NMR Spectral Study of Proton Transfer in Amitriptyline Hydrochloride–Chlordiazepoxide Hydrochloride Combinations in Dipolar Aprotic Solvent

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Abstract □ The singlet resonance due to the two equivalent methyl groups of amitriptyline hydrochloride in dimethyl sulfoxide-d_6 solution changed into a doublet with the addition of chlordiazepoxide hydrochloride. A spin decoupling experiment revealed that the doublet originated because of the emergence of observable spin–spin coupling between the tertiary amine proton and the methyl groups. The phenomenon was interpreted to be due to the nature of proton transfer caused by the relative magnitudes of the basicities of the amines in these compounds, which were determined by the inductive and steric effects of the substituents, leading to the formation of hydrogen-bonded ion-pairs in the aprotic diluent. The enthalpy of the exchange process was 6.4 ± 0.5 kcal/mole.

Keyphrases □ NMR spectroscopy—proton transfer in amitriptyline hydrochloride–chlordiazepoxide hydrochloride mixtures, dipolar aprotic solvent □ Amitriptyline hydrochloride—effect of chlordiazepoxide hydrochloride on NMR spectra, proton transfer in a dipolar aprotic solvent □ Chlordiazepoxide hydrochloride—effect of amitriptyline hydrochloride on NMR spectra, proton transfer in a dipolar aprotic solvent

The relative magnitudes of the basicities of amines in solution essentially determine the outcome of the competition between the bases for available acidic protons. In an analytical study with a formulation containing amitriptyline hydrochloride and chlordiazepoxide free base, it was observed that mixtures of the hydrochloride salts of amitriptyline and chlordiazepoxide provide an interesting example of the proton exchange equilibrium caused by the relative orders of basicities of the amines in these compounds. However, since hydrogen bonding plays a vital role in acid–base behavior leading to the formation of many types of aggregates, which vary widely in stability, the protic solvents such as water and alcohols are not suitable for such an investigation because they are actively involved in hydrogen bonding and exchange reactions.

EXPERIMENTAL

The hydrochloride samples were prepared in NMR grade dimethyl sulfoxide-d_6 (99.8 atom % D), which was shaken with molecular sieves type 13 X. Spectra were obtained with an NMR spectrometer[1] equipped with accessories for performing double-resonance and temperature-dependence experiments.

RESULTS

The NMR spectra of chlordiazepoxide hydrochloride and amitriptyline hydrochloride in dimethyl sulfoxide-d_6 are presented in Figs. 1A and 1B. Table I contains the assignments of the NMR spectra of the hydrochloride salts and free bases of amitriptyline and chlordiazepoxide. The NMR spectrum (Fig. 1C) shows that the singlet resonance due to the two equivalent *N*-methyl groups of amitriptyline hydrochloride changes strikingly into a doublet when chlordiazepoxide hydrochloride is added to the amitriptyline hydrochloride solution. The doublet is observed when the mole ratio of chlordiazepoxide hydrochloride to amitriptyline hydrochloride is ≥0.1. The doublet (splitting ~4.5 Hz) collapses into a singlet (Fig. 2) when the tertiary amine proton resonance region (δ ~10.5–11.5 ppm) in amitriptyline hydrochloride is saturated by the double-resonance technique.

[1] Varian XL-100/Nicolet TT-100.

Table I—Assignments[a] of the PMR Spectra of Amitriptyline Hydrochloride and Free Base and of Chlordiazepoxide Hydrochloride and Free Base

Compound	Proton	δ, ppm (in Dimethyl Sulfoxide-d_6) HCl Salt	Free Base
Amitriptyline	$>CH_2 \times 4$	2.3–3.5 (m)	2.0–3.4 (m)
	$-CH_3 \times 2$	2.64 (s)	2.0 (s)
	$=CH-$	5.82 (t, 7.5 Hz)	5.8 (t, 7.5 Hz)
	Aromatic	6.9–7.4 (m)	$6.9–7.3_5$ (m)
	HCl	10.8–11.4 (b)	
Chlordiazepoxide	$-CH_3$	3.28 (s)	2.83 (d, 4.5 Hz)
	$>CH_2$	5.0 (s)	4.4 (s)
	Aromatic	$6.3–7.0_5$ (m)	6.3–7.7 (m)
	>NH	11.4 (b)	8.08 (q, 4.5 Hz)
	HCl	11.4 (b)	

[a] Multiplicities and, where applicable, coupling constants in Hertz are noted within parentheses; s = singlet, d = doublet, t = triplet, m = multiplet, and b = broad peak.

