Dynamic Aspects of the Stereochemistry of Phenothiazines in Solution. Part 2.[1] Segmental Motion and Conformational Analysis of the Side-chain in Promazines

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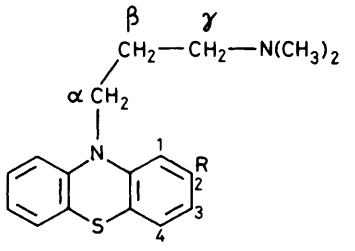
Some promazines and the model compound *NN*-dimethyl-3-phenylpropylamine have been studied, as protonated and unprotonated species, by ^1H and ^{13}C n.m.r. spectroscopy. ^{13}C T_1 relaxation times have shown that the mobility of the dimethylaminopropyl chain in chloroform solution varies depending on whether the chain is protonated or unprotonated, and whether it is attached to a phenyl or to a tricyclic system. Segmental motions were also detected in two compounds. The conformation of the side-chain has been deduced for different solvents. Remarkably, for protonated promazines the preferred conformations are *gauche* for the C_α-C_β fragment and *trans* for the C_β-C_γ in chloroform as well as in water solution, whereas the model compound exists preferentially in the fully extended *trans–trans*-conformation. In dioxan and in DMSO the population of the α,β-*gauche* forms decreases. The presence and the nature of the 2-substituent do not appear to have any effect, whereas the tricyclic system is predominant in the stabilization of the α,β-*gauche* form. This has been related to the ability of these molecules to aggregate in solution with a vertical stacking-type association.

The possibility that phenothiazine drugs adopt different conformations in solution may play an important role in determining the selectivity of their interaction with the biogenic amines pathway.[2] Although there may be links between the action of antipsychotic agents and the dopamine system,[2b] the mechanism by which these drugs act is still largely unknown. It has been shown that antipsychotic drugs, *e.g.* trifluoperazine and chloropromazine, are inhibitors of specific forms of phosphodiesterase and adenylate cyclase, besides a number of other enzymes, and that this inhibition must be related to the binding of the drug to the calcium-dependent protein activator of all these enzymes, calmodulin.[3] Actually, phenothiazine drugs bind selectively to the activator-protein calmodulin.[3] The effects of this specific binding on the conformation and on the calcium binding sites of calmodulin have been studied in solution by n.m.r. spectroscopy.[4]

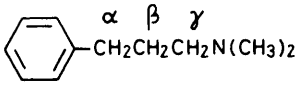
Although the importance of mobility in drug receptor interactions is now acknowledged, the dynamic features of phenothiazine drugs have not been systematically investigated. By contrast a number of papers have been devoted to studies of the solid state.[5–7] From some measurements of dipole moments, it has been concluded [8] that phenothiazine drugs exist in solution as fixed conformers, except for some cases in which a mixture of different conformers might be present. Furthermore Barbe *et al.* argued that each conformation is responsible for a particular biological activity, thus allowing a classification of these agents on the basis of their molecular geometry. This picture is somewhat naïve and certainly an oversimplification. In addition, the n.m.r. data given [8] to support the dipole moment results are incomplete and questionable.

An accurate n.m.r. study of the conformational equilibria in 5-substituted 10,11-dihydrodibenz[*b*,*f*]azepines has been published by Abraham *et al.*; [9] the preferred conformation of the flexible *NN*-dimethylaminopropyl side-chain has been determined for imipramine.

Using the same method, we have examined, in different solvents, some promazines and *NN*-dimethyl-3-phenylpropylamine as a model compound. Furthermore, to obtain more information on the mobility of the side-chain, we measured the T_1 relaxation times of the methylene carbon



(1) R = H
(2) R = Cl
(3) R = $COCH_3$



(4)

atoms. Our plan was to ascertain whether the tricyclic system may influence the conformation and the mobility of the chain for protonated and unprotonated species, and in particular whether the presence and the nature of the 2-substituent (which is so important for the biological activity) have an effect on the conformation in solution.

