extended from hydrocarbons to oxygen-containing compounds. The general structure trends of experimental ^{13}C shifts in alcohols, aldehydes, ketones, and carboxylic acids are reproduced satisfactorily by the theory, which tends to exaggerate the degree of electronic polarization generated by oxygen substitution into a hydrocarbon framework. The chemical shift anisotropies computed by the theory are in reasonable agreement with the sparse experimental data available.

The theory accounts satisfactorily for the experimentally demonstrated reduction of shielding of a carbonyl carbon due to hydrogen bonding to the carbonyl oxygen. Carbonyl-carbonyl interactions consistent with known dilution shifts can be rationalized by the calculations. The role of intermolecular interactions with the formate ion can be qualitatively accounted for in calculations in which these interactions are modelled.

Comparison of computed shifts and electron density elements reveals correlations only within a very narrow range of cases, e.g., within the set of four carbons of phenol or phenoxide.

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Conformational Analysis by Nuclear Magnetic Resonance. Nitrogen-15 and Carbon-13 Spectra of Lactams[1a]

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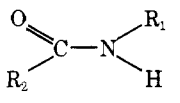
Abstract: The 5-, 6-, 7-, 8-, 9-, and 13-numbered lactams have been studied as simple models for the amide linkage. ^{15}N and ^{13}C spectra have been determined in various solvents and assignments made for the majority of the resonance lines. The ^{15}N and ^{13}C carbonyl chemical shifts show no apparent relationship to the amide ring size or to each other. The 9-membered lactam, azacyclononanone, exists as an equilibrium mixture of cis and trans conformers, the relative amounts of which depend on solvent and temperature. Carbon-13 NMR spectra of pure *trans*-azacyclononanone have been obtained from solutions of the solid at −45 °C in $CDCl_3$. At −35 °C two trans conformers are seen; at room temperature approximately equal quantities of the cis and trans isomers are present in ethanol, dimethyl sulfoxide, and tetrachloroethylene, whereas the cis conformer predominates in chloroform. Further increases in temperature cause rapid (on the NMR time scale) equilibrium between the two isomers such that a single averaged spectrum is seen at 120 °C. From the coalescence temperature of the carbonyl peaks the free energy of activation for the interconversion of *cis*- and *trans*-azacyclononanone is found to be 17 kcal/mol. This value is discussed in terms of the twofold periodic potential function for the distortion of amides from a coplanar conformation.

Lactams afford an excellent model system for a study of the amide group constrained in various conformations, such as might be found in cyclic peptides, large polypeptides, and proteins. We have made a systematic investigation of the ^{15}N and ^{13}C spectra of a series of closely related lactams to determine the relationship between ring size and conformations and the chemical shifts of ^{15}N and ^{13}C.

In open-chain N-monosubstituted amides, the trans conformation, **1**, is strongly preferred over the cis, **2**, as shown by dipole-moment measurements, as well as infrared, ultraviolet, and Raman spectroscopy.[2] Rotation about the C-N bond at room temperature is slow, the barrier to rotation being about 20 kcal mol^{-1} as determined by numerous NMR measurements[3] and, as first suggested by Pauling,[4] is ascribed to the partial double-bond character of the amide C–N bond.



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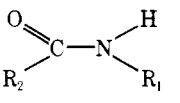
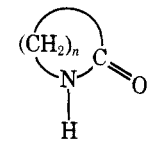


2

In small-ring lactams, **3–6**, the amide group must, of necessity, adopt the cis conformation **2**. As the large rings approach the conformations of straight chain amides, they are, of course, found in the trans conformation **1**. The cross-over point, first detected by Huisgen[5] using infrared spectroscopy,



3, $n=3$ 7a(cis), $n=7$
4, $n=4$ 7b(trans), $n=7$
5, $n=5$ 8, $n=11$
6, $n=6$

is at the nine-membered ring and it is on this compound, 1-aza-2-cyclononanone (7) that we have focused much of our attention in the present study.

