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NMR AND MOLECULAR MODELLING BASED CONFORMATIONAL ANALYSIS OF SOME *N*-ALKYL 1- AND 2-BENZAZEPINONES: USEFUL CENTRAL NERVOUS SYSTEM AGENT DESIGN MOTIFS

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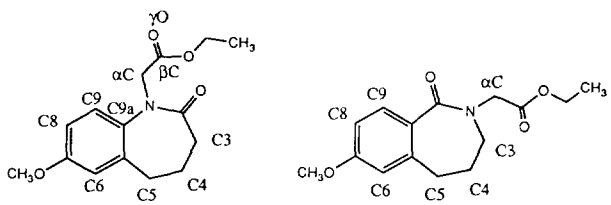
Variable temperature ^{1}H NMR lineshape analyses and ^{13}C NMR spin-lattice relaxation time studies were undertaken to characterise the conformationally dynamic N-alkyl 1- and 2-benzazepinones **1** and **2**. For **1**, dynamic interchange between two mirror-image puckered forms of the azepine ring occurs with a barrier of 56 kJ mol^{-1}. There is a significantly greater degree of ring flexibility in the case of **2**. Molecular modelling studies were used to examine the degree of sidechain flexibility in these compounds.

Benzazepinone-based structures have been successfully employed by several groups as peptide mimetics and constraints, and in the development of new central nervous system active agents [1-8]. The solution conformations of these structures are therefore of particular interest for the design of improved analogues and for understanding the structural basis of their activities. The *N*-alkylated benzazepinones **1** and **2** described here were synthesised as intermediates in a study of *N*-terminally constrained mimics of the endogenous neuropeptide leucine enkephalin [8]. Significant broadening of the ring and αCH_2 resonances in the room-temperature 500 MHz ^{1}H NMR spectrum of **1** prompted a closer examination of the dynamic equilibria associated with this ring system.



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MATERIALS AND METHODS

Compounds: The *N*-alkylated benzazepinones **1** and **2** were prepared and characterised as previously described [8].

NMR Spectroscopy: NMR spectra were recorded on a Bruker AMX 500 instrument in deuteriochloroform solution containing tetramethylsilane as the internal standard. The error limits cited are based on the indicated error range in the coalescence temperature and an assumed error of ± 10% in δν.

Molecular Modelling: Macromodel Version 3.1 [9] was used for modelling studies. The structures of **1** and **2** were constructed using standard bond lengths and angles, with torsion angles derived from measurements made on Dreiding models. Dihedral angles were varied systematically using increments of 20°, this degree of incrementation having been established as sufficient to identify all low-energy conformations [10]. Bond angle variation was permitted over the range ± 10° and a ring closure bond tolerance of 1.5 ± 0.5 Å for each of the saturated azepine ring bonds. An energy filter of 84 kJ mol^{-1} was employed to reject unlikely conformations. Each resultant conformation was optimised using the MM2 program forcefield, with forcefield parameters as provided with the Macromodel package.

RESULTS AND DISCUSSION

A series of ^{1}H NMR spectra of **1** recorded over the range 214 to 330 K revealed a marked temperature dependence of both ring and sidechain proton resonances (Figure 1). The broad signals observed in room temperature spectra were sharpened at higher temperature, indicating conformational averaging. Conversely, as the temperature was lowered, the broad resonances of the C3 and C5 methylene protons were each split into two multiplets, as were the Cα protons. At

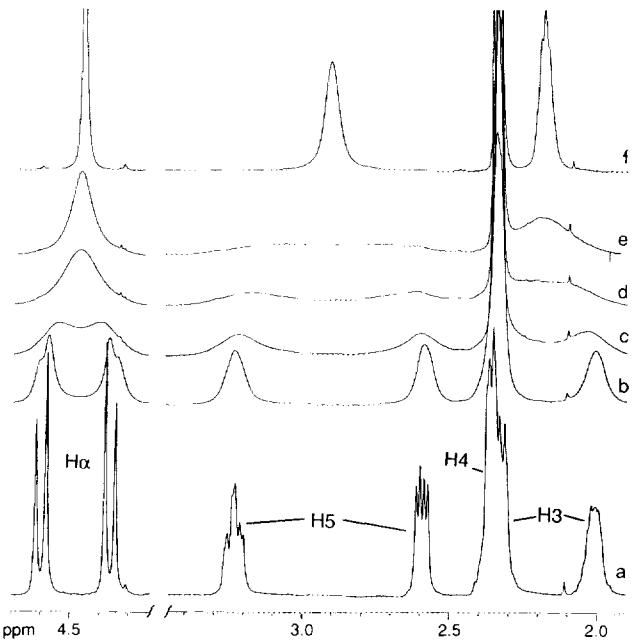

Figure 1. Partial 500 MHz ^{1}H NMR spectra of **1** at (a) 240 K, (b) 265 K, (c) 280 K, (d) 288 K, (e) 293 K, (f) 330 K.

the lowest temperature examined these signals are well resolved and the spectra are indicative of a single conformation in which the individual methylene protons have different environments to each other. As temperature is increased their environments interchange at increasing rates, leading to conformationally averaged spectra at higher temperatures. The energy barrier to the conformational processes responsible for this dynamic behaviour was estimated from the observed coalescence temperature, T_c, using Equation 1 [11], where $\delta\nu$ is the difference in resonance frequencies of the interconverting protons.

$$\Delta G^{\neq} = 19.14T_c[9.97 + \log(T_c/\delta\nu] \qquad \text{(Equation 1)}$$

Table 1 shows T_c and $\delta\nu$ values for various sites in the molecule and the derived values for the energy barriers. The similarity of the $\Delta G^{\neq}$ values for the ring and sidechain methylenes suggests a single process is responsible for interconversion between conformers. The process responsible for the dynamic changes in the NMR lineshape is most likely ring inversion of the seven-membered azepine ring. Symmetry considerations show that a ring inversion process which transposes the pucker on the azepine ring from one face of the benzene ring to the other results in interchange of the environments of the individual methylene protons on C3, C4 and C5. Figure 2 schematically illustrates the process.

The observed changes in the spectra of **1** with temperature are in contrast to the ^{1}H NMR spectra of the regioisomeric 2-benzazepin-1-one, **2**, which showed no freezing-out of conformations in the temperature range examined (220-330 K). A similar ring inversion process can, in principle, occur in this compound but it appears to have a significantly lower barrier. This is readily explained in terms of reduced hindrance to ring inversion because the N-R substituent is not in as crowded an environment as in **1**.

To further characterise the intramolecular mobility of **1** and its regioisomer, **2**, ^{13}C spin-lattice relaxation times were determined. These measurements provide a means of quantifying much faster motions (ie., on the nanosecond timescale) than those detected in the VT experiments [12]. The assignment of protonated carbon resonances for compounds **1** and **2** was carried out using a combination of ^{13}C DEPT NMR spectra and two-dimensional ^{13}C,^{1}H correlated spectra.

Table 1. Chemical shifts, coalescence temperatures and calculated energy barriers for **1**

Proton	δ ppm (240 K)	δν (Hz)	T_c (± 2 K)	$\Delta G^{\neq}$ (kJ mol^{-1})
Hα	4.36, 4.60	120	283	56.0 ± 0.6
H3	2.01, 2.32	155	288	56.4 ± 0.7
H4	2.35, 2.35	---	---	---
H5	2.59, 3.23	320	294	55.9 ± 0.7

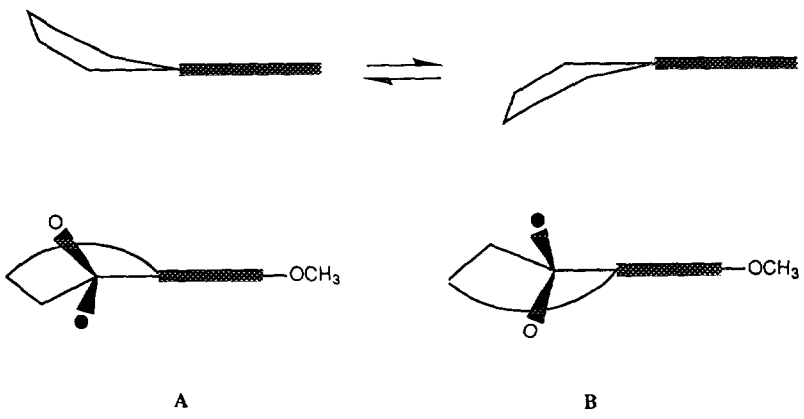

Figure 2. Schematic representation of conversion of azepine ring. To illustrate the interchange of methylene environments, the two protons on C5 are represented as open and filled circles. In (A) the pro-R proton (●) is in the plane of the ring and the pro-S proton (O) is axial. In the mirror image form (B), the environments are interchanged.

All ^{13}C NMR spectra were recorded well above (320 K) the coalescence temperatures described previously. The analysis of relaxation data in terms of molecular dynamics is simplified if relaxation is dominated by the dipolar mechanism, as is usually the case for protonated carbons. To validate this assumption for a given system the contribution of dipolar relaxation may be assessed from the observed nuclear Overhauser enhancement (NOE), as shown in Equation 2.

$$T_1^D/T_1^{obs} = 1.99/(NOE-1) \qquad \text{(Equation 2)}$$

where T_1^D and T_1^{obs} are the dipolar and observed relaxation times, respectively. T_1^D is related to molecular motion as described in Equation 3, where the γ's are the gyromagnetic ratios of the ^{13}C and ^1H nuclei, $r_{C\text{-}H}$ is their internuclear separation, and $J(\omega)$ is the spectral density function [13,14].

$$1/NT_1^D = (1/10)\left[\gamma_C^2\gamma_H^2\hbar^2/r_{C\text{-}H}^6\right]\left[J(\omega_C - \omega_H) + 3J(\omega_C) + 6J(\omega_C + \omega_H)\right] \qquad \text{(Equation 3)}$$

For the isotropic tumbling of a rigid molecule in solution with correlation time τ_c, it can be shown that $J(\omega) = \tau_c$ if motion is in the so-called extreme narrowing limit ($\omega_2\tau_c < 1$), in which case Equation 3 can be simplified [14] to :

$$1/NT_1^D = \left[\gamma_C^2\gamma_H^2\hbar^2/r_{C\text{-}H}^6\right]\tau_C \qquad \text{(Equation 4)}$$

From Equation 4 it can be seen that for molecules tumbling rapidly in solution, there is an inverse correlation between spin-lattice relaxation time and the degree of motion. Motion is quantified by τ_C, which is the 'effective' correlation time and includes contributions from overall and internal molecular motions. For the molecules under study internal motions only contribute significantly

Table 2. ^{13}C chemical shifts and NT_1 values of **1** and **2**

Carbon	δ (ppm)		NT_1 (s)[a]	
	1	**2**	**1**	**2**
C3	32.7	48.1	1.58	1.44
C4	28.5	29.4	1.48	1.64
C5	30.0	30.6	1.43	1.64
C6	114.7	114.1	1.51	1.64
C8	112.4	111.7	1.56	1.49
C9	123.3	130.8	1.48	1.59
CH_3O	55.5	55.3	7.44	7.00
αCH_2	50.1	49.6	1.87	2.06
$\underline{CH_2}CH_3$	61.3	61.2	6.42	7.00
$CH_2\underline{CH_3}$	14.1	14.2	9.85	9.81

a. The estimated error in the T_1 values is 10%.

to the effective correlation time (and hence T_1) if they are fast relative to the overall motion, ie only sub-nanosecond internal motions, if present, will be detected.

The NOE measurements for all protonated carbons in **1** and **2** were found to be within 10% of the theoretical maximum value of 2.99, confirming that the dipolar mechanism constitutes the dominant influence on spin lattice relaxation. The measured values for T_1 therefore reflect solely dipolar relaxation, and the reported NT_1 values in Table 2 may be used to compare the relative mobilities at the various protonated sites in **1** and **2**. It is clear from the data for **1** that, within experimental error, the NT_1 values are identical at all sites in the phenyl and azepine rings, ie., there is no internal flexibility of the saturated ring on the nanosecond timescale. By contrast, there is a degree of fast segmental motion present in the sidechain, as judged by the successively higher NT_1 values down the sidechain. An identical result is observed for **2**. The NT_1 data thus unequivocally show that any differences in flexibility of the azepine ring in **1** and **2** occurs on the millisecond timescale (as determined by the VT lineshape experiments) and not on the nanosecond timescale. The lack of fast internal motion for the methylene carbons in **1** and **2** contrasts with the high degree of internal flexibility reported in the central seven-membered ring of tricyclic antidepressant drugs [15,16].

Theoretical molecular mechanics calculations were undertaken to help define the preferred conformation of the azepine ring in **1** and **2**, and to confirm that the dynamic NMR behaviour arises from ring pucker rather than conformational restriction within the sidechain. The low energy conformation for the ring system of **1** is shown in Figure 3. The figure also shows the energy as a function of rotation around the first two bonds of the sidechain, τ_1 and τ_2. From this it is seen that the sidechain minimum energy conformation corresponds to dihedral

