Theoretical and NMR Structural Characterisation of a Polymorphic Azo Dye

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Abstract

Proton and carbon-13 NMR studies in solution and carbon-13 CP-MAS NMR in the solid state of a polymorphic azo dye derived from 2-amino-5-nitrothiazole were performed and the results are discussed and compared with theoretical data from quantum chemical calculations. The polymorphism of this compound has conformational character, two conformers coexist in CDCl₃ solution and in solid state, as shown by NMR

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1. Introduction

Heterocycles are extensively used in disperse dye chemistry either for textile or non-textile uses. Non-textile applications of heterocyclic dyes include their use in reprography, functional dye and non-linear optical systems, photodynamic therapy and lasers [1].

Azo dyes containing heterocyclic rings lead to brighter and often deeper shades than their carboxylic analogues [2] and they are still very important for applications such as disperse dyes for polyester fibres [3].

When the synthesis of this type of dye was accomplished [4], it was found that some of them, namely compound (**1**) (figure 1) showed two sets of proton resonances in deuteriochloroform solutions, even after repeated purification. These two independent proton resonances seem to indicate the presence of two forms (two conformers, **A** and **B**) of (**1**). The presence of both forms is not due either to hindered rotation about the amide bond, or a *cis-trans* isomerization since the latter would need higher energy. Chippendale *et al.* described polymorphism for Disperse Red 278 (**2**) (figure 1), which presents broad carbon resonances for C-2 and C-6, in CDCl₃ and CD₂Cl₂, due to slow exchange between two conformers [5]. Lyčka published an excellent review on the NMR of

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azo dyes [6], where cases of polymorphism and the characterisation of the corresponding polymorphs by NMR were discussed.

Figure 1. Structures of compounds **1** and **2**

2. Experimental

¹H NMR spectra were recorded on a Varian Unity Plus and on a Bruker Avance 300 with operating frequencies of respectively 299.94 and 300.13 MHz for ¹H and 75.42 and 75.47 MHz for ¹³C. Chemical shifts (δ) are reported in ppm, coupling constants (J) are in hertz and the internal standard was TMS. ¹³C Assignments were made with the aid of 2D gHSQC and gHMBC (delays for one bond and long-range *J* C/H couplings were optimised for 145 and 7 Hz, respectively) experiments. HSQC spectra give ¹J_{C/H} correlations while HMBC spectra allowed the establishment of connectivities between coupled proton and carbons two to three bonds away from each other. ¹³C CP-MAS NMR spectra were recorded at 100.62 MHz on a Bruker Avance 400 spectrometer with the following conditions: 5 s of recycle delay, 1.5 ms of time contact, 90° pulse of 5 μs and AQ of 33.8 ms. In the dipolar dephasing experiment a contact time of 50 μs was used.

3. Quantum Chemical calculations

The structures were firstly optimized using the semi-empirical PM3 method, based on the AMPAC 6.56PC software [7]. The structures were then refined using a DFT method (B3LYP/6-31G**), aiming a better description of the electronic structure [8]. The Berny analytical gradient was used in this optimisation. The requested convergence limit on RMS density matrix was 1 x 10⁻⁸ and the threshold values for the maximum force and the maximum displacement were 0.000450 and

0.001800 a.u., respectively. The results were viewed using Gaussview (03W) [9]. From the refined structures, the geometric and thermodynamic parameters for both forms, for the isolated (AMPAC) and solvated (Hyperchem) [10] molecule (chloroform, dimethylsulfoxide, methanol), and for transition state (TS), only for the isolated molecule, were calculated using PM3 method. The conversion between the **A** and **B** forms was obtained by the rotation of the C(14)-C(12)-N(11)-N(8) dihedral angle (**Figure 2, Inset**), using the PM3 method and the facilities of AMPAC. The DFP/BFGS optimizer was used in these calculations. The solvated molecules (in chloroform, methanol and dimethylsulfoxide) were modeled using the facilities of Hyperchem 5.11 (UHF calculation, gradient 0.1000 kcal/Å mol, Polak-Ribiere optimisation algorithm). The simulations using solvent molecules involved at least 14 solvent molecules distributed in the neighborhood of one molecule, with the minimal distance of 2.3 Å. From $\Delta^\circ H_f$ and ΔS (reaction entropy) furnished in the output of AMPAC, $\Delta^\circ G_f$ and $\Delta^\# G^\circ$ (**A** $\leftrightarrow$ **B** conversion) could be estimated. $\Delta^\circ S_f$ could be estimated from the expression,

