JOURNAL

OF THE AMERICAN CHEMICAL SOCIETY

VOLUME 106, NUMBER 24 NOVEMBER 28, 1984

Carbon-13 NMR in Liquid-Crystal Solutions. Hindered Rotation in 4-(Dimethylamino)pyrimidine

B. M. Fung,* Robert V. Sigh, and Martha M. Alcock

Contribution from the Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73069. *Received May 29, 1984*

Abstract: The hindered rotation of the dimethylamino group in 4-(dimethylamino)pyrimidine in a liquid-crystal solution (Merck ZLI 2142) was studied from 232 to 263 K by natural-abundance carbon-13 NMR. The enthalpy of activation ($\Delta H^{\ddagger}$) in the liquid-crystal solution is 53 ± 1 kJ mol^{-1}, which is 50% higher than that in CD_2Cl_2 (35 ± 1 kJ mol^{-1}) and about the same as that in CD_3OD (52 ± 1 kJ mol^{-1}). It is possible that the ordering forces of the liquid-crystal solvent may favor the planar ground state of the solute more than the bulkier transition state, causing the barrier of rotation to be unusually high.

NMR is a powerful technique in the study of conformational equilibria and rate processes. Most investigations using dynamic NMR spectroscopy have been carried out in the liquid phase.[1,2] Although some work in gases,[3] solids,[4] and liquid crystals[5-11] has been reported, dynamic NMR studies in these phases are not extensive. Liquid crystals have bulk mechanical properties similar to those of liquids, but their molecules are subject to local ordering forces due to strong intermolecular interactions. The local ordering of liquid crystals has a pronounced effect on the motional behavior of solute molecules, and in some cases it may also affect their conformational behavior. For example, we have found that the solvent-dependent conformational equilibria between the tetrahedral form and the planar form of some nickel(II) complexes shift completely to the planar side in liquid-crystal solutions.[12] Here we report the effect of liquid-crystal solvents on the barrier to hindered rotation about a C–N bond.

In previous dynamic NMR studies of liquid-crystal solutions, the solute molecules investigated often have high symmetry. For example, the valence isomerism of bullvalene,[6] the bond shift equilibrium of cyclooctatetraene,[7,10b] and the ring inversions of *s*-trioxane,[10a] cyclohexane,[11a] and *p*-dioxane[11b] in liquid crystals have been studied. The liquid-crystal solvents do not seem to have any pronounced effect on the dynamic behavior of these systems because the equilibrium processes involve little or no change in the bulk shapes of the solute molecules. On the other hand, if the transition state has a bulkier conformation or a different steric requirement, the ordering forces of the liquid-crystal solvent may have a considerable effect on the dynamic and/or equilibrium process.

The use of proton NMR to study dynamic processes in liquid crystal solutions has been limited to very simple or highly symmetric compounds, because extensive dipolar couplings make the proton spectra very complex and difficult to interpret.[8,10] To the best of out knowledge, the only example of using dynamic NMR to study hindered rotation about a C–N bond in a liquid-crystal solution is an *estimation* of the exchange rate from the proton NMR spectra of *N,N*-dimethylformamide.[7] The deuterium NMR spectra of deuterated solute molecules dissolved in liquid crystals are much easier to interpret because the dominant effect is the quadrupolar interaction.[6b,11] An additional advantage of deuterium NMR is that the large quadrupole splittings (usually several kHz) make it possible to study fast dynamic processes up to a rate of 10^6 s^{-1}.[6b,11] However, the synthesis of deuterated compounds of moderately complex structures is not always straightforward, and the types of molecules to be studied are often limited.

We have recently shown[13,14] that natural-abundance carbon-13 NMR in liquid-crystal solutions can be conveniently studied by

