Nuclear Magnetic Resonance Spectra of Psychotherapeutic Agents

V*—Conformational Analysis of 1,3,4,5-Tetrahydro-2*H*-1,5-benzodiazepin-2-ones†

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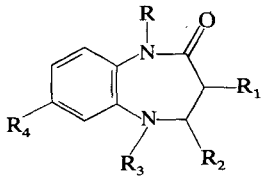
The conformational analysis of biologically active lofendazam (7-chloro-5-phenyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one) is carried out by means of lanthanide shift reagent assisted ^{1}H NMR spectroscopy: the lanthanide induced shift computer simulation suggests that in deuteriochloroform the heterocyclic ring of lofendazam assumes a cycloheptene-like chair conformation, where 1-N moves away from trigonal stereochemistry to a very flattened pyramidal structure. At room temperature the conformational equilibrium is markedly shifted (85%) towards the conformer showing pseudoaxial H-1 and 5-Ph. The remarkable influence of steric requirements in controlling conformation, and the importance of 3- and/or 4-methyl groups in hindering the ring inversion at room temperature, have been verified by conformational analysis of suitable analogous 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones.

Several benzodiazepine derivatives are psychotherapeutic agents, widely used for the treatment of anxiety and neuroses including psychosomatic disturbances; moreover, they show low toxicity, minimal effect on respiration and lack of autonomic side effects. It is to be expected that these products will remain in therapeutic use for many years.[1]

Our structure–activity relationship (SAR) studies concerning psychotherapeutic drugs,[2–5] such as 1,4- and 1,5-benzodiazepine derivatives, led us to suggest that their biological activity—strictly related to the stereochemistry of the heptatomic ring in addition to the presence of 'pharmacophoric' substituents—is improved by the existence of the drug in solution as only one conformer which does not interconvert at room temperature. Therefore, it seems important to investigate both the steric and electronic factors which may control conformation. For instance, it was verified for 1,3-dihydro-2*H*-1,4-benzodiazepin-2-ones that a 3-methyl substituent has a highly restricting effect on conformational inversion,[6] while the increment from the size of the 1-substituent appears to be not very relevant in enhancing the activation enthalpy of the inversion of the heterocyclic ring.[7] An accurate evaluation of the factors which affect the conformational equilibrium might lead to useful previsions concerning the structural characteristics necessary in a benzodiazepine derivative to enhance its biological activities and, possibly, narrow the spectrum of action.

On the basis of the aforementioned considerations, we undertook the study of the 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one system—occurring in biologically active lofendazam (**1**)[8]—with the aim of investigating its stereochemistry and evaluating the factors which eventually influence the conformational equilibrium.



	R	R_1	R_2	R_3	R_4
1	H	H	H	Ph	Cl
2	Me	H	Me	Me	H
3	H	H	Me	H	H
4	H	H	Me	Me	H
5	H	Me	H	Me	H
6	H	H	H	H	H
7	H	H	H	Me	H

RESULTS AND DISCUSSION

At room temperature, the ^{1}H NMR spectrum of lofendazam (**1**) in $CDCl_3$ solution shows that, in the higher field region, the 3-CH_2—4-CH_2– protons resonate as an A_2B_2 system, exactly symmetrical about its midpoint: the pattern of the half-spectrum approximates to a triplet of the A_2X_2 limit, where the A nuclei are

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Table 1. Chemical shifts, measured and simulated LIS, for 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones[a]

