A theoretical study of epibatidine

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A theoretical study of the conformational profile of epibatidine and its protonated form has been carried out using molecular mechanics (CVFF and CFF91), semiempirical (AM1) and *ab initio* (RHF/6-31G* and B3LYP/6-31G*) methods. Six minima have been found for the neutral molecule and four for the protonated one with small rotational barriers between them. The stability of the minima has been explained using the AIM methodology. Finally, the NMR shieldings of the different conformers found have been calculated with the GIAO method and used to assign some of the ambiguous experimental signals.

Introduction

Epibatidine is an alkaloid that was first isolated by Daly and co-workers in 1974 from extracts of the skin of the Ecuadorian poison frog, *Epipedobates tricolor* of the family Dendrobatidae, in very small amounts. Its chemical structure was established by Daly in 1992 as *exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]-heptane.[1] Epibatidine has attracted considerable interest because it appears to be the first compound exhibiting analgesic activity as a selective and potent nicotinic receptor agonist. Thus, epibatidine was found to have very high affinity for neuronal nicotinic receptors where it acts as a potent agonist with selective activity at different nicotinic receptor subtypes.[2,3]

Previous studies on the nicotinic pharmacophore,[4,5] had postulated a specific geometric arrangement between the three essential components required for agonist activity: a cationic center (*e.g.* the pyrrolidine nitrogen), a hydrophobic region and an electronegative atom that likely participates as a hydrogen bond acceptor (*e.g.* the pyridine nitrogen atom of nicotine).

Molecular modeling studies indicated that although epibatidine can mimic the structure of (*S*)-(−)-nicotine, its N–N distance is somewhat longer than the one found in nicotine, and exceeds that of a proposed nicotinic receptor pharmacophore.[6,7]

Most of the molecular modeling studies have been carried out using the neutral form of the molecules. However, it is known that some ligands interact with their corresponding receptors in the protonated form, especially when there are basic centers in the molecule as is the case with epibatidine and all the ligands of the nicotinic receptors.

In the present article, the conformational profile of the neutral and protonated forms of epibatidine (Fig. 1) has been studied by means of molecular mechanics, semiempirical and *ab initio* (molecular orbital and hybrid HF-density functional) methods. The relative energies of the minima have been compared and explained on the basis of intramolecular hydrogen bonds using the Atoms in Molecules (AIM) theory. Finally, the NMR spectroscopic experimental characteristics of epibatidine have been compared with the calculated ones, using the GIAO perturbation method, for the different minima structures.

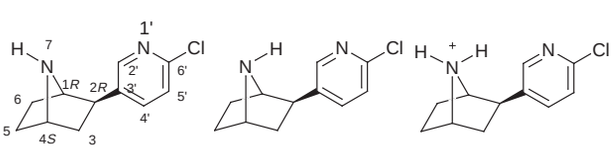

Fig. 1 Neutral (forms A and B) and protonated epibatidine with numbering used through the text.

Results and discussion

The rigidity of the epibatidine structure allows us to apply different techniques in the study of its conformational profile. In this case, molecular dynamics (MD) and systematic bond rotation have been performed with the molecular mechanics methods. For the semiempirical and *ab initio* methods, only systematic bond rotations have been carried out.

The total and relative energies of the minima found with the different methods are gathered in Table 1. The same number of conformations has been found in the MD and systematic bond rotation methods with the CVFF and CFF91 force fields. In contrast, a comparison of the number of different minima is very dependent on the method used to calculate the energetic profile. Thus, they range between four to six minima in the neutral molecule and from three to four in the protonated epibatidine. In both cases, the CVFF force field is unable to locate several of the minima since it underestimates the repulsive barrier generated by the N–H moiety (B form, Fig. 1) in the rotation of the pyridine ring.

These results together with those reported in the literature (eight conformers were found[7] for the neutral form with the MM2 force field and only two in the protonated epibatidine[8] with the AM1-hyperchem method) indicate the importance of a preliminary validation of the molecular mechanics force field used.

