Anal. Calcd for $C_{20}H_{14}F_8O_2$: C, 68.50; H, 6.04. Found: C, 68.25; H, 6.30.

The other chromatography fractions were combined and concentrated. The residue was sublimed (50°, 1 mm, 2 hr) to give ester (1.03 g, free of hydroxyl band in the ir spectrum). This sample was reduced with lithium aluminum hydride (0.5 g) by standard procedures; the products were isolated by glpc (Dow 710, 5 ft × 0.25 in., 147°, 97 cc/min of He): phenyl-*t*-butylcarbinol (0.36 g, t_R = 9.5 min; $[\alpha]^{20.8}D$ 30.2 ± 0.3° (*c* 5.10, acetone)) and 2-phenyl-3,3,3-trifluoro-1-propanol (0.34 g, t_R = 4.3 min; $[\alpha]^{26.4}D$ −4.32 ± 0.11° (*c* 5.10, chloroform)). The maximum rotation of this latter compound is about $[\alpha]^{20}D$ −38° (chloroform).[24]

(*RS*)-Methylethylcarbinyl (*S*)-α-(Tetrafluorosuccinimido)propionate (XI).[23] Methylethylcarbinyl *l*-alaninate[38] (3.37 g) was added to tetrafluorosuccinyl chloride[39] (6 ml) in a flask, kept at 25° with a cooling bath, while a positive nitrogen pressure was maintained. The mixture was frozen, and the flask was evacuated on a vacuum line. After distilling the volatile materials (1 mm), the system was attached to a diffusion pump; on heating the residue to 100°, the product (2.69 g, 34.2%) collected in a liquid nitrogen cooled trap, t_R = 8.7 min (SE-30, 6.5 ft × 0.25 in., 200°, 60 cc/min of He). This material is very sensitive to moisture.

Anal. Calcd for $C_{11}H_{13}F_4NO_4$: C, 44.15; H, 4.38; N, 4.68. Found: C, 43.97; H, 4.59; N, 4.72.

Acknowledgment. We thank Dr. Lois Durham and Dr. Yoko Kanazawa for their unfailing efforts and cooperation in collecting the nmr spectral data.

(38) R. Rometsch and W. Kuhn, *Helv. Chim. Acta*, **29**, 1483 (1946).

(39) J. J. Padbury and E. L. Kropa, U. S. Patent 2,502,478 (1950).

Carbon-13 Magnetic Resonance Studies of Amino Acids and Peptides[1]

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Contribution from the Department of Chemistry, University of California, Berkeley, California. *Received December 29, 1967*

Abstract: The carbon-13 nuclear magnetic resonances of glycine, diglycine, triglycine, alanine, and alanylglycine have been measured as a function of pH using a double-resonance technique. The resonance positions of these amino acids and peptides can be derived from a suitable set of substituent parameters empirically obtained from carbon-13 measurements on amines and carboxylic acids. The α-carbon resonances are determined by the carbon charge density. We show that charge densities derived from the Del Re–Pullman semiempirical molecular orbital theory can be used to predict the α-carbon resonance positions in the amino acids. The carbon-13 resonances are large compared with the proton resonances. It should eventually be possible to use the large chemical shifts and narrow line widths typical of carbon-13 for macromolecular chemical and conformational studies. The pH-induced carbon-13 shifts in the amino acids are indicative that protonation of the amino group to give NH_3^+ is accompanied by transmission of negative charge from the hydrogen through the carbons to the NH_3^+ group. The carbon charge density remains essentially constant. The charge densities on the carbons may actually become slightly more negative upon protonation. A similar behavior is observed in methylamine. The opposite appears true on ionization of the carboxyl group to give COO^-. Ionization is accompanied by transmission of positive charge from the hydrogen through the carbons to the COO^- group. The carbon charge density remains essentially constant. These experimental observations are consistent with the self-consistent field predictions of charge transmission in NH_4^+ and H_3O^+ from the hydrogens in NH_3 and H_2O through the nitrogen and oxygen to the added proton. The nitrogen and oxygen *electronic* charge densities remain constant or increase upon protonation.

In recent studies of protein folding by proton magnetic resonance, Sternlicht and Wilson[2] and MacDonald and Phillips[3] have observed large upfield shifts of methyl and methylene protons in the folded enzyme. These shifted resonances arise primarily from valine, leucine, and isoleucines in the ring-current fields of neighboring aromatic residues. The detailed pattern of these upfield shifts are significant clues to the protein conformation. Unfortunately, there is no unambiguous method of assigning the resonances. If the X-ray crystal structure is known, and if the structure in solution is essentially identical with the crystal, an approximate reconstruction is possible using the Bovey–Johnson ring-current tables.[4] In the case of lysozyme[2] a reconstruction in quite good agreement with the observed spectrum was indeed achieved by this procedure. However, despite the apparent success achieved in the lysozyme study, any conclusions must be treated with caution. The proton intrinsic line widths in proteins are broad, typically 15 cps or more, while the chemical shifts are small. The methyl resonance positions of the valine, leucine, and isoleucine residues in the unfolded configuration, for example, occur within ~0.1 ppm of each other. The ring-current fields of the aromatic type residues of phenylalanine, tryptophan, and histidine are not presently known. Even if these were known accurately, the intrinsically broad proton lines and the small chemical shifts in the unfolded state make an interpretation of the folded configuration very difficult.

In view of these difficulties we have begun a program of measuring the carbon-13 (C^{13}) nmr of the amino acids and peptides. The C^{13} chemical shifts are generally more than an order of magnitude greater than

(1) This paper was presented in part at the Pacific Conference on Chemistry and Spectroscopy, Anaheim, Calif., Oct 30, 1967.

(2) H. Sternlicht and D. Wilson, *Biochemistry*, **6**, 2881 (1967).

(3) C. C. MacDonald and W. D. Phillips, "Magnetic Resonance in Biological Systems," Pergamon Press, New York, N. Y., 1967, pp 3–27; *J. Am. Chem. Soc.*, **89**, 6332 (1967).

(4) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

those of protons. The dipolar interaction makes the major contribution to the intrinsic line widths in macromolecules. The C^{13} gyromagnetic ratio is $\approx {}^1/_4$ that of the proton. Consequently, we expect the C^{13} line widths in macromolecules to be approximately ${}^1/_{16}$ that of protons, once the carbon-13 is proton decoupled. The two great difficulties present in the proton studies, *i.e.*, broad lines and small chemical shifts, are rendered more tractable in the C^{13} case. Practical applications of C^{13} nmr to protein systems are, of course, contingent on having an inexpensive C^{13} source for enrichment beyond the present 1% natural abundance.

In this paper we present a pD study of the C^{13} resonances of glycine, diglycine, triglycine, alanine, and alanylglycine using a double-resonance technique. From these measurements we can make realistic estimates of the carbon-13 resonances of valine, leucine, and isoleucine. In view of the scarcity of C^{13} data in general, our results and conclusions should also be of considerable value to many readers who have no strong biochemical interests.

Experimental Section

A. Equipment and Operational Details. The double-resonance technique enables one to determine the carbon-13 resonance frequencies with proton sensitivity. This technique has been used in the past with considerable success in carbon-13 studies[5-7] and is capable of giving the resonance accurate to a cycle. The method of operation is to locate a C^{13} satellite in the proton spectrum, to "sit" on this satellite, and sweep the C^{13} oscillator. A decrease in the satellite intensity is observed[5] when the C^{13} frequency equals the transition frequency between two C^{13} levels, one level being in common with the C^{13} satellite in the proton spectrum. The decrease, or dip, in the satellite intensity arises from a breakup[5] of the satellite line into a multiplet pattern. Maximum accuracy of measurement is obtained by using the minimum C^{13} radiofrequency power that will produce a detectable dip in the satellite intensity. C^{13} spectra obtained in this fashion are referred to as indor spectra.

The experimental setup is illustrated in Figure 1. An internal lock system (home built) is used in proton detection at 60 MHz. Sample tubes (10 mm) were spun using a Wilmad modified spinner. A General Radio GR 1164 frequency synthesizer, externally referenced to the proton transmitter, was voltage swept. The frequency counter, the frequency synthesizer, and the magnetic field are locked to the proton transmitter crystal. Consequently, the proton frequency is effectively 60 MHz to within a few hundreds of a hertz and need not be measured during the course of the experiment. A very stable audio oscillator (Wave Tek Model 111) is used in the analytical channel of the internal lock system in order to reduce "drift" noise encountered when one sits on a C^{13} satellite line. The sensitivity achieved using the double-resonance technique was quite good. Neither computer enhancement nor proton decoupling was necessary in the present study done with natural abundance C^{13}. For more complicated spectra systems, as occurs in the case of valine, leucine, and isoleucine, proton decoupling the C^{13} satellite of interest from more distant protons is often necessary to simplify the spectra. In addition, these systems often require computer enhancement and $C^{12}H$, *i.e.*, main peak, suppression using difference spectroscopy[6] techniques.

