to give 98.4 g of distillate. Analysis of this material by gas chromatography showed it to contain 90.6% 1-cyanooctane corresponding to a 95% yield.

When the reaction was repeated in the absence of decane, a 94% yield of 1-cyanooctane was obtained in 97% purity.

Reaction of Chloroform with 1-Octene with Sodium Hydroxide. Chloroform (37.5 g, 0.31 mol) was added dropwise to a stirred mixture of 75 g (0.67 mol) of 1-octene, 140 g of 50% NaOH solution (1.75 mol of NaOH), and 5.0 g (0.01 mol) of "tricaprylylmethylammonium chloride" at such a rate that the temperature was maintained at 45–50° (*ca.* 0.5 hr). After the addition was complete, the reaction mixture was stirred at room temperature for 1 hr. The organic layer was separated and washed with H_2O (two 200-ml portions); the last wash gave an emulsion which was broken by the addition of 10 ml of saturated NaCl solution). Analysis of the organic layer by glc showed it to contain 59% 1-octene, 9.6% chloroform, and 31% products. Distillation through a 12-in. vacuum-jacketed Vigreux column gave 28 g of product fraction, bp 49° (0.2–0.5 mm), and was shown by glc analysis to contain two components. These were separated by preparative glc. The major component (93%) was the expected 2-hexyl-1,1-dichlorocyclopropane as shown by comparison of its ir and nmr spectra and its glc retention time with those of a sample prepared by the method of Weinberg.[15] The minor component (7%) was identified by its nmr and mass spectra as 2-pentyl-3-methyl-1,1-dichlorocyclopropane. The isolated yields of the two products were 60 and 4%, respectively, based on unrecovered chloroform.

Permanganate Oxidation. 1-Decene (28 g, 0.2 mol) was added to a stirred mixture of 50 ml of benzene, 5 g (0.01 mol) of "tricaprylylmethylammonium chloride," 125 g (0.8 mol) of $KMnO_4$, and 100 ml of H_2O at such a rate that the temperature was maintained at 40–45° (0.5 hr). After addition was complete the mixture was stirred for an additional 0.5 hr. Excess permanganate was destroyed by addition of sodium sulfite solution. The reaction mixture was filtered to remove MnO_2 and acidified with dilute HCl. The MnO_2 was washed with 100 ml of benzene, which was also used to wash the aqueous phase of the filtrate. The combined benzene solutions were shaken with 100 ml of 10% NaOH solution. The aqueous alkaline phase was washed with ether and then acidified with hydrochloric acid. The carboxylic acid which was separated was taken up in 100 ml of ether, and the ethereal solution was dried (Na_2SO_4). Evaporation of ether left 29 g (91%) of nonanoic acid (98% purity by glc).

Acknowledgment. The author wishes to acknowledge the financial support of the Continental Oil Company, the valuable assistance of Mr. Chester M. Haworth, and the helpful discussions of Drs. Flynt Kennedy, Allan J. Lundeen, and Paul H. Washecheck.

(15) H. E. Weinberg, *J. Org. Chem.*, **24**, 264 (1959).

Carbon-13 Nuclear Magnetic Resonance Studies of Heterogeneous Systems. Amino Acids Bound to Cationic Exchange Resins[1]

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Abstract: The ^{13}C nmr spectra of several protonated and deuterated amino acids bound to cationic exchange resins (cross-linked polystyrenes with sulfonic acid groups) have been measured using pulsed Fourier transform techniques. In many cases narrow, 1–10-Hz wide ^{13}C resonances were observed for the amino acids in this heterogeneous system. In those cases where they were measured accurately Overhauser enhancement factors of about 3 were obtained for the protonated amino acids. Large differences between T_2 (transverse relaxation time) and T_1 (spin–lattice relaxation time) were observed. T_2 was considerably shorter than T_1 and was sensitive to the degree of cross-linking in the resin, whereas T_1 was insensitive to the degree of cross-linking. Deuterium-decoupled, deuterated amino acids on the resins often showed ^{13}C line widths which were three–four times narrower than those from proton-decoupled, protonated amino acids. These observations are discussed in terms of an anisotropic model for the resin-bound amino acids which assumes a nonzero time-averaged dipolar interaction. The resin method is shown to have particular value in (1) shortening the T_1 values of quaternary carbons and other carbons that do not have hydrogens bonded to them, thus facilitating their detection by pulsed Fourier transform techniques, and (2) distinguishing these carbons from carbons which are bonded to hydrogens.

There is presently considerable interest in using ^{13}C nmr for organic and bioorganic investigations. Instrumental advances based on pulsed Fourier transform techniques[2–4] have provided significant signal-to-noise improvement per unit time relative to conventional continuous-wave nmr. Measurements are now possible which could not readily be done in the past. However, carbons that are not bonded to hydrogens remain difficult to detect because of their relatively long spin–lattice relaxation (T_1) times.[2,4,5] The driven equilibrium Fourier transform (DEFT)[5,6] and modifications thereof[7] represent instrumental methods for circumventing the low signal-to-noise ratios which are often characteristic of carbons that have long T_1 values. The instrumentation required, however, is more complex than that needed for pulsed Fourier transform.

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(1) A preliminary report was presented April 1970 to the Eleventh Experimental Nuclear Magnetic Resonance Conference, Mellon Institute, Pittsburgh, Pa.

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(3) R. R. Ernst, *Advan. Magn. Resonance*, **2**, 1 (1966).

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(5) E. D. Becker, J. A. Ferretti, and T. C. Farrar, *J. Amer. Chem. Soc.*, **91**, 7784 (1969).

(6) R. L. Streever and H. Y. Carr, *Phys. Rev.*, **121**, 20 (1961).

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In addition, the DEFT procedure is restricted to natural abundance or low ^{13}C enrichment samples where carbon–carbon J coupling is unimportant.[8] It would be of considerable help to have alternative methods for detecting carbons that are not bonded to hydrogens which would be simple to use and applicable to a large number of different types of compounds.

Recently we began a ^{13}C nmr study of amino acids bound to cationic exchange resins for purposes of (1) shortening the T_1 relaxation times of quaternary carbons and other carbons that do not have hydrogens bonded to them, thus facilitating their detection, and (2) distinguishing these carbons from those carbons which are bonded to hydrogens. In these studies amino acids were measured, but it is obvious that many other molecules that could be adsorbed to resins could have been investigated. Our studies have revealed that high-resolution nmr measurements in solid–liquid systems are possible. For example, we were able in many cases to obtain fairly narrow, 1–10-Hz wide ^{13}C resonances for the resin-bound amino acids. We feel that the ^{13}C nmr technique may well have considerable value for chemists interested in biopolymers, catalysis, and in heterogeneous systems such as biological membranes.

Experimental Section

Nmr Equipment and Operational Details. The proton spectra were obtained on a Varian T-60 spectrometer, where the probe temperature was approximately 40°. The ^{13}C 15-MHz spectrometer was constructed in this laboratory, and the probe temperature was *ca.* 25°. Pulsed Fourier transform techniques were used to accumulate the ^{13}C spectra. We were thus able to detect simultaneously all the resonances in the spectrum, which in the ^{13}C case extends over a range of 200 ppm. In this technique a repetitive series of *ca.* 15.08-MHz pulses perturbs the spins from their equilibrium position aligned either parallel or antiparallel to the magnetic field. Each pulse rotates the spins an angle α away from the field direction. This produces a free induction decay, *i.e.*, a transverse magnetization component at right angles to the field which eventually decays to zero as the spin system relaxes back to equilibrium. The spins are then again perturbed by a new pulse and the process is repeated. Experimentally, the transverse component is recorded as a "ringdown," *i.e.*, as a superposition of exponentially decaying sinusoids, which when Fourier transformed give the conventional frequency spectrum. The sinusoidal frequencies are the resonance frequencies relative to the pulse frequency, while the decay times, T_2, determine the widths. This technique provides a significant reduction in the accumulation time required relative to the conventional continuous wave method.[2,3] Deuterated and protonated amino acids bound to resins were contained in 5-mm sample tubes. The accumulation time required per sample varied from a few minutes for ^{13}C enriched material to several hours when measuring deuterated amino acids at the 1% natural abundance of ^{13}C. A 60-MHz noise generator was used to decouple all protons simultaneously from the carbons, and a coherent 9.2-MHz decoupler was used to decouple the deuterium resonances simultaneously. In the case of the protonated samples the magnetic field was locked to an internal D_2O resonance, while in the deuterated sample study the magnetic field was locked to an external H_2O sample. A detailed description of an earlier version of the spectrometer appears in a previous publication.[9]

The present spectrometer differs from the earlier one in several respects. First, a Fabri-Tek 1070 averager which had 4096 memory locations rather than a Varian 1024 channel accumulator was used to accumulate the spectrum. Second, the pass band of the V 4311 receiver was increased from several hundred hertz to 6.5 kHz. Third, a Nelson-Ross spectrum analyzer (PSA 011) was used to magnitude transform[10] the ringdown, whereas earlier we could only record the ringdown. After having made these modifications, we could simultaneously detect and transform all the ^{13}C resonances of our sample with better than 2-Hz resolution in many cases, and have only a moderate band pass attenuation across the frequency spectrum.