The comparison of the relative energy values of the minima indicate that for all the methods used the A conformations (Fig. 1) are more stable than the B conformations in the neutral system. The two molecular mechanics force fields used predict that the absolute minimum corresponds to **1**, while **2** is only 0.1 or 0.2 kcal mol^{-1} less stable depending on the force field used. The quantum mechanics methods predict that conformer **2** is the most stable the relative energy being **1** 0.2 kcal mol^{-1} with the AM1 method and 0.9 kcal mol^{-1} in the *ab initio* calculations. Similarly, the MM and semiempirical methods predict relative energies of the B conformers below 1 kcal mol^{-1} while the *ab initio* conformers are between 2.5 and 3.5 kcal mol^{-1}.

In the case of the protonated epibatidine, a similar trend as in the neutral case is observed: the MM minima are within the 0.5 kcal mol^{-1} range, the AM1 are around 1.3 kcal mol^{-1} and the *ab initio* slightly over 2.0 kcal mol^{-1}. In the present cases, the molecular mechanics and semiempirical methods provide

Table 1 Computed energies and relative energies (kcal mol^{-1}) found for the neutral and protonated epibatidine

	NH Disp.[a]	CVFF		CFF91		AM1		RHF/6-31G*		B3LYP/6-31G*	
		E/kcal mol^{-1}	E_{rel}	E/kcal mol^{-1}	E_{rel}	H_f/kcal mol^{-1}	E_{rel}	E/hartree	E_{rel}	E/hartree	E_{rel}
1	**A**	63.18	0.00	−7.88	0.00	41.90	0.18	−992.48645	0.95	−996.68376	0.93
2	**A**	63.42	0.24	−7.76	0.12	41.72	0.00	−992.48797	0.00	−996.68525	0.00
3	**B**	63.66	0.48	−6.97	0.91	42.66	0.94	−992.48242	3.48	−996.67991	3.35
4	**B**			−6.84	1.05	42.37	0.66	−992.48304	3.10	−996.68117	2.56
5	**B**	63.80	0.62	−7.06	0.82	42.30	0.58	−992.48324	2.97	−996.68140	2.41
6	**B**			−7.40	0.49	42.51	0.79	−992.48251	3.43		
7				3.90	0.47	187.99	0.62	−992.86562	0.65	−997.05991	0.95
8		94.04	0.00	3.47	0.04	187.37	0.00	−992.86666	0.00	−997.06143	0.00
9		94.27	0.22	3.44	0.01	188.08	0.71	−992.86505	1.01	−997.05980	1.02
10		94.46	0.42	3.43	0.00	188.71	1.34	−992.86341	2.04	−997.05783	2.26

[a] See Fig. 1.

Table 2 Conformational barriers for the rotation of the pyridine ring (kcal mol^{-1}). (Difference between the lowest minimum and the highest hill for each conformational profile)

	Neutral A form	Neutral B form	Protonated
CVFF	4.3	3.7	2.0
CFF91	2.8	2.2	1.3
AM1	2.8	1.3	1.9
RHF	4.8	1.4	2.2

smaller relative energies of the minima than the *ab initio* calculations.

The study of the conformational profile of the rotation of the pyridine ring in epibatidine and its protonated form suggests a low interconversion barrier between the different minima (Fig. 3). The largest barrier found in the A form and protonated epibatidine corresponds to the *ab initio* calculations (Table 2). Qualitatively all the methods agree that the larger barrier corresponds to the rotation in the A N–H disposition (ranging between 4.8 and 2.8 kcal mol^{-1}). This result indicates that the most important rotational barriers are generated by the close interaction of the CH groups of the azabicycloheptane and those of the pyridine and not by NH and the pyridine ring in the B form and protonated species.

In addition, we have calculated the nitrogen inversion barrier of 7-azabicyclo[2.2.1]heptane obtaining values of 12.0 and 11.4 kcal mol^{-1} at the RHF/6-31G* and B3LYP/6-31G* levels, respectively. The unusually high value of this barrier in comparison to monocyclic compounds of the same pyramidal geometry at nitrogen had already been pointed out by different authors and was referred to as the "bicyclic effect".[9,10] Our results are in good agreement with the experimental barrier of the *N*-methylated compound (13.8 kcal mol^{-1}).[11] This barrier constitutes the larger one for epibatidine indicating that at room temperature, conversion of the A form to the B form can easily occur, and additionally, to any of the conformers due to the rotation of the pyridine ring. Thus, from a conformational point of view, epibatidine at room or biological temperatures is a mixture of all the minimum conformations described plus some populations of intermediate situations.