$$\Delta^\circ S_f = \Delta S - \sum \Delta S_{\text{elements}}$$

in which $\Delta S_{\text{elements}}$ were obtained from S_{element}° data [11]. Particularly, ΔS was estimated from thermodynamic calculation using AMPAC with the combination of the keywords PM3 PRECISE FORCE GRAD THERMO and ROT=1.

The activation energy rate constant and the A-factor from Arrhenius equation, were estimated from thermodynamic and kinetic equations based on transition state theory [12],

$$E_a = \Delta^\# H^\circ + 2RT$$

$$k_r = \frac{k_B T}{hc_0} \exp(-\Delta^\# G^\circ / RT)$$

$$A = \frac{k_B T \exp(2)}{hc_0} \exp(\Delta^\# S^\circ / R)$$

For solvated species, $\Delta^\circ G_r$ was estimated considering that both species possess the same $\Delta^\circ S_f$ value ($\Delta^\circ G_r = \Delta(\Delta^\circ H_f)$).

4. Results and Discussion

Figure 2 presents the structure of both conformers, and their charge distribution.

The results from optimisation show that both species are almost planar, due to a significative electron delocalisation between the diethylamino and the nitro group, mainly for **B** conformer. The conversion from **A** to **B** implies in a magnification of this effect, principally in the region in which the intramolecular hydrogen bond occurs (**Figure 2**). This results in a more polar species. In fact, the dipole moment calculated for **B** (10.90 Debye) is slightly higher than the value obtained for **A** (10.55 Debye).

Figure 2 Optimised structure and Mulliken atomic charges of A (a) and B (b) conformers in the vacuum, obtained using a DFT method (B3LYP-31G**). The charges were omitted for practically all hydrogens. Inset: structure corresponding to the A-conformer with numbered atoms.

In the conversion from **A** to **B**, significative changes are observed in the charge over the atoms. The analysis of these variations indicates that the **A**→**B** conversion induces a considerable displacement of the electronic density towards the region in which the intramolecular hydrogen bonding occurs. The bonds over the molecule are significantly shortened when compared with typical bond lengths (**Table 1**), and the considerable planarity (**Table 2**), are additional evidences of electron delocalisation over almost the whole molecule. The electronic coupling between N(24) and the aromatic ring implies in the participation of the non-bonding p-orbital of nitrogen in the π system of the aromatic ring, and gives to the nitrogen a partial sp^2 character. This characterizes an intramolecular charge-transfer state [13].

Table 1 Bond lengths (Å) estimated using DFT method, compared with typical bond lengths of equivalent bonds, for both forms of the isolated molecule.

Bond	A	B	Typical bond lengths for non-ICT compounds
N(24)-C(19)	1.368	1.367	1.448 – 1.480
C(12)-N(11)	1.363	1.362	1.451 - 1.471
N(11)-N(8)	1.287	1.295	1.228 – 1.231
N(8)-C(6)	1.376	1.378	1.435
C(2)-N(7)	1.425	1.424	1.487 – 1.513

The formation of intramolecular hydrogen bonding will favour the existence of the **B** form. For the isolated molecule, the intramolecular hydrogen bond formed between the H(22) and the N(11) is practically in-plane, with a dihedral angle H(22)-N(17)-C(13)-C(12) of 0.621° . The calculated length of the hydrogen bond is 1.95 Å, a small value for hydrogen bonding [14]. Despite this small value, it is coherent with the expected, considering that with the solvation and consequent solute-solvent interactions, this bond length tends to become larger. In fact, this was observed in the simulations: the values calculated for the solvated molecule were 2.42, 2.03 and 2.26 Å, respectively in chloroform, dimethylsulfoxide (DMSO) and methanol. For the solvated molecules, some loss of planarity is observed, due to solute-solvent interactions (**Table 2**). However, considering the calculated lengths for hydrogen bond, this is not sufficient to affect its stability.

