Pulsed Nuclear Magnetic Resonance Study of Molecular Motion in Solid Imipramine Hydrochloride, Desipramine Hydrochloride, and Iminobibenzyl

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Abstract: NMR relaxation times T_1, $T_{1\rho}$, and T_{1D} and second moments M_2 were measured for solid imipramine hydrochloride (I), desipramine hydrochloride (II), iminobibenzyl (III), and iminobibenzyl-*d* (IV). Studies were made from 98 K to the melting point of the solid. Methyl reorientation was observed in I and II, characterized by activation barriers of 2.8 and 1.9 kcal/mol, respectively. Evidence was obtained that the barriers are different in the two molecular forms of I present in the solid. Flexing of the ethano bridge in II and III has been characterized by barriers of 0.7 and 1.2 kcal/mol, respectively, while in IV this process was observed to be ~1 kcal/mol. Flexing of the ethano bridge in I was also observed. Inversion at nitrogen, characterized by a barrier of 4.6 kcal/mol in III and ~4 kcal/mol in IV, was observed. Factors relating drug activity to molecular conformation and motional processes are discussed.

Introduction

In general, those compounds which have been classified as the most potent pyschotropic drugs are those which consist of a tricyclic framework with an aminoalkyl substituent on the central ring.[2a] Those derivatives which have a central six-membered ring are usually antipsychotic agents. The phenothiazine derivative, chlorpromazine, is the most widely prescribed drug and the standard to which all other neuroleptics are compared.[2b]

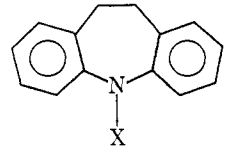
Enlargement of the central ring to a seven- or eight-membered ring system, or structural modification of a 6,6,6 system to increase the twist angle[3] of the rings, enhances clinical antidepressant effects. In contrast to potent neuroleptics, which require a tertiary amino group separated from the ring system by a three-carbon chain, some very active antidepressants have secondary amino groups, and other compounds have a side chain consisting of two carbon atoms. In addition, neuroleptic activity is markedly increased by a substituent, such as chloro or trifluoromethyl, meta to the atom which has the attached side chain. This trend is not as striking when comparing antidepressant agents, although 3-chlorimipramine has been reported to be more potent than imipramine in inhibiting uptake of 5-hydroxytryptamine.[4,5]

The prototype for tricyclic antidepressants is imipramine. Imipramine undergoes at least two metabolic reactions, including N-demethylation to give the major product, desipramine. It is unclear whether imipramine derives its activity, at least in part, from this metabolite.[6] However, the potency of desipramine has been reported to be $^1/_5$ that of imipramine when comparing the inhibition of 5-hydroxytryptamine.[4]

It appears that one of the most important properties possessed by a pyschotropic drug is its ability to affect the metabolism and influence the reactivity of biogenic amines. Several theories have been postulated to explain the reactivity of neuroleptic agents, all based upon stereochemical and conformational arguments. These theories have been advanced on the premise that neuroleptics produce their clinical effects by blocking the transport of dopamine at receptor sites.[7,8] For example, it has been suggested that chlorpromazine is able to block dopamine receptor sites due to the similarity between dopamine and part of the chlorpromazine molecule.[9] Parameters such as distance between aromatic rings and the distance between amine and ring centers have been invoked to predict drug potency.[10] It has also been suggested that the ring substituent exerts a van der Waals attractive force on the basic amino group of the side chain, which may directly influence the overall conformation of the molecule.[11] In addition, it has been proposed that an available lone pair of electrons on a heteroatom in the central ring enhances reactivity at the receptor site.[12]

It is believed that tricyclic antidepressants influence the metabolism of norepinephrine and 5-hydroxytryptamine by blocking the reuptake of these amines into the neuron.[12,13] In general, it has been observed that increased antidepressive action occurs among compounds in which the ring system is prevented from adopting a stereochemical conformation capable of interacting with a planar surface (perhaps a receptor surface).[14] In addition, it has been proposed that structural features of imipramine and desipramine are similar to the conformation of norepinephrine at the biological site.[15] Thus, arguments similar to those used for neuroleptic compounds may be introduced eventually to explain the reactivity of antidepressant agents.

The current attempts to relate drug activity to structural requirements can be broadened in scope by including information on structural flexibility. Thus pulsed NMR relaxation measurements are being utilized by us to study molecular motion in tricyclic compounds in the solid state. We recently reported the results on a series of eight-membered central ring systems[16-18] and we now wish to report the results from the study of imipramine hydrochloride (I) and desipramine hydrochloride (II) as well as iminobibenzyl (III), an inactive



I, X = $C_3H_6N(CH_3)_2H^+Cl^-$
II, X = $C_3H_6NH_2CH_3^+Cl^-$
III, X = H
IV, X = D

precursor of these drugs. As a comparison study, results are also reported for iminobibenzyl-*d* (IV).

