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SOLVENT DEPENDENCY OF ROTATIONAL BARRIERS IN ETHAMIVAN AND COMPARISON TO NIKETHAMIDE

LARRY S. ROSENTHAL, EILEEN CURRAN, JOHN W. BEAN and DONALD J. NELSON*
Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA 01610, U.S.A.

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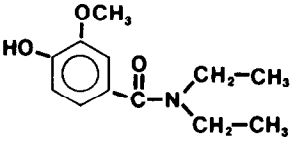
Abstract—Carbon-13 nuclear magnetic resonance (NMR) techniques were employed to examine the effects of solvent environment on rotational barriers in two drugs known to cause widespread stimulation in the mammalian central nervous system: ethamivan and nikethamide. Total NMR bandshape analysis was performed for the exchanging alkyl carbon resonances of these compounds as a function of temperature in six solvent systems: D_2O, CH_3OD, CH_3CH_2OD, $CDCl_3$, C_6D_6 and CF_3CH_2OH. The rate constants for rotation about the amide bond obtained in this way were used to calculate free energy ($\Delta G‡$), enthalpy ($\Delta H‡$) and entropy ($\Delta S‡$) of activation parameters for this process. Our results indicate that the magnitude of rotational barriers is affected markedly by (1) the size and polarity of the solvent molecules, and (2) the nature of the aromatic ring system attached to the amide grouping. Comparative interpretation of the thermodynamic parameters in light of the structures of nikethamide and ethamivan (in the various solvent systems examined) has further clarified the manner in which hydrogen bonding interactions between solvent molecules and the carbonyl oxygen of these analogues stabilize transition state conformers.

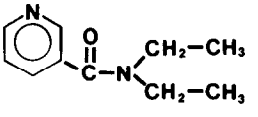
Ethamivan (*N,N*-diethyl-4-hydroxy-3-methoxybenzamide) and nikethamide (*N,N*-diethylnicotinamide) are two drugs known to cause widespread stimulation in the mammalian central nervous system (see structures in Fig. 1). Acting through the carotid body, both compounds enhance respiration and also raise blood pressure by stimulating medullary centers [1]. Both ethamivan and nikethamide are postulated to interact with muscarinic receptors in the medulla. Like the acetylcholine binding site of nicotinic receptors, the acetylcholine binding site of muscarinic receptors is thought to possess an anionic site and a hydrogen bond donor site [2–5]. The exact nature and specificity of ethamivan and nikethamide binding to muscarinic medullary receptors are unknown. Because both drugs share common structural features, these two molecules are ideally suited for solution studies aimed at the elucidation of the relationship between internal molecular motions and solute : solvent interactions. A clearer understanding of this fundamental relationship, which dictates the effective solution structure of these drugs, will aid our understanding of how these drugs interact specifically with neural receptors.

While alkyl substitutions on the amide nitrogen have only modest effects on the rotational energy barrier, this is not the case for substitutions on the aromatic ring. Sattler and Schunack [6, 7], for example, have found that methylation of the pyridyl ring of nikethamide at carbons 2 and 4 (the carbonyl carbon of the amide bond is attached to the ring at carbon 3) increases the $\Delta G‡$ value by about 2 kcal/mol (from 15.2 kcal/mol to 17.5 kcal/mol and 17.3 kcal/mol, respectively, in carbon tetrachloride). Methylation at carbon 5 decreases $\Delta G‡$ slightly (from 15.2 kcal/mol to 14.9 kcal/mol in deuterochloroform). They attribute these results to electronic effects caused by pyridyl ring methylation (i.e. methylation at carbons 2 and 4 of the pyridyl ring increases electron density at the amide carbon—nitrogen bond, and methylation at carbon 5 of the pyridyl ring has an electron withdrawing effect at that same bond).

The present study is an extension of our previous work [8, 9], and its primary goal was to obtain an understanding of the role of the aromatic ring system in determining the magnitude of the thermodynamic activation energy barriers to rotation about the



Ethamivan



Nikethamide

Fig. 1. Molecular structures of ethamivan and nikethamide.

* Author to whom correspondence should be addressed.

amide bond of ethamivan in solvents of varying polarity. The activation energy barriers determined for ethamivan are compared to those obtained previously for nikethamide [8, 9].