The problem of the *extra-intra*-configuration at the thiazine nitrogen atom was previously approached by an n.m.r. study [1,10] which showed that both nitrogen-inversion and ring-inversion processes are fast at temperatures higher than −60 °C; however, evidence was given [1,10] that the population of the *extra*-conformer (nitrogen ligand pseudo-axial) increases significantly when the ligand at the thiazine nitrogen is heavier than proton, *i.e.* methyl, acetyl,[11] or an alkyl chain. A model of the fast librational motion originating from nitrogen-inversion and ring-inversion processes in tricyclic molecules with a folded structure like phenothiazines has been described.[12] In their treatment Baldo *et al.* suggest the use of the T_1 values of the aromatic hydrogen-bearing carbons to verify the proposed fast librational motion. Unfortunately the

Table 1. ^{13}C and ^{1}H chemical shift assignments for the dimethylaminopropyl chain in (1)—(4) [a]

	C_α	C_β	C_γ	N-Me	H_α	H_β	H_γ	N-Me
(1)	45.4	25.3	57.1	45.6	3.90	1.97	2.38	2.13
(1), HCl	44.1	21.7	55.3	42.7	4.07	2.35	3.12	2.66
(2)	45.5	25.0	56.9	45.5	3.88	1.96	2.38	2.08
(2), HCl	44.3	21.7	55.3	42.8	3.90	2.20	2.96	2.52
(3)	45.4	24.9	56.9	45.4	3.95	1.96	2.39	2.20
(3), HCl	43.9	21.6	55.0	42.6	4.10	2.27	3.16	2.71
(4)	33.7	29.5	59.3	45.4	2.64	1.79	2.28	2.20
(4), HCl	32.5	25.8	57.3	42.9	2.70	2.13	3.05	2.80

[a] 0.5M-Chloroform solutions. Chemical shifts are in p.p.m. (δ) from internal Me_4Si.

Table 2. Conformational analysis of the *NN*-dimethylaminopropyl side-chain [a]

		$CDCl_3$			D_2O			Dioxan			DMSO		
Compound	Fragment	*N*	Δ*E*	% *trans*	*N*	Δ*E*	% *trans*	*N*	Δ*E*	% *trans*	*N*	Δ*E*	% *trans*
(1)	C_α–C_β	13.9	0.15	39				13.8	0.11	37	13.5	−0.03	32
	C_β–C_γ	14.3	0.33	46				13.4	−0.08	30	14.0	0.20	41
(2)	C_α–C_β	13.9	0.15	39				13.9	0.15	39	13.4	−0.08	36
	C_β–C_γ	13.9	0.15	39				13.2	−0.19	27	13.7	0.06	35
(3)	C_α–C_β	13.9	0.15	39				13.9	0.15	39	13.5	−0.03	32
	C_β–C_γ	14.3	0.33	46				13.6	0.01	34	13.9	0.15	39
(4)	C_α–C_β	15.5	0.86	67					[c]		15.4	0.81	65
	C_β–C_γ	12.9	−0.36	22							13.5	−0.34	32
(1), HCl	C_α–C_β	12.3	−0.85	11	12.1	−1.11	7 [d]	12.7	−0.50	18	13.9	0.15	39
	C_β–C_γ	16.0	1.13	76	16.0	1.13	76 [d]	15.0	0.63	58	15.5	0.86	67
(2), HCl	C_α–C_β	12.2	−0.97	9	12.2	−0.97	9	13.0	−0.30	23	13.9	0.15	39
	C_β–C_γ	16.0	1.13	76	16.2	1.25	79	15.5	0.86	67	15.5	0.86	67
(3), HCl	C_α–C_β	12.4	−0.75	13	12.2	−0.97	9	13.0	−0.30	23	13.4	−0.08	36
	C_β–C_γ	15.7	0.96	70	16.2	1.25	79	15.5	0.86	67	15.8	1.02	73
(4), HCl	C_α–C_β		[b]		15.1	0.68	60		[c]		15.5	0.86	67
	C_β–C_γ	16.5	1.47	85	16.4	1.39	83				16.2	1.25	79

[a] $N = J + J'$; Δ*E* (excess of energy of the *gauche* form) is in kcal mol^{-1}. The accuracy of *N* is within ±0.1 Hz, which corresponds to an error of ±2% in the % *trans* values. Compounds (1)—(4) are insoluble in D_2O. [b] Partially overlapped by the NCH_3 signal. [c] Not measured. [d] Values for (1),HCl at 80 °C are: C_α–C_β *N* 12.0, Δ*E* −1.28, % *trans* 6.0; C_β–C_γ *N* 15.7, Δ*E* 0.96, % *trans* 70.

interpretation of the experimental values of promazines and their hydrochlorides cannot be based only on such a model.

