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^{17}O and ^{1}H-^{15}N Heteronuclear Multiple Quantum Coherence (^{1}H-^{15}N HMQC) NMR of Linear Amides: Evidence of an Out-of-Plane (Torsion Angle) Deformation of the Amide Bond and Pyramidicity at the Amide Nitrogen

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Abstract: ^{17}O and ^{1}H-^{15}N heteronuclear multiple quantum coherence NMR studies of linear amides demonstrate the ready occurence of an out-of-plane (torsion angle) deformation of the amide bond and pyramidicity at the amide nitrogen for the sterically hindered trans-isomer of N-tert-butylformamide. Solvent accessibility, steric hindrance and, thus, torsion angle deformation is shown to be strongly solvent dependent.

INTRODUCTION

The amide group is a crucial structural element in peptides and proteins and its geometry is of prime importance in defining three dimensional structures of polypeptides and proteins [1-3]. Therefore, amides have been extensively investigated with a variety of spectroscopic techniques. The presence of N-alkyl substitution of amides, such as N-alkylformamides, is of particular interest because of the resulting increase in population of the cis- isomer and the restriction in conformational freedom[4-6]. In conformational studies of peptides and proteins, it is generally assumed that the peptide unit is rigidly planar due to its partial double bond [1-3]. However, several X-ray diffraction analyses of model amides, peptides and proteins have indicated that a non-planar deformation of an amide (peptide) group is quite common[7-10]. It is, therefore, not unreasonable that out-of plane (torsion angle deformation) of the amide bond has been extensively studied by a variety of spectroscopic techniques in solution[11-14]. However, the results obtained were quite controversial, and it is clear that there exists a need for new methodologies. In view of the strategic role of the oxygen and nitrogen atom in the amide bond, ^{17}O[15-17] and ^{15}N[18-20] NMR can be considered as obvious candidates. We demonstrate here, for the first time, that the combined use of ^{17}O and ^{1}H-^{15}N heteronuclear multiple quantum coherence NMR is a highly effective tool to investigate torsion angle deformation of the amide bond and pyramidicity at the amide nitrogen.

RESULTS AND DISCUSSION

^{17}O NMR Studies

^{17}O NMR has begun to receive considerable attention as a powerful method for detection and study of steric effects on molecular structure in organic systems, despite the low natural abundance (0.037%) and the quadrupolar properties of the ^{17}O nucleus[15-17]. Thus ^{17}O NMR chemical shifts have been found to be more sensitive to structural variation than those of ^{13}C, and relationships between $\delta(^{17}O)$ and torsion angle of oxygen functional groups, such as aryl ketones and aldehydes, aryl carboxylic esters, acids and amides, have been reported[16,21,22]. However, no reports have so far been published for cis and trans amides.

The cis isomer is present in N-methylformamide (NMF) to an extent of ~12% in CD_2Cl_2 and $CDCl_3$ solutions and increases to ~18% and 15% for N-ethylformamide (NEF) in CD_2Cl_2 and $CDCl_3$ solutions, respectively. The cis and trans forms (in percent of the total solute concentration) were obtained from the relative integrals of the respective resonances in the ^1H NMR spectra. For tert-butylformamide (TBF) the amount of the cis form is strongly solvent dependent. It is about 25% in CD_3CN and 45% and 48% in CD_2Cl_2 and $CDCl_3$ solutions, respectively. Unequivocal assignment of the trans isomer was achieved by 2D ^1H-^1H NOESY experiments which demonstrate the through space proximity of the C(O)H and NH protons. The significant increase in the population of the cis isomer of tert-butylformamide can be easily rationalized as the result of increasing steric effects between the tert-butyl group and the amide oxygen atom.

In CH_3CN the cis and trans ^{17}O amide resonances of N-methylformamide and N-ethylformamide were poorly resolved, even when using resolution enhancement techniques[23]. The situation is reversed with N-tert-butylformamide where a large chemical shift separation, Δ, is observed for the trans and cis amide resonances (Figure 1, Table I). The use of shielding differences has the advantage of providing an internal standard, although for ^{17}O most of the change seems to reflect the deshielding of the trans resonance. Once intermolecular self-association has been eliminated by concentration dependence, the only possible mechanism for the strong deshielding of the trans isomer of N-tert-butylformamide is an out-of-plane (torsion angle) deformation of the amide bond.