(1) Jackman, L. M., Cotton, F. A., Eds. "Dynamic NMR Spectroscopy"; Academic Press: New York, 1975.
(2) Mann, B. E. *Prog. NMR Spectrosc.* **1977**, *11*, 95.
(3) (a) Ross, B. D.; True, N. S. *J. Am. Chem. Soc.* **1983**, *105*, 1382. (b) Ross, B. D.; True, N. S. *J. Am. Chem. Soc.* **1983**, *105*, 4871. (c) Spring, C. A.; True, N. S. *J. Am. Chem. Soc.* **1983**, *105*, 7231. (d) Ross, B. D.; True, N. S.; Decker, D. L. *J. Phys. Chem.* **1983**, *87*, 89. (e) Chauvel, J. P., Jr.; True, N. S. *J. Phys. Chem.* **1983**, *87*, 1622. (f) Chauvel, J. P., Jr.; Conboy, C. B.; Chew, W. M.; Matson, G. B.; Spring, C. A.; Ross, B. D.; True, N. S. *J. Chem. Phys.* **1984**, *80*, 1469.
(4) Lyerla, J. R.; Yannoni, C. S.; Fyfe, C. A. *Acc. Chem. Res.* **1982**, *15*, 208.
(5) Emsley, J. W.; Lindon, J. C. "NMR Spectroscopy Using Liquid Crystal Solvents"; Pergamon Press: Oxford, 1975.
(6) (a) Yannoni, C. S. *J. Am. Chem. Soc.* **1970**, *92*, 5237. (b) Poupko, R.; Zimmermann, H.; Luz, Z. *J. Am. Chem. Soc.* **1984**, *106*, 5391.
(7) Anderson, J. M.; Lee, A. C.-F. *J. Magn. Reson.* **1970**, *3*, 427.
(8) Courtieu, J.; Grunelle, Y.; Gonord, P.; Kan, S. K. *Org. Magn. Reson.* **1974**, *6*, 151.
(9) Luz, Z.; Meiboom, S. *J. Chem. Phys.* **1973**, *59*, 1077.
(10) (a) Poupko, R.; Luz, Z. *J. Chem. Phys.* **1981**, *75*, 1675. (b) Moseley, M. E.; Poupko, R.; Luz, Z.; *J. Magn. Reson.* **1982**, *48*, 354. (c) Luz, Z. *Isr. J. Chem.* **1983**, *23*, 305.
(11) (a) Luz, Z.; Naor, R.; Meirovitch, E. *J. Chem. Phys.* **1981**, *74*, 6624. (b) Poupko, R.; Luz, Z. *Mol. Phys.* **1982**, *76*, 5662. (c) Poupko, R.; Luz, Z. *Mol. Phys.* **1982**, *46*, 891.
(12) Fung, B. M., to be published.
(13) Fung, B. M. *J. Magn. Reson.* **1983**, *55*, 475.
(14) Fung, B. M. *J. Am. Chem. Soc.* **1983**, *105*, 5713.

0002-7863/84/1506-7301$01.50/0

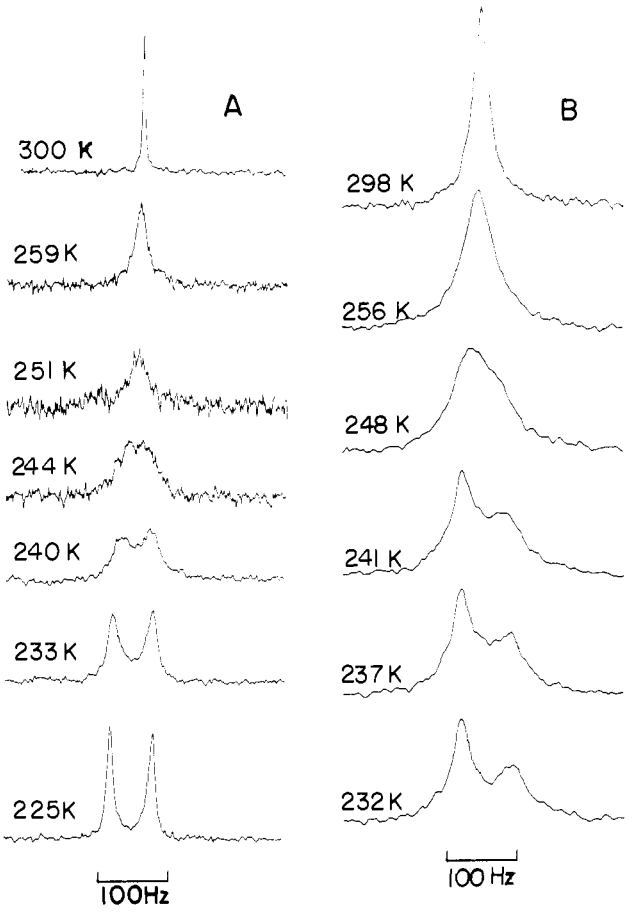


Figure 1. Proton-decoupled carbon-13 NMR spectra of the methyl group of 4-(dimethylamino)pyramidine at 75.43 MHz and different temperatures: (A) in CD_2Cl_2; (B) in liquid-crystal ZLI 2142. The spectra above 260 K are shown for comparison only and are not used in the calculation.

