perimental and predicted spectra of a given isomer is clearly much greater than that between the experimental spectrum of one isomer with the predicted spectrum of any other isomer. The rather close agreement between observed and calculated spectra for *cis*- and *trans*-decalin is taken as additional evidence that these compounds do have all chair ground states.

The parameters in Table III will greatly facilitate ^{13}C chemical shift assignments of the spectra of other isomers of this series and of other compounds of this general type. For example, the many isomers produced by hydrogenation of polycyclic aromatic hydrocarbons should be readily identified by this technique. It is anticipated that the parameters developed in this work will provide a basis for conformational identification of systems which otherwise are not readily characterized. Furthermore, the parameter set provides a reasonable basis for estimating the shifts of the parent hydrocarbons for such important systems as the steroids, thereby providing a basis for elucidating other substituent parameters important in these systems.

Acknowledgment. This research was suported by the National Institutes of Health under Grant No. GM-08521. D. K. D. was the recipient of a National Science Foundation traineeship during a part of the time in which this work was accomplished.

pH-Dependent Nuclear Magnetic Resonance Spectra of ^{15}N-Enriched Glycine. Line Shape and Relaxation Studies[1a]

Robert A. Cooper,[1b] Robert L. Lichter, and John D. Roberts*

Contribution No. 4522 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California 91109.
Received September 7, 1972

Abstract: The influence of pH on the line shapes, chemical shifts, and relaxation times of the ^{15}N resonances in aqueous solutions of 95%-enriched ^{15}N glycine is discussed. Below pH 6.4, the observed ^{15}N and ^{1}H spectra of glycine clearly reflect chemical-exchange modulation of the ^{15}N–^{1}H scalar interaction. The observed increase in the scalar relaxation, as well as the concomitant decrease in the nuclear Overhauser enhancement of the ^{15}N resonance as the pH increases, can also be explained on the basis of chemical exchange. A comparative study of ^{15}N-enriched glycine and ethyl glycinate shows that the ^{15}N chemical-shift changes found for glycine in the acid region reflect the electronic changes at the carbonyl function. Furthermore, the observed differences in the line shapes for the two compounds correlate well with the differences in pK_a's for the $^{+}NH_3$ group. The changes in line shape for both the decoupled and undecoupled ^{15}N spectra of glycine and its ethyl ester above pH 6.4 suggest that ^{15}N chemical-shift averaging occurs as the result of exchange between the protonated and deprotonated forms.

The question of the effects of chemical exchange on ^{15}N nmr spectra is of special importance to the use of ^{15}N resonances in the study of biological systems. In an earlier paper,[2] the nuclear Overhauser enhancement of ammonium-^{15}N chloride resulting from irradiation of the protons was shown to be pH dependent, as expected for chemical exchange. In addition, it was noted that the proton-decoupled ^{15}N signals of ammonium chloride disappeared in basic solution in an apparently anomalous way. Other reports have shown that both chemical shifts[3] and $^{1}J_{^{15}NH}$ splittings[4] are pH dependent as the result of chemical exchange. Surprisingly, the reported[5] ^{15}N relaxation studies have ignored the possible contributions of chemical exchange to the total spin–lattice relaxation time. The purpose of this paper is to delineate the effects of chemical exchange on ^{15}N resonances through the pH dependence of the line shapes, chemical shifts, nuclear Overhauser enhancements, and nuclear relaxation rates of ^{15}N-enriched glycine.

Experimental Section

Glycine-^{15}N. Glycine, enriched to 99% with ^{15}N (Bio Rad), was used, for the most part, without further purification. For relaxation studies, the amino acid was passed through a column packed with Dowex IA chelating resin to remove paramagnetic ions that might be present. The T_1's obtained before and after this treatment were identical. The samples were thoroughly degassed with N_2 before the spectra were taken.

Ethyl Glycinate-^{15}N Hydrochloride. Glycine, enriched in ^{15}N (0.069 g), was added to 3.0 ml of absolute ethanol in a 5-ml pear-shaped flask fitted with a reflux condenser. Gaseous hydrogen chloride was bubbled slowly into the solution. The temperature rose quickly and the glycine dissolved. The flow of hydrogen chloride was continued for an additional 20 min. The reaction mixture was then poured into a petri dish and the solvent removed under reduced pressure at room temperature. The yield was 0.11 g (87%) and the melting point was 142–144° (lit.[6] 144°).