In the case of the antidepressants, the central ring is expected to undergo a relatively low activation energy flexing which may be detectable. In addition, it is of interest to determine whether motion associated with the side chain can be observed. Both these motions are much too rapid to detect by use of NMR line-shape analysis.[19] Finally, we hope to determine the extent

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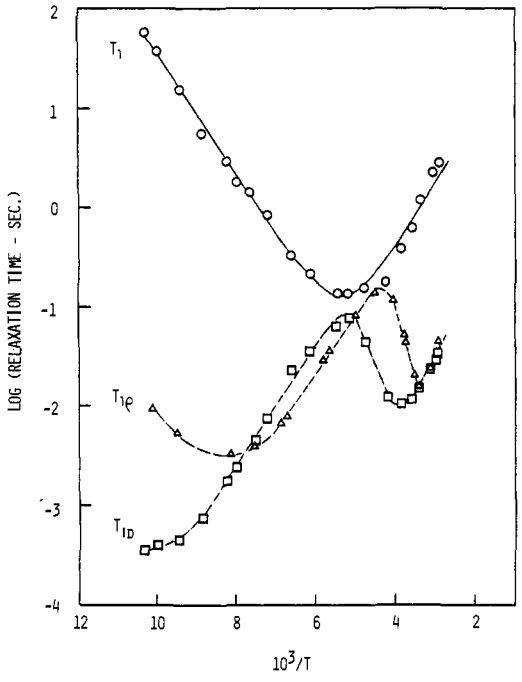

Figure 1. Log relaxation times, T_1, $T_{1\rho}$, and T_{1D}, vs. reciprocal temperature for solid imipramine hydrochloride. The solid line for T_1 is calculated as described in the text. The dashed lines are arbitrarily drawn through the data points.

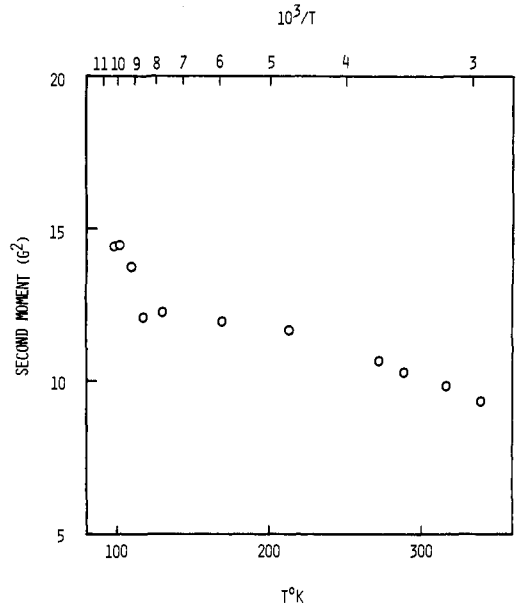

Figure 2. Second moment vs. temperature for solid imipramine hydrochloride.

to which motions exhibited by the unsubstituted tricyclic framework are influenced by the addition of an aminopropyl side chain.

Experimental Section

Imipramine hydrochloride, 5-(3,3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b,f*]azepine hydrochloride (I), and desipramine hydrochloride, 5-(3-methylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b,f*]azepine hydrochloride (II), were obtained from CIBA Pharmaceuticals. Commercially available iminobibenzyl (III) was sublimed several times before use. Iminobibenzyl-*d* (IV) was prepared according to the following procedure. To 5 g of III was added a 10% excess of BuLi in 25 mL of THF and the mixture stirred for 3 h. After evaporation of the THF, anhydrous ether was added and the mixture hydrolyzed with 15 mL of D_2O. The ether layer was separated and dried over Na_2SO_4, and the ether was then evaporated. The white, crystalline product (III) was further purified by sublimation. The extent of the deuterium exchange reaction was verified by ^1H NMR and mass spectral data.

Polycrystalline samples were placed in glass containers for study. ^1H NMR measurements[16,20] of the Zeeman spin-lattice relaxation time (T_1), the rotating frame spin-lattice relaxation time ($T_{1\rho}$), the dipolar relaxation time (T_{1D}), and the second moment (M_2) were made using a Polaron (Watford, England) high-power pulsed NMR spectrometer operating at 60 MHz, as described previously.[16] The Bloch decays were found to be Gaussian within experimental error for the polycrystalline samples. Experimental uncertainty in M_2 values is ±0.5 G^2. For the $T_{1\rho}$ measurements, H_1 = 28.6 G. Computer calculations were done on an IBM 370/168 computer.