Experimental

N.m.r. spectra were measured with a Varian XL-100-15 spectrometer operating at 35 °C. Chemical shifts are in p.p.m. (δ) from internal Me_4Si. The concentrations for the ^{1}H spectra were 0.2M in $CDCl_3$, 0.15M in dimethyl sulphoxide and in dioxan. Much more dilute solutions in D_2O were used (0.015M) in order to reduce the relaxation rates and so obtain sufficiently resolved signals. Measurements at different concentrations have also been performed in $CDCl_3$ from 0.5 to 5×10^{-3}M and in D_2O from 3×10^{-2} to 5×10^{-3}M but significant conformational change has not been detected. An external reference was used to measure the dilution shifts in $CDCl_3$ solution; no correction was made for bulk magnetic susceptibility effects as these are expected to be small. The free base (1) did not show any significant dilution effect, whereas the hydrochlorides of (1) and (2) gave the following dilution shift values to low field: α-CH_2 0.12—0.14, β-CH_2 0.14—0.18; γ-CH_2 0.02—0.06, N-CH_3 0.05—0.07 p.p.m. For the ^{13}C spectra the concentration used was 0.5M in $CDCl_3$. The ^{1}H and ^{13}C signals were assigned by the single-frequency selective decoupling technique and by the protonation effect (Table 1).

T_1 Measurements were performed by the inversion recovery method by using a (180–*t*–90–*T*) pulse sequence. The pulse width corresponding to a 90° flip angle is 44 μs. Separate experiments were performed for the low- and high-field regions, with the radiofrequency carrier set at low and high field, respectively, in order to minimize errors arising from the pulse imperfections. In all cases the values of the most critical parameter S_∞ (intensity of the completely relaxed signal) were determined from an average of three separate measurements carried out under the same conditions.

The ' homospoil ' accessory was used to correct for non-linear phase errors. T_1 Values were determined from sets of 7—12 spectra by the common procedure of semilogarithmic fitting. The least-squares method was then used to fit a straight line to the experimental data; the standard deviations given in Tables 3 and 4 indicate the accuracy of the T_1 values, but do not include possible systematic errors. However, these errors should be constant for each sequence of experiments and thus should not affect the discussion below. We argue that in our case the systematic errors considered, when values from different solutions are compared, do not exceed 10—15%. The ^{1}H n.m.r. spectra of the $-N-CH_2-CH_2-CH_2-NMe_2$ chain (AA'BB'CC' type) have been analysed to pick up the parameters $N = |J + J'|$ for each CH_2CH_2 fragment, following the approach of Abraham *et al.*[9] Thus the AA'CC' part has been considered, which appears either as simple triplets or as deceptively simple patterns; also in the latter case the separation of transitions 1 and 3 can be easily measured, to give the required value of *N* for each fragment. Appreciable long-range interactions between A and C protons have not been detected by decoupling experiments. The existence of three-bond ^{14}N–N coupling constants, which have been observed [13] in similar systems, does not affect the results, since such interactions involve the B protons only. The introduction of the

Table 3. T_1 Relaxation times (s) of the side-chain carbon atoms [a]

Compound	α-CH_2	β-CH_2	γ-CH_2	N-$(CH_3)_2$	$COCH_3$
(1)	1.06 ± 0.02 (0.999)	1.21 ± 0.02 (0.999)	1.54 ± 0.03 (0.999)	1.62 ± 0.05 (0.997)	
(1), HCl	0.34 ± 0.01 (0.997)	0.41 ± 0.06 (0.999)	0.46 ± 0.04 (0.999)	1.03 ± 0.02 (0.998)	
(3)	*b*	0.93 ± 0.01 (0.999)	1.15 ± 0.02 (0.999)	*b*	3.82 ± 0.06 (0.999)
(3), HCl	0.22 ± 0.01 (0.998)	0.22 ± 0.01 (0.999)	0.24 ± 0.01 (0.999)	0.67 ± 0.01 (0.999)	1.97 ± 0.06 (0.996)
(4)	3.59 ± 0.05 (0.999)	3.54 ± 0.07 (0.998)	3.37 ± 0.06 (0.998)	2.44 ± 0.03 (0.999)	
(4), HCl	1.09 ± 0.02 (0.998)	1.00 ± 0.02 (0.998)	0.79 ± 0.01 (0.999)	1.15 ± 0.03 (0.998)	