Huisgen[5] showed the five- to eight-membered lactams have the cis conformation and the ten-membered and larger lactams have the trans conformation. The nine-membered lactam was reported as trans in the crystalline state, but in dilute chloroform solution as an equilibrium mixture of "a little trans and considerable cis". In a more recent infrared study, Swenson and Chen[6] found four N-H stretching bands and attributed these to two cis and two trans conformations for the nine-membered lactam, **7**. Their study indicated that temperature and solvent had little effect on the percentages of the conformers. These workers were apparently unaware of the work of Hallam and Jones[7] who had previously carried out a similar investigation of the seven-, nine-, and ten-membered lactams in 24 different solvents. These workers also noted four N-H stretching bands for **7** in nonpolar solvents which they attributed to two cis and two skew conformations. In the most polar solvent (dimethyl sulfoxide, DMSO) the quartet of bands merged into one broad band which led Hallam and Jones to conclude that in this solvent only one solvated conformer predominates. Most recently Svoboda and co-workers[8] in still another infrared study of the nine-, ten-, and eleven-membered lactams have also noted four N-H stretching vibrations for **7** in dilute carbon tetrachloride solutions and have determined the percentage of each conformer (8 and 21% for the trans (skew) conformers and 24 and 47% for each of the two cis conformers). The trans conformation of the solid 9-membered lactam is firmly established by the x-ray crystallographic analysis of Winkler and Dunitz.[9]

Experimental Section

Lactams were commercially available (Aldrich) and were used without further purification except for 1-aza-2-cyclononanone (**7**) which was slowly crystallized from benzene–hexane. The **^{15}N spectra** were taken with a Bruker WH-180 spectrometer at 18.25 MHz employing 25 ml of 2 M solutions in 2.5-cm o.d. spinning-sample tubes containing a 5-mm concentric tube filled with a 1.0 M solution of enriched $H^{15}NO_3$ in D_2O which function as reference and lock. Samples were proton noise decoupled and exhibited full negative nuclear Overhauser enhancement. A 20-μs pulse and 2-s repetition rate was employed (80 μs = 90° pulse); quite good signal-to-noise ratios were obtained after 500 pulses. With 4 W of decoupling power, the sample temperature was about 30 °C depending on the solvent. Chemical shifts are reported in ppm upfield from $H^{15}NO_3$ and are considered accurate to ±0.2 ppm. The ^{13}C spectra were obtained with a Varian XL-100 spectrometer in 12-mm diameter tubes containing a 4-mm capillary filled with D_2O or DMSO-d_6 for the deuterium lock or were run in $CDCl_3$ solution which provided the lock. Spectra were proton noise decoupled. Chemical shifts are reported in ppm downfield from TMS and are considered accurate to ±0.1 ppm. The temperature at coalescence in the variable temperature spectrum of **7** was measured with a small thermometer in place of the 4-mm capillary and is probably accurate to ±0.5 °C over the period of time necessary to acquire the spectrum. The temperatures shown on Figure 1 are only approximate, being read from the variable temperature control device of the XL-100.

Results and Discussion

^{15}N Spectra. The ^{15}N chemical shifts of **3–8** and the straight-chain amide **9** are given in Table I. Like the basicity