Results

Imipramine Hydrochloride. Experimental values of the relaxation times[20] T_1 (spin-lattice), $T_{1\rho}$ (rotating frame spin-lattice), and T_{1D} (dipolar) for I are presented in Figure 1. Experimental values of the second moment for I are presented in Figure 2. The analysis of experimental results will proceed as previously described in detail by us.[16-18] In Figure 1, T_1 exhibits a relaxation minimum at $10^3/T$ = 5.4 (~185 K) due to molecular motion. $T_{1\rho}$ also exhibits a relaxation minimum at $10^3/T$ = 8.3 (~120 K), and T_{1D} appears to be near a relaxation minimum at the lowest temperature. These three relaxation minima are all associated with the same motion, which we believe to be methyl group reorientation. The transition in M_2 below 110 K in Figure 2 is also associated with this motion. The T_1 data in Figure 1 can be roughly fitted by a BPP-type expression[21]

$$\frac{1}{T_1} = \frac{2}{3}\gamma^2 M_{2\text{mod}}\left[\frac{\tau_c}{1+\omega_0^2\tau_c^2} + \frac{4\tau_c}{1+4\omega_0^2\tau_c^2}\right] \quad (1)$$

where γ is the gyromagnetic ratio for protons, $M_{2\text{mod}}$ is the portion of the second moment M_2 which is modulated by the motion, ω_0 is the Larmor frequency in the laboratory magnetic field, and τ_c is the correlation time of the motion. The solid line T_1 in Figure 1 is calculated assuming an Arrhenius expression $\tau_c = (8.1 \times 10^{-13}) \exp(2.8 \times 10^3/RT)$ s and $M_{2\text{mod}}$ = 4.5 G^2. It can be seen in Figure 1 that the agreement between calculated and observed T_1 values for $10^3/T < 5$ is rather poor. The observed T_1 behavior might correspond to a double minimum[18,22] (i.e., contributions from two different motions), although two distinct minima are not resolved. The gradient of the low-temperature arm of T_1 corresponds to a motion with E_A = 2.8 kcal/mol, but the high-temperature arm of T_1 corresponds to a second motion with $E_A \sim 5$ kcal/mol. The gradients of $T_{1\rho}$ and T_{1D} between $10^3/T$ = 5 and 9 correspond to E_A = 2.8 kcal/mol. The activation energy can also be estimated from the positions of the T_1 and $T_{1\rho}$ minima;[16] $E_A \sim$ 4 kcal/mol is obtained. We believe that these effects could be a result of contributions from two motions of comparable rate. This point will be discussed below.

An additional relaxation process is evident in both T_{1D} and $T_{1\rho}$ for $10^3/T < 5$. From the gradient of $T_{1\rho}$, we estimate $E_A \geq 7$ kcal/mol for this process. Both minima are quite shallow, which corresponds to a very weak relaxation. The T_{1D} and $T_{1\rho}$ minima indicate that a relatively sharp additional M_2 transition should be observed for $T > 250$ K. It can be seen in Figure 2 that if there is a relatively sharp transition in M_2 near 250 K, its magnitude is $\leq 1 G^2$. No effects attributable to this process can be seen in T_1; apparently a weak T_1 minimum for this process occurs at much higher temperatures than shown in Figure 1.

One additional feature of Figure 2 is the gradual decrease in M_2 observed over the temperature range 100-300 K. This is indicative of a very low activation barrier process. No effects attributable to this process can be observed in Figure 1.

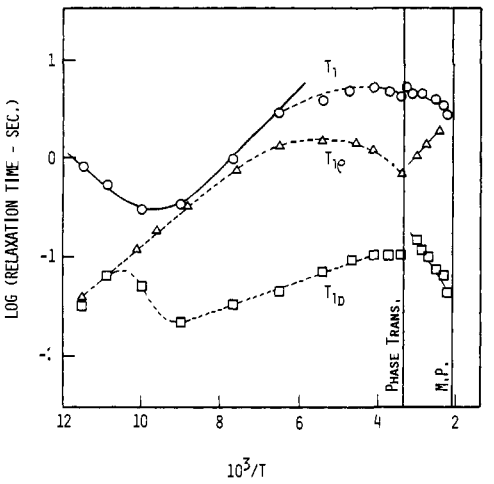

Figure 3. Log relaxation times, T_1, $T_{1\rho}$, and T_{1D}, vs. reciprocal temperature for solid desipramine hydrochloride. The solid line for T_1 is calculated as described in the text. The dashed lines are arbitrarily drawn through the data points.