All spectra shown in this paper are uncorrected original data and should be multiplied by appropriate pass band intensity correction factors, F.[11] We indicate the F values when they are significant in the appropriate figures. Intensity distortions within a spectrum can also occur because of differences in T_1 relaxation times and differences in the degree of Overhauser enhancement.[12,13] In our case distortions in relative intensities because of T_1 differences probably did not exceed a factor of 1.5 across the spectrum of any resin-bound amino acid.[14] The Overhauser factors were not determined accurately except in a few samples. In general, all carbons appear to have very similar enhancement factors. We discuss this further below.

Two methods were used to determine line widths, $\Delta\nu$. The preferred method was to measure T_2, the time required for the magnitude of the free induction decay to decrease by a factor of 0.368 relative to its initial value [$\Delta\nu = (\pi T_2)^{-1}$]. When this method was not practical, $\Delta\nu$ was determined from the magnitude transformed spectrum. In the latter case, non-Lorentzian lines are observed which are $\sqrt{3}$ broader at half-maximum than the usual Lorentzian absorption lines obtained from continuous wave nmr. The reported widths in Tables II and III or quoted in the text were corrected for this $\sqrt{3}$ factor. Generally, we tried to obtain the most accurate line widths consistent with good signal-to-noise ratios across the entire spectrum. However, if the sample contained some resonances that were very narrow while others were broad, our "best" instrument settings were often such that the line widths of the narrow resonances had an appreciable broadening introduced by the resolution limitations of the spectrum analyzer, for which only approximate corrections could be made. If more accurate values were required, the sample was rerun at different settings more appropriate to measurement of narrow line widths. In the deuterated amino acid measurements, an additional instrumental broadening estimated to be 1–2 Hz was introduced because of the external lock and the coherent decoupler. The T_1 relaxation measurements were done using the standard 180° pulse followed by a 90° pulse.[15] Oxygen degassing by bubbling argon through the samples for several hours had no effect. All chemical shifts are reported relative to the α-carbon of glycine, pD = 7.0, which occurs 86.5 ppm above benzene.[9]

Sample Preparation. Glycine, L-alanine, L-glutamic acid, L-cysteine, and L-cystine (all A-Grade chromatographically pure) were supplied by Calbiochem Corp. D,L-Alanine-*2,3,3,3-d_4* and glycine-d_5 were supplied by International Chemical and Nuclear Corp. The alanine (lot No. 20188) was nominally 97% deuterium labeled. However, we found it to be ~65% labeled. L-Glutamic acid-*2,3,3,4,4-d_5* was supplied by Merck Sharp and Dohme. The amino acids were dissolved at room temperature in D_2O, and then passed through a Chelex-100 column to remove any paramagnetic ion impurities. The pD was next adjusted to ~5.5 with HCl, and the amino acid solution was added to a 0.5 cm × 1 cm resin column. Common amino acid stocks were used for the different resins. The resins, AG 50W-X4 (200–400 mesh) and AG 50W-X8 (100–200 mesh), were supplied by Bio-Rad Laboratories and had a polystyrenesulfonic acid matrix which was either 4% or 8% cross-

(8) R. Freeman, private communication.

(9) W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Amer. Chem. Soc.*, 92, 680 (1970).

(10) The magnitude transform of the ringdown is the square root of the sum of the cosine transform squared and the sine transform squared. It is also equal to the square root of the sum of the dispersion signal squared and the absorption signal squared. We could only do a magnitude transform with the Nelson-Ross analyzer because this analyzer could not make use of phase information.

(11) This factor contains contributions from (a) the receiver, (b) the low pass filters introduced after the receiver to reduce aliasing noise, and (c) the Nelson-Ross spectrum analyzer.

(12) A. Abragam, "Principles of Magnetic Resonance," Oxford University Press, London, 1961, p 299 ff.

(13) K. F. Kuhlmann, D. M. Arant, and R. K. Harris, *J. Chem. Phys.*, **52**, 3439 (1970).

(14) Appreciable intensity distortions can occur if there are large differences in T_1 and if the repeat period, T, of the pulse sequence is short compared with the longest T_1. These distortions[2,4] are maximal for $\alpha = \pi/2$. In our study all carbons generally had short T_1 times when bound to the resins ($T_1 \gtrsim 1$ sec). Our flip angle α was generally less than $\pi/2$ (α varied from $\pi/6$ to $\pi/2$), and our T values were greater than 0.2 sec.

(15) H. Y. Carr and E. M. Purcell, *Phys. Rev.*, **94**, 630 (1954).

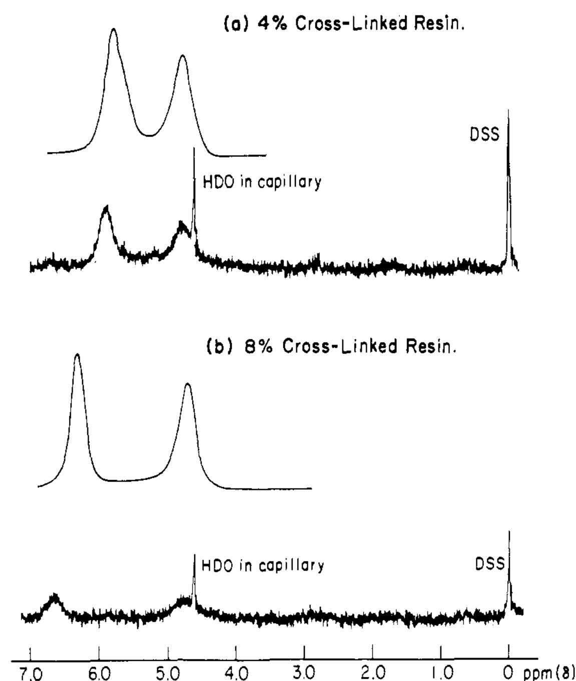

Figure 1. The proton nmr spectra at 60 MHz of (a) AG 50WX4 resin in D_2O and (b) AG 50WX8 resin in D_2O. The resonances from a capillary containing DSS in D_2O placed in the sample tubes show homogeneity beats characteristic of good field homogeneity.

linked by divinylbenzene. An analysis provided by the supplier indicated negligibly small, trace amounts of paramagnetic ion impurities. The bottle resin was regenerated by standard wash procedures.[16a] A spectrographic analysis of the treated resin performed for us by Drs. J. G. Conway and G. V. Shalinoff of the Lawrence Radiation Laboratory indicated only a negligibly small amount of paramagnetic ion impurities, consistent with the original original Bio-Rad analysis. After binding the amino acids to the resins (~0.3–1.0 mequiv of amino acid per milliliter of wet resin), the samples were washed with D_2O to remove any H_2O or exchanging protons that might interfere with the proton nmr measurements. The external pD of the 8 and 4% resin samples were ~4.1 and 5.9, respectively. These measurements were made using a glass electrode inserted into 10-ml supernatant solutions containing 1-ml resin beds.[16b]

Histidine labeled at the C_2 imidazole carbon (50–60% ^{13}C) was synthesized. Two such samples were prepared: one had a proton bonded to the C_2 carbon; the other had a deuterium bonded to the C_2 carbon (50–60% ^{13}C, 90% ^{2}H). The synthetic procedures were as follows.

