Pyrrolizidine Alkaloids Necine Bases: II. Conformational Analysis of Free Bases

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ABSTRACT: Molecular mechanics calculations were applied to the conformational analysis of two diasteroisomers, the pyrrolizidine alkaloids (PAs) retronecine and heliotridine. The application of reoptimized parameters for H bonding corrected the tendency of MM3(92) calculations to give unrealistic $H \cdots O$ distances for intramolecular OH interactions occurring in both diasterisomers. Inversions in the H-bond direction of *exo*-retronecine and in the relative stability of heliotridine *endo–exo* conformers were also observed with the application of the new parameters. A set of probable conformers was obtained for each diasterisomer, based on conformational and Boltzmann population analysis. Only *exo*-puckered conformers were found in the retronecine set, whereas both *exo*- and *endo*-puckered conformers were obtained for heliotridine. Transition state conformations supplied arguments supporting the design of models for H-bond interconversion in the case of *exo*-retronecine and for the *exo–endo* interconversion of heliotridine. Reactivity behaviors and ^{1}H-NMR data of both diasterisomers were elucidated in light of the theoretical results. © 1998 John Wiley & Sons, Inc. J Comput Chem 19: 1853–1861, 1998

Keywords: pyrrolizidine alkaloids; necine bases; retronecine; heliotridine; conformational analysis; molecular structure; hydrogen bond; molecular mechanics

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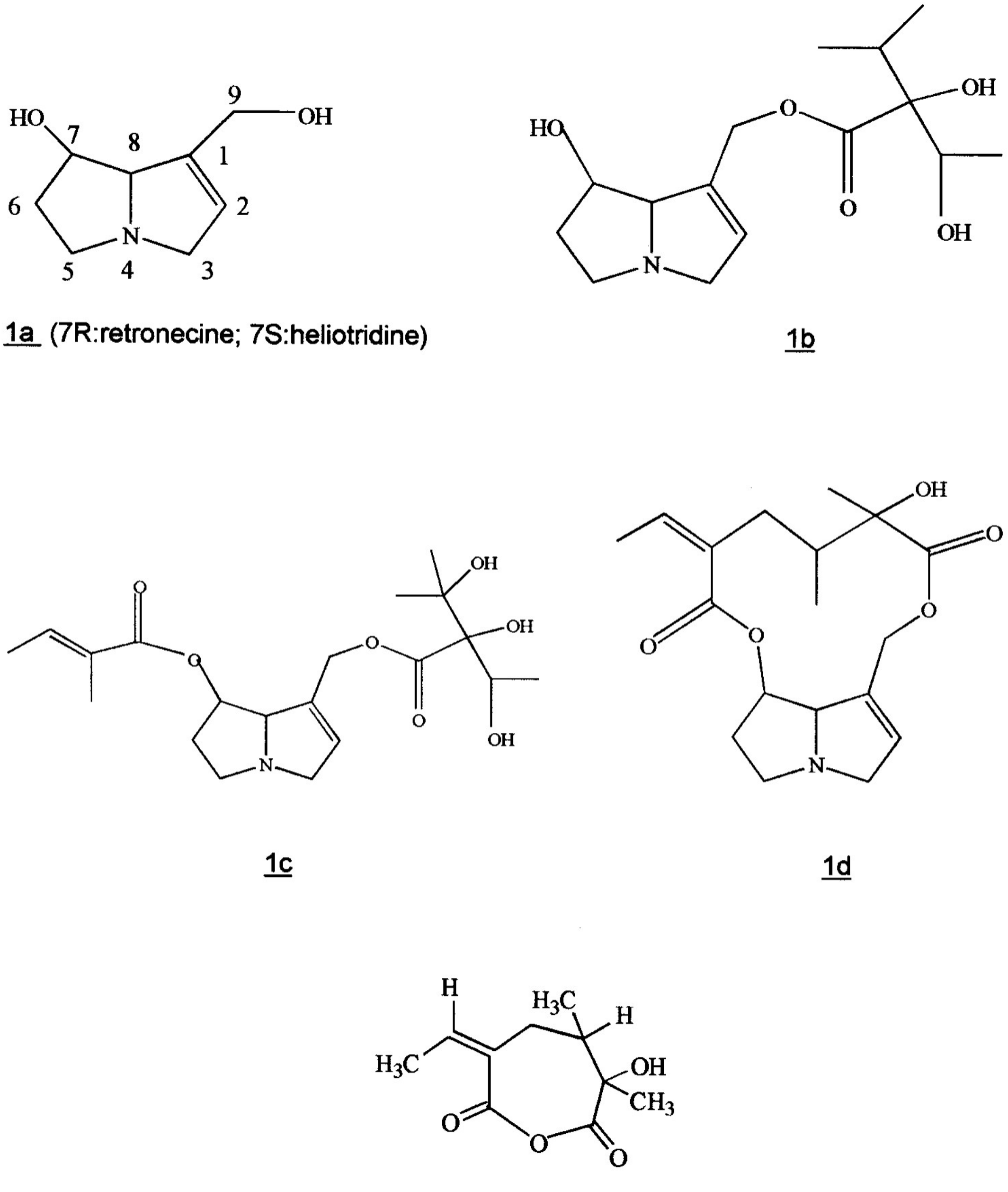
Introduction