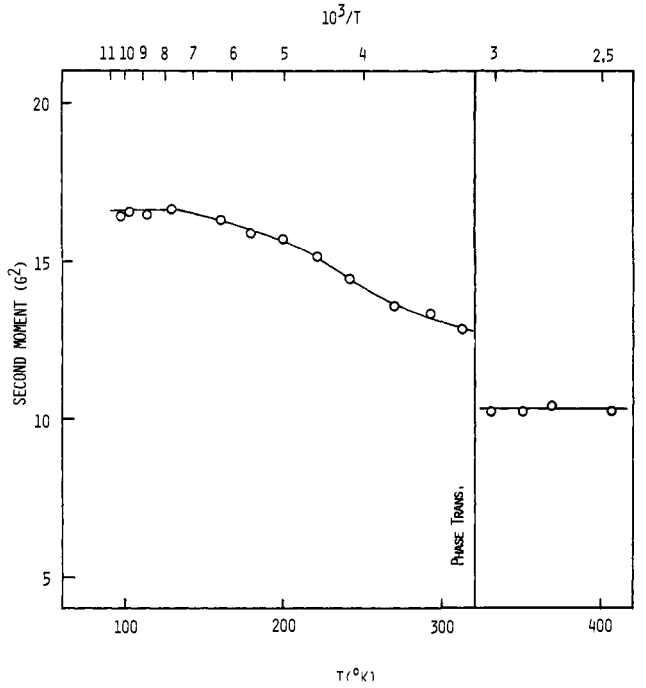

Figure 4. Second moment vs. temperature for solid desipramine hydrochloride.

Desipramine Hydrochloride. Relaxation data for II are presented in Figure 3 and second moment data are presented in Figure 4. In Figure 3, T_1 exhibits a relaxation minimum centered at $10^3/T = 9.75$ (~103 K) due to methyl group reorientation. The solid line for T_1 in Figure 3 is calculated from eq 1 with $\tau_c = (1.5 \times 10^{-13})\exp(1.9 \times 10^3/RT)$ and $M_{2mod} = 1.8$ G². The gradient of $T_{1\rho}$ between $10^3/T = 8$ and 11 corresponds to $E_A = 1.8$ kcal/mol, which indicates that $T_{1\rho}$ is also controlled by methyl reorientation in this temperature region.

Between $10^3/T = 3.5$ (phase transition) and 10, T_{1D} is controlled by a second motion with $E_A = 0.7$ kcal/mol. T_{1D} appears to exhibit a very weak relaxation minimum at $10^3/T \sim 9$. Contributions from this motion to T_1 and $T_{1\rho}$ can also be seen between the phase transition and $10^3/T \sim 6$. A very gradual M_2 transition associated with this second motion can be seen in Figure 4 between 100 K and the phase transition at about 320 K.

Abrupt changes and discontinuities associated with a phase transition[16] can be seen in both Figures 3 and 4. In the high-temperature phase, T_{1D} appears to be controlled by a third process with $E_A = 3.4$ kcal/mol, while $T_{1\rho}$ probably contains contributions from both second and third processes.

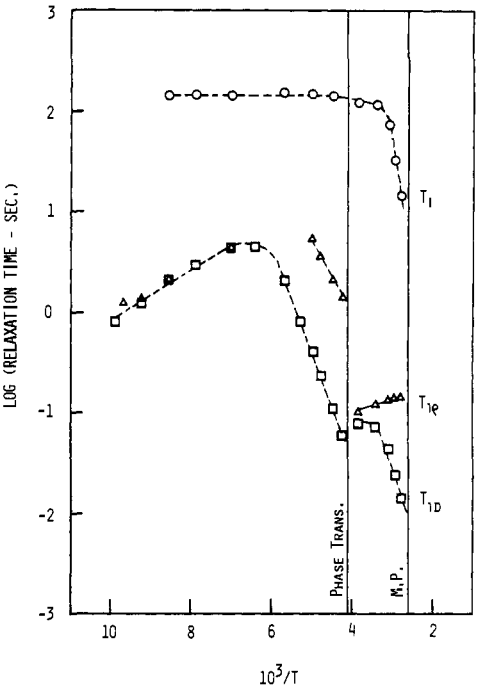

Figure 5. Log relaxation times, T_1, $T_{1\rho}$, and T_{1D}, vs. reciprocal temperature for solid iminobibenzyl. The solid line for T_1 is calculated as described in the text. The dashed lines are arbitrarily drawn through the data points.