[^{13}C]**Sodium cyanide** was prepared by the method of Jeanes[17] from [^{13}C]barium carbonate (Isomet Corp., 50–60% ^{13}C). An aqueous solution of [^{13}C]NaCN was obtained, and the cyanide was recovered quantitatively as [^{13}C]AgCN by precipitation with dilute aqueous $AgNO_3$ solution.

[^{13}C]**Sodium thiocyanate** was prepared from [^{13}C]AgCN as follows. The [^{13}C]AgCN (4.3 g) was added to a solution of 4.8 g of NaI in 75 ml of acetone, and the slurry was heated at reflux for 15 min with vigorous stirring. Sulfur (2.1 g) was added, and the mixture was heated at reflux for 4 hr. After cooling, the slurry was washed with about 100 ml of acetone through a filter to remove the AgI which has precipitated. The filtrate was taken to dryness at reduced pressure, and 30 ml of H_2O was added. Excess sulfur and the remaining AgI, both insoluble in water, were removed by filtration. The filtrate and washings (about 20 ml more of H_2O) were taken to dryness at reduced pressure. A nearly quantitative yield (2.6 g) of [^{13}C]NaSCN remained as a white, crystalline solid. Greenberg and Rothstein[18] have previously shown that NaCN may be converted to NaSCN in quantitative yield by heating with sulfur in acetone.

[*2*-^{13}C]L-**2-Thiohistidine** was prepared under a stream of nitrogen from 11.4 g of γ-keto-L-ornithine dihydrochloride[19] and 2.6 g of [^{13}C]NaSCN by the method of Heath, Lawson, and Rimington.[20] The [*2*-^{13}C]-L-2-thiohistidine was converted to [*2*-^{13}C]-L-histidine diflavianate as described by Ashley and Harington.[21] This diflavianate was converted to [*2*-^{13}C]-L-**histidine dihydrochloride** by ion exchange using Dowex-1 (Cl^- form) resin. The yield of [*2*-^{13}C]-L-histidine dihydrochloride was 1.1 g (15% based on the amount of [^{13}C]NaSCN used). The ir spectrum (Nujol) corresponded to that of authentic L-histidine dihydrochloride which was prepared from L-histidine hydrochloride (Eastman Organic Chemicals). The proton nmr spectrum at 60 MHz in D_2O corresponded exactly to that of the authentic unlabeled material except that the doublet (J_{HCNCH} = 1.5 Hz) for the proton at the C_2 position of the histidine at δ 8.73 was *ca.* half the normal size and two new doublets, each *ca.* half the size of the doublet at δ 8.73, appeared at δ 6.88 and 10.60. Thus, for the proton at the C_2 position $J_{^{13}CH}$ = 223 Hz. Moreover, the peak at δ 7.51, corresponding to the proton at the C_4 position of the histidine, showed new shoulders which indicate that $J_{^{13}CNCH} \cong 6$ Hz.

[*2*-^{13}C]-L-**2-Deuteriohistidine** and L-**2-deuteriohistidine** were prepared from the appropriate nondeuterated histidines by exchange in D_2O (Bio-Rad Laboratories, 99.88% deuterium) as described by Meadows, *et al.*[22] The exchange reaction was followed by observing the decrease of the peak for the proton at the C_2 position by nmr spectroscopy. Under the conditions used no deuterium exchange was observed to occur at either the C_α, C_β, or C_4 positions of the histidine.

Results

The proton spectra of the 4 and 8% resins prior to addition of the amino acids are shown in Figure 1. All samples were contained in 5-mm nmr tubes. The resin beds were 3–4 cm above the receiver coil to eliminate vortexing inhomogeneities upon spinning. Two broad resonances of approximately equal area are observed in each resin (Figure 1). The widths are relatively insensitive to the degree of cross-linking. Paramagnetic ions are not responsible for the broad lines observed since chemical analysis indicated negligible quantities of such ions. Nor is this broadening due to magnetic field inhomogeneities introduced by the heterogeneous (water-solid) sample.

The resonances from capillary DSS (sodium 3-trimethysilyl-1-propanesulfonate) in D_2O introduced into the sample remained narrow with no detectable change in width (Figure 1). The resonance at *ca.* δ 4.80 is HDO. Its shift relative to the capillary HDO is probably due to volume magnetic susceptibility differences. The origin of the downfield resonance (δ 6.0–6.8) is not certain. It is characteristic of the $H^+(D^+)$ form and is absent in the Na^+ form of the resin. In the 8% resin, for example, it disappeared between external pD = 4.15 and pD = 4.7 as measured with a glass electrode. Its position, width, and intensity relative to the upfield HDO resonance change upon amino acid binding; in general, it shifts toward the upfield resonance and broadens. On the other hand, the widths of the bound amino acids appear to be insensitive to the position and amount of the downfield resonance (Figure 2a,b). If one adds a few drops of H_2O to the D_2O resin suspension, both the upfield and downfield resonances increase equally in intensity

(16) (a) F. Helfferich, "Ion Exchange," McGraw-Hill, New York, N. Y., 1962, p 91; (b) p 92.

(17) J. K. Jeanes, *Science*, **118**, 719 (1953).

(18) D. M. Greenberg and M. Rothstein, *Methods Enzymol.*, 4, 678 (1957).

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(21) J. Ashley and C. Harington, *ibid.*, 2586 (1930).

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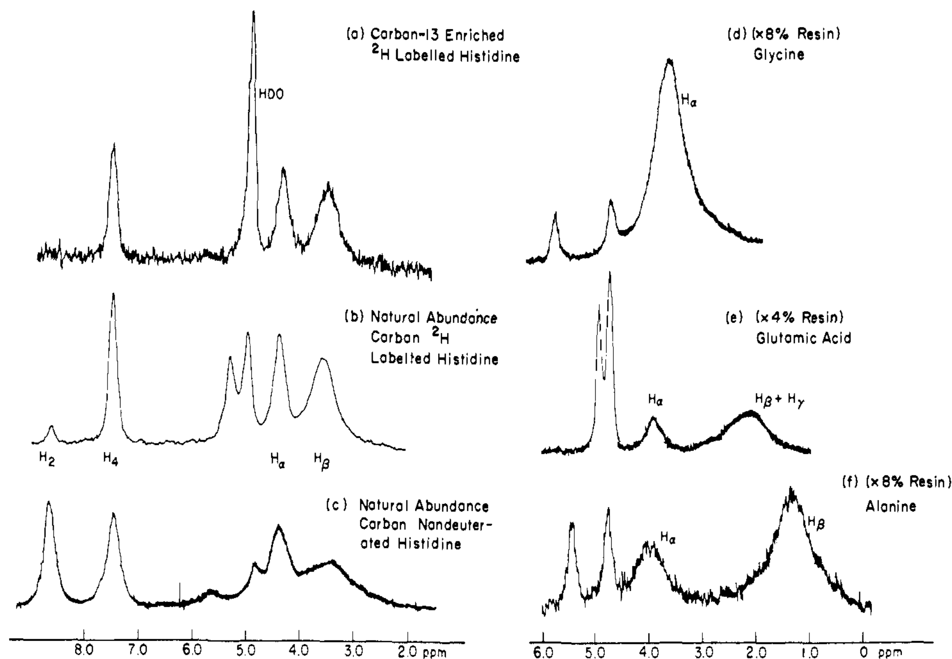

Figure 2. The proton nmr spectra at 60 MHz of amino acids bound to the AG 50WX4 resin: (a) ^{13}C and ^{2}H labeled [$^{13}C_2$–^{2}H]histidine; (b) ^{13}C unlabeled and ^{2}H labeled histidine; (c) unlabeled histidine; (d and e) glycine, glutamic acid, and alanine, respectively.

