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INVESTIGATION OF SOLVENT EFFECTS ON THE STABILITY AND ^{15}N NMR SHIELDING OF HALLUCINOGENIC HARMINE USING THE PCM MODEL AND NBO INTERPRETATION**A. Tahan¹, N. Ahmadinejad²**¹*Semnan Branch, Islamic Azad University, Semnan, Iran*

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Density functional theory (DFT) and Tomasi's polarized continuum model (PCM) are applied for the investigation of the solvent structure and its dielectric constant effects on the relative stability and nuclear magnetic resonance (NMR) tensors of hallucinogenic harmine. All of the used computational methods indicate that the structural stability in protic polar solvents is higher than that in aprotic polar and nonpolar solvents, and the most stable structure was observed in methanol. Also, the natural bond orbital (NBO) interpretation demonstrates that with an increase in the solvent dielectric constant the resonance energy for $\text{LP}(\text{N}_9) \rightarrow \sigma^*$ and π^* interactions of the structural pyrrole ring increases while the $\text{LP}(\text{N}_9)$ occupancy decreases, and the highest resonance energy and the lowest occupancy are observed in water and methanol. On the other hand, NMR calculations show that there are the lowest values of $\Delta\sigma_{\text{dir}}$ in water and methanol media for both nitrogen nuclei of the harmine structure.

Keywords: harmine, polarized continuum model (PCM), NMR, NBO interpretation.**INTRODUCTION**

Harmine [$\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$] is a well known member of the β -caroline alkaloid family (Fig. 1). β -Carboline belongs to the class of indole alkaloids and consists of the pyridine ring fused to the indole skeleton. They are available in a broad range of medicinal plants and are endogenously produced in human and animal tissues as a product of secondary metabolism [1–4]. They have different biological properties such as hypotensive, hallucinogenic or antimicrobial activities [5]. In the literature it has been reported that harmine and other β -carbolines have antioxidative properties. Therefore, they interfere the action of reactive oxygen species and protect the nervous system [6]. Many studies were performed on harmine and its derivatives from the view point of pharmacological and medicinal aspects [7–10 and references therein]. Researchers carried out the analysis and separation of β -carboline alkaloids by HPLC, UV-Vis spectrophotometric and mass spectrometry techniques [11, 12]. Since harmine can be a metabolite of harmane, their separations were studied via HPLC and micellar electrokinetic chromatography techniques [13]. Natural and chemically modified cyclodextrins (CDs) have been widely used to improve the resolution of chromatographic separations and capillary electrophoresis [14, 15]. Many methods have been used to describe the nature and type of the interactions but just NMR can differentiate the part of the analyte involved in the interactions with the CD cavity [16–18]. On the other hand, the effect of substituents such as chlorine and bromine on the photochemistry and acid-base properties of some derivatives of β -carboline alkaloids was investigated using DFT [19, 20]. Unfortunately, the other effective theoretical studies on the structures of interest have

not been performed yet. Since, the solvent effects on the structure and properties of harmine and its derivatives have not been investigated experimentally and theoretically, we modeled the harmine structure using DFT methods and the polarizable continuum model (PCM) in the different media. The basic interest of the present research is to analyze the effects of the solvent structure and its dielectric constant on the stability and the NMR chemical shielding tensors of heterocyclic structures and the NBO interpretation of the structural factors. Finally, we evaluated the PCM model reliability.

COMPUTATIONAL DETAILS

The geometrical optimizations were performed on harmine modeled using the B3LYP/6-311++G** method. Energy minimum molecular geometries were located by minimizing the energy with respect to all geometrical coordinates without imposing any constraints. The nature of the stationary points for the structure under study has been fixed by the imaginary frequencies. For the minimum state structures, only real frequency values were accepted. Single-point energy calculations were performed on the optimized structure at the MP2/6-311++G**, MP2/6-31++G**, and MP2/6-31G* levels of theory.

Bulk effects were modeled on the title structure using the self-consistent reaction field (SCRF) method based on a continuum model with the uniform dielectric constant (ϵ). Tomasi's polarized continuum model (PCM) [21] defines the cavity as a union of a series of interlocking atomic spheres.

The NBO analysis was then performed on the optimized structure at the B3LYP/6-311++G** level of theory via the NBO 3.1 program in the gas and solution phases [22, 23]. The lone pair orbital occupancies of nitrogen atoms in the optimized structure and the stabilization energies associated with LP (1) N \rightarrow σ^* or π^* delocalizations were calculated using the NBO analysis.

