Conformation of 7-Chloro-5-phenyl-d_5-3(*S*)-methyldihydro-1,4-benzodiazepin-2-one in Solution

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7-Chloro-5-phenyl-d_5-3(*S*)-methyldihydro-1,4-benzodiazepin-2-one (**1a**) was synthesized and its conformation in solution determined using a computer assisted LIS-NMR method. It was found, with Pr(fod)-d_{27} as a shift reagent, that a lanthanide coordinates to a carbonyl oxygen at a 2.02 Å distance (bond angle 158.07°, torsional angle 39.53°), while the substrate **1a** adopts an expected quasi-boat conformation with a C(3)-methyl group exposed in a quasi-equatorial position.

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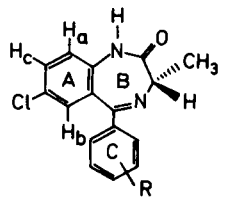
The title compound (**1**, CRC 1011) was found to possess the highest antianxiety and sedative properties within a series of C(3)-chiral 1,4-benzodiazepines which were recently prepared and tested (3,4). Proceeding further with the study of this therapeutically interesting chiral compound, we investigated its metabolism *in vitro* and *in vivo* and compared it to that of its 3R-enantiomer (5-7). Also, the stereoselective binding of **1** to human serum albumin (HSA) was studied (8,9). At this stage we become interested in obtaining a detailed insight into the conformation of **1** in solution.

Crystal structures have recently been determined for some achiral 1,4-benzodiazepines (10,11) and racemic 3-hydroxy derivatives (12,13). However, there is always an element of uncertainty when interpretation of chemical behaviour of the molecules in solution is based on conformations observed in the solid state. To be valid, such interpretations should rely on conformational data obtained with solutions. However, to the best of our knowledge, no study on the conformation of 1,4-benzodiazepines in solution has appeared thus far.



R
1 - 5xH
1a - 5xD

We have studied the conformation in solution of **1** using the NMR-LIS method, according to Armitage (14). In these experiments we encountered difficulties with the all-H compound **1**, because eight of its aromatic protons could not be completely resolved at any ratio of the lanthanide shift reagent to substrate. Therefore, the deuterated 5-phenyl-d_5 compound **1a** was prepared (as described in Experimental) and assignement of the three aromatic protons in ring A for this compound was made without difficulty.

The assignement was based on a comparison of the 360 MHz spectrum of **1a** with a calculated spectrum obtained by means of a computer program. A similar procedure was carried out with the 60 MHz spectrum, obtained from a 0.15*M* solution of **1a** in deuteriochloroform in the presence of 0.008*M* of Pr(fod)$_3$. In the latter case, the calculation gave a second order spectrum of 15 lines, three of which had low intensity. The results are presented in Table 1.

Once assigned, aromatic protons H_a, H_b and H_c, as well as the C(3)-proton and protons of the methyl group were used to determine the structure of the **1a** Pf(fod)$_3$ complex in solution. The bound shifts for these protons were obtained according to Armitage (14) by plotting the reciprocals of the induced shifts *vs.* substrate concentration at constant LSR concentration, and calculating the slopes by linear regression (Table 2). Some difficulties were encountered in fitting the data for the induced shifts of the aromatic protons, as shown by the respective coefficients of correlation.

All experimental values for the bound shifts were compared with those calculated from the McConnell-Robertson equation (15) $(3\cos^2\theta - 1)/r^3$, which considers the pseudocontact contribution and is generally valid for proton nmr spectra (16). The binding of substrate to LSR was considered to be of the 1:1 type, whereas 2:1 binding was excluded considering the bulkiness of the substrate. The acceptability of the results was discussed (17) in terms of the agreement factor $R = [\Sigma_i w_i(obs_i - calc_i)^2/\Sigma_i w_i(obs_i)^2]^{1/2}$, where w_i is the weight applied to the *i*-th observation (unitary weights were actually used): obs_i and $calc_i$ are the observed and calculated shifts.