This experiment conclusively proves that the doublet in the NMR spectrum of amitriptyline hydrochloride is caused by the protons in the methyl groups becoming spin-coupled to the tertiary amine proton when chlordiazepoxide hydrochloride is added. This finding is confirmed by the experiments where the doublet collapses into a singlet when the amine proton is deuterated by the addition of deuterium oxide. The splitting does not arise from nonequivalent *N*-methyl groups or from stereoisomers protonated on opposite sides of the nitrogen atom.

The spin–spin coupling originates because of the emergence of the following predominant rate-determining step (Scheme I) in the acid–base equilibrium of amitriptyline hydrochloride in the presence of chlordiazepoxide hydrochloride in dimethyl sulfoxide-d_6 solvent:

$$N\text{:}\cdots HCl \rightarrow N^+H\cdots Cl^-$$

Scheme I

where the N:- - -H bond is electrostatic with no charge transfer and the N^+H bond is covalent with charge transfer.

With amitriptyline hydrochloride, the proton donor and acceptor are both strong enough so that the proton shifts toward the nitrogen nucleus under the condition where slightly more than one acid is attracted (probably by aggregation) per amine molecule. The product, symbolized by $N^+H\cdots Cl^-$, is called a hydrogen-bonded ion-pair (1). Thus, the transfer of acidity between the two compounds results in the neutral molecule changing into a positive ion where the intermolecular distance between the nitrogen and the proton is smaller than in the neutral molecule.

Since the doublet formation indicating that protonation has occurred on the nitrogen atom is a reflection of ion-pair formation, the criterion of splitting is a qualitative indication of the covalent character of the bond between the nitrogen atom and proton. Since the precise character of the sp^3 orbital occupied by the lone pair of electrons of the nitrogen atom depends on the groups linked to this atom, the energy of the N^+H bond produced and the thermodynamic stability of the positive ion depend on the nature of these groups.

The spin–spin coupling is an interaction that provides two magnetic environments for the *N*-methyl protons, depending on the spin state of the N^+H proton. Whether or not the *N*-methyl protons of amitriptyline appear as a doublet depends on the lifetime of the spin states of the N^+H protons. Development of the theory for intermediate rates of exchange was first published by Gutowsky *et al.* (2). Later extension of the theory