B. Compounds. The amino acids and peptides used were obtained commercially. D_2SO_4 or NaOD were used to adjust the pD values. pD's were measured on a Beckman zeromatic pH meter All meter readings were converted to pD by adding 0.40 unit, the standard conversion correction. All measurements were done on saturated solutions in D_2O.

C. Chemical Shift Values. We internally lock on the first upper or lower side band of a suitable lock proton. Only the C^{13} center band frequency is detected in our double resonance experiment. We first obtain the C^{13} chemical shift of carbon i, $\delta_{C^{13}}(i)$ [relative to TMS], and then convert these values to shifts relative to proton-decoupled benzene.

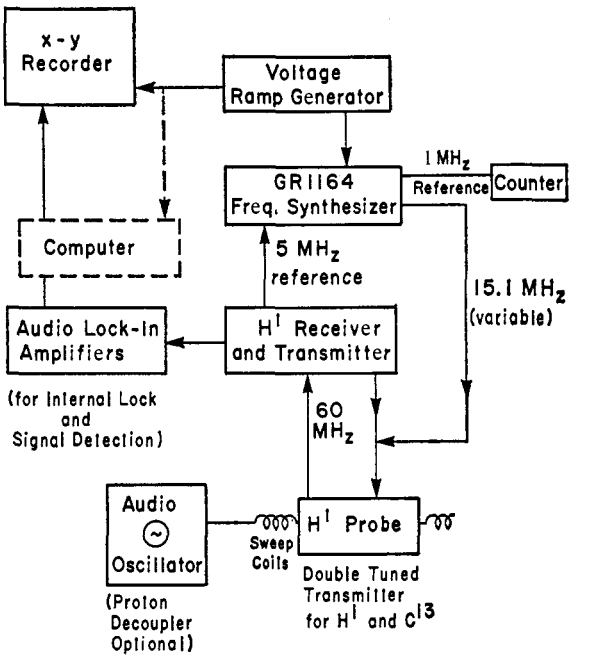


Figure 1. Instrumentation for C^{13} detection *via* the H^1–C^{13} satellites in the proton spectrum.

$$\delta_{C^{13}}(i)\ [\text{relative to TMS}] = \frac{\Gamma_i - 3.9769331}{3.9769331} + \delta_H(L) \quad (1a)$$

$$\Gamma_i = \frac{60.0000000\ \text{MHz}\ (\pm)\ \text{lock frequency}}{f_c(i)} \quad (1b)$$

$$\delta_{C^{13}}(i)\ [\text{relative to benzene}] = 129.0 \pm 0.1\ \text{ppm} + \delta_{C^{13}}(i)\ [\text{relative to TMS}] \quad (1c)$$

The derivation of (1a–c) is straightforward. The constant 3.9769331, measured by Grant, *et al.*,[8] is the ratio of the proton-decoupling frequency of the TMS methyls to the decoupled methyl C^{13} frequency. The proton transmitter frequency is effectively 60 MHz to within a few hundredths of a hertz, as indicated above. This value is inserted in (1b). The lock frequency is added with a positive (+) or negative (−) sign to the proton transmitter frequency, depending on whether we use an upper or lower side-band lock, respectively. In the amino acid measurements we lock on an upper side band at a lock frequency equal to 1671.0 cps. $f_c(i)$ is the C^{13} resonance frequency as measured in the double-resonance experiment outlined above. $f_c(i)$ depends on the lock frequency, the lock side band used, and the lock proton. Γ_i, however, depends only on the "lock" proton and is independent of "lock" frequency, "lock" side band, and variations in laboratory spectrometers. Consequently, Γ_i will be the same for all laboratories using the same lock proton. (A Γ_i tabulation is, therefore, a logical way of reporting and comparing double-resonance experiments.)

$\delta_H(L)$ is the shift of the "lock" proton relative to TMS, and is negative for a "lock" proton at a lower field relative to TMS. Since our studies are carried out in aqueous solution, DSS [3-(trimethylsilyl)-1-propanesulfonate], rather than TMS, is used to internally reference (but not lock) our proton spectra. It is generally accepted that TMS and DSS are interchangeable with respect to referencing proton chemical shifts. To the extent that this is true, (1a) gives the C^{13} shifts relative to TMS rigorously and accurately.

In our measurements we lock either on an HDO peak (δ_H −4.90 ± 0.15 ppm, pD 1–12) or on the trifluoroacetic acid proton in an external capillary (δ_H −10.80 ± 0.02 ppm). (If HDO is

(5) W. A. Anderson and R. Freeman, *J. Chem. Phys.*, **37**, 85 (1962).
(6) R. Freeman, *ibid.*, **43**, 3087 (1965).
(7) J. K. Becconsall and P. Hampson, *Mol. Phys.*, **10**, 21 (1965).
(8) W. R. Woolfenden and D. M. Grant, *J. Am. Chem. Soc.*, **88**, 1496 (1966).

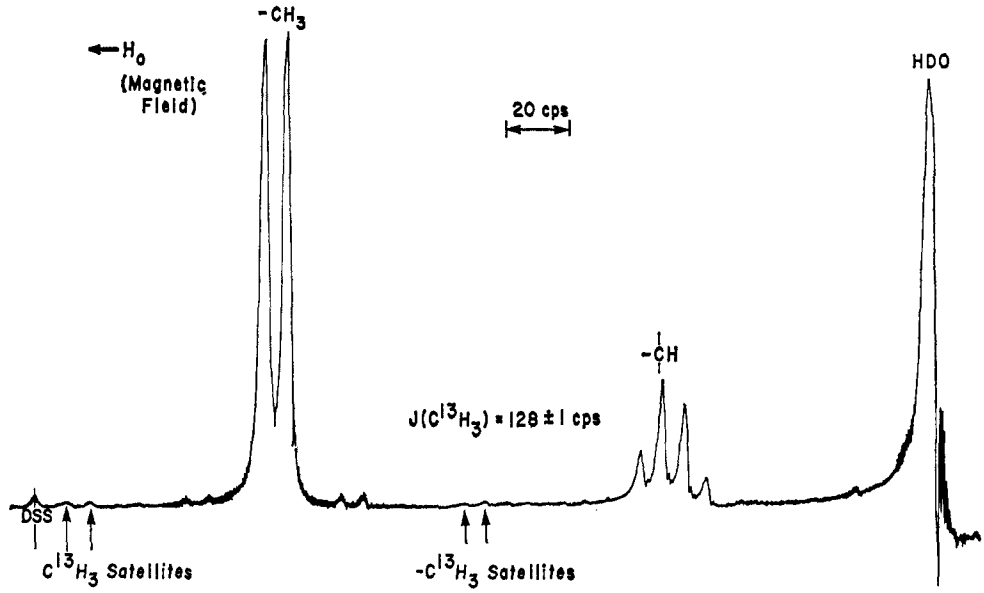


Figure 2. Alanine, pD 11.2, locked on TFA.

used as the lock, 60-cycle and 120-cycle side bands about the lock will often interfere with the C^{13} satellite measurements.) The only purpose of the "lock" proton is to hold the field constant and common for proton and carbon-13. Therefore, it makes no difference whether the proton is a solution proton (*i.e.*, an internal proton) or a proton in an external capillary. All shifts are referenced to DSS (TMS) which is internal to the solution. Consequently, susceptibility corrections need not be made for locking on an external capillary proton.

Our results are all given relative to benzene. The interested reader can convert these results back to a Γ_i and $f_c(i)$ for either a HDO or TFA proton lock in the double-resonance experiment by using (1a–1c).