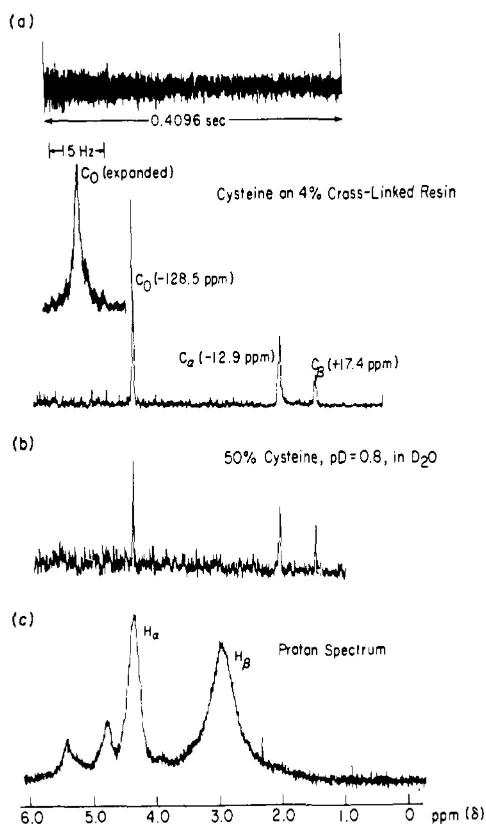

Figure 3. (a) The ^{13}C free-induction decay of cysteine on the 4% resin and its magnitude transform; $\alpha = \pi/2, T = 1.0$ sec, 13,000 accumulations. (b) The ^{13}C magnitude transform of 50% cysteine in D_2O, pD = 0.8; $\alpha = \pi/4, T = 13$ sec, 32 accumulations. Our magnitude transform resonances are non-Lorentzian and consequently have widths that are a factor of $\sqrt{3}$ broader than the widths that would have been obtained from conventional slow passage continuous wave measurements. The instrument intensity correction factors are F = 1.0, 1.6, and 1.7 for C_0, C_α, and C_β, respectively. (c) The proton nmr spectrum at 60 MHz of cysteine on the 4% resin.

(Figure 1). The downfield resonance clearly arises from an exchanging proton, and, on the basis of the amount present, it would appear to be a chemically shifted low pD water resonance. Previous investigators,[23] using methods other than nmr spectroscopy, have also identified two types of water within the swollen ion exchange resin; one they refer to as water of hydration about the H^+ counterion and the other as "free" water. These studies indicate, however, a smaller amount of "water of hydration" than we apparently have observed. In accord with our findings the "water of hydration" would be expected to be distinguishable from the "free" water by a relatively low-field chemical shift in the nmr spectrum, which is characteristic of a low pD environment. No further work was done to establish the bases for the two water resonances observed in these resins. ^{13}C resonances from the resin could not be detected.

The amino acids are bound completely to the resin and have very slow chemical exchange rates. As expected, there were no detectable amino acid resonances from the liquid phase above the resin suspension. In Figure 3 we show the ^{13}C spectra of a 50% aqueous solution of cysteine (–SH) in D_2O and the corresponding spectra when the cysteine is bound to the 4% resin. The resonance positions for cysteine are seen to be within a hertz the same whether in acid solution or bound to the resin. The carboxyl, C_0, carbon has a measured width of 1.8 ± 0.3 Hz on the resin which was probably resolution limited by the spectrometer. This indicates little or no paramagnetic ions are present since these should bind to and selectively broaden the carboxyl carbon. Within experimental error, the α and β carbon widths, which were 9 ± 2 and 14 ± 2 Hz, respectively, are proportional to the number of bonded protons. In solution, all three carbons have Overhauser enhancement factors of

(23) F. Helfferich, ref 16, p 104 ff.

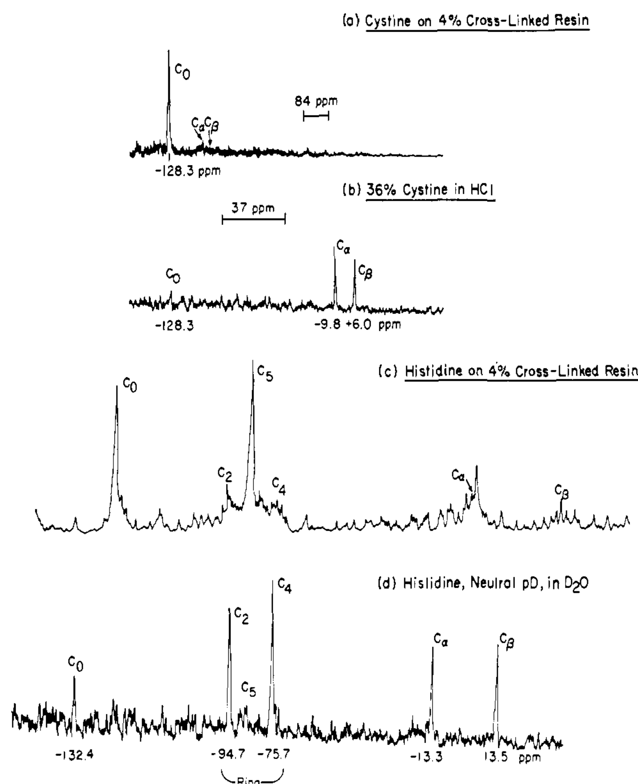

Figure 4. The ^{13}C nmr spectra of (a) cystine on the 4% resin; $\alpha = \pi/2, T = 0.8$ sec, 40,000 accumulations; (b) a 35% solution of cystine in dilute HCl; $\alpha = \pi/2, T = 5.0$ sec, 2100 accumulations; (c) histidine on the 4% resin; $\alpha = \pi/2, T = 0.5$ sec, 14,000 accumulations; (d) histidine at neutral pD in D_2O; $\alpha = \pi/3, T = 5.0$ sec, 4096 accumulations. In this last case the resonances are filter broadened. Within experimental error the chemical shifts in c are the same as the shifts in d. Spectra c and d have different frequency scales.

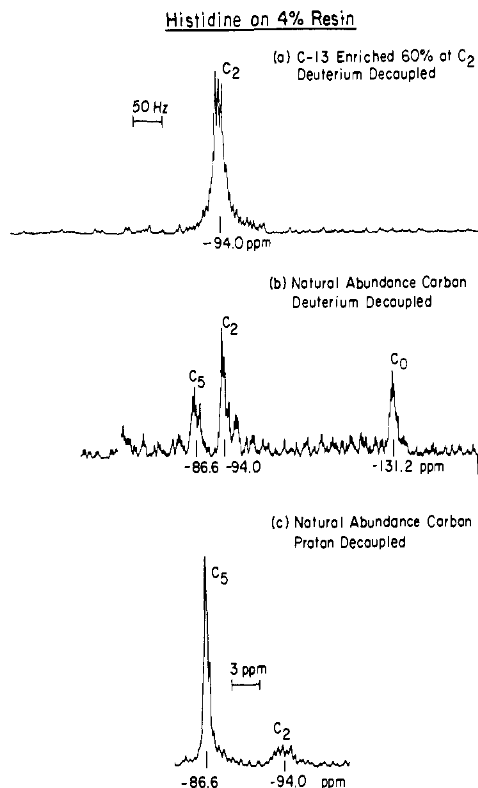

Figure 5. The ^{13}C nmr spectra of histidine (90% deuterium labeled at the C_2 position) on the 4% resin: (a) C_2 position enriched with [13]C (50–60 atom %), deuterium decoupled; $\alpha = \pi/4, T = 0.5$ sec, 16,000 accumulations. The resonance is broadened by a long-range J coupling of ~6 Hz between the C_2 carbon and the proton on the C_4 position of the imidazole ring. (b) Natural abundance ^{13}C, deuterium decoupled; $\alpha = \pi/4, T = 1.0$ sec, 100,000 accumulations. (c) Natural abundance ^{13}C, proton decoupled; $\alpha = \pi/4, T = 1.0$ sec, 35,000 accumulations. The C_5 carbon is Overhauser enhanced while the C_2 carbon is broadened by the partial collapse of the ^2H–$^{13}C_2$ multiplet pattern (see text). The narrowing of this C_2 resonance by ^2H decoupling is evident from a and b.

approximately 3, the maximal value possible.[12,13] On the resin all the carbons again appear to have very similar Overhauser enhancement factors. However, we did not make an accurate determination of these values for cysteine. The proton spectrum of cysteine is shown for comparison in Figure 3c.