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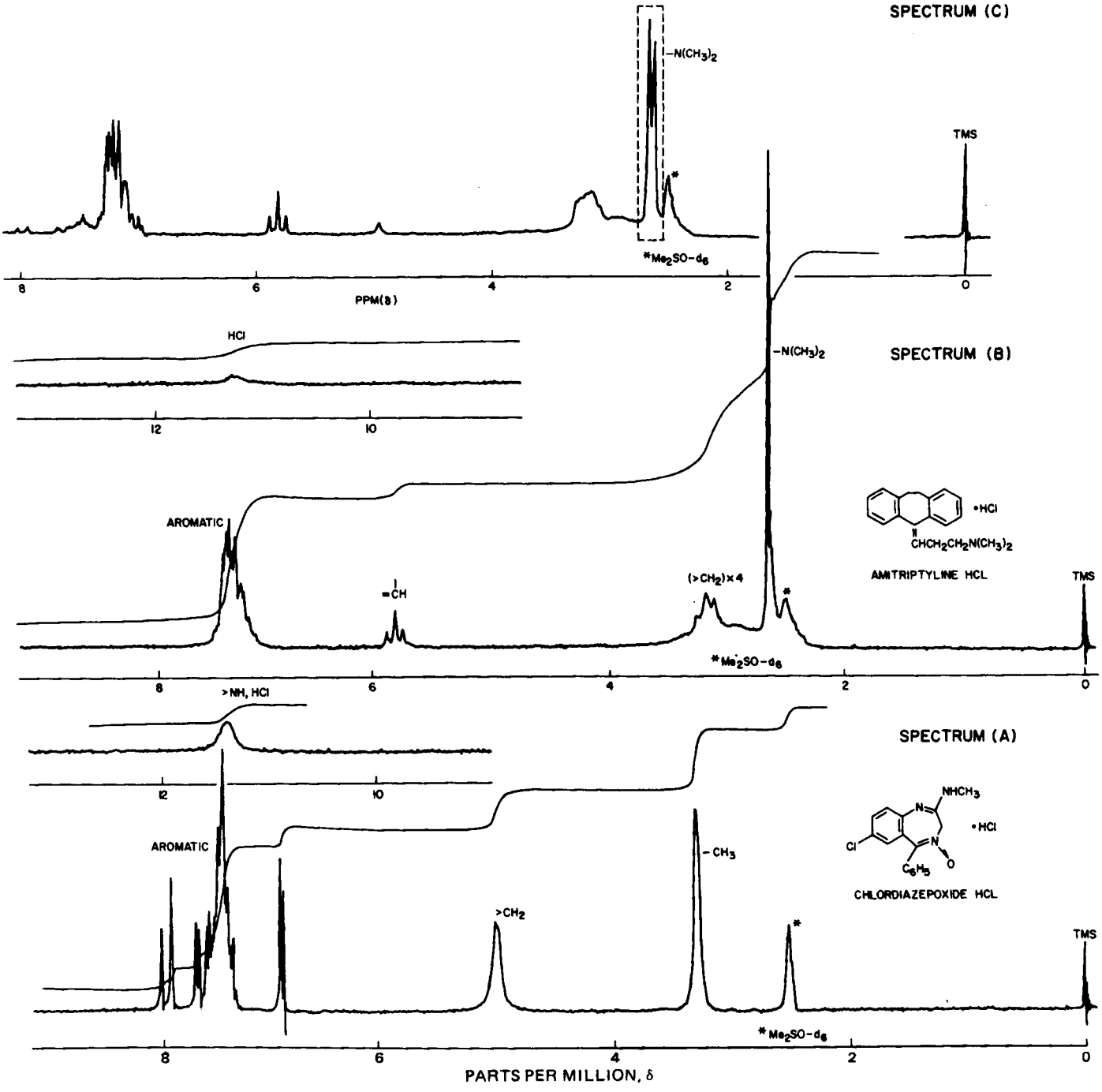

Figure 1—*PMR spectra (A) of chlordiazepoxide hydrochloride (50 mg) in 0.5 ml of dimethyl sulfoxide-d_6 solvent, (B) of amitriptyline hydrochloride (50 mg) in 0.5 ml of dimethyl sulfoxide-d_6 solvent, and (C) of the combination of amitriptyline hydrochloride (50 mg) and chlordiazepoxide hydrochloride (5 mg) in 0.5 ml of dimethyl sulfoxide-d_6 solvent. The doublet is marked with a dashed rectangle. (TMS = tetramethylsilane.)*

(3) was not very general in that it did not account for an asymmetric pair of lines. Rogers and Woodbrey (4) developed a general treatment in which a precise measure of lifetime, τ, is obtained from the line shape parameter, p. In the case of a doublet, the lifetime is given by the following expression:

$$2\pi\tau|J| = \pm[2p \pm 2(p^2 - p)^{1/2}]^{1/2} \quad \text{for } \sqrt{2}\pi\tau|J| > 1 \quad \text{(Eq. 1)}$$

where p is the ratio of the mean of the peak maxima to the minimum between them and J is the spin–spin coupling constant.

At the point of coalescence, the lifetime is given by:

$$2\pi\tau|J| = \sqrt{2} \quad \text{(Eq. 2)}$$

The proton exchange rates are obtained from the relation:

$$r = \frac{1}{\tau} \quad \text{(Eq. 3)}$$

The exchange process can be accelerated by raising the temperature (Fig. 3). Increasing the temperature increases r since a heat of activation, ΔH, is associated with it according to the Arrhenius equation:

$$r \propto \exp(-\Delta H/RT) \quad \text{(Eq. 4)}$$

where T is the absolute temperature and R is the gas constant.