Relative solvent effects on the ^{15}N NMR shielding of the pyridine and pyrrole rings of the harmine structure were calculated using the corresponding nuclear shielding in cyclohexane as the reference. Direct ($\Delta\sigma_{\text{dir}}$) and indirect ($\Delta\sigma_{\text{ind}}$) solvent effects are obtained with a slight modification of the method used by Cammi et al. [24]. Instead of deriving $\Delta\sigma_{\text{ind}}$ from the difference of the PCM optimized shielding and the PCM shielding of the molecule held at the geometry optimized in vacuum, it is obtained from the shielding calculated in vacuum for a molecule whose geometry is optimized in solution. Thus,

$$\begin{aligned}\Delta\sigma_{\text{dir}} &= \sigma_{\text{sol}}(R_v) - \sigma_{\text{cyc}}(R_v), \\ \Delta\sigma_{\text{ind}} &= \sigma_{\text{vac}}(R_s) - \sigma_{\text{vac}}(R_{\text{cyc}}),\end{aligned}$$

where $\sigma_{\text{sol}}(R_v)$ is the value of the nuclear shielding computed in solution but with the solute with the geometry optimized in vacuum, and $\sigma_{\text{vac}}(R_s)$ is the value of the nuclear shielding in vacuum but with the solute geometry optimized in solution. $\sigma_{\text{cyc}}(R_v)$ and $\sigma_{\text{vac}}(R_{\text{cyc}})$ are the corresponding parameters for calculations with cyclohexane.

All calculations in the present work were performed using the GAUSSIAN program [25].

RESULTS AND DISCUSSION

Energy results. The relative energy values (ΔE_{rel}) of the Harmine structure were reported in Table 1 at different levels of the theory and media. The results show that the structural stability of harmine depends on the solvent structure and its dielectric constant. However, the structural stability (based on the reduction of relative energy values) in protic polar solvents (water, ethanol, and methanol) is more than that in aprotic (dimethyl sulfoxide (DMSO) and nitromethane) and non-polar solvents (cyclohexane, carbon tetrachloride, chloroform, and acetone). Furthermore, the most stable structure was observed in the methanol medium at the B3LYP/6-311++G**//B3LYP/6-311++G** level of theory. The comparison of ΔE_{rel} values at the different levels of theory shows that the solvent effect on the relative stability was observed more obviously at the MP2/6-311++G**//B3LYP/6-311++G** level than at the other levels. The interesting result obtained indicates that changes in the harmine relative energy in different media is the same as the Gibbs free energy of solvation (ΔG_{sol}). The

Table 1

Calculated relative energy values ($\text{kcal}\cdot\text{mol}^{-1}$), dipole moments (μ in Debye), and Gibbs free energy of solvation (ΔG_{sol} in $\text{kcal}\cdot\text{mol}^{-1}$) for the harmine structure at different levels of theory and solvent dielectric constants (ϵ)

Method	MP2/6-31G*// B3LYP/6-311++G**		MP2/6-31++G**// B3LYP/6-311++G**		MP2/6-311++G**// B3LYP/6-311++G**		B3LYP/6-311++G**//B3LYP/6-311++G**			
	$-E_{\text{el}}$	ΔE_{rel}	$-E_{\text{el}}$	ΔE_{rel}	$-E_{\text{el}}$	ΔE_{rel}	$-E_{\text{el}}$	ΔE_{rel}	μ , D	ΔG_{sol}
1	685.1949	13.2405	685.3311	13.6170	685.5456	16.0643	687.5277	15.1230	4.1775	—
2.023	685.2009	9.4754	685.3387	8.8479	685.5534	11.1697	687.5354	10.2912	4.6963	-4.82
2.228	685.2017	8.9734	685.3330	12.4247	685.5477	14.7465	687.5297	13.8680	4.7590	-1.21
4.9	685.2071	5.5848	685.3369	9.9774	685.5517	12.2364	687.5335	11.4834	5.1886	-3.64
20.7	685.2053	6.7144	685.3421	6.7144	685.5569	8.9734	687.5386	8.2831	5.5655	-6.81
24.55	685.2127	2.0708	685.3465	3.9533	685.5650	3.8906	687.5460	3.6396	6.2513	-10.75
32.63	685.2160	0.0000	685.3528	0.0000	685.5712	0.0000	687.5518	0.0000	6.2972	-14.69
38.2	685.2131	0.8049	685.3394	4.4645	685.5542	6.7013	687.5360	6.2394	5.6281	-5.14
46.7	685.2131	1.8198	685.3401	7.9694	685.5547	10.3539	687.5374	9.0361	5.6403	-6.01
78.39	685.2144	1.0040	685.3464	4.0161	685.5628	5.2711	687.5460	3.6396	6.3699	-11.01