The cysteine chemical shifts clearly indicated that the local[24] pD in the neighborhood of the amino acid is significantly lower than the external pD (pD $\gtrsim$ 5). Similar differences were observed for all the other amino acids, with the exception of histidine. In Table I we list the chemical shifts of several resin-bound amino acids. The observed shifts indicate a low local pD environment, pD < 2, in which 75–100% of the COO^- groups are deuterated. In the case of resin-bound histidine, however, the chemical shift values indicated a higher local pD value (pD > 3).

The results for cystine (–S–S–) are shown in Figure 4. Effectively complete immobilization on the resin occurs because of the binding of both $-ND_3^+$ groups to the sulfonate moieties. Only a 20 ± 3 Hz carboxyl resonance was observed (Figure 4a). No proton signals could be detected because of the extensive broadening. The resin significantly shortens the T_1 times of the amino acids. For example, no difficulty was encountered in detecting the carboxyl on the resin using $\alpha = \pi/2$ pulses and a repeat period $T = 0.8$ sec. However, in aqueous solution (no resin) (Figure 4b)

(24) F. Helfferich, ref 16, p 86 ff.

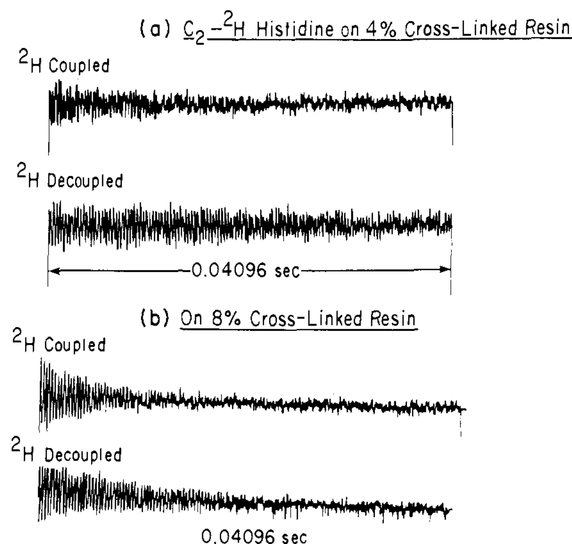

Figure 6. The free-induction decay spectra of $^{13}C_2$–^2H histidine on the 4 and 8% resins showing line narrowing upon deuteriun decoupling.

the carboxyl carbon had a sufficiently long T_1 that its intensity was still significantly attenuated when we used an $\alpha = \pi/2$ pulse and a much longer repeat period, $T = 5.0$ sec.

Table I. Representative Shifts, δ, of Resin-Bound Amino Acids[a]

Resin-bound amino acid	$\delta_{\mathrm{pH}\ 7}$[a]	δ resin	$\Delta = \delta_{\mathrm{resin}} - \delta_{\mathrm{pH}\ 7}$	Δ_{calcd}[b]	% ionization of COOD
Glycine[c]					
C_0	−131.0	−127.5	3.5	3.4	75
C_α	0.00	2.0	2.0	2.1	
Alanine[c]					
C_0	−134.3	−130.2 ± 0.2	4.3 ± 0.2	4.5	100
C_α	−9.1	−6.6	2.5	3.5	
C_β	25.2	26.7 ± 0.4	1.5 ± 0.4	1.8	
Glutamic acid[d]					
C_0	−133.1	−129.5	3.6	3.6	75
$C_{0\delta}$	−139.8	−134.0	5.8	4.7	100
C_α	−13.2	−9.8	3.4	3.1	
C_β	8.0	12.8	4.8	2.8	
C_γ	14.4	18.1	3.7	4.3	

[a] Chemical shifts are relative to the C_α of glycine, pD = 7.0; W. Horsley and H. Sternlicht, *J. Amer. Chem. Soc.*, **92**, 680 (1970). [b] Shift parameters from (i) W. Horsley and H. Sternlicht, *ibid.*, **90**, 3738 (1968); (ii) R. Hagan and J. D. Roberts, *ibid.*, **91**, 4504 (1969). [c] 8% cross-linked resin. [d] 4% cross-linked resin.

The resin and solution spectra of natural abundance ^{13}C histidine are shown in Figures 4c and 4d. On the resin the carboxyl carbon, C_0, and the quaternary imidazole carbon, C_5, are appreciably narrower than the rest of the carbons, and consequently are readily distinguishable from those carbons that are bonded to hydrogen. The Overhauser enhancement factors of all the carbons appear to be very similar on the resin. We could only determine the C_0 and C_5 line widths accurately; the remaining widths had a large margin of error (Figure 5). In solution (no resin) the T_1's of C_0 and C_5 are long, and consequently the C_0 and C_5 intensities are attenuated relative to the other carbons.

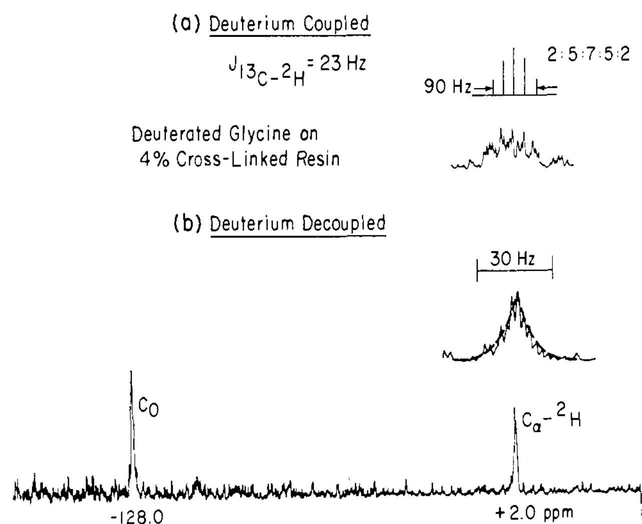

Figure 7. The natural abundance ^{13}C nmr spectra of glycine-d_5 on the 4% resin. The deuterium coupled multiplet is shown in a. The deuterium decoupled spectrum is shown in b. The intensity correction factors are F = 1.1 for C_0 and 1.8 for C_α.

The measurements on these resin systems clearly indicate that if a hydrogen is bonded to a carbon, broad carbon resonances will often be obtained. Furthermore, the resulting widths are comparable to the proton resonance widths. In an effort to see how narrow we could make the carbon resonances, and at the same time learn more about the anisotropic motions of the bound amino acids, we examined a number of deuterated amino acids and prepared histidine samples that were ^{13}C enriched at the C_2 position. Representative spectra are shown in Figures 2 and 5–8 and the results are summarized in Tables I–III. The ^{2}H

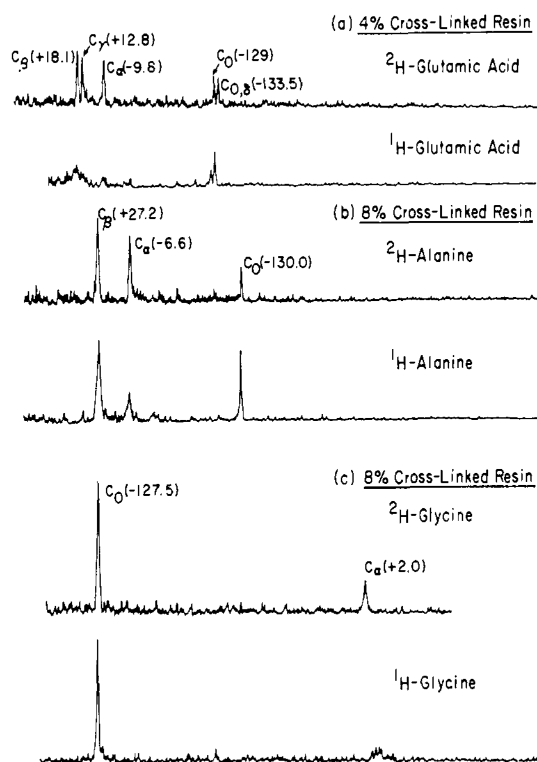

Figure 8. Comparison of the natural abundance ^{13}C nmr spectra of several deuterated amino acids with those of the corresponding protonated forms. The intensity correction factors, F, for both glutamic acid and alanine are near 1.0. The glycine spectra are on a different frequency scale, where F = 1.5 for C_0 and 1.7 for C_α. Approximately 50,000–100,000 accumulations were required for the deuterium samples.