The six minima found in the neutral form are depicted in Fig. 2 and a selection of the geometrical parameters of the minima found are gathered in Table 3. The minima in the neutral form can be divided into three pairs (**1**–**2**, **3**–**6** and **4**–**5**) which differ in the disposition of the pyridine ring that is rotated 180° on a given pair. The conformations in the protonated epibatidine **7**–**10** are similar to the **3**–**6** conformations of the neutral form, respectively.

The geometry of the different conformers can be defined with two parameters: the disposition of the N–H moiety (A or B as defined in Fig. 1) and the pyridine rotation angle. The N–N distance that has been used in different pharmacophoric

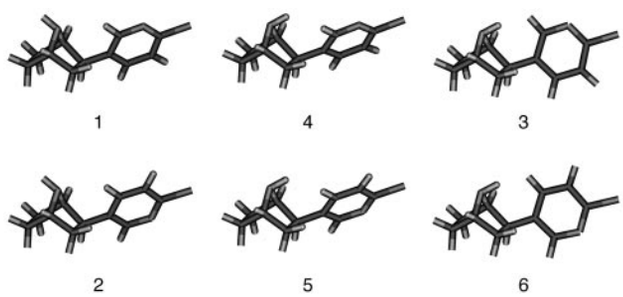

Fig. 2 Conformations found in the neutral epibatidine.

models shows two extreme values in the conformations obtained, *ca.* 4.5 and 5.5 Å. The first one corresponds to the case where the two nitrogens are on the same side of the molecule and the second when they are on opposite sides. Even though the value of the N–N distance has been considered sufficiently important in the literature to justify different pharmacophoric models, it is clear that the small relative energy values of the different conformers and the small rotational barriers indicate the presence of structures in all the ranges previously mentioned.

The Atoms in Molecules (AIM) methodology, based on the analysis of the electron density, has been shown to be a powerful tool to study the hydrogen bond and other weak interactions.[12,13,14] In the conformations obtained for epibatidine, two kinds of non covalent interactions are encountered (defined as an atomic interaction line between a given pair of atoms) (Table 4). The first one corresponds to a hydrogen bond between a C–H group of the pyridine moiety and the sp^3 nitrogen of the azabicycloheptane in the A conformers **1** and **2**. The second one corresponds to a H···H interaction between the pyridine and the azabicycloheptane moiety, mainly with the N–H group.

The intramolecular hydrogen bonds, although weak, as shown by their long H···N distances and small electron density at the bond critical points (ρ_{bcp}), are able to explain the stability of the A conformers *vs.* the B forms. In the case of the molecular mechanics methods, a similar conclusion can be reached using a simple electrostatic model. At the same time, the absence of the N–H group pointing towards the pyridine ring reduces the number of minima from four in the B conformers to two in the A forms, with all the methods except the CVFF molecular mechanics forcefield.

The second interaction (H···H) has traditionally been associated with repulsive interactions. In the present case, the protonated conformers show a closer H···H distance than the neutral ones. To verify the possible effect of a charged moiety in the electronic cloud of an aromatic moiety, the simple amonium···benzene model has been studied, placing the ammonium in the same plane of the benzene and with a hydro-

Table 3 Selected distances (N7–N1′) in Å and torsion angles (C1C2C3′C2′) in degrees for the conformational minima found