using a spin-echo sequence to reduce solvent interference and phase-alternated broad-band decoupling to increase the efficiency of proton decoupling. The application of these techniques makes it feasible to use dynamic carbon-13 NMR to study moderately complex solute molecules in liquid-crystal solutions. For the initial study of this subject, we have investigated the hindered rotation of the dimethylamino group in 4-(dimethylamino)pyrimidine. The reasons for choosing this system will be discussed below.

Experimental Section

4-(Dimethylamino)pyrimidine was synthesized according to a procedure in the literature.[15] CD_2Cl_2 was obtained from Stohler Isotopes, Waltham, MA. The liquid-crystal solvent (ZLI 2142) was obtained from EM Chemicals, Hawthorne, NY.

All carbon-13 NMR measurements were performed on a Varian XL-300 spectrometer with a 5-mm probe. In taking the spectra of the liquid-crystal solutions, a spin-echo sequence ($90°-\tau-180°-2\tau-180°-\tau-$acquisition) was used in order to reduce the solvent interference.[13,14] The value of τ was 80 μs. This is much shorter than the shortest lifetime for a discrete rotational state (calculated $\tau = 1/k = 732$ μs) and the spin-echo sequence is not expected to affect the line shape. This point was confirmed by using other values of τ in the experiment. For nuclear Overhauser enhancement and decoupling, proton irradiation was turned on before the 90° pulse and during acquisition, with a duty cycle of 40% to reduce radio frequency heating. The radio frequency field strength was $\gamma B_2/2\pi = 12$ kHz. A phase-alternated broad-band decoupling sequence specially developed for liquid crystals (ALPHA-26)[16] was used to increase the efficiency of decoupling. The temperature of the probe was calibrated with a methanol sample under conditions identical with those at which the spectra were obtained (same radio frequency power and duty cycle). The decoupler coil was used as a receiver coil for taking the proton spectra of the methanol sample.

(15) Brown, D. J.; Short, L. N. *J. Chem. Soc.* **1953**, 331.
(16) Fung, B. M.; Mui, D. S. L.; Bonnell, I. R.; Enwall, E. L. *J. Magn. Reson.* **1984**, *58*, 254.

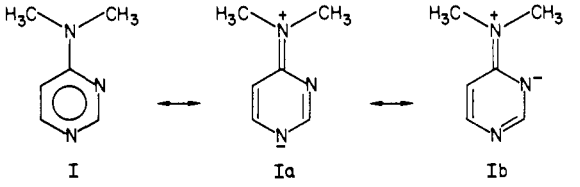
Table I. Thermodynamic Parameters for Hindered Internal Rotation of the Dimethylamino Group in 4-(Dimethylamino)pyrimidine

	$\Delta H^{\ddagger}$, kJ mol^{-1}	$\Delta S^{\ddagger}$, J mol^{-1} K^{-1}	$\Delta G^{\ddagger}_{250}$, kJ mol^{-1}
CD_3OD[a]	52 ± 1	−8 ± 4	54 ± 1
$(CH_3)_3CNH_2$[b]	49 ± 2	+46 ± 6	38 ± 3
$CHCl_3$[b]	45 ± 2	−21 ± 4	50 ± 2
CD_2Cl_2	35 ± 1	−39 ± 3	50 ± 1
ZLI 2142	53 ± 1	20 ± 6	48 ± 2

[a] Reference 21. [b] Reference 20.

