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Nuclear magnetic resonance of amides

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We investigated several secondary and tertiary amides in our studies of the peptide bond by the n.m.r. technique.

Amides are simple molecules containing a peptide bond which can be considered as a fragment of a polypeptide chain. Considerations of resonance (1) lead to the conclusions that the C-N bond has a considerable amount of double bond character and accordingly among the internal rotational states having the C-N bond as axis, only the cis and trans positions are favored.

The 60 Mc/s proton magnetic resonance spectra of three unsymmetrically substituted amides are shown [1, 2] in figure 1. For each non equivalent proton group two resonances are present in each spectrum, having the same intensity ratio and separated by chemical shift. These spectra are consistent with the presence of rotational isomers which are interconverting slowly at room temperature.

Table I.

	$\varnothing CHCH_3 NCH_3 CHO$	$\varnothing CH_2NCH_3CHO$	$\varnothing_2 CHNCH_3 CHO$
Isomer Ratio	2.33	1.08	2.00
ΔE (Kcal/mole) . . .	11.0 $\pm$ 1.5	11.9 $\pm$ 2	11.4 $\pm$ 1.5
ν_0 (sec^{-1})	10^7	10^7	10^8

The exchange theory of Gutowsky et al. [3] has been used to obtain the equation of the line shape for an unsymmetrical collapsing doublet, and the relative numerical solutions have been obtained by using an electronic computer. Comparing spectra taken at different temperatures with

