Pyrrolizidine Alkaloids Necine Bases: *Ab Initio*, Semiempirical, and Molecular Mechanics Approaches to Molecular Properties

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ABSTRACT

The structural stabilities of endo and exo conformations of retronecine and heliotridine molecules were analyzed using different *ab initio*, semiempirical, and molecular mechanics methods. All electron and pseudopotential *ab initio* calculations at the Hartree-Fock level of theory with 6-31G* and CEP-31G* basis sets provided structures in excellent agreement with available experimental results obtained from X-ray crystal structure and ^{1}H-NMR (nuclear magnetic resonance) studies in D_2O solutions. The exo conformations showed a greater stability for both molecules. The most significant difference between the calculations was found in the ring planarity of heliotridine, whose distortion was associated with the interaction between the O(11)H group and the C(1)-C(2) double bond as well as with a hydrogen bond between O(11)H and N(4). The discrepancy between pseudopotential and all-electron optimized geometries was reduced after inclusion of the innermost electrons of C(1), C(2), and N(4) in the core potential calculation. The MNDO, AM1, and PM3 semiempirical results showed poor agreement with experimental data. The five-membered rings were observed to be planar for AM1 and MNDO calculations. The PM3 calculations for *exo*-retronecine showed a greater stability than the endo conformer, in agreement with *ab initio* results. A good agreement was observed between MM3 and *ab initio* geometries, with small differences probably due to hydrogen

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bonds. While *exo*-retronecine was calculated to be more stable than the endo conformer, the MM3 calculations suggested that *endo*-heliotridine was slightly more stable than the exo form. © 1996 by John Wiley & Sons, Inc.

Introduction

Pyrrolizidine alkaloids (PAs) are a class of natural products including about 350 structures isolated from plants (especially *Senecio, Eupatorium*—Asteraceae, *Crotalaria*—Fabaceae, and *Heliotropium*—Boraginaceae).[1–3] About 3% of all angiosperms contain these alkaloids.

The natural pyrrolizidine (1-azabicycle-[3.3.0]-octane) moiety of these alkaloids, called a necine base, is almost always an unsaturated 1-hydroxymethylpyrrolizidine (Fig. 1a). These bases have

different patterns of hydroxylation, unsaturation, and stereochemistry. Generally, they are esterified at C9 and/or C7 with necic acids and can occur as monoesters (Fig. 1b), diesters (Fig. 1c), or macrocyclics (Fig. 1d). The alkaloids occur in plants mainly as the *N*-oxide.[5]

PAs present a wide spectrum of biological activities.[2,6] Alkaloids with 1,2-unsaturated necine bases are hepatotoxic and genotoxic. These PAs are also important chemical mediators in interactions among lepidopterans (Arctiidae moths and Danainae and Ithomiinae butterflies) and plants. Lepidopterans in the larval or adult stage form obtain these alkaloids from plants and use them
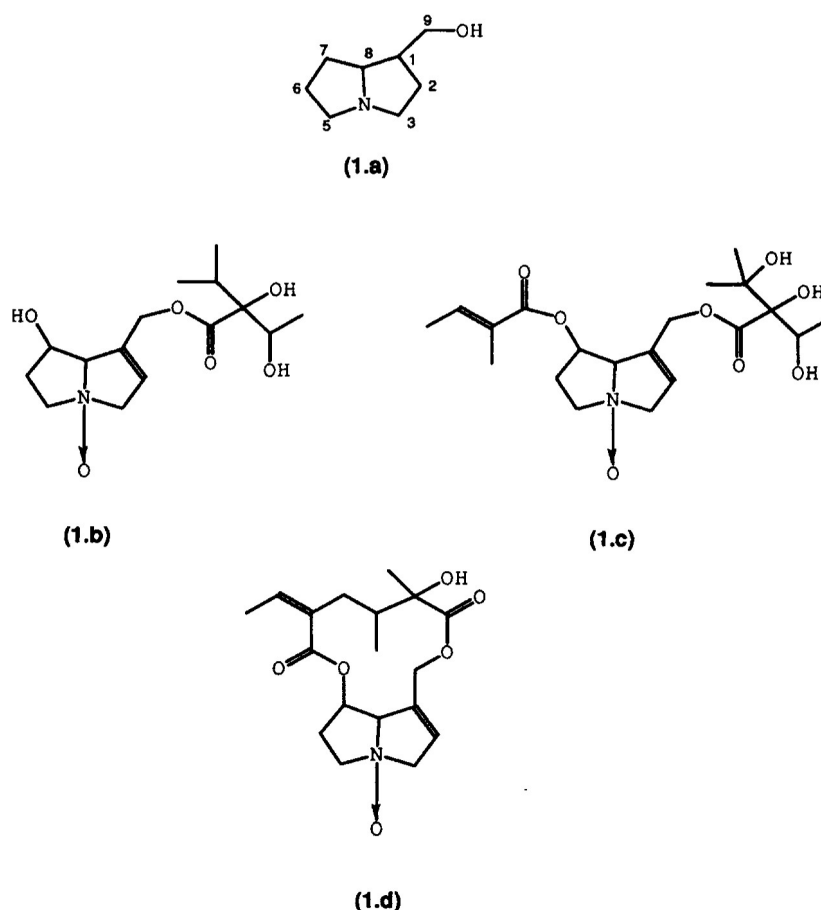