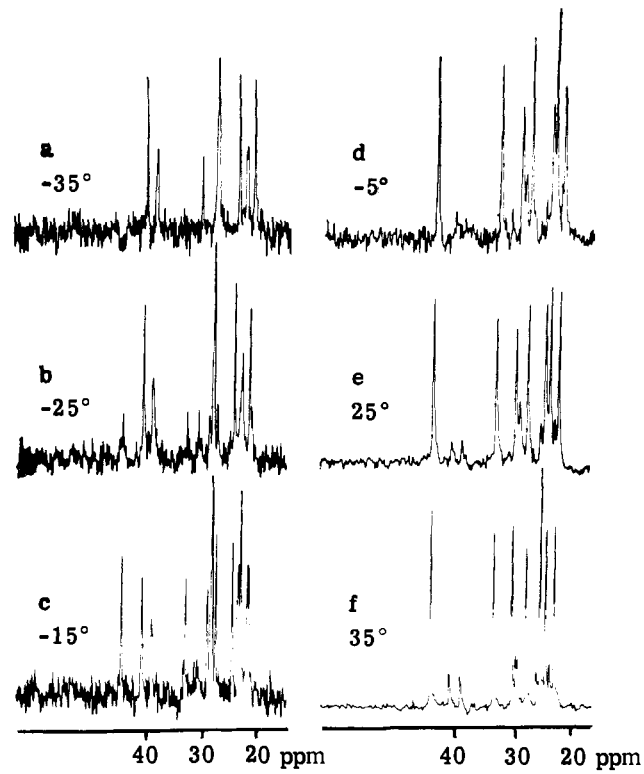
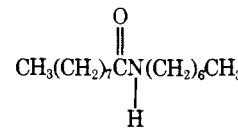


Figure 1. The upfield region of the carbon-13 spectrum of azacyclononanone at various temperatures in $CDCl_3$.



9

constants,[5] the ^{15}N shifts show no apparent relationship to the amide ring size. It is noteworthy that the cis and trans forms of **7** show a 2–3-ppm difference in their chemical shifts, but it would be difficult, a priori, to assign the two peaks to the cis and trans conformers. We have made the assignments on the basis of the relative peak sizes of the two isomers in chloroform where the cis isomer predominates (see below).

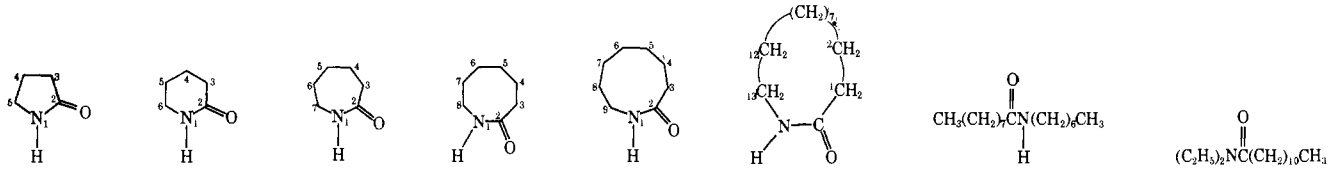
We have measured the ^{15}N spin-lattice relaxation times of the two isomers of **7** in 95% ethanol by the progressive saturation method.[10] They are virtually identical: 7.6 s for the cis isomer and 8.0 s for the trans. The NOE's for both isomers are also virtually identical indicating that the nitrogen atom of the cis isomer does not participate in any special relaxation mechanisms by virtue of a particularly crowded conformation.

The chemical shifts of the lactams show no regular or predictable solvent shifts. This behavior is similar to *N*-methylacetamide[11,12,13] which has ^{15}N shifts varying from 269 to 275.5 ppm upfield from $H^{15}NO_3$ depending on the solvent and concentration.

^{13}C Spectra. Johnson and Jankowski[14] assigned ^{13}C resonances to specific carbons in the five- and seven-membered lactams, **3** and **5**, and we agree with these assignments. The shifts in Table I are within ± 0.2 ppm of those reported except for the carbonyl peaks which we find 0.4 ppm further downfield.

We have assigned peaks in **4**, **6**, **7**, and **8** by analogy with the reported[14] assignments for **3**, **5**, and **10** (Table I). The most downfield peak is obviously the carbonyl. It will be noted that there is not a simple monotonic change in the carbonyl chemical shift with ring size. In this respect, these lactams are just as puzzling as the corresponding cyclic ketones. The next most