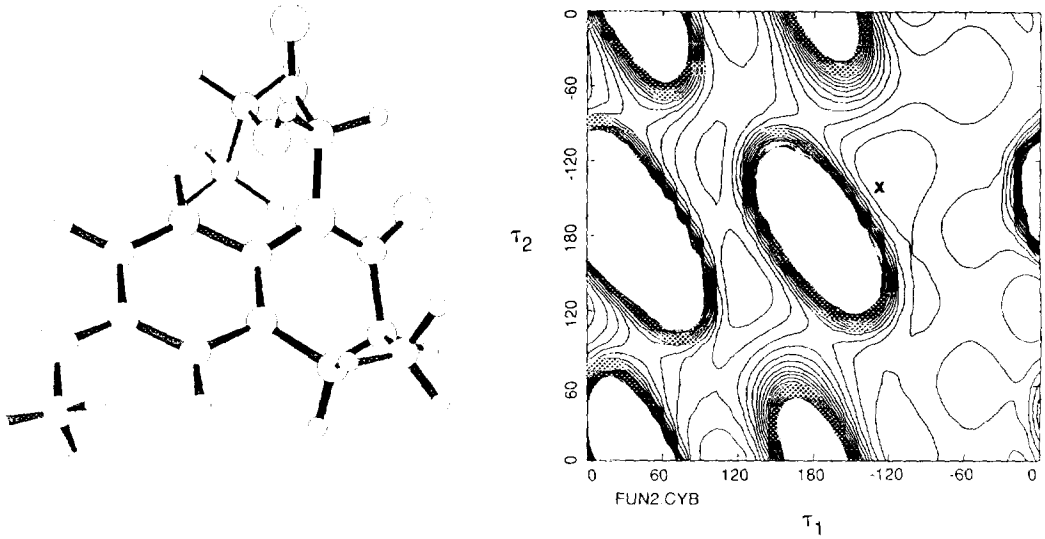

Figure 3. Global minimum energy conformation of **1** and energy contour plot for the rotation of the alkyl side chain of **1** about torsion angles τ_1 (9a-N-αC-βC) and τ_2 (N-αC-βC-γO) in increments of 10°. Contours correspond to 5 kJ mol^{-1} steps in energy. The global minimum energy is depicted by **X**.

angles of τ_1 = -130° and τ_2 = -140°, but that rotation to other conformations may occur with low energy.

In conclusion, the ^1H NMR temperature-dependence studies indicate the presence of a dynamic equilibrium involving puckering of the heterocyclic ring of **1** between mirror image forms. This occurs on a millisecond timescale, but is significantly faster in the case of **2**. The greater rigidity of **1** relative to **2**, suggests it may be a more suitable motif for the construction of turn-mimics in drug design applications. ^{13}C NMR spin-lattice relaxation time determinations show that any internal molecular motion of the azepine ring in both **1** and **2** occurs at a rate comparable to, or slower than, the rate of overall molecular tumbling in solution.

ACKNOWLEDGMENT

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REFERENCES

1. Smith, R.G., Cheng, K., Schoen, W.R., Pong, S-S., Hickey, G., Jacks, T., Butler, B., Chan, W.W-S., Chaung, L-Y.P., Judith, F., Taylor, J., Wyvratt, M.J. and Fisher, M.H. (1993) Science 260, 1640-1643.
2. Nicholls, I.A., Brinkworth, R.I., Alewood, P.F., Morrison, S.F. and Andrews, P.R. (1993) J. Chem. Research (M) 2810-2826; *ibid* S 408-409.
3. Bock, M.G., Di Pardo, R.M., Verber, D.F., Chang, R.S.L., Lotti, V.J., Freedman, S.B. and Friedinger, R.M. (1993) Bioorg. Med. Chem. Lett. 3, 871-874.

4. Busacca, C.A. and Johnson, R.E. (1993) Tetrahedron Lett. 33, 165-168.
5. Petterson, I., Liljefors, T. and Bøgesø, K. (1990) J. Med. Chem. 33, 2197-2204.
6. Nicholls, I.A. (1990) The Design, Synthesis and Biological Evaluation of Novel Central Nervous System Active Agents, PhD thesis, University of Melbourne.
7. Ball, J.B., Wong, M., Capuano, B., Gulbis, J.M., Mackay, M.F. and Alewood, P.F. (1990) J. Heterocyclic Chem. 27, 279-286.
8. Nicholls, I.A. and Alewood, P.F. (1994) Bioorg. Chem. 22, 300-317.
9. Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caulfield, C., Chang, G., Hendrickson, T. and Still, W.C. (1990) J. Comput. Chem. 11, 440-467.
10. Lipton, M. and Still, W.C. (1988) J. Comput. Chem. 9, 343-355.
11. Gale, D.W., Craik, D.J. and Brownlee, R.T.C. (1988) Magn. Reson. Chem. 26, 275-277.
12. Levy, G.C., Lichter, R.L. and Nelson, G.L. (1980) Nuclear Magnetic Resonance Spectroscopy, 2nd edn., p. 221, Wiley, New York.
13. Deslauriers, R. and Smith, I.C.P. (1976) in Topics in Carbon-13 Spectroscopy (G.C. Levy, Ed.), Vol. 2, pp. 1-80.
14. Govil, G. and Hosur, R.V. (1978) in New Results from NMR (P. Diehl, E. Fluck and R. Kosfeld, Eds.), Vol. 20, pp. 99-100, Springer-Verlag, Berlin.
15. Munro, S.L., Andrews, P.R., Craik, D.J. and Gale, D.J. (1986) J. Pharm. Sci. 75, 133-141.
16. Casarotto, M. and Craik, D.J. (1991) J. Phys. Chem. 95, 7093-7099.