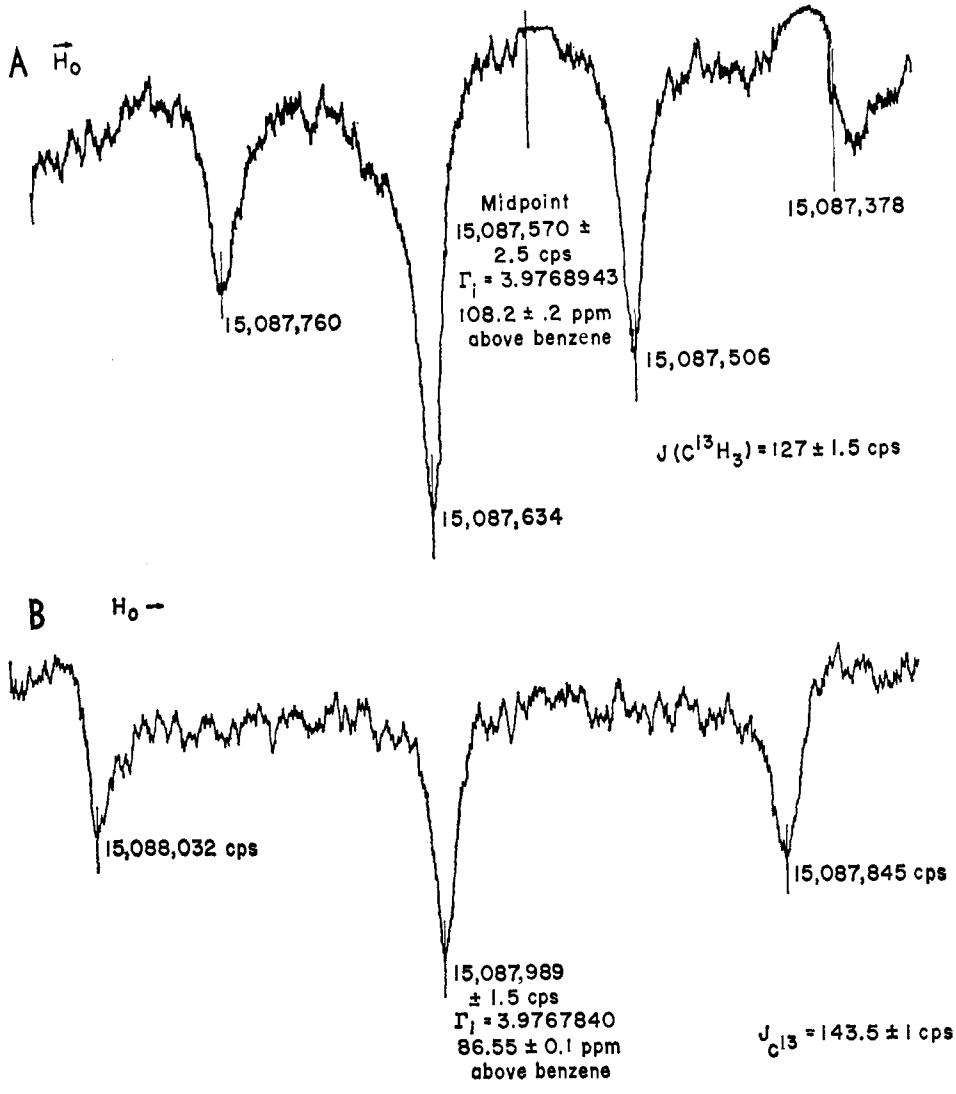
Results

A typical proton spectrum and some sample indor spectra are shown in Figures 2 and 3. All spectra are single sweep using a 10-sec time constant. The sweep rate of the C^{13} transmitter was approximately 0.3 cps/sec. The proton spectra generally have small spinning side bands from the main $(C^{12})H$ peaks. If one reduces the C^{13} radiofrequency power, the indor line widths decrease, and the dip intensity remains constant, until the width is somewhat greater than the proton line widths, whereupon the dip intensity decreases. By using as low C^{13} radiofrequency as is practical, and narrowing the C^{13} sweep range, one can measure the resonances accurately to $\sim\pm 0.5$ cps under the most favorable circumstances.

Glycine and alanine (methyl) generally show the expected triplet and quartet patterns corresponding to the C^{13} coupling with two and three protons, respectively. The carbon-13 proton coupling constants obtained from the indor are in excellent agreement with measurements from the proton spectra. In the example shown in Figure 3, the $C^{13}H_3$ resonance of alanine is asymmetric and deviates from the expected 1:3:3:1 quartet intensity. In fact, one of the upfield lines shows a dispersion-like line shape rather than a negative absorption characteristic of indor spectra. This asymmetry is typical of those cases where one does not "sit" exactly on the center of the carbon-13 satellite line in the proton spectrum. Locating the exact center turns out to be very tedious in practice. Small spinning side bands from the main $C^{12}H$ peaks and carbon-13 satellites from other proton groups in the spectrum often overlap or distort the carbon-13 satellite of interest (in fact, sometimes the satellite is so obscured in the proton spectrum that the indor technique is used to locate the satellite). We generally accept slightly asymmetric spectra similar to the above, since there is no difference, to within $\sim$1–3 cps, in the midpoint resonance position between these and the symmetric indor spectra.

We present the results of our pD study in Figures 4–7. In the case of the dipeptides we use α to denote the carbon bonded to the terminal carboxyl, and β the carbon bonded to the terminal amino group. In triglycine (g_3), α denotes the carboxyl-terminal methylene, while γ denotes the N-terminal methylene. Sheinblatt[9] has done a pH proton study of glycine, diglycine, and triglycine. Since his results were referenced to an external H_2O sample, we thought it would be of value to also present the proton pD results referenced to internal DSS. Glycine was measured in some detail from pD 1 to 12. The pK values for the NH_3^+ and COOH groups were found from the carbon-13 and proton resonances to be 9.75 and 2.35, respectively, in excellent agreement with the more routine methods.[10] Because of the tedious nature of the measurements, we did not carry out as an extensive pD study of di- and triglycine and alanine as we did of glycine. Measurements were taken to establish pD trends, and curves were drawn through the data using pK estimates. The results, nevertheless, clearly establish that, in all cases when the molecule loses a proton, the proton resonances shift upfield, while the carbon-13 shifts downfield. The titration curves are often asymmetric: the carbon-13 shifts are greater when a NH_3^+ proton is lost than when a COOH proton is lost. The carbon-13 proton coupling constants are shown as a function of pD in Figure 7. There is a pronounced increase in the glycine $J(C^{13}H_2)$ during the protonation of the amino group (p$K \approx 9.7$).

(9) M. Sheinblatt, *J. Am. Chem. Soc.*, **88**, 2123, 2845 (1966).
(10) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press Inc., New York, N. Y., 1958, pp 452.



GLYCINE (-$C^{13}H_2$-), 400 cps SWEEP, pD = 6.5, HDO UPPER SIDEBAND LOCK

Figure 3. (A) C^{13} resonances of alanine ($C^{13}H_3$), 500-cps sweep, pD 11.2, TFA upper side-band lock. (B) C^{13} resonances of glycine ($C^{13}H_2$), 400-cps sweep, pD 6.5, HDO upper side-band lock.

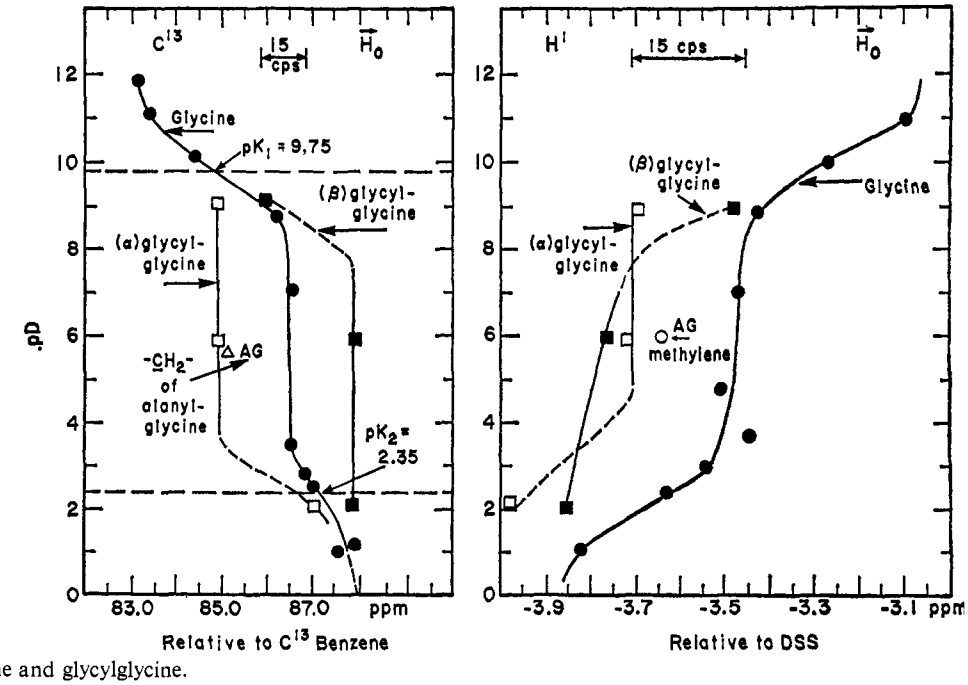


Figure 4. Glycine and glycylglycine.