[a] 0.5M-Chloroform solutions. Values in parentheses are the correlation coefficients. [b] Not determined because α-CH_2 and N$(CH_3)_2$ signals are coincident.

observed N values into the equation[9] $\Delta E = E_g - E_t = RT\ln[2(N - N_g)/(N_t - N)]$ gives ΔE, provided that N_g and N_t are known. Suitable values of N_g and N_t are those given by Abraham *et al.*,[9] derived from the model compounds $Bu^tCH_2CH_2\overset{+}{N}Me_3$, which is entirely in the *trans*-conformation (N_t 17.37) and piperidine, which can exist only in the *gauche*-form (N_g 11.67 Hz). Then the molar fractions of each conformer, n_t and n_g, are deduced from the relation $n_g/n_t = 2\exp^{-\Delta E/RT}$. The accuracy of N is within ±0.1 Hz, which corresponds to a maximum error of ±0.1 kcal mol^{-1} and ±2% for ΔE and for the % *trans*-value, respectively.

Results and Discussion

The data given in Table 2 show the energy differences between *gauche*- and *trans*-conformations and the populations of the *trans*-form in different solvents for compounds (1)—(4) and their hydrochlorides. Inspection of Table 2 indicates that in the case of unprotonated promazines (1)—(3) there is no great preference for any conformation in all the solvents examined (30—40% of *trans*-isomer). On the other hand protonation of the amino-nitrogen favours the *gauche* forms of the C_α–C_β and the *trans*-form of the C_β–C_γ fragment. The most interesting result is the marked preference for the α,β-*gauche* conformations in the hydrochlorides of (1)—(3) both for chloroform and for water solution (ΔE *ca.* 1 kcal mol^{-1}; *ca.* 90% *gauche*-isomers). These values are constant for a large range of concentration (see Experimental section). The same preference for different media such as $CDCl_3$ and D_2O is even more surprising if we consider that in dioxan the *gauche*-population decreases and in DMSO the three rotamers are equally populated.

These results differ from those reported by other authors,[8b] from theoretical calculations,[14] and from the results obtained in the solid state.[5,6] In fact from the numerous *X*-ray analyses of promazines, the chain appears to be always fully extended (*trans-trans*-conformation) when the amino-nitrogen is protonated.[6] When the amine is free, the preferred conformations in the solid state is α,β-*trans*-β,γ-*gauche*.[5] There is only one report [15] of an α,β-*gauche*-β,γ-*trans*-conformation found in the solid state for a dimethylaminopropyl chain, that is chlorimipramine hydrochloride. It is obvious that the crystal packing forces strongly affect the conformational equilibrium of such a system where the energy differences between conformers are small.

In order to establish whether the presence of the tricyclic system is a determining factor for stabilizing, in solution, the α,β-*gauche*-forms of the hydrochlorides we have analysed the model compound (4). For the hydrochloride of (4), the *trans-trans*-conformation is highly preferred, while the unprotonated species shows a predominant *trans*-conformation for the C_α–C_β fragment,[7] and no preference for C_β–C_γ. Therefore the tricyclic system in some way determines the stabilization of the *gauche*-form, whereas no effect can be ascribed to the 2-substituent on the conformational preference of the chain.

The mobility of the dimethylaminopropyl chain in solution also varies depending on whether the chain is attached to a phenyl or to a tricyclic system, as appears from the relaxation time T_1 of the methylene carbons (Table 3).

The first observation is that the T_1 values for the salts in general are much shorter than those of the corresponding free bases showing that the alkyl chain is, in the salts, significantly anchored by the ionic site. This is a consequence of the electrostatic interactions and leads, as expected, to some inhibition of motion.