4.1 NMR analysis

The ^1H NMR spectrum of compound (**1**) in deuteriochloroform presents two sets of proton resonances, which are presumably due to two forms (A and B conformers) that coexist in equilibrium in an approximately 1:1 proportion at room temperature (**Figure 3**).

Table 2 Dihedral angles for the isolated and solvated conformers.

	Conformation	C(14)-C(12)- N(11)-N(8) (degrees)	C(27)-N24- C(19)-C(15) (degrees)	C(28)-N24- C(19)-C(15) (degrees)	H(22)-N(17)- C(13)-C(12) (degrees)
Isolated ^a	A	2.457	5.604	-174.427	
	TS	100.000	-	-	-
	B	176.172	4.806	-175.237	0.621
Solvated in chloroform	A	178.73	13.291	158.356	22.096
	B	178.43	12.032	156.876	28.549
Solvated in dimethylsulfoxide	A	-5.095	-25.718	-171.032	7.796
	B	-177.562	-17.594	-171.069	-11.184
Solvated in methanol	A	-0.682	20.981	-116.369	14.191
	B	-0.434	-49.627	86.278	28.825

The parameters for the solvated molecules were calculated using the PM3 semi-empirical method.

^aDFT B3LYP/6-31G*

Figure 3. ¹H NMR spectrum of compound (1) in deuteriochloroform at 223 K.

In order to get some insights about this equilibrium, we have acquired ¹H NMR spectra of this compound at low temperatures (until 223 K) and found only a slight decrease in the **B**:**A** ratio (45:55) when the solution reached 218 K. The NH proton resonances appear at δ 9.09 and 12.65 ppm; the latter value was assigned to a form where the NH proton was involved in an intramolecular hydrogen bond.

The distance between N(11) and H(22) (**Figure 2**), equal to 2.11 Å, suggests that the NH in conformer **A** may also be involved in an intramolecular hydrogen bonding. If this occurs the bond should be weaker since a 5-membered ring is formed.

When the ^1H NMR spectrum was acquired on DMSO- d_6 at temperatures above 323 K only one set of signals is observed, which presumably means that only one of the conformers is present or the interchange between them is much faster than the NMR time scale. The signal corresponding to NH is broad and with an intermediate resonance value of δ 10.43 ppm (**Figure 4**).

Figure 4. ^1H NMR spectrum of compound (**1**) in DMSO- d_6 at 343 K.

The assignment of proton and carbon resonances of both conformers started by the connectivities found in the HMBC spectrum of (**1**) (**Table 3**). The NH resonance of the **B** conformer (δ 12.65 ppm) showed correlations with the carbon resonances of C-1, C-3 and CON and then the analysis of the HSQC spectrum allowed the assignment of H-3 8.32 (d, J 2.7). In the HMBC spectrum H-3 of this conformer showed correlations with C-1, C-2 and C-5, which allowed the assignment of H-5 (HSQC spectrum). Also in the HMBC spectrum, H-5 presents correlations with C-1 and C-3 while H-6 correlates with C-4 and C-2; the latter was already assigned and allowed the unequivocal assignment of this proton resonance to the **B** conformer.

The assignments of all proton and carbon resonances of the **A** conformer were made in the same way, but starting from the corresponding NH resonance (δ 9.09 ppm).

In order to confirm the NMR data of both **A** and **B** conformers of compound (**1**) in solution, a solid state ^{13}C NMR spectrum of this sample was acquired. The analysis of the CP-MAS NMR spectrum confirms the presence of two conformers and the carbon resonances obtained in this spectrum fits quite well with those of the solution spectrum (**Table 3**). The assignment of the non-protonated carbons (*i.e.*, with no directly bound hydrogens) was confirmed by the dipolar dephasing experiment.