(1.a)

(1.b)

(1.c)

(1.d)

FIGURE 1. Some structures of necine bases: (a) 1-hydroxymethylpyrrolizidine, (b) 1,2-unsaturated *N*-oxide monoester, (c) 1,2-unsaturated *N*-oxide diester, and (d) 1,2-unsaturated *n*-oxide macrocyclic diester.

for protection against predation and as pheromone precursors.[7,8]

All natural 1,2-unsaturated macrocyclic PAs show the 7R configuration. However, natural 7-OH monoesters show both 7R and 7S configurations in plants,[3] while in lepidopterans the usual configuration is 7R.[9-16] Trigo[15] verified that the 7R configuration was predominant (and sometimes 100%) in butterflies, even when feeding on plants containing 7S molecules. He suggested that stereochemical inversion at C(7) could occur enzymatically and depend on the conformation of the necine bases of these alkaloids. ^{1}H-NMR studies suggested that the two most common necine bases have typical conformations: exo buckled in 7R-(+)-retronecine (Fig. 2a), and exo (Fig. 2c) and endo buckled (Fig. 2d) (1:2) in 7S-(+)-heliotridine.[17] The puckering of these compounds is defined by the relative position of the C6 atom and the unsaturated ring C(1)-C(2)-C(3)-N(4)-C(8) with

relation to the plane defined by atoms N(4)-C(5)-C(7) (Fig. 2). The puckering is endo (Figs. 2b and 2d) when the atoms are found on the same side of this plane and exo (Figs. 2a and 2c) when they are on opposite sides.[18]

Retronecine and heliotridine represent the structures of the necine bases in almost all 1,2-unsaturated PAs. Additional information on the molecular structure of these two compounds is strongly desirable for ecological research. The absence of any theoretical information on these two substances suggests that various approaches with molecular orbitals and molecular mechanics methods should be tried. This work compares the performance of *ab initio*, semiempirical, and molecular mechanics analyses of the conformational stability of retronecine and heliotridine. A choice among these methods should represent a compromise between accuracy and computational costs in the study of larger PA molecules. The results will
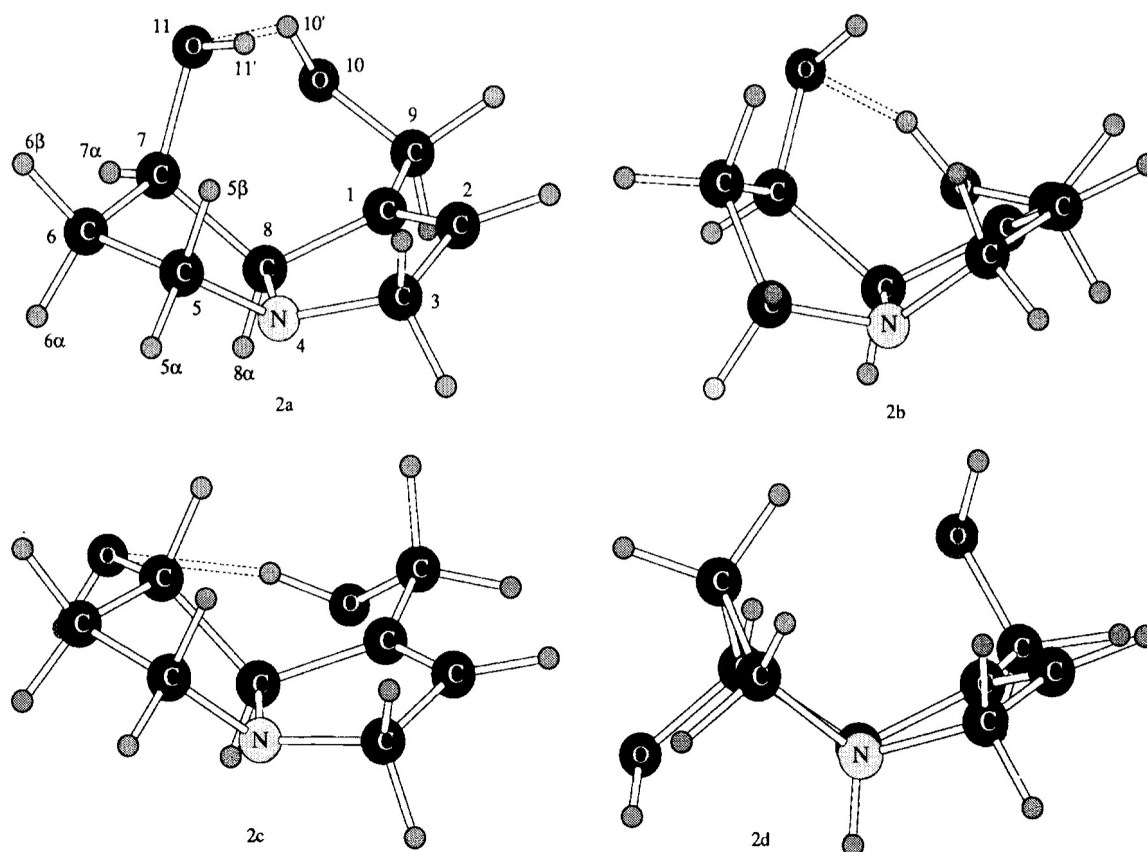

FIGURE 2. Conformations of *exo* (a) and *endo* (b) retronecine and *exo* (c) and *endo* (d) heliotridine showing the presence of hydrogen bonds and the numerical index of the hydrogens in the saturated ring.

be analyzed by statistical methods, comparing the calculated data with available experimental information.