Let us consider first the model compound (4) and its hydrochloride. T_1 Values for (4) are particularly long, thus indicating high mobility. Since any local motion must approximate or exceed the overall tumbling rate of the molecule to be observed, it is difficult in this case to detect a segmental motion, if any, of the chain. Actually the quite similar T_1 values of the methylene carbons indicate that this motion is absent, or is inefficient. By contrast, in the salt of (4), the charged nitrogen acts as a strong molecular anchor which reduces the mobility sufficiently to observe some degree of segmental motion even along such a short chain. More pronounced segmental motion along a four-carbon chain has been observed for n-butylammonium ion in CH_2Cl_2.[16] The smaller T_1 differences in our case are nevertheless significant, as they are reproducible and exceed the standard deviations. The less efficient segmental motion is justified, for the salt, because the free end of the chain is here represented by a phenyl instead of a methyl group.

For the salts of (1) and (3), T_1 values of the α-, β-, and γ-carbons are very short and quite similar to each other. The small variations observed are of the same order of magnitude as the experimental error. Thus any local motion cannot be detected. As the overall molecular reorientation in such a solvent is also greatly reduced we argue that the failure to detect any segmental motion means that this motion is probably absent. Therefore the tricyclic system is as good an anchor as the ionic site. This conclusion is indirectly confirmed by the results for the free base (1): the increasing values from the α- to the γ-carbon show the existence of segmental motion. In that motion, the *NN*-dimethylamino-group acts as the free-end chain, while the tricyclic system is the molecular anchor.

Some motional processes have also been detected in the solid state [7b] for chloropromazine. They are associated with the reorientation of methyl groups (2.5 kcal mol^{-1} activation barrier) and with side-chain motions (3.9 kcal mol^{-1} barrier). A model for the chain motion has been considered only for the unprotonated species; in this model the framework is essentially stationary and the most important motion involves the internal portion of the chain. The apparent absence of motion for the salt has been attributed to the presence of the hydrogen bond N–H ••• Cl.

Table 4. T_1 Relaxation times (s) and ^{13}C chemical shift (δ) values for hydrogen-bearing aromatic carbons of promazines (1) and (1), HCl [a]

	Relaxation times T_1	
	(1)	(1), HCl
C-1	1.26 ± 0.05	0.68 ± 0.01
C-3	1.07 ± 0.06	0.59 ± 0.01
	Chemical shifts	
C-1	115.4	115.9
C-2	127.3	127.5
C-3	122.3	123.0
C-4	127.1	127.5

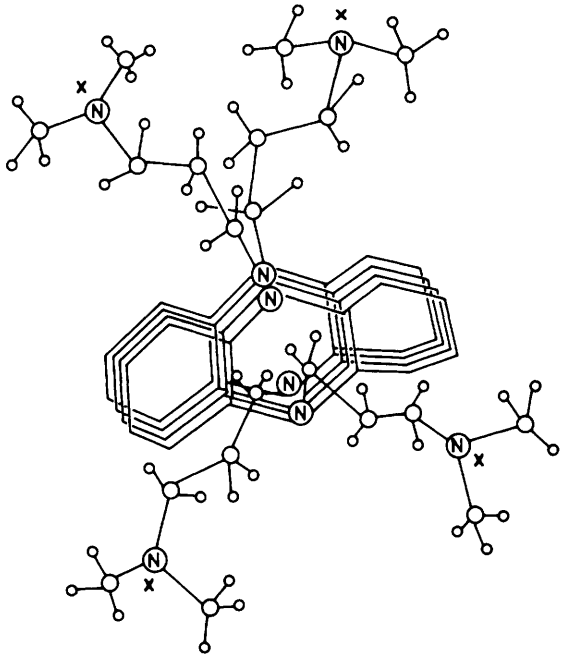
[a] 0.5M-Chloroform solutions. T_1 values for C-2 and C-4 were not determined because their signals were too close or coincident. The assignments are as in ref. 10.