Procedures. The pH measurements were made with a Radiometer Model 26 pH meter and a combined glass reference electrode (Radiometer Copenhagen). Hydrochloric acid and potassium hydroxide were used to adjust the pH.

(1) (a) Supported by the Public Health Service, Research Grant No. GM-11072 from the Division of General Medical Sciences, and the National Science Foundation; (b) National Institutes of Health Postdoctoral Fellow, 1971–1972.

(2) R. L. Lichter and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 3200 (1971).

(3) R. L. Lichter, "Determination of Organic Structures by Physical Methods," Vol. 4, F. C. Nachod and J. J. Zuckerman, Ed., Academic Press, New York, N. Y., 1971, Chapter 4.

(4) R. L. Lichter and J. D. Roberts, *Spectrochim. Acta, Part A*, **26**, 1813 (1970).

(5) T. Saluvere and E. Lippmaa, *Eesti NSV Tead. Akad. Tiom., Fuus., Mat.*, **20**, 91 (1971); E. Lippmaa, T. Saluvere, and S. Laisaar, *Chem. Phys. Lett.*, **11**, 120 (1971).

(6) T. Curtius and F. Goebel, *J. Prakt. Chem.*, [2] 37, 157 (1888).

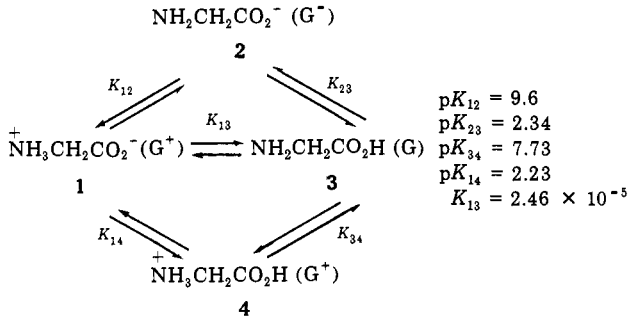
The ^{15}N spectra were recorded with a DFS-60 frequency sweep spectrometer operating at 6.07 MHz.[2] The coupled and decoupled spectra were obtained using high H_1 fields and a 4 Hz/sec sweep rate, while a 40 Hz/sec sweep rate was employed for the relaxation studies. Proton-noise decoupling (H_2) was achieved by mixing the outputs from a Hewlett-Packard 5100A frequency synthesizer and a Hewlett-Packard 3722A noise generator with the bandwidth set at 600 Hz. The decoupling frequency was set for maximum signal intensity and this changed somewhat with pH.

The ^1H spectra were recorded on standard Varian A60A and 220-MHz spectrometers. The proton-decoupling experiments were carried out on a Varian T-60 spectrometer.

Results and Discussion

The chemistry of glycine is complicated by the fact that it can exist in several forms: the glycine cation (G^+), $^+NH_3CH_2CO_2H$; the zwitterion ($G^\pm$), $^+NH_3CH_2CO_2^-$; the neutral amino acid form, $NH_2CH_2CO_2H$ (G); and the glycine anion (G^-), $NH_2CH_2CO_2^-$. The concentrations of these forms are highly pH dependent: the cation predominates at very low pH's; the zwitterion is the major form at pH 6.4; and the anion prevails in very basic media. At pH 2.34, the concentrations of G^+ and $G^\pm$ are equal and are by far the dominant forms, while equal amounts of $G^\pm$ and G^- are found at pH 9.6.[7] These compounds, as well as their respective equilibrium constants, are summarized in Scheme I. This system has been previously

Scheme I



studied, using ^1H nmr of glycine-^{14}N.[8] As expected, G^+ was found to be the principal species below pH 2.4 and $G^\pm$ became dominant at higher pH's. The hydrogens on nitrogen exchange intermolecularly and with the solvent. The exchanges are base catalyzed, with water being the principal active base at low pH's, while hydroxide is the important base at higher pH's. In neutral solution, the carboxylate function of the zwitterion appears to be an active participant in the exchange process.