Pyrrolizidine alkaloids (PAs) are a class of naturally occurring compounds found mostly in the plant families *Asteraceae*, *Boraginaceae*, *Fabaceae* (*Crotalaria*), and *Orchidaceae*.[1–3] There were only 27 known PAs in 1950, but the most recent review indicated 373 structures isolated from more than 560 species of plants.[3] These alkaloids generally occur as esters of an eight-carbon bicyclic necine base with a five- to ten-carbon necic acid. The necine bases (1a) are formed by two fused five-membered rings with a nitrogen in the ring junction (Fig. 1). Radioactive experiments with ^{3}H characterized putrescine as the specific percursor for retronecine,[4,5] a typical necine base. Experiments employing deutered, marked putrescine in the biosynthetic environment elucidated the stereochemistry of retronecine,[6] where the nitrogen lone pair of electrons, always cis to the C(8)—H bond, gives a concave–convex conformation to the two rings.



1a (7R:retronecine; 7S:heliotridine)

1b

1c

1d

1e

FIGURE 1. Some structures of 1,2-unsaturated pyrrolizidine alkaloids (PAs). (1a) Necine bases, (1b) monoester, (1c) diester, (1d) macrocyclic diester, and (1e) the senecic acid.

The necine bases can be esterified by necic acids at the C(9) and/or C(7) hydroxyls, giving, for instance, monoesters (1b), open diesters (1c), and macrocyclic diesters (1d). Experiments with ^{14}C and ^{3}H associated the biogenesis of the necic acids to common amino acids such as threonine,[7,8] isoleucine,[7,8] and valine.[9] In macrocyclic PAs, like senecionine (1d), the necic acids were observed to contain five or six carbons in the main chain (1e), which formed an 11- or 12-membered ring with retronecine. PAs occur in plants mostly as N-oxides, whose high polarity plays an important role in transport and storage.[10] Hartmann and Witte[3] suggested a useful classification of PAs based on their biogenetic pathways.

Two major features of PAs contributed to increased interest in these alkaloids. One feature is the heptatoxic potential of the widely distributed 1,2-unsaturated necine bases[3,11]; many plants containing PAs are used as food by humans. The second feature that has received the attention of chemical ecologists is the role of PAs as chemical mediators in plant–insect communication.[3,12–14]

Recently, a novel scenario for insect acquisition of PAs was proposed, including the evolution of a probable enzymatic system, which would oxidize and reduce 1,2-unsaturated monoesters of the lycopsamine type 1b before transport to the insect integument.[15] The inversion of the 7S center (and also of the 3′OH belonging to the necic acid portion) was observed in butterflies and moths.[13,15] The failure of this probable enzymatic system to carry 7S PAs led to their inversion to transportable 7R configuration PAs.

Computational chemistry could provide, in the future, information useful for understanding the role of PAs in plant–insect relationships. A first approach to the use of computational methods to understand necine base structures was recently published.[16] Double-zeta basis sets at the Hartree–Fock and molecular mechanics levels provided structures in good agreement with available experimental results obtained from x-ray crystal analysis and ^{1}H-NMR studies in D_2O solutions. On the other hand, semiempirical methods failed to reproduce the puckered character of the saturated ring, giving an almost planar conformation.

One aspect of great interest is the possibility of PAs to form intramolecular hydrogen bonds. *Ab initio* and molecular mechanics-optimized geometries of the free bases retronecine and heliotridine (1a) defined intramolecular H-bond interactions between the hydroxyls at C(7) and C(9).[16] Preliminary calculations on the N-oxides also revealed the possibility of intramolecular H-bond formation between the hydroxyls and between the O(11)H and the oxygen of the N-oxide. It is well known that interactions involving enzymatic systems and substrates can be mediated by H bonds, because they provide strong short-range interactions.[17] In this sense, understanding of H-bond formation in necine bases could help to explain their role in possible interactions between these bases and necic acids in the biosynthesis of macrocyclic PAs.

Conformation analysis is an important technique for understanding the behavior of molecular structures containing several degrees of freedom.[18] In necine bases, the puckered character of the saturated ring, the rotation of C(1)—C(9) bond, and even the intramolecular OH interactions can be discussed in terms of conformational analysis. The high number of possible conformations limits the choice of computational method to one of lower cost. Molecular mechanics has proven to be an economical and trustworthy method in conformational analysis studies of ring systems.[19] At the same time, the corrections made in the H-bond parameters and equation of the MM3 force field[20] provided more reliable results for these intramolecular interactions.