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Table 1

Experimental and Calculated Frequencies of Aromatic Protons in Compound **1a** Appearing on the 360 and 60 MHz Spectra, the Latter Recorded in the Presence of 0.008*M* Pr(fod)$_3$)

360 MHz (Deuteriochloroform)				60 MHz (0.15*M* **1a** + 0.008*M* Pr(fod)$_3$)			
Shifts (Hz)	Coupling Constants (Hz)	Frequency, Hz (a) Calcd.	Measured	Shifts (Hz)	Coupling Constants (Hz)	Frequency, Hz Calcd.	Measured
H_a 2555.40	*ortho* 8.6	2693.64	2693.50	H_a 405.50	*ortho* 8.6	455.41 (c)	
H_b 2631.59	*meta* 2.4	2691.24	2691.07	H_b 423.90	*meta* 2.4	445.95	455.80
H_c 2687.98	*para* 0.5 (b)	2685.05	2684.88	H_c 439.80	*para* 0.5 (b)	443.57	443.80
		2682.65	2682.45			437.74	437.40
		2633.02	2632.69			435.04	435.00
		2632.51				425.57 (c)	
		2630.62	2630.48			425.29	424.80
		2630.11				424.71	
		2559.81	2559.71			422.92	423.00
		2559.30				422.33	
		2551.25	2551.09			413.45	414.00
		2550.71	2551.09			412.86	
						404.92	405.00
						404.33	
						393.22 (c)	

(a) All frequencies are related to TMS as internal standard. (b) This coupling constant was chosen on the basis of analogy with a similar spin systems, as it is not visible in the actual spectrum. (c) Small intensity lines.

Table 2

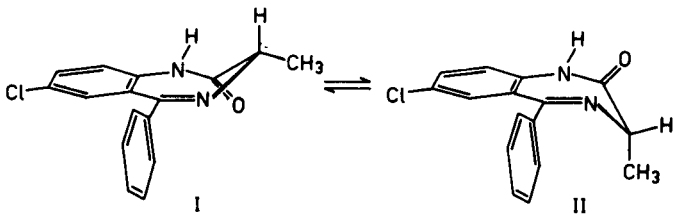
Characteristics of the Plot of the Induced Shifts *vs.* Concentration of **1a**

Proton, or Group of Protons	Intercept	Slope	Coefficient of Correlation
CH_3	-0.028	-10.932	0.999
C(3)H	-0.023	-10.064	0.998
H_a	-0.0002	-3.016	0.995
H_b	-0.042	-1.904	0.994
H_c	-0.011	-2.008	0.994

The geometries of the possible conformations for **1a** were calculated using structural data for diazepam (18), as well as the standard bond lengths, bond angles (19), and dihedral angles, as estimated from Dreiding models. For the three equivalent methyl protons, a mean position was adopted assuming free rotation of the group as a whole.

Conformations I and II in Scheme I are proposed as being likely. The possibility of I⇌II interconversion under the experimental conditions (35.0 ± 0.1°) may be ruled out because no evidence was found for the existence of two conformers of **1a** in various solvents, and in the presence of LSR, at this temperature. Lehn, *et al.* (20), found by DNMR that *N*(1)-demethyldiazepam possesses an inversion barrier of 12.3 kcal./mole, and the presence of the additional *N*(1)-methyl group in diazepam raises this barrier to 17.6 kcal./mole. We assume that a methyl group situated at C(3) has an even higher restricting effect on conformational inversion. This inversion requires the transposition of the methyl group from a pseudo-equatorial position in the more stable diastereomeric conformation I, to the pseudo-axial position in the less stable conformation II.

SCHEME 1.



Carbonyl oxygen, and the nitrogen atoms N(1) and N(4) have been proposed as possible binding sites for praseodymium upon addition of the reagent. The three parameters (ρ-bond length, ϑ-bond angle, and θ-torsional angle), which define the position of the praseodymium atom were varied until the minimum value was obtained only for conformation I, with oxygen as the binding site; in the other cases either non-acceptable bond distances or too high R values were obtained. Subsequently the agreement factor for conformation I of the substrate-Pr(fod)$_3$ complex was minimized with respect to other parameters pertaining to the seven-membered ring, *i.e.*, torsional angles for the ring as well as torsional and bond

Table 3

Calculated and Experimental Bound Shifts, Parameters (ρ, ϑ and φ) and Agreement Factor for the Two Conformations of the Complex **1a**-Pr(fod)$_3$