The change in the relative population of the above species with pH, as well as the exchange process, has a profound effect on the ^{15}N chemical shifts of glycine, as exemplified in Table I. The change in chemical shift from pH 0.5 to 6.6 is most likely due to changes in the polarity of the carboxy function in going from predominantly G^+ to $G^\pm$ by analogy to ^{13}C results,[9] and the comparatively large upfield shift at pH 13.6 surely reflects the predominance of the glycine anion, *i.e.*, by direct electronic changes at the nitrogen itself ($-^+NH_3 \rightarrow -NH_2$). Assuming the shifts at pH's 0.5 and 6.6 reflect the predominance of G^+ and $G^\pm$, respectively, the

(7) E. J. Cohn and J. I. Edsall, "Proteins, Amino Acids and Peptides," Reinhold, New York, N. Y., 1943, p 99.
(8) M. Sheinblatt and H. S. Gutowsky, *J. Amer. Chem. Soc.*, 86, 4814 (1964).
(9) W. J. Horsley and H. Sternlicht, *ibid.*, 90, 3738 (1968).

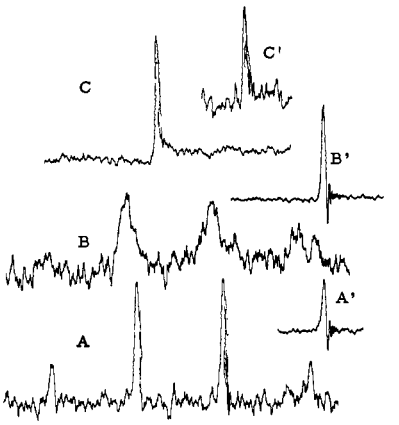


Figure 1. Coupled and decoupled ^{15}N spectra of enriched glycine as a function of pH: A, below −0.5; B, −0.3; C, 6.4. The decoupled signals (marked with primes) were stored on the CAT 1024 in the subtract mode and are actually negative signals. The sweep width for each was 500 Hz.

Table I. Chemical Shifts of ^{15}N-Enriched Glycine as a Function of pH

pH	Chemical shifts[a]
0.5	346.1 $^+NH_3CH_2CO_2H$
2.8	344.9
3.7	344.3
6.0	344.3
6.3	344.1
6.6	344.0 $^+NH_3CH_2CO_2^-$
13.6	356.5 $NH_2CH_2CO_2^-$

[a] Chemical shifts are in ppm upfield from external 10 M $H^{15}NO_3$. All the measured shifts, except at pH 13.6, are of the proton-decoupled ^{15}N resonance.

chemical shift observed at pH 2.8 is compatible with complete chemical-shift averaging of the nitrogen between G^+ and $G^\pm$, *i.e.*, the difference in ^{15}N chemical shifts between G^+ and $G^\pm$ (79.7 radians/sec) is much smaller than the rate of exchange between the two forms (*vide infra*). The shift at pH 2.8, equidistant between those observed at pH's 6.6 and 0.5, reflects the equal concentration of the two forms (pK_{a1} of glycine = 2.34). If these small variations of chemical shifts for glycine in acid media are due to changes in ionization only at the carboxyl function, the position of the ^{15}N signal of ethyl glycinate should be invariant with pH over the same pH range. It is observed that the ^{15}N shift of ethyl glycinate remains 347 ppm upfield from external $H^{15}NO_3$ from pH 0.5 to 7.3. At higher pH's, the ester begins to be hydrolyzed.

Line-Shape Studies below pH 6.4. The chemical-shift variations in the acid region were ascribed to the gradual change in the relative concentration of G^+ and $G^\pm$ with pH and their interconversion *via* proton exchange at the carboxy function. This does not imply that exchange of the protons on nitrogen is not occurring, but simply that the concentrations of the resulting conjugate bases (G and G^- from G^+ and $G^\pm$, respectively) are too small to have a significant effect on the chemical shifts. In fact, chemical exchange of the ammonium protons does occur at just the proper rate to affect the ^{15}N–^1H scalar interaction. These effects are demonstrated in Figure 1, which shows the proton-coupled and decoupled ^{15}N spectra of glycine at pH's below −0.5 (12.0 M HCl), −0.3, and 6.4. At pH 1.0,