In this second article of this series (the first is ref. 16), conformational aspects of PAs are investigated. The characterization of the probable conformers of two necine bases, retronecine and heliotridine, is the primary objective. Geometrical aspects of the conformers (i.e., like ring puckering and intramolecular H bonds) are described, and their influence on conformational stability and on conformer interconversion is discussed. Special attention is given to the role of the (7R, 7S)—OH epimeric center in H-bond formation. The influence of corrected H-bond parameters on the optimized geometries is also discussed.

Methods

The MM3(92) program[21] was employed in all calculations and the conformers were generated with a routine for stochastic search.[22] In a first approximation, H-bond interactions in the free necine bases retronecine and heliotridine were analyzed through the two distinct parameterizations of the MM3(92): the original, and one in which the H-bond parameters were reoptimized.[20] The strength of the hydrogen bonds is discussed in terms of OH ⋯ OH distances. The remaining parameters and options of the program were used as default.

In the conformational search procedure, a preliminary test case with retronecine revealed the optimal number of trials in the stochastic search routine. This number was fixed at 100 for all stochastic search calculations, because no dissimilar conformation was observed when a greater number of trials was utilized in the test case. No difference in the generated conformational set was observed when the starting conformation was an *endo* or *exo* one. In this sense, 200 conformations were generated for each diasteroisomer.

After generating the conformational set, a subset of dissimilar conformations was selected. The criterion of dissimilarity was the difference between steric energies of the conformers. Steric energy differences smaller than 0.01 kcal mol^{-1} were not considered significant and the conformers were classified as similar. The vibrational frequencies of each dissimilar conformer were calculated and the structures were classified as energy minimum or transition states. The steric energy of the conformers was used as a parameter for the Boltzmann energy population analysis. The temperature was fixed at 298.15 K and the probability of finding a conformer was evaluated for an interval of 99%.

A general picture of the conformer geometry may be drawn in terms of ring conformations, mainly for the saturated ring, and the relative position of the hydroxyls. For retronecine, the *exo*-puckered characteristic of the saturated ring combined with H-bond interactions led us to employ the dihedral-drive option for rotating dihedral angles in predefined intervals. Each C—O bond was rotated in intervals of 30° and 144 more conformations were generated for retronecine. A model for the interconversion of the H bonds in *exo*-retronecine and another for the interconversion of *exo–endo* conformers in heliotridine were proposed.

Results and Discussion

Results of equilibrium geometries for some conformers of retronecine and heliotridine are presented, where the new H-bond parameterization was employed. The results are compared with those obtained previously,[16] employing the original parameterization for a restricted set of conformers.

HYDROGEN-BOND PARAMETERIZATION

The tendency for overestimating the strength of H bonds by MM3(92) was observed when comparisons with distances between hydrogen and oxygen derived from *ab initio* calculations were made.[16] The directional term added to the H-bond function in MM3(92) was not sufficient to give reliable results; thus, reoptimization of the parameters was recently implemented in MM3(94).[20] The new parameterization provided a better description of this property in several compounds containing C, N, and O, where *ab initio* 6-31G** calculations at the Hartree–Fock and MP2 levels were employed as reference. Thus, the new H-bond parameters have been applied in the MM3(92) force field for the geometry optimization of retronecine and heliotridine.

Table I presents the results of steric energies, bond distances for H ⋯ O and O ⋯ C, and the average error (AE) between parameters of molecular geometry (bond distances, bond angles, and dihedral angles). The AEs are calculated from eq. (1):

$$AE = \sqrt{\frac{\sum_{i=1}^{N} (y_i^{NP} - y_i)^2}{N}} \qquad (1)$$

where y_i^{NP} and y_i are the results for each geometrical parameter obtained from the calculations with the new parameterization set and with the original set, respectively (see ref. 16 for the definition of the geometrical parameters). Steric energies and bond distances for the original parameterization set are shown in Table I (in italics).

A large difference between the properties of the two sets of results could be seen. An increase in the values of H ⋯ O bond distances appeared when the reoptimized parameters were employed. These results agree with the known tendency of increase in the intermolecular H ⋯ O bond distances of smaller systems.[20] Another change could be observed in the relative stability of the *exo–endo* conformations of heliotridine, which will be discussed later.

For all three conformers, a reduction in the steric energies was also observed, which indicates a higher stability of the newly optimized structures. The reduction of the steric energies was a consequence of finding new conformational states when the reoptimized parameters and the stochastic search were applied together. In fact, the H-bond orientation was inverted in each conformer

TABLE I.
Steric Energies (kcal mol^{-1}), Bond Distances H ··· O and O(11) ··· C(9) (Å), and Average Error between Parameters of Molecular Geometry, Obtained with Corrected H-Bond Parameters (in Roman) and with Original H-Bond Parameters from Ref. 16 (in Italics).