Methods

All the *ab initio* calculations were carried out with the Gaussian 92 program.[19] The standard 6-31G* basis set was used for most of the computations. Calculations were also done with the compact effective potential basis sets (CEP-31G*).[20] All the structures were fully optimized at the Hartree-Fock level of theory using internal coordinates; some tests were also carried out using cartesian coordinates. The results were identical with both coordinate systems.

The Gaussian 92 program was also used for the calculations of retronecine and heliotridine with three semiempirical methods: MNDO,[21] PM3,[22] and AM1.[23] Tight convergence criteria were employed in the geometrical optimization of the semiempirical hamiltonians. The MNDO, PM3, and AM1 parameters are those from the original references.[21-23]

The MM3/92 molecular mechanics program[24] was used to study the same molecules. Default parameters were used in the MM3 force field calculation.[25] The geometries were also fully optimized either at the semiempirical level or with the molecular mechanics model.

The similarity among the equilibrium geometries was analyzed using the average error (AE) as

$$AE = \sqrt{\dfrac{\displaystyle\sum_{i=1}^{N'} (y_i^{6\text{-}31G^*} - y_i)^2}{N}} \qquad (1)$$

where $y_i^{6\text{-}31G^*}$ refers to the ith geometric parameters calculated with the 6-31G* basis set and y_i to the ith parameter calculated using any of the other methods.

The different magnitude of the geometrical parameters suggests that a reasonable analysis should be made considering the bond lengths, bond angles, and dihedral angles separately. Another reason for this separation is the source of experimental data. Some experimental data are from X-ray crystal analysis,[26] and others are from D_2O solution structures from ${}^1H$-NMR.[17]

To evaluate the distortion of both five-membered rings from the planar conformation, only two dihedral angles of each ring were analyzed; the two other dihedral angles were used to discuss the fusion of the ring, as described later. Even though degeneracy should be present among the bond angles defined by the z-matrix, all angles were used in the comparison among the methods. The bond distance comparisons were also based on values defined by the z-matrix obtained as indicated in Figure 2a.

Results and Discussion

ALL-ELECTRON *AB INITIO* CALCULATIONS

Figures 3a and 3b show the bond lengths for *exo*-retronecine and *endo*-heliotridine obtained from X-ray diffraction experiment (values in parentheses)[26] and calculated at the Hartree-Fock level of theory using the 6-31G* basis set. The average difference between experimental and theoretical results for both molecules is approximately 0.014 Å. It is well known that *ab initio* bond lengths (r_e) and X-ray diffraction results (r_α) are different types of measurements. However, considering that the experimental error is around 0.005 Å and that Hehre et al.[27] report for several organic molecules differences in the range of 0.01 Å to 0.02 Å between 6-31G* (r_e) and X-ray diffraction bond distances, the calculated deviation in this work indicates a good agreement between the bond lengths obtained by the two methods.

X-ray diffraction does not provide reliable bond distances involving hydrogen atoms to compare with our *ab initio* results. However, typical CH and OH bond lengths obtained from microwave and electron diffraction measurements[28] are close to our *ab initio* results.

Theoretical bond angles are compared with experimental data in Table I. Once again, there is good agreement between the X-ray results and *ab initio* calculations, with an average difference around 1° for retronecine and 1.4° for heliotridine, expected at this level of calculation.[27] In both molecules, the largest errors involve the N(4) atom at the A-N(4)-B vertices. The N(4) atom occupies one of the two positions of ring condensation; the HO(10) atoms form an intermolecular hydrogen bond with the lone pair of N(4),[29] which can shift N(4) downward, diminishing the A-N(4)-B bond angles in the crystal structure. Smaller A-N(4)-B angles are also observed in two retronecine monoester PAs[30] and in one heliotridine monoester[29]
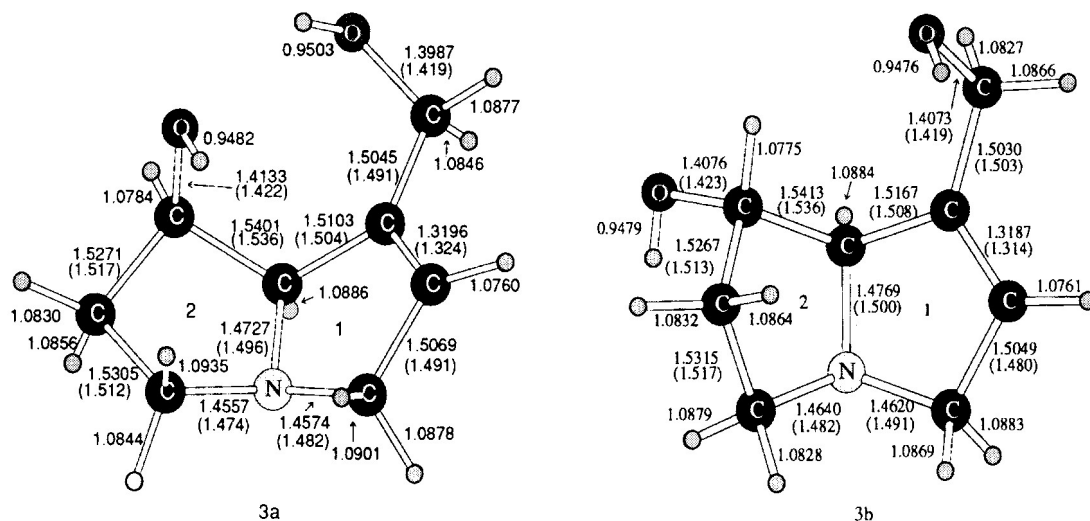
FIGURE 3. Bond distances (in Å) of exo-retronecine (a) and endo-heliotridine (b) calculated at the Hartree-Fock level of theory with the 6-31G* basis set and obtained from X-ray results (values in parentheses). Numbers 1 and 2 identify the five-membered rings in these compounds.

