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Structural characterization of saturated pyrrolizidine alkaloids from *Heliotropium transalpinum* var. *transalpinum* Vell by NMR spectroscopy and theoretical calculations

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ABSTRACT

The chemical investigation of *Heliotropium transalpinum* var. *transalpinum* Vell. (Boraginaceae) led to the isolation of transalpinecine (**1**), a novel pyrrolizidine alkaloid, in addition to known alkaloids subulacine (1β-2β-epoxy-1α-hydroxymethyl-8α-pyrrolizidine) (**2**), and 1α-2α-epoxy-1β-hydroxymethyl-8α-pyrrolizidine (**3**). The structures of the isolated compounds were elucidated based on spectroscopic data and theoretical calculations.

Heliotropium species (Boraginaceae) have been long used in Brazilian folk medicine for the treatment of gout, rheumatism, and as anti-inflammatory and healing agents.[1,2] The main constituents of *Heliotropium* species, pyrrolizidine alkaloids (PAs), are responsible for several biological activities, including antitumoral, anti-microbial, and anti-viral activities.[3–7] Phenolic compounds, terpenoids, and quinones have also been reported in this genus.[8–10] In this work, we describe the isolation and structure elucidation of a new PA, transalpinecin (1β-2β-dihydroxy-1α-hydroxymethyl-8α-pyrrolizidine) (**1**) in addition to alkaloids, 1β-2β-epoxy-1α-hydroxymethyl-8α-pyrrolizidine, namely, subulacine (**2**), and its isomer 1α-2α-epoxy-1β-hydroxymethyl-8α-pyrrolizidine (**3**) from the aerial parts of *Heliotropium transalpinum* var. *transalpinum* (Fig. 1). Compound **3** was previously synthesized[11,12]; however, this is the first report of its occurrence as a natural product. Due to the structural similarity of alkaloids **2** and **3**, the assignment of their NMR spectra based only on substituent effects is difficult, so we decided to run theoretical calculation in order to corroborate the experimental results.

The ^{1}H and ^{13}C NMR data for isolated alkaloids **1**, **2**, and **3** are presented in Table 1. Alkaloids **2** and **3** contain an epoxy ring at C-1 and C-2. This type of pyrrolizidine alkaloid is usually found in the *Crotalaria* genus (Leguminoseae),[13] but it has been detected in *Heliotropium* genus as a minor alkaloid.[14,15]

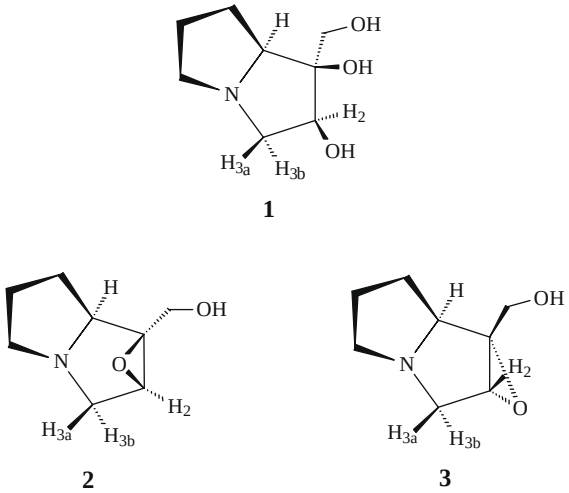

Figure 1. Saturated pyrrolizidine alkaloids.

The new alkaloid, 1β-2β-hydroxy-1α-hydroxymethyl-8α-pyrrolizidine (**1**), was isolated as an amorphous yellow powder. Its

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Table 1
^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD) spectral data for compounds **1**, **2**, and **3**[a]

Position	1		2		3	
	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C
1		82.3		70.9		83.7
2	4.81 d (3.3)	84.4	3.95 sl	63.4	4.59 d (3.6)	65.2
3	3.86 d (12.0)	59.2	3.79 dd (13.8, 1.2)	54.0	3.81 dd (3.6, 12.9)	62.3
	3.38 d (12.0, 3.3)		3.38 d (13.8)		3.89 d (12.9)	
5	3.06 dt (910.8, 3.3)	55.9	2.97 m	58.4	3.23 m (11.4)	56.2
	3.36		3.62 m		3.50 m (11.4, 6.3)	
	Overlapped					
6	1.95 m	28.0	1.96 m	27.1	2.08 m	27.9
	2.16 m		2.24 m		2.23 m	
7	1.88 m	24.6	2.19 m	26.2	2.00 m	25.0
	2.26		2.26 m		2.30 m	
8	3.99 dd (4.8, 8.7)	72.3	4.45 dd (6.3, 8.1)	67.9	4.31 dd (4.2, 8.7)	72.1
9	3.90 d (12.3)	63.9	3.97 d (12.9)	60.2	3.93 d (11.4)	64.0
	3.61 d (12.3)		3.78 d (12.9)		3.69 (11.4)	

[a] Assignments based on ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra.