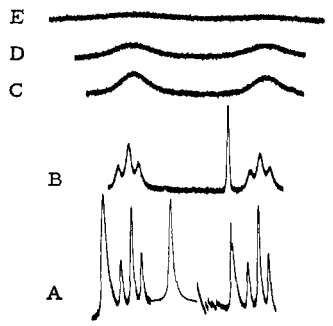


Figure 2. NH_3 proton spectra of ^{15}N-enriched glycine as a function of pH: A, below −0.5 (12 *M* HCl); B, −0.2; C, 0.0; D, 0.3; and E, 0.5.

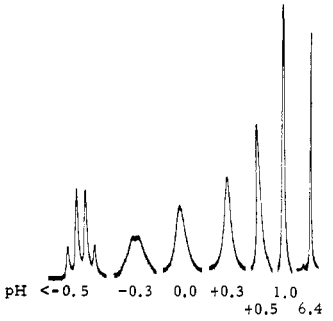


Figure 3. α-Methylene proton spectra of ^{15}N-enriched glycine as a function of pH.

the ^{15}N quartet coalesces to a broad single line. On further addition of base, this line broadens, then vanishes in the baseline noise, and finally reappears as a broad signal at pH 6.0, which sharpens at pH 6.4, as shown in Figure 1c.

The pattern of the proton-coupled ^{15}N signals is consistent with the expected effects of chemical-exchange modulation of the ^{15}N–^{1}H scalar interaction. Broadening of the quartet commences (pH −0.3) as the rate of chemical exchange, τ_e^{-1} (where τ_e is the preexchange time), approaches the spin–spin coupling constant, A, 464 radians/sec. Coalescence to a broad, single-line absorption occurs when $A^2\tau_e^2 \simeq 1$, and a sharpened signal is expected for exchange rates where $A^2\tau_e^2 \ll 1$ (Figure 1c). Sharp ^{15}N signals can also be achieved by proton decoupling which, like rapid exchange, averages out the ^{15}NH scalar interactions to zero. The similar line widths of the decoupled and coupled signals in Figures 1a and 1c suggest, for the conditions of Figure 1c, complete averaging of the scalar interaction by chemical exchange, while for those of Figure 1a, no appreciable perturbation for the undecoupled signals is noted. The line broadening in the coupled spectrum at pH −3.0 (Figure 1b) is clearly due to modulation of the scalar interaction by chemical exchange.

Accurate exchange rates could not be extracted from the ^{15}N resonances because the rapid-passage technique was employed. This is not the case for the corresponding spectra of the protons on nitrogen and the α-methylene protons of glycine whose spectra are presented in Figures 2 and 3, respectively. The H–N–C–H coupling constant (37 radians/sec) is much smaller than the ^{15}N–H splitting (466 radians/sec), and it

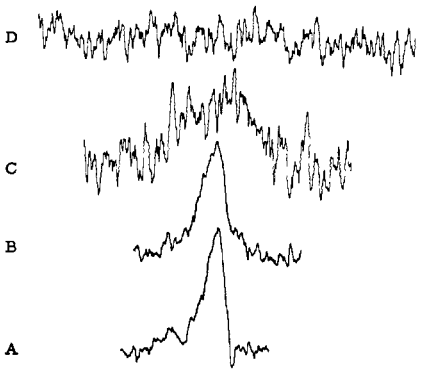


Figure 4. Coupled ^{15}N spectra of enriched glycine at pH 6.4 as a function of glycine concentration: A, 1.0 *M* (100 scans); B, 0.5 *M* (200 scans); C, 0.35 *M* (500 scans); and D, 0.25 *M* (1300 scans). The sweep width in each was 100 Hz. The number of scans in each case was adjusted to offset the inherent changes in sensitivity with changes in the glycine concentration.

averages at an exchange rate well below that required to collapse the ^{15}N–H doublet. Above pH 1.0, the amine proton signal is either buried under, or averages into, the strong water absorption.

