Biochemical Pharmacology, Vol. 35, No. 6, pp. 1011–1017, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

SOLVENT INTERACTIONS WITH *N,N*-DIALKYLNICOTINAMIDES AND THEIR EFFECTS ON ROTATIONAL BARRIERS

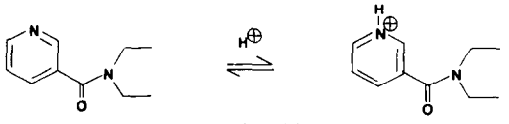
JOHN W. BEAN and DONALD J. NELSON*
Department of Chemistry, Sackler Sciences Center, Clark University, Worcester, MA 01610;
and
GEORGE E. WRIGHT
Department of Pharmacology, University of Massachusetts Medical Center, Worcester, MA 01605, U.S.A.

(*Received* 18 *June* 1985; *accepted* 26 *August* 1985)

Abstract—Carbon-13 nuclear magnetic resonance techniques were employed to examine the effects of solvent environment on rotational barriers in a series of molecules structurally-related to the analeptic, nikethamide: *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine. Total bandshape analysis was performed for the exchanging alkyl carbon resonances of these compounds as a function of temperature in four solvent systems: D_2O, CH_3OD, CH_3CH_2OD and $CDCl_3$. 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 rotational barriers are less affected by the nature of the alkyl chain attached to the amide nitrogen than by the size and polarity of the solvent molecules. Interpretation of the thermodynamic parameters in light of both nikethamide analogue structure and solvent type has further clarified the manner in which hydrogen bonding interactions between solvent molecules and the carbonyl oxygen of these analogues stabilize transition state conformers.

Ionic and hydrogen-bonding interactions are two very important forces that contribute to the proper binding of a pharmacologically-active substance to a receptor site. The acetylcholine receptor, for example, is thought to possess an anionic center that interacts with the positively charged quaternary ammonium group of acetylcholine, as well as a hydrogen-bond donor site that is postulated to interact with the carbonyl oxygen of the neurotransmitter [1–4]. Support for the critical importance of both types of interactions for acetylcholine binding to its receptor is the finding that dimethylbutyl acetate, an uncharged analog of acetylcholine, is physiologically inert [2]. In addition, trimethyl pentyl ammonium ion and ethyl choline ether have been shown to possess, respectively, only 1.4 and 1.5% of the potency of acetylcholine in eliciting clam heart contraction [5].

The muscarinic acetylcholine receptor site, to which the analeptic nikethamide (see structures) binds, is also thought to possess an anionic site and a hydrogen-bond donor site [3, 4].



Nikethamide

* Address all correspondence to: Dr. Donald J. Nelson, Department of Chemistry, Clark University, Sackler Sciences Center, 950 Main St., Worcester, MA 01610, U.S.A.

The distance separating these two binding sites has been estimated to be about 4.4 Å, based on the distance between the ether oxygen and the quaternary nitrogen of the rigid analog muscarine. Nikethamide, most likely in its cationic form (see structures), binds preferentially to respiratory centers in the medulla, with considerably less effect on peripheral muscarinic or nicotinic acetylcholine receptors [6]. The reason for reduced affinity of nikethamide for the less sensitive acetylcholine receptor sites is unknown, but it is most likely a result of subtle structural differences between it and the more sensitive medullary receptor.

In the present study we employed nuclear magnetic resonance techniques to explore the effects of solvent environment on the rate of rotation about the carbon–nitrogen partial double bond in a series of molecules that are structurally-related to nikethamide: *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine, a compound in which the alkyl groups are joined. This work is an extension of a previous study on the effect of solvent polarity on rotational barriers in the parent molecule, nikethamide [7]. In that study, it was shown that internal rotation was more restricted in the more polar solvents. In the more polar solvents, the free energy of activation for rotation about the carbonyl carbon—nitrogen bond, $\Delta G‡$, was increased significantly, presumably reflecting a higher degree of hydrogen bond formation between the solvent molecules and the carbonyl oxygen of the nikethamide molecule. In the present study, we

have further clarified the manner in which hydrogen bonding interactions between solvent molecules and the carbonyl oxygen function to stabilize a transition state conformer.

The critical role that the solvent environment plays in internal rotatory events has been indicated clearly by the ^{1}H- and ^{13}C-NMR studies of Ross *et al.* [8–11] on amide bond rotation in gas phase systems. They demonstrated that, on the average, the free energy of activation of rotation about an amide bond is about 2 kcal/mole less in the gas phase than in a solvent such as CCl_4. The lower energy threshold for rotation in the gas phase was specifically attributed to two sources: (1) reduced electrostatic interactions, and (2) a reduced internal solvent "pressure", i.e. a lower viscosity. In the present study on amide bond rotation in solution systems, we have been able to discriminate amongst various solvents with respect to the dielectric contribution to the free energy of activation barrier.

METHODS

The nicotinamides, *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine, were prepared by reacting nicotinoyl chloride with the appropriate amine, i.e. dimethylamine, di-*n*-propylamine and piperidine, in the presence of aqueous sodium hydroxide. The product was extracted from the aqueous solution with chloroform, the chloroform was removed by rotary evaporation, and the resulting oil was distilled *in vacuo*. The purities of the products were assayed by TLC, and they were identified by their nuclear magnetic resonance and infrared spectra, and their characteristic melting and/or boiling points [12].

The nicotinoyl chloride employed in the above syntheses was prepared by refluxing nicotinic acid with thionyl chloride followed by distillation of the excess thionyl chloride. The resulting crystals of nicotinoyl chloride were rinsed with hexane and filtered dry.

Solutions of the nicotinamides employed for measurement of rotational energy barriers were made to 1.0 mole/100 ml in the appropriate solvent. This high ratio of solvent to solute molecules was employed to minimize solute–solute interactions. All reagents and solvents were purchased from the Sigma Chemical Co. (St. Louis, MO).

Carbon-13 Fourier transform 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). The internal thermocouple was calibrated using the temperature-dependent chemical shifts of ethylene glycol (above room temperature) and methanol (below room temperature). A temperature calibration curve was then constructed by a least squares analysis. All spectra were proton decoupled, and the chemical shifts of the coalescing carbon resonances were recorded relative to the carbonyl carbon resonance.

Magnetic resonance spectra were calculated by the "complete bandshape method" [13, 14]. The lineshape equation, implemented on an IBM-9000 computer, was designed to simultaneously simulate multiple sets of coalescing resonances. The resonance intensities of all the calculated spectra were thereby "normalized". In brief, calculated NMR spectra were obtained as follows: experimental frequencies and linewidths, as well as a projected rate constant, were fed into the 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 times could be adjusted to obtain the best fit to the experimental spectrum. Figure 1 illustrates the method for 1-nicotinoyl piperidine in deuterated ethanol. Experimental and calculated spectra at four temperatures are shown. Agreement between each

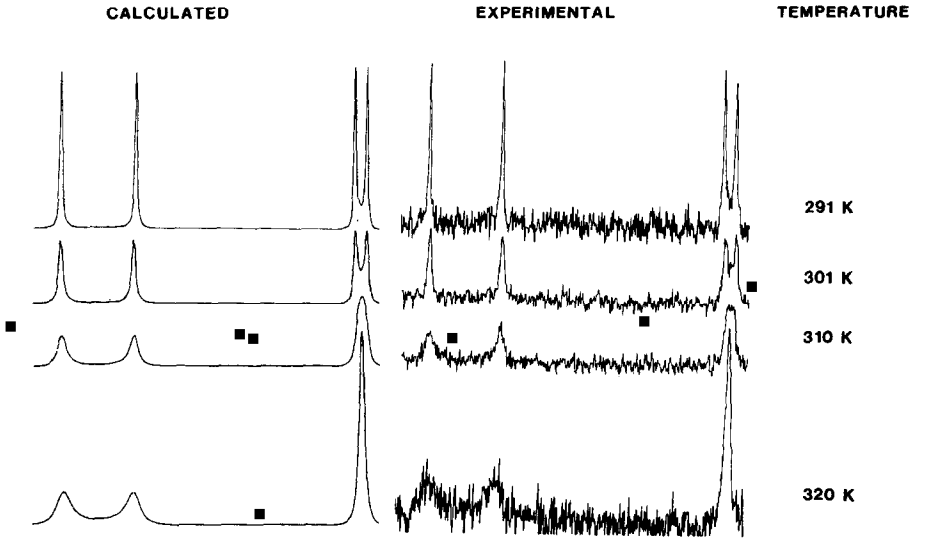


Fig. 1. Calculated and experimental ^{13}C-NMR spectra of 1-nicotinoyl piperidine dissolved in deuterated ethanol. Rate constants of 250 sec^{-1}, 150 sec^{-1}, 70 sec^{-1}, and 35 sec^{-1} were used to calculate spectra at 320 K, 310 K, 301 K, and 291 K respectively.

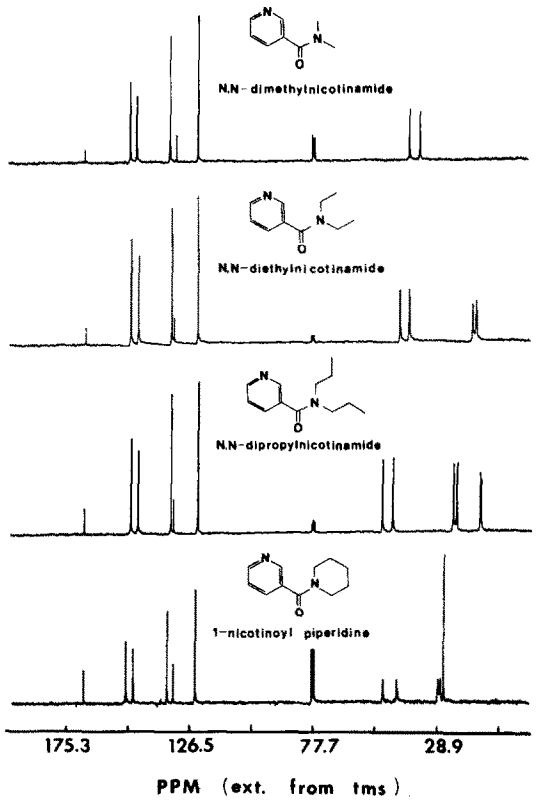


Fig. 2. ^{13}C-NMR spectra of dialkylnicotinamides in chloroform. All compounds exhibit characteristic carbonyl carbon (170 ppm) and pyridyl ring carbon (150–120 ppm) resonances. The triplet at 77.7 ppm is the chloroform resonance. The alkyl substituents of the amide nitrogen resonate upfield of chloroform.

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 the compound under study. At higher temperatures, the increasing rates become larger, which translates into broader NMR signals and a smaller chemical shift difference between signals derived from chemically-equivalent, but magnetically non-equivalent, carbon atoms. At very high temperatures, complete coalescence of signals is observed. Values of $\Delta G\ddagger$, the free energy of activation for rotation about the carbonyl carbon—nitrogen partial double bond, were calculated from the rate constants (one rate constant per temperature value) of the fitted spectra using the Eyring equation [15]:

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

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 $\Delta H\ddagger$; $\Delta S\ddagger$ can be calculated from the y-intercept of the plot. $\Delta G\ddagger$ 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 the nicotinamides dissolved in chloroform are shown in Fig. 2. The carbonyl carbons of these compounds resonated at about 170 ppm. The five pyridyl ring carbons resonated in a band roughly spanning the 150–120 ppm spectral region. Upfield of the chloroform resonance (77.7 ppm) were the alkyl carbon resonances. Due to the presence of the partial double bond character of the carbonyl carbon—nitrogen bond in each of these compounds, the carbonyl carbon and oxygen, the amide nitrogen, and carbon-3 of the pyridyl ring all existed in a plane with the two methyl (or methylene) carbon atoms (derived from the two alkyl chains) bonded to the amide nitrogen atom. As a consequence of this configuration, the resonance associated with the methyl (or methylene) carbon closest to the electron-withdrawing oxygen atom, i.e. *cis* to oxygen, was shifted downfield relative to the *trans* carbon. As the alkyl substituent(s) was increased in length (e.g. from a methyl group to an ethyl group), progressive deshielding occurred at the carbons nearest the amide bond, and the *cis* and *trans* carbon resonances shifted further downfield. Also, chemically-equivalent carbon atoms further removed from the amide nitrogen resonated further upfield with reduced chemical shift differences. In *N,N*-diethylnicotinamide, for example, the methyl carbons were magnetically more "equivalent" than were the methylene carbons because they were further removed from the anisotropic effects of the carbonyl oxygen.

An increase in temperature increased the rate of rotation about the carbon—nitrogen partial double bond in each of the compounds illustrated in Fig. 2. As was shown in Fig. 1, this increase in the rate of rotation with increasing temperature manifested itself first as a progressive line broadening and culminated with the coalescence of a pair of carbon resonances. This effect was observed, at high temperatures, because the two carbons exchanged positions often enough so that they become magnetically equivalent on the NMR experimental time scale.

The degree to which temperature affected the rate of rotation about this carbon—nitrogen bond was indeed solvent dependent. Figure 3 presents Eyring plots ($\ln k/T$ vs $1/T$) for *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine in four solvent systems: D_2O, CH_3OD, CH_3CH_2OD, and $CDCl_3$. Similar $\Delta G\ddagger$ values were obtained for each of the compounds in a particular solvent (see Table 1). A higher value for $\Delta G\ddagger$ corresponds to a more hindered rate of rotation about the carbon—nitrogen bond. It is clear from both the Eyring plots and from the $\Delta G\ddagger$ values that rotation about the carbon—nitrogen bond of the nicotinamide compounds was more hindered in D_2O than in any of the other solvents.

Entropy and enthalpy of activation values for the various *N,N*-dialkylnicotinamides in the four solvent systems examined are given in Fig. 4. Entropy of activation values ($\Delta S\ddagger$) ranged from -1.75 ± 2.1 calories/degrees for the dimethyl analog in D_2O to -22.82 ± 2.13 calories/degree for the di-*n*-propyl analog in CH_3CH_2OD. The $\Delta S\ddagger$ values for all the compounds were similar in a particular solvent. However, $\Delta S\ddagger$ values were much lower in the alcohols than in $CDCl_3$ and were highest in D_2O. Values for

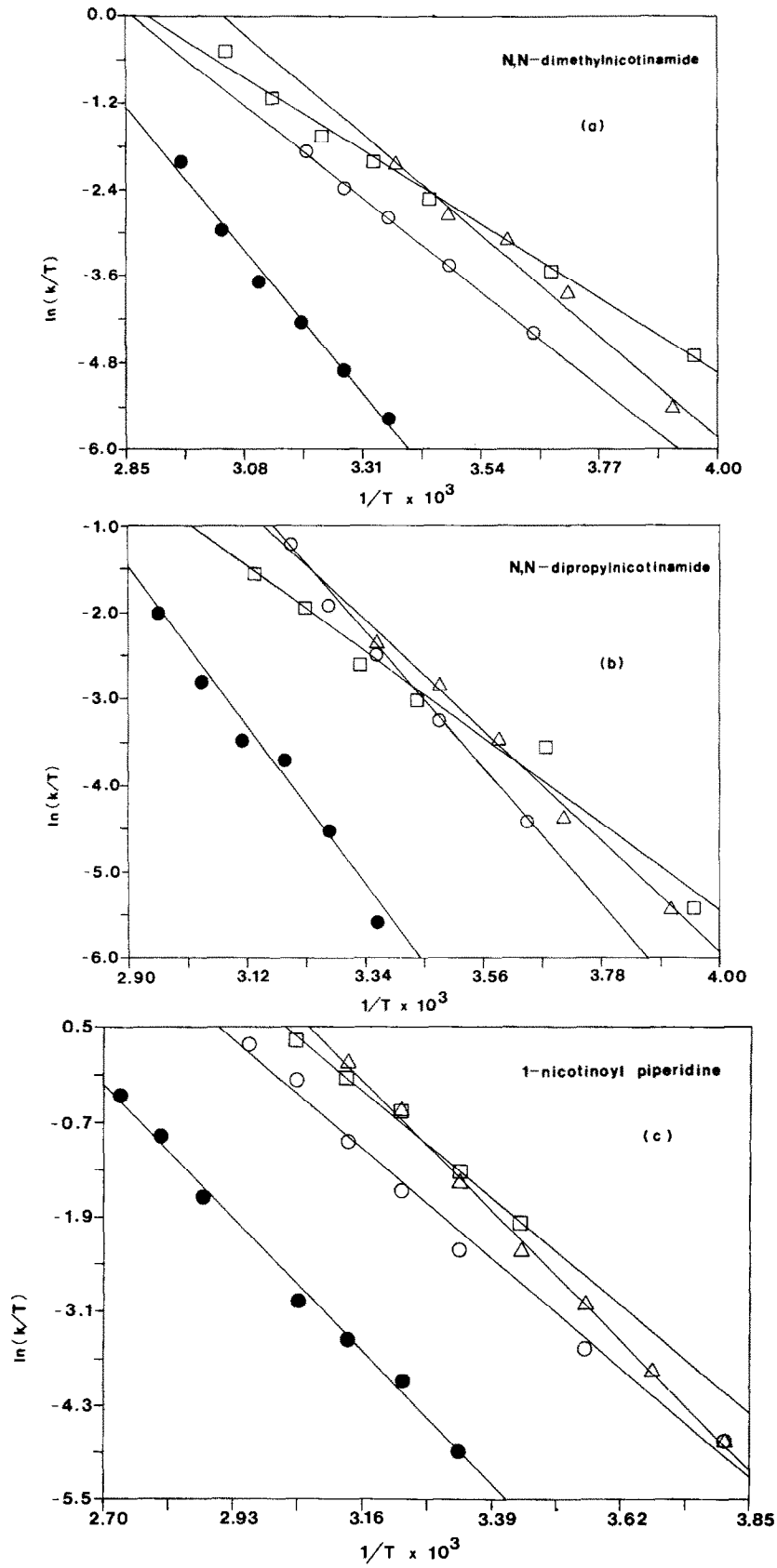


Fig. 3. Plots of $\ln(k/T)$ vs $(1/T)$ for 1.0 mole/100 ml *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide, and 1-nicotinoyl piperidine dissolved in D_2O (●), CH_3OD (○) CH_3CH_2OD (□), and $CDCl_3$ (△). $\Delta H‡$ can be obtained from the slope of the regression line, and $\Delta S‡$ can be obtained from its y-intercept.

Table 1. Values of $\Delta G‡$ for the three dialkylnicotinamides at three different temperatures solvated in the indicated solvent

Nicotinamide	K	$\Delta G‡$ (kcal/mole)			
		D_2O	CH_3OD	CH_3CH_2OD	$CDCl_3$
Dimethyl-	330	17.6	16.3	16.1	15.6
	310	17.5	16.0	15.7	15.4
	280	17.5	15.5	15.0	15.1
Dipropyl-	330	17.4	15.7	16.4	15.9
	310	17.4	15.6	15.9	15.6
	280	17.3	15.5	15.2	15.2
Piperidyl-	330	17.4	15.9	15.4	15.2
	310	17.3	15.6	15.2	15.1
	280	17.0	15.3	14.8	15.1

Standard deviations for the listed $\Delta G‡$ values were less than 0.2 kcal.

the enthalpy of activation, $\Delta H‡$, were large and positive for all the compounds tested. Again, as was observed for $\Delta S‡$, $\Delta H‡$ was greatest in D_2O, followed by $CDCl_3$ and was lowest in the alcohols.

DISCUSSION

Solvents can affect activation energies for internal rotations through electrostatic interactions and internal pressure effects [8]. We reported earlier that hydrogen bonding of the solvent to nikethamide stabilizes the conformer, reducing the rate of rotation about the carbon—nitrogen bond by increasing the free energy of activation for this rotation [7]. This conclusion was based upon the observed higher $\Delta G‡$ values and slower rates of rotation about that bond in nikethamide in solvents more capable of hydrogen bond formation.

In the present study, we report the same trend in a series of nikethamide analogues. The negative entropies of activation for rotation about the carbon—nitrogen bond are indicative of highly ordered solvent–solute complexes in the transition state, i.e. hydrogen bonding interactions between solvent and nikethamide analog are facilitated in the transition state. This conclusion is readily accounted for upon examination of molecular models of the various nicotinamides. In the preferred conformers of each

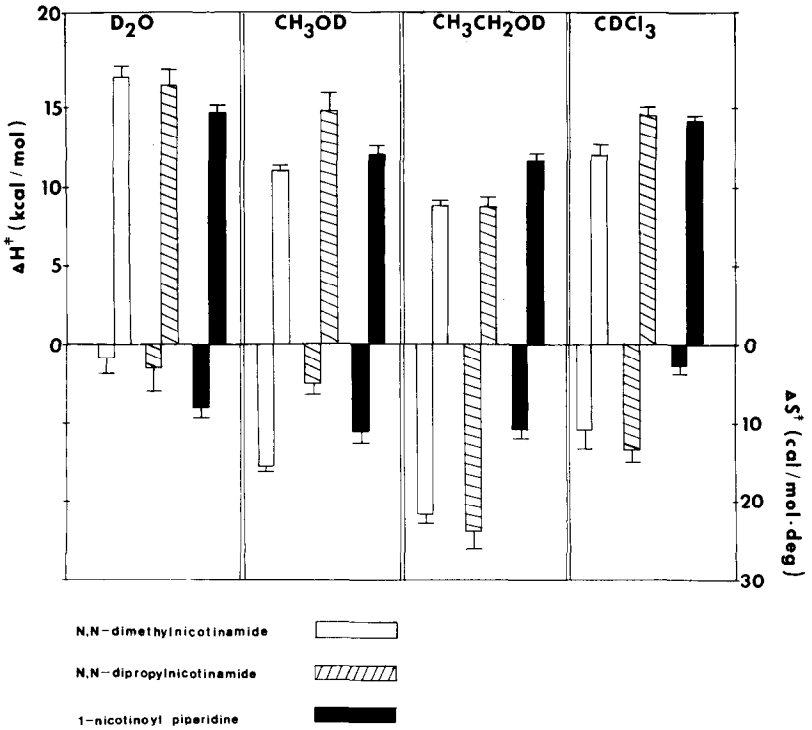
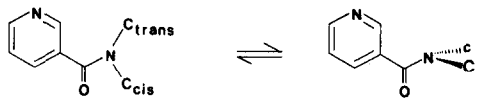


Fig. 4. Bar graph representing the entropy and enthalpy of activation values for rotation about the carbon—nitrogen partial double bond for *N,N*-dimethylnicotinamide (clear bar), *N,N*-di-*n*-propylnicotinamide (slashed bar), and 1-nicotinoyl piperidine (solid bar). Notice the lower enthalpy and entropy of activation values for each compound in the alcoholic solvents.

N,N-dialkylnicotinamide, the carbonyl oxygen, a likely site for strong hydrogen bonding interactions with the solvent, is sterically crowded by one of the two carbons (derived from either a methyl group or a methylene group of a larger alkyl chain) directly bonded to the amide nitrogen. By contrast, in the transition state the steric challenge is diminished considerably, as the carbonyl oxygen is more accessible for hydrogen bonding interactions, as depicted in the following structures:



ground state transition state

Figure 4 presents values for entropy of activation, $\Delta S\ddagger$, and enthalpy of activation, $\Delta H\ddagger$, for the three *N,N*-dialkylnicotinamides in four solvent systems. Considering $\Delta S\ddagger$ first, it is particularly interesting that the largest (negative) values observed for $\Delta S\ddagger$ were recorded when ethanol was used as a solvent. This finding is indicative of the presence of a facilitated hydrogen bonding interaction between the $-OD$ moiety of the solvent and the carbonyl oxygen in the transition states of the *N,N*-dialkylnicotinamides. Also note that there is a correlation between the size of the solvent molecules possessing the $-OD$ moiety and the magnitude of $\Delta S\ddagger$ (i.e. $\Delta S\ddagger$: $CH_3CH_2OD > CH_3OD > D_2O$). Thus, CH_3CH_2OD binds to —C═O in the transition state much more strongly than in the ground state conformation, whereas D_2O binds to —C═O in the transition state only marginally better than to the ground state. CH_3OD, as expected, is intermediate between CH_3CH_2OD and D_2O. Thus, it appears that increasingly negative $\Delta S\ddagger$ values with increasing size of solvent molecule, as is experimentally observed, are entirely predictable from steric considerations alone. Entropy of activation values for alkylnicotinamides dissolved in chloroform are consistent with this observation despite the fact that their $\Delta S\ddagger$ values were not as large as in CH_3CH_2OD. The deuterium of $CDCl_3$ was not as efficient a hydrogen bond donor as the deuteriums bonded to the electronegative oxygen atoms of D_2O, CH_3OD and CH_3CH_2OD, and thus $CDCl_3$ was less efficient at stabilizing the transition state.

The enthalpies of activation for rotation about the carbon—nitrogen bond of these compounds support the conclusions derived from the calculated values for $\Delta G\ddagger$ and $\Delta S\ddagger$. Overall, $\Delta H\ddagger$ values were large and positive, reflecting the generally high energy of activation barrier which must be surmounted to effect rotation about the carbonyl carbon—nitrogen bond of the *N,N*-dialkylnicotinamides. The lower $\Delta H\ddagger$ values for all the compounds in deuterated ethanol is indicative of more favorable solvent-transition state conformer interactions compared to the other solvent systems.

Dielectric effects appear to be relatively important in this system. The $\Delta G\ddagger$ values reported here for *N,N*-dimethylnicotinamide, *N,N*-di-*n*-propylnicotinamide and 1-nicotinoyl piperidine fall in the proper order and roughly the right proportions as one would predict from the dielectric constants of the various solvents: H_2O, 78.54; CH_3OH, 32.63; CH_3CH_2OH, 24.30; $CHCl_3$, 4.806 (all values measured at 20°) [16]. The thermodynamic values, $\Delta H\ddagger$, $\Delta S\ddagger$ and $\Delta G\ddagger$, are also influenced by internal pressure effects of the solvent upon the particular dialkylnicotinamide. The solvent viscosities are temperature dependent, and a decreased viscosity was reflected in decreased hindrance to rotation about the amide bond and, consequently, lower measured $\Delta G\ddagger$ values. For example, ethanol has a viscosity of 0.82 centipoise at 0° and 0.456 centipoise at 40°, whereas chloroform has a viscosity of 0.70 centipoise at 0° and 0.500 centipoise at 40° [17]. Therefore, one observes a relatively larger variation in the $\Delta G\ddagger$ value for these compounds dissolved in ethanol than in chloroform (see Table 1).

The results of the present investigation show no clear correlation between the magnitude of $\Delta G\ddagger$ and the size of the alkyl group attached to the amide nitrogen, i.e. in a particular solvent system at a particular temperature all *N,N*-dialkylnicotinamides had similar rotational free energy barriers (see Table 1). Whereas di-alkyl substitutions on the amide nitrogen had only modest effects on the rotational barrier, this was not the case for substitutions on the pyridyl ring. Sattler and Schunack [18, 19], for example, found that methylation of the pyridyl ring at carbons 2 and 4 increases the $\Delta G\ddagger$ value by about 2 kcal/mole (from 15.2 kcal/mole to 17.5 kcal/mole and 17.3 kcal/mole, respectively, in $C_2D_2Cl_4$), whereas methylation at position 6 decreases $\Delta G\ddagger$ slightly (from 15.2 kcal/mole to 14.9 kcal/mole in $CDCl_3$). These values are in general agreement with the results reported here, although the literature data were determined solely from the coalescence temperature of the ^{13}C magnetic resonance signals, i.e. not the complete bandshape method used here.

In summary, the solvent environment has a significant effect upon the rotation about the carbon—nitrogen partial double bond of several nikethamide analogs. Although the exact nature of the muscarinic receptor site for nikethamide is not known, the results of this and our previous study [7] suggest a model for the interaction between this receptor and nikethamide. The muscarinic receptor most likely possesses, among other groups, a hydrogen bond donor group in the ligand binding site, perhaps a hydroxyl group by analogy with the structure of acetylcholinesterase [20], and may have difficulty binding to nikethamide when the latter is in the planar ground state conformation because of steric crowding caused by the *cis* ethyl carbons. If nikethamide, or nikethamide analog, were to undergo a slight twisting (say, 10–15°) about the carbonyl carbon—nitrogen bond, the molecule could still maintain a high degree of partial double bond character in the amide bond; however, the ligand: receptor steric crowding would be alleviated, enabling the hydrogen bonding attractive forces between the receptor hydrogen bond donor and the ligand —C═O group to reach a magnitude sufficient to offset the increased $\Delta G\ddagger$.

Acknowledgements—This research has been supported by Grants 1230 and 1729 from The Council for Tobacco Research—U.S.A., Inc. The NMR data were obtained at the Worcester Consortium NMR Facility. This material is based upon work supported by the National Science Foundation under Grant DMR 8108697. The authors are also grateful to Prof. Wen-Yang Wen (Chemistry Department, Clark University) for many helpful discussions.

REFERENCES

1. E. J. Lien, E. J. Ariens and A. J. Beld, *Eur. J. Pharmac.* **35**, 245 (1976).
2. A. S. V. Burgen, *Br. J. Pharmac.* **25**, 4 (1965).
3. C. Chothia, *Nature, Lond.* **225**, 36 (1970).
4. C. Chothia and P. Pauling, *Nature, Lond.* **226**, 541 (1970).
5. J. H. Welsh and R. Taub, *J. Pharmac. exp. Ther.* **103**, 334 (1950).
6. R. L. Volle and G. B. Volle, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman), pp. 363–4. Macmillan, New York (1970).
7. J. W. Bean and D. J. Nelson, *Biochem. Pharmac.* **33**, 2145 (1984).
8. B. D. Ross, N. S. True and D. L. Decker, *J. phys. Chem.* **87**, 89 (1983).
9. B. D. Ross, N. S. True and G. B. Matson, *J. phys. Chem.* **88**, 2675 (1984).
10. B. D. Ross, L. T. Wong and N. S. True, *J. phys. Chem.* **89**, 836 (1985).
11. B. D. Ross and N. S. True, *J. Am. chem. Soc.* **106**, 2451 (1984).
12. Y. Hukushima, *J. chem. Soc. Japan* **61**, 121 (1940).
13. H. S. Gutowsky and C. H. Holm, *J. chem. Phys.* **25**, 1228 (1956).
14. J. Sandstrom, *Dynamic NMR Spectroscopy*. Academic Press, London (1982).
15. H. Eyring, *Chem. Rev.* **17**, 65 (1935).
16. R. C. Weast (Ed.), *C.R.C. Handbook of Chemistry and Physics*, 61st Edn, p. E55. CRC Press, Boca Raton, FL (1980).
17. R. C. Weast (Ed.), *C.R.C. Handbook of Chemistry and Physics*, 61st Edn, p. F52. CRC Press, Boca Raton, FL (1980).
18. H-J. Sattler and W. Schunack, *Arch Pharm., Weinheim* **309**, 222 (1976).
19. H-J. Sattler and W. Schunack, *Arch. Pharm., Weinheim* **309**, 439 (1976).
20. D. E. Koshland Jr., *Science* **142**, 1533 (1963).