Results

The methyl part of the carbon-13 NMR spectra of 4-(dimethylamino)pyramidine (I) in CD_2Cl_2 and in the liquid-crystal solvent ZLI 2142 at different temperatures are shown in Figure 1, parts A and B, respectively. In the liquid-crystal solution at



I Ia Ib

low temperature, the two methyl peaks have different widths. The upfield peak is broader than the peak at lower field. This is likely due to a small dipolar interaction between the 3-nitrogen and the methyl carbon that is cis to it. The dipolar coupling between the 3-nitrogen and the *trans*-methyl carbon and that between the 1-nitrogen and the two methyl carbons are likely to be negligible because the distances between them are large and the gyromagnetic ratios of the nuclei are small. The dipolar couplings between the methyl carbons and the directly bonded nitrogen seem to be very small due to rapid quadrupole relaxation or accidentally small ordering factors, or both. This point was affirmed by examining the spectra of a similar compound, *N,N*-dimethylaniline (II), in the same solvent. The line width of the methyl carbons in II at 258 K was 24 Hz, which is comparable to those of other compounds without nitrogen.

In order to obtain the rate of the hindered rotation, we analyzed each spectrum by fitting the amplitude as a function of frequency to an appropriate rate equation by a non-linear least-squares program. This method (total line-shape analysis) is the best procedure of obtaining the rate from dynamic NMR spectra.[17] The rate equation used for analyzing the spectra in CD_2Cl_2 is the standard Gutowsky–Holm[18] equation. The two methyl peaks in the spectra of the liquid-crystal solution have different widths, and the equation given by Williams and Brown[19] was used for the analysis.

In the calculation of the exchange rate, it is necessary to know the line width of the peaks in the absence of exchange. For the spectra in CD_2Cl_2, we use the generally adopted assumption that the line width of the methyl groups at any temperature is the same as the average of those of other carbon atoms in the same molecule at the same temperature.[1] For the spectra in the liquid-crystal solution, we assume that the line width of the low-field peak in I is the same as the line width of the methyl carbons in II in the same solvent under identical conditions. In view of the similarity in the structure of I and II, this assumption appears to be well justified. The width of the high-field peak was treated as a variable in the low-temperature spectra, restricting the intensities of the two peaks to be the same in the least-squares calculation. At higher temperatures at which the two peaks merge, the width of the low-field peak was obtained by extrapolating the low-temperature data, using the temperature dependence of the line width of II.

The results of the total line-shape analysis are presented in Figure 2 as an Eyring plot. The thermodynamic parameters of

(17) Binsch, G., in ref 1, p 45.
(18) Gutowsky, H. S.; Holm, C. H. *J. Chem. Phys.* **1956**, *25*, 1228.
(19) Williams, K. C.; Brown, T. L. *J. Am. Chem. Soc.* **1966**, *88*, 4134.

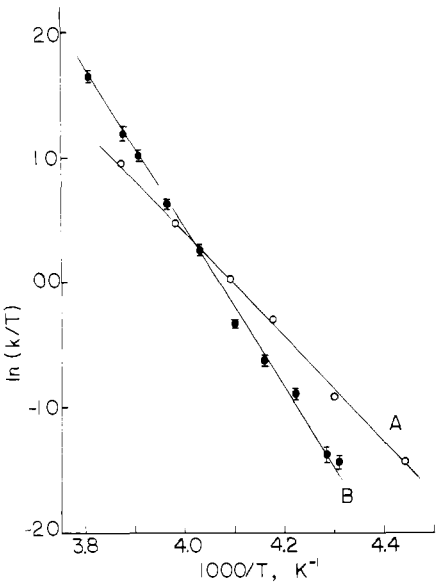
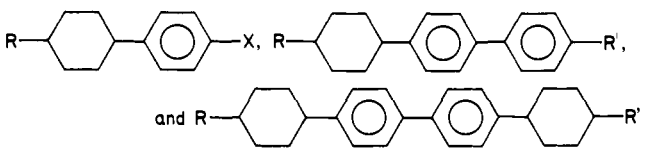


Figure 2. Erying plots for hindered rotation of the dimethylamino group in 4-(dimethylamino)pyrimidine: (A) in CD_2Cl_2 (the errors are within the limits of the circles); (B) in ZLI 2142. The lines are drawn from weighted linear least-squares calculations.

the activation process of the hindered rotation are given in Table I, together with data in several other solvents.[20,21]