Table I. Chemical Shifts of Lactams and Amides



3 4 5 6 7 8 9 10

Atom Ring Size	3	4	5	6	7-cis	7-trans	8	9	10[a]
				^{15}N Chemical Shifts (ppm upfield from HNO_3)					
N[b]	259.3	259.7	256.4	256.3	253.4	256.4	255.5	256.5	
N[c]	260.1	261.3	257.4	257.5	254.4	256.5	256.0		
N[d]					251.5	254.2		254.6	
				^{13}C Chemical Shifts (ppm downfield from TMS)[d]					
C=O	179.8	173.1	179.9	178.3	177.5	175.9	174.1		
α to C=O	30.4	31.5	36.9	32.3[h]	32.2	37.4	36:6		33.1
β to C=O	20.8[e]	20.9	23.4	24.7	22.4	23.2	24.1		25.5
γ to C=O		22.3[f]	30.7[g]						29.5
γ to N			30.7[g,h]						
β to N	20.8[e]	22.3[f]	29.8[h]	25.8	23.7	24.7	24.7		
α to N	42.5	42.0	42.7	41.8	42.5	39.3	39.1		
Not assigned				32.1[h]	29.1	28.6	28.4		29.5
				28.1	27.1	28.2	26.9		
					24.5	25.3	26.4		
							26.0		
							25.3		
							24.7		

[a] Data and assignments from L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley-Interscience, New York, N.Y., 1972, p 473. [b] 2.0 M in $CHCl_3$. [c] 2.0 M in dimethyl sulfoxide. [d] 2.0 M in 95% ethanol. [e] Same carbon. [g] Same carbon. [h] Assignments could be reversed.

downfield peaks are those carbons α to N (range 39.1 to 42.7 ppm) followed by those α to the carbonyl (range 30.4 to 37.4 ppm). The most *upfield* peaks in these spectra we have assigned to the carbon β to N and the next most upfield to the carbon β to the carbonyl group. At the present time we are unable to make more definitive assignments for the other carbons, but it is obvious from the wide range of shifts displayed by these compounds that steric and conformational effects play a large part in determining these chemical shifts.

Variable Temperature Spectra of 7a and 7b. X-ray crystallography[9] shows that **7** exists exclusively as the trans conformer in the solid. If the barrier to trans → cis isomerization is sufficiently high it should be possible to dissolve crystalline **7** at low temperature and observe its ^{13}C spectrum before it has sufficient time to isomerize. The upfield region of the ^{13}C spectrum of **7** obtained by dissolving the solid in $CDCl_3$ which had previously been cooled to −45 °C is shown in Figure 1a. Seven well-resolved peaks are seen, as expected for the seven aliphatic carbons of the trans isomer along with a single carbonyl peak at 175.8 ppm. At −25 °C (Figure 1b), the seven peaks of the trans compound predominate but new peaks are beginning to show because of slow nonequilibrium formation of the cis configuration. The −15 °C spectrum shows about equal quantities of both isomers (and two carbonyl peaks of equal intensity) and by the time the −5 °C spectrum was run, the seven largest peaks are those of the cis compound which is obviously the thermodynamically stable isomer at this temperature. Further increases in temperature (Figures 1e and 1f) bring about a reappearance of the resonances of the trans isomer, this time in slow (on the NMR time scale) equilibrium with the cis isomer. Most of the chemical shifts of the trans compound are the same at high and low temperature, with the notable exception of the peak at about 32 ppm in Figure 1a which will be discussed later.

At 35 °C the populations of the cis and trans isomers of **7** are about equal in ethanol, dimethyl sulfoxide, and tetrachloroethylene whereas in chloroform the cis isomer is favored,

Table II. Carbon-13 Chemical Shifts of 1-Aza-2-cyclononanone (7) at 30 and 120 °C in $Cl_2C{=}CCl_2$

