Proton Coupled Carbon-13 Magnetic Resonance Spectra. The Simple Amides

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The carbon-13 magnetic resonance spectra of a selection of simple amides are reported, with special emphasis placed upon the application of proton coupled spectra to the problem of peak assignment.

Because of their relationship to the biologically important polypeptide macromolecules, the amides have been the subject of numerous studies using diverse spectroscopic methods. As a result of this intensive study, a great deal is known regarding the molecular and electronic structure of these molecules.[1] Thus, the amides are essentially planar, with the nitrogen–acyl bond restricted to two rotameric states which are separated by an energy barrier of about 20 kcal/mol. Proton magnetic resonance spectroscopy has been extensively used to study these conformations and their interconversions.

The amides have also received the attention of carbon-13 magnetic resonance (^{13}C nmr) spectroscopists. Using double resonance techniques, it has been shown that *N*-methyl carbons which are syn[2] to the carbonyl oxygen in *N*,*N*-dimethylamides are shielded relative to the anti by 3–5 ppm.[3] A more recent publication[4] has concentrated largely upon the relaxation times of the carbons of simple amides, and it is clear that T_1 measurements will be a useful aid in distinguishing the syn and anti α-carbon resonances of such molecules as *N*,*N*-dibutylformamide.

As part of a more extended investigation of ^{13}C nmr spectra of polypeptides,[5] we have undertaken a brief survey of the amides. The major thrust of our study has been to develop additional methods by which the carbon resonances of more complex molecules may be assigned. Our prior experiences with the esters[6] suggested that carbon–proton coupling might be useful in this regard. The present paper describes our progress in the measurement of proton coupled ^{13}C nmr spectra of amides.

Experimental Section

All compounds were commercially available and were used without purification. *N*-Deuterioamides were prepared by treatment with deuterium oxide or methanol-d_1 followed by distillation.

Carbon-13 nuclear magnetic resonance spectra were measured on a Varian XL-100 spectrometer modified for Fourier transform spectroscopy in a manner which has been previously described.[7] Carbon chemical shifts were measured in aqueous solutions (10% v/v) relative to internal (2–5%) 1,4-dioxane. The chemical shifts so measured were than related to external carbon disulfide on the basis of the chemical shift of 1,4-dioxane measured relative to that standard (126.2 ppm). Proton coupled ^{13}C nmr spectra were measured using neat solutions when possible. For crystalline amides, proton coupled spectra were measured in aqueous solutions.

For the preliminary coherent decoupling experiments reported in this paper, decoupling power was adjusted to the minimum necessary for complete decoupling. On our basic Varian XL-100 spectrometer,[7] this generally corresponded to about 110 dB on the low power range.

Results and Discussion

Carbon Chemical Shifts.—Carbon chemical shifts reported in this paper are presented in Tables I and II. To facilitate later comparison of these spectra to those of peptides and amino acid derivatives,[5] aqueous solutions were used in all measurements. In many cases, peak assignments were derived from the literature.[3,4] These assignments were confirmed and extended using carbon–proton coupling constants measured in this study (*vide infra*).

TABLE I

CARBON CHEMICAL SHIFTS[a] IN SIMPLE AMIDES

Registry no.	Amide	C=O	—NCH$_3$— anti	—NCH$_3$— syn	CCH$_3$
75-12-7	Formamide	26.1			
123-39-7	*N*-Methylformamide				
	(trans)	28.2		168.3	
	(cis)[b]	25.0	164.7		
68-12-2	*N*,*N*-Dimethylformamide	28.1	155.9	161.4	
60-35-5	Acetamide	15.6			171.4
79-16-3	*N*-Methylacetamide	18.3		166.8	171.0
127-19-5	*N*,*N*-Dimethylacetamide	19.0	154.6	157.5	172.2

[a] Measured relative to external carbon disulfide. [b] Spectrum measured as a 25% aqueous solution.

TABLE II

CARBON CHEMICAL SHIFTS[a] OF FORMYL- AND ACETYLSARCOSINES

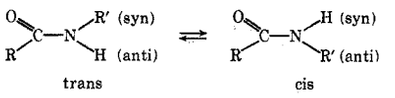
Registry no.	Sarcosine	COOH	NCH$_2$	NCH$_3$	O ‖ –CN	CH$_3$C
38456-66-5	*N*-Formylsarcosine					
	(trans)	20.5	146.5	157.0	27.3	
	(cis)	19.4	141.5	161.9	26.7	
5888-91-5	*N*-Acetylsarcosine					
	(trans)	19.4	143.1	155.3	18.0	172.2
	(cis)	19.5	140.4	158.0	17.9	172.4

[a] All chemical shifts measured relative to carbon disulfide.