* The mentioned values are the dielectric constant for vacuum, cyclohexane, carbon tetrachloride, chloroform, acetone, methanol, ethanol, nitromethane, dimethylsulfoxide (DMSO), and water media respectively.

values of both parameters are higher in protic solvents than in aprotic and non-polar ones and the highest ΔG_{sol} value about $-14.69 \text{ kcal}\cdot\text{mol}^{-1}$ is in methanol. By plotting the relative energy versus ΔG_{sol} , the following equation was derived (Fig. 2):

$$\Delta E_{\text{rel}} = 0.999\Delta G_{\text{sol}} + 14.5.$$

$$\text{Coefficient of variation} = 0.925.$$

The dipole moment is the first derivative of the energy with respect to the applied electric field. It is a measure of the asymmetry in the molecular charge distribution. We reported the dipole moment of harmine in Table 1 at the B3LYP/6-311++G** level of theory in different solvents. The results reveal that with increasing solvent dielectric constant, the dipole moment of the title structure increases because of increasing solvent-solute interactions. Hence, this structure has the most asymmetric charge distribution in an aqueous solution. Solvents that do not follow this trend are aprotic nitromethane and DMSO (dimethyl sulfoxide). The NBO interpretation indicates that with an increase in the solvent dielectric constant, the resonance energy for $\text{LP}(\text{N}_9) \rightarrow \sigma^*$ and π^* interactions of the structural pyrrole

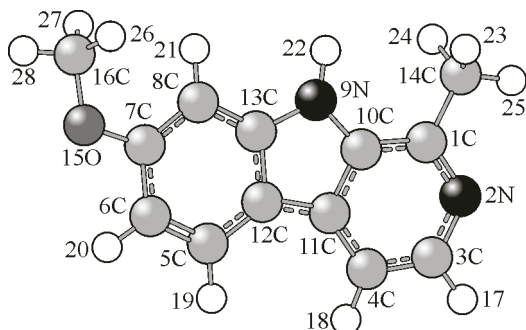


Fig. 1. Optimized structure of hallucinogenic harmine

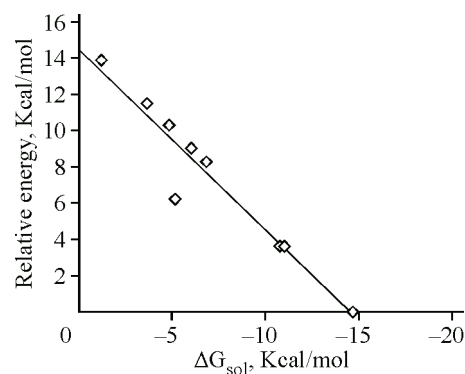


Fig. 2. Linear dependence of the relative energy on ΔG_{sol} in different solvents

Table 2

Calculated lone pair occupancies and total resonance energies ($E(2)$ in kcal·mol⁻¹) of the harmine compound using the NBO analysis at the B3LYP/6-311+G**//B3LYP/6-31G* level of theory and different solvent dielectric constants (ϵ)

ϵ	Parameter NBO	Occupancy	$\Sigma E(2)$, kcal/mol	ϵ	Parameter NBO	Occupancy	$\Sigma E(2)$, kcal/mol
1	LP (1) N ₂	1.91565	23.13	24.55	LP (1) N ₂	1.92193	22.39
	LP (1) N ₉	1.66709	68.15		LP (1) N ₉	1.64273	74.13
2.023	LP (1) N ₂	1.91782	22.92	32.63	LP (1) N ₂	1.92208	22.38
	LP (1) N ₉	1.66007	69.81		LP (1) N ₉	1.64178	74.36
2.228	LP (1) N ₂	1.91806	22.9	38.2	LP (1) N ₂	1.92179	22.51
	LP (1) N ₉	1.65921	69.99		LP (1) N ₉	1.64398	73.54
4.9	LP (1) N ₂	1.91993	22.7	46.7	LP (1) N ₂	1.92181	22.52
	LP (1) N ₉	1.65225	71.59		LP (1) N ₉	1.64393	73.57
20.7	LP (1) N ₂	1.92151	22.54	78.39	LP (1) N ₂	1.92231	22.38
	LP (1) N ₉	1.64545	73.13		LP (1) N ₉	1.63996	74.85

ring increases while the LP(N₉) occupancy decreases, and the highest value of the resonance energy and the lowest occupancy were observed in water and methanol (Table 2). As we know, the overlap of p orbitals in aromatic rings causes an increase in π -electron clouds. The π -electron clouds contain six electrons. The delocalization of the nitrogen lone pair of the pyrrole ring (LP (N₉)) and its participation in ring currents lead to the development of aromaticity and an increase in the ring stability, and finally, the structural stability. These findings are in good agreement with the energy data and they can be the probable structural reasons for the harmine stability in methanol and water solvents. In addition, the Table 2 data show that with an increase in the solvent dielectric constant the resonance energy for LP(N₂) \rightarrow σ^* and π^* interactions of the structural pyridine ring decreases while the LP (N₂) occupancy increases, and the lowest value of the resonance energy and the highest occupancy were observed in water and methanol. The probable reason for this phenomenon can be the aromaticity of the pyridine ring. Aprotic nitromethane and DMSO solvents again did not follow the mentioned trends in all cases.