spin–lattice relaxation rates were not sufficiently rapid so as to decouple the deuteriums from the carbons. For example, in some cases a resolvable ^{2}H–^{13}C multiplet was observed as in glycine on the 4% resin (Figure 7), or a collapsed multiplet as in histidine on both the 4 and 8% resins (Figure 6). In all cases an

Table II. A Comparison of the ^{13}C Line Widths, $\Delta\nu$, of the Protonated Form (^1H) of the Resin-Bound Amino Acids with the Corresponding Deuterated Form (^2H)

Resin-bound amino acid	$\Delta\nu$, Hz (on 4% resin)			$\Delta\nu$, Hz (on 8% resin)		
	$^1H^a$	$^2H^b$	$^1H/^2H$	$^1H^a$	$^2H^b$	$^1H/^2H$
Glycine						
C_0	<1.6	<1.6		<3	<3	
C_α	11 ± 1	5 ± 1 (uncorr) 4 ± 1 (corr)[c]	2.2 ± 0.6 (uncorr) ~3 (corr)[c]	40 ± 5	11	3.7 ± 0.4
Alanine[d]						
C_0				≲6	≲6	
C_α				37	14	~2.7
C_β				47	12	~4.0
Glutamic acid[e]						
C_0	≲4	≲3				
$C_{0\delta}$	≲4	≲3				
C_α	34	4–5	~7–8			
C_β	Not resolved	~7				
C_γ	Not resolved	~7				
Histidine						
C_2 (50–60% ^{13}C enriched)	80 ± 15[f]	9 ± 1[g]	9 ± 2 5.5 ± 1.5) (normalized)[h]	190 ± 30	30 ± 5[g]	6.5 ± 2.0 4 ± 1.5) (normalized)

[a] Proton-decoupled measurements done on protonated samples. [b] Deuterium-decoupled measurements done on deuterium-labeled samples. [c] Estimated correction for lock drift inherent in an external lock when measurement extended over many hours. [d] Alanine bound to the 4% resin was not measured. [e] Glutamic acid bound to the 8% resin was not measured. [f] Measurements done on protonated histidine in D_2O. [g] Measurements done on deuterium-labeled histidines. [h] $^1H/^2H$ value of 9 ± 2 divided by the 1.6 discrepancy factor discussed in the text. This discrepancy factor of ~1.6 is evident in Figure 2b,c. We also observed this discrepancy factor in the C_0 and C_5 carbon resonances. For example, in the 4% resin the C_0 and C_5 widths of the deuterium-labeled histidine were 8 and 3 Hz, respectively, while in protonated histidine they were 12 and 6 Hz, respectively.

Table III. Spin–Lattice Relaxation Time (T_1), Transverse Relaxation Time (T_2), and Overhauser Enhancement Factor of Resin-Bound Histidine

Type of resin-bound histidine	T_2, sec				
	Proton decoupled	Deuterium coupled	Deuterium decoupled	T_1, sec	Overhauser enhancement factor[a]
[$^{13}C_2$–^2H]Histidine					
On 4% resin	0.01 ± 0.002	0.01 ± 0.002	0.038 ± 0.0009	0.5 ± 0.02	1.25 ± 0.1 (uncorr)[b] 0.9 ± 0.1 (corr)
On 8% resin	0.006 ± 0.001	0.006 ± 0.001	0.011 ± 0.0015	0.39 ± 0.01	1.20 ± 0.1 (uncorr) 0.9 ± 0.1 (corr)
[$^{13}C_2$–^1H]Histidine					
On 4% resin	0.0038 ± 0.0004			0.083 ± 0.005	3.6
On 8% resin	0.0016 ± 0.00025			0.067 ± 0.004	3.2 ± 0.3
In D_2O solution (no resin)	≳0.3			1.0	

[a] Observed in the proton-decoupled spectrum. [b] The sample was 90% deuterium labeled. The residual 10% protons at the C_2 position were Overhauser enhanced a factor of 3.

external 9.208-MHz source was used to decouple the deuteriums completely from the carbons. In this connection, compare the two spectra of [C_2–^2H]histidine shown in Figure 5b,c. In one, the sample is deuterium decoupled, and in the other it is proton decoupled. The narrowing of the C_2 resonance upon deuterium decoupling is evident. The collapse of the ^2H–^{13}C multiplet pattern upon ^2H irradiation is also evident in the glycine-d_5 case (Figure 7).

A number of interesting observations were made. First, as anticipated, the ^2H decoupled deuterated amino acids had narrower widths than the ^1H decoupled protonated amino acids. However, the narrowing was not as large as expected. Factors of 3–4 narrowing were often observed rather than the expected 10–15. Second, in the one protonated amino acid case where the Overhauser enhancement factor could be determined accurately, *i.e.*, in the [^{13}C–^1H]histidine resin sample, a value of 3 was obtained within experimental error (Table III). Third, in those few cases where T_1 was measured accurately[25] large differences between T_2 and T_1 were evident. T_2 was considerably shorter than T_1. In addition, T_1 changed only slightly with the degree of cross-linking whereas T_2 changed appreciably (Table III). The significance of these observations is discussed below.

Discussion and Conclusions

We observed from the above measurements that binding of molecules to resins can be a useful technique in ^{13}C nmr for purposes of detecting and distinguishing carbons without directly bonded protons from those with directly bonded protons. The T_1 relaxation times are significantly reduced on the resin relative to the

(25) Although only the T_1 and Overhauser factors for the ^{13}C-enriched histidine were determined accurately, we estimate the other amino acids had T_1 times less than 1 sec, and Overhauser enhancement factors near 3. This is inferred from the α and T values used, and the number of accumulations required to obtain a given signal-to-noise ratio.

solution values. In pulsed Fourier transform nmr this means that short repeat times ($T < 1$ sec) and optimal α values near $\pi/2$ can be used. These are conditions that permit good signal-to-noise ratios with computer accumulation. In the case of the deuterated amino acids not bound to the exchange resin the T_1 times were so long in aqueous solution that, even in concentrated solutions, at best only extremely weak resonances could be detected. This was true also for the ^{13}C enriched histidine samples where we used a range of α values ($\alpha \sim \pi/2$ to $\alpha \ll \pi/2$) and $T \sim$ 1–10 sec in an effort to detect the $^{13}C_2$ carbon. The T_1 in solution clearly must have exceeded 1 min. When we shortened the T_1 by binding the deuterated amino acids to resins, the increase in the signal-to-noise ratio was dramatic (Figure 5b).

In the case of the protonated amino acids where the T_1 times were not excessively long in aqueous solution ($T_1 \sim$ 1–10 sec), the advantage we could gain from the shorter T_1 on the resin was offset by the fact that the amino acids could be prepared at very high concentrations in acid solution, whereas the concentration on the resin could not exceed 2 mequiv/ml of wet resin bed. However, in other areas of research, either the quantity of material and/or the solubility of the material in solution may be limited. In such cases, there is clearly an advantage to using the resin technique even for protonated samples. In fact, the material could often be concentrated on the resin.

At present different types of carbons are often distinguished by proton decoupling experiments.[26] That is, carbons directly bonded to proton will split into a multiplet extending over a wide frequency range when the proton decoupling power is turned off. On the other hand, resonances from carbons not directly bonded to protons will either remain as singlets or display multiplet patterns confined to a small frequency interval. Any multiplets that appear when the proton decoupling power is turned off obviously reduce signal intensity. However, if one uses the resin technique one can still proton decouple. The carbons are then distinguished on the basis of their width differences.