The most significant source of carbon chemical shift differences in amides is that due to the carbonyl group. *N*-Methyl carbons which are syn to the carbonyl oxygen are invariably shielded relative to the anti case. There appears to be some question regarding the source

(1) M. B. Robin, F. A. Bovey, and H. Basch in "The Chemistry of Amides," J. Zabicky, Ed., Interscience, New York, N. Y., 1970, Chapter 1.

(2) Throughout the subsequent discussion syn will be used to describe groups which are on the same side of the amide linkage as the carbonyl oxygen. The term anti will denote the opposite geometry. Such a convention will leave the terms cis and trans to describe the two stable conformers around the amide function.



(3) W. McFarlane, *Chem. Commun.*, 418 (1970).

(4) G. C. Levy and G. L. Nelson, *J. Amer. Chem. Soc.*, **94**, 4897 (1972).

(5) D. E. Dorman and F. A. Bovey, in preparation.

(6) D. R. Bauer, D. E. Dorman, and J. D. Roberts, in preparation.

(7) H. Sternlicht and D. M. Zuckerman, *Rev. Sci. Instrum.*, **43**, 525 (1972).

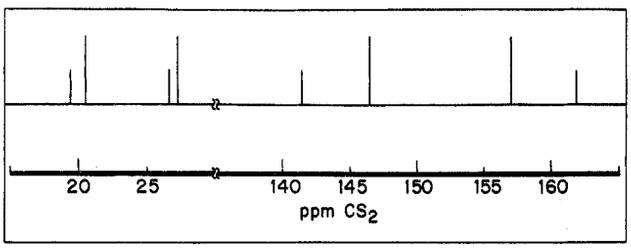


Figure 1.—Schematic representation of the ^{13}C nmr spectrum of *N*-formylsarcosine. The more intense set of peaks represents the spectrum of the major or trans conformer. The back-to-back pattern shown in the upfield resonances is typical of the spectra of sarcosine derivatives.

of this shielding proximity effect. McFarlane,[3] citing evidence derived from proton chemical shifts and one-bond carbon–proton coupling,[8] attributed it to the electric field associated with the carbon–oxygen bond. More recently[4] the proximity effect of the carbonyl oxygen has been discussed in terms of steric compression. Whatever the source of this effect, it is thought to extend its influence even to the terminal methyl carbons of *N*,*N*-di-*n*-butylformamide.[4]

The existence of such an effect may have important implications in the ^{13}C nmr study of the conformations of peptides. An indication of this is apparent in the spectra of *N*-acylsarcosines (Table II). Even in the absence of any data other than the carbon chemical shifts of these compounds, full and reliable peak assignments may be made. The case of *N*-formylsarcosine will be used as an example. The aqueous solution of this compound shows the presence of the two unequally populated conformers, cis and trans. Within

cis trans

the spectra of each of these conformers the two upfield resonances may be differentiated by off-resonance decoupling.[9] On the basis of the shielding effect of the carbonyl group we would predict that (1) the *N*-methyl resonance of the cis conformer would be upfield relative to the trans; and (2) the *N*-methylene resonance of the trans conformer would be the more shielded. This should lead to a back-to-back pattern in the upfield carbon resonances, as has been observed for similar compounds using proton magnetic resonance spectroscopy.[10] This is indeed observed in the ^{13}C nmr spectrum of this compound, as is schematically shown in Figure 1. Similar methods of assignment have proven useful in the ^{13}C nmr spectra of proline derivatives.[5]

Proton Coupled ^{13}C Nmr Spectra.—A previous study[6] of the proton coupled ^{13}C nmr spectra of ethers and esters demonstrated that geminal and vicinal carbon–proton coupling constants could be easily measured in simple compounds. Such data were found to be useful in the conformational analysis of these systems. Corresponding measurements on amides should be useful in making peak assignments. Unfortunately, the spectra of amides are subject to problems not present in the earlier investigation.[6] The presence of the ^{14}N nucleus in amides, for example, might be expected to lead to additional complexities in their spectra. The presence in many amides of an exchangeable N proton is another potential complication. Because virtually every carbon nucleus in these simple amides is spin coupled to the N proton, any exchange phenomena could lead to irreproducible results. This problem can be avoided by exchanging the N protons with deuterium, a device which also leads to simplification of the proton coupled spectra.