Molecule	Energy	r(O ··· C)	r(H ··· O)	AE-r_g (mÅ)	AE-a_g (deg.)	AE-d_g (deg.)
Exo-retronecine	26.75	3.468	2.015	3.5	0.86	2.99
	29.31	*3.266*	*1.795*			
Endo-retronecine	30.99	3.154	2.024	4.9	1.25	10.7
	32.18	*3.226*	*1.776*			
Exo-heliotridine	29.08	3.618	2.085	4.0	1.27	4.39
	32.08	*3.489*	*1.983*			

when the reoptimized parameters were employed. The new parameterization gave H(11′) ··· O(10) interactions for the three conformers, whereas H(10′) ··· O(11) interactions were obtained with the original parameters.

The application of reoptimized parameters for H bonding corrected the tendency of MM3(92) calculations to give distorted values for this property in the two necine bases. The sets of probable conformers for each diasteroisomer were qualitatively different. Only *exo* conformers with H-bond interactions were identified at the 99% population interval for the retronecine conformational set. In the case of heliotridine, *endo* and *exo* conformers were observed. For the *exo* conformer, an H-bond was observed, whereas, for the *endo* conformers, no such interaction was identified. The results are discussed separately for each diasteroisomer.

CONFORMATIONAL ANALYSIS OF RETRONECINE

Figure 2 shows the ball-and-stick model for two disimilar *exo*-retronecine conformers and the

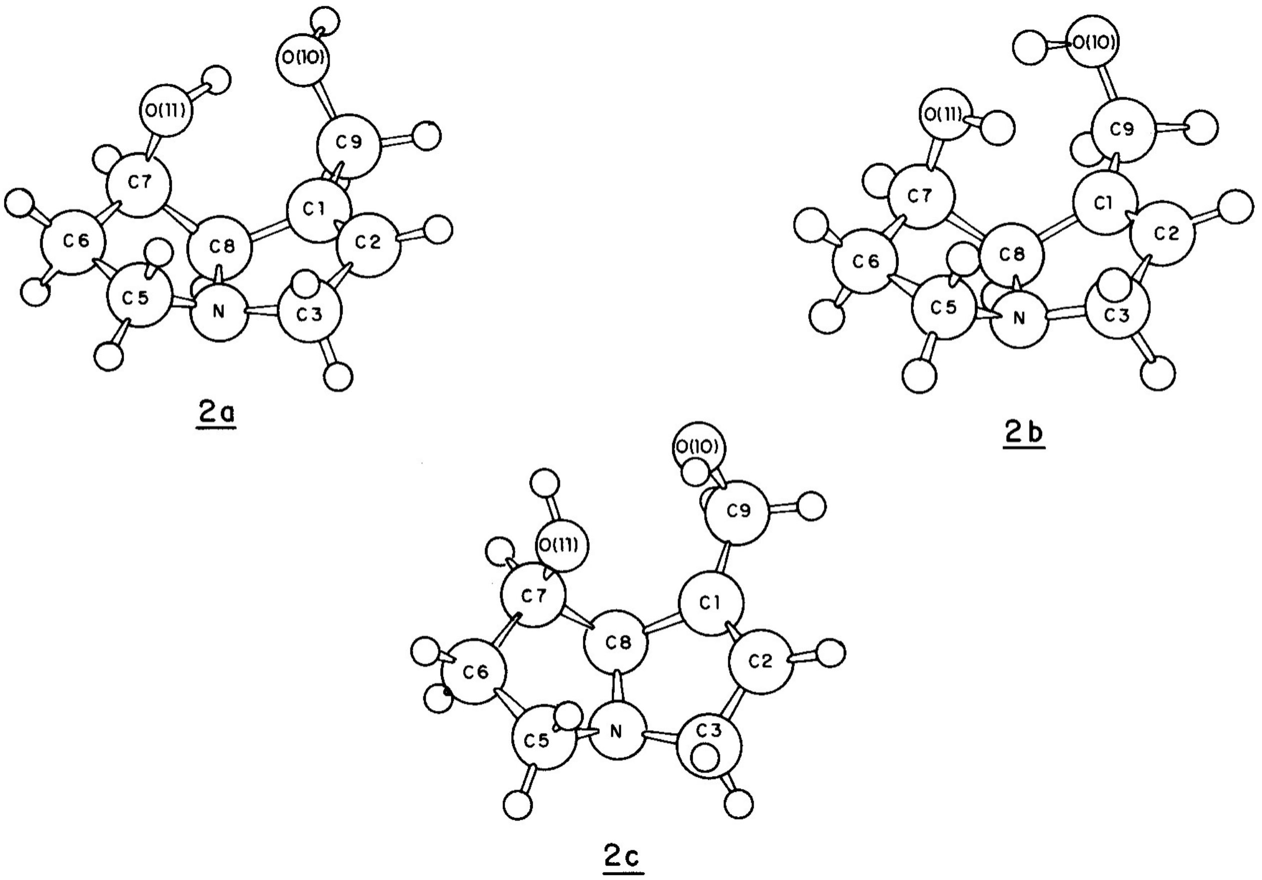


FIGURE 2. Some structures of retronecine obtained from conformational analysis.