30 °C δ, ppm	Isomer	120 °C	Assignment
178.0	Cis	176.8	C=O
176.7	Trans		
44.1	Cis	42.6	α to N
40.8	Trans		
38.6	Trans	35.8	α to C=O
33.9	Cis		
31.1	Cis	30.8	
30.7	Trans		
30.0	Trans	29.3	
29.2	Cis		
27.4	Trans	27.0	
26.5	Cis		
25.7	Cis and trans	26.5	β to N
25.4	Trans	25.1	β to C=O
24.4	Cis		

as seen in Figure 1f. If a tetrachloroethylene solution of the nine-membered lactam is heated above room temperature, profound changes in the spectra occur as noted in Figure 2. In Table II are given the chemical shifts of the 15 (of a possible 16) well-resolved lines of **7** in tetrachloroethylene at room temperature. (This spectrum is not illustrated.) At 120 °C the spectrum consists of just eight lines (Figure 2h shows the upfield region) which must arise from the cis and trans isomers in rapid equilibrium. The chemical shifts of these eight peaks are the averages (with the exception of the peak at 26.5 ppm) of the chemical shifts of specific pairs of peaks in the room temperature spectrum as detailed in Table II.

It is of interest to determine the free energy of activation for the interconversion of the cis and trans isomers in this lactam. In tetrachloroethylene the populations of the isomers are approximately equal at room temperature. The chemical shifts of the two carbonyl peaks were measured as a function of temperature. At the coalescence temperature (64 °C) the

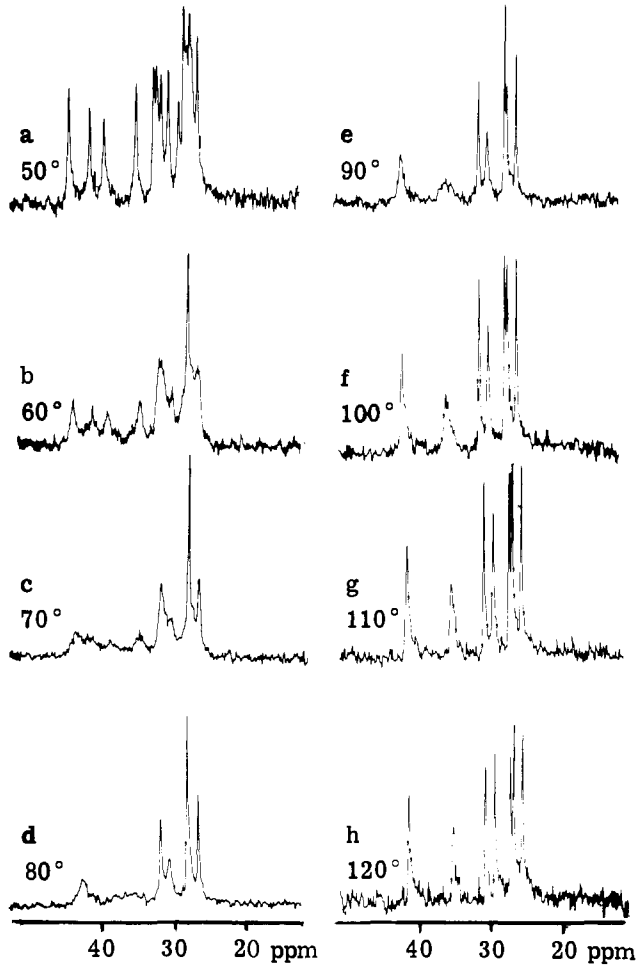


Figure 2. The upfield region of the carbon-13 spectrum of azacyclononanone at various temperatures in $Cl_2C{=}CCl_2$.

peaks are calculated by extrapolation to be 31 Hz apart which corresponds to an exchange rate, k_1, of 69 s^{-1} according to the equation

$$k_1 = \pi(\nu_A - \nu_B)/\sqrt{2} \qquad (1)$$

From absolute reaction rate theory we have

$$k_1 = \kappa(kT/h) \exp - \Delta G^{\ddagger}/RT \qquad (2)$$

in which κ is the transmission coefficient (assumed to be unity), k the Boltzmann constant, T the absolute temperature, and $\Delta G^{\ddagger}$ the free energy of activation, from which we calculate $\Delta G^{\ddagger}$ to be 17 kcal/mol.[15] This same value is found when dimethyl sulfoxide is employed as the solvent. Although E_a, the activation energy, which is best obtained by a complete lineshape analysis, and $\Delta G^{\ddagger}$ are not strictly comparable, we note that $\Delta G^{\ddagger}$ is lower than the generally accepted 20 kcal/mol E_a for rotation about the C–N bond of straight-chain amides. Steric interactions in this nine-membered ring enhanced by the rigidity of the planar amide linkage are reasonably expected to raise the ground-state energy of both the cis and trans conformers by comparison with straight-chain amides, and if this strain is relieved in the transition state, then the overall $\Delta G^{\ddagger}$ will be less than for a straight-chain amide.