Compound	H-1	1-Me	3-CH_2–	3-Me	4-CH_2–	4-Me	H-5	5-Me	*K*	W_a	AF
	8.91		2.63		3.78						
1	9.06		14.97		6.57				82516	0.85	0.0224
	9.05		14.77 14.99		7.04 6.45						
	8.99		2.80		3.		3.67				
6	7.87		8.01		4.10		1.80		43612	0.52	0.0200
	7.86		8.09 8.13		3.87 3.96		1.90				
	9.06		2.55		3.56			2.83			
7	9.98		9.43		4.85			1.73	51717	0.51	0.0812
	9.50		9.59 9.63		4.50 4.58			3.02			
			H-3ax H-3eq		H-4ax H-4eq					W_A	
		3.40	2.19 2.49		3.80	1.13		2.82			
2		13.23	13.96 17.26		9.13	3.71		3.39	86493	0.98	0.0708
		12.74	13.31 17.64		8.90	5.44		3.58			
	8.69		2.56 2.76		4.09	1.37					
3	12.69		10.49 11.53		6.51	4.99			60295	1.00	0.0854
	12.65		9.10 12.27		6.35	4.03					
	9.12		2.30 2.61		3.91	1.21		2.85			
4	9.22		7.48 9.92		6.47	4.39		2.12	48891	1.00	0.1157
	10.25		7.38 9.95		5.15	3.27		2.12			
	8.70		3.22	1.09	3.45 3.72			2.77			
5	5.52		2.37	7.64	5.34 1.12			1.55	34653	1.00	0.2234
	7.50		2.78	6.44	4.55 1.53			1.60			

[a] Figures in the first row indicate chemical shifts (δ) of undoped spectra; figures in the second row indicate the observed molar induced shifts; figures in the third row indicate calculated molar induced shifts; *K* is the pseudocontact constant; W_A is the molar fraction of form A; W_a is the molar fraction of conformer a; AF is the agreement factor.

equally coupled with both the B nuclei, and the proton chemical shifts are pairwise coincident (Table 1). The two-proton triplet at lower field (δ3.78) is attributed to the 4-CH_2– protons because of their proximity to the 5-nitrogen atom: this assignment is supported by the observed shifts in the presence of lanthanide shift reagent (LSR) (*vide infra*). In a flexible ring asymmetric compound such as **1** these ^{1}H NMR characteristics can be explained on the basis of an existing conformational equilibrium, with the result that the observed resonance data are averaged values resulting from the parameters of the limiting conformations.

The ^{1}H NMR parameters of some lofendazam analogues, 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones **2–5** all methyl substituted on the 3- or 4-carbon atom, were than examined: derivatives **2, 4** and **5** were new compounds (see Experimental). The spectra of **2–5** show that these compounds all exist as only one conformer which does not interconvert at room temperature: the methylene protons at C-3 and C-4 in **2–4** and **5**, respectively, resonate as an AB part ($\nu_0\delta$ *c.* 16 Hz) of an ABX system, further split by the spin–spin coupling of the X proton with the adjacent methyl group. The assignment of the resonances to the axial and equatorial protons is straightforward from the observed vicinal coupling constants (see Tables 1 and 2). These results compared with ^{1}H NMR data of **1**, whose methylene protons constitute two different pairs of magnetically equivalent nuclei, confirm its existence as two rapidly equilibrating conformers in deuteriochloroform solution and at ambient temperature; these conclusions are supported by variable temperature ^{1}H NMR experiments.[9]

The necessary information on the stereochemical characteristics of the two limiting conformations of **1** was achieved with the help of the lanthanide shift reagent (LSR) Eu(fod)$_3$. The application of LSR in NMR spectroscopy affords considerable spectral simplification, and cogent information on the stereochemistry of the lanthanide/substrate complex may be achieved according to the McConnell–Robertson equation,[10] which correlates the lanthanide induced shifts (LIS) to the geometric parameters of various protons.

By adding Eu(fod)$_3$ to the deuteriochloroform solution of **1** all the signals are shifted towards lower field, with an almost linear dependence of the LIS on the ligand/substrate (L/S): it is noteworthy that the 3-CH_2– protons, which resonate at higher field than 4-CH_2– in the absence of Eu(fod)$_3$, are more deshielded by LSR owing to their greater proximity to the carbonyl oxygen, which represents the preferred site of complexation for Eu^{3+}. At L/S = 0.13, therefore, the

Table 2. Coupling constants (Hz) for 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones (2–5)

Compound	J(3a3e)	J(3a4a)	J(3e4a)	J(3a4e)	J(4a4e)
2	12.3	9.1	5.4		
3	13.7	7.6	4.4		
4	12.5	8.9	5.3		
5		9.4		6.7	12.4

Table 3. Coupling constants (Hz) and molar fractions of conformer a for 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones (1, 6, 7) as deduced by LAOCN3 analysis (for proton identification, see Fig. 1)