transition state between them. In one case (2a), the H bond was formed by the interaction of O(11)H ⋯ O(10) with an H ⋯ O distance of 2.015 Å. This was the most stable *exo* conformer, with steric energy of 26.75 kcal mol^{-1}. The other conformation (2b) shows an inversion of the H-bond orientation, O(10)H ⋯ O(11), where the H ⋯ O distance was 2.059 Å. It was also an energy minimum and the steric energy was higher than the 2a conformer, 28.37 kcal mol^{-1}. The steric energy difference between 2a and 2b was 1.61 kcal mol^{-1}. No other conformer with steric energy lower than 2a was found.

Conformer 2a had a Boltzmann population distribution abundance close to 94% in the set of probable conformers; all other possible conformers represented only 6% probability. The search and dihedral-drive routines did not find any conformer without H-bond interaction that could belong to the set of probable conformers. This permanent H-bond interaction, combined with an *exo*-puckered orientation of the saturated ring, help to explain some experimental results with retronecine.

Conformer 2a agrees with ^{1}H-NMR experimental results.[21] Only an *exo*-puckered conformation was proposed to be present in D_2O solution, based on coupling constants of vicinal hydrogens and on the Karplus equation, which relates these dihedral angles to vicinal hydrogens.[23] No evidence of H-bond formation could be obtained from the ^{1}H-NMR measurements, but the investigators affirmed its presence in both *exo* and hypothetical *endo* conformations of retronecine. The existence of only *exo* conformers in D_2O solution was attributed to the pseudoaxial orientation of the OH on C(7). In this conformation, the repulsion between O(11) and C(9) could be minimized. In fact, this repulsion interaction was overestimated for both conformers, as can be seen from the values derived for *exo* and *endo* conformers in the conformational analysis (see Table I). The analysis of the C(9) ⋯ O(11) repulsion term calculated by MM3 did not show any correlation with the oscillations in the steric energies of the conformers. On the other hand, the absence of an H-bond interaction was followed by an increase in the steric energy of the conformers. This tendency was even observed for the *exo* conformers, where an H bond was not formed. Thus, H-bond formation in *exo*-puckered conformations of retronecine is strongly correlated to their stability.

The calculated steric energies for *endo*-puckered conformations were much greater than the values obtained for the probable conformers. Thus, the thermodynamic criterion of relative stability can explain the presence of only *exo* conformers in D_2O solution.

In saturated-ring conformations, no significant differences were found between the dihedral angles of vicinal hydrogens of conformers 2a and 2b and those proposed previously.[21] The mean difference between the experimental and MM3 values for the torsional angles was 3.2° and 2.5° for conformers 2a and 2b, respectively (Table II). Although the comparison was made between two distinct concepts of geometric parameters (one based on an empirical equation and the other on the theoretical concept of r_g, see ref. 24 for useful discussion), the results show similar proposed conformations.

A second aspect relating theoretical results with experimental characteristics of retronecine was the restricted rotation of the C(1)—C(9) bond. The H bond gave a pseudo-seven-membered ring O(11)—C(7)—C(8)—C(1)—C(9)—O(10)—H(12/13, depending on the direction of the H bond). Because this pseudo-ring prevents free rotation of the C(1)—C(9) bond, no direct hindrance of the C(1)—C(2) double bond was observed from the convex side of the fused rings. The pseudo-seven-membered ring also created steric hindrance on the concave side of the fused rings (see Fig. 2). This explains the steroselective synthesis of (1S)-platynecine from (7R)-retronecine. The hydrogenation reaction was catalyzed by Pd-C,[25] which had a complexation with the double bond that is favored the convex side of the fused rings, where little steric hindrance was present. Both hydrogens were inserted from the convex side, and the chiral

TABLE II.
Dihedral Angles (Deg.) between Vicinal Hydrogens of *Exo*-Retronecine (2a and 2b), Calculated by Molecular Mechanics and Obtained by ^{1}H-NMR.