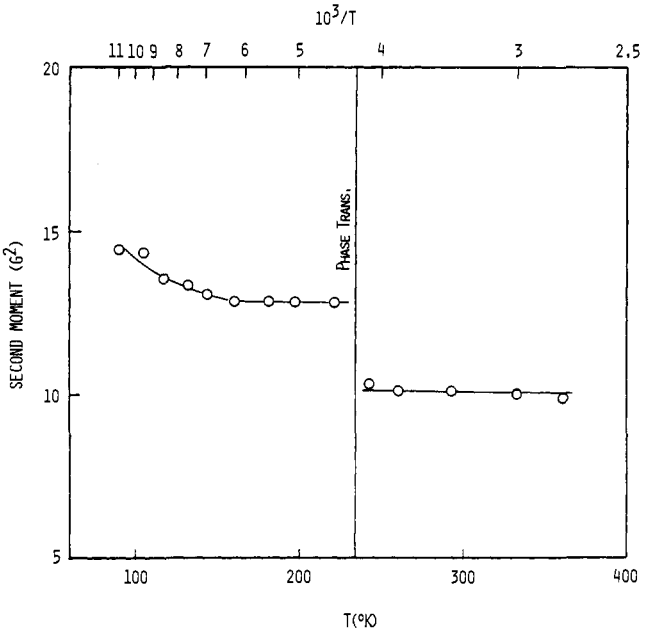

Figure 6. Second moment vs. temperature for solid iminobibenzyl.

Iminobibenzyl. Relaxation data for III are presented in Figure 5 and second moment data are presented in Figure 6. Evidence for a phase transition can be seen at $10^3/T \sim 4.1$ (240 K) in both Figures 5 and 6. In the low-temperature phase, two separate motions can be observed in both $T_{1\rho}$ and T_{1D}, while T_1 is independent of temperature. The behavior of both $T_{1\rho}$ and T_{1D} for $10^3/T > 6$ corresponds to high-temperature arms of relaxation minima, characterized by $E_A = 1.2$ kcal/mol. The data in Figure 6 indicate that an M_2 transition occurs below ~150 K; only the high-temperature portion of the

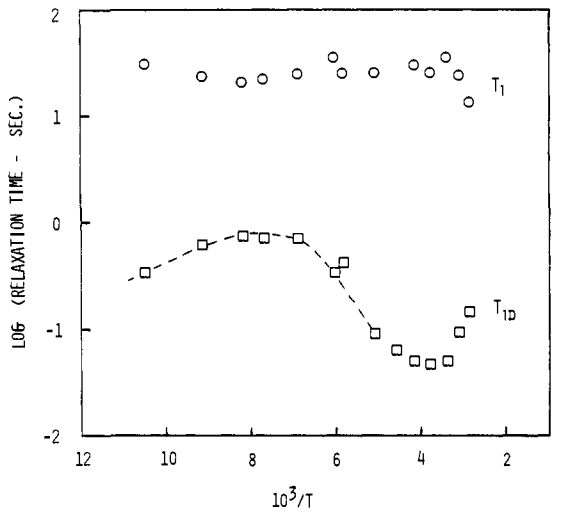

Figure 7. Log relaxation times, T_1, $T_{1\rho}$, and T_{1D}, vs. reciprocal temperature for solid iminobibenzyl-*d*. The dashed lines are arbitrarily drawn through the data points.

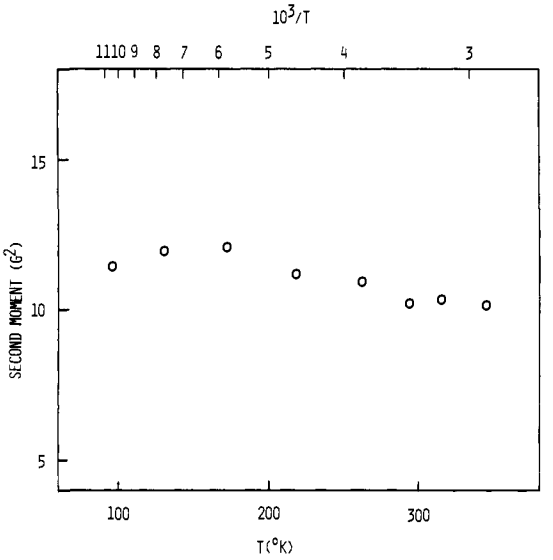

Figure 8. Second moment vs. temperature for solid iminobibenzyl-*d*.

transition can be seen in Figure 6. This transition must be associated with the relaxation observed in $T_{1\rho}$ and T_{1D} for $10^3/T > 6$, and this indicates that $T_{1\rho}$ must be near a relaxation minimum at $10^3/T = 10$. The relaxation process is thus very weak, which accounts for its failure to affect T_1.