Compound	J(AA')	J(AB)	J(AB')	J(A'B)	J(A'B')	J(BB')	W_a[a]
1	−13.67	9.31	5.34	5.34	7.03	−13.57	0.81
6	−14.00	8.14	5.45	5.45	8.14	−14.00	0.50
7	−14.02	8.18	5.27	5.27	8.07	−13.99	0.52

[a] Calculated from the coupling constants (see text).

four methylene protons resonate as only one line, and at L/S greater than 0.25 an absorption pattern approximating to an A_2X_2 system is observed. The related coupling constants (Table 3), unaltered during the LSR addition, indicate that the conformational equilibrium is unaffected by LSR addition, so that the stereochemical information concerning the complexes can be reasonably extended to the uncomplexed molecules.[11]

For **1**, the theoretical LIS of the protons in several model structures, intermediate between the possible limiting situations of the cycloheptadiene and cycloheptene type (1- and 5-N trigonal or pyramidal, respectively) were computer simulated, using a topological approach to the LSR complexation with the carbonyl group.[12] (The geometrical parameters of the model structures were deduced from Dreiding models, suitably modified to simulate the progressive flattenings of both nitrogen atoms.) This is based on the simplifying assumption that LSR complexes only at the diastereomeric chemically accessible sites (A and B), i.e. the two lone pairs of the carbonyl oxygen. These two positions, A and B, in the 2-carbonyl plane are indicated by values of $\varphi = 140°$ and 220°, respectively, where φ is the Eu—O—C-2 angle (the lanthanide—oxygen internuclear distance is taken as 3 Å). The populations of conformers **1a** and **1b** (Fig. 1) were calculated using the following equation

$$\Delta\nu_i = K[W_a(W_A{}^aG_A{}^a + W_B{}^aG_B{}^a) + W_b(W_A{}^bG_A{}^b + W_B{}^bG_B{}^b)]$$

where $\Delta\nu_i$ are the pseudocontact lanthanide induced shifts; K is the pseudocontact constant of the McConnell–Robertson equation; W_a and W_b are the conformer molar fractions; $W_A{}^a$, $W_A{}^b$, $W_B{}^a$, $W_B{}^b$ refer to the molar fractions of the forms with the lanthanide in positions A and B, respectively; and $G_A{}^a$, $G_A{}^b$, $G_B{}^a$, $G_B{}^b$ are the corresponding geometric factors. By means of a minimization procedure[3] we used calculated G values in the different model structures, and varied K and W_a (W_b) to obtain the best agreement—minimum AF[3]—between the calculated and observed LIS. The principal magnetic axis of the lanthanide substrate complex was assumed to coincide with the orientation of the bond from Eu^{3+} to the coordinating oxygen atom. The validity of this assumption is supported by the fact that minimal deviations of the effective magnetic axis from the assumed orientation, and no significant improvement of the fit,[5] were obtained with the use of two additional parameters, φ and ω,[13] needed to define the 'true' orientation of the magnetic axis of the complex. The results show that the two rapidly equilibrating conformers of **1** are chair-shaped, with a very flat pyramidal form of 1-N (elevation of the 1-nitrogen atom in the range of 0.15 Å from the plane defined by its three bonded atoms); an analogous geometrical change holds for 5-N, whose lone pair conjugation with the phenyl group and condensed aromatic ring flattens the nitrogen pyramid.[14] The analogy with the stereochemistry of *N*-desmethyldiazepam,[3] clobazam and triflubazam is remarkable.[4] Conformer **1a** is markedly preferred (85%) at room temperature. This conformational result is supported by the analysis of the vicinal coupling constants: an iterative analysis of the methylene region of the spectrum of **1** using the LAOCN3 program,[15] in the absence of $Eu(fod)_3$ and at various L/S ratios, was carried out. The agreement between the observed and computer simulated spectra confirms the correctness of the analysis, the assignment of the protons and, also, that the observed coupling constants are unaltered by the addition of $Eu(fod)_3$. The J(AB) and J(A'B') values obtained (Table 3) were used to calculate the conformer percentages, using limiting values of ^3J(HH) (ax–ax) = 9.98 and ^3J(HH) (eq–eq) = 6.30 Hz resulting from the iterative LAOCN3 analysis of the spectrum of **1** at low temperature.[9]