The values of the lifetime and proton transfer rate of the positive ions at different temperatures, which are calculated from Eqs. 1–3, are listed in Table II. The doublet collapses into a singlet when the probe temperature is raised to 50° or higher (ambient probe temperature is 32°). The activation energy associated with the exchange process is obtained from the slope of the plot of:

$$\ln\frac{r(322)}{r(T)} = -\frac{\Delta H}{R}\left(\frac{1}{322} - \frac{1}{T}\right) \quad \text{(Eq. 5)}$$

The ΔH value is determined to be 6.4 ± 0.5 kcal/mole. In this example,

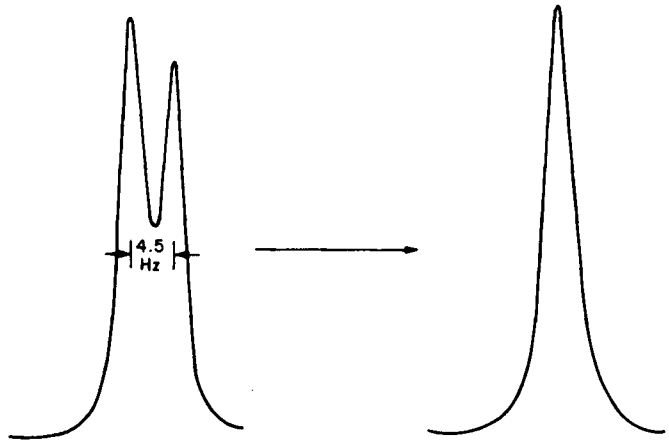

Figure 2—*Transformation of the doublet into a singlet when the tertiary amine proton resonance is decoupled.*

the spin–spin splitting averages to zero by the transfer process having a mean lifetime in the various states of <0.05 sec. Incidentally, the chemical shifts of the singlet and doublet due to the methyl groups of amitriptyline in the forms of associated complex and ion-pairs, respectively, are essentially the same (2.64 ppm), probably because (5) of the similar wave functions of the species rather than lack of sensitivity of the nuclei to changes in electron densities in the two forms of the compound.

It is also obvious from Figs. 1B and 1C that the *N*-methyl doublet splitting, which stands out as an observable effect caused by the intermolecular competition for acidic protons in this experiment, is not detectable in the following combinations since the amount of available exchangeable acidic protons is below the necessary threshold: amitriptyline hydrochloride and chlordiazepoxide base, amitriptyline base and chlordiazepoxide hydrochloride, and amitriptyline base and chlordiazepoxide base. This finding is confirmed by the doublet splitting in the competition-free model experiment where 5 μl of 0.01 *N* HCl is added to 20 mg of amitriptyline hydrochloride in dimethyl sulfoxide-d_6 solvent.

DISCUSSION

The overall acid–base behavior of compounds and, therefore, the observed competitive behavior of amines for acidic protons are determined by the relative proton donor–acceptor affinities and the role of intermolecular hydrogen bonding in dimethyl sulfoxide-d_6. The relative orders of basicities of amines, which can be considered as nucleophilic agents whose exact nature depends on the position of the unshared electron pair at the coordinatively active nitrogen atom, are determined (6) by the inductive and steric effects of the substituents attached to this atom. The introduction of electron donor groups with positive inductive effects into the tertiary amine molecule increases the basicity, and the introduction of electron acceptor groups with negative inductive effects lowers the basicity. Thus, in one direction, the increasing substitution of the nitrogen atom in going from the primary to tertiary amine results in an increase in base strength because of the electron-donating property of the alkyl group. In the other direction, the increasing steric hindrance due to the bulk of the alkyl groups decreases the apparent basicity. With amitriptyline, there is a fairly large amount of positive inductive effect on the tertiary nitrogen owing to the methylene and two methyl groups. The doublet splitting indicates that the steric hindrance is not large enough to neutralize this increase of basicity. In chlordiazepoxide, on the other hand, the positive inductive effect induced by the methyl group on the nitrogen atom of the secondary amine is reduced by the neighboring unsaturation in the ring. Thus, a significant difference in the electronic contributions to base strengths exists in the two compounds. The imines of chlordiazepoxide, which are expected to be comparatively weak in the relative order of basicity, will have a negligible influence on the competitive acid–base behavior in this study because the competition for the acidic protons obviously will be restricted mainly to the secondary amine proton of chlordiazepoxide and the tertiary amine proton of amitriptyline.