which present hydrogen bonds with N(4). So the larger discrepancy between 6-31G* and X-ray diffraction values for these angles could be related to interactions present in the crystal structure.

A significant deviation between calculated and experimental bond angle is also observed for C(8)-C(7)-O(11): Retronecine and heliotridine present

TABLE I.
Bond angles (in Degrees) of Retronecine and Heliotridine Calculated at the Hartree-Fock Level of Theory with the 6-31G* Basis Set and Obtained from X-Ray Analysis.[26]

Angle	Retronecine			Heliotrine		
	ab initio	Δ	Exp.	ab initio	Δ	Exp.
C5-N4-C3	117.9	2.8	115.1	116.8	2.5	114.3
C8-N4-C3	109.9	1.5	108.4	108.8	1.3	107.5
C8-N4-C5	110.1	2.9	107.2	108.7	2.9	105.8
C8-C1-C2	110.0	-0.8	110.8	110.0	-0.9	110.9
C9-C1-C2	127.4	0.7	126.7	126.4	-1.8	128.2
C9-C1-C8	122.5	0.5	122.0	123.6	2.7	120.9
C3-C2-C1	111.9	-0.1	112.0	111.6	-0.7	112.3
C2-C3-N4	103.8	-0.8	104.6	104.5	-0.5	105.0
C6-C5-N4	104.0	0.0	104.0	105.7	0.3	105.4
C7-C6-C5	102.9	0.0	102.9	102.3	0.8	101.5
C6-C7-O11	111.9	0.8	111.1	112.0	0.3	112.3
C8-C7-O11	112.9	3.9	109.0	111.5	4.7	106.8
C8-C7-C6	101.8	-0.1	101.9	102.8	-0.6	103.4
C1-C8-N4	104.3	0.0	104.3	104.4	0.2	104.2
C7-C8-N4	105.5	-1.2	106.7	106.2	-0.5	106.7
C7-C8-C1	117.0	-0.2	117.2	117.4	2.1	115.3
C1-C9-O10	113.4	-0.1	113.5	113.0	-0.5	113.5

The difference between theoretical and experimental bond angles (θ) was assigned as $\Delta = \theta_{theoretical} - \theta_{experimental}$.

deviations of 3.9° and 4.7°, respectively. The X-ray data suggest that OH groups present an antiparallel conformation that is characteristic of these diasteroisomer crystal structures.[26] The absence of antiparallel conformation in retronecine and heliotridine esters is related to a larger C(8)-C(7)-O(11) angle[29, 30] in better agreement with our ab initio results for necine basis. In this sense, the antiparallel orientation of the OH groups in the crystal structures of the necine bases provides a smaller C(8)-C(7)-O(11) angle than in similar alkaloids. The intermolecular hydrogen bond between O(10)H and N(4) stabilizes the crystal structure and possibly compensates for the C(8)-C(7)-O(11) angular torsion, which is related to the antiparallel orientation. ^{1}H-NMR studies of retronecine in aqueous solution showed the presence of intramolecular hydrogen bonds in both the endo and exo conformations.[17] These results indicate a large flexibility of the OH group orientation in the necine bases, depending on the molecular environment in which the diasteroisomer is enclosed.

Table II shows the dihedral angles of ring conformations obtained from crystal structures[26] for both diasteroisomers and calculated at Hartree-Fock level with the 6-31G* basis set. A strong exo-puckered conformation of retronecine is observed in the crystal structure, while heliotridine presents an endo-puckered conformation. The retronecine structure presents an average difference between ab initio and experimental results of 2.4°, while for the heliotridine the average differ-

TABLE II.

Dihedral Angles (in Degrees) of Retronecine and Heliotridine Calculated at the Hartree-Fock Level of Theory with 6-31G* and Obtained from X-Ray Analysis.[26]

Angle	exo-retronecine			endo-heliotridine		
	ab initio	Δ	Exper.	ab initio	Δ	Exper.
C5-N4-C8-C1	129.6	5.6	124.8	119.4	-5.9	125.3
C7-C8-N4-C3	-125.6	-0.9	-124.7	-133.5	-13.9	-119.6
N4-C5-C6-C7	-36.0	4.3	-40.3	34.6	-6.4	41.0
C8-C7-C6-C5	38.8	-0.6	39.4	-36.8	1.3	-38.1
C1-C8-N4-C3	-1.8	-1.7	-0.1	-8.8	-11.6	2.8
C2-C3-N4-C8	2.5	3.3	-0.8	8.1	10.5	-2.4

The difference between theoretical and experimental diedral angles (ϕ) is assigned as $\Delta = \phi_{theoretical} - \phi_{experimental}$.