	CVFF		CFF91		AM1		RHF/6-31G*		B3LYP/6-31G*	
	N–N	C1C2C3′C2′	N–N	C1C2C3′C2′	N–N	C1C2C3′C2′	N–N	C1C2C3′C2′	N–N	C1C2C3′C2′
1	4.725	55.1	4.456	58.9	4.474	66.1	4.478	55.5	4.469	55.6
2	5.672	−126.3	5.516	−121.4	5.501	−113.5	5.501	−124.5	5.520	−124.4
3	4.741	54.7	4.629	14.2	4.624	11.3	4.723	17.3	4.739	25.6
4			4.593	68.5	4.622	77.9	4.717	79.0	4.712	78.2
5	5.681	−127.8	5.520	−109.4	5.490	−101.7	5.536	−102.5	5.551	−101.6
6			5.593	−168.5	5.578	−169.8	5.687	−156.4		
7			4.545	15.9	4.541	8.8	4.606	9.4	4.571	12.3
8	4.777	73.2	4.458	80.0	4.534	81.5	4.576	87.9	4.505	85.1
9	5.583	−105.9	5.326	−97.6	5.395	−96.9	5.298	−88.0	5.285	−88.7
10	5.689	−147.2	5.487	−170.7	5.455	−175.5	5.410	−179.7	5.510	−169.4

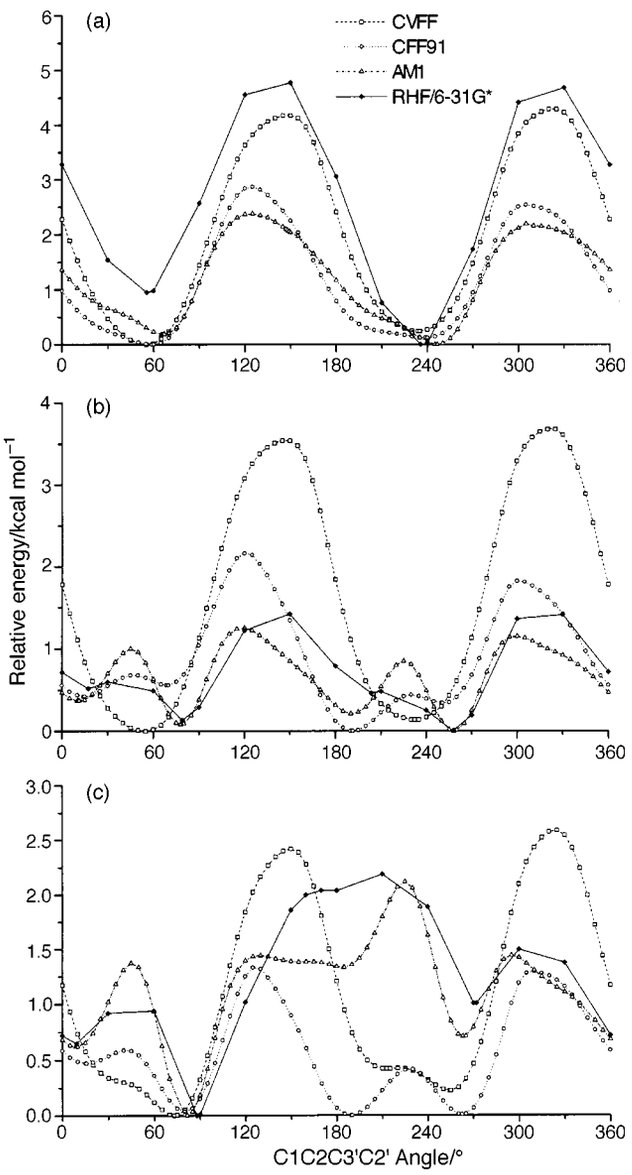

Fig. 3 Conformational profile of the (a) neutral A form (b) neutral B form and (c) protonated epibatidine. The lowest minima in each case were used as a reference value for each method.

gen atom pointing towards one of the hydrogens of the benzene. The optimized structure shows close H···H distances (Table 4) similar to those observed in the protonated epibatidine. The interaction energy of the ammonium···benzene complex is −0.44 kcal mol^{-1} including the basis set superposition error (BSSE) at the RHF/6-31G* level of calculation which indicates that the present interactions can be attractive due to the polarizing effect of the positive charge on the electronic distribution of the aromatic ring.

The analysis of the dipole moments of the conformers shows that the more polar ones are the most stable (Table 5). Assuming a simple solvation model, the more polar structure should be more stabilized resulting, in this case, in a larger energetic difference between the B and A minima. However, since a weak C–H···N hydrogen bond seems to be responsible for the higher dipole moment and larger stabilization of the A conformers, the specific solute–solvent interaction can play a very important role in the energetic profile of these conformers in solution.

