)-(*O*-*β*-glucosyl)-hydroxy-sarpagan-17-oic acid (**1**), reserpine (**2**) and yohimbic acid (**9**) were the most active, inhibiting the contractions by 87.9%, 86.3%, and 81.2%, respectively, followed by *α*-yohimbine (**10**) (76.0%) and isoreserpiline (**8**) (52.2%) (see Fig. 7) indicating a possible antihypertensive activity. It is worth mentioning that this effect supports the antihypertensive efficacy of the indole alkaloids since the vascular tonus is maintained by the noradrenergic activity [45]. Additionally, alkaloids **1**, **2** and **9** are expected to exhibit activity related to male sexual dysfunction as premature ejaculation, for instance, since drugs that inhibit the vas deferens contractions and neurotransmission could retard ejaculation [46].

In a previous study [47], sarpagan-type alkaloids, like compound (**1**), demonstrated to reduce the mechanical and electrical activity of vascular smooth muscle by decreasing Na^{+} and Ca^{2+} conductance, which could also justify its inhibitory activity in the mice vas deferens reported herein. Relatively low concentration (100 nmol/L) of yohimbine (**10**) can reduce the noradrenergic component of neurogenic twitches of the vas deferens by its nonspecific *α*-adrenergic blocking properties [48]. The neurotransmission in the epididymal segment of the mice vas deferens is mainly sympathetic with both *α*-1 and *α*-2 postsynaptic adrenoceptors expressed and mediating contraction [49]. Yohimbic acid (**9**), reserpine (**2**) and its congeners such as isoreserpiline (**8**) reduce the neurogenic twitches of the epididymal segment of the mice vas deferens by its sympatholytic activity [50]. The molecular mechanism involved for reserpine (**2**) is the inhibition of the binding of monoamines such as noradrenaline and dopamine to the vesicular monoamine transporters (VMATs) proteins in the storage vesicles [51].

In contrast, alkaloids isocarapanaubine (**3**) and corynanthine (**11**) exhibited an acute neuroexcitatory effect (see Fig. 8) inducing contractions in the tissue by 298.1% and 613.5%, respectively, suggesting an antihypotensive activity. Alkaloid **11** has *α*-1 adrenergic receptor affinity higher than *α*-2 adrenergic receptor affinity and was shown to potentiate sympathetic nerve stimulation in the isolated cervical ganglia of dogs if the dose was increased sufficiently [52]. We have found that at 10 μM, this compound, can also potentiate the sympathetic neurotransmission in the mice vas deferens probably by a partial *α*-1 adrenergic agonist. This is the first report on the increase in noradrenergic response induced by isocarapanaubine (**3**), but its mechanism of action was not addressed in the present study.

The maximal inhibitory response and concentrations necessary to induce half of the maximal inhibitory effect (IC_{50}) are shown in Table S2 (supplementary material). According to protocol 2, the muscular response to the administration of 10^{-5} M noradrenaline was inhibited by **2** (36.5%), **7** (37.7%), **8** (51.1%) and more efficiently by **9** with a maximal inhibitory effect of 82.4% (see Fig. 9).

4. Conclusions

A new glycosylated sarpagan alkaloid 21(*R**)-(*O*-*β*-glucosyl)-hydroxy-sarpagan-17-oic acid **1** was isolated from the roots of *R. ligustrina* along with eleven known alkaloids **2–12**. Based on the results, three different types of action in the noradrenergic neurotransmission were observed. Compounds **1**, **2** and **9** presented a dose-related, reversible and potent inhibitory effect in the neurotransmission while compounds **3** and **11** presented acute neuroexcitatory effects. On the other hand,

