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COMMUNICATIONS

A ^{1}H nmr study of the amide-bond conformational equilibrium in pirenzepine

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The hindered rotation about the exocyclic amide bond of pirenzepine has been studied using ^{1}H nuclear magnetic resonance spectroscopy. The ratios of the two slowly interconverting conformations (1 : 1·76 at 2 °C and 1 : 2·37 at 30 °C) and the rate of interconversion (1 ± 0·5 s^{-1} at 20 °C and 247 ± 20 s^{-1} at 80 °C with $\Delta G^{\ddagger}_{80}$ = 71 KJ mol^{-1}) have been measured at various temperatures. The observed rapid rates of interconversion allow us to eliminate the rate of conformational selection of forms II and III as a major contributor to the slow phase of the binding kinetics observed when pirenzepine binds to muscarinic receptors from rat brain (Stockton, Birdsall, Hulme and Burgen, unpublished results).

Pirenzepine (I), a novel muscarinic antagonist, is used clinically in the treatment of peptic ulcers and acts by selectively inhibiting gastric secretion stimulated by the vagus while having fewer side effects than non-selective antagonists such as atropine (Hammer & Giachetti 1982; Jaup et al 1982). This behaviour accords with the findings of Birdsall and co-workers who have found that unlike most muscarinic antagonists, pirenzepine binds with different affinities to different subclasses of receptor binding site in different tissues (Birdsall et al 1980; Hammer et al 1980) and furthermore that the kinetics of binding are more complex than those expected for a simple bimolecular association process (Stockton, Birdsall, Hulme and Burgen, unpublished results). If the binding process involves the selection of an active conformation or conformations of pirenzepine from a mixture of conformations in free solution, then a knowledge of the populations of these conformational states and the rates of interconversion between them could be useful in understanding the binding. In the case of pirenzepine, one possible source of conformational isomerism is the expected hindered rotation about its exocyclic amide bond leading to the slowly interconverting Structures II and III. In this paper we report nmr measurements which detect and characterize this equilibrium.

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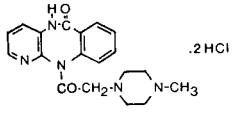

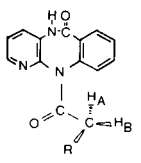

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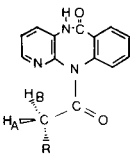

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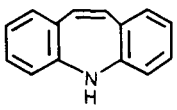
Methods

Pirenzepine dihydrochloride was kindly supplied by Dr R. Hammer (Dr Karl Thomas GmbH) and was used without further purification. It was examined as a 30 mM solution in ^2H_2O (pH 1·71) at a series of temperatures. $^2H_20$ (99·85% ^2H) ws obtained from Norsk Hydroelektrisk. The ^{1}H nmr spectra were acquired using a Bruker WH 270 spectrometer operating in the Fourier transform mode. In a typical experiment 64 free induction decays were accumulated in 8K data points, using a spectral width of 4200 Hz and an acquisition time of 0·96 s. Chemical shifts were measured with respect to 1,4-dioxane (1 mM) and quoted relative to 4,4-dimethyl-4-silapentane-sulphonate (DSS), by the addition of 3.71 ppm.

The method of saturation transfer was used to estimate the rates of conformational interconversion (Forsén & Hoffman 1963). This method involved saturating a signal in the minor conformation for t seconds and measuring the modified insensity, I_t, of the corresponding signal in the major conformation. For long values of t an equilibrium intensity I_∞ is observed where

$$I_\infty = I_0\rho/(\rho + k) \qquad (1)$$

I_0 being the intensity in the absence of irradiation, ρ the relaxation rate of the observed proton and k the rate



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constant for the interconversion from the major to the minor conformer. The approach to this equilibrium value is a simple exponential function of t

$$I_t = I_0 k \exp[-t(\rho + k)]/(\rho + k) + I_0\rho/(\rho + k) \quad (2)$$

Measured values of I_t could be fitted to equations (1) and (2) to give values for ρ and k.

Results and discussion

The ^{1}H nmr spectrum of a 30 mM aqueous solution of pirenzepine at 10 °C (Fig. 1a) provides clear evidence for a mixture of two forms. In the region 3·8–4·4 ppm there are eight signals arising from two AB quartets from protons in two non-equivalent methylene groups (-$COCH_2$-protons); the sharp signals at ~3·07 ppm are from two non-equivalent N-CH_3 groups whilst in the aromatic region (7·5 to 8·3 ppm) there are signals corresponding to two sets of non-quivalent aromatic protons. In each case the relative intensity of the two sets of signals is the same (1·92 : 1) indicating that they arise from the same two forms of pirenzepine. The ratio of the two forms changes reversibly with temperature over the range studied (2–89 °C) which indicates the presence of a mixture of two slowly interconverting conformations. From the changes in equilibrium constant with temperature reported in Table 1, values for $\Delta H°$ (7·1 ± 0·4 kJ mol^{-1}) and $\Delta S°$ (30·1 ± 1·7 kJ mol^{-1}) were determined. Interconversion between the two conformations was further confirmed by transfer of saturation experiments: selective irradiation of either of the A° signals (see Fig. 1a) resulted in a decrease in intensity of the corresponding A signal as a result of the exchange of saturated nuclei between the A and A° sites. It is interesting that the more shielded methylene proton in one form becomes the less shielded proton in the other form. The -$COCH_2$-protons are experiencing by far the largest shielding differences (~0·4 ppm) between the two forms which strongly supports the idea that the two conformations observed in the nmr spectrum arise from hindered rotation about the amide bond (Structures II and III).