In the low-temperature phase for $10^3/T < 6$, a second relaxation process is observed in $T_{1\rho}$ and T_{1D}. $T_{1\rho}$ was not measured between $10^3/T = 5$ and 7 for technical reasons. The gradient of T_{1D} corresponds to $E_A = 4.6$ kcal/mol. For reasons to be discussed below, we believe that this process is motion of the imino proton (i.e., inversion at nitrogen). For this reason, we have also studied N-deuterated iminobibenzyl (IV) to be described below.

In the high-temperature phase, both $T_{1\rho}$ and T_{1D} presumably contain contributions from more than one process. The T_1 behavior gives evidence of a third relaxation process, just below the melting point. The gradient of T_1 (the low-temperature arm of a relaxation minimum) corresponds to $E_A = 9$ kcal/mol. It is possible that T_{1D} is also controlled by this process near the melting point.

Iminobibenzyl-*d*. Relaxation data and second moment data for IV are presented in Figures 7 and 8. Compounds III and IV present an interesting contrast, in that no evidence of a phase transition was obtained for IV. For $10^3/T > 7$, T_{1D} is controlled by a low activation barrier process, with the gradient corresponding to $E_A \sim 1$ kcal/mol. This behavior is essentially the same as that for III.

For $10^3/T < 7$, T_{1D} exhibits a very weak relaxation minimum, characterized by $E_A \sim 4$ kcal/mol. If the relaxation process is inversion at nitrogen, then deuterium substitution is expected to substantially decrease the strength of the interaction,[23] giving rise to a weak minimum as observed. Unfortunately, the corresponding minimum in III is obscured by the phase transition, and a direct comparison of minimum depths cannot be made.

The behavior of T_1 in Figure 7 is similar to that of III, independent of temperature, but the values are consistently lower for IV by almost an order of magnitude.

The relaxation data for IV suggest that M_2 should exhibit a gradual transition (decrease) across the entire temperature range shown in Figure 8, associated with the 1 kcal/mol motion. In addition, a second transition is anticipated at temperatures >250 K, associated with the 4 kcal/mol motion; this second transition is expected to be of small magnitude so as to be not observable by our method. It can be seen in Figure 8 that M_2 does exhibit a gradual decrease from about 12 G^2 to about 10 G^2 with increasing temperature, although the effect is partially obscured by experimental scatter.

Table I. Calculated Second Moments (G^2) for Imipramine Hydrochloride

structure	M_2 intra	M_2 inter	M_2 total
rigid lattice	17.2	2.6	19.8 ± 2
methyl reorient	11.8	1.8	13.6 ± 2
methyl reorient + bridge flexing[a]	9.8	1.3	11.1 ± 2

[a] Calculation assumes that the methylene interproton vector changes by 90°, which gives the maximum reduction in M_2 for that contribution (see ref 16).

Analysis of Second Moment Data. The assignments of relaxation minima to specific motions can be made in favorable cases by consideration of second moment data.[16-18] The "rigid lattice" value of M_2 is that value observed only if all motion is sufficiently slow so that the NMR line is not motionally narrowed. This value can be calculated from the Van Vleck equation[24]

$$M_2 = \frac{715.9}{n} \sum_{j>k} r_{jk}^{-6} \qquad (2)$$

in which M_2 is in G^2, n is the number of protons in the sample, and r_{jk} is the distance between protons j and k in Å. Equation 2 can be evaluated most conveniently from x-ray crystallographic data. To our knowledge, only the structure of I has appeared in the literature, and we have performed the calculation as described previously[16-18] from the crystallographic data.[25] The results (rigid lattice) are presented in Table I. It can be seen in Figure 2 that the first motion discussed above (low-temperature motion) results in a lowering of M_2 to ~12.0 G^2, which is the plateau between about 120 and 200 K. We wish to compare this observed value (12.0 G^2) with values calculated from $M_2(rl)$ by use of reduction factors[16-18] for specific motions. Inspection of molecular models for this compound indicates that anticipated motions are flexing of the ethano bridge in the central ring and methyl group reorientation. We have calculated[26] M_2 values in the presence of these motions and the results are presented in Table I. If one compares the apparent M_2 plateau in Figure 2, 12.0 ± 0.5 G^2, with the values in Table I, it can be seen that rapid methyl group reorientation is required to fit the observed data. It is also apparent that no conclusion can be drawn as to the extent of M_2 reduction due to ring flexing. The high-temperature motion

Table II. Estimated and Observed Second Moments (G^2) for Desipramine Hydrochloride

structure	M_2 estd	M_2 obsd
rigid lattice	19.4	
methyl reorient	15.7	16.5
methyl reorient + bridge flexing[a]	12.8	10.3–12.8

[a] Calculation assumes that the methylene interproton vector changes by 90°, which gives the maximum reduction in M_2 for that contribution (see ref 16).

in Figure 1 is difficult to assign. Its small effect on M_2, its shallow $T_{1\rho}$ and T_{1D} minima, and its $E_A \geq 7$ kcal/mol suggest that it could be either a small motion of the side chain or a libration.