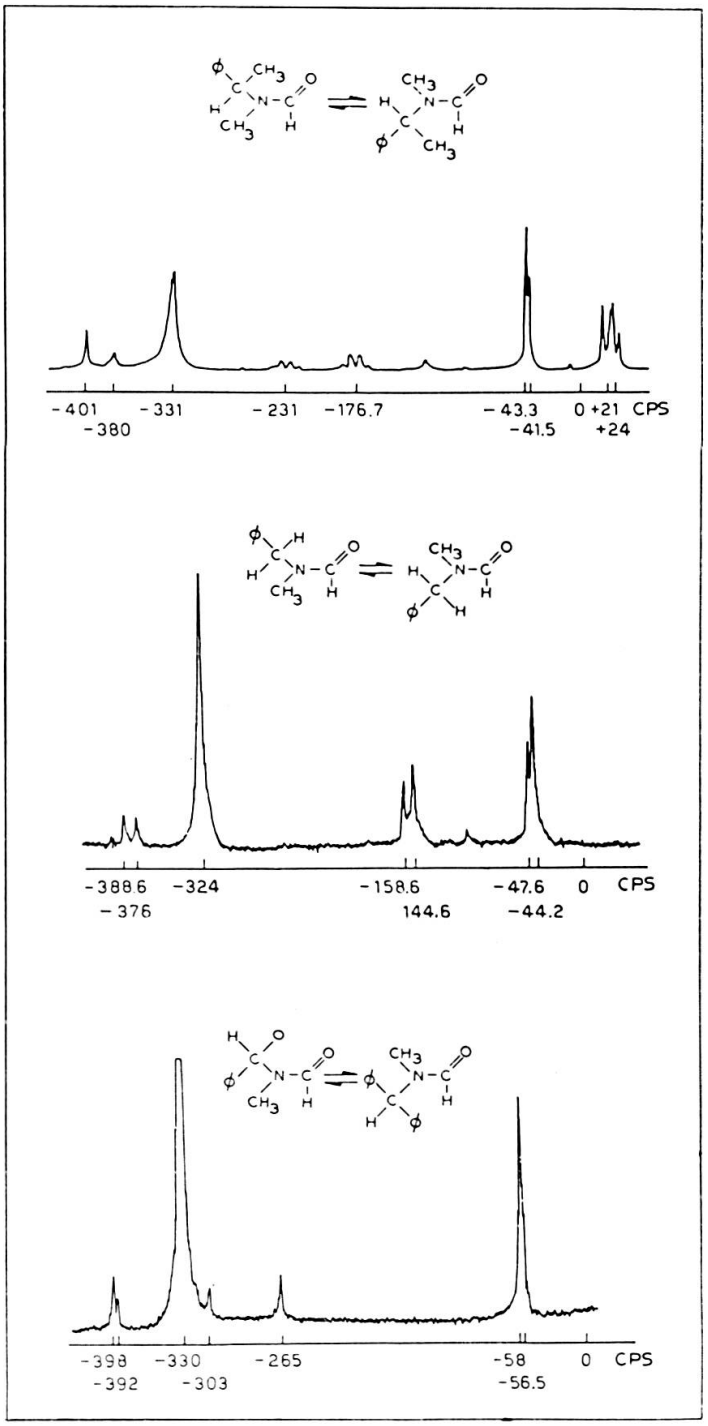


Fig. 1.

Proton magnetic resonance spectra of tertiary amides at 60 Mc/s and 29° C.
The external field increases from left to right.
Acetone is used as the external reference standard.

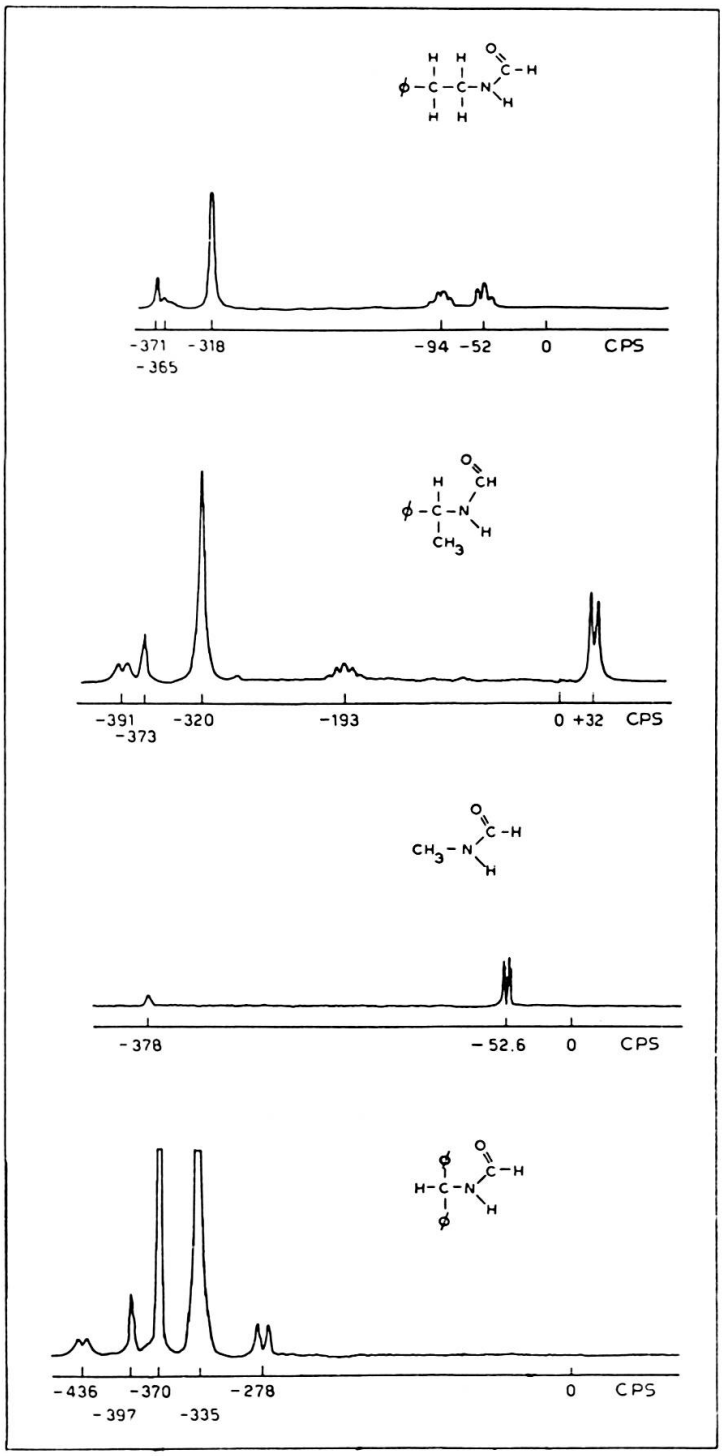


Fig. 2.
Proton magnetic resonance spectra of secondary amides at 60 Mc/s and 29° C.
The field increases from left to right.
Acetone is used as the external reference standard.

theoretical line shapes, the mean lifetimes of the formyl protons between rotations have been evaluated as a function of temperature. Activation energies ΔE and frequency factors ν_0 were thus determined for all the amides studied. The results obtained are summarized in Table I.