It is largely due to problems of this nature that the coupling constant data of Tables III and IV must be considered approximate. Even in view of these limitations, however, the present data are sufficient to provide important information for peak assignments and conformational analysis. Thus, as shown in Figure 2, the coupling between the *N*-methyl carbon and formyl proton nuclei of *N*-methylformamide is strongly dependent upon the dihedral angle about the nitrogen–acyl bond. In the trans isomer, wherein the dihedral angle between the two coupled nuclei is 180°, this vicinal carbon–proton coupling constant is about 5 Hz. For the cis conformer, corresponding to a dihedral angle of 0°, the coupling is too small to be resolved under the conditions of the experiment. Clearly such differences

TABLE III

COUPLING[a] INVOLVING THE CARBONYL CARBON

Amide	$^1J_{CH}$	$^2J_{CCH}$	$^3J_{CNH}$	$^3J_{CNCH}$
Formamide	192.8		2.4, *ca.* 5.5	
N-Methylformamide-d_1				
(trans)	191.5			3.1
(cis)	189.1			*ca.* 4.3
N-Methylformamide				
(trans)	191.7		*ca.* 3.7	*ca.* 3.7
(cis)	189.1		*b*	*b*
Acetamide[c]		5.9	2.65, 2.65	
N-Methylacetamide-d_1		6.1		3.5
N-Methylacetamide		5.9	3.7	3.7

[a] All coupling constants are in hertz, and are accurate within ±0.2 Hz. [b] Not resolved. [c] Measured in saturated aqueous solution.

TABLE IV

COUPLING[a] INVOLVING *N*-METHYL CARBONS

Amide	$^1J_{CH}$	$^2J_{CNH}$	$^3J_{CNCH}$
N-Methylformamide-d_1			
(cis)			*b*
(trans)			5.1
N-Methylformamide			
(cis)	137.3	*b*	*b*
(trans)	137.9	2.8	4.8
N,*N*-Dimethylformamide			
(syn) CH_3	138.5		*ca.* 3.4,[c] *ca.* 3.4[d]
(anti) CH_3	138.9		*ca.* 3.4,[c] *ca.* 2.0[d]
N-Methylacetamide	138.2	2.7	

[a] All coupling constants are reported in hertz, and are accurate to approximately ±0.2 Hz. [b] Not resolved. [c] Vicinal coupling between the carbon of one methyl group and the protons of the other. [d] Vicinal coupling between *N*-methyl carbons and the formyl proton.

(8) W. T. Raynes and T. A. Sutherley, *Mol. Phys.*, **18**, 129 (1970); **17**, 547 (1969).

(9) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).

(10) F. A. Bovey, J. J. Ryan, and F. P. Hood, *Macromolecules*, **1**, 305 (1968).

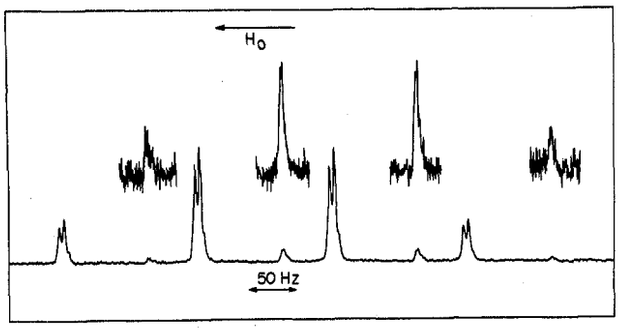


Figure 2.—The proton coupled spectrum of the *N*-methyl carbons of *N*-methylformamide. Minor peaks, which are shown at higher gain in the insets, are those of the minor cis conformer.

in coupling are of potential use in peak assignment and conformational analysis in these systems.

A more striking dihedral dependence is shown by acetamide (*cf.* Figure 3). Here, the methyl carbon is coupled observably to only one of the N protons ($^3J_{CCNH}$ = 7.1 Hz). In the spectrum of *N*-methylacetamide, the acetyl methyl carbon resonance is coupled to none but the directly attached protons. Because this latter compound is known to be 100% trans,[11] these results indicate that the methyl carbon of acetamide is coupled only to the syn N proton.

One-bond carbon–proton coupling constants also have occasional application to the problem of peak assignment. In the spectrum of *N*-methylacetamide, for example, the two methyl resonances differ by less than 5 ppm, a surprisingly small difference. The indicated assigment (Table I) can be supported, however, by the proton coupled ^{13}C nmr spectrum. In the latter spectrum the one-bond carbon–proton coupling constant for the high-field methyl resonance is found to be approximately 128 Hz, while that of the low-field methyl quartet is about 138 Hz. The same coupling constants can be conveniently measured from the ^{13}C side bands of the proton spectrum, in which there is no question of assignment.[11] Using this method, the low-field methyl carbon resonance can be related to the *N*-methyl proton resonance, thereby confirming the above assignment.