^{17}O NMR chemical shifts are usually discussed with respect to contributions from a diamagnetic and a paramagnetic term[24-26]. It can be assumed for good reasons that the diamagnetic term is independent of change in the chemical environment of the oxygen atom. Thus the shift differences are essentially induced by the paramagnetic term δ^P. The latter is usually evaluated following the average excitation energy approximation[27]

$$\delta^P \propto \langle r^{-3} \rangle_{2p} \frac{1}{\Delta E} \left[Q_{AA} + \sum_{B \neq A} Q_{AB} \right]$$

where the expression $\langle r^{-3} \rangle_{2p}$ is the average value of the inverse cube of the distance of the 2p electrons from the nucleus; ΔE is the lowest excitation energy in the electronic spectrum of the compound concerned, and Q_{AA} and Q_{BB} are defined in terms of the charge density and bond order matrix and, thus, represent a measure of multiple bonding to the nucleus being studied. Thus deshielding is expected for an out-of-plane deformation of the amide bond which leads to greater double bond character and reduced electron density on oxygen (Scheme 1, Structure I [28,29]). Further, decreasing charge density at the oxygen is expected to lead to a contraction of the 2p orbitals and thereby a decrease in shielding. This is exactly what is observed experimentally for trans-N-tert-butylformamide. It is therefore evident that steric interactions in the trans-N-tert-butylformamide are characterized by an out-of-plane

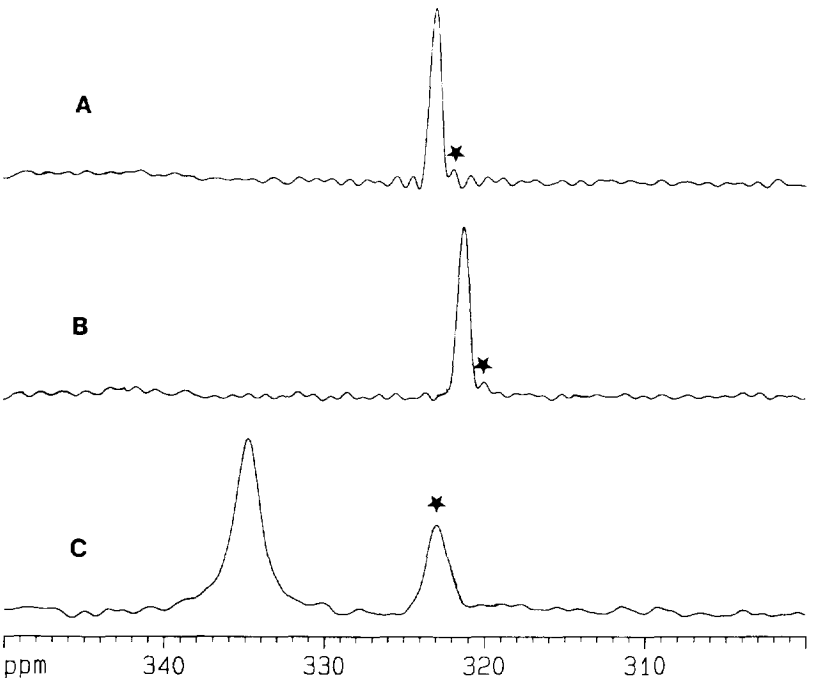


Figure 1. ^{17}O NMR spectra (54.4 MHz) in natural abundance of the amides of Table 1, concentration 0.1 M in CH_3CN, at 30° C, using a Brüker AMX-400 MHz instrument. (A) N-methylformamide, T_{acq}~ 18 ms, number of scans 2224000, after resolution enhancement by a Gaussian-exponential function (LB=-25 Hz; GB=0.7). (B) N-ethylformamide, T_{acq}~18 ms, number of scans 2539000, after resolution enhancement by a Gaussian-exponential function (LB=-25 Hz; GB=0.99). (C) N-tert-butylformamide, T_{acq}~ 18 ms, number of scans 2500000, after multiplication of the FID with an exponential function (LB=30 Hz). The asterisk denotes the resonance position of the cis isomer.