molecular formula was deduced by HRESIMS to be $C_8H_{15}NO_3$ due the presence of the $[M+H]^+$ peak at *m/z* 174.1181 (calcd for $C_8H_{15}NO_3$ 173.2136). The EI-MS mass fragmentation pattern peaks at *m/z* 98, 83, and 70 were characteristic of the 1,1-disubstituted-pyrrolizidine skeleton.[15] The presence of a saturated 1,1,2-trisubstituted-pyrrolizidine skeleton was supported by comparing ^{1}H and ^{13}C NMR data from **1** (Table 1) with those reported for related PAs.[16–18] The ^{13}C NMR and DEPT spectral data exhibited eight carbons, including two methine, five methylene, and one quaternary carbon. The evidence for the presence of a hydroxyl group at C-2 was based on the methine carbon signal at δ_C 84.4, correlated to the signals at δ_H 3.99 (H-8), δ_H 3.61, and 3.90 (H-9) in the HMBC spectra. Additionally, the multiplicity observed for H-2 and COSY data was in agreement with the proposed structure. The ^{1}H–^{1}H COSY showed correlation between the signal at δ_H 4.81 (*d*, *J* = 3.3 Hz, H-2) with δ_H 3.38 (dd, J_{gem} = 12.0 and *J* = 3.3 Hz, H-3a) and at δ_H 3.99 (dd, *J* = 4.8 and *J* = 8.7 Hz, H-8) with δ_H 1.88 (*m*, H-7a), confirming the connection between C-2/C-3 and C-7/C-8. The downfield signal at δ_C 82.3 was assigned to C-1, a quaternary carbon, which was confirmed by its ^{3}*J*-HMBC coupling with the signal at δ_H 3.86 (H-3b) and at δ_H 3.61/3.90 (H-9a/H9b) and at δ_H 1.96/2.16 (H-6a/H-6b). The stereochemistry was established by NOE experiments. NOE interactions between H-2/H-3a/H-9 and H-8/H-7a/H-6a/H-9 were evident. Thus, the structure of **1** was established as 1β,2β-dihydroxy-1α-hydroxymethyl-8α-pyrrolizidine.

Alkaloid **2** spectral data (EI-MS, HR-ESIMS, ^{1}H and ^{13}C NMR) agreed with those reported for subulacine, 1β,2β-epoxy-1α-hydroxymethyl-8α−pyrrolizidine, which was isolated from *Crotalaria* and *Heliotropium* genus.[12,13] Its complete assignments are reported in this work.

Alkaloid **3** was obtained as an amorphous yellow powder. The fragmentation pattern observed in the EI-MS spectra of **3** at *m/z* 124 and 70 was consistent with the presence of a 1,2-epoxy-pyrrolizidine skeleton.[14] The HRESIMS of **3** showed a $[M+H]^+$ ion at *m/z* 156.1400 corresponding to the molecular formula $C_8H_{13}NO_2$, similar

Table 2
Structural parameters[a], energy, and dipole moment for alkaloids **2** and **3**

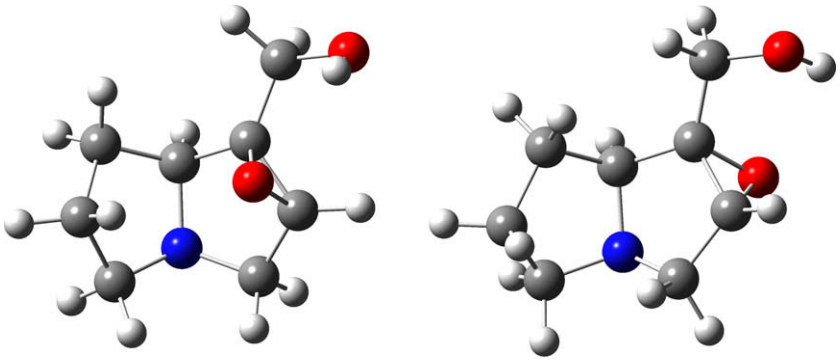
Parameters	2	3
∠ (C_2–C_3–N)	106.3	104.6
ϕ (H_2–C_2–C_3–$H_{3\alpha}$)	139.7	108.1
ϕ (H_2–C_2–C_3–$H_{3\beta}$)	78.0	46.8
μ (D)	0.82	2.56
E (hartrees)	−517.836348	−517.837760

[a] Bond angles and dihedral angles are in degrees.

Table 3
Interaction energies (kcal/mol) to donor and acceptor orbitals for C-1, C-3, and C-8 in compounds **2** and **3**

	2			3		
	C-1	C-3	C-8	C-1	C-3	C-8
NBO donor	48.45	26.03	37.14	49.30	26.87	36.75
NBO acceptor	60.39	19.63	30.72	57.29	21.35	27.54
Difference[a]	−11.94	6.40	6.42	−7.99	5.52	9.21

[a] Difference = NBO donor−NBO acceptor.



2 **3**

Figure 2. Lowest energy conformations for compounds **2** and **3**.