METHODS

Ethamivan was obtained from the Fluka Chemical Corp. (Hauppauge, NY) and nikethamide from the Sigma Chemical Co. (St. Louis, MO). Both compounds were used without further purification. Solutions of ethamivan were made to 3.0 mol% (i.e. 3.0 mol/100 ml solvent) in chloroform ($CDCl_3$), benzene (95% C_6D_6) (Stokler/Kor Chemical Co., Cambridge, MA), D_2O, methanol (MeOD), ethanol (EtOD), and non-deuterated 2,2,2-trifluoroethanol (Sigma Chemical Co.). Similar solutions were made for nikethamide in D_2O, benzene, chloroform and 2,2,2-trifluoroethanol.

Carbon-13 Fourier transform nuclear magnetic resonance (NMR) spectra were obtained on a Bruker WM-250 spectrometer operating at 5.8 Tesla (corresponding to a carbon-13 resonance frequency of 62 MHz). Precise temperatures were determined and maintained with the aid of an internal thermocouple previously calibrated by employing the temperature-dependent chemical shifts of ethylene glycol (above room temperature) and methanol (below room temperature). A temperature calibration curve was then constructed by least squares analysis. All spectra were proton decoupled, and the chemical shifts of the methyl and methylene carbons were recorded with respect to the standard tetramethylsilane (TMS).

For the determination of rate constants, decoupled magnetic resonance spectra were calculated according to the complete bandshape method [10, 11] using an IBM-9000 computer. With this method, one can

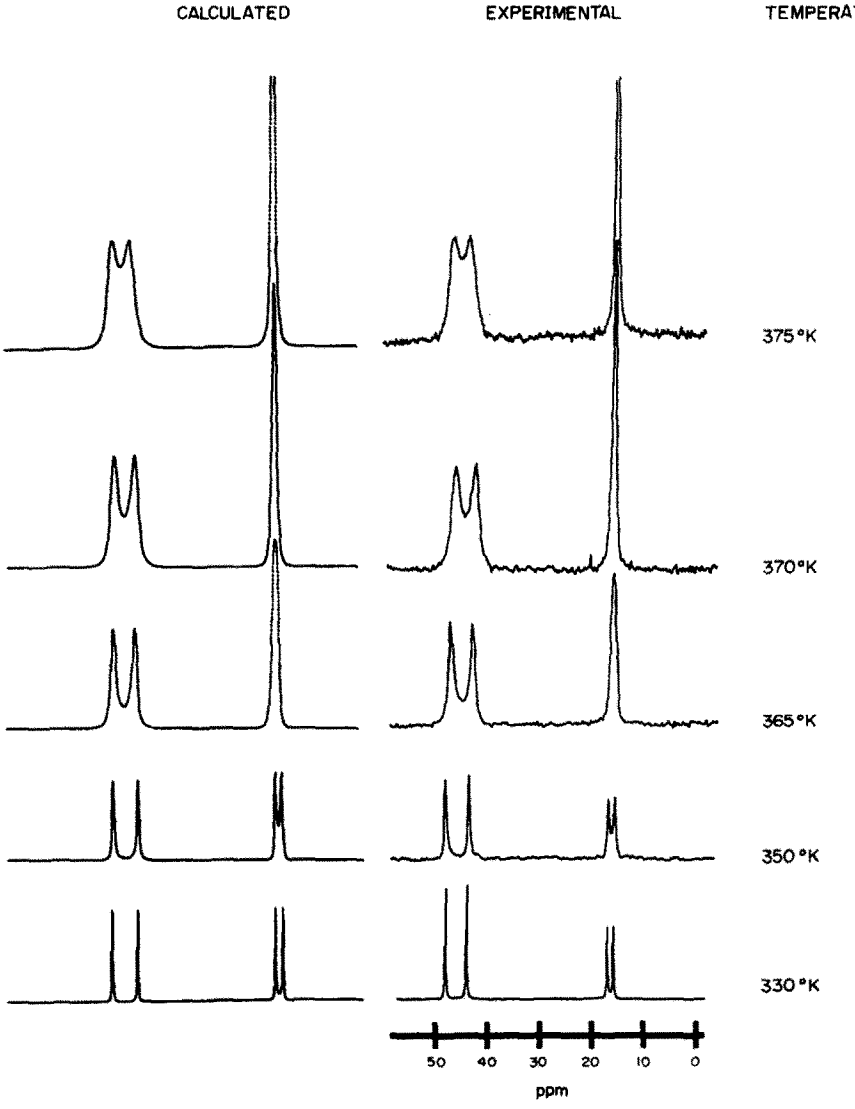


Fig. 2. Experimental and calculated carbon-13 NMR spectra for nikethamide in the solvent D_2O. The rate constants 30 sec^{-1}, 70 sec^{-1}, 175 sec^{-1}, 265 sec^{-1}, and 370 sec^{-1} were employed to calculate spectra at 330°K, 350°K, 365°K, 370°K, and 375°K respectively.