Discussion

The data in Table I show that the barrier to the restricted rotation of the dimethylamino group in I, $\Delta H^{\ddagger}$, has a considerable dependence on the solvent. The ground state of I is probably planar or very close to planar. The charge-separated forms Ia and Ib would have major contributions to the ground state. Polar solvents, especially those which can act as hydrogen donors, are expected to stabilize the charge-separated forms, causing the barrier to rotation to increase. This was observed for *N,N*-dimethylacetamide[22] and *N,N*-dimethylbenzamide.[23] In the present case, $\Delta H^{\ddagger}$ for the hindered rotation of the dimethylamino group in I in different solvents indeed decreases in the order of $CD_3OD > (CH_3)_3CNH_2 > CHCl_3 > CD_2Cl_2$ (Table I).[20,21] The liquid-crystal solvent ZLI 2142 is a mixture of *trans*-4-alkylcyclohexyl compounds having the structures



where R and R′ are alkyl groups and X is a cyano or an alkoxyl group. These compounds do not act as hydrogen donors, and their overall polarities are quite small. However, $\Delta H^{\ddagger}$ for the hindered rotation of the dimethylamino group of I in ZLI 2142 is 50% higher than that in CD_2Cl_2 and about the same as that in CD_3OD.

The relatively large positive entropy of activation ($\Delta S^{\ddagger}$) for I in $(CH_3)_3CNH_2$ and ZLI 2142 is rather surprising. One possible reason for a positive $\Delta S^{\ddagger}$ is due to the contribution of the internal rotation of the methyl groups to the partition function.[3e,f] Because of a reduction the *intra*molecular steric interaction in the nonplanar transition state, the barrier to the internal rotation of the methyl group in this conformation is expected to be lower than that in the ground state. As a result, the partition function of the transition state can be much larger than that of the ground state and lead to a positive $\Delta S^{\ddagger}$. However, we do not understand why this factor might be important in the solvents $(CH_3)_3CNH_2$ and ZLI 2142 but not important in CD_3OD, $CHCl_3$, and CD_2Cl_2. We note only that the first two solvents in which $\Delta S^{\ddagger}$ of I is positive have more complex molecular structures. An alternative explanation is that the positive values of $\Delta S^{\ddagger}$ simply mean that the rotation of the dimethylamino group proceeds through a less ordered state in these solvents which have complex molecular structures. Finally, the most plausible explanation of the positive value of $\Delta S^{\ddagger}$ is the following. In condensed phases, the activation parameters are not determined by the solute molecules alone; the contribution of the surrounding solvent molecules must also be taken into account. In a liquid-crystal solution, the solvent molecules surrounding a bulky transition state of the solute are less well ordered. This loss in solvent ordering may be the major contributing factor to the positive value of $\Delta S^{\ddagger}$.

Many investigators have pointed out that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ obtained from an Arrhenius plot or an Eyring plot of the NMR results are subject to more experimental error than $\Delta G^{\ddagger}$.[23-28] This is especially true if results obtained by different methods of data analysis are compared. It has also been shown that ΔH° and ΔS°, or $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, are influenced more by solvent effects[29] than are ΔG or $\Delta G^{\ddagger}$. It is argued that the latter is not very sensitive to intermolecular interations,[29] with possible exception of strong hydrogen bonding which affects the electron distribution of the solute molecule. In other words, $\Delta G^{\ddagger}$ is a good measure of the intramolecular contribution to the barrier to rotation but $\Delta H^{\ddagger}$ may be more affected by intermolecular interaction.[28] The values of $\Delta G^{\ddagger}$ of I in $CHCl_3$, CD_2Cl_2, and ZLI 2142 are essentially the same within experimental error, which is consistent with the argument that $\Delta G^{\ddagger}$ is a good criterion for the intramolecular contribution to the barrier to rotation. It is to be noted that the comparison of $\Delta G^{\ddagger}$ at the coalescence temperature, as practiced by some authors, is much less preferable than the comparison at a given temperature, because the former is dependent upon the resonance frequency.