TABLE III.

Dihedral Angles (in Degrees) of Vicinal Hydrogens of exo-Retronecine and endo-Heliotridine Calculated at the Hartree-Fock Level of Theory with 6-31G* and Obtained from ^{1}H-NMR Study in D_2O Solution.[17]

Angle	exo-retronecine		endo-heliotridine		exo-heliotridine	
	ab initio	Exper.	ab initio	Exper.	ab initio	Exper.
5α,6α	42.3	40	36.8	45	45.2	40
5α,6β	79.7	84	159.0	167	76.8	84
5β,6α	161.6	165	82.6	78	165.1	165
5β,6β	39.6	41	39.6	43	43.2	41
6α,7α	42.6	40	80.0	76	164.9	162
6β,7α	78.4	84	41.0	45	43.1	40
7α,8α	29.1	27	91.8	95	147.0	150

ence is significantly higher, 6.8°. In the first case, the X-ray conformational structure of retronecine has been reproduced well by *ab initio* calculation, which indicates a weaker exo-puckered character than the crystal structure. In other words, the N(4)-C(5)-C(6)-C(7) and C(8)-C(7)-C(6)-C(5) dihedral angles are smaller than the experimental values. This tendency is also observed for the endo-puckered character of heliotridine. *Ab initio* results overestimate the nonplanar conformation of ring 1 observed for this diasteroisomer in the crystal structure. The differences between *ab initio* and experimental data for C(5)-N(4)-C(8)-C(1) and C(7)-C(8)-N(4)-C(3) dihedral angle values, $\approx -5.9°$ and $-13.9°$, also indicate a small distortion in the ring junction, which is still smaller for retronecine (4.8° and 0.9°, respectively). Considering that the features of the ring junction are controlled by intermolecular hydrogen bonding in the crystal environment, the differences between experimental and *ab initio* data may be attributed to molecular packing forces.

Experimental information on the structure of retronecine and heliotridine was also obtained by ^{1}H-NMR studies in D_2O solutions.[17] These studies indicated the presence of a rigid exo-puckered conformation of retronecine and two alternate conformations (2:1 exo/endo) for heliotridine in aqueous solution. The Karplus revised equation[31] was employed to provide the relative conformation of vicinal hydrogens bonded to the puckered ring: The dihedral angles were calculated from the vicinal hydrogen coupling constants obtained from the ^{1}H-NMR measurements of the diasteroisomers in D_2O solution. Table III compares the results from the experimental angles with our *ab initio* calculations. The α hydrogens refer to the cis structure

regarding the hydrogen located at C(8), and the β hydrogens are the trans hydrogens corresponding to the hydrogen in C(8) (see Fig. 2). The average deviation between theoretical and hydrogen conformations in exo-retronecine, endo-heliotridine are 3.1°, 5.0°, and 3.4°, respectively. These deviations show that theoretical conformations of the vicinal hydrogens in the saturated rings are in excellent agreement with the structure obtained in solution using the Karplus equation, even though its parameters were optimized for aliphatic alkane molecules.[31]

Bond distances and bond angles between the O(10)H and O(11)H groups are shown in Table IV. The endo conformation of heliotridine does not show any significant interactions between these groups since the atoms are separated by more than 5 Å. However, the distance of 2.902 Å between H(11') and N(4) and the orientation of the hydroxyl group O(11)H reveal an interaction between N(4) lone pair and the hydroxyl proton. On the other hand, there is a great proximity between H(10')-O(11) atoms forming an angle of 98.5° with C(7) in the exo conformation of heliotridine. For

TABLE IV.

Bond Distances (in Å) and Bond Angles (in Degrees) between OH Groups exo and endo Retronecine and Heliotridine Calculated at the Hartree-Fock Level of Theory with 6-31G*.

	exo-retronecine		endo-heliotridine	
	endo	exo	endo	exo
R(O11-H10)	2.025	2.127	4.804	2.240
Angle(C7-O11-H10)	101.3	96.6	-	98.5
Total Energy	-514.63234	-514.63655	-514.62975	-514.63470

Total energies in atomic units for the conformers are also presented.

retronecine, the OH interactions are stronger for both ring conformations, where endo puckering gives the closest O(11)-H(10′) distance among the conformations.

Based on Dreiding models, Culvenor et al.[17] suggested that an intramolecular hydrogen bond was formed by *endo-* and *exo-*retronecine conformations, while no hydrogen bond should be observed for heliotridine.[17] The *ab initio* results gave a similar picture for the OH interaction in retronecine. However, the theoretical calculations predict the formation of a hydrogen bond for the exo-puckered heliotridine conformation. The difference between the endo and exo total energies of retronecine and heliotridine at the equilibrium geometries showed a greater stability of the exo-puckered conformation of retronecine (2.6 kcal mol^{-1}) and the exo-puckered form of heliotridine (3.1 kcal mol^{-1}). The presence of a hydrogen bond in the exo-puckered conformation of heliotridine is correlated with its greater stability compared to the endo-puckered conformation. The opposite is observed for retronecine. The strongest hydrogen bond occurs in the endo-puckered conformation, showing a smaller stability than the exo-puckered structure. In this sense, the strength of the hydrogen bond is not the most significant factor for the relative stability of these conformers, agreeing with the conclusions of the ^{1}H–NMR studies.[17]