simultaneously simulate multiple sets of coalescing carbon resonances. In brief, the simulated spectra were obtained as follows: experimental frequencies and linewidths, as well as a "projected" rate constant, were fed into the computer program to yield a set of contracted Lorentzian line shape functions. The line shape functions were then multiplied over the experimental frequency range to yield a calculated spectrum whose rate constant and transverse relaxation time could be adjusted to obtain the best fit to the experimental spectrum. Figure 2 illustrates the simulation procedure for nikethamide in the solvent D_2O at a number of temperatures. Agreement between each calculated spectrum and the corresponding experimental spectrum at a particular temperature was achieved by adjusting the rate constants for rotation about the carbonyl carbon—nitrogen bond in nikethamide. In practice, the "fitting" process was continued until the computer simulated spectrum was superimposable, by eye, with the experimental spectrum obtained at a particular temperature. Although we estimate the error (in the projected rate constant) to be no higher than 10% for each individual fit, it should be noted that no thermodynamic parameters (i.e. $\Delta H\ddagger$ or $\Delta S\ddagger$) were determined from one single point; rather they were obtained from the slopes and intercepts of multipoint Eyring plots (see below), each characterized by a correlation coefficient $r > 0.99$. It can be seen from Fig. 2 that at high temperatures the rates of rotation become larger (i.e the NMR signals from chemically equivalent but magnetically non-equivalent carbon atoms become broader with a smaller chemical shift difference). At very high temperatures, complete coalescence of NMR signals was observed. The free energy of activation, $\Delta G\ddagger$, for rotation about the amide bond was calculated from rate constants derived from the simulated spectra (one rate constant per temperature value) using the Eyring equation [12]:

$$\ln(k/T) = -\Delta H\ddagger/RT + \Delta S\ddagger/R + \ln(k_B/h)$$

where k_B is the Boltzmann constant, h is Planck's constant and R is the universal gas constant. Plots of $\ln(k/T)$ versus $1/T$ (i.e. an Eyring plot) were constructed. The slope of each plot multiplied by the gas constant yields the enthalpy of activation. $\Delta H\ddagger$; the entropy of activation, $\Delta S\ddagger$, can be calculated from the y-intercept of the plot. The free energy of activation, $\Delta G\ddagger$, at a particular temperature can then be calculated from $\Delta H\ddagger$ and $\Delta S\ddagger$.

RESULTS

The ^{13}C-NMR spectra, at ambient temperature, of ethamivan and nikethamide, in the solvent 2,2,2-trifluoroethanol (TFE), are shown in Fig. 3, panels A and B respectively. The carbonyl carbon of

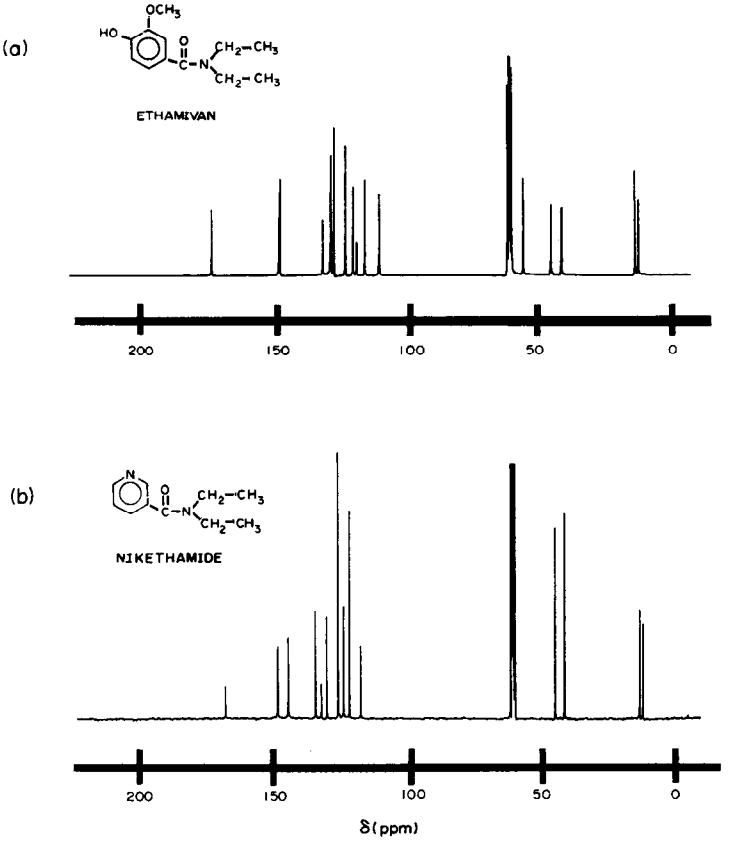


Fig. 3. Proton decoupled carbon-13 NMR spectra of (A) ethamivan and (B) nikethamide in the solvent 2,2,2-trifluoroethanol.