Hydrogens[a]	Conformer 2a	Conformer 2b	Experimental
5α, 6α	40.5	38.7	40
5α, 6β	80.6	82.2	84
5β, 6α	161.9	160.0	165
5β, 6β	40.7	39.1	41
6α, 7α	43.6	41.1	40
6β, 7α	77.6	79.8	84
7α, 8α	32.1	29.4	27

[a] See ref. 16 for definitions of orientations.

ity of C(1), determined by the O(10)H orientation [the same as O(11)H], was antiparallel to the C(8)H bond.

A third noteworthy feature associated with experimental evidence was the occurrence of macrocyclic PAs derived (where both hydroxyls are esterified, forming a new ring) only from retronecine.[3] The biosynthesis of PAs indicated the formation of esterified compounds in the roots and/or leaves of plants from necine bases and necic acid precursors (see ref. 3 for detailed discussion). The formation of the seven-membered pseudo-ring gave a conformation favorable to effective attacks on the hydroxyls to form a macrocyclic diester. The kinetics of macrocyclic formation should be faster if an H-bond interaction is present. In fact, retronecine was observed to react with ferrocene boronate, producing a seven-membered macrocycle, but no reaction was seen between heliotridine and ferrocene boronate.[26] Once again, the seven-membered pseudo-macrocycle formation was related to the reactivity features of retronecine.

The restriction of the probable conformational set to only *exo*-puckered conformers with different H-bond orientations poses an important question regarding the interconversion of H bonds. Two models for this interconversion can be formulated. One is directly related to an enzymatic environment, or even to a protic solvent solution. In this case, the interconversion mechanism involves the migration of protons from one oxygen to the other in a concerted fashion. For O(11)H $\cdots$ O(10) to O(10)H $\cdots$ O(11) interconversion, the solvent interaction with the hydroxyls could provide proton donors to O(11)H and proton acceptors to O(10)H. These interactions establish a specific channel for proton migration from O(11) to O(10). Enzymatic conditions could also supply favorable electrostatic potential, and even proton donors and acceptors, through its amino acid components, to mediate the H-bond interconversion. This mechanism involves the proposal of charged species, preventing the application of the molecular mechanics method.

The second model for H-bond interconversion involves C—O bond rotation. In this model, the interconversion follows the mechanism of simultaneous rotation of C(7)—O(11) and C(9)—O(10) bonds. From the molecular mechanics approach, the simultaneous rotations can be modeled by the step-by-step rotation of each bond. The dihedral-drive option was used for this purpose. In fact, only one maximum between 2a and 2b was sufficient to describe this interconversion mechanism. The transition state 2c, with a steric energy of 29.67 kcal mol^{-1}, satisfied the conditions of this intermediate between the conformers. Hydroxyl interactions were not directed to each other, but were antiparallel with H $\cdots$ O distances larger than those observed in the H bonds of 2a and 2b.

Although the molecular mechanics method could not provide information about a possible enzymatic intermediate for H-bond interconversion, the low energy cost of an enzymatically mediated process should be associated with a low energy intermediate. The rotational model involves a higher energy intermediate, 2.92 kcal mol^{-1} over that of 2a, so the enzymatically mediated H-bond interconversion should be faster.

CONFORMATIONAL ANALYSIS OF HELIOTRIDINE

Ten different conformers were found in the 99% confidence interval of the Boltzmann population distribution. Six conformers were classified as energy minima and four as transition states. Both *endo* and *exo* conformations were detected in the set. Intramolecular H bonds were only observed among *exo* conformers, correlated with the smaller energy values in the set. The *exo* conformer, 3a, energetically the most stable, formed an H bond between H(11′) and O(10) larger than that of *exo* retronecine (around 2.085 Å). The *endo* conformer, 3b, did not form an H-bond and its hydroxyls were pointed away from the fused rings. A probable *exo–endo* interconversion should go through an intermediate with strained rings, like 3c, energetically more favorable than the planar ring forms that were 9 kcal mol^{-1} above the *exo* conformer.

The corrected H-bond parameterization and the conformational search inverted the provision of the relative stability for *exo* and *endo* conformations. In the previous calculation,[16] the *endo* conformer was predicted to be more stable, whereas, in this work, the *exo* conformer (3a) was 1.01 kcal mol^{-1} more stable than *endo* (3b). The conformational analysis of this diasteroisomer again showed a correlation between the presence of H bonds and the relative stability of the conformers. In heliotridine, this H bond could also be identified as the main factor for structure stabilization, instead of the minimization of the repulsive interaction between O(11) and C(9), as suggested by Culvenor et al.[21] This distance was calculated to be in the range of 3.1–3.2 Å, such that its