PSEUDOPOTENTIAL, SEMIEMPIRICAL, AND MM3 CALCULATIONS

The comparison between all-electron *ab initio* calculations and experimental structural data for heliotridine and retronecine showed good agreement. However, in biological systems, heliotridine and retronecine are part of more complex molecules with several biological activities in different systems. The large size of these molecules imposes computational restrictions on *ab initio* calculations. The results presented earlier showed that the polarized double-zeta basis set provided structures in good agreement with experimentally determined geometries. Consequently, it would be convenient to use basis sets with this characteristic. Pseudopotentials allow the use of polarized double-zeta basis sets, changing the innermost basis functions to a core potential. In this sense, double-zeta plus polarization basis sets could be preserved at the Hartree-Fock level for calculations of alkaloids at lower computational costs than equivalent all-electron calculations.

Two other classes of computational methods more economical than *ab initio* can also be envisioned to evaluate the structural characteristics of heliotridine and retronecine: semiempirical and molecular mechanics models. These have been extensively reported in the literature[21–24] and compared with *ab initio* calculations in conformational studies of five- to eight-membered rings.[32] Thus, three different semiempirical models (MNDO,[21] PM3,[22] and AM1[23]), and the Allinger molecular mechanics force field model[25] were applied in the retronecine and heliotridine structures optimization.

Table V shows the AE results for calculations of all bond lengths, bond angles, and dihedral angles by the various methods for both diasteroisomers. The dihedral AE values were calculated for the angles listed in Table II considering the puckered character of rings 1 and 2 and the relative angle between them, as shown in Figure 2. Table V shows good agreement between equilibrium geometries of *exo-*retronecine calculated with the CEP-31G* and 6-31G* basis sets. The *ab initio* CEP effective potential calculations with double-zeta plus polarization basis sets are in better agreement with the *ab initio* all-electron results than any of the semiempirical methods. However, two geometric parameters of *exo-*retronecine gave a poor result when compared with all-electron calculation: (1) the intramolecular hydrogen bond, and (2) the C(1)-C(2) double bond. These two geometric parameters calculated with CEP-31G* were larger than those predicted by the calculations using the 6-31G* basis set. For the intramolecular hydrogen bond, the distance between H(10′) and O(11) was 0.055 Å larger than 6-31G*, while the C(1)-C(2) double bond was larger by 0.023 Å. The small AE (see Table V) comparing the pseudopotential and

TABLE V.

Average Error for Bond Distance (in Å), Bond Angles, and Dihedral Angles (in Degrees) of Retronecine and Heliotridine Calculated at the Hartree-Fock Level with 6-31G* Basis Set.

	*exo-*retronecine			*endo-*heliotridine		
	Distance	Angle	Dihedral	Distance	Angle	Dihedral
CEP	0.93 10^{-2}	0.37	1.19	1.08 10^{-2}	1.02	15.4
AM1	2.75 10^{-2}	2.63	25.2	2.80 10^{-2}	2.35	23.5
PM3	2.13 10^{-2}	2.08	10.3	2.10 10^{-2}	1.81	12.6
MNDO	2.54 10^{-2}	2.15	16.4	2.54 10^{-2}	2.46	26.2
MM3	2.04 10^{-2}	1.39	3.02	2.06 10^{-2}	1.71	5.34

the all-electron results shows that the most significant factors governing the nature of these structures are determined by the valence interactions.

A large deviation is observed for dihedral angles of *endo*-heliotridine calculated with CEP-31G*. The interactions between two different molecular regions of *endo*-heliotridine were observed to affect significantly the conformations of rings 1 and 2. The first is the bond length of the C(1)-C(2) double bond and the orientation of the O(10)-H(11) group; with the double bond calculated with CEP-31G*, this gives a distortion of ring 1. A simple test supporting the importance of this interaction was attempted. The OH group was changed to an H, and the geometry of the modified molecule was optimized. The resulting structure showed an excellent agreement between all-electron and pseudopotential calculation. The AEs for this deoxy compound for bond distances, bond angles, and dihedral angles were 0.94×10^{-2} Å, $0.32°$, and $3.17°$, respectively. These AEs are considerably better than those calculated for *endo*-heliotridine, which presented the values of 1.22×10^{-2} Å, $2.11°$, and $15.35°$ (see Table V). The CEP-31G* equilibrium geometry of the deoxy compound also presented a C(1)-C(2) double bond length 0.025 Å larger than the respective all-electron result. A large distortion of ring 1 was not observed for the deoxy compound. The force field of the olefinic carbons described by pseudopotential seems to be strongly affected by the presence of the hydroxyl group, which reduces the 1,4 repulsive interaction between C(2) and O(10). The distance C(2)··O(10) in *endo*-heliotridine is 3.436 Å for the 6-31G* equilibrium geometry, while CEP-31G* gives a larger value of 3.519 Å. At the same time, the electronic density population of the double bond could be stabilized by the presence of the hydroxyl proton.