ethamivan resonated slightly downfield (174.6 ppm from TMS) from the carbonyl carbon of nikethamide (170.9 ppm). The aromatic ring carbons of both drugs resonated in the region from 150 to 119 ppm. At ambient (and lower) temperatures, the two methylene carbons in both compounds resonated with similar chemical shifts (one methylene carbon at about 45 ppm, the other at about 41 ppm). Similarly, at ambient (and lower) temperatures, the two methyl carbons of both drugs resonated separately (at about 14 and 12.5 ppm). The methylene and the methyl carbon resonances in these two drugs are "split" because of the presence of the partial double bond character of the carbonyl carbon—nitrogen bond. The partial double bond character resulted in a special configuration: the carbonyl carbon and oxygen atoms, the amide nitrogen atom, the ring carbon attached to the carbonyl carbon atom, and the two methylene carbon atoms bonded to the amide nitrogen atom all exist in a plane. As a consequence of this configuration the resonance associated with the methylene carbon closest to the electron-withdrawing oxygen atom, i.e. cis to oxygen, was shifted downfield relative to the trans carbon. Also, the two methyl carbons in both drugs resonated separately, further upfield, with reduced chemical shifts (as the two methyl carbons are at greater distance than the two methylene carbons from the electron-withdrawing carbonyl oxygen atom).

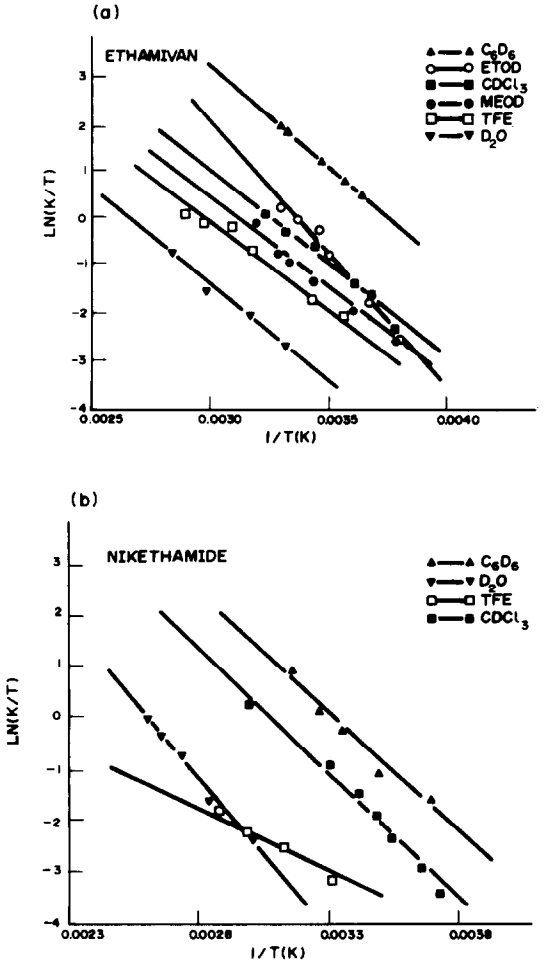


Fig. 4. Eyring plots for (A) ethamivan in CH_3OD, CH_3CH_2OD, D_2O, C_6D_6, CF_3CH_2OH, and $CDCl_3$ and (B) nikethamide in D_2O, C_6D_6, CF_3CH_2OH and $CDCl_3$.

The rate of rotation about the partial double bond in both ethamivan and nikethamide can be increased by providing sufficient energy (i.e. by increasing the temperature). The consequence of this operation for the drug nikethamide in the solvent D_2O can be seen in Fig. 2. As the temperature of a solvated sample of nikethamide was slowly increased, a progressive line broadening of the two resonances associated with the methylene carbons and the two resonances associated with the methyl carbons was observed. At a high enough temperature, the two methylene carbon resonances coalesced into a single resonance peak, as did the two methyl carbon resonances (i.e. at high temperature the two methylene carbons become magnetically equivalent on the NMR time scale, as do the two methyl carbon atoms).

Eyring plots (i.e. ln k/T vs $1/T$ plots) for rotation about the amide bond in ethamivan in the solvents CH_3OD, CH_3CH_2OD, D_2O, C_6D_6, CF_3CH_2OH and $CDCl_3$ are shown in Fig. 4A; similar plots for nikethamide in the solvents D_2O, C_6D_6, CF_3CH_2OH and $CDCl_3$ are shown in Fig. 4B. Enthalpy of activation ($\Delta H‡$), entropy of activation ($\Delta S‡$) and free energy of activation ($\Delta G‡$) values for both molecules in the various solvents, obtained from the Eyring analysis, are given in Table 1.

DISCUSSION

The rate of internal rotation about the carbonyl carbon—nitrogen bond in both ethamivan and nikethamide is dependent primarily on temperature. At high temperatures sufficient energy may be available to overcome the pi bonding interactions, and rotation is facilitated. However, as is clear from the current studies, solvent properties can affect dramatically the magnitude of free energy rotational barriers. If atoms in the transition state conformer have increased charge separation (relative to atoms in the ground state conformer), polar solvents will increase the rate of internal rotation about the amide bond [9]. Conversely, if there is a decrease in charge separation in atoms of the transition state conformer, relative to the ground state conformer, non-polar solvents will lead to greater rates of internal rotation. Thus, it is the relative differences in solute : solvent interactions between the transition state and the thermodynamically more stable ground state conformers that contribute to the differences in the heights of the free energy of activation barriers.

Another important factor responsible for dictating the heights of rotational barriers, which can in fact be included as part of the solvent polarity effect, has to do with hydrogen bonding interactions between the drug and solvent molecules. Specifically, the accessibility of the principal hydrogen bond acceptor group on the drug (i.e. the carbonyl oxygen atom)

Table 1. Thermodynamic activation parameters

		Solvent					
		D_2O	CH_3OD	CH_3CH_2OD	CF_3CH_2OH	$CDCl_3$	C_6D_6
ETHAMIVAN	$\Delta S‡$ (kcal/mol-°K)	−0.026	−0.022	−0.096	−0.026	−0.018	−0.014
	$\Delta H‡$ (kcal/mol)	7.93	7.93	11.16	7.17	8.83	8.92
	$\Delta G‡$ (kcal/mol)[b]	16.0 ± 0.5	14.8 ± 0.4	14.1 ± 0.3	15.2 ± 0.4	14.5 ± 0.3	13.2 ± 0.3
NIKETHAMIDE	$\Delta S‡$ (kcal/mol-°K)	−0.016			−0.032	−0.013	−0.083
	$\Delta H‡$ (kcal/mol)	11.84			6.54	11.08	11.74
	$\Delta G‡$ (kcal/mol)[b]	16.9 ± 0.5	15.5[a]	15.1[a]	16.4 ± 0.4	15.1 ± 0.4	14.3 ± 0.6

[a] Data from Ref. 9.
[b] Calculated at 310°K.

to various hydrogen bond donor groups on the solvent is critically important in determining the magnitude of rotational barriers. One can envision an ethamivan transition state conformer as having both methylene carbons (of the *N*-alkyl groups) in a plane perpendicular to the plane containing the amide nitrogen and carbonyl carbon and carbonyl oxygen atoms. Hydrogen bond donor groups in certain solvents may have better access to the principal hydrogen bond acceptor group in the transition state conformer of ethamivan (or in nikethamide) than in the ground state conformer. This would result in a large (negative) entropic contribution (i.e. a large positive $-T\Delta S‡$ term), effectively increasing the free energy of activation barrier.

Entropies of activation for ethamivan

Hydrogen bonding between drug and solvent molecules appears to be one of the more important factors in dictating entropy of activation values. It seems clear that solvents capable of hydrogen bond donation form hydrogen bonds to the carbonyl oxygen of the drug molecules (i.e. ethamivan and nikethamide), thus stabilizing the ground state conformers, with concomitant reduction in the rate of rotation about the amide bond. This conclusion is supported by the observed higher free energy of activation values in solvents more capable of hydrogen bond formation. In our experiments, an Eyring analysis giving a *positive* $\Delta S‡$ would indicate that the hydrogen bond to the amide oxygen was ruptured (as the temperature was increased) and did not reform during rotation. This is clearly not the case for ethamivan (or nikethamide) (Table 1). The *negative* entropies of activation for rotation about the amide bond (observed in all solvents examined) are indicative of highly ordered solvent–drug complexes in *both* the ground state and transition state conformers, with the transition state conformer–solvent complexes apparently more rigidly structured than the ground state–solvent complexes.

In general, the more polar solvents had significantly larger activation entropies than the less polar solvents, consistent with the interpretation above. However, when comparing one solvent with another, care must be exercised, since from these experiments it is not possible to determine whether the observed differences in stabilization of the transition state conformer relative to the ground state conformer (i.e. difference in $\Delta S‡$ from one solvent to another) were due to an increased stabilization of the transition state or to an increased destablization of the ground state.

While increased polarity generally leads to increased $\Delta S‡$ values, an apparent anomaly was observed with the polar solvent ethanol, which gave the smallest activation entropy value. An interesting comparison can be made between ethanol and trifluoroethanol (which gave one of the largest activation entropies), and perhaps the difference in properties between these two molecules will help explain the measured differences in activation entropies. Although ethanol and trifluoroethanol have similar molecular structures, boiling points, and dielectric constants, these two solvents differ significantly in acidity/basicity, and in their solvating

abilities. Mukherjee and Grunwald [13] examined the acid/base properties of both molecules, as well as their capacities for hydrogen-bonding, and found that trifluoroethanol is a far better hydrogen bond donor than ethanol. Conversely, ethanol was found to be a far better hydrogen bond acceptor than trifluoroethanol. Furthermore, the pK_a of the hydroxyl group of ethanol in water has been reported to be 16.0 [14], whereas the hydroxyl pK_a of trifluoroethanol in water is appreciably lower (12.5 at 25°) [13]. Therefore, as a weaker acid, the hydroxyl hydrogen of ethanol may be less available, relative to the hydroxyl hydrogen of trifluoroethanol, for participation in hydrogen bonding with hydrogen bond acceptor groups, such as the carbonyl oxygen of ethamivan. Thus, the larger, negative activation entropy for hindered rotation in ethamivan in the solvent trifluoroethanol may be indicative of hydrogen bonding interactions between the transition state conformer and trifluoroethanol that are more favorable than those which exist between the same transition state conformer and ethanol.

Enthalpies of activation for ethamivan

The enthalpies of activation for rotation about the carbonyl carbon—nitrogen bond of ethamivan were large and positive in all solvents examined (see Table 1). The large and positive enthalpies of activation principally reflect the significant energy required to disrupt the pi bonding interaction associated with the amide bond. Our results indicate that ΔH‡ values vary widely with solvent type; however, care must be taken in attempting to interpret the observed solvent dependency. One source of the solvent dependency of activation energy values relates to differences in the nature of "non-bonded" interactions (i.e. electrostatic, van der Waals, hydrogen-bond) which occur between the different solvents and the drug containing the amide bond of interest. Different types and strengths of non-bonded interactions will result in different degrees of pi-bonding character. In addition, some of these interactions may be altered or disrupted completely during the rotation process. Another source of the solvent dependency of activation energy values relates to solvent viscosity [15–18], which varies with temperature. In the present study we have not corrected for viscosity effects. We have discussed the viscosity effect in a previous paper [9].

Our results for ethamivan indicated that relatively non-polar solvents (such as chloroform and benzene) result in large activation enthalpies, whereas more polar solvents (such as water, methanol and trifluoroethanol) give rise to smaller activation enthalpy barriers. The larger activation enthalpy barriers associated with the non-polar solvents would be consistent with lower rates of rotation (at a particular temperature). It would also be indicative of increased charge separation between the atoms of the amide linkage in the transition state conformer. Anomalous enthalpy behavior was noted in the solvent ethanol. The enthalpy of activation in ethanol (11.165 kcal/mol) was more than two kcal/mol larger than in any other solvent employed, perhaps indicative of the solvent ethyl moiety (i.e. CH_3CH_2—) playing a larger role in solute:solvent interactions than previously expected. This is consistent with the measured entropy of activation found in ethanol, which indicated the rather weak hydrogen bond donor activity of ethanol (compared to methanol and trifluoroethanol). We must keep in mind, however, that hydrogen bonding (or lack of hydrogen bonding) may not dominate in every instance, as other non-bonded interactions between solvent and solute molecules (electrostatic and van der Waals interactions), and viscosity effects also play a role in dictating barrier heights.

Free energies of activation for ethamivan

The free energies of activation (calculated at 310°K) for rotation about the carbonyl carbon—nitrogen bond of ethamivan were also very large and positive (see Table 1). Table 2 presents values for the dielectric constants of the solvents examined. Bearing in mind that the dielectric constant is most certainly not the only relevant parameter in determining the height of rotational barriers, the data in Tables 1 and 2 indicate a general correlation between solvent polarity and the magnitude of the free energy barrier to rotation about the amide bond in ethamivan. However, this is only a "trend", as trifluoroethanol had a slightly "higher" ΔG‡ value (at 310°K) and ethanol a slightly "lower" ΔG‡ value (at 310°K) than would be predicted from dielectric constant information alone. [Note also that, for ethamivan in ethanol, the entropy and enthalpy parameters seem to have "compensated for one another", producing a ΔG‡ value more in line with its relative "polarity."]

The relationship between solvent polarity and solvent hydrogen bond donation propensity in dictating thermodynamic activation barrier magnitudes seems to be particularly important. The formation of a hydrogen bonded network (between solute and sol-

Table 2. Calculated ΔG‡ values for ethamivan

Solvent	Dielectric constant (298°K)[a]	$\Delta G‡_{298°K}$ (kcal/mol)
H_2O	78.54	15.73
CH_3OH	32.63	14.58
CF_3CH_2OH	26.14	14.86
CH_3CH_2OH	24.32	14.02
$CHCl_3$	4.806	14.25
C_6D_6	2.273	13.00

[a] All dielectric constants were taken from Ref. 19 except the values for CF_3CH_2OH and CH_3CH_2OH, which were taken from Ref. 13.

vent molecules) in the transition state that is more "structured" than that found in the "ground state" would result in larger free energy barriers for internal rotation about the amide bond. Complexes of ethamivan in polar solvents with a high propensity *for hydrogen bond donation* have higher internal rotational barriers than complexes of ethamivan in *either* polar solvents with a low propensity for hydrogen bond donation or in relatively non-polar solvents with no propensity for hydrogen bond donation. Note that the largest free energy of activation barrier for ethamivan was observed in D_2O (having the highest dielectric constant of the solvents employed and having high propensity for hydrogen bond donation), while the lowest barrier was measured in the solvent benzene (having the lowest dielectric constant of the solvents employed and having no propensity for hydrogen bond donation). Benzene interactions with ethamivan are likely to consist mainly of aromatic ring stacking, leaving the diethylamide group relatively "free" of direct/close contact with solvent. The hydrophobic effect and viscosity effects may be important in determining barrier heights in solvents such as benzene.

Comparison of activation parameters for ethamivan and nikethamide

Rotational barriers in both ethamivan and nikethamide were examined in the solvents D_2O, CF_3CH_2OH, $CDCl_3$ and C_6D_6, and the thermodynamic activation parameters are compared in Fig. 5. The results are summarized as follows:

(1) Activation entropy values in the solvents D_2O, $CDCl_3$ and C_6D_6 were larger (i.e. more negative) for ethamivan than for nikethamide (Fig. 5A). Only in the solvent CF_3CH_2OH was the activation entropy larger for nikethamide than for ethamivan.

(2) Activation enthalpy values in the solvents D_2O, $CDCl_3$ and C_6D_6 were significantly larger (i.e. more positive) for nikethamide than for ethamivan (Fig. 5B). In the solvent CF_3CH_2OH the enthalpy values were comparable, although slightly larger for ethamivan than for nikethamide.

(3) Activation free-energy values (calculated at 310°K) in *all four solvents* were markedly (i.e. >0.6 kcal/mol) larger for nikethamide than for ethamivan (Fig. 5C). For the four solvents examined in this comparison, there was a direct correlation between the magnitude of the free energy barrier and solvent polarity (i.e. greater polarity gave rise to larger free-energy barrier) for both compounds.

An examination of the structures of ethamivan and nikethamide (see Fig. 1) together with the data represented in Fig. 5C indicates clearly that the replacement of the pyridine ring in nikethamide by a 4-hydroxy-3-methoxy-benzamide ring in ethamivan facilitated rotation about the carbonyl carbon—nitrogen bond (i.e. the thermodynamic barrier is lower in ethamivan). Panels A and B in Fig. 6 depict computed low energy conformations and net atomic charges in ethamivan and nikethamide *in vacuo*. An examination of the net atomic charges revealed that the carbonyl carbon and amide nitrogen atoms of nikethamide had a higher net charge than on these same atoms in ethamivan (i.e. atomic charge separation is greater in nikethamide—the carbonyl carbon has a higher net positive charge and the amide nitrogen has a higher net negative charge). According to the electronic model proposed by Jackman [20], withdrawal of electron density from the carbonyl carbon results in the stabilization of the ground state conformer and destabilization of the transition state conformer (in which the carbonyl carbon is relatively more electrophilic). The net result is an increase in the barrier to internal rotation if the aromatic ring system (i.e. ring plus substituents) has a greater electron withdrawing capability (as is the case with the pyridine ring system in nikethamide) [21]. In addition, more energy is required to weaken the solvent:solute interactions leading to rotation about the amide bond in nikethamide than in ethamivan. The calculated dipole moment for nikethamide (1.276 debye) was substantially higher than that for ethamivan (0.468 debye), consistent with greater solvent–drug interactions and higher rotational barriers.

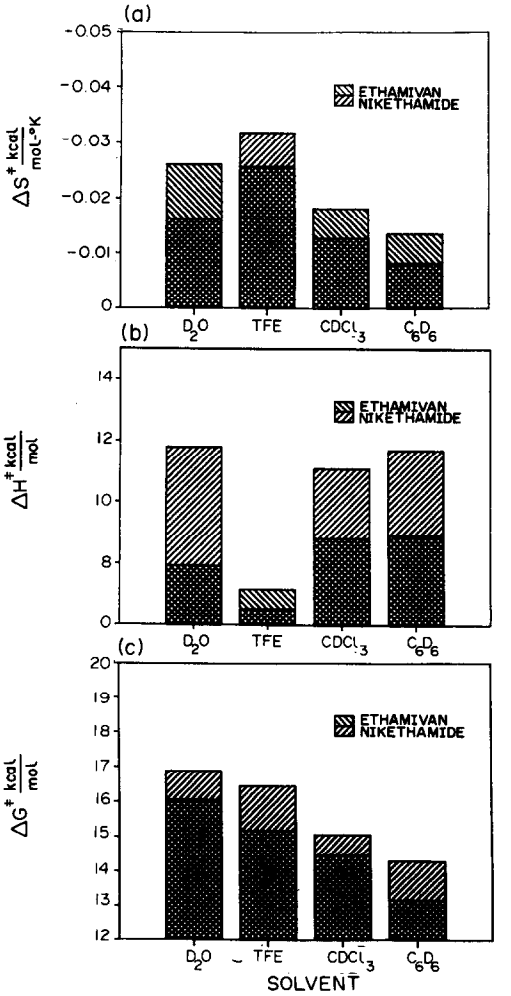


Fig. 5. Double bar graphs depicting (A) the activation entropies (kcal/mol-°K), (B) the activation enthalpies (kcal/mol) and (C) the activation free energies (kcal/mol) for rotation about the amide bonds in ethamivan and nikethamide in the solvents: D_2O, CF_3CH_2OH, $CDCl_3$ and C_6D_6.

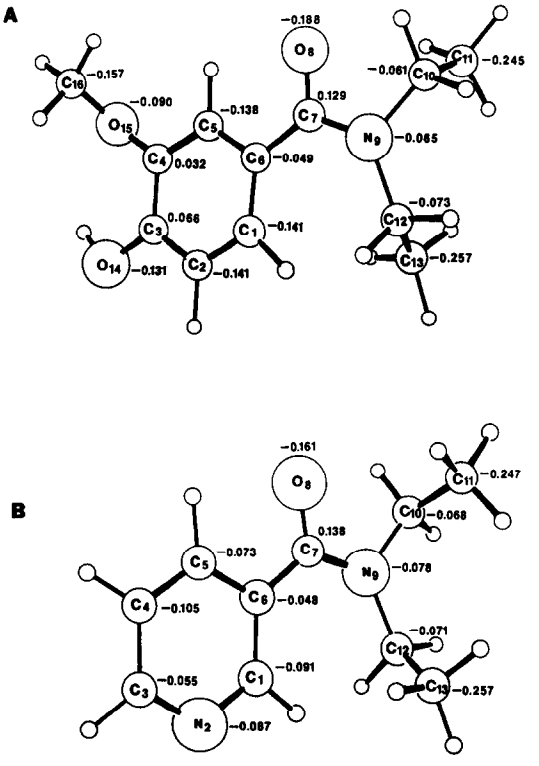


Fig. 6. Low energy conformations and *in vacuo* calculations of atomic charges on (A) ethamivan and (B) nikethamide.

Current research is directed towards the determination of rotational barriers in a wide range of substituted *N,N*-diethyl benzamides, in order to better understand the inductive and mesomeric influences on thermodynamic activation barriers in this important class of pharmacological agents.

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