Conformations of 1-Aza-2-cyclononanone (7). Infrared analysis of dilute CCl_4 solutions of **7** at room temperature have been interpreted as indicating two different cis and two different trans conformers.[8] In the lowest temperature ^{13}C spectra of **7** (Figure 1a), although there are seven aliphatic carbon peaks, they are by no means of equal intensity or line width. Peaks 2, 4, and 6 have greater line widths than peaks 1, 3, and 5 and we have, on occasion, partially resolved each of the

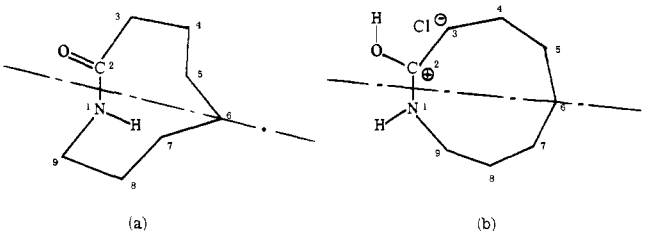


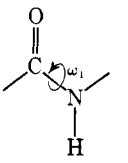
Figure 3. (a) Conformation of **7** determined by x-ray crystallography.[9] (b) Conformation of the hydrochloride of **7** as determined by x-ray crystallography.[9]

broader lines into a doublet. Also, as noted before, the peak at 32 ppm in the −35 °C spectrum (the lowest intensity peak) does not appear in the mixture of isomers recorded at −15 °C (Figure 1c). We conclude therefore that at low temperatures **7** does exist as two trans conformers which are interconverting slowly on the NMR time scale. It remains for low-temperature infrared analysis to determine if these are the same two trans conformers found at room temperature. As the temperature is raised from −15 to 25 °C, relatively minor changes in chemical shifts occur. In the 35 °C spectrum a new peak is resolved, but we can see no unequivocal NMR evidence for a mixture of four conformers as indicated by the infrared spectra. Furthermore the spectra of **7** in DMSO (like those in ethanol and tetrachloroethylene) which clearly shows peaks for both the cis and trans conformers is at variance with the conclusions of Hallam and Jones[7] who report that **7** in this solvent probably exists as one solvated conformer. Our work is also at variance with that of Swenson and Chen[6] who concluded temperature and solvent have little effect on the percentages of the conformers. We find the cis isomer predominates at 25 °C in $CHCl_3$ (Figure 1e), while in ethanol, DMSO, and tetrachloroethylene the two isomers are present in about equal amounts. The conformation of **7** in the crystal is shown in Figure 3a. The compound has a 4.9 Å periodicity which arises from association of the amide into infinite chains by N—H···O=C hydrogen bonding in the crystal. Undoubtedly one of the conformations detected in our −35 °C spectrum must resemble this closely. Winkler and Dunitz[9] also determined the crystal structure of the hydrochloride of **9** (Figure 3b) and found it to be a regular polygon much as shown in Figure 3b. Both of these ring skeletons have an approximate twofold axis passing through the midpoint of the N(1)–C(2) bond and C(6). To the extent that Figure 3b resembles the cis amide, it is tempting to ascribe the single sharp peak which is so prominent in Figures 2b–d to C(6) which acts as a pivot point while all the other atoms in the ring undergo motions which change their magnetic environments and lead to broad lines as the temperature rises.

One of the principal tenets of protein chemistry is that the amide bond is trans and coplanar in polypeptides and proteins. Only recently has the necessity for taking into consideration the possible nonplanarity of peptide bonds arisen.[16] Crystal structures of some dipeptides, cyclic peptides, and globular proteins show deviations from coplanarity of up to 16°.[17]

One of the principal conclusions reached by Winkler and Dunitz[9] in their x-ray study of azacyclononanone (**7**) was that the simple twofold periodic potential function does not lead to reasonable energies for nonplanar amide groups. This torsional function[19] with barrier height of 20 kcal/mol of the form was

$$V_\omega = \tfrac{1}{2}K_\omega(1 - \cos 2\omega)$$
$$K_\omega = 20 \text{ kcal/mol} \qquad (3)$$



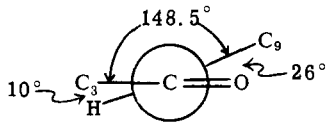


Figure 4. Conformation of the amide portion of **7-trans** in the solid state.[9]

thought to severely underestimate the degree of nonplanarity achievable at a given energy cost.

Pullman, on the other hand, has carried out ab initio self-consistent field molecular orbital calculations for the rotational barrier in formamide[17] and found that the computed total energy as a function of the torsional angle follows the theoretical curve (eq 3) extremely well, deviating by not more than 0.4 kcal/mol up to $\omega = 25°$.

Dunitz found the amide group in the trans isomer of **7** to have the conformation shown in Figure 4. If this is the conformation of the trans isomer in solution, it would appear from our work that this degree of distortion is achieved by the expenditure of approximately 3 kcal/mol in this compound. From eq 3 and a distortion of 26°, we calculate 3.8 kcal/mol. We conclude therefore in contrast to Dunitz and in agreement with Pullman that eq 3 probably does not err significantly in describing the energetics of the amide group. The form of this potential function is of great interest because of the important part it plays in theoretical calculations of polypeptide conformations.

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Proton Localization in Chemical Ionization Fragmentation[1]

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Abstract: The positional integrity of the ionizing proton in fragmentation reactions in chemical ionization mass spectrometry has been examined as a function of sample proton affinity and reaction temperature. In order to distinguish hydrogen atoms originating in the sample from protons transferred from reagent gas, a series of cyclohexyl-d_{11} compounds was used. It is found that hydrogen rearrangements may occur before fragmentation and the amount of scrambling increases with proton affinity of sample and with decreasing temperature. The ease of fragmentation of a series of cyclohexyl derivatives has been related to the proton affinity of the leaving group.

In studies of the chemical ionization mass spectra of deuterium-labeled compounds it has been found that the isotope labels may partially scramble with hydrogen atoms before fragmentation reactions occur.[3-5] Consequently, it has been suggested that the proton transferred in ionization need not be specifically localized in the molecule.[4] To investigate this further we have studied the effects of temperature and proton affinity on the randomization of the ionizing proton.

Cyclohexyl bromide, cyclohexanol, cyclohexyl mercaptan, and cyclohexylamine provide a range of proton affinities and all four compounds form $C_6H_{11}^+$ fragment ions by elimination of appropriate small neutral molecules under methane chemical ionization. In order to distinguish hydrogen atoms originating in the sample from protons transferred from methane reagent gas, the $C_6H_{11}^+$ fragment ions were compared to the corresponding $C_6D_{11}^+$ ions formed by the fully ring deuterated analogues of these compounds at reaction temperatures of 100, 150, and 200 °C.

$$c\text{-}C_6H_{11}X \xrightarrow{(H^+)} \underset{m/e\ 83}{C_6H_{11}^+} + HX$$

$$c\text{-}C_6D_{11}X \xrightarrow{(H^+)} \underset{m/e\ 94}{C_6D_{11}^+} + HX$$

$$X = Br, OH, SH, NH_2$$

Exchange of deuterium atoms out of the ring will be reflected in formation of $C_6D_{10}H^+$ or $C_6D_9H_2^+$ ions in addition to $C_6D_{11}^+$ ions.

Field[6] has predicted that the extent of fragmentation in which a small neutral molecule is eliminated in chemical ionization will be inversely proportional to the proton affinity of