The second interaction which affects the ring structure occurs between the secondary O(11)-H and N(4). The absence of O(10)H and O(11)H interaction, as observed in the endo but not in the exo conformation, and the distortion of ring 1 produce an approximation between N(4) and H(11') of 2.634 Å in the CEP-31G* equilibrium geometry, while the value for 6-31G* is 2.902 Å. The N(4)··H(11') bond distance in the deoxy compound is predicted to be 3.199 Å and 3.132 Å for CEP-31G* and 6-31G*, respectively. This comparison shows that the CEP calculation gives a strong interaction between O(10)H and the C(1) and C(2) atoms, modifying more significantly the ring conformation than in the 6-31G* results. However, the absence of the OH group also changed the N(4)··H(11') distance

by 0.230 Å with the 6-31G* basis set. The interaction between O(10)H and the C(1)-C(2) double bond and between N(4) and H(11') could also depend on the influence of the basis set used. Thus we propose a solution that combines both pseudopotential and all-electron estimates for different atoms of *endo*-heliotridine.

The nitrogen compact effective potential can also be an important factor favoring N(4) and H(11') approximation. Changing only the pseudopotential of N(4) to an all-electron 6-31G* basis set gives an increment of 0.086 Å for the N(4)·· H(11') distance in relation to the calculation using pseudopotential in all the other atoms. This combined calculation presented an AE of $9.92°$ for the dihedral angles, which is $5.43°$ smaller than the AE from the CEP-31G* result in all atoms. The AEs of bond lengths and bond angles have also been diminished to 1.06×10^{-2} and $1.90°$, respectively.

To verify the interactions responsible for the molecular distortions of the molecular structures calculated with CEP-31G*, a second combined calculation was attempted. The N(4), C(1), and C(2) pseudopotentials were changed to the respective all-electron 6-31G* basis set, while the CEP-31G* core potential basis set was used for the remaining C and O atoms of *endo*-heliotridine. The distortion in the condensed ring was drastically reduced, which was confirmed by the AE value for the first two dihedral angles of Table II. A deviation of $3.83°$ was verified against a value of $18.65°$ in the original CEP-31G* calculation. An overall AE for dihedral angles of $3.14°$ was obtained using the pseudopotential and all-electron combined calculation, which is in good agreement with the value of $3.17°$ obtained for the deoxy compound. The other two AE values for distances and bond angles were 0.82×10^{-2} Å and $1.86°$, respectively. The absence of O(10)H··C(1)-C(2) and N(4)··H(11') interactions in *exo*-heliotridine is correlated to AEs similar to those obtained by the equilibrium geometry of the second modified calculation—namely, 0.93×10^{-2} Å, $0.44°$, and $3.11°$ for bond distances, bond angles, and dihedral angles, respectively. These results reinforce the importance of C(1)-C(2)·· O(10)H and N(4)··H(11') interactions in driving the distortions observed in the condensed rings and the need for treating C(1), C(2), and N(4) with explicit all-electron calculations to obtain well-adjusted pseudopotential calculations at this basis set level.

The analysis of the semiempirical results (Table V) shows that PM3 provided the smallest AE for both diasteroisomers (compared to the MNDO and

AM1 methods), indicating a greater similarity between PM3 equilibrium geometries and the *ab initio* one. AM1 showed the worst fit to the *ab initio* equilibrium geometry of retronecine, while MNDO yielded the poorest fit of bond angles and dihedral angles for the heliotridine equilibrium geometry.

The great AE values for the dihedral angle can be attributed to the weak interactions that drive these geometrical parameters. The first two dihedral angles in Table II responsible for the relative angle between rings 1 and 2 are well reproduced by AM1 and PM3. However, MNDO dihedral angles showed a difference of 10° for the first angle, while the second angle was well adjusted for both diasteroisomers. The AE of the last two dihedral angles of Table 2 showed some similarity with that of the first two angles. So the great total dihedral AE value of AM1 for retronecine is related to the dihedral angles that define the puckered character of ring 2. In the case of heliotridine, these angles contributed to a large AE value for AM1 in comparison with the PM3 and MNDO results.

The well-known inability of AM1 to reproduce the nonplanar character of five-membered rings[32] justifies the largest AE for the dihedral angles of retronecine when compared with other methods. Although this failure is still present in heliotridine, the better adjustment for the remaining four dihedral angles calculated with AM1 implies a smaller AE value than the MNDO results. In this sense, the exo- and endo-puckered character of retronecine and heliotridine was well reproduced by PM3 when compared with the all-electron calculation. This factor agrees with the correction introduced in the parameterization of PM3 to reproduce the nonplanar character of five-membered rings.[33]

Table V also presents AEs for MM3 calculations. The MM3 results for retronecine provided once more a smaller AE than heliotridine. However, in both cases the experimental dihedral angles of Table II were not as well reproduced by MM3 as by the *ab initio* calculations using the 6-31G* basis set.

For retronecine, the MM3 calculations predicted a greater stability of the exo conformer over the endo, by about 2.8 kcal mol^{-1}. The *endo*-heliotridine conformation was predicted to be 0.6 kcal mol^{-1} more stable than the exo conformation. The order of stability of *exo*-retronecine (difference in total energy) is the same in MM3 and *ab initio* calculations. However, the MM3 results for he-

liotridine show a small difference in energy favoring the endo conformation in opposition to the *ab initio* results. Among the contributions to the total energy of MM3, the most sensitive component regarding the conformers was the torsional energy. The torsional contribution was observed to be lower by approximately 2 kcal mol^{-1} in the two most stable conformers (exo or endo) for retronecine and heliotridine. A partition of the torsional energy showed a significant change in the magnitude of N(4)C(8)C(7)O(11) component. The O(11)H in *exo*-retronecine and *endo*-heliotridine was revealed to be axially oriented, which could produce a smaller torsional energy to N(4)C(8)C(7)O(11) than the semiequatorial orientation of *endo*-retronecine and *exo*-heliotridine. Consequently, the most stable conformers of both disteroisomers presented axial O(11)H conformation.