Table 3 ^1H and ^{13}C NMR data (δ , ppm) and HMBC and HSQC correlations of part of the **A** and **B** conformers of (**1**) in deuteriochloroform at 293.15 K.

Positions	δ_{H}	δ_{C}	HSQC	HMBC	δ_{C} (solid state)
NH (B)	12.65	-	-	C-1, C-3, CON	-
		131.6 or			
1 (B)	-	131.7	-	-	130.9
2 (B)	-	138.0	-	-	141.3
3 (B)	8.32 (d, J 2.7)	100.8	C-3	C-1, C-2, C-5	102.0
4 (B)	-	155.3	-	-	155.6
5 (B)	6.62 (dd, J 2.7 and 9.4)	109.1	C-5	C-1, C-3	113.0
6 (B)	7.56 (d, J 9.4)	140.2	C-6	C-2, C-4	141.8
CON (B)	-	175.1	-	-	173.4
NH (A)	9.09	-	-	C-1, C-3, CON	-
		131.6 or			
1 (A)	-	131.7	-	-	132.5
2 (A)	-	142.9	-	-	143.8
3 (A)	8.08 (d, J 2.7)	100.0	C-3	C-1, C-2, C-5	102.0
4 (A)	-	156.5	-	-	156.1
5 (A)	6.53 (dd, J 2.7 and 9.8)	110.3	C-5	C-1, C-3	113.6
6 (A)	7.98 (d, J 9.8)	121.3	C-6	C-2, C-4	122.2
CON (A)	-	172.5	-	-	173.2

The ^1H and ^{13}C NMR spectrum of compound (**1**) in deuteriochloroform also presents two sets of resonances for the methynic group (4'-CH) of the thiazole ring (δ_{H} 8.52 and 8.56 ppm; δ_{C} 144.3 and 144.7 ppm). The carbon resonance of C-2' of this thiazole ring appears at δ_{C} 181.9 ppm whereas the resonance of C-5' does not appear at room temperature, but below 273 K it is possible to observe two signals (for both conformers) very close to those of 4'-CH. For example at 253 K the resonances of 4'-CH appear at δ_{C} 144.6 and 145.0 ppm and those of C-5' at δ_{C} 144.1 and 144.7 ppm.

4.2.Energetics

The energy barrier calculated for the interconversion is low in both directions. The analysis of thermodynamic data (**Table 4; Figure 5**) shows that the conversion from **B** to **A** is the preferential process. The activation energy (21.84 kJ/mol), calculated for **A**→**B** conversion of the isolated molecule, from the thermodynamic data, must be at least two times higher than the opposite conversion (10.79 kJ/mol). This last value is of the expected magnitude of the energy released during the formation of hydrogen bonding [14,15]. These values must suffer changes due to solvation, mainly considering protic and viscous solvents, in which is expected an additional stabilisation of the **B** form. It is evident that the conversion needs very small energy amount to

occur, which explains why the interconversion is verified even at low temperatures, as experimentally observed. An estimate of the minimal energy needed for the **B**↔**A** conversions shows that they are at the level of rotational transitions (microwave region), being that **B**→**A** conversion occurs at a frequency near 3×10^{13} Hz, and the opposite at 5×10^{13} Hz. Based on these frequencies an estimate of the **B/A** ratio could be obtained, being 40:60, which agrees well with the experimental data. The magnitude of A-factor is also another evidence of the effectiveness of these conversions even at extremely low temperatures. The calculated value for the conversion occurring at 298 K is equal to $4.6 \times 10^{13} \text{ s}^{-1}$, a typical value found in *cis-trans* isomerizations [16]. Even at low temperatures, as for example 218 K, in which some NMR measurements were done, the A-factor practically did not change ($3.4 \times 10^{13} \text{ s}^{-1}$). This result shows that the entropy variation between the transition state and **A** or **B** forms must be null or very near zero. This is an interesting aspect, which shows that these conversions do not need positive changes in the activation entropy to occur. This result agrees with the data calculated using a semi-empirical method (**Table 4**). Additionally, the rate constant for the **B**→**A** conversion, is equal to $5.9 \times 10^{11} \text{ s}^{-1}$, and the value for the reverse conversion, both at 298 K, is $6.9 \times 10^9 \text{ s}^{-1}$. Even at low temperatures, this process continues to be favoured. At 218 K, for example, these values are respectively 4.3×10^{11} and $5.1 \times 10^9 \text{ s}^{-1}$.

