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CONFORMATIONAL ANALYSIS OF AN ANXIOLYTIC AGENT: TANDOSPIRONE IN AQUEOUS SOLUTION

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Abstract: The conformations of tandospirone (**1a**) and related compounds in aqueous solution have been studied by ^{1}H NMR, IR experiments and MM calculations. The significant contribution to the folded conformer of tandospirone was found in aqueous solution. © 1997 Elsevier Science Ltd.

Tandospirone[1] (**1a**) (Fig. 1) is an arylpiperazine compound that binds to the 5-HT_{1A} receptor with high affinity[2], characterized as a partial agonist and classified as an anxiolytic and antidepressant in a variety of pharmaceutical tests.[3] It was found that **1a** was much less potent than buspirone at the D_2 receptors[4] and did not bind at either the benzodiazepine or the GABA receptor sites.[5] Although **1a** bound to the 5-HT_{1A} receptor selectively, a detailed understanding of the receptor binding mode is unknown. In a series of the hetero-arylpiperazines, it was reported[7] that the lipophilic character of the imide portion is needed for high 5-HT_{1A} affinity, while another hypothesis[8] suggested that steric factors play an important role. These results encouraged us to analyze the conformations of **1a** in aqueous solution, as our hypothesis expected that the active conformations of **1a** were closely related with its conformations in aqueous solution. It is the purpose of this paper to describe the conformational analysis of **1a** in aqueous solution using ^{1}H NMR, IR experiments and MM calculations.

2D-NOESY and 1D-NOE difference experiments[9] were conducted in both deuterated chloroform and buffer solution. Three types of NOE pattern of **1a**, and the free base of tandospirone (**1b**) were observed in aqueous and chloroform solution (Fig. 2). The NOEs of H_e-H_h and H_d-H_f (Fig. 2 (A)) were observed in **1a** in

chloroform solution. The enhancements of the H_b (3 %), H_j (3 %), and H_m (4 %) resonances were observed for **1a** in aqueous solution when H_n was irradiated (Fig. 2 (B)). In the case of **1b**, in chloroform solution, significant NOEs were not observed (0.5 % >). The intensities of the NOEs for **1a**, in chloroform solution, were gradually reduced as the temperature rose from 20℃ and the significant NOEs disappeared at 43 ℃. The intensity of the NOEs for **1a** in aqueous solution were gradually decreased by raising the temperature and the significant NOEs disappeared at 48 ℃. The pattern of the NMR resonances for the alkyl chain region for **1a** in chloroform and an aqueous solution was changed by temperature. The significant NOEs of **1a** in chloroform and aqueous solution at high temperature were essentially identical to those of **1b** in chloroform solution at room temperature. From these results, it can be seen that the conformations of **1a** in aqueous and chloroform solution became more flexible as the temperature was raised. These results suggest that the conformations of **1a** in chloroform solution and in aqueous solution at room temperature were different from that of **1b** in chloroform solution.

The existence of restricted conformations of **1a** in chloroform solution which give rise to the NOEs shown in Fig. 2 (A) was explained by invoking an intramolecular hydrogen bond, which was investigated by IR experiments of **1a** and **1b**, in chloroform solution. described as follows [10,11]. IR spectral analysis showed that the absorption of the imide carbonyl in a diluted solution were 1693 cm^{-1} for **1a** and 1703 cm^{-1} for **1b**. Thus the C=O absorption band was shifted to a lower frequency ($\Delta\nu$=10 cm^{-1}) as a result of the intramolecular hydrogen bond present in **1a**(Fig.3). Compound (**3**) (Fig. 1) was synthesized to evaluate the effect of this intramolecular hydrogen bond, as a comparable intramolecular hydrogen bond is not possible. Indeed, the IR spectrum showed that the absorption of the imide carbonyl of a dilute solution in chloroform was 1704 cm^{-1}. Hence, the spectral analysis supported the intramolecular hydrogen bond in **1a**, in chloroform solution.