Table 3

NMR parameters of ¹⁵N nuclei (isotropic chemical shielding, σ_{iso} , and $\Delta\sigma_{\text{dir}}$ values (ppm)) of the harmine structure at the B3LYP/6-311++G**//B3LYP/6-31G* level of theory and different solvent dielectric constants (ϵ)

ϵ	σ_{iso}		ϵ	$\Delta\sigma_{\text{dir}}$	
	² N	⁹ N		² N	⁹ N
1	-83.9488	131.6290	24.55	-180.6384	122.1098
	—	—		-74.6348	-11.7398
2.023	-101.0306	133.8379	32.63	-182.2711	122.8249
	0.0000	0.0000		-76.5859	-11.9926
2.228	-103.0874	134.1800	38.22	-130.0339	138.7398
	-1.4811	0.3332		-27.5046	5.5468
4.9	-71.3073	128.0075	46.7	-128.4742	138.6381
	-14.9394	2.7855		-27.8927	5.6348
20.7	-128.3112	138.2323	78.39	-178.0195	123.1643
	-25.7438	5.1448		-80.1530	-12.4576

NMR chemical tensors. In this paper, the effects of the solvent dielectric constant and intramolecular interactions on the ^{15}N NMR shielding parameters of harmine at the B3LYP/6-311++G** level of theory have also been presented (Table 3). The obtained results show that the ^{15}N NMR shielding tensors are strongly affected by the chemical environment, the solvent structure, and its dielectric constant respectively. Hence, their values are different for the nitrogen atom of the pyridine ring (N_2) and the nitrogen atom of the pyrrole ring (N_9) in the same medium and N_9 has a higher value of the chemical shielding (σ_{iso}) than N_2 . On the other hand, the NBO analysis reveals that N_9 has a higher resonance energy and lower occupancy than N_2 in all media, i.e. the resonance interactions produced the strong chemical shielding in this position. As mentioned in section 2, the total solvation effect is composed of two distinct contributions: $\Delta\sigma_{\text{dir}}$ and $\Delta\sigma_{\text{ind}}$. The first contribution is directly related to the intensity of the solvent reaction field used in the PCM calculation, whereas the second is due to the relaxation of the molecular geometry of the solute brought about by the solvent. The results presented show that the lowest $\Delta\sigma_{\text{dir}}$ values were observed in the water and methanol media for both nitrogen nuclei. Also, the results show that the direct solvent effect on N_9 is higher than that on N_2 . It is worth mentioning that the $\Delta\sigma_{\text{ind}}$ value is zero in all media. The mentioned result was also checked at the Hartree-Fock (HF) level with different basis sets and was reproduced (the data are not presented here).

CONCLUSIONS

This work is a brief investigation of the reliability of Tomasi's polarizable continuum model (PCM) in exploring the solvent effect on the relative stability and ^{15}N NMR shielding of the harmine structure. The results presented show that PCM can differentiate between the structure and energy of a heterocyclic compound in polar and non-polar, aprotic and protic solvents. However, it cannot exactly recognize the structural and energy differences in the solvents having a similar structure, such as water and methanol. Our approach (the B3LYP level of theory with the 6-311++G(*d,p*) basis set), however, does not take into account the relaxation of the molecular geometry of the solute brought about by the solvent. Hence, the $\Delta\sigma_{\text{ind}}$ value is zero in all media. However, the PCM model combined with DFT methods could not predict precisely the $\Delta\sigma_{\text{ind}}$ value for a molecule with an average size such as harmine. Furthermore, our computations show that the electronic energy values (E_{el}) of harmine in the liquid phase decreases with an increase in the dielectric constant and the improvement of the basis set leads to an increase in the relative stability of the compound under study. The NMR computations indicate that N_9 has a higher value of the chemical shielding (σ_{iso}) than N_2 . On the other hand, the NBO analysis reveals that N_9 has a higher resonance energy and lower occupancy than N_2 in all media. However, it can be deduced that with an increase in the contribution of nitrogen lone pairs in resonance interactions, the NMR chemical shielding values around them increase.

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