In the case of II, the above procedure cannot be used since crystallographic data are not available. We have attempted to estimate M_2 values for II from those of I with appropriate corrections for different numbers of protons. The estimated rigid lattice M_2 is 19.4 G^2. If the T_1 minimum in Figure 3 is attributed to methyl reorientation, then the M_2 plateau at 100 K in Figure 4 (16.5 G^2) should be compared with the estimated value in the presence of methyl rotation (15.7 G^2). In addition, if the T_{1D} minimum in Figure 3 is attributed to flexing of the ethano bridge, then the M_2 transition in Figure 4 is also due to this motion and the anticipated plateau for $T > 300$ K probably lies between 10.3 and 12.8 G^2; unfortunately, the phase transition interferes. The estimated M_2 value in the presence of rapid methyl reorientation and bridge flexing is 12.8 G^2. This is the maximum reduction assuming a 90° flex angle. These results are summarized in Table II. The good agreement between estimated and observed M_2 values indicates that our assignments are reasonable; however, in light of the approximations made, the agreement must be considered fortuitous.

In the case of III, only the intramolecular contribution can be estimated by the procedure used for II. The intermolecular contribution for III is unknown, although it is expected to lie between 2.5 and 6 G^2, based on analogous systems in our studies. In addition, only a portion of the M_2 transition is observed in Figure 6; it is anticipated that the rigid lattice value is reached well below 100 K. For these reasons, we will not present estimated M_2 values for III and IV. Assignments of motions must then be based primarily on the observed activation barriers. We believe that the low-temperature (1.2 kcal/mol) process is due to bridge flexing and that the second (4.6 kcal/mol) process is inversion at nitrogen. The high-temperature (9 kcal/mol) process seen in T_1 just below the melting point is probably due to molecular reorientation or libration.

The same motions detected in III can also be seen in IV. It is of interest to note that the discontinuities due to a phase transition in III are clearly absent in IV.

Discussion

The barrier to methyl reorientation in I is 2.8 kcal/mol, which is comparable to barriers observed in the previously studied compounds.[16-18] The features in Figure 1 also give an indication that there may be a contribution from a second process, possibly with a higher barrier. The x-ray results[25] indicate that there are two molecules A and B within the asymmetric unit for imipramine hydrochloride. These two molecules exhibit respectively trans-trans and trans-gauche configurations of the dimethylaminopropyl chain. This suggests that the two processes contributing to the methyl minima in I are a result of the two different chain configurations. The barrier to methyl reorientation in II is about 1 kcal/mol lower than for I. This undoubtedly reflects the fact that the methyl-methyl interaction is greater than the methyl-proton interaction.

Table III. Activation Barriers (kcal/mol)

	I	II	III	IV
methyl reorientation	2.8[a]	1.9		
bridge flexing	*b*	0.7	1.2	~1
nitrogen inversion			4.6	~4

[a] Indication of two processes. [b] Not observable.

The results for bridge flexing indicate that the addition of the aminopropyl chain to the tricyclic framework lowers the activation barrier. The bridge flexing is expected to be present in I also, even though it cannot be observed in Figure 1, since the stronger methyl relaxation dominates. Evidence for this motion is the gradual M_2 transition in Figure 2 and also the disorder in the ethano bridge observed in the x-ray study.[25]

The barrier to inversion at nitrogen is 4.6 kcal/mol for III. This process has also been observed in our previous studies[16-18] with solids, in which motion of a methyl group is involved. On this basis, inversion at nitrogen in I and II might be expected to occur rapidly in solution. Our failure to detect such a process in the solid for I and II is probably due to the fact that it would involve a substantial reorientation of the entire chain in the solid. Activation barriers for these processes are summarized in Table III.

It is surprising that substitution of deuterium for protium in III and IV apparently causes enough of a perturbation to affect the phase transition. However, we have observed that phase transitions in organic solids are quite common. They are characterized by small enthalpy changes and by small changes in M_2; thus, they probably involve slight changes in packing, and the presence or absence of a phase transition of this type will no doubt depend upon a number of subtle structural features.