It can be seen that the activation energies for the process of reorientation are not affected to a great extent by the different substituents, as compared to the isomer ratios.

Proton magnetic resonance spectra of four secondary amides [2] are shown in figure 2. Noticeable are the narrow line widths of the N-H resonances in the first, second and fourth spectrum. Thus it seems that the planar bond configuration is rather effective in averaging out the quadrupolar field at the N^{14} nucleus.

In β-phenylethylformamide a coupling (J = 5.9 cps) between the two methylene proton groups splits their resonances into two triplets, but a further coupling (J = 5.9 cps) of the methylene protons next to the nitrogen with the N-H proton, splits the triplet at -94 cps into four lines of relative intensities 1:3:3:1.

In α-phenylethylformamide the C-H proton is coupled both to the $C\text{-}CH_3$ (J = 6,9 cps) and the N-H protons (J = 6,9 cps), giving the 1:4:6:4:1 quintuplet at — 193 cps.

The spectrum of N-methylformamide shows spin-spin couplings of the N-H proton with the $C\text{-}CH_3$ (J = 4.6 cps) and the formyl proton (J = 0.4 cps). The N-H resonance is possibly broadened by proton exchange and lies beneath the formyl line.

The spectrum of the N-benzhydrylformamide shows the existing coupling (J = 8.4 cps) between the N-H and the C-H protons. The line at -370 cps is due to the solvent used, N,N-dimethylformamide.

From the relative positions of the formyl resonances of the secondary and tertiary amides one could try to assign absolute configurations to the isomers.

In fact comparing the first two spectra in figure 1 with the first two in figure 2, one can see that the formyl resonances of the secondary amides (lines at — 371 and — 373 cps, fig. 2) are very close to the formyl resonances of the isomers of the tertiary amides which fall at a higher field (lines at — 376 and — 380 cps, fig. 1). This can be associated with the presence of only one isomeric species for the secondary amides.

Further work has been planned to solve the problem of cis, trans-isomerism of the secondary amides, which is closely related with the determination of the configuration of the peptide bond.

The question as to whether amides protonate on oxygen or nitrogen has remained open for many years. There are two possible configurations of a protonated amide (fig. 3, A and B). From considerations of resonance, configuration A should be preferred.

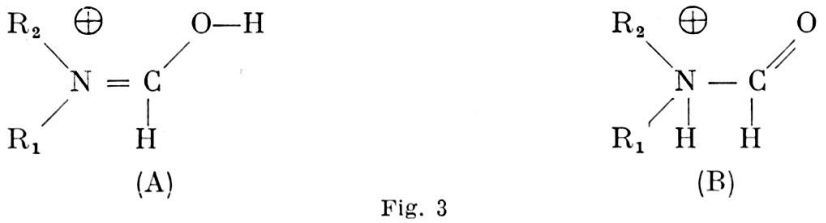


Fig. 3

In conjunction with n.m.r. studies [5], we have determined the degree of protonation of N,N-dimethylformamide (DMF), N-methylformamide