The measurements also show that high-resolution spectra can be obtained in heterogeneous systems. In the case of the deuterated amino acids, the lines were sufficiently narrow and the chemical shifts sufficiently large at 14 kG that had we worked with a mixture of the amino acids we would have been able to distinguish and determine the amount of each amino acid in the mixture. We observed that the major factor for proton and carbon line width broadening was the proton nuclear spin dipolar interaction with the bonded carbon or with a near-neighbor proton, while ^{13}C chemical-shift anisotropies and intermolecular dipolar interactions with the "rigid" resin lattice were not generally important. If we replaced protons with deuteriums, we could achieve a factor of 3–8 line narrowing in the ^{13}C spectra (Figure 8, Table II). If a proton were effectively isolated from other protons, again relatively narrow lines would be observed. The C_2 proton of histidine in D_2O solvent is more than 3 Å away from its nearest proton neighbors (the C_4 protons), and has a width of 18 Hz on the 4% resin. In contrast with this, the two C_β protons were ~50 Hz wide, while the C_2 carbon had a width of ~80 Hz.

(26) E. Wenkert, A. O. Clouse, D. W. Cochran, and D. Doddrell, *J. Amer. Chem. Soc.*, 91, 6879 (1969).

The C_0 and C_5 carbons have relatively narrow line widths. The factors responsible for their widths are not known. They may arise from a dipolar interaction with a C_α proton, or C_β and C_4 protons, respectively. This is a reasonable conjecture since these carbons are appreciably Overhauser enhanced which implies that their T_1 times are determined by a proton dipolar interaction.[12,13] It is also possible that ^{13}C chemical-shift anisotropies and intermolecular dipolar interactions make some contributions to their width.

We will now show that the anisotropic motions that persist when the amino acids are bound to the resins result in a nonzero time-averaged dipolar interaction, and that it is the nonzero value which is primarily responsible for the observed widths of carbons bonded to hydrogen or deuterium. Intuitively one expects anisotropic motion. Evidence for it comes from the three observations mentioned above which we will now discuss in some detail. Our interpretation was guided by earlier nmr work on adsorbed molecules on clays and gels.[27] In particular, we found the extensive proton nmr study of benzene adsorbed to silica gel done by Woessner[28] most useful.

I. The ^{13}C resonances of deuterated amino acids were often observed to be a factor of 3 to 4 narrower than the protonated analogs.

In solution one generally assumes that a molecule undergoes an isotropic diffusional rotational motion which can be characterized by an angular correlation time, τ_c. If the rotational motion is sufficiently rapid, and if the dipolar interactions dominate the relaxation rates, one obtains the well-known expression[29] (eq 1)

$$T_1^{-1} = T_2^{-1} = {}^4/_3\gamma_c^2\gamma_{\{H,D\}}{}^2\hbar^2S(S+1)r^{-6}\tau_c \quad (1)$$

for a carbon interacting with a proton or deuterium a distance r away, where $\hbar$ is Planck's constant divided by 2π, $\gamma_{\{H,D\}}$ is the gyromagnetic constant of the hydrogen or deuterium ($\gamma_H = 2.6752 \times 10^4$ and $\gamma_D = 0.4106 \times 10^4$ radians G^{-1} sec^{-1}), and $S = {}^1/_2$ for H and 1 for D. T_1 and T_2 decrease as τ_c increases. If τ_c becomes comparable to or less than the resonance frequency of the nucleus measured, the relaxation expressions change.[29] That is, a transition region occurs where T_1 ceases to decrease as τ_c increases. For still larger τ_c, T_1 turns about and increases as τ_c increases. T_2, however, continues to decrease as τ_c increases. One readily calculates from eq 1 or from the frequency modified relaxation expressions[29] that a factor of 10–15 narrowing of the ^{13}C line width should be observed if we replaced a bonded proton with a deuterium.

If the tumbling motion becomes anisotropic, or if there is internal rotation about internuclear axes, one must use a number of different correlation times to describe the relaxation rates. This is discussed in some detail by several authors.[30,31] At any rate a $\gamma_c^2\gamma_{\{H,D\}}{}^2r^{-6}$ dependence for T_2 is still predicted, and a factor of 10–15 narrowing is again expected upon

(27) K. J. Packer, *Progr. Nucl. Magn. Resonance Spectrosc.*, 3, 87 (1969).
(28) D. E. Woessner, *J. Phys. Chem.*, 70, 1217 (1966).
(29) H. G. Hertz, ref 27, p 159 ff.
(30) D. E. Woessner, *J. Chem. Phys.*, 37, 647 (1962).
(31) D. Wallach, *ibid.*, 47, 5259 (1967).

replacing a proton with a deuterium. These conclusions are based on the tacit assumption that the correlation times which determine T_2 are much shorter than T_2. If these times become comparable to or larger than T_2, the situation changes.[28] Consider the case of a molecule locked in position and oriented in a strong magnetic field, H_0. In the case of a ^{13}C nucleus a distance r from a proton a doublet splitting of magnitude $\gamma_C\gamma_H\hbar^2r^{-3}|1 - 3\cos^2\theta|$ is obtained in the ^{13}C spectra where θ denotes the angle r makes with H_0. If the hydrogen is replaced by a deuterium, a triplet results with a splitting between extremities equal to $2\gamma_C \cdot \gamma_D\hbar^2r^{-3}|1 - 3\cos^2\theta|$. If the molecule were now to undergo an anisotropic rotational reorientation at a rate slow compared with T_2, then one can show using arguments similar to those used by Woessner[28] that

$$(T_2^{-1})_{C-H} \propto \gamma_C\gamma_H\hbar^2r^{-3}|\langle 1 - 3\cos^2\theta(t)\rangle| \quad (2a)$$

$$(T_2^{-1})_{C-D} \propto 2\gamma_C\gamma_D\hbar^2r^{-3}|\langle 1 - 3\cos^2\theta(t)\rangle| \quad (2b)$$

$$(T_2^{-1})_{C-H}/(T_2^{-1})_{C-D} = \gamma_H/2\gamma_D = 3.25 \quad (2c)$$

The inverse of T_2 now falls off as r^{-3} and depends only on the first power of the proton or deuterium γ value. The brackets indicate an appropriate time average over the reorientational motion. When $\langle 1 - 3\cos^2\theta(t)\rangle$ has a value very close to zero, Woessner refers to the motion as pseudoisotropic.[28] As the reorientation times become more rapid and the reorientation angular amplitudes become larger, the T_2 ratio increases from 3.25 to a value of 10–15, as mentioned above.

In Table I we see that many of the T_2 ratios are close to the predicted value of 3.25. The T_2 ratio of 6:59 found for histidine is probably not correct (Table II). The proton widths of protonated histidine were ~1.6 times wider than the proton widths of the deuterated [C_2-^2H]histidine.[32] If we renormalize by dividing the T_2 ratio by 1.6, a value close to 4 is obtained. Glutamic acid also shows a value significantly larger than 3.25. This may be due to the T_2's being in a transition region where neither eq 1 nor eq 2 apply.

II. Overhauser enhancement factors near 3 are observed. A plausible model for the anisotropic motion of the bound amino acid is one in which the amino acid undergoes internal rotation about one or more internuclear axes while bound to the sulfonate group. (In fact, the important internal rotation may be about the R–SO_3^-, D_3N^+–R′ bond.) At the same time the resin lattice undergoes a slow "wobbling" type motion. The rapid internal rotations dominate the T_1 relaxation rate while the slow "wobbling" motion dominates T_2. The overall motion is pseudoisotropic in the sense suggested by Woessner.[28,33] Woessner has used a very similar model to explain the proton T_1 and T_2 results for benzene adsorbed to silica gel.[28]

The Overhauser enhancement factor, H, depends on the rotational correlation times, τ_c, which determine T_1. That is, as τ_c increases and T_1 enters the turnabout region mentioned above, H is predicted to decrease[12,13] from its maximal value of 3. In the case of isotropic rotational motion at 14 kG, H decreases from a maximal value of 3 for $\tau_c \lesssim 1 \times 10^{-9}$ sec to a value of 1.33 for $\tau_c \sim 1 \times 10^{-8}$ sec. The fact than an Overhauser factor equal to 3 is observed for both the 4 and 8% resins indicates that T_1 must be determined by a correlation time that is short compared with 1×10^{-8} sec.