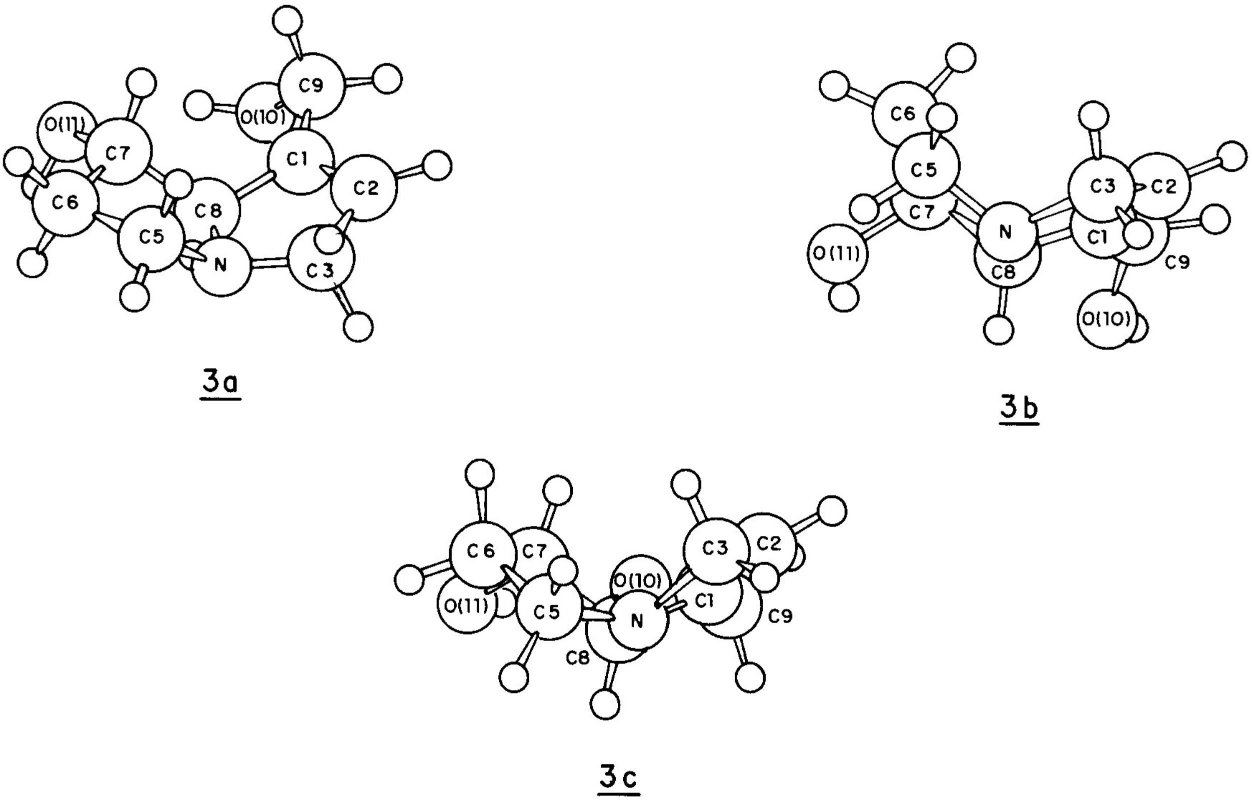


FIGURE 3. Some structures of heliotidine obtained from conformational analysis.

influence on the relative stability—based on the energetic partition of the components of the molecular mechanics force field—of the conformers was significantly lower than the H-bond strength.

The ^{1}H-NMR results indicated a 1:2 (*exo*:*endo*) ratio of the conformers in aqueous solution. On the other hand, the Boltzmann population analysis gave an inverted ratio, 2:1, in favor of the *exo* conformer. In the case of heliotridine, the conditions of solvation could stabilize the *endo* conformation. It would be useful to analyze the ^{1}H-NMR spectrum of this diastereoisomer in another solvent with a low possibility of forming an intermolecular H-bond with the alkaloid, in order to verify the relative participation of *exo*:*endo* conformers.

The fact that heliotridine does not present natural and synthetic macrocycle esters seems to be related to the various orientations assumed by the O(10)H in the *endo* conformation. This prevents sterically favorable orientations for interactions with the carbonyls of the necic acids, like the seven-membered pseudo-rings of *exo*-retronecine. However, the *exo* conformation could form the pseudo-rings, through intramolecular H bonding, indicating, theoretically, favorable conditions for formation of macrocycles. Hence, it would be useful to investigate the synthesis of heliotridine-derived macrocycles under controlled conditions, which could favor the *exo* conformations in the reaction environment.