In the spectrum of *N*-acetylsarcosine, the chemical shifts of the carbonyl nuclei were found to be rather similar (*cf.* Table II). By correlation with the spectrum of *N*-formylsarcosine, the peaks near 19.5 ppm were assigned to the carboxyl resonance. Confirmation of this assignment was derived from the proton coupled ^{13}C nmr spectrum. The acetyl carbonyl carbon of *N*-acetylsarcosine would be expected to be coupled to the protons of both methyl groups and the *N*-methylene group, as well as to the ^{14}N nucleus. The carboxyl carbon, however, would be coupled only to the adjacent methylene protons. In the proton coupled ^{13}C nmr spectrum, the resonances near 19.5 ppm appeared as triplets ($^2J_{CCH} \cong 5.7$ Hz), thereby confirming their assignment to the carboxyl carbons.

These results, taken in conjunction with the data in Tables III and IV, indicate that proton coupled ^{13}C nmr spectra may provide important information regarding the peak assignment and conformational analysis in such systems. More recent results indicate

(11) L. A. LaPlanche and M. T. Rogers, *J. Amer. Chem. Soc.*, **86**, 337 (1964).

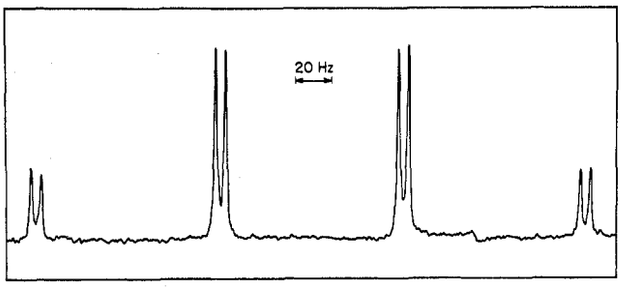


Figure 3.—The proton coupled methyl resonance of acetamide. Each portion of the widely split quartet shows evidence of vicinal coupling to only one N proton.

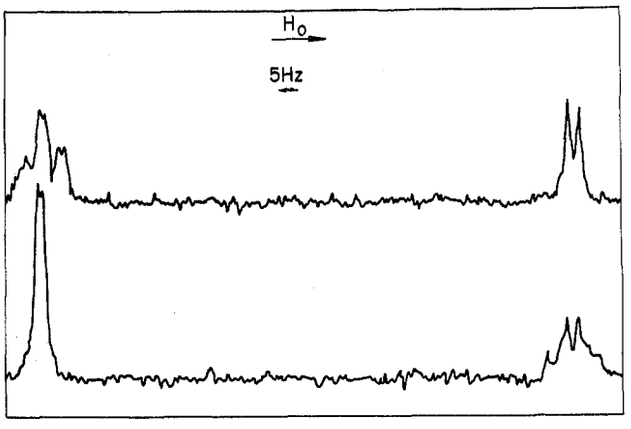


Figure 4.—Coherent proton decoupling experiments with *N,N*-dimethylformamide. In the upper trace, the decoupling frequency has been set on the upfield *N*-methyl proton resonance. The upfield carbon resonance shows residual vicinal coupling to the formyl proton. The lower trace was obtained when the decoupling frequency was moved to the lower *N*-methyl proton resonance.

that proper control of the temperature at which the spectra are taken leads to much narrower lines and thus more precise coupling data. Continuing experiments designed to investigate these effects, and to evaluate the application of carbon–proton coupling constants to problems in conformational analysis, are in progress.

Coherent Proton Decoupling.—In many amides, it may be desirable to measure a particular coupling constant in the absence of any other carbon–proton coupling. As an example of such a situation, we may consider the case of *N,N*-dimethylformamide. Through measurements of the coupling between the *N*-methyl carbons and the formyl proton, one can detect dihedral angle effects in such systems. Unfortunately, each of the methyl carbons in *N,N*-dimethylformamide is also coupled to six methyl protons, and the resonances of these spectra are accordingly complex.

It is possible in this system, however, to decouple the *N*-methyl proton resonances without significant perturbation of the formyl proton resonance (*cf.* Figure 4). Under such conditions the residual coupling is easily estimated. As observed for the simpler cases, the coupling constant for the syn *N*-methyl carbon was larger (3.4 Hz) than that of the anti ($\leq$2.0 Hz). It is similarly possible to decouple only the formyl proton, thus facilitating the measurement of the coupling between each methyl carbon and the protons of the other methyl group. This vicinal coupling was approxi-

mately the same (3.4 Hz) for each methyl carbon. Similar techniques may find application in the analysis of the conformations of *N*-formyl derivatives of greater complexity.