(torsion angle ω) deformation of the amide bond to relieve van der Waals interactions. These interactions appear to be strongly solvent dependent since Δ is 20.5 and 16.9 ppm in $CHCl_3$ and CH_2Cl_2 solutions respectively, which is significantly larger than the respective value of 11.9 ppm in CH_3CN solution. It has been reported that specific CH···O hydrogen bond interactions play a significant role in CH_2Cl_2 and $CHCl_3$ solutions[30-33]. It is therefore evident that in CH_2Cl_2 and, especially, $CHCl_3$ solution maximization of solvation of the trans CO amide group is retained at the expense of significant increase in the ω torsion angle of the amide plane to minimize van der Waals interactions. To our knowledge this is the first example of a limited solvent accessibility and solvent induced torsion angle distortion of the trans amide bond.

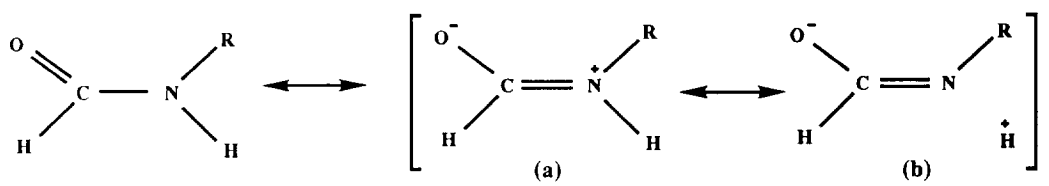
In CH_2Cl_2 and $CHCl_3$ solution there is an increase in the linewidth of the trans isomer relative to the cis; this might indicate a significant increase in the nuclear quadrupolar coupling constant (NQCC) and therefore in the electric field gradient at the oxygen nucleus of the trans isomer. It is usually assumed that the largest orbital contribution to the electric field gradient is due to the lone pair MO,

Table I. ^{17}O Chemical Shifts of NMF, NEF and TBF and Chemical Shift Differences, Δ, of the Two Isomers in CH_3CN, CH_2Cl_2 and $CHCl_3$ at 0.1 M [a]

Compound	Solvent	δ(^{17}O) trans	cis	Δ
NMF	CH_3CN	323.2	322.2	1.0[b]
	CH_2Cl_2	321.9	319.8	2.1
	$CHCl_3$	318.3	315.9	2.4
NEF	CH_3CN	321.4	320.2	1.2[b]
	CH_2Cl_2	321.5	318.9	2.6
	$CHCl_3$	316.0	312.0	4.0
TBF	CH_3CN	334.9	323.0	11.9
	CH_2Cl_2	335.3	318.4	16.9
	$CHCl_3$	330.4	309.9	20.5

[a] Chemical shifts were found to be practically independent of concentration below 0.1 M.

[b] The cis resonance was purely resolved even after Gaussian-exponential filtering[23] (Figure 1).



Structure I **Structure II**

Scheme 1

followed by the π MO and then by the σ bond MO[17,34]. Therefore, it can be assumed that the electric field gradient is increased in the trans isomer because of the increase in the π-bond order of the CO bond. Although a rigorous interpretation of the relative ratio of the NQCCs would require detailed molecular orbital calculations, it is clear that the NQCCs are important structural parameters in addition to the ^{17}O chemical shifts and reveal that the charge distributions about the cis and trans amide oxygen atoms of N-tert-butylformamide differ significantly.