Next, the anisotropy effect of **1a** in an aqueous solution was investigated in two ways, one was by a comparison of the chemical shifts of the bicyclo ring moiety between **1a** and the model compound (**2**), and the other was by the comparison of the temperature dependent anisotropy effect of **1a** with **2**. The NOE experiments in **2** showed similar NOE patterns to that of **1a** except in the NOE enhancement between pyrimidine and the bicyclo moiety (Fig.2 C), which indicated that the conformation of **2** is similar to that of **1a**. The chemical shifts of H_j and H_m in **1a** shifted to higher field ($\Delta\delta$ -0.017 and -0.018 ppm) compared with that of **2**. In contrast, H_n shifted to lower field ($\Delta\delta$ +0.016 ppm) and no shift change($\Delta\delta$ 0.002 ppm >) was observed for H_i and $H_{k,l}$ (Table 1). No anisotropy effect ($\Delta\delta$ 0.002 ppm >) could be observed for both **1a** and **1b** in chloroform solution. The chemical shifts of all the protons of **1a** in aqueous solution at 48 ℃ were essentially identical to those of **1b** in chloroform solution at room temperature. Moreover, there was no

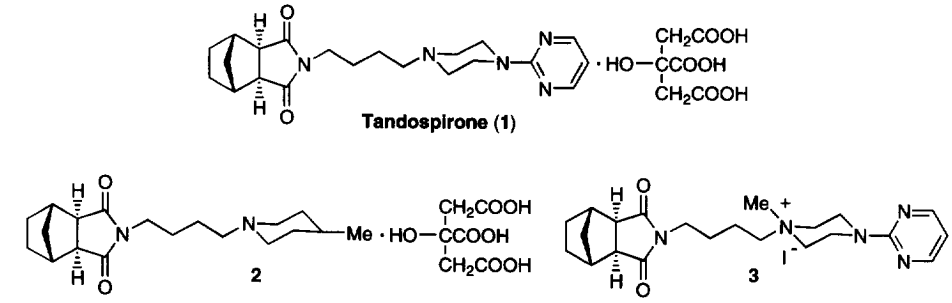

Fig. 1 Molecular formulas of tandospirone and related compounds.

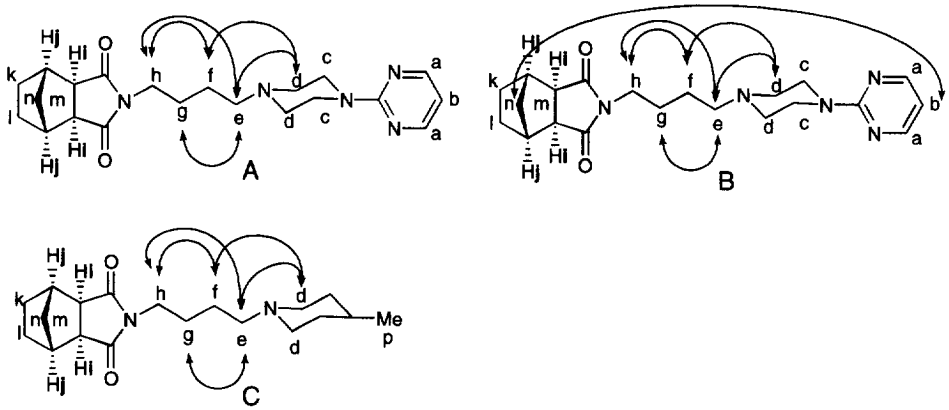

Fig. 2 Intramolecular NOEs of tandospirone and compound (**2**) at room temperature. Significant NOE signals are indicated: (A) tandospirone in chloroform solution, (B) tandospirone in aqueous solution, (C) compound (**2**) in aqueous solution.

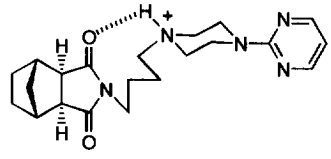

Fig. 3 Proposed bent, intramolecular hydrogen-bonded conformation of tandospirone.

anisotropy effect observed for **1a** in aqueous solution at 48 ℃. These chemical shift changes indicated that H_j and H_m for **1a** in aqueous solution, are present in the inside of the pyrimidine ring and that H_n existed in the outside of the ring. Although it could be considered that both H_i and $H_{k,l}$ also existed in the outside of the pyrimidine ring, the distance between these protons and the ring would be too long to detect any anisotropy effect. It has been that the bridge-head of the bicyclo ring and the plane of the pyrimidine ring face each other in a folded conformer in aqueous solution. Janson *et al.* have reported[12] the relationship between the small chemical shift differences (0.01-0.29 ppm) and the structures of phthalocyanines and Norman *et al.* have reported[13] that two aromatic rings face each other by π–π interaction in the folded conformations of isoindolinone derivatives.

The study of the coupling constants of the protons in alkyl chains and piperidine rings may also be effective in discussing the conformational differences between the aqueous and chloroform solutions. However, this data could not be obtained due to the multiplicity and overlapping nature of the resonances. The conformational contributions to **1a** were then investigated using molecular modeling. Monte Carlo conformational searches (800 steps) were conducted using Macromodel[14] with the AMBER* force field. The conformational contributions for **1a** and **1b** were modeled in the protonated and nonprotonated forms respectively and investigated using the parameter for either water or chloroform continuum. All flexible bonds were allowed to rotate and every generated conformation was minimized up to 2000 iterations. The results showed three general families of conformations, namely, extended (D), bent (E) and folded (F) conformations, as shown in Fig. 4. For **1a**, the conformations of the type E in Fig. 4, preferred in both chloroform and water continuum [$\Delta\Delta$H (kJ/mol), water, D: 3.8, E: the most stable, F: 6.3; chloroform, D: 4.2, E: the most stable, F: 8.4]. The conformations of the type F were more preferential in water continuum. However, in **1b** many conformations were predicted in approximately the same energy level in both continua mainly due to the free rotation of the alkyl chain[$\Delta\Delta$H (kJ/mol), water,D: the most stable, E: not found, F: 2.4; chloroform, D:the most stable, E: not found, F:6.3]. For example, 70 conformations were found within 1kJ/mol of the lowest energy conformation in chloroform. As the conformations of types E and F in Fig. 4 were consistent with those of types A and B in Fig. 2 respectively, these conformational searches suggested that the bent conformation of tandospirone is stabilized by an intramolecular hydrogen bond in chloroform solution. Although, the stability of the conformation of type F in aqueous solution, was not well reproduced, the contribution of the hydrophobic interactions[15] between the bicyclo ring and the pyrimidine ring was observed.

These computational results together with NMR and IR data indicate that the bent conformer contributed to the conformations of **1a** in chloroform solution due to an intramolecular hydrogen bond and the folded

Table 1. ^{1}H NMR chemical shifts of tandospirone and compound (**2**).
(500 MHz, pH 7.4, 30°C in 50 mM phosphate buffer)

	tandospirone	compound (**2**)	Δ (δtandospirone-δcompound (**2**))
H_i	2.800, s	2.800, s	0.000
H_j	2.621, s	2.638, s	-0.017
$H_{g,k,l}$	1.605-1.674, m	1.603-1.676, m	-
$H_{k,l}$	1.372, d, J=7.6 Hz	1.371, d, J=7.6 Hz	+0.001
H_m	1.271, d, J=11.3 Hz	1.289, d, J=11.3 Hz	-0.018
H_n	1.046, d, J=11.3 Hz	1.030, d, J=11.3 Hz	+0.016

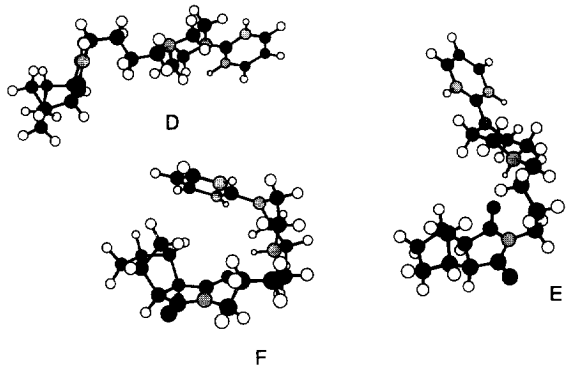

Fig.4 Representative low energy conformations of tandospirone: (D) extended conformation of nonprotonated form in chloroform, (E) bent conformation of protonated form in chloroform with a hydrogen bond, (F) folded conformation of protonated form in aqueous solution with a hydrogen bond and hydrophobic interaction.

conformer contributed to the conformations of **1a** in aqueous solution due to hydrophobic interactions[15] between the bicyclo ring moiety and the pyrimidine ring. The folded conformer of **1a** in aqueous solution and related data are of interest in connection with its structure activity relationship.

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(9) A sample was dissolved in 0.6 ml of phosphate buffer solution (50 mM sodium phosphate, pH 7.4). Both the 1D and 2D NMR spectra were recorded on JEOL A 500 spectrometer. The chemical shifts (in ppm) were referenced to 2-trimethylsilyl-2,2,3,3,-tetradeuteriopropionic acid, sodium salt as the internal standard. Phase sensitive 2D NMR spectra were recorded as 256 t_1 blocks of 1024 complex points each in the t2 dimension and averaged 16 scans per block during the recycle delay of 3.0 sec. for the NOESY. The data sets were processed using the EDL (JEOL Inc., Tokyo, Japan) on a VAX 3200 workstation.

(10) IR spectra were recorded at room temperature on a Perkin Elmer 1600 FT-IR spectrometer using a KBr solution cell with an optical pathlength of 0.1mm. 1mg of Samples were dissolved in 10ml of chloroform.

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