Figure 5. Graph of reaction path for the conversion between **B** and **A** for an isolated molecule, obtained by the rotation of the C(14)-C(12)-N(11)-N(8) dihedral, using PM3 method.

Table 4 Thermodynamic data for the **A**↔**B** conversion of (**1**), for isolated and solvated species, calculated using DFT (B3LYP) and semi-empirical (PM3) methods.

Substituent	Form	$\Delta^{\circ}H_f$ (kJ mol ⁻¹)	$\Delta^{\circ}S_f$ (kJ mol ⁻¹ K ⁻¹)	$\Delta^{\circ}G_f$ (kJ mol ⁻¹)	$\Delta^{\#}G^{\circ}$ (kJ mol ⁻¹)	$\Delta^{\circ}G_r^{**}$ (kJ mol ⁻¹)
Isolated	A	182.16	-1.31	572.74	16.88	11.05
	TS*	199.04	-1.31	589.62	-	-
	B	193.21	-1.31	583.79	5.83	-
(solvated: CHCl ₃)	A	-273.51	-	-	-	6.76
	B	-266.75	-	-	-	-
(solvated: DMSO)	A	-580.71	-	-	-	15.54
	B	-565.17	-	-	-	-
(solvated: CH ₃ OH)	A	-716.28	-	-	-	5.26
	B	-711.02	-	-	-	-

*TS = Transition State; ** (**A**→**B**)

Further investigations were directed towards modelling both forms in solvent cages. This approach aims to reproduce solute-solvent interactions on a molecular microscopic level, considering the solvent as a discontinuum of individual solvent molecules, in strong contrast to the approach in which physical parameters such as dielectric constant or refractive index, which are macroscopic solvent parameters, are used. Theoretical evaluations of the thermodynamic data for both forms show a lower stability for the **B** form.

Some additional information on the energy barrier was obtained by modelling the molecules in association with CHCl₃, CH₃OH and DMSO. These data show as expected, an additional stabilisation for both conformers due to solvation effects, presented as an increase in the difference of enthalpy of formation. The $\Delta^{\circ}G_r$ for **A**→**B** conversion presents changes which should be attributed to solvent interactions, mainly intermolecular hydrogen bonding. In methanol and chloroform the energy barrier is significantly lower than the calculated for the isolated form. Differently, for the solvated form in DMSO, the value is 41% higher, indicating that the **B** form is being benefited by the formation of intramolecular hydrogen bonding. Additionally, as solvent viscosity exerts an important role on conformational changes of molecules, mainly if the solvent is highly viscous [17-19], it is expected that this conversion be more affected by the viscosity of DMSO than of methanol or chloroform, since that it is considerably higher (1.987 cp, whereas for methanol and chloroform are respectively 0.544 and 0.537 cp, at 298 K [20]), which explains the increase in the energy barrier.

5. Conclusions

The azo dye which is described in this paper exists in two forms, **A** and **B**, interchangeable by rotation around the (N=N)-phenyl bond. At room temperature they coexist in 1:1 ratio but at 218 K the major form is **A** (55:45), which is in agreement with our theoretical results (60:40).

The theoretical data show that the **A** form is the more stable and low activation energy must be expected for **B**→**A** conversion. The analysis of the calculated A-factors indicates that the conversion occurs as a cis-trans isomerization, and that it does not need positive changes in the activation entropy to occur.

The simulations in solution indicate that the **B** form is favoured by the solvation in DMSO, due to the formation of intermolecular hydrogen bonding. It is also expected an effect due to solvent viscosity on the conversion.

Additionally, the results suggest that at higher temperatures in DMSO-d₆ solution the only form which is observed may be attributed to the **B** conformer.

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