The large shielding difference between the methylene protons in a particular conformation indicates that there is also hindered rotation about the CO-CH_2 bond as shown in Structures II and III. The large shielding difference between the same methylene protons (H_A or H_B) in the two conformations (0·42 and 0·32 ppm at 10 °C) could then be explained by the individual protons having different orientations with respect to the aromatic rings in the two conformations and thus experiencing different ring current shifts. An alternative view that shielding differences arise from the methylene protons having different orientations with respect to the neighbouring carbonyl group seems less likely since the sterically favoured rotamer about the CO–CH_2 bond would be expected to have the carbonyl oxygen symmetrically placed between the methylene protons.

A direct measurement of the interconversion rate between the two conformers was obtained using a transfer of saturation experiment (Forsén & Hoffman 1963). As described earlier, by measuring the intensity of one of the A signals as a function of the time of irradiation at the corresponding A° signal, it was

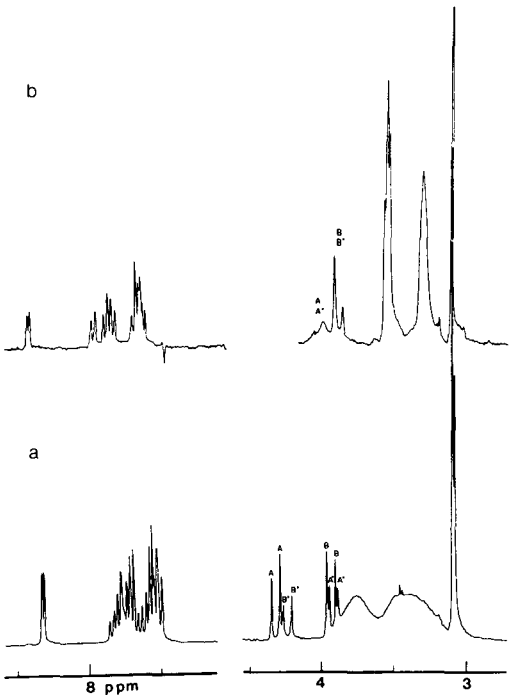

FIG. 1. The 270 MHz ^{1}H nmr spectra of pirenzepine dihydrochloride at pH 1.71 (a) at 10 °C (b) at 89 °C.

Table 1. Ratios of the amide bond conformers of pirenzepine as a function of temperature (pH 1·71[a]).

T (°C)	K = [AB]/[A°B°]
2	1·76
5	1·86
10	1·92
15	1·97
20	2·14
25	2·22
30	2·37

Errors in K ± 0·05.

[a] The ratio of conformers at pH 7·1 were not significantly different from those at pH 1·71.

possible to estimate the rate constant ($1 \pm 0{\cdot}5\ s^{-1}$ at 20 °C) for interconversion from the major to the minor form. Rates of interconversion at higher temperatures were estimated from analysis of the line shapes. At temperatures above 40 °C the methylene signals begin to broaden and they eventually coalesce at 60–70 °C as a result of the exchange process. At 89 °C (see Fig. 1b) a single averaged AB pattern is observed as conditions for fast exchange are approached (the AA° signals which have a larger chemical shift separation than the BB° signals have not fully coalesced at this temperature). At 89 °C the two sets of aromatic and N-CH_3 signals have also coalesced to give a single set of averaged signals. From the line shape analysis (Gutowsky & Holm 1956; Martin et al 1980), rates of interconversion from the major to minor form were estimated at $34 \pm 5\ s^{-1}$ at 50 °C and $247 \pm 20\ s^{-1}$ at 80 °C: $\Delta G^{\ddagger}_{80}$ is thus 71 kJ mol^{-1}. These values are fairly typical for rotation about an *NN'*-disubstituted amide bond (Siddall & Stewart 1969).

In addition to the slow rotation about the amide bond there is also a more rapid conformational interconversion taking place within the piperazine ring. The signals from the piperazine ring protons (3 to 4 ppm) are exchange broadened even at 10 °C: at this temperature the amide bond interconversion rate $<1\ s^{-1}$ and cannot be contributing to this effect. The exchange broadening almost certainly arises from rapid chair-to-chair interconversions simultaneous with nitrogen inversions within the piperazine ring as previously observed for *NN'*-dimethylpiperazines by Sudmeier & Occupati (1968). Such molecules exist predominantly (>90%) in a diequatorial *trans* conformation when both ring nitrogens are protonated. Model building studies on pirenzepine indicate that the tricyclic moiety cannot be planar and probably exists in a butterfly type structure (cf. related structures such as dibenz [b,f]-azepine IV). Drake & Jones (1977) have reported results of crystal structure studies on this compound which indicate a dihedral angle of 145° between the almost planar aromatic rings and with the heterocyclic ring in a boat conformation. Whether or not there is any conformational interconversion within the non-aromatic ring of pirenzepine cannot be deduced from the present nmr experiments.

The conformational information obtained in these nmr studies does not provide an explanation for the kinetic results of Birdsall and co-workers. In the recent study of kinetics of binding of pirenzepine to muscarinic receptors from rat brain (Stockton, Birdsall, Hulme and Burgen, unpublished results) a rapid initial binding process was observed followed by a slow process having a half-life of several minutes. Now that we know that the interconversion rate for the amide bond rotation is relatively fast ($>1\ s^{-1}$ at 30 °C) we can eliminate this as a major contributor to the slow phase of the binding process. However, it seems likely that one of the conformational forms will bind preferentially to the receptors, and the design of pirenzepine analogues existing predominantly in one or other of the two forms could lead to more tightly binding drug molecules which might have different selectivity for muscarinic receptors from different tissues.

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