The O(11)$\cdots$H(10') distances, characteristic of a hydrogen bond, in both exo and endo conformations of retronecine were shorter than in the all-electron calculation—1.795 Å and 1.776 Å, respectively. These results suggest that the hydrogen bonds calculated with MM3 are stronger than the *ab initio* ones. The exo conformation of heliotridine showed a similar tendency when MM3 was compared with the all-electron calculation. No strong OH interaction was observed in *endo*-heliotridine, but the MM3 distance between H(11') and N(4) was 2.676 Å, considerably shorter than the all-electron result of 2.902 Å. The MM3 thus suggests a stronger interaction between H(11') and N(4) than the all-electron *ab initio* calculation. The stronger hydrogen bonds indicated by MM3 are another factor responsible for the greater conformational stability of *exo*-retronecine and *endo*-heliotridine, in relation to the *ab initio* results. The better agreement of the conformational structures between all-electron *ab initio* calculations and the X-ray data and the poor description of the hydrogen bond by MM3 suggest that the *ab initio* results are simulating more appropriately these molecules in the experimental environment than MM3. However, as mentioned earlier, different types of bond lengths are being compared. While *ab initio* results are providing r_e, MM3 calculates r_g and both sets of parameters are compared with X-ray bond lengths (r_α). The MM3 program was written to reproduce experimental geometries often in error by less than 0.005 Å. The observed discrepancies with respect to the experimental data were attributed to the hydrogen bonds and probably to the molecular environment. Our calculations were

carried out with the MM3(92) program. A better agreement between MM3 and experimental structures could perhaps be achieved by the use of a newer version of the MM3 program (MM3(94)), in which hydrogen bonds were reparameterized.

A better evaluation of the discrepancies between MM3 and *ab initio* results will be also possible by the calculations with MM3(94). This new version includes a routine conveniently adapted to calculate r_e. In contrast with MM3 program, *ab initio* calculations are intended to achieve high accuracy after a careful study of the dependency of the calculated properties with the basis set truncation and electronic correlation effects. The reasonable agreement between all electrons or pseudopotential *ab initio* calculations and X-ray data suggest either that the cancellation of error at the Hartree-Fock level of theory is adequate to represent adequately the experimental data or that the calculation is really representing the correct molecular structure of the molecules. A detailed investigation of the new features of MM3(94) and the performance of the *ab initio* calculations using different basis sets and correlation effects is in progress.

Conclusion

The structural stability of *endo*- and *exo*-retronecine and heliotridine molecules was analyzed using different *ab initio*, semiempirical, and molecular mechanics methods. The all-electron *ab initio* calculations at the Hartree-Fock level of theory with the 6-31G* basis set provided structures in excellent agreement with available experimental results obtained from X-ray crystal structure and ^{1}H-NMR studies in D_2O solutions. The *exo*-retronecine and *exo*-heliotridine conformers presented a greater stability than the endo conformations by approximately 2.6 kcal mol^{-1} and 3.1 kcal mol^{-1}, respectively.

In an attempt to minimize computational costs while preserving the level of accuracy of all-electron calculations, *ab initio* calculations were also carried out with the compact effective potential suggested by Stevens et al.[20] The use of pseudopotential reduced the number of basis functions from 191 to 169, and the overall agreement between all-electron and pseudopotential was excellent. The pseudopotential calculations also showed that *exo*-retronecine and *exo*-heliotridine are more stable than the endo structures by 2.4 kcal mol^{-1} and

2.6 kcal mol^{-1}. The most significant difference between the calculations was observed in the ring planarity of heliotridine. This ring distortion of heliotridine was associated with the interaction between the O(10)H(11) group and the C(1)-C(2) double bond as well as with a hydrogen bond formed between O(11)H and N(4). The inclusion of all electrons for C(1), C(2), and N(4) reduced the discrepancies between pseudopotential and all-electron calculations. However, further studies involving pseudopotential are need to analyze in detail the dependence of the calculations with the CEP basis set as well as the pseudopotential.

Semiempirical calculations with MNDO, AM1, and PM3 gave a poor description of the conformation of both diasteroisomers in relation to the experimental results. The five-membered rings were observed to be planar for AM1 and MNDO calculations. The hydrogen bonds and the difficulty of methods like AM1 in reproducing the nonplanar structure of five-membered rings may be responsible for these discrepancies. The PM3 calculations provided a strong endo-exo character for the diasteroisomers, with a relative stability of *exo*-retronecine of only 0.5 kcal mol^{-1} over the endo conformer, and indicated that the heliotridine molecule was more stable in the endo conformation. These results indicate the inconvenience of studying the endo-exo conformational stability of these compounds without an adequate reparameterization of the semiempirical methods.

A good agreement was observed between MM3 and *ab initio* geometries; the differences were mainly due to the overestimation of the hydrogen bonds by MM3. While *exo*-retronecine was calculated to be more stable than the endo structure by approximately the same difference in the total energy observed by all-electron *ab initio* results, the MM3 calculations suggested that *endo*-heliotridine was slightly more stable than the exo form.

Acknowledgments

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