III. Large differences between T_2 and T_1 are observed. T_1 changes only slightly with the degree of cross-linking whereas T_2 changes appreciably. All resin-bound amino acids, for example, showed a factor of 2–3 increase in width when the degree of cross-linking changed from 4 to 8%.

This observation is readily explained on the basis of the above anisotropic rotational model. To a first approximation, we suggest that the rapid internal rotational motion which probably determines T_1 is little affected by increasing the degree of cross-linking, whereas the wobbling motion of the resin lattice, which determines T_2, becomes more restricted in amplitude. This results in a factor of 2–3 increase in the time-averaged dipolar interation, eq 2, when the degree of cross-linking increases from 4 to 8%.

If the motions were isotropic, one can readily calculate from Table III and the detailed relaxation expressions in the literature[29] that in the case of histidine, T_1 is determined by a τ_c correlation time which equals $\sim 1.5 \times 10^{-9}$ sec and 2×10^{-9} sec, respectively, for the 4 and 8% resins. While these τ_c values are consistent with the large H factors observed, they must be regarded as only approximate since the motions of the amino acids on the resins are not isotropic.

An independent estimate of τ_c can be made from the *deuterium* T_1 times. If one uses an isotropic rotational model to define an "approximate" τ_c, then[35]

$$(T_1)^{-1} = {}^3/_8\left(\frac{e^2qQ}{\hbar}\right)^2\tau_c \quad (3)$$

$e^2qQ/\hbar$ is typically[35,36] 180–200 kHz; eQ is the deuterium quadrupole moment, while eq is the bond electric field gradient at the deuterium nucleus. The histidine deuterium T_1's can be indirectly inferred from a comparison of the ^{2}H coupled and decoupled carbon line widths (Figure 6). The broadening due to the deuterium spin–lattice relaxation is simply the difference between the ^{2}H coupled and decoupled carbon widths. This difference is 20 ± 5 and 26 ± 5 Hz, respectively, for the 4 and 8% resins (Figure 7 and Table III). This implies that the deuterium T_1 times, like the carbon T_1 times, do not change with the degree of cross-linking. We use Pople's equation (3.5)[37] to estimate the deuterium T_1. This equation

(32) This discrepancy in widths is not understood. It appears in part due to the differences in ionic strength. The deuterated histidine had an appreciable amount of Na^+ introduced during the base-catalyzed exchange of the C_2 proton with deuterium. Adding an approximately equivalent amount of NaCl to a resin-bound sample of protonated histidine caused some line-width narrowing.

(33) Our observation of pseudoisotropic motion in ^{13}C nmr may not be the first example in the literature. Duch and Grant[34] reported the ^{13}C spectra of bulk *cis*-1,4-polyisoprene. The line widths were ~10 Hz or less and do not show an r^{-6} dependence. For example, the α-carbon in this polymer was the only carbon with no protons bonded to it, yet its width could not have been more than a factor of 2–3 narrower than the remaining carbons which had one or more hydrogens bonded to them. If the system was pseudoisotropic with widths going as r^{-3} (eq 2 above) the nearest neighbor protons may contribute significantly to the α carbon T_2, and its observed width relative to the other carbons would be more reasonable.

(34) M. W. Duch and D. M. Grant, *Macromolecules*, 3, 165 (1970).

(35) J. A. Glasel, *J. Amer. Chem. Soc.*, 91, 4569 (1969).

(36) B. M. Fung, I. Y. Wei, and P. L. Olympia, Jr., to be published. These authors find that e^2qQ/h has the values 165–175, 185–195, and 200–210 kHz, respectively, in deuterioalkane, -alkene, and -alkyne samples.

requires as input the difference between the ^2H coupled and decoupled carbon widths and the J coupling constant between the C_2 carbon and bonded deuterium. This coupling constant is 35 Hz. One obtains a $(T_1)^{-1}$ value equal to $\sim$320 sec^{-1}. Substituting this value in eq 3 gives a value of 5×10^{-10} sec for τ_c. This is within a factor of 3–4 that estimated from the ^{13}C T_1 times. A similar type of argument applies to glycine (Figure 6) where we infer from the partially resolved multiplet (Figure 7) that T_1^{-1} for the α-deuterium is approximately 120 sec^{-1}. This estimate together with the fact[36] that $e^2qQ/h \sim 165$ kHz implies a τ_c value of $1{-}2 \times 10^{-10}$ sec.

In conclusion, we see that a model which assumes anisotropic motion on the resin can explain the nmr results. We deduce that a short correlation time of the order of 1×10^{-9} sec or less at room temperature determines the T_1 and H values. The line widths, or T_2 values, are determined by a long correlation time. A "wobbling" motion of the resin lattice may be responsible for this long correlation time. It would have been of some interest to study the T_2 ratios of the deuterated and protonated amino acids as a function of temperature to see if this ratio could be increased from 3 to 10 or 15 with increasing temperature. Unfortunately, we did not have a suitable temperature probe for this type of study. The model we propose is a first approximation. Previous work[27,28] done on molecules adsorbed on surfaces supports its basic features. However, the previous studies also revealed features of considerably greater complexity.[27] For example, instead of one short and one long correlation time determining T_1 and T_2, respectively, these studies show a distribution of short and long correlation times.

(37) J. A. Pople, *Mol. Phys.*, 1, 168 (1958).

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The Kinetics of Iodination of Sodium Phenylpropiolate

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Abstract: The addition of iodine to sodium phenylpropiolate was studied in aqueous solution. At moderate iodide ion concentrations the reaction was found to be termolecular, involving iodine, iodide ion, and sodium phenylpropiolate with an activation energy of 15.3 kcal/mol, and an activation entropy of −23.5 eu. Analysis of the rate constants at low iodide ion concentrations revealed a three-term expression $k_{obsd} = k_t(I^-)/(K_1 + I^-) + k'/(K_1 + I^-) + k''/(I^-)(K_1 + I^-)$. In this expression k_t is the rate constant for the termolecular reaction, k' the rate constant for a bimolecular reaction involving molecular iodine, and the constant k'' is the rate constant for a reaction possibly involving the hydrated iodine cation (H_2OI^+). K_1 is the equilibrium constant for the triiodide ion. Product analyses, salt effects, the relative rates of reaction of phenylpropiolic acid and its anion, the effect of pH, activation parameters, and solvent isotope effects were investigated and are not inconsistent with the proposed mechanism.

The kinetics of the addition of iodine to sodium phenylpropiolate in water was studied in 1933 by Moelwyn-Hughes and Legard.[3] These authors found that at most concentrations the reaction was first order in sodium phenylpropiolate and triiodide ion, which they assumed to be the active iodinating species at high iodide ion concentrations. At low concentrations ($I_2 \leqq 0.0025$ M) the rate of the reaction increased sharply, and they proposed hypoiodous acid as the predominating species. Calculations of the rate constants at two different low iodide ion and iodine concentrations gave results consistent with this proposal.

The early authors kept the ratio of concentrations of sodium phenylpropiolate, iodine, and potassium iodide constant throughout all the experiments (1:1:4) and thus were unable to study the effect of each of these independently of the others. They did not study the effect of added salts, the nature of the products, or the pH dependence of the reaction, which is particularly important if HOI were involved at low iodide ion concentrations. In view of the current interest in additions to acetylenic compounds,[4,5] it seemed worthwhile to study the reaction in greater detail, particularly since it seemed that the reactive species at low concentrations had not been sufficiently identified by the early authors.

(1) Taken in part from the Ph.D. Dissertation of M. H. Wilson, Bryn Mawr College, May 1970; National Science Foundation Trainee, 1967–1968; United States Steel Fellow in Chemistry, 1968–1970.
(2) To whom inquiries should be addressed.
(3) E. A. Moelwyn-Hughes and A. R. Legard, *J. Chem. Soc.*, 424 (1933).

Results

The Order of the Reaction. The rate of the reaction is first order with respect to both sodium phenylpropiolate (A) and stoichiometric iodine and the experi-

(4) P. B. D. de la Mare and R. Bolton, "Electrophilic Additions to Unsaturated Systems," Elsevier, New York, N. Y., 1966.
(5) R. C. Fahey, *Top. Stereochem.*, 3, 237 (1968).