The same flattened pyramidal structure of 1-N as in **1** was found in derivative **2**, while compounds **3–5** show sp^3 hybridization of both nitrogen atoms. These stereochemical characteristics were deduced from a computational procedure in which the LIS values observed for each hydrogen were matched with those calculated in the different model structures (see above) according to the following simplified formula

$$\Delta\nu_i = K(W_AG_A + W_BG_B)$$

where W_A and W_B are the molar fractions, and G_A and G_B are the geometrical factors corresponding to the forms with the lanthanide in positions A and B, respectively.

The 1-N flattening from a pyramidal to a *quasi*-planar structure (verified in **1–2**) induces a puckering distortion[16] of the 3-CH_2—4-CH_2– moiety of the heptatomic ring (Fig. 2) with a consequent decrease of the dihedral angle between the two vicinal pseudoequatorial bonds; these are more nearly eclipsed in a

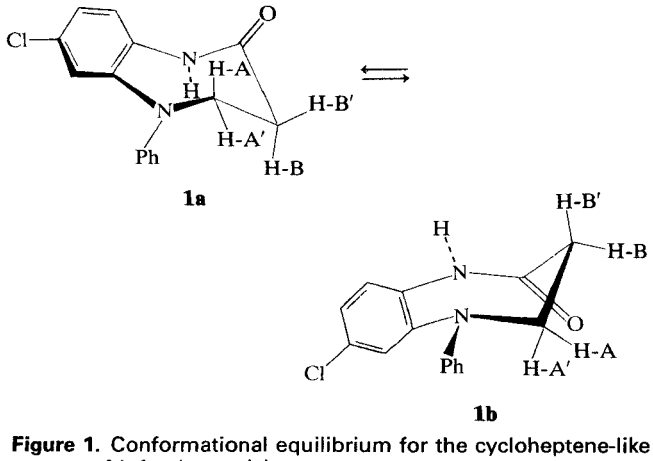

Figure 1. Conformational equilibrium for the cycloheptene-like system of lofendazam (**1**).

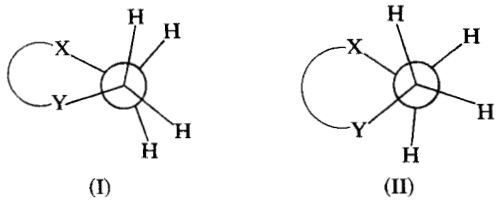

Figure 2. Puckering distortion of the 3-CH_2—4-CH_2– moiety in a cycloheptadiene-like system (II), and in a cycloheptene-like system (I).

cycloheptadiene-like system (II, trigonal 1-N) than in a cycloheptene-like system (I, pyramidal 1-N). When pseudoequatorial 3- or 4-substituents are present, as in compounds **3–5**, the ring distortion (II) is unfavoured by steric hindrance, and the pyramidal structure is the one adopted by the amide nitrogen atom. However, when a 1-methyl group is present, as in **2**, we suggest that the flattened pyramidal structure of 1-N arises as a good compromise between the electron-donating effect of the methyl group—which should stabilize the planar structure of 1-N—and the loss of steric hindrance which takes place when 1-N assumes a pyramidal structure.

The substituents in 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones **2–5** are all pseudoequatorial in the chair-shaped conformation observed at room temperature, according to both LIS stereochemical results and the magnitude of the vicinal spin–spin coupling constants (Table 2). On the contrary, the preferred conformer **1a** of lofendazam shows a pseudoaxial H-1 and 5-Ph. This conformational preference of the phenyl group is explainable according to the steric requirements of the bulky substituent and the electronic repulsions between the 5-lone pair electrons and the 1,2-amide π system, which is analogous to the results already reported for 1-methyl-5-phenyl-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepin-2,4-diones.[4] The 5-phenyl pseudoaxial preference is also supported by the 5-lone pair delocalization towards the π system of the phenyl substituent.