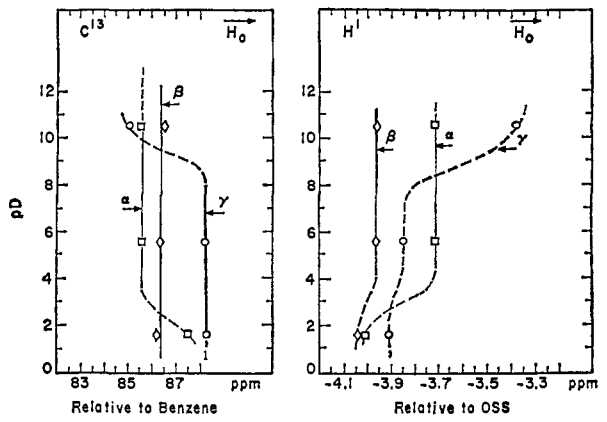


Figure 5. Triglycine.

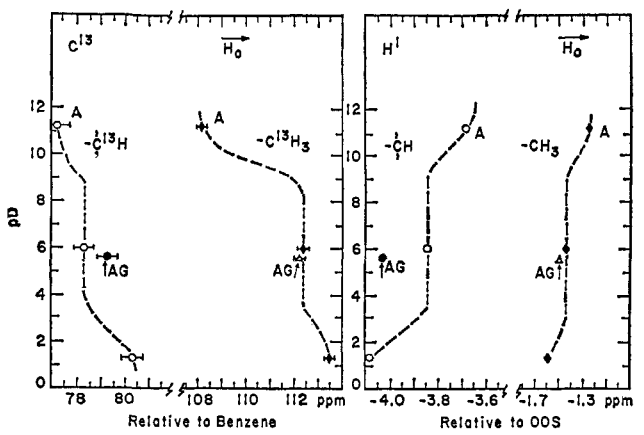


Figure 6. Alanine (A) and alanylglycine (AG).

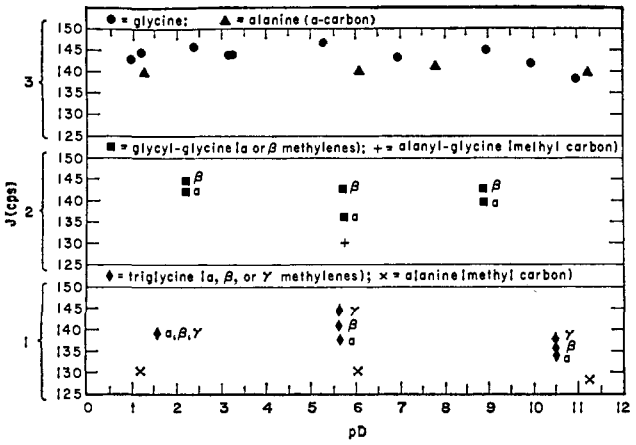
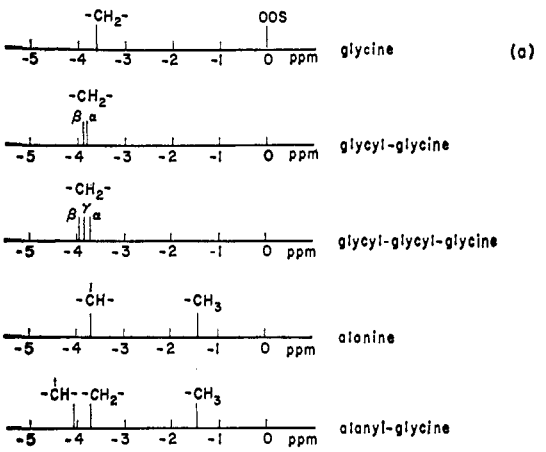
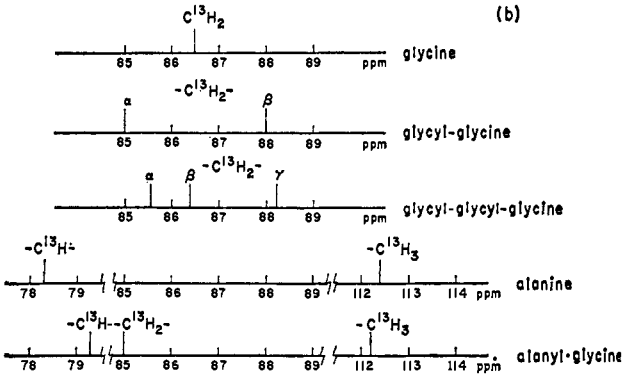


Figure 7. H^1–C^{13} coupling constants as a function of pD.

Carbon-13 magnetic resonance, like proton magnetic resonance,[9] can be used in the nondestructive analysis of sequences in the di- and tripeptides. In diglycine the α- and β-methylene resonances at neutral pD's are symmetrically placed about the glycine resonance at $\approx$86.5 ppm—the α-methylene resonance occurring downfield of the β-methylene. Furthermore, the α- and β-methylene shift with pD are independent of each other. In triglycine, neutral pD, the middle, *i.e.*, β-methylene has essentially the same resonance frequency as glycine at neutral pD. The α and γ resonances are, respectively, downfield and upfield of the β resonance, as expected from the diglycine results.



(a)



(b)

Figure 8. Comparison of H^1 and C^{13} chemical shifts in ppm (pD 6) relative to DSS and benzene.

The α carbon of alanine occurs 8.5 ppm downfield of the α carbon of glycine, while the methyl carbon-13 of alanine occurs $\approx$25 ppm above glycine. These C^{13} shifts are large indeed compared with protons (Figure 8). Despite the large shifts that occur in the carbon-13 spectra, the shifts may not be very dependent on neighbor residues. For example, the methylene carbon-13 of alanylglycine, is within $\approx$0.15 ppm of (α) glycylglycine. This degree of neighbor sensitivity is comparable to what is observed in the proton spectra.

Discussion

A. The Amino Acids as Substituted Hydrocarbons. Grant and coworkers have found that if one uses a suitable set of carbon-13 chemical shift parameters then (1) the resonances of many substituted alkanes can be predicted very accurately from the parent hydrocarbon resonance;[11] (2) the parent hydrocarbon resonances in turn can be derived from methane.[12] For example, in the case of the alkanes, if a proton is replaced by a methyl, one measures an $\sim$9 ppm downfield shift in the α carbon, an $\approx$9 ppm downfield shift in the β carbon, an $\approx$2.5 ppm upfield shift for the γ carbon, and small downfield shifts for the δ and ϵ. Methane occurs at 131 ppm above benzene. Ethane occurs at $\approx 131 + (-9)_\alpha = 122$ ppm; the end carbons of propane occur

(11) T. D. Brown, Ph.D. Thesis, University of Utah, Salt Lake City, Utah, 1966.

(12) D. M. Grant and E. G. Paul, *J. Am. Chem. Soc.*, 86, 2984 (1964).

Table I. "Predicted" Carbon-13 Shifts (ppm) of Several Amino Acids[a] Relative to the α Carbon of Glycine at 86.5 ppm above Benzene

Amino acid	$C_1 = C_\alpha$	C_2	C_3	C_4	C_2'
Alanine (NC_1C_2) C_0	−8 (−8.0 ± 0.4 obsd)	**+23.0** (25.9 ± 0.2 obsd)	...	...	...
Valine C_3 $(NC_1C_2C_3)$ C_0	−26.5	+4	**+25**	...	...
Leucine C_4 $(NC_1C_2C_3C_4)$ C_0	−13.5	−3	+19.0	**+20**	...
Isoleucine C_2' $(NC_1C_2C_3C_4)$ C_0	−24	−1	+17.5	**+30**	**+27**

[a] The methyl carbons are in bold type.

at $\approx 131 + (-9)_\alpha + (-9)_\beta = 113$ ppm while the middle carbon occurs at $\approx 131 + 2(-9)_\alpha = 113$ ppm.