At this point, it is important to remember that the dipolar aprotic solvents such as dimethyl sulfoxide-d_6 are not truly inert but show proton donor–acceptor behavior, thus affecting quantitative relationships (7) observed in studying interactions between bases and acids. The aprotic solvents are thus admirably suited for ascertaining differentiating kinds of acid–base behavior, which are masked in amphiprotic and other active solvents. These solvents display significant and differing hydrogen bonding tendencies since the interactions of principal interest in such solvents are the much stronger interactions between dissolved species, including long-range Coulomb effects caused by the general electrostatic

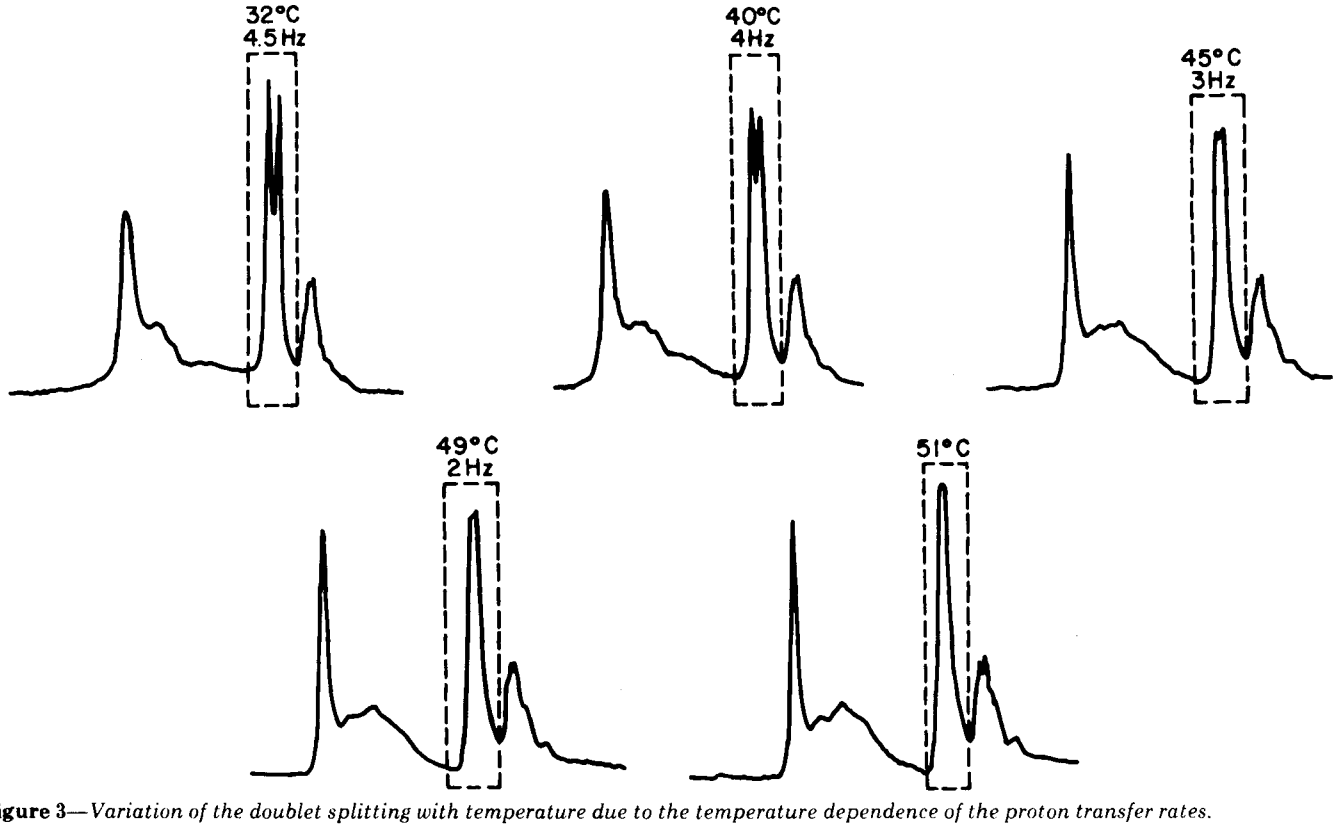

Figure 3—*Variation of the doublet splitting with temperature due to the temperature dependence of the proton transfer rates.*

Table II—Temperature Dependence of the Proton Exchange Rate (r) and Mean Lifetime (τ) of the Positive Ion

Temperature	$2\pi\tau\lvert J\rvert$[a]	τ, sec	r, sec^{-1}
32°	2.5	0.09	11
40°	2.0	0.07	14
45°	1.6	0.06	17
49°	1.4	0.05	20

[a] $|J|$ = 4.5 Hz.

solvation of the ammonium cation or the ion-pair by both the static and induced moments of the diluent molecules. Consequently, intermolecular hydrogen bonding assumes greater importance in aprotic solvents than in leveling solvents in the detection of competitive basic sites.

The relative degree of dissociation exhibited by amitriptyline hydrochloride and chlordiazepoxide hydrochloride in dimethyl sulfoxide-d_6 results from two opposing effects: the positive inductive effect, which tends to increase the stability of the salts, and the steric influence, which tends to decrease the stability. The observed base strengths are determined by the relative ease by which the respective amine hydrochlorides can form aggregates of ion-pairs by intermolecular hydrogen bonding (8). The tertiary amine hydrochlorides have one proton, which is presumably bonded to the chloride and unavailable for participation in aggregate formation. This condition delays aggregation until a higher concentration of acidic protons is reached by transfer of acidity caused by the migration of acidic protons from chlordiazepoxide molecules to