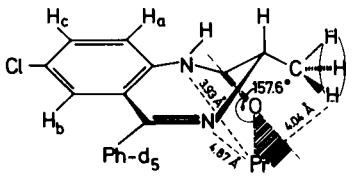
		Conformation I					Conformation II		
	Binding Site	O		N(1)	N(4)	O	N(1)	N(4)
	Expt.	Calcd.	Calcd.					
CH_3	10.93	10.93	10.93					
C(3)H	10.06	10.05	10.06					
H_a	3.02	3.24	3.02					
H_b	1.91	1.91	1.91					
H_c	2.01	1.50						
ρ (a)		2.07	2.02	4.86	7.64	0.312	8.15	7.36
ϑ		157.60 (b)	158.07 (b)	16.65 (e)	122.9 (g)	162.34 (b)	41.32 (e)	117.01 (g)
φ		45.82 (c)	39.53 (c)	121.23 (f)	357.67 (h)	244.92 (c)	66.55 (f)	9.49 (h)
R (d)		3.6	0.0	7.1	8.0	2.6	1.5	9.5

(a) Bond distance reported in Å. (b) Pr-O-C(3) bond angle reported in degrees. (c) Dihedral angle reported in degrees of C(2)-N(1) relative to Pr-O, measured anticlockwise along the direction C(2) to O. (d) Reported in percentages (e) Pr-N(1)-C(2) bond angle reported in degrees. (f) Dihedral angle reported in degrees of C(2)-C(3) relative to Pr-N(1), measured anticlockwise along the direction C(2) to N(1). (g) Pr-N(4)-C(5) bond angle reported in degrees (h) Dihedral angle reported in degrees of C(5)-C(5a) relative to Pr-N(4), measured anticlockwise along the direction C(5) to N(4).

angles for the substituents on C(3). Of these, only the N(4)-C(3)-H-bond angle was varied from the initial standard value 109.47° to 110.13°, while the initial values for other angles were those corresponding to the minimum value of R. Calculated and experimental bond shifts, the three parameters for LSR, and the agreement factor values are given in the Table 3.

Bound shift for H_c resulted to be the less accurate and it was responsible for R value, since it collapsed to zero when this bound shift was not taken into account in the calculations, as shown in the Table 3. It is noteworthy in this case the position of praseodymium did not change appreciably from that calculated when all five protons were taken into account. This discrepancy is presumably due to the difficulties encountered in getting reliable experimental bound shifts for the H_c proton.

In conclusion, the existence of conformation I in deuteriochloroform solution fo CRC 1011 was established, and the amide (carbonyl) oxygen was identified as the binding site for praseodymium (Figure 1). Due to a



higher stability of conformation I, we propose that this may be the biologically active conformation capable of existence even in the aqueous environment of biological tissue. However, the torsional angle of the 5-phenyl group and the potential influence of its change on the biological activity remains to be explored.

EXPERIMENTAL

Melting points were determined on a Kofler microheating stage and are not corrected. If spectra were run on a Perkin Elmer M 297 with potassium bromide pellets. Thin layer chromatography was performed on aluminium plates precoated with silica gel 60F 254 (Merck); column chromatography was performed on a silica gel 0.05-0.2 mm (Merck) column. Optical rotations were measured on a Perkin Elmer M 141 spectropolarimeter. Nmr spectra were recorded and LIS measurements were performed on a Perkin Elmer R 12 instrument.

LIS Measurements.

Tris[2,2-bis(trideuteriomethyl)-1,1,1-trideuterio-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato] praseodymium (III) [Pr(fod)$_3$-d_{27}] and tris[2,2-bis(trideuteriomethyl)-1,1,1-trideuterio-6,6,7,7,-8,8,8-heptafluoro-3,5-octanedionato europium (III) (Eu(fod)$_3$-d_{27}), both from Aldrich (uvasol grade), were purified by sublimation at 0.005-0.1 mm and kept in a paraffin-sealed vessel stored in a vacuum dessicator. All manipulations with these reagents, solvents, and compound **1a** were carried out in a glovebox which was continuously flushed with dry nitrogen during use. The stock solution of the lanthanide reagent, Pr(fod)$_3$-d_{27}, was prepared by dissolving 84.2 mg. in 5.0 ml. of deuteriochloroform which contained 2% TMS. The stock solution of substrate was prepared by dissolving **1a** (868.5 mg.) in 5.0 ml. of deuteriochloroform (2% TMS). Thereafter a constant amount (0.500 ml.) of lanthanide stock solution was measured into each of a series of six 1.0 ml. graduated flasks. Then, varying volumes of the substrate stock solution were added to each flask, and finally the volume of liquid was made up to 1.0 ml. by addition of deuteriochloroform (2% TMS). Each solution was then placed into a separate nmr tube, termostated before measurements were taken, and the spectra were recorded at 35 ± 0.2°. The six final

solutions had the following molarities in substrate: 0.25, 0.20, 0.15, 0.10, 0.08, and were 0.008*M* referred to Pr(fod)$_3$-d_{27}.

Calculations.

Programs for linear regression, simulation of spectra and calculation of the bound shifts were set up in our laboratories. With the latter program, the minimization of the agreement factor was accomplished in two successive steps: a broad search with a Monte Carlo technique (21), followed by a minimization with the SIMPLEX algorithm (22).

2-*N*-Acetylamino-5-Chlorobenzophenone-d_5 (**2**).

Bromobenzene-d_5 (Aldrich) (5.0 g., 30.8 mmoles) was dried before use with a molecular sieve (4Å). The dried substance was dissolved in twice absolutized ether (40 ml.), and this solution was added dropwise, with stirring under nitrogen, to a Grignard dispersion of magnesium (710 mg., 30.8 mmoles) in absolute ether to which a crystal of iodine was added. The addition was extended over 2 hours with continual stirring and nitrogen flow, maintaining a reflux temperature. Stirring and heating under reflux were continued for 1 hour after the addition was completed. The ethereal solution was separated by decantation, residual magnesium was washed with dry ether, and the combined ethereal mixture was added dropwise to a solution of 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (5.86 g., 30.0 mmoles, prepared as described in reference (3)) in a solvent mixture of ether-benzene (20 ml./40 ml.) cooled in an ice-water bath. After 2 hours of stirring in the cold, the ice-water bath was removed, and stirring was continued overnight at room temperature. The resulting mixture was once more cooled and acidified to *p*H 2 with 25 ml. of aqueous hydrochloric acid (1:4). The acid mixture was allowed to assume room temperature, and after 2 hours of stirring, it was extracted with benzene (3 x 50 ml.). The extracts were washed with dilute sodium hydroxyde and water, dried (sodium sulfate) and evaporated. The crude product (6.18 g.) was crystallized from benzene-cyclohexane (10 ml./20 ml.), to obtain 4.86 g. (57%) of pure **2**, m.p. 109-110°.

2-Amino-5-chlorobenzophenone-d_5 (**3**).

Compound **2** (4.86 g., 17.5 mmoles) was hydrolyzed by heating under reflux in a mixture of 96% ethanol (30 ml.) and hydrochloric acid 1:1 (30 ml.) over a period of 4.5 hours. After partial evaporation, water (20 ml.) was added. The *p*H was adjusted to 12 with concentrated ammonia, and the resulting mixture was extracted with chloroform (4 x 50 ml.). The extracts were washed with water, dried, evaporated, and the residual oil was crystallized from 50 ml. of hot ethanol and 40 ml. of water. Yellow crystal, 2.86 g. (70%), m.p. 98-99°, were obtained.

7-Chloro-1,3-dihydro-3*S*-methyl-5-phenyl-d_5-2*H*-1,4-benzodiazepin-2-one (**1a**).

Compound **3** (3.2 g., 13.5 mmoles) and dicyclohexylcarbodiimide (DCC) (3.05 g., 14.8 mmoles) was dissolved in 10 ml. of methylene chloride, and the solution was cooled in an ice-bath. To the cold solution, *N*-carbobenzoxy-*S*-alanine (3.31 g., 14.85 mmoles), dissolved in methylene chloride (10 ml.), was added dropwise with stirring. After 2 additional hours of stirring, the reaction mixture was left overnight in a refrigerator. Precipitated dicyclohexylurea (3.08 g., 100%) was filtered off, the filtrate was evaporated to dryness, the residue was dissolved in 40% hydrogen bromide in acetic acid (30 ml.), and the solution was stirred for 2 hours at room temperature. Then, 700 ml. of ether was added, and the precipitated oil was separated by decantation of the upper layer. On addition of ether (150 ml.), the oil was converted into an amorphous powder, from which the liquid was decanted. The residue was dissolved in concentrated aqueous ammonia (30 ml.) after 3 hours of stirring at room temperature; the solution was then extracted with methylene chloride (3 x 30 ml.). The combined extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. Crude **1a** (3.7 g.) was purified by column chromatography (100 g. of silica gel) using methylene chloride-acetone (9:1) as the mobile phase. Five ml. fractions were collected. From fractions 52-91, 2.92 g. of pure **1a** was obtained, which was crystallized from acetone-water (25 ml./30 ml.) to give 2.5 g. of the purified product with m.p. 196-197°; $[\alpha]_D^{22}$ = +155° (c = 1.65 in chloroform); ir: 3220, 1600, 1475 cm^{-1}.

Although recently (23) a procedure for the preparation of the intermediate **3** was reported, we prefer to describe here the complete procedure for preparation of **1a**, since optimization of the conditions already described by us (3) and others (23) are included.

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REFERENCES AND NOTES

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(2) Present address: Istututo di Chimica, Universitá degli Studi di Trieste, 34127, Trieste, Italy.

(3) V. Šunjić, F. Kajfež, I. Stromar, N. Blažević and D. Kolbah, *J. Heterocyclic Chem.*, 10, 591 (1973).

(4) V. Šunjić, J. Kuftinec and F. Kajfež, *Arzneim.-Forsch.*, 25, 340 (1975).

(5) D. Kolbah, N. Blažević, M. Hannoun, F. Kajfež, T. Kovac, S. Rendić and V. Šunjić, *Helv. Chim. Acta*, 60, 265 (1977).

(6) S. Rendić, V. Šunjić, F. Kajfež and N. Blažević, in "Microsomes and Drug Oxidations", V. Ulrich, Ed., Pergamon Press, Oxford and New York, 1977, pp. 622-627.

(7) I. Vukušić, Thesis, University of Zagreb, 1978.

(8) T. Alebić-Kolbah, F. Kajfež, S. Rendić, V. Šunjić, A. Konowal and G. Snatzke, *Biochem. Pharmacol.*, 1979, in press.

(9) A. Konowal, G. Snatzke, T. Alebić-Kolbah, F. Kajfež, S. Rendić and V. Šunjić, *ibid.*, in press.

(10) A. Camerman and N. Camerman, *J. Am. Chem. Soc.*, 94, 268, (1972).

(11) G. Gilli, V. Bertolasi, M. Sacerdoti and P. A. Borea, *Acta Crystallogr. B*, 33, 2664 (1977).

(12) G. Gilli, V. Bertolasi, M. Sacerdoti and P. A. Borea, *ibid.*, 34, 2826 (1978).

(13) For compound **1**, the following crystallographic parameters are determined; monocline space group $P2_1$; a = 20.86(3), b = 7.52(1), c = 14.86(2), β = 109.68(5)°, V = 2198.5 Å^3, z = 6, M = 284.7, D_c = 1.290 g. cm^{-3}, MoKα, λ = 0.7107 Å; unpublished data: M. Sikirica and I. Vicković, Laboratory of General and Inorganic Chemistry, Faculty of Science, University of Zagreb.

(14) J. M. Armitage, G. Dunsmore, L. D. Hall and A. G. Marshall, *Can. J. Chem.*, 50, 2119 (1972).

(15) H. M. Mc Connell and R. E. Robertson, *J. Chem. Phys.*, 29, 1361 (1958).

(16) O. A. Gansow, P. A. Loeffler, R. E. Davis, R. E. Lekinski and M. R. Willcott III, *J. Am. Chem. Soc.*, 98, 4250 (1976).

(17) R. E. Davis and R. M. Willcott III, in "Nuclear Magnetic Resonance Shift Reagents", E. Siever, Ed., Academic Press, Inc., New York and London, 1973, p. 146.

(18) Diazepam is WHO name for 7-chloro-5-phenyl-1-methyl-2*H*-dihydro-1,4-benzodiazepin-2-one, which may be regarded as the *C*(3)-demethyl-*N*(1)-methyl structural analogous to **1**. The authors gratefully acknowledge that detailed crystallographic data for diazepam were supplied by Dr. W. Oberhänsli, Forschungsabteilung, F. Hofmann-La Roche, Basel, Switerland.

(19) J. A. Pople and D. L. Beveridge, "Approximate Molecular Orbital Theory", McGraw-Hill, Inc., London and New York, 1970, pp. 110-113.

(20) J. M. Lehn and P. Linscheid, *Bull. Soc. Chim. France*, 992 (1967).

(21) J. James in "Monte Carlo for Particle Physicists", Section 6.1, in "Methods in Subnuclear Physics", Vol. IV, Part 3, M. Nikolic, Ed., Gordon and Breach Publishers, 1974.

(22) J. A. Nelder and R. Mead, *Comput. J.*, 7, 308, (1967).

(23) A. F. Fentiman and R. L. Flotz, *J. Labelled Compd. Radiopharm.*, 13, 579 (1977).