The fast librational motion due to nitrogen-inversion and ring-inversion processes has been studied by Baldo *et al.*[12] Table 4 shows the T_1 values of some aromatic carbons for protonated and unprotonated promazine (1).

One would expect that such libration would result in a shortening of relaxation time for C-1 more than for C-3. But the opposite occurs. Obviously many factors other than libration motion contribute to the relaxation of the aromatic carbons, for instance the number of hydrogens in the α-position, or the particular conformation of the side-chain which brings the methylene protons closer to the *peri*-position (C-1), or even the structure and size of the aggregates present in solution. Therefore we argue that T_1 values of promazines are not the best experimental parameters for verifying the proposed [12] model of librational motion in tricyclic systems.

The effect of temperature on the conformation of the side-chain has been tested in the interval +35—+80 °C in D_2O, for promazine (1) hydrochloride. The ^1H spectra of (1) hydrochloride show constant N values, corresponding to a constant preference for the α,β-*gauche*- and β,γ-*trans*-conformations. Abraham *et al.* in their n.m.r. study of protonated and unprotonated imipramine [9] found a conformational preference quite similar to that in promazines, but in D_2O at 80 °C the population of the α,β-*gauche*-forms is considerably reduced. This has been attributed to a solvent rather than to a temperature effect. Therefore we conclude that the *gauche*-form for the salts is slightly more stabilized when the chain is attached to the phenothiazine than to the dihydrodibenzazepine system.

These results allow some consideration of the factors which induce the protonated chain to assume the α,β-*gauche* form in solution, instead of the fully extended *trans-trans*. The stabilization of the *gauche*-form can be correlated with the ability of these molecules to aggregate by a vertical stacking-type association. Phenothiazine drugs are suggested to form, in water solution, micelles of *ca.* 10—12 monomers, vertically stacked, with the alkyl chain in an alternating mode.[17] Our n.m.r. measurements with D_2O were also performed below the critical micelle concentration (cmc), but intermediate aggregates can be present at concentrations which are several orders of magnitude below the cmc.[18] With such a vertical stacking model, the α,β-*gauche* conformations of the *N*-alkyl chain allow the hydrophobic regions of the molecule (the aromatic core and the methylene groups of the chain) to be more 'protected' from water in a three-dimensional structure. Moreover it allows a greater separation of the nitrogen



Schematic representation of a vertical-stacked aggregate of four monomeric units of a promazine salt, in alternating mode, with the alkyl chains in α,β-*gauche*- and β,γ-*trans*-conformations

positive charges, thus decreasing electrostatic interactions, and this results in a stabilization of the system. A possible arrangement of four monomeric units is sketched in the Figure; however the above arguments are still valid if the rings are not perfectly aligned in the stacks. Imipramine hydrochloride should also form micelles or aggregates in water, but to a lesser extent than promazines [17] and this might be related to the lower population of the *gauche*-forms in water.

The interpretation of the data for rather 'weak' polar media, like $CDCl_3$, is more difficult. Actually the *gauche*-form appears as stable as in D_2O. The presence of ion pairs or higher aggregates in $CDCl_3$ has been postulated for imipramine.[19] For promazine hydrochlorides we have observed in $CDCl_3$ (although less pronounced than in D_2O) a dilution shift to low field, which indicates a vertical stacking-type association. The similar results obtained for chloroform and water solutions (*i.e.* conformational preference and n.m.r. dilution shift) would throw some doubt on the formation of micelles in water. Although the distinction between micellar and non-micellar aggregates may not be clear enough, a vertical stacking-type association appears to be sufficiently proved in both solvents. With such a structure, the arrangement of the chain in the *gauche*-conformations allows a better distribution of the polar heads, which decreases the electrostatic interactions.

With dilution, we should expect changes in N values, if dilution leads to monomeric species, but the maximum dilution which still allows measurement of N is not enough to reach the monomer. However, changes in N values were observed in DMSO and dioxan. Actually these hydrophilic solvents (even if dioxan is classified as a non-polar solvent) can be involved in hydrogen bonding with NH^+, and are expected to have a disaggregating effect. The interaction between solvent molecules and the alkyl chain, disturbing the vertical-stacking aggregation to form more complicated equilibria, results in a destabilization of the α,β-*gauche*-form.

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