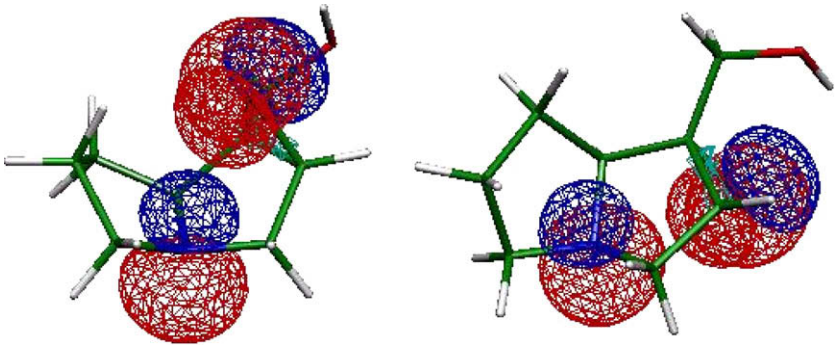


Figure 3. Lone pair interactions for nitrogen and oxygen in compounds **2** and **3**.

to subulacine (**2**). The ^{1}H and ^{1}H–^{1}H COSY NMR data of **3** were closely related to those of compound **2**. As observed for compound **2**, the ^{13}C NMR and DEPT spectra of **3** exhibited eight signals: two CH, five CH_2, and one quaternary carbon. The principal difference between these compounds was the deshielding observed for C-1 (δ_C 83.7), C-3 (δ_C 62.5), and C-8 (δ_C 72.1) in the ^{13}C NMR spectra of **3** (Table 1), comparing to that of subulacine (**2**). These data suggest that compound **3** is an isomer of subulacine (**2**), probably containing the epoxy group in the α-position. Thus, compound **3** was determined to be 1α-2α-epoxy-1β-hydroxymethyl-8α-pyrrolizidine.

Based on the structural similarity of alkaloids **2** and **3**, molecular modeling studies were performed to corroborate the experimental results. The lowest energy conformations adopted by **2** and **3** are shown in Figure 2. The stability of these conformations is probably due to a hydrogen bond between O–H and the oxygen atom of the epoxy group. Some structural parameters obtained from optimization calculations are presented in Table 2. These data show that changing the position of the epoxy group leads to a major modification of the dipole moment of these compounds. The theoretical dipole moment values are in accordance with experimental observations. When eluted through a silica column, the retention time of compound **2** was lower than that of **3**, which is in agreement with its lower dipole moment (Table 2).

The NMR data also showed important differences between alkaloids **2** and **3**. The vicinal coupling is $^{3}J_{H3\alpha\text{-}H2}$ = 1.2 Hz in **2** while in **3** the observed coupling is $^{3}J_{H3\beta\text{-}H2}$ = 3.6 Hz. These observations are in accordance with the calculated dihedral angles between H-3α/H-3β and H-2 (Table 2). The dihedral angle (H_2–C_2–C_3–$H_{3\beta}$) in **2** and (H_2–C_2–C_3–$H_{3\alpha}$ in **3** are very close to 90°; thus, no coupling is observed between these protons.

Although the difference between the structures of **2** and **3** is only on the epoxy group stereochemistry, it observed large deviations in the chemical shifts of some carbons, mainly C-1, C-3, and C-8. The chemical shifts for these carbons in **2** are shielded around 10 ppm each compared with the same chemical shifts in **3**. In order to investigate these shielding effects, the orbital interactions were calculated using natural bond orbital (NBO) theory. These interactions may reflect the electron transference (hyperconjugative effect) between the orbital localized on the atoms, so it is possible to determine the difference between the interaction energy of donor and acceptor orbitals. In other words, it is possible to evaluate the change in electron density over a specific atom. Table 3 summarizes the total interaction energy for donor and acceptor orbitals. The difference between them indicates the atom capacity to act as donor or acceptor of electronic density.

Analyzing the data in Table 3, we observe that C-1 accepts more electron density in **2** than in **3**, shielding this atom in **2**. Furthermore, we observe that C-8 is a stronger donor in **3** than in **2**, and is thus more deshielded in **3**, which is in agreement with the observed chemical shifts (Table 1). The differences in donor/acceptor energies for C-3 in **2** and in **3** are negligible (below 1 kcal/mol), meaning that the hyperconjugative effect could not drive the shielding in C-3. Therefore, the influence of steric factors on chemical shifts was investigated. The lone pair orbitals from NBO analysis for nitrogen and oxygen atoms were plotted (Fig. 3) to visualize the repulsive interactions between them.

The nitrogen and oxygen lone pairs in compound **2** cause a steric compression under H-3α and H-3β, shielding C-3. Conversely, the repulsive interaction between the lone pairs on nitrogen and oxygen in compound **3** increases their distance and, consequently, diminishes the steric compression. To check these suppositions, the bond angles C_2–C_3–N (Table 2) of both compounds were compared. The bond angle is smaller in **3** (104.6°) than in **2** (106.3°) in order to decrease the repulsive interaction between the lone pairs.

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Supplementary data

Supplementary data (experimental procedures and computation details) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.074.

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