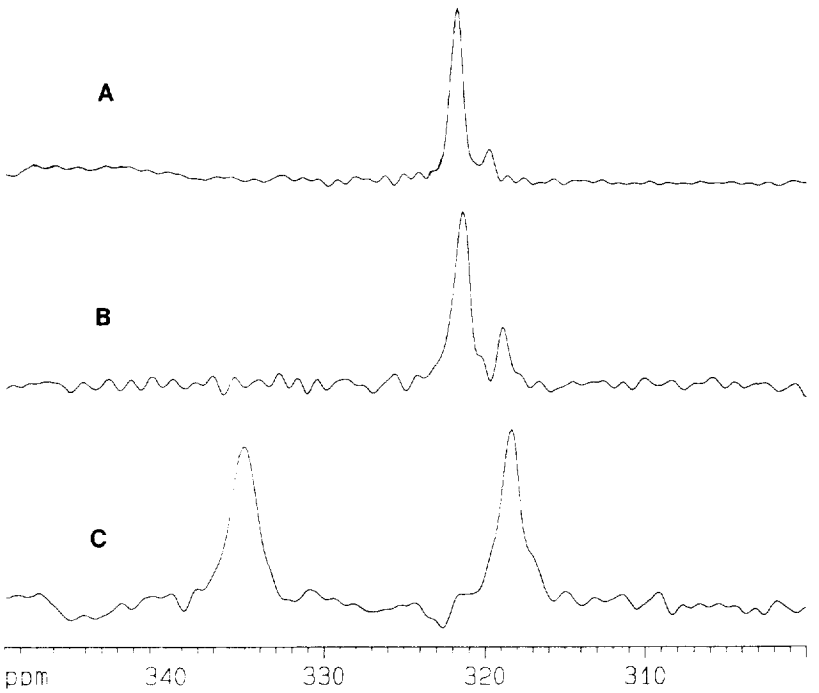


Figure 2. ^{17}O NMR spectra (54.4 MHz) in natural abundance of the amides of Table 1, concentration 0.1 M in CH_2Cl_2, at 30°C, using a Brüker AMX-400 MHz instrument. (A) N-methylformamide, T_{acq}~ 18 ms, number of scans 1740000. (B) N-ethylformamide, T_{acq}~ 18 ms, number of scans 2000000. (C) N-tert-butylformamide, T_{acq}~ 18 ms, number of scans 1250000, after multiplication of the FID with an exponential function (LB=30 Hz). In all cases the resonance at low frequency (more shielded) corresponds to the cis isomer.

^{15}N NMR Studies

^{15}N NMR spectroscopy is of considerable potential value for the structural analysis of peptides; however, the general utility of this spectroscopic tool has been hampered by the lack of natural abundance, the low sensitivity, and often inconvenient relaxation properties of the ^{15}N nucleus[13,18-20]. It has been demonstrated that the indirect detection of nitrogen chemical shifts from the proton resonances permits a significant gain in sensitivity over the direct observation of the ^{15}N signals by a theoretical factor of $(\gamma_{^1H}/\gamma_{^{15}N})^{5/2} \sim 306$[35-37].

Figure 3 shows the ^{1}H-^{15}N multiple quantum coherence NMR spectrum of an equimolar mixture of N-methylformamide, N-ethylformamide and N-tert-butylformamide in 0.5 M in $CDCl_3$. The pulse sequence used is that proposed by Bax et al.[38] Contrary to ^{17}O NMR spectra, there are considerable substituent effects which result in a strong deshielding along the series t-Butyl>Et>Me, which may be reasonably explained by structural changes involving an increase in π-bonding of the nitrogen atom through its lone electron pair when either a stronger electron donor is introduced or the π orbital system is extended over the R group[39] (Scheme 1, Structure II). The ^{15}N chemical shift separation, Δ, of

the trans and cis isomers of NMF and NEF is +2.5 and +1.8 ppm respectively. The situation is reversed for NBF which indicates a negative value Δ=-1.5 ppm. Similarly, the NH ^{1}H resonance of trans-NBF is strongly shielded by ~0.5 ppm while the cis ^{1}H resonances are within ~0.16 ppm for the amides studied. The conformational dependence of the ^{15}N chemical shifts upon the torsion angle ω can be rationalized in terms of the relative contribution of the resonance structures of the peptide bond I and II (Scheme 1). Non-planarity and pyramidicity at the amide nitrogen tends to favour the structure I; this results in increased electron charge density on the amide nitrogen and NH proton and thus increased shielding of both the ^{15}N and ^{1}H NMR signals. This is in excellent agreement with our ^{15}N-^{1}H HMQC results (Figure 3). The ω dependence of $\delta(^{15}N)$ could also been explained by the change in hybridization of the nitrogen atomic orbitals when going from a strictly planar peptide group, with partial sp^2 hybridization of the nitrogen orbitals, to a non-planar form where there is predominantly sp^3 hybridization of the nitrogen valence electrons[40]. Since the three principal hybridization states of nitrogen give rise to shielding in the sequence $sp^3>sp>sp^2$ [20], an increase in shielding is expected upon increase in the pyramidicity at the amide nitrogen. This is in agreement with our results.