Finally, the ^{13}C-NMR shieldings have been calculated and correlated with the experimental ones (Table 6). The experimental assignation of the signals was not unambiguous and the signals at 137.6 and 123.8 ppm were assigned to C-5′ and C-4′ or *vice versa* depending on the authors.[15,16] Good linear correlations ($R^2_a > 0.995$ for all the conformers) can be found between the calculated data for all the conformers and the experimental ones if C-5′ is assigned to the 123.8 signal and the C-4′ to the 137.6 signal. In addition, the calculated shieldings allow the 30.0 and 31.2 ppm experimental signals to be assigned to the C-5 and C-6 atoms, respectively.

It is worth mentioning the poor results obtained at the B3LYP/6-31G* level for the ^{13}C-NMR shielding of the C-2′ atom that is attached to a chlorine atom. In order to check if this is a problem of this system or a systematic error of the calculation level, the corresponding shielding values for the 2-chloropyridine have been calculated at the RHF/6-31G*, RHF/6-311++G**, B3LYP/6-31G* and B3LYP/6-311++G** levels.[17] In the two cases where the B3LYP functional was used, the signal corresponding to C-2 clearly deviates from the linear relationship with the experimental data.[18] This was not the case for the RHF calculations. All these results indicate that the problem is due to the B3LYP functional and not to the system itself.

A comparison of the variation of the ^{1}H-NMR shieldings with the conformation shows that the larger variations in the neutral molecules correspond to the atoms that form the hydrogen bond. Thus, the variation is about 1.3 ppm in H-2′ and 1.2 ppm in H-4′. As regards the variation in the ^{13}C-NMR, the largest effect (over 5 ppm) in the neutral molecules corresponds to the atoms attached to the hydrogen which form the hydrogen bond, C-4′ and C-2′. In the charged epibatidine, the presence of the charged moiety modifies the shielding of these two carbons up to 13 ppm.

The small conformational barriers found for epibatidine and the inherent differences between the calculated and experimental NMR data (the calculations consider isolated molecules in the gas phase and the experimental ones reflect the effect of the surrounding solvent molecules) prevent the assignation of the preferred conformation on the basis of the experimental data.

Table 4 Non-covalent interactions found using the AIM methodology. Electron density at the bond critical points, ρ_{bcp}, in e au^{-3} and Laplacian of the electron density, $\nabla^2\rho_{bcp}$, in e au^{-5}

		RHF/6-31G*			B3LYP/6-31G*		
Conf.	Atoms involved	Interatomic distance	ρ_{bcp}	$\nabla^2\rho_{bcp}$	Interatomic distance	ρ_{bcp}	$\nabla^2\rho_{bcp}$
1	N7 · · · H2′	2.593	0.0105	0.0361	2.549	0.0116	0.0357
2	N7 · · · H4′	2.583	0.0108	0.0368	2.535	0.0119	0.0365
3	H7 · · · H2′	—			2.209	0.0075	0.0299
4	H7 · · · H2′	2.347	0.0058	0.0240	2.289	0.0067	0.0255
5	H7 · · · H4′	2.316	0.0058	0.0247	2.273	0.0067	0.0256
6	—						
7	—						
8	H3eq · · · H2′	2.121	0.0107	0.0487	2.127	0.0109	0.0466
	H7 · · · H2′				2.189	0.0088	0.0340
9	H3eq · · · H4′	2.125	0.0105	0.0474	2.120	0.0107	0.0456
10	H1 · · · H4′	2.150	0.0102	0.0478			
$NH_4 \cdots C_6H_6$		2.126	0.0055	0.0248			

Table 5 Dipole moment (D) for the conformers obtained with the *ab initio* methods

	RHF/6-31G*	B3LYP/6-31G*
1	5.72	5.48
2	5.65	5.50
3	4.33	4.06
4	3.99	3.84
5	3.66	3.59
6	3.25	

Table 6 Calculated at the RHF/6-31G* level (absolute values) and experimental (relative to TMS) shielding of the carbon atoms in the different conformers