Another point to be discussed in relation to $\Delta G^{\ddagger}$ is the following. In analogy to amides, it is likely that the transition state of I would have a larger effective molecular volume than the ground state.[3d] Since

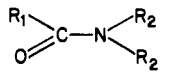
$$(\partial \Delta G^{\ddagger}/\partial P)_T = \Delta V^{\ddagger} \quad (1)$$

one would expect that $\Delta G^{\ddagger}$ increases as the internal pressure of the system increases. This was indeed observed for *N,N*-dimethyltrifluoroacetamide when the data in gaseous and liquid phases are compared.[3d] When the medium changes from liquid to liquid crystal, the solute molecules are partially ordered. However, the ordering force is anisotropic and it may not correspond to a simple increase in internal pressure. The fact that $\Delta G^{\ddagger}$'s are more or less the same in different solvents (except $(CH_3)_3CNH_2$; Table I) indicates that the "internal pressure" of a liquid-crystal solvent is not substantially different from that of an ordinary liquid.

Since $\Delta H^{\ddagger}$ is a physically accurate description of the rotational barrier and reflects the effect of intermolecular interaction,[28,29] we conclude that the data in Table I indicate that I has a much higher rotational barrier in ZLI 2142 than in CD_2Cl_2. It is true that $\Delta H^{\ddagger}$ is more subject to experimental errors than $\Delta G^{\ddagger}$ is, and a comparison of data obtained from different laboratories must be cautioned.[23-28] However, the data of I in CD_2Cl_2 and ZLI 2142 were both obtained in our laboratory, and the method of data analysis is the same. Therefore, the calculated values of $\Delta H^{\ddagger}$ would be affected by the same kind of possible systematic errors,

(20) Katritzky, A. R.; Tiddy, G. J. T. *Org. Magn. Reson.* **1969**, *1*, 57.
(21) Riand, J.; Chenon, M.-T.; Lumbroso-Bader, J. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1248.
(22) Ando, I.; Jinno, N.; Nishioka, A. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2639.
(23) Jackman, L. M.; Kavanagh, T. E.; Haddon, R. C. *Org. Magn. Reson.* **1969**, *1*, 109.
(24) Drakenberg, T.; Dahlquist, K.-I.; Forsen, S. *Acta Chem. Scand.* **1970**, *24*, 694.
(25) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 219.
(26) Shoup, R. R.; Becker, E. D. *J. Phys. Chem.* **1972**, *76*, 71.
(27) Liljefors, T. *Org. Magn. Reson.* **1974**, *6*, 144.
(28) Piccinni-Lepardi, C.; Fabre, O.; Zimmermann, D.; Reisse, J. *Can. J. Chem.* **1977**, *55*, 2679.
(29) Laidler, K. J. *Trans. Faraday Soc.* **1959**, *55*, 1725.

and a comparison of the data in at least these two solvents should be justified. The 50% difference in $\Delta H^{\ddagger}$ far exceeds the experimental error and is likely to reflect a genuine difference in the solvent effect. The most likely reason for the unusually high barrier to rotation of I in the liquid-crystal solution may be due to the ordering forces of the solvent rather than electronic forces. In its transition state, the plane of the dimethylamino group is perpendicular to that of the pyrimidine ring. The solute molecule in this conformation would disturb the ordering of the solvent molecules in its vicinity more than the ground state would. Therefore, the strong ordering forces of the liquid-crystal solvent would not favor the transition state and would cause the rotational barrier of I to increase. Although the explanation is a conjecture without sophisticated statistical calculations as a proof, it seems to be a reasonable one from the point of view that in liquid-crystal solutions planar molecules usually have larger ordering factors than globular molecules. It is for this reason that we chose to start our investigation on hindered rotation in liquid crystals using compound I rather than the classical examples of amides. If R_1



III

in III is small (e.g., H or CH_3), the transition state in which the R_2—N—R_2 plane is perpendicular to the R_1—C=O plane may not perturb the ordering of the liquid-crystal solvent much more than the planar ground state does. On the other hand, if R_1 is large (e.g., phenyl), the ground state may be quite bulky and even non-planar and would differ little from the transition state in perturbing the solvent ordering.

In the discussion presented above, we have treated the system as having a single transition state in which the dimethylamino group is perpendicular to the ring in I. Actually, in any condensed medium, the existence of several transition states with the solute interacting differently with the solvent should be considered. The values of $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ are ensemble averages.[30] In other words, the neighboring packing effect of the liquids[31] would affect the activation parameters in addition to the polarity and hydrogen-bonding character of the solvent. This effect is quite different in liquid crystals and is highly anistropic. The details of the packing effect of liquid crystals on molecular conformations and dynamics remain to be investigated. Our observation of an unusually large value of $\Delta H^{\ddagger}$ for the hindered rotation of I in liquid-crystal solutions is an interesting experimental result, and similar studies on other systems should be pursued.

In summary, we have demonstrated for the first time that natural abundance carbon-13 NMR can be used to study dynamic processes in liquid-crystal solutions. It was observed that $\Delta H^{\ddagger}$ of the hindered rotation of the dimethylamino group in 4-(dimethylamino)pyrimidine in the liquid-crystal solvent ZLI 2142 is 50% higher than that in CD_2Cl_2. The most likely reason for this is that the non-planar transition state perturbs the ordering of the liquid-crystal solvent and becomes less favorable so that the barrier to rotation increases over that in CD_2Cl_2.

Acknowledgment. This work was in part supported by the National Science Foundation under Grant No. CHE-8318840. The Varian XL-300 spectrometer was purchased through a grant from the National Science Foundation (CHE-8113507). We acknowledge Drs. Nancy S. True and Alex Pines for helpful discussions.

Registry No. 4-(Dimethylamino)pyrimidine, 31401-45-3; ZLI-2142, 87004-30-6.

(30) Stillinger, F. H.; Weber, T. A. *Science (Washington, D.C.)* **1984**, *225*, 983.

(31) (a) Chandler, D.; Pratt, L. R. *J. Chem. Phys.* **1976**, *65*, 2925. (b) Pratt, L. R.; Chandler, D. *J. Chem. Phys.* **1978**, *68*, 4202. (c) Hsu, C. S.; Pratt, L. R.; Chandler, D. *J. Chem. Phys.* **1978**, *68*, 4213.

On-Line Peptide Sequencing by Enzymatic Hydrolysis, High Performance Liquid Chromatography, and Thermospray Mass Spectrometry

H. Y. Kim, D. Pilosof, D. F. Dyckes, and M. L. Vestal*

Contribution from the Department of Chemistry, University of Houston, Houston, Texas 77004.
Received June 15, 1984

Abstract: On-line procedures for peptide sequencing using columns containing immobilized enzymes, carboxypeptidase Y (CPY) and trypsin, and an LC column, coupled to a thermospray mass spectrometer, are described. After on-line hydrolysis by immobilized CPY, both the amino acids, sequentially cleaved from the C terminus of the peptides, and the residual peptides are carried into the thermospray ion source by a continuous buffer flow and detected as molecular ion species. Use of both positive and negative ion modes gives additional flexibility to detect these molecules. For large peptides, on-line proteolytic hydrolysis into peptide fragments using immobilized trypsin can be employed along with separation by an LC column before hydrolysis by immobilized CPY. Immobilized trypsin alone can be used with LC separation for peptide mapping. These procedures provide rapid and convenient sequencing methods with high sensitivity. Total experimental times are usually less than 30 s for small peptides and detection limits extend to the low picomolar range.

Introduction

"Soft ionization" mass spectrometric techniques for sequencing peptides and for detection of biological peptides have found increasing use recently. Unlike conventional mass spectrometry, these techniques permit the analysis of thermally labile and/or nonvolatile molecules, usually without derivatization. Of the several "soft ionization" techniques, plasma desorption,[1] field desorption (FD),[2-5] fast atom bombardment (FAB),[6-10] and thermospray liquid chromatography/mass spectrometry (LC/

(1) Hakansson, P.; Kamensky, I.; Sundqvist, B.; Fohlam, J.; Peterson, P.; McNeal, C. J.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1982**, *104*, 2948.

(2) Beckey, H. D.; Hoffmann, G.; Maurer, K. M.; Winkler, H. V. *Int. J. Mass Spectrom. Ion Phys.* **1969**, *2*, 500.

(3) Winkler, H. V.; Beckey, H. D. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 391.

(4) Matsuo, T.; Katakuse, I.; Matsuda, M. *Adv. Mass Spectrom.* **1980**, *8*, 990.

(5) Matsuo, T.; Matsuda, H.; Katakuse, I.; Wada, Y.; Fujita, T.; Hayashi, A. *Biomed. Mass Spectrom.* **1981**, *8*, 25.

0002-7863/84/1506-7304$01.50/0