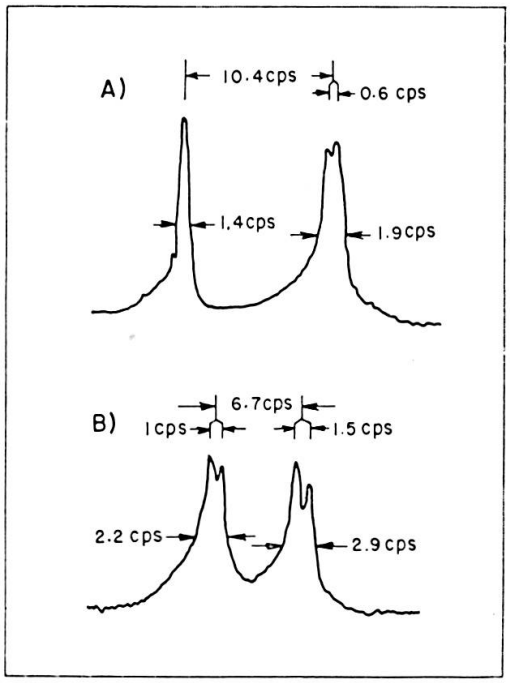


Fig. 4.
Nuclear magnetic resonance spectra of N-methyl protons in DMF at 60 Mc/s and 29° C.
A) pure DMF; B) 0.4 M DMF in 100% H_2SO_4.
The magnetic field increases from left to right.

(NMF) and several other amides in 100% H_2SO_4 by means of cryoscopic measurements. It was thus established that all these amides are monoprotonated in 100% H_2SO_4.

The n.m.r. spectra of A and B (fig. 3) should be clearly differentiable. In fact if an amide is N-protonated, there should be free rotation about the central C-N bond, so any sign of rotational isomerism would disappear. O — protonation would yield a spectrum similar to that of the pure amide, with some modifications reflecting the change of the double bond character of the C-N bond.

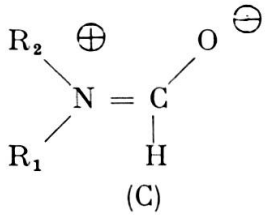
Figure 4 A) illustrates the methyl proton resonance pattern of DMF, and figure 4 B) the one of a 0.4 M solution of DMF in 100% H_2SO_4, $DMFH^+$. The main splitting is due in both cases to chemical shift, for the molecule is planar and one of the methyl groups is cis and the other is trans to the formyl proton. The observed fine structure arises from couplings of the cis and trans methyl protons with the formyl proton.

If one goes from the first to the second spectrum, the coupling constants of these cis and trans couplings increase from 0.5 to 1.2 and from 0.75 to 1.7 cps, respectively.

As DMF has found to be monoprotonated in 100% H_2SO_4, these spectra clearly show that DMF protonates chiefly on oxygen.

To check if the double bond character of the C-N bond has been increased by O-protonation, activation energies for the processes of reorientation about the C-N bonds of DMF and $DMFH^+$ have been measured, following a new procedure (App. B, ref. 5) which takes into account the fine structure of the doublets. For this purpose a new Dewar-jakleted probe has been designed [6] for the high temperature measurements.

The results obtained of 9.6 $\pm$ 1.5 and 12.7 $\pm$ 1.5 Kcal/mole respectively for DMF and $DMFH^+$ are reasonable, for the rotational barrier of $DMFH^+$ could exceed that of pure DMF, since the resonance configuration A (fig. 3) would be more important in stabilizing $DMFH^+$ than would configuration C (fig. 5) in stabilizing DMF.



(C)

Fig. 5.

The increase in the formyl-methyl proton coupling constants in $DMFH^+$ compared to DMF also indicates the increase of the double bond character of the C-N bond [7].

That even other amides, secondary amides included, protonate on oxygen in 100% H_2SO_4 may be deduced by looking at the n.m.r. spectra of the pure and monoprotonated NMF [5]. Here also there is the preservation of the main splitting (due to spin-spin coupling with the N-H proton) and an enhancement of the methyl-formyl proton coupling (from 0.4 to 1.1. cps). Also from the spectra of the other amides studied [5] one can reach the same conclusions.

Qualitatively the criterion for hydrogen bonding should follow that found for protonation, hydrogen bonding to oxygen being more favorable than to nitrogen. Similarly an increased rotational barrier should be found for H-bonded amides over the non associated species.

Intermolecular hydrogen bonding in proteins is assumed to be partially responsable for maintaining the structural stability necessary for biological activity. In addition to holding the system in a specific shape, the hydrogen bonds could also strengthen the individual amide linkages which are associated, by analogy, with our results.

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