If a substituent other than a methyl group is introduced, one must use a different set of chemical shift parameters. In the amine case these shifts[12] relative to the parent hydrocarbon are, respectively, −29, −11.4, and +4.6 ppm for a carbon α, β, and γ to the amino group. In methylamine the carbon resonance is predicted at $\approx 131 - 29 = 102$ ppm (observed: 100.5 ppm); in ethylamine, the α carbon is predicted to be at $\approx 122 - 29 = 93$ ppm, the β carbon at $\approx 122 - 11.4 = 110.6$ ppm (observed: 91.8 and 109.7 ppm, respectively).

In the case of a COOH group substituent, we found from measurements on neat acetic, propionic, and butyric acids that the shift parameters relative to the parent hydrocarbon are approximately −21.5, −2, and +2.5 ppm, respectively, for a carbon α, β, and γ to the COOH group. (Measurements on ≈ 3 *M* acetic acid in D_2O were also done to determine the sensitivity of the α-shift parameter to concentration. We obtained −23 ± 0.5 ppm at this lower concentration.) The amino acids are geminally disubstituted hydrocarbons. To the extent that the shift parameters are additive for a geminal disubstitution involving amino and carboxyl groups, one predicts for the un-ionized form of the amino acids that the α carbons of glycine and alanine and the methyl carbon of alanine will occur, respectively, at: 131 − 29 − 21.5 = 80.5 ppm; 122.8 − 29 − 21.5 = 72.3 ppm; 122.8 − 11.4 − 2 = 109.4 ppm. If we make the assumption that at neutral pD, the dipolar form of the amino acids predominates, we can estimate the resonances of the un-ionized form to an accuracy of about 1 ppm from Figures 4–6.

$$\text{un-ionized} = \text{anionic} + \text{cationic} - \text{dipolar}$$
$$[NC = (NC^-) + (N^+C) - (N^+C^-)]$$

We obtain 85, 79, and 109.5 ppm, respectively, for the "experimental" shifts of the α carbons of glycine and alanine and the methyl carbon of alanine in the un-ionized state. (In the dipolar state the experimental values are 86.5, 78.4, and 112.5 ppm, respectively.)

The "predicted" and "observed" shift values are in fairly good agreement. The α carbon of alanine, for example, is predicted to be ≈ 8 ppm downfield of glycine. This is what is observed within the experimental error. The "observed" α-carbon shift values, however, are ≈ 6 ppm upfield from the predicted values. It would appear that if one uses the shift parameters derived from monosubstituted hydrocarbons, one would overestimate the α-carbon shifts relative to the parent hydrocarbon by 6 ppm in the case of the amino acids.

In Table I we give the "predicted" resonances for alanine, valine, leucine, and isoleucine at neutral pD relative to glycine observed at 80.5 + 6 = 86.5 ppm using (1) Grant's shift parameters (with refinements) for hydrocarbons,[12] (2) a suitable set of chemical shift parameters (Table II) for substituted hydrocarbons.

Table II. Chemical Shift Parameters (ppm)

Family of compounds	Carbon position	Parameters
Amines	α	−29.0
	β	−11.4
	γ	+4.6
	δ	−0.6
	ϵ	−0.5
Carboxylic acid (-COOH)	α	−21.5
	β	−2
	γ	+2.5
	δ	−0.6[a]
	ϵ	−0.5[a]

[a] Not experimentally determined. These shift parameters were assumed to be essentially the same as the amines and alcohols. The magnitude of the δ- and ϵ-shift parameters may actually be slightly overestimated.

The amine values are derived from Brown's thesis.[11] A 6-ppm upfield correction has been added to all the α-carbon shifts (including glycine's). The predicted values in Table I are expected to be accurate within a few parts per million. Measurements on valine, leucine, and isoleucine are currently in progress in this laboratory and will be reported shortly.

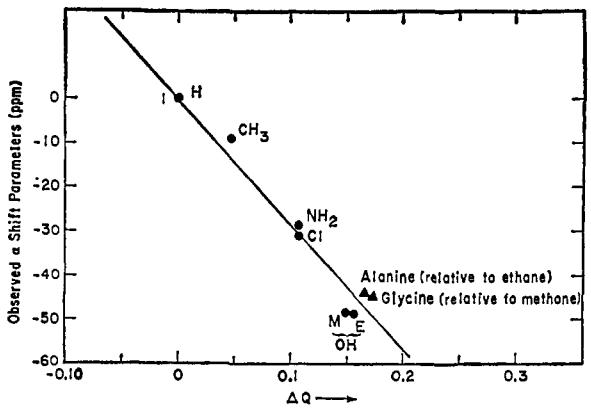


Figure 9. α-Shift parameters *vs.* ΔQ. ΔQ denotes the charge density change of the α carbon in the substituted hydrocarbon relative to the parent hydrocarbon.

A number of interesting observations can be made from Table I. Firstly, the α carbon of glycine in the amino acid series is analogous to the methane carbon in the hydrocarbon series. The amino acids are disubstituted hydrocarbons. The shift of the α carbon in the amino acids relative to glycine is predicted to be essentially the same as the shift relative to methane of the corresponding carbon in the parent hydrocarbon. Secondly, the carbon-13 resonances from the four amino acids listed in Table I are predicted to extend over 50 ppm. This contrasts with the 3-ppm spread observed in the proton resonances. The C^{13} methyl shifts of valine, leucine, and isoleucine, in particular, are predicted to extend over 10 ppm. This contrasts with the proton methyl spread of about 0.1 ppm. It should eventually be possible to use these large carbon-13 shifts relative to protons for conformational and chemical studies of proteins in solutions.

B. The α-Chemical Shift Parameters. The α-shift parameters of substituted hydrocarbons are generally inductive in origin.[11,13,14] This was shown quite clearly by Brown[11] who plotted the shifts of the α carbon (relative to the parent hydrocarbon) against the electronegativity of the substituent. The electronegativity values were essentially that determined by Dailey and Shoolery.[15] This was done for alcohols, amines, chlorides, bromides, iodides, and cyanides. The α-shift parameters plotted linearly against the electronegativity, with the α resonance shifting downfield with increased substituent electronegativity. Furthermore, the charge change of the α carbon relative to the parent hydrocarbon could be estimated using Pauling's procedure.[16] Brown found that a plot of the α-shift parameter *vs.* the α-carbon charge change gave[17] a straight line with slope 200 ppm/electron, in excellent agreement with the empirical value of $\approx$160 to 190 ppm/electron deduced by Spiesecke and Schneider[18] and Lauterbur[19] from shift studies of the series $C_5H_5^-$, C_6H_6, $C_7H_7^+$, and $C_8H_8^{2-}$.

Del Re[20] and Smith, *et al.*,[21] have developed a simple semiempirical method for calculating inductive effects and charge distributions in saturated systems. This semiempirical molecular orbital method has been applied by Pullman and coworkers to heteronuclear molecules in general[22] and amino acids[23] in particular. Recently, a number of more rigorous approaches to calculations of charge distribution in saturated systems have been developed.[24,25] However, in view of the ease of application and the extensive use of the semiempirical approach in the literature, we thought it would be of some interest to compare the observed shift parameters with charge density changes calculated from the semiempirical method.[20,23] The semiempirical approach for heteroatoms uses a set of inductive parameters, derived in part from electronegativities and checked against molecular dipole moments and bond dissociation energies, to modify the coulomb and exchange integrals of hydrocarbons. One might expect to obtain reasonably accurate charge density changes using the semiempirical method because of its builtin bias in that direction, a consequence of the induction parameters having been empirically related to the bond electronegativities. The α-shift parameters depend primarily on the electronegativities of the substituents. To the extent that the set of inductive parameters are derived self-consistently and have physical validity, one would expect to be able to use the semiempirical method to predict the α-shift parameters from the charge density changes. Ideally, a linear dependence of shifts on charge density changes with slope $\approx$160–200 ppm/electron is expected. Significant deviations from linearity, or from a slope $\approx$160–200 ppm/electron, would cast doubt on the internal consistency of the inductive parameters and on the physical validity of the semiempirical method. In Figure 9 we plot the observed α-shift parameter as derived from Brown's thesis against the charge density change, ΔQ, as determined from the semiempirical method. ΔQ denotes the charge density change of the α carbon in the substituted hydrocarbon relative to the parent hydrocarbon. The α carbon charge densities in substituted hydrocarbons are given in the papers by Del Re[20] and Del Re, *et al.*[23] The plot, except for point E, is actually based on the charge