In conclusion, the results from this study indicate that these tricyclic systems are extremely flexible molecules with low activation motions occurring in the solid state. Although these results do not offer definitive information concerning a preferred conformation at the biological site, nevertheless it is likely that the observed bridge flexing is the mechanism by which the molecule achieves the nonplanarity which is believed to be a requirement for drug activity.[13] From these results, it is not clear whether bridge flexing is affected by removal of one of the methyl groups from the terminal amine. However, it appears that conformation of the side chain does exert a direct influence on methyl group reorientation, in the case of imipramine. In order to obtain more information concerning motional processes in tricyclic systems, we are currently studying some 6,6,6 systems including two potent neuroleptics, as well as other 6,7,6 systems.

Acknowledgments. B.A.S. acknowledges the support of NIH Research Grant ROINS10903 from the National Institute of Neurological Diseases and Stroke. We wish to thank Drs. E. R. Corey and J. Y. Corey for many helpful discussions. We also wish to thank McDonnell Douglas Astronautics Co. for the use of a storage oscilloscope and CIBA Pharmaceuticals Co. for supplying the samples used in this study.

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Circular Dichroism and the Conformation of Sugars Having Vicinal Diacylamino Substituents

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Abstract: The circular dichroism spectra of carbohydrates vicinally substituted by acetamido groups differ fundamentally from those of the common 2-acetamido-2-deoxy sugars as a result of mutual coupling effects. The calculations presented in this paper follow a method due to Schellman and co-workers which has been widely used in peptide CD calculations and which treats both the exciton coupling and static field effects in a consistent manner. The calculations predict, in agreement with experiment, that the 189-nm amide π–π^* transition is split into two oppositely signed CD bands of approximately equal magnitude near 180 and 200 nm. A small n–π^* band is predicted near 210 nm. The sign and magnitude of the bands depend on the dihedral angles defining the orientation of the amides with respect to the hexapyranose ring. It is shown that the calculations yield two independent parameters which may be compared with experiment: the product of the splitting with the rotational strength for the exciton bands and the total rotational strength for the n–π^* band. Comparison of the calculated results with the experimental spectra of 2,3-diacetamidoglucose and of the glycopeptide linkage compound, 2-acetamido-1-*N*(4-*l*-aspartyl)-2-deoxy-β-D-glucopyranosylamine, leads to the conclusion that both amide residues of the latter compound are oriented such that the relationship between the amide proton and the pyranose CH proton is trans while in the former the relationship is cis.

I. Introduction

2-Deoxy-2-acetamido sugars are common constituents of the complex oligosaccharide chains which are covalently attached to glycoproteins and glycolipids. Containing from 2 to 15 sugar units, these chains exhibit highly varied sequence and linkage often including branched structures. Although the biosynthetic pathway leading to these oligomers is exceedingly complex, little is known of their biological function. Even less is known of their three-dimensional conformation and of the forces governing their interactions with immunoglobulins, lectins, and hormones. The amide chromophore of the acetamido sugars serves as a convenient ultraviolet optical probe for conformational studies. The system whose CD[12] is treated in this work serves as a model for the region of covalent attachment of the sugar chain to the peptide chain of the "serum-type" glycoproteins.

The CD of 2-acetamido-2-deoxyhexoses has been the subject of both theoretical and experimental study. These carbohydrates, which have a single amide function, show a CD band near 209 nm which is assigned to the electrically forbidden amide n–π^* transition. The rotational strength arises mainly from a one-electron mechanism in which the asymmetric field of the atoms near the amide induces optical activity in the chromophore.[1] Since the major electrostatic perturber is the hydroxyl at C-3, changes in the solvent which modify the orientation of this group have a striking solvent effect on the CD spectrum.[2] In addition to the band near 209 nm, methyl glycosides of compounds such as GlcNAc and GalNAc show CD bands in the 185–192-nm region which are due to the π–π^* transition of the amide chromophore which has a strong absorption band at 189 nm. The magnitude and position of these shorter wavelength bands depends in a regular way on the configuration of the glycosidic linkage.[3] Similar results are found for oligosaccharides composed of *N*-acetylamino sugars such as the chitin oligosaccharides.[3,4]

The CD spectra of sugars vicinally substituted with two amide residues contrast sharply with the spectra of the ordinary 2-acetimido-2-deoxy sugars. Unlike the two amide residues of chitobiose, the chromophores of diacetamido sugars are sufficiently close to interact by mutual coupling much as occurs between adjacent amide residues of a regular polypeptide. The CD spectra of at least two biochemically interesting diacetamido sugars have appeared in the literature. 2,3-Diacetamidoglucose isolated from the lipopolysaccharide of several strains of photosynthetic bacteria has been studied by Keilich et al.[5] They report a spectrum having a strong negative band at 198 nm and a weak positive band near 222 nm. By analogy to polypeptide CD spectra, we may assign the 198-nm band

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