The perturbation of the ^{1}H line shapes in Figures 2 and 3 for the amine proton exchanges is expected to be governed by eq 1, with slow sweeps and in the limit of

$$\frac{1}{T_2} - \frac{1}{T_2^0} = \frac{A^2\tau_e^2}{4}\left(1 + \frac{1}{1 + 9\omega_{IS}^2\tau_e^2}\right) \qquad (1)$$

rapid exchange ($A^2\tau_e^2 < 1$),[10] where ω_{IS} is the chemical-shift separation of the coupled spins in radians/sec (δ_{HNCH} = 642 radians/sec), and $1/T_2$ and $1/T_2^0$ are the respective full widths at half height (in radians/sec) for the signals observed during, and in the absence of, exchange.[10] The signals of the α-methylene protons in Figure 3 were used to determine the exchange rates, taking $(T_2^0)^{-1}$ as the line-width measurement at pH < -0.5.[11] The exchange rates were the same at pH's −0.2 and 0.0 (11.5 and 11.7 sec^{-1}, respectively) and increased steadily with pH to 33.6 and 105 sec^{-1} for pH 0.3 and 0.5, respectively. The increase with pH may reflect increased concentration of the zwitterion $G^{\pm}$, in which the carboxyl function can act as an additional base. Extrapolation to higher pH's yields exchange rates which satisfy the condition for total averaging of the ^{15}N–^{1}H scalar interaction at pH 6.4 ($A^2\tau_e^2 < 1$) noted earlier.

At a given pH, decreasing the glycine concentration should decrease the exchange rate, if the exchanges involve two or more glycines in the rate-determining step. Figure 4 shows the coupled ^{15}N signal of glycine at pH 6.4 as a function of glycine concentration, and the gradual broadening of the signal with decreasing concentration of glycine is as expected for a change in the rate of exchange. In addition, as in the experiments where the pH was raised, changes in glycine concentration had no effect on the line shapes of the decoupled

(10) (a) I. Solomon and M. Bloembergen, *J. Chem. Phys.*, 25, 261 (1956); (b) S. Alexander, *ibid.*, 38, 1787 (1963); (c) *ibid.*, 40, 2741 (1964); (d) E. Grunwald, C. F. Jumper, and S. Meiboom, *J. Amer. Chem. Soc.*, 84, 4664 (1962).

(11) Interestingly, the changes in τ_e with pH are such that the increase in $9\omega_{IS}^2\tau_e^2$ on going to 220 MHz gives a ^{1}H spectrum of glycine at pH −0.2, similar to that observed at pH < -0.5 at 60 MHz. Analogous differences were observed for the other pH values shown in Figure 3.

signals at pH 6.4. The spectra in Figure 4 are quite similar to those found with constant glycine concentration (0.5 M) and decreasing the pH from 6.4 to 5.5.

Comparison of the pH dependence of the ^{15}N spectra of enriched glycine and its ethyl ester, the latter being a stronger acid ($pK_{a2}(G^{\pm}) - pK_a(\text{ester}) = 1.9$),[12] showed that the full widths at half-height for the ^{15}N resonances of glycine and ethyl glycinate at pH 6.0 and 3.7, respectively, were similar, and each was broader than the corresponding proton-decoupled resonances. For both substances, addition of equal amounts of acid caused the ^{15}N signal to disappear into the base-line noise. The difference in pH units for comparable line widths closely approximates the difference in pK_a's of the two species. The similarities in line shape as a function of pH, aside from the pH shift, suggest that in this pH range, intramolecular exchange in glycine (K_{13} in Scheme I) is not the dominant cause of line broadening.

Line-Shape Studies above pH 6.4. The similar line widths found for the undecoupled and decoupled ^{15}N signals of glycine at pH 6.4 (Figure 1c) indicate complete averaging of the nitrogen–hydrogen scalar interaction. Therefore, one might not expect further change in the coupled spectra as the pH is increased, because the expected increase in rate of chemical exchange should have no additional effect on the scalar interaction. This expectation is not borne out by Figure 5, which shows the coupled spectra of ^{15}N glycine from pH 6.4 to 13.5. Furthermore, the strong negative proton-decoupled signal also broadens at pH 8.3, then vanishes into the base line, and finally returns as a barely discernable negative signal at pH 13.5.