(13) P. C. Lauterbur, *Ann. N. Y. Acad. Sci.*, **70**, 841 (1958).
(14) H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 722 (1961).
(15) B. P. Dailey and J. N. Shoolery, *J. Am. Chem. Soc.*, 77, 3977 (1955).
(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, pp 97–98.
(17) The charge density change on the carbon is believed to primarily affect the paramagnetic term of the chemical shielding expression [J. A. Pople, *Mol. Phys.*, 7, 301 (1963–1964)]. The paramagnetic term is due to the induced electronic currents about the nucleus. These currents result from mixing of the ground and excited states by the magnetic field. The paramagnetic term is approximated (J. A. Pople, reference above) by an expression of the form

$$-K\langle 1/r^3\rangle_{2p}/\Delta E$$

where K is a positive constant that is relatively insensitive to charge density changes on the carbon. The effect of the charge density change is primarily on the $\langle 1/r^3\rangle_{2p}/\Delta E$ product. ΔE is a mean excitation energy, and $\langle 1/r^3\rangle_{2p}$ is the mean $1/r^3$ value of a 2p carbon atom (r denotes the distance of the 2p electron from the carbon nucleus). $\langle 1/r^3\rangle_{2p}$ is believed to be particularly sensitive to charge density change. Increasing the electronic charge density on the carbon causes the 2p electron, on the average, to be further away from the carbon nucleus; $\langle 1/r^3\rangle$ decreases and the paramagnetic term becomes less negative, the shielding increases, and the resonances shift to higher field with increasing electronic charge density.
(18) H. Spiesecke and W. G. Schneider, *Tetrahedron Letters*, **14**, 468 (1961).
(19) P. C. Lauterbur, *J. Am. Chem. Soc.*, **83**, 1838, 1846 (1961).
(20) G. Del Re, *J. Chem. Soc.*, 4031 (1958).
(21) R. P. Smith, T. Ree, J. L. Magee, and H. Eyring, *J. Am. Chem. Soc.*, **73**, 2263 (1951).
(22) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, New York, N. Y., 1963.
(23) G. Del Re, B. Pullman, and T. Yonezawa, *Biochim. Biophys. Acta*, **75**, 153 (1963).
(24) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966).
(25) R. Hoffmann, *ibid.*, **39**, 1397 (1963).

change of the α carbon of the methane substituents relative to methane, but should hold generally. For example, point M is derived for methanol relative to methane, while point E is derived from ethanol relative to ethane. These authors do not give the charge density in methane and ethane explicitly. We calculate[26] the carbon charge density in methane and ethane to be -0.164 and -0.117, respectively, using the Del Re–Pullman procedure and inductive parameters.[20] Point 1 has an α-shift parameter of zero and a ΔQ of zero, by definition. The best line, that will fit the data and pass through point 1, has a slope of -280 ± 10 ppm/charge.

Figure 9 can be used to estimate the α-carbon resonances of the amino acids. For example, the charge density change of the dipolar form alanine and glycine relative to ethane and methane are 0.166 and 0.169, respectively, according to the semiempirical approach.[23] For these charge changes, the total α shifts relative to the parent hydrocarbon are predicted to be -47.5 and -48.5 ppm, respectively. The alanine α-carbon resonance would be expected at $122.8 - 47.5 = 75.3$ ppm (observed: 78.5 ± 0.4 ppm); the glycine α resonance at $131.2 - 48.5 = 82.7$ ppm (observed: 86.5 ± 0.15 ppm). The predicted and observed shifts relative to the parent hydrocarbon agree within $\approx 8\%$.

The semiempirical method is thus seen to give a linear plot of α shift *vs.* charge density change. Furthermore, it is possible to fairly accurately predict α shifts from the calculated charge density changes, as illustrated for glycine and alanine. This suggests that the method is internally consistent although refinements are in order. The slope of -280 ppm/charge of Figure 9 is 40–60% larger in magnitude than we feel should be the true value[11,18] of -160 to -200 ppm/charge. The reason for this discrepancy between slopes and what form the refinement should take are not clear to us. It would be premature to conclude that the Del Re–Pullman semiempirical approach underestimates[27] the actual charge density and charge density change in (saturated) substituted hydrocarbon systems by 40–60%.

A few words concerning the β- and γ-shift parameters are in order. These parameters are not understood at present. The β-shift parameters are very often independent of the substituent. For example, in the case of an OH, NH_2, CH_3, Br, Cl, or I substituent, the β-shift parameter has a value of $\approx -10 \pm 1$ ppm. In the case of a COOH substituent, however, we obtained a value of ≈ -2 ppm. A small β-shift parameter has also been observed by Grant and coworkers (unpublished results) in the case of C≡N. Why the β-shift parameters of the carboxyl and cyanide groups should behave differently is not clear to us. It has been suggested that the β and γ parameters involve contributions from inductive effects, electron delocalization, and steric effects. Grant and Cheny,[28] in fact, were able to calculate the γ-shift parameter in hydrocarbons and explain peculiarities in the methylbenzene carbon-13 spectra in terms of steric induced shifts. (If steric effects are indeed important, as seems to be the case, a protein molecule would be expected to show pronounced strain or steric induced shifts upon folding. This would provide further insights in the conformation although interpretations may initially be difficult.)

C. The Anomalous Carbon-13 Shifts with pD. The carbon-13 pD results (Figures 4-6) are curious in two respects. Firstly, the changes in the α resonances upon protonation of the amino group (or ionization of the COOH group) are surprisingly small. For example, if we replace a hydrogen in a hydrocarbon by an amino group, the α carbon is observed to shift approximately 30 ppm downfield. This is unquestionably due to an inductive effect. The neutral, yet more electronegative, amino group induces a positive charge density change on the carbon. Protonating the amino group of the amino acids introduces a $+1$ charge, yet this charge is relatively ineffective in changing the carbon charge density as evidenced by the small chemical shift change relative to the unprotonated amino group. The small shift that is observed brings us to the second curious aspect. The shifts observed with pD suggest that the charge density changes on *all* the carbons are in the opposite direction from what one would have expected from the inductive model only. For example, we would conclude from the inductive model that the positively charged amino group draws in electronic charge from the neighboring carbon, which in turn draws in electronic charge from the hydrogens of carbons bonded to it. Presumably, the primary induction of electronic charge, C → N, dominates the sum total of the secondary inductions H → C–N or C → C–N, and all nuclei should show a positive charge density increase. One would consequently expect a downfield shift of all resonances in both the C^{13} and H^1 spectra corresponding to a decrease in electron shielding; however upfield shifts are observed in the carbons. In a similar fashion, one would predict from inductive considerations that the carbon-13 and proton resonances should shift upfield upon ionization of the carboxylic acid group (pD $\approx$ 2.3). Downfield shifts are observed in the carbon-13 case.

This anomalous behavior is not peculiar to the amino acids. It occurs, for example, in the amines, in the carboxylic acids, and in phosphoric acid[29] systems. In methylamine we have measured an upfield shift of 1.5 ± 0.4 ppm in the C^{13} resonance upon protonation (101.5 ppm at pH 13, and 103.0 ppm at pH 2.8). The proton resonance, on the other hand, shifts downfield by 0.5 ppm upon protonation. In the case of propionic acid[30] all the carbons shift downfield, and all the protons upfield, upon ionization of the COOH group. The carboxyl carbon shifts 4.7 ppm, the α carbon 3.5 ppm, the β carbon 1.7 ppm. Essentially the same α- and β-carbon shifts are observed in our amino acid studies.

(26) The Del Re–Pullman procedure assumes a CH bond dipole between 0.2 and 0.28 D. The hydrogen, therefore, has a charge density between 0.04 and 0.05.

(27) A comparison of the semiempirical charge densities with those obtained from self-consistent field calculations shows that the semiempirical method does not consistently underestimate charge density. For example, in NH_3, the semiempirical charge densities on the atoms are indeed a factor of 280/200 smaller than Clementi's *ab initio* calculation [E. Clementi, *J. Chem. Phys.*, 47, 2323 (1967)]. However, in the case of H_2O, the semiempirical method charge densities are overestimated by a factor of about 3 relative to the *ab initio* calculation [J. Rosenfeld, *ibid.*, 40, 384 (1964)]. Nevertheless, it may be that for (saturated) substituted hydrocarbons, the particular choice of indicative parameters used leads to an underestimation of charge density changes.

(28) D. Grant and B. Cheny, *J. Am. Chem. Soc.*, **89**, 5315, 5319 (1967).

(29) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, **235**, 3250 (1960).

(30) J. D. Roberts, private communication.