The evaluation of the ^{1}H NMR data of compounds **6** and **7** (Tables 1 and 3) provides information on the greater importance of steric factors in determining the unusual pseudoaxial conformational preference of 5-Ph in **1**. Both **6** and **7** exist as rapidly equilibrating mixtures of conformers at room temperature, and these cycloheptene-like chair-shaped conformers contribute almost equally to the equilibrium (1-N shows a very flattened pyramidal structure, as in **1** and **2**). These quantitative results (obtained independently from LIS data and vicinal coupling constant analysis, see Tables 1 and 3) suggest that the 5-Ph pseudoaxial preference in **1** can be essentially ascribed to the steric hindrance between the two *ortho*-hydrogens of the 5-Ph and H-6 of the fused benzene ring when the 5-phenyl group is pseudoequatorially situated.

CONCLUSIONS

The pharmacological activities of 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-ones are not fully known at present: to the best of our knowledge only lofendazam has been widely tested as a psychotherapeutic drug. Nevertheless, based on the above reported conformational results, we propose that the interesting biological activity of lofendazam (**1**) can be ascribed—in addition to the known importance of the 7-Cl group—to the marked conformational preference, observed in $CDCl_3$, for conformer **1a** where 5-Ph is pseudoaxially directed. This is analogous to our previous findings concerning biologically active clobazam and triflubazam.[4]

EXPERIMENTAL

^{1}H NMR spectra were obtained at 60 MHz (Varian T60A) at a probe temperature of 34 °C, for solutions *c.* 0.3 M in deuteriochloroform (TMS as internal standard) containing increasing amounts of $Eu(fod)_3$ up to a value of 0.4 moles of ligand (L) per mole of substrate (S). $Eu(fod)_3$ was purchased from B. H. Schilling. The lanthanide shift reagent was added stepwise from a stock solution (*c.* 300 mgml^{-1}) using a 50 μl syringe. Each signal was followed and the LIS were found to be directly proportional to the L/S ratio present. A least-squares fit for the experimental points was used to obtain the observed molar LIS. Calculations related to the simulation of the experimental LIS data were performed on an IBM 370/115 computer, using the topological program for the LSR interaction with the carbonyl group.[12] The spectra were analysed with the aid of a version[15] of the LAOCN3 program modified by us to run on our IBM computer, and to include a subroutine for plotting calculated spectra on a line printer.

Satisfactory elemental analyses were obtained for all compounds. Melting points were determined on a Kofler apparatus, and are uncorrected. Compounds **1**, **3**, **6** and **7** were prepared as previously described[8,17–19] and were pure by TLC.

Methylation of 3

Compound **3** (3 mmoles), 15 mmoles of MeI and 0.5 g K_2CO_3 in DMF were refluxed for 5 h to give a crude material which, after filtration and solvent removal, was purified by preparative TLC on silica gel plates.

Elution with ethyl acetate/light petroleum (8:2) afforded 0.2 g of 4,5-dimethyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (**4**), m.p. 137–138 °C (from methanol) (Calc. for $C_{11}H_{14}N_2O$: C, 69.45; H, 7.42; N, 14.72. Found: C, 69.40; H, 7.48; N, 14.69).

0.2 g of 1,4,5-trimethyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (**2**), m.p. 69–70 °C (from methanol) were also eluted (Calc. for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.90; N, 13.71. Found: C, 70.33; H, 8.03; N, 13.76).

3,5-Dimethyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (5)

o-Phenylenediamine (5.4 g), methacrylic acid (6.45 g) and 5.5 N hydrochloric acid (7.5 ml) were heated together at 100 °C for 4 h.

Basification with concentrated aqueous ammonia gave 3-methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (49%) m.p. 209–211 °C from methanol (Calc. for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90. Found: C, 67.89; H, 6.94; N, 15.75).

3-Methyl-1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one (3 mmoles), 7 mmoles of MeI and 0.5 g K_2CO_3 in DMF were then refluxed for 5 h; the reaction mixture was filtered, the solvent removed *in vacuo*, and the residue recrystallized from methanol to give, almost quantitatively, **5**, m.p. 156–158 °C (Calc. for $C_{11}H_{14}N_2O$: C, 69.45; H, 7.42; N, 14.72. Found: C, 69.32; H, 7.27; N, 14.74).

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