Concluding Remarks

The reoptimized parameters of H bonds corrected the MM3(92) tendency of giving unrealistic values for intramolecular H bonds in medium-sized organic molecules. Reactivity behaviors and ^{1}H-NMR data of PAs were elucidated through the analysis of geometrical and steric energy properties of the conformers. The existence of only macrocyclic PAs derived from retronecine, the 7R diasterisomer, can be justified by the strong H-bond interaction observed in this alkaloid. The impossibility of synthesizing macrocycles from heliotridine, the 7S diasteriosmer, can be questioned because molecular mechanics predicts a stable *exo* conformer with an appropriate steric orientation to react with dicarboxylic necic acids. Molecular mechanics data showed permanent intramolecular H bonds in retronecine, which provides an argument supporting the presence of short-range, strong interactions between PAs and possible enzymatic systems. Thus, this theoretical method can be used as a trustworthy framework from which to elucidate mechanisms of interactions involving PAs in synthetic and biological environments.

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References

1. A. R. Mattocks, *Chemistry and Toxicology of Pyrrolizidine Alkaloid*, Academic Press, New York, 1986.
2. A. F. M. Rizk, In *Naturally Occurring Pyrrolizidine Alkaloids*, A. F. M. Rizk, Ed., CRC Press, Boca Raton, 1991, p. 1.
3. T. Hartmann and L. Witte, In *Alkaloids: Chemical and Biological Perspectives*, *Vol.* 9, S. W. Pelletier, Ed., Pergamon Press, Oxford, 1995, p. 153.
4. D. J. Robins and J. R. Sweeney, *J. Chem. Soc. Perkin Trans. I*, 3083 (1981).
5. H. A. Kahn and D. J. Robins, *J. Chem. Soc. Chem. Commun.*, 146 (1981).
6. (a) E. K. Kunec and D. J. Robins, *J. Chem. Soc. Chem. Commun.*, 1450 (1985); (b) E. K. Kunec and D. J. Robins, *J. Chem. Soc. Perkin Trans. I*, 1089 (1987).
7. D. J. Robins, N. M. Bale, and D. H. G. Crout, *J. Chem. Soc. Perkin Trans. I*, 2082 (1974).
8. D. H. G. Crout, N. M. Davies, E. H. Smith, and D. Whitehouse, *J. Chem. Soc. Perkin Trans. I*, 671 (1972).
9. J. A. Devlin and D. J. Robins, *J. Chem. Soc. Perkin Trans. I*, 1329 (1984).
10. A. Ehmke, K. Von Borstel, and T. Hartmann, *Planta*, **176,** 83 (1988).
11. C. C. J. Culvenor, J. A. Edgar, M. V. Jago, A. Outteridge, J. E. Peterson, and L. W. Smith, *Chem.-Biol. Interact.*, **12,** 299 (1976).
12. J. R. Trigo, L. Witte, K. S. Brown Jr., T. Hartmann, and L. S. Barata, *J. Chem. Ecol.*, **19,** 669 (1993).
13. K. S. Brown Jr. and J. R. Trigo, *The Alkaloids*, **47,** 227 (1995).
14. A. G. Orr, J. R. Trigo, L. Witte, and T. Hartmann, *Chemoecology*, **7,** 68 (1996).
15. (a) J. R. Trigo, *Alcalóides Pirrolizidinicos em Borboletas Ithomiinae. Alguns Aspectos em Ecologia Quimica*, Doctoral Thesis, Instituto de Quimica, Univ. Est. de Campinas, Campinas, SP, Brazil (1993); (b) J. R. Trigo, L. E. S. Barata, and K. S. Brown, Jr. *J. Chem. Ecol.*, **20,** 2883 (1994).
16. M. Giordan, J. R. Trigo, and R. Custodio, *J. Comput. Chem.*, **17,** 156 (1996).
17. (a) L. Paulling, *The Nature of the Chemical Bond*, 3rd Ed., Cornell University Press, New York, 1972; (b) G. A. Jeffrey and W. Saenger, *Hydrogen Bonding in Biological Structures*, Springer, Berlin, 1991.
18. E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, *Conformational Analysis*, Wiley, New York, 1965, p. 147.
19. D. M. Ferguson, I. R. Gould, W. A. Glauser, S. Schroeder, and P. A. Kollman, *J. Comput. Chem.*, **13,** 525 (1992).
20. J.-H. Lii and N. L. Allinger, *J. Phys. Org. Chem.*, **7,** 591 (1994).
21. C. C. J. Culvenor, M. L. Heffernan, and W. G. Woods, *Austral. J. Chem.*, **18,** 1605 (1965).
22. M. Saunders, *J. Chem. Soc.*, **109,** 3150 (1987).
23. M. Karplus, *J. Am. Chem. Soc.*, **85,** 2870 (1963).
24. N. L. Allinger, X. Zhou, and J. Bergsma, *J. Mol. Struct. (Theochem)*, **312,** 69 (1994).
25. R. Adams and E. F. Rogers, *J. Am. Chem. Soc.*, **63,** 537 (1941).
26. C. J. W. Brooks, W. J. Cole, and D. J. Robins, *Heterocycles*, **28,** 151 (1989).