There seem three possible explanations for this pattern: first, the possibility of contamination by paramagnetic impurities from the added base; second, increased association of the glycine species as the pH is raised; and third, chemical-shift averaging.

The first of these possibilities seems unlikely because lyophilization of the glycine solution at pH 9.4, which might be expected to render paramagnetic impurities insoluble, followed by addition of acid, regenerated the ^{15}N signal without the line broadening expected for contamination by paramagnetic salts. The second possibility, increased aggregation of the glycine, could attenuate the Overhauser effect[13] and induce broadening of the coupled signal,[14] but the degree of aggregation necessary to achieve these effects would seem to involve a substantial increase in viscosity of the solution, which was not observed. The remaining alternative, ^{15}N chemical-shift averaging, is probably the most tenable.[15] The principal species involved in the exchange are expected to be the glycine anion (δ_{NH_2} 356 ppm) and zwitterion (δ_{+NH_3} 344 ppm). Although a complete treatment would necessitate considering all four species of Scheme I, an A $\rightleftarrows$ B ($G^{\pm} \rightleftarrows G^-$) type exchange should suffice in the pH range considered.[16]

From the previous kinetic[8] data on glycine-^{14}N, it is

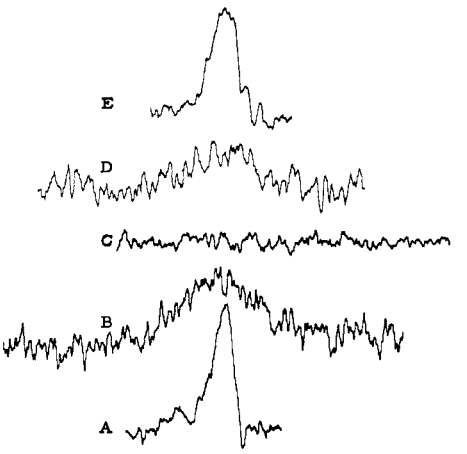


Figure 5. Coupled ^{15}N spectra of enriched glycine as a function of pH: A, 6.4; B, 8.3; C, 9.4; D, 13.2; and E. 13.5. The sweep widths are 100 Hz.

$$\underset{\delta_N\ 344\ \text{ppm}}{{}^{+}NH_3CH_2CO_2^-} + B \rightleftarrows \underset{\delta_N\ 356\ \text{ppm}}{NH_2CH_2CO_2^-} + {}^{+}BH$$

reasonable to assume that the exchange rate at pH 6.4 is such that the ^{15}N resonances of $G^{\pm}$ and G^- have coalesced but not necessarily sharpened, that is, short of the rapid exchange limit ($\tau_e^2\omega^2G^{\pm}$, $G^- < 1$). Under this assumption, the degree of line broadening may be described by eq 2,[17] where P_A and P_B are the relative

$$\frac{1}{T_2} = \frac{1}{T_2^0} + P_A^2P_B^2\Delta\omega_{IS}^2(\tau_A + \tau_B) \qquad (2)$$

populations of $G^{\pm}$ and G^-, respectively, $\tau_A = P_A/(P_A + P_B)$, and ω_{IS} is the chemical-shift difference of ^{15}N between $G^{\pm}$ and G^-. Other things being the same, the maximum broadening results when the relative populations of $G^{\pm}$ and G^- are the same (at pH 9.6). At the two extremes, pH 6.4 and 13.6, $G^{\pm}$ and G^-, respectively, are dominant ($\sim$99.999%). Thus, as observed, the broadening will be small at pH's 6.4 and 13.6. The degree of broadening at pH 8.3 is reasonable because here G^- is about 10% of the total glycine concentration.[17] However, the system is more complex than this because the pH determines, besides the equilibrium concentrations, the rates of exchange. For this reason, the ^{15}N signal was lost in the noise at pH 10.6, where the relative concentrations of $G^{\pm}$ and G^- are the same as that at pH 8.3 with G^- dominant.