At present it is premature to attempt to quantify the expected correlation between torsion angle, ω, and ^{17}O chemical shift differences, Δ, for N-tert-butylformamide due to lack of X-ray structural data and complications arising from an interplay of steric effects and solvent induced hydrogen bonding interactions. However the fact that N-tert-butylformamide in CH_3CN, which does not form a hydrogen bond with the amide oxygen, shows a Δ value of 11.9 ppm compared to 1.1 and 1.4 ppm for NMF and NEF, respectively, strongly suggests that the deviation from planarity must be very substantial.[41] Interestingly an analysis of crystal structure data of a limited number of peptides indicates that the degree of pyramidicity at the nitrogen atom and the dihedral angle ω have significant values and are correlated. Thus, an increase in the degree of pyramidicity at the nitrogen atom will manifest itself as an increase in the torsion angle ω. This is in excellent agreement with our combined ^{17}O and ^{1}H-^{15}N HMQC results of trans-N-tert-butylformamide.

Two important conclusions emerge from the present work. First, ^{17}O and ^{15}N NMR studies demonstrate the ready occurence of non-planar distortions of an out-of-plane (torsion angle) deformation of the amide bond of the sterically hindered trans-N-tert-butylformamide. Second, steric hindrance and thus torsion angle deformation is shown to be strongly solvent dependent. It is evident from these results that the combined use of ^{17}O and ^{1}H-^{15}N heteronuclear multiple quantum coherence NMR is a highly effective tool in the study of torsion angle deformation of the amide bond and pyramidicity at the amide nitrogen.

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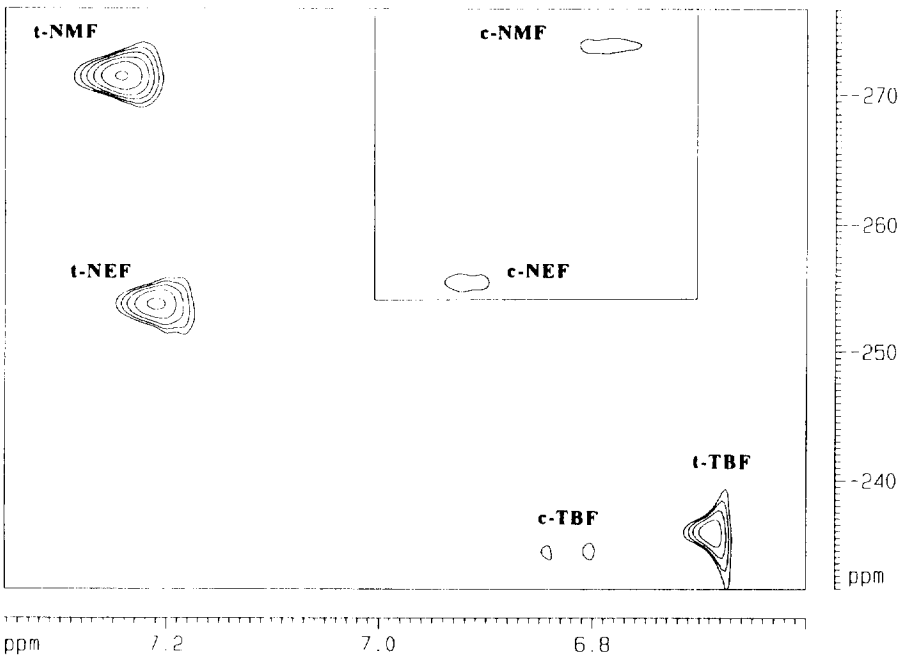

Figure 3. 2D ^{1}H-^{15}N heteronuclear multiple quantum coherence (2D ^{1}H-^{15}N HMQC) NMR spectrum, with broad-band ^{15}N decoupling, of a mixture of N-methylformamide, N-ethylformamide and N-tert-butylformamide, 0.5 M in $CDCl_3$, at 30°C, using a Brüker AMX-400 MHz instrument. Time domain data size 256 x 64 points and 1360 scans per free induction decay. Square sine bell window function in both dimensions. Acquisition time in the t_2 dimension was 0.19 s. The delay time between scans was 0.50 s. Data accumulation time was 12 h. Box indicates the region of the spectrum of the cis-NMF and cis-NEF isomers plotted with a lower contour level.

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