C	**1**	**2**	**3**	**4**	**5**	**6**	Exp.[a]
C-1	146.3	146.3	150.1	145.6	145.4	148.9	62.8
C-2	161.3	163.0	161.9	159.8	161.3	162.8	44.5
C-3	163.5	163.9	161.8	166.0	165.0	161.1	40.3
C-4	153.2	153.2	152.4	153.0	153.0	152.7	56.5
C-5	174.3	174.3	175.9	175.4	175.6	176.2	31.3[b]
C-6	172.6	172.7	173.8	174.1	174.0	173.6	30.2[b]
C-4′	63.4	62.0	62.0	61.7	66.4	67.7	137.5
C-5′	83.5	81.1	80.9	81.4	81.4	81.4	123.8
C-6′	51.1	50.4	51.2	50.8	50.6	51.3	148.8
C-2′	52.1	55.6	59.0	57.5	53.6	53.4	148.6
C-3′	65.0	65.2	65.6	65.8	65.9	65.5	140.7

[a] Taken from ref. 15. [b] Interchangeable signals in the experimental report.

Conclusions

A full conformational analysis of epibatidine and its protonated form has been carried out by studying the energetic profile of the rotation of the pyridine ring and the nitrogen inversion of the azabicycloheptane with molecular mechanics (CVFF and CFF91), semiempirical (AM1) and *ab initio* methods (RHF/6-31G* and B3LYP/6-31G*). Six minima have been found for the neutral molecule and four for the protonated one within a small energetic range (less than 3 kcal mol^{-1}). The barrier obtained for the pyridine rotation is very small, less than 5 kcal mol^{-1} in all the cases, and the nitrogen inversion barrier is around 12.0 kcal mol^{-1}. These values indicate that from a conformational point of view, epibatidine is a mixture of all the conformational minima and some population of the intermediate structures.

The relative energy of the minima has been explained using the AIM methodology. Thus, a intramolecular hydrogen bond is observed in the lower energy minima. Other intramolecular H · · · H interactions have been found and analysed.

Finally, the NMR shieldings of all the minima found have been calculated with the GIAO perturbation method. The calculated results have been used to assign some ambiguous experimental signals.

Methods

The molecular mechanics methods were performed with the CVFF[19] and CFF91[20] force fields implemented in the Discover program.[21]

The molecules studied were built, initially, with standard bond lengths and angles by using the molecular modeling package Insight II.[22] The structures were energy minimized using the steepest descendent and conjugate gradient method until the root-mean-square (RMS) gradients were less than 0.01 and 0.001 kcal mol^{-1} Å^{-1}, respectively. A conformational search using molecular dynamics studies was performed at constant temperature with an integration step of 1 fs. The structures were initialized at 1500 K followed by 20 ps of equilibration and 40 ps of simulation. From this simulation step four hundred structures were stored every 0.1 ps and each one of them was subjected to energy minimization until the RMS gradient was less than 0.001 kcal mol^{-1} Å^{-1}. In addition, a relaxed conformational profile of the rotation was generated in 5° steps in the A and B forms of the neutral (Fig. 1) and in the protonated epibatidine. The minima found by this procedure were optimized as mentioned before.

The quantum mechanics AM1 semiempirical calculations[23] were performed with the MOPAC package[24] and the *ab initio* calculations (RHF/6-31G*[25] and B3LYP/6-31G*[26,27]) with the Gaussian-94 program.[28]

The conformational profile of the pyridine rotation was repeated with the AM1 semiempirical method and the *ab initio* RHF/6-31G*. In the last case, the step size was 30°.

The adequate structures of the conformational profile were re-optimized to obtain the conformational minima. In the case of the AM1 Hamiltonian, the EF minimization algorithm and the PRECISE keyword, which increases the precision of the electronic and geometrical parameters by two orders of magnitude, were used. The *ab initio* minima were obtained using the Berny minimization algorithm.

Additionally, each minimum found at the RHF/6-31G* level was re-optimized with the hybrid HF-density functional method B3LYP and the 6-31G* basis set.

The Atom in Molecule (AIM) analysis[29] was carried out with the AIMPAC program package.[30]

Finally, the NMR shielding was calculated using the GIAO perturbation method[31] as implemented in the Gaussian-94 program.

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