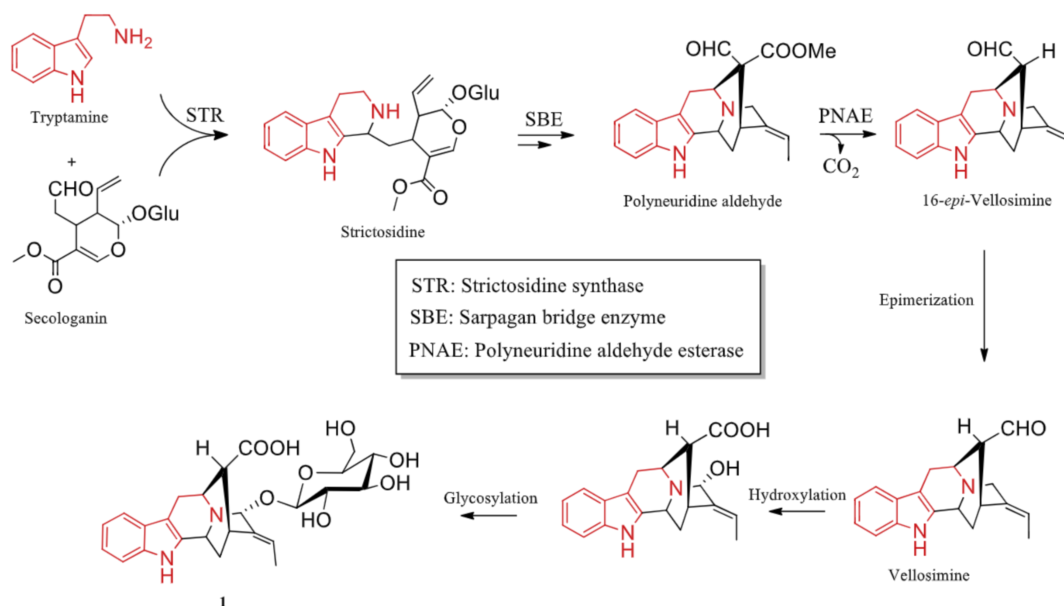

Fig. 6. Reasonable biogenetic pathways for alkaloid **1**.

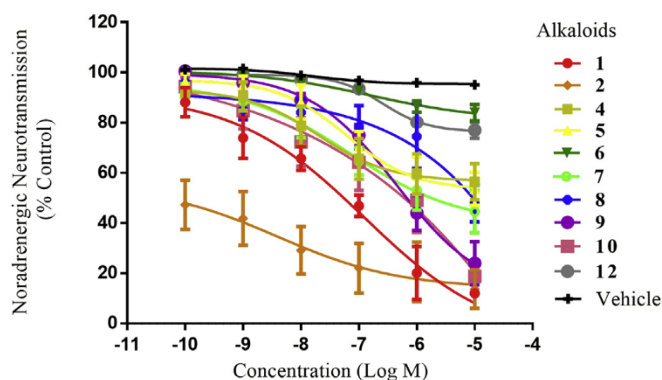

Fig. 7. Concentration-response curves (10^{-10} to 10^{-5} M) to the *Rauwolfia ligustrina* alkaloids (**1**, **2**, **4–10** and **12**) in the noradrenergic neurotransmission elicited by electrical field stimulation of the mice vas deferens.

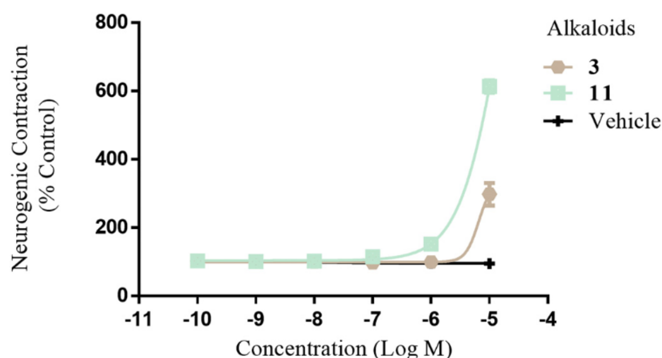

Fig. 8. Excitatory effect in the noradrenergic neurotransmission induced by **3** and **11**.

compound **10** displayed an effective post-synaptic inhibitory activity. Such compounds could be useful for the study of the role of noradrenergic neurons, since they can be both stimulated and inhibited in a reversible form, for example.

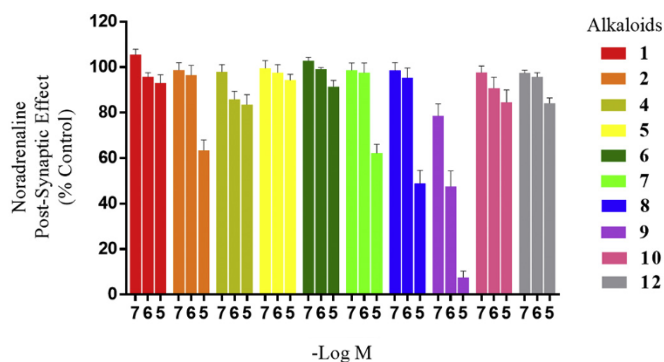

Fig. 9. Effect of single concentrations of *Rauvolfia ligustrina* alkaloids **1**, **2**, **4–10** and **12** (10^{-7}, 10^{-6} or 10^{-5} M) in the muscular contracture evoked by 10^{-5} M noradrenaline in the mice vas deferens.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary information (^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, NOESY, and HRESIMS spectra) is available free of charge at doi:https://doi.org/10.1016/j.fitote.2020.104545

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