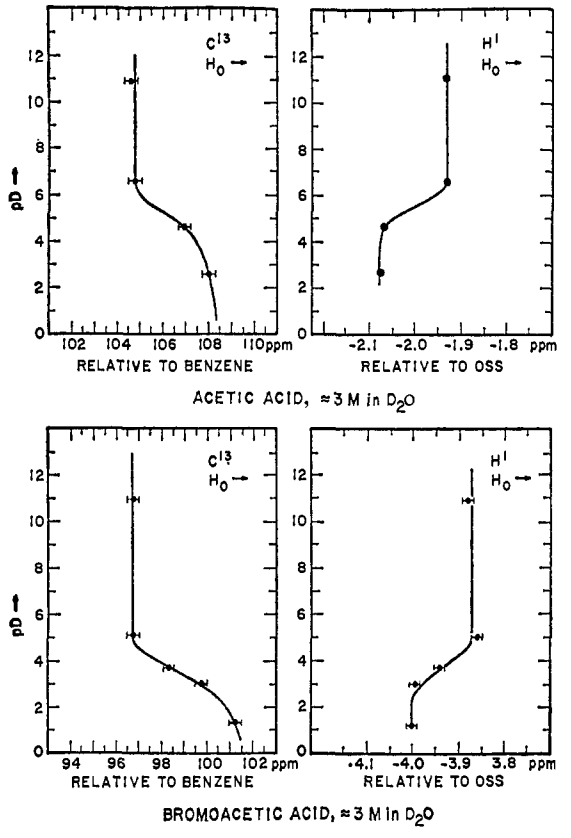


Figure 10. A comparison of the bromoacetic acid carbon-13 and proton shifts with acetic acid as a function of pD.

The carbon-13–proton coupling constants of both the α and β carbons of the amino acids are observed to increase upon protonation of the amino group as shown in Figure 7. (In methylamine a similar behavior is observed. J increases from 135 to 142 ± 1.5 cps.) The increase is much larger for the α than for the β carbon. However, no change within the experimental error is observed in the coupling constants upon ionization of the COOH group. Changes in the coupling constants in hydrocarbons are generally indicative of changes in the bond hybridization.[31] For heteronuclear systems other factors such as charge density changes on the carbon are important.[32]

We do not think a hybridization change is responsible for the anomalous shifts of the carbon-13 for the following reasons.

(1) The methyl carbon in alanine shifts upon amino group protonation by the same amount as the α carbon of glycine despite the smaller change in its coupling constant.

(2) The anomalous carbon-13 shift observed upon COOH ionization is not accompanied by any detectable change in the coupling constant within the experimental error of ±1.5 cps. Nevertheless, the shifts that do occur are often comparable in magnitude to that observed upon amino group protonation.

In view of these considerations, and the fact that the proton and carbon shifts are always in opposite directions, we think that a C–H bond polarization arising from the positive (NH_3^+) or negative (COO^-) charged group is most probably responsible for the anomalous C^{13} shifts rather than a hybridization change. This hypothesis is further substantiated by the glycylglycine and triglycine resonances (Figures 4 and 5). In the case of glycylglycine, the α- and β-methylene groups, bonded respectively to COO^- and NH_3^+, have resonances which are *symmetrically* placed about the resonance of the neutral, *i.e.*, dipolar, form of glycine. Furthermore, the α-methylene shift with pD is independent of the β-methylene shift with pD. The α- and β-methylene shifts have limiting values which coincide with those of glycine.

In Figure 10 we present a comparison of the C^{13} and proton shifts in acetic acid and bromoacetic acid at ≈ 3 M concentration in D_2O as a function of pD. The polarizability of the C–Br bond is four times larger than that of the C–H bond.[33] The proton shifts with pD upon ionization is the same (0.15 ppm upfield) in the two samples. The carbon-13 shift with pD of the bromoacetic acid, however, is 60% greater than that of acetic acid. A greater C^{13} shift upon ionization in the bromoacetic acid case was indeed expected. The negative charge on the carboxyl polarizes the C–Br and C–H bonds, so that the bromine or hydrogen gains electronic density whereas the carbon loses electron density. Since the C–Br bond polarizability is largest, the carbon would lose more electron density in the bromoacetic acid case. Consequently a larger pD-induced downfield shift in the bromoacetic acid C^{13} resonance relative to acetic acid is expected. (Whether the observed 60% increase in the downfield shift of the C^{13} can indeed be ascribed to the above mechanism is uncertain at present.)

The polarization effect we are referring to can occur both through the bonds as an inductive effect and through space as an electric field effect. Consider the following illustrative example involving CH_3–X. A $+q_0$ charge is introduced into the X substituent. The methyl group relaxes from its initial charge distribution in the absence of q_0 to a new distribution. We describe this relaxation by a series of "virtual" charge adjustments. (a) A charge q_0 is introduced on X. (b) q_0 polarizes the C–X bond, drawing in the electronic charge, $-\delta q_1$, from the carbon. (c) The positive charge, $+q_0 - \delta q_1$, on X produces an electric field. This electric field polarizes each CH bond and induces a charge, δq_E, on every hydrogen, and a $-3\delta q_E$ charge on the carbon. (d) Each CH bond now "sees" at the carbon a charge density change equal to $\delta q_1 - 2\delta q_E$. (The carbon sp^3 orbital participating in the C–X bond has undergone a δq_1 charge density change; the other two sp^3 orbitals participating in C–H bonds each have a charge density change equal to $-\delta q_E$.) This excess charge polarizes each C–H bond and induces a $+\delta q_{11}$ and $-\delta q_{11}$ charge, respectively, on the hydrogen and carbon. The total charge density changes are: X, $q_0 - \delta q_1$; C, $\delta q_1 - 3(\delta q_{11} + \delta q_E)$; H, $\delta q_{11} + \delta q_E$.

The reason for the small shift of the α carbon upon protonation of the amino group or ionization of the COOH becomes clear if $3(\delta q_{11} + \delta q_E) \approx \delta q_1$.

If $3(\delta q_{11} + \delta q_E) > \delta q_1$ a negative charge density change occurs at the α carbon upon introducing a positive q_0 at X. If a negative charge density change does develop at the α carbon, a propagation of negative charge down the carbon chain is plausible. For ex-

(31) J. N. Shoolery, *J. Chem. Phys.*, 31, 1427 (1959).
(32) D. Grant and W. Litchman, *J. Am. Chem. Soc.*, 87, 3994 (1965).
(33) K. G. Denbigh, *Trans. Faraday Soc.*, 36, 936 (1940).

ample, if one of the protons of the methyl group of CH_3–X ($+q_0$) is replaced by a carbon to give a substituted ethane, the charge density on the β carbon would be

$$\approx(\delta q_{11} + \delta q_E) - 3(\delta q_{11}^{\beta} + \delta q_E^{\beta})$$

δq_{11}^{β} denotes the charge density change on the β hydrogen because of the "through-bond" induction effect of q_0, while δq_E^{β} denotes the charge density change on the β hydrogen from the "through-space" induction effect of q_0. If $3(\delta q_{11} + \delta q_E) > \delta q_1$, making the total charge density change on the α carbon negative, it is reasonable to expect that $3(\delta q_{11}^{\beta} + \delta q_E^{\beta}) > \delta q_{11} + \delta q_E$, making the charge density change on the β carbon negative. This effect could propagate down a chain of carbons. (The "through-space" induction, if not initially dominant, could become increasingly more important, since it would decrease less rapidly down the chain than the "through-bond" induction. This could occur in nonpolar solvents having low dielectric constants.) In general, what we can end up with is a transmission of electronic charge from the hydrogens, through the chain carbons whose charge densities remain essentially unaffected (possibly acquiring a small negative charge), unto the positive charge substituent, X ($+q_0$). This mechanism is expected to be operative in many systems, causing anomalous shifts with pH, for example, in the resonances of atoms whose valences are greater than one. The phosphoric acid system[29] is probably one such example.

A similar effect might be expected in the $NH_3 + H^+ \rightarrow NH_4^+$ system. A proton appears at the lone pair of the nitrogen, forms a bond, and induces a charge δq_1 on the nitrogen. Virtual charge adjustments similar to steps b–d above occur. The net charge on the nitrogen is $\delta q_1 - 3(\delta q_{11} + \delta q_E)$. If $3(\delta q_{11} + \delta q_E) \approx \delta q_1$, the three initial hydrogens in ammonia would be observed to transmit electronic charge to the added proton, while the nitrogen charge density remains essentially unchanged. This is indeed found by Clementi[34] from his *ab initio* calculations. (The charge densities of N and H in NH_3 were calculated to be: N, −1.08; H, +0.36. In NH_4^+ the densities were calculated to be: N, −1.10; H, +0.53. The nitrogen density remains essentially constant upon protonation.)