amitriptyline molecules, particularly in a high dielectric constant solvent like dimethyl sulfoxide-d_6. When this happens, owing to the mobility of protons (9) within the hydrogen bonds, proton transfer can occur within the hydrogen bond representing the joining of the Brönsted acid–base pair N- - -HCl to yield the polar form N^+H- - -Cl^-.

At this time, the tertiary amine hydrochlorides are induced to form aggregates of ion-pairs (7) by intermolecular hydrogen bonding in response to the need to stabilize the ion-pairs of the salt. Therefore, the proton exchange equilibrium shifts toward the direction of reduced proton mobility of the tertiary amine proton of the amitriptyline salt, which results in the covalent N^+H bond being time resolved in the NMR spectrum. This phenomenon is reinforced by the fact that the tertiary amine forms stronger N^+H- - -Cl^- bonds than does the secondary amine due to the greater concentration of positive charge in the tertiary ammonium ion (10). The stronger the cation–anion hydrogen bonding, the less is the extent of the aggregation of the resulting ion-pairs by electrostatic solvation. While the number of molecules forming an aggregate is not determined in this example, it is disrupted when the temperature is raised. The existence of a doublet splitting requires that the N-methyl protons with fixed orientations of the magnetic moment vector remain coupled with the spin state of the N^+H group for a time greater than τ (Table II). The value of the heat of activation for the exchange process (~6.4 kcal/mole) probably leads to the suggestion of dimeric aggregates being the means of effecting exchange (11).

In conclusion, NMR spectroscopy is a useful tool for investigating proton exchange equilibrium between compounds possessing basic sites that compete for acidic protons, particularly in an aprotic solvent. An indication of the formation of ion-pairs and, hence, the covalent character of the nitrogen–proton bond are provided by the appropriate line splitting, which also gives an estimate of the mobility of protons existing in the exchange environment influenced by varied types of intermolecular hydrogen bonding and solute-solvent interactions.

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