Conclusion

One of the most important carbon-13 chemical shift effects seen in the amides is a proximity effect associated with the carbonyl oxygen. Thus, *N*-alkyl carbons which are syn to this oxygen are strongly shielded. While at present this effect has only been recognized for carbon nuclei attached to the nitrogen of amides or the ether oxygen of esters,[6] there appears to be no reason why a similar effect cannot occur at the β carbon of amino acids. Such an effect could have important application to the conformational analysis of such systems.

Carbon–proton coupling appears to hold promise of useful applications in the conformational analysis and peak assignment for at least the simple amides, and may be useful in further investigations into the shielding effect of the carbonyl group in small molecules. Such experiments are currently in progress.

Carolenin and Carolenalin, Two New Guaianolides in *Helenium autumnale* L. from North Carolina[1,2]

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The major sesquiterpene lactones found in *Helenium autumnale* L. collected during the summer in North Carolina were not the pseudoguaianolide helenalin or the norsesquiterpene lactone, dihydromexicanin E, but were the new guaianolides, carolenin and carolenalin. The structures of carolenalin and carolenin have been shown to be **1** and **13** on the basis of chemical transformations and spectral evidence.

The constituents of *Helenium autumnale* L. collected from different populations were previously examined and reported to contain helenalin,[7-10] dihydromexicanin E,[11-12] helenium lactone,[13] 2-acetyl flexuosin A,[14] autumnolide,[14] tenulin,[15] mexicanin I,[15] and flexuosin A.[15] In the course of a search for convenient supply of the pseudoguaianolide helenalin for investigation of the relationship between the sesquiterpene lactone structure and the antitumor or cytotoxic activity,[16-19] we had occasion to extract a batch of *Helenium autumnale* L., collected during the summer in the vicinity of Durham, N. C. We report herein the isolation and structural elucidation of two new guaianolides, carolenalin and carolenin.[20]

Carolenalin and carolenin, isolated from a chloroform extract of the finely ground plant material by fractionation involving successive solvent partitions and silica gel chromatography, were assigned structure **1** and **13**, respectively, on the basis of the following chemical transformations and spectral evidence.

Carolenalin (**1**) was isolated as an oil in 0.4% yield and had infrared bands at 3500, 1760, and 1640 cm^{-1}, thus indicating the presence of a hydroxyl group, a γ-lactone carbonyl, and a carbon–carbon double bond. The nmr spectrum of carolenalin (Table I) is in accord with the structure **1**. The vinyl methyl protons at C-10 appeared as a broad singlet at δ 1.75 and the methyl groups at C-11 and C-4 were seen, respectively, as a doublet (J = 7.5 Hz) at 1.19 and a sharp singlet at 1.08. Other nmr signals were seen at δ 5.35 (1 H, m, H-9), 5.09 (1 H, m, H-8), and 3.74 (1 H, t, J = 3.0 Hz, H-3) which was shifted downfield to 4.79 (q, J = 3.0, 5.25 Hz) in the monoacetate **2** and 5.45 (t, J = 3.0 Hz) in the diacetate **3** as described below.

Acetylation of **1** with acetic anhydride in pyridine for 3 days at room temperature yielded approximately equal amounts of a monoacetate **2** (mp 160–161°, $C_{17}H_{24}O_5$) and a diacetate **3** (mp 146–148°, $C_{19}H_{26}O_6$),[21] indicating the presence of two hydroxyl groups. Mass spectral peaks at m/e 308 (M^+), 290 (M − 18) (M − H_2O), 248 (M − 60) (M − AcOH), and 230 (M − 78) (M − H_2O and AcOH), and ir absorption at 3580 (OH) and 1740 cm^{-1} (acetyl C=O) showed that **2** was a

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(19) K. H. Lee, R. Meck, C. Piantadosi, and E. S. Huang, *ibid.*, **16**, 299 (1973).

(20) The plant material used in the present work contained neither helenalin nor dihydromexicanin E, although the latter compound was isolated before from the same plant species in almost the same season and at the same site.[11]

(21) The formation of a diacetate (**3**) with acetic anhydride–pyridine suggested that a vicinal diol moiety was present, since under this mild reaction condition **3** could only be formed *via* a neighboring group participation mechanism.