The methylamine–methylammonium nmr results are also indicative of a transmission of electronic charge from the amino hydrogens through the carbon to the protonating hydrogen. In methylamine (40% in H_2O, pH 13.0) the C^{13} and H^1 resonances occur −30 and −2 ppm below methane, respectively. Protonation of the amino group shifts the C^{13} upfield to −28.0 ppm and the H^1 downfield to −2.5 ppm below methane, respectively. This is suggestive that the methyl protons have experienced an increase in charge density upon amino protonation, while the carbon has undergone only a very small, probably negative, charge density change. We would be very interested to see an *ab initio* calculation done on methylamine and methylammonium ion.

Rosenfeld[35] has done a self-consistent field calculation of H_2O, H_3O^+, and H_4O^{2+}. The results of his calculation are reproduced in Table III. This calculation, like all theoretical calculations at present, is subject to uncertainties as to how to subdivide overlap population. Nevertheless, here again a transmission of electronic charge from the initial hydrogen (through the oxygen) to the added proton is predicted. In going from H_2O to H_3O^+, the initial hydrogens undergo a +0.33 net charge density change, whereas the oxygen undergoes a −0.13 net charge change. The pH-induced carbon-13 shifts in the amino acids (Figures 4–6) are thus indicative that protonation of the amino group to give NH_3^+ (or ionization of the carboxyl groups to give COO^-) is accompanied by transmission of negative (positive) charge from the hydrogen through the carbons to the NH_3^+ (COO^-) group. The carbon charge densities remain essentially constant,[36] probably becoming slightly more negative (positive) upon protonation (ionization).

Table III. The Self-Consistent Field Charge Distribution in Some Oxygen Hydrides

	Charge on H	Charge on O
H_2O	+0.133	−0.266
H_3O^+	+0.464	−0.391
H_4O^{2+}	+0.639	−0.555

We now estimate carbon-13 shifts as a function of electric field. Buckingham[37] calculated the electric field contribution to the C–H proton chemical shift to be

$$\Delta\sigma_H = -2 \times 10^{-12} E_{11}\,(\text{cgs}) = -A_H E_{11}$$

Subsequent estimates[38] vary from -2.5 to $-3.5 \times 10^{-12} E_{11}$. E_{11} is the field component along the bond direction. A positive E_{11} is directed from the carbon to the hydrogen and draws electron density from the hydrogen to the carbon, decreasing the electron shielding about the hydrogen and causes a downfield shift in the proton resonance.

The constant A_H was derived using perturbation theory.[37,38] We find the following approach conceptually simpler, and more useful for our purposes. The constant electric field polarizes the C–H bond, inducing a dipole moment, μ. Let b_{11} denote the bond polarization along the bond axis. $|\mu|$ is given by

$$|\mu| = |e|\delta q_E R = b_{11} E_{11}$$

R is the C–H bond length, $|e|$ the electronic charge, and δq_E is the induced charge density on H. δq_E is given by

$$\delta q_E = \frac{b_{11}}{|e|R} E_{11}$$

The electron at the H atom moves essentially in a 1s

(34) E. Clementi, *J. Chem. Phys.*, 47, 2323 (1967).
(35) J. L. J. Rosenfeld, *ibid.*, 40, 384 (1964).

(36) In our interpretation of the pH effects on chemical shifts, we have assumed that the carbon-13 shifts predominantly depend on the carbon charge density. We have neglected (1) changes in bond order and (2) delocalization effects arising from deviations from perfect pairing as discussed by D. Grant and B. Cheney [*J. Am. Chem. Soc.*, 89, 5319 (1967)] in their valence bond treatment of the chemical shift. Protonation of the nitrogen lone pair in methylamine, for example, can change the N–C_α bond order, and if this change is large, the chemical shift could be significantly affected. There is some theoretical evidence that changes in the N–C_α bond order are indeed large in the case of pyridine, where upfield shifts in C_α are observed upon protonation (D. Grant, *ibid.*, in press).
(37) A. D. Buckingham, *Can. J. Chem.*, 38, 300 (1960).
(38) J. I. Musher, *J. Chem. Phys.*, 37, 34 (1962).

hydrogen orbit. The shielding in a hydrogen atom is the Lamb value of 17.6 ppm. Decreasing the electronic charge density by an amount δq_E would produce a downfield shift.

$$\Delta\sigma_H = -17.6 \times 10^{-6}\delta q_E = \frac{-17.6 b_{11}}{|e|R} E_{11} \times 10^{-6}$$

Using $R = 1.09 \times 10^{-8}$ cm, $b_{11} = 0.79 \times 10^{-24}$ cm^3 from the Denbigh tables[33] we obtain $\Delta\sigma_H = -2.6 \times 10^{-12} E_{11}$ (cgs), in excellent agreement with literature estimates. For the shift of the carbon in the C–H bond, we would use the experimental value of ≈200 ppm/electron rather than the hydrogen value of 17.6 ppm/electron

$$\Delta\sigma_{C-H} = A_{C-H}E_{11} = \frac{200 b_{11}^{H}}{|e|R_{C-H}} E_{11} \times 10^{-6} = 2.9 \times 10^{-11} E_{11}$$

The carbon of the C–H bond shifts upfield when the hydrogen shifts downfield. Using this procedure we can derive the electric field constant A_{C-X} for a series of bonds, C–X (Table IV). b_{11}^{X} is taken from Denbigh's paper;[33] $\Delta\sigma_{C-X} = A_{C-X}E_{11}$ (cgs).

Table IV. The Electric Field Constant, A_{C-X} (cgs), as a Function of Bond Substituent

X	$b_{11}^{X} \times 10^{24}$, cm^3	R_{C-X}, Å	$A_{C-X} \times 10^{11}$
H	0.79	1.09	3.0
C	1.88	1.54	5.1
N	1.6	1.47	4.5
Br	5.0	1.76	12.0
Cl	3.7	1.90	8.2

If the effective dielectric constant is equal to 1, a +1 charge located on the X–C bond axis of XCH_3, at a distance of 1.8 Å from the carbon, produces a field with a component, $E_{11} = 0.55 \times 10^6$ esu, along each CH bond. The electronic charge density on each hydrogen would decrease an amount $\delta q_E = 0.08$, while the carbon electronic density would increase by an amount, $3\delta q_E$. Assuming a 200-ppm shift/electron in the C^{13} resonance, the total carbon shift from the electric field polarization of the three CH bonds by the +1 charge would be 48 ppm upfield. The actual observed shift (≈2–4 ppm upfield) upon protonation of methylamine and the amino acids is much less, although a +1 charge has been introduced at an effective distance of 1.8 Å from the carbon. The effective dielectric constant for our aqueous systems is probably nearer[39] to 3 than 1. If so, δq_E now would be 0.025, and an increase of $3\delta q_E$ in the *electronic* charge density should produce a 16-ppm upfield shift. The total charge density change on the carbon upon protonation is not $-3\delta q_E$ but rather $\delta q_1 - 3(\delta q_E + \delta q_{11})$. A small shift of 2–4 ppm upfield can occur if $3(\delta q_E + \delta q_{11}) \approx \delta q_1$. We are presently examining C^{13} shifts upon protonation or ionization as a function of dielectric constant and bond polarizability.

The above analysis indicates that electric field perturbations on the C^{13} resonances can, under favorable circumstances, be quite large. Large electric field effects in the C^{13} resonance, as compared with the proton resonances, have been reported.[7] In an earlier paper[2] we examined the electric field effect on the proton resonances of helical polypeptides. A net electric field (relative to the random coil) would arise from the permanent dipoles of the peptide units in the helical configuration. We estimated that this effect might produce ≈0.5 ppm difference between the α hydrogens of right- and left-handed helices. Polypeptides have carbonyl groups. The observed differences in the proton resonances between right- and left-handed helices[40] can apparently be ascribed to the carbonyl magnetic anisotropy, which produces a comparable but dominant shift in the opposite direction from that of the electric field. In the carbon-13 case the electric field effects on shifts are expected to be considerably larger than for the proton case and should dominate the carbonyl magnetic anisotropy effect since the latter should be approximately the same as in the proton case (≈1 ppm).

If indeed large shift differences between the carbons of right- and left-handed helices are observed because of electric field perturbations, one may be able to use this eventually in macromolecular conformation studies.

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(39) C. Tanford, *J. Am. Chem. Soc.*, **79**, 5348 (1957).
(40) J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967).