The changes in exchange rate with pH account for the very weak, negative proton-decoupled signal observed at pH 13.6. The small Overhauser effect suggests a larger $T^{-1}{}_{1sc}$ (*vide infra*) which, in turn, implies a much faster rate of chemical exchange at pH 13.6 than found at pH 6.4. To get this effect, the rate of chemical exchange has to be in the vicinity of $\omega_{N,H}$, the chemical-shift difference between ^{15}N and ^1H in radians/sec, 3.4×10^8 sec^{-1}. This is comparable to what would be expected from the rate constants determined previously.[8] It is important to note that, unlike exchange modulation of the ^{15}NH scalar interaction, chemical-shift averaging affects both the proton coupled and decoupled ^{15}N resonance. Evidence in support of the postulated behavior is provided by the observation of a

(12) (a) M. Robson, *Nature (London)*, **208**, 265 (1965); (b) O. Emerson and P. Kirk, *J. Biol. Chem.*, 87, 597 (1930); (c) J. Edsall and M. Blanchard, *J. Amer. Chem. Soc.*, **55**, 2337 (1933).

(13) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).

(14) A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, Chapter 10.

(15) Chemical-shift averaging is reviewed in: C. S. Johnson, *Advan. Magn. Resonance*, **1**, 33 (1965); A. Hoffman, *ibid.*, 4, 88 (1970).

(16) Exchange will not remove the ^{15}N–C–H splitting but, because this splitting is certainly small ($\sim$0.5 Hz),[4] it will be neglected.

(17) *Cf.* G. C. Y. Lee, J. H. Prestegard, and S. I. Chan, *J. Amer. Chem. Soc.*, **94**, 951 (1972), and references cited therein.

Table II. Nuclear Overhauser Effect and Relaxation Times for ^{15}N-Enriched Glycine as a Function of pH

pH	η DO	η SO	T_1	T_{1d}	T_{1sc}
<−0.5	−4.85	−4.13	12.7 ± 1.0	13.1 ± 1.1	>460
−0.3	−2.9		11.8 ± 2.0	13.7 ± 2.0	85.0 ± 7
6.4[a]		−2.15			62

[a] The value for T_{1sc} was determined from the observed SO and eq 3–6, assuming T_{1d} was the same as determined for pH −0.3.

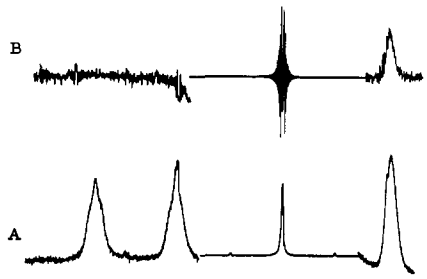


Figure 6. Proton spectra (at pH 0) of ^{15}N-enriched glycine, without (A) and with (B) decoupling of the water signal.

strong, negative decoupled signal for *N*-acetylphenylalanine over the entire pH range. Here, exchange of the amide proton is slower than the nmr time scale and none of the effects of pH observed with the free amino acids are expected to occur.

Nuclear Overhauser and Relaxation Studies. Chemical exchange of the amine protons of amino acids should strongly influence both homo- and heteronuclear Overhauser effects.[18] A homonuclear Overhauser experiment can be carried out with glycine by observing the ^1H resonance of the amine protons while irradiating the proton resonance of water with a strong H_2 field. Figure 6 shows the results of this experiment at pH 0. The loss in intensity of the amine proton resonance on decoupling the water resonances results because, under these conditions, the rate of exchange is faster than the proton relaxation rate in glycine, and the saturation of the water protons by the H_2 field is transferred to the ^{15}N–^1H protons of the glycine. As expected, aside from an intrinsic loss in intensity when the decoupler is employed, the resonances of the α-methylene protons in glycine, which are not involved in the exchange, are observed in both the absence and presence of the H_2 field. The results suggest that at pH 0.0, water is involved in the exchange process, but this does not demand that water is the only effective base. For example, both of the following exchange processes would lead to the same overall result.

$$G^+ \text{ (or } G^{\pm}) + H_2O \rightleftharpoons G \text{ (or } G^-) + H_3O^+$$

$$G^+ + G^{\pm} \rightleftharpoons G + G^+ \overset{H_2O}{\rightleftharpoons} G^+ + H_3O^+$$

The heteronuclear Overhauser effect, η, is strongly dependent on the rate of chemical exchange. Neglecting the contributions made by spin rotation and chemical-shift anisotropy to T_1,[19] η may be defined as in eq 3, