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The stereochemistry in solution of phthalazino[2,3-*b*]phthalazine-5,12-(14*H*,7*H*)dione, a synthetic anti-inflammatory drug, has been studied by proton magnetic resonance using the paramagnetic shift reagent Eu(fod)₃. A topological approach to the computer simulation has been applied and it has been found that the preferred conformation of the computer under study is the planar one.

Diftalone (phthalazino[2,3-*b*]phthalazine-5,12-(14*H*,7*H*)-dione), antiinflammatory agent (1-2), was previously synthesized (3). The purpose of the present paper is to compare the conformation of the title compound observed for the crystalline state (4) with that found in solution.
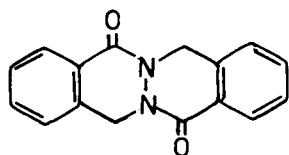
It has been shown by X-ray diffraction (4) that the molecule of diftalone consists of two symmetrical parts connected through the N-N bond; the dihedral angle between the two moieties is 115.2°. The two nitrogen atoms are in an imperfect sp² hybridization state with a displacement of 0.16 Å for N(6) and 0.17 Å for N(13) from the plane defined by the three atoms bound to them.

X-ray diffraction reveals the conformation in the solid state. However, for many compounds, it can be expected that solvent-solute interactions may produce various conformational changes. Since many biologically active substances show their activity in liquid environments, it is of great interest to ascertain the stereochemistry of these molecules in solution.

The application of lanthanide shift reagents (LSR) (5-6) in the ¹H nmr analysis and the simulation of the observed lanthanide induced shifts (LIS) using computational procedures (7-14) appears to us as a valuable method for the elucidation of molecular structure in solution. We have used Eu(fod)₃ [tris(1,1,1,2,2,3,3,-heptafluoro-7,7-dimethyl-4,6-octanedionate)europium] as a shift reagent and have applied to the title compound a topological approach (11-12,14), at first used for substrates containing a carbonyl group. In diftalone, two identical functional groups are present, but the reliability and utility of this approach to the interaction of LSR with bifunctional substrates has been confirmed (13). This fact (13) was demonstrated in that 2:1 or 1:1 complexes could be both analyzed in the same way, at least on the nmr time scale, at ambient temperature, in which the exchange of lanthanide ion is a

fast process, and the doped spectrum represents an averaged spectrum of free and complexed substrate. Besides, if both functional groups are identical, there should be no problem with group competition and it can be assumed that on an average they are equally complexed (15). The topological approach (12) reveals that the lanthanide (Ld) complexed only with the two oxygen lone-pairs, which reside in diastereomeric environments represented by values of the C-O-Ld internuclear angle (ψ) of 140° (A) and 220° (B).
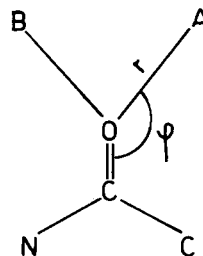

Figure 1

The distance lanthanide-oxygen is fixed at 3.0 Å, which is the most widely accepted value (11,12), and the C=O distance is taken to be equal to 1.22 Å according to the title compound diffractometric data (4).

The molar fraction of the two diastereomeric complexed substrates were determined by computer program (12), in which the LIS values observed for each proton were matched with those calculated according to the following formula,

$$\Delta \nu_{i,obs} = K(W_A G_A + W_B G_B) \quad (1)$$

where W_A and W_B are the molar fractions, and G_A and G_B the geometrical factors corresponding to the forms with the Ld in positions A and B, respectively (Figure 1). The constant K is the pseudocontact constant of the McConnell-Robertson equation (16),

$$\Delta \nu = K[(3\cos^2 \chi - 1) r^{-3}] \quad (2)$$

where (χ is the O-Ld-H internuclear angle and r is the corresponding Ld-H distance. The term in square brackets represents the geometrical factor (G). Direct analytical solution of equation 1 yields the molar fraction (W_A) and the pseudocontact constant (K).

The difference between observed and calculated LIS is expressed in terms of error by means of the Hamilton (17) agreement factor (AF).

$$AF = \sqrt{\frac{\Sigma(\Delta\nu_{i,obs} - \Delta\nu_{i,calcd})^2}{\Sigma_i(\Delta\nu_{i,obs})^2}} \quad (3)$$

The minimum value of AF is assumed to determine the most likely molecular geometry of the complex (11).

With this computational procedure we have tried to determine the molar fraction of A and B and the conformation of the title compound in solution, since the possibility of deformation of two nitrogen atoms toward a configuration different from that observed in the crystalline state could not be excluded.

In the ^{1}H nmr spectrum in deuteriochloroform, the methylene protons resonate as a singlet (Table I) also at low temperature. In order to exlude an accidental isocrony, the spectrum of the title compound was recorded also in acetone-d_6 and benzene-d_6, but no difference was observed. The compound exists therefore in a conformation in which the methylene protons are enantiotopic and the sharp signal can be due either to a planar conformation or to a mean value resulting from an exchange between two conformers in equilibrium for a rapid N-inversion.

The use of LSR, coupled with simulation of the observed LIS and the recording of the low temperature spectrum, allows us to observe that the compound under investigation prefers the planar conformation in solution.

In fact, the calculated LIS for the other possible conformers, obtained by altering the two nitrogen atoms stereochemistry (from N-trigonal to N-pyramidal), are too far away to be reconciled with the experimental values, while for the planar conformer, the AF is low (0.0168) (Figure 2) and the LIS calculated are nearly coincident with the experimental one.
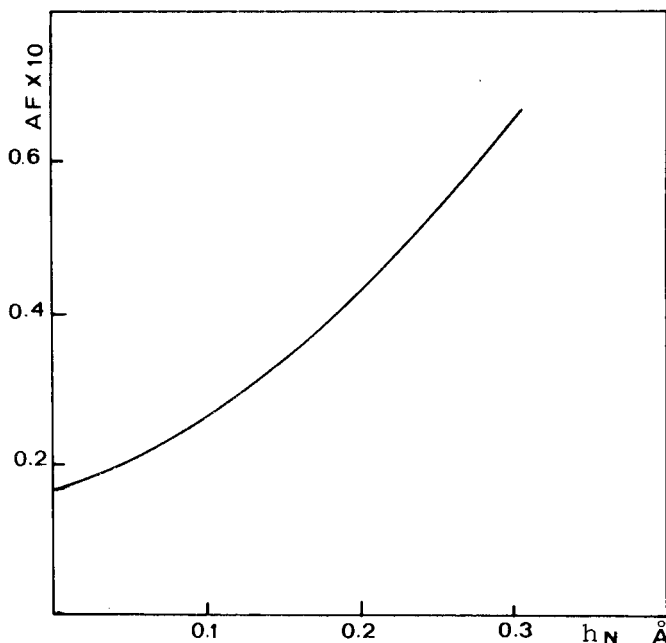

Figure 2 - Plot of agreement factor values (A.F.) as a function of the elevation of the two nitrogen atoms.

Besides the minimum A.F. and the calculated LIS, as output the program (14) yields the molar fraction W_A of the form with ψ at 140°. The value of 0.91 for the molar fraction W_A (Table I) of the complexed compound under investigation reflects a steric situation in which the lanthanide site of complexation A is the less sterically demanding. Since the carbonyl oxygen is the preferred site of complexation for Eu^{3+}, comparable LIS have been observed for methylene and ortho-protons (H_3) of two phenyl groups, while for the other aromatic signals, no remarkable shift was observed in the presence of $Eu(fod)_3$.

Table I

Chemical Shifts, Measured and Simulated LIS and Preferred Location of Lanthanide for Diftalone (in Deuteriochloroform)
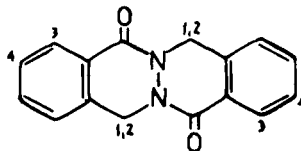
Protons	δ (ppm)	LIS (ppm)		K	W_A(a)	AF
		Obsv.	Calcd.			
$H_{1,2}$	5.23	8.68	8.80	753	0.91	0.0168
H_3	8.03	9.05	8.91			
H_4	7.40	1.98	2.08			

(a) Molar fraction of the form with the lanthanide attached at $\psi = 140°$.

EXPERIMENTAL

The compound used in this study was extracted from the corresponding drug Aladione® (Lepetit) with chloroform and crystallized from ethanol. The product had m.p. 220-221°.

All ^{1}H nmr spectra were observed on a Varian T-60A spectrometer at 60 MHz. The spectra at a probe temperature were recorded in deuterio-chloroform, acetone-d_6 and benzene-d_6. The low temperature spectrum was recorded in deuteriochloroform even at −80°. All chemical shifts value are given in (δ) ppm relative to internal tetramethylsilane.

The tris(1,1,1,2,2,3,3,-heptafluoro-7,7-dimethyl-4,6-octanedionate)-europium Eu(fod)$_3$ was added stepwise, using a 50 μl. syringe, up to a value of *ca.* 0.4 [Lanthanide]/[Substrate] ratio on samples whose molarity is 0.5M in 0.3 ml. of deuteriochloroform. Each signal was followed in the spectra and the LIS were found to be directly proportional to the [L]/[S] ration present. A least square fit of the experimental points was used to obtain the observed molar LIS.

The geometrical features of the possible conformatins were calculated using Dreiding models and taking into account X-ray data, for the conformer present in the solid state. Calculations relative to the simulation of the experimental LIS data were carried out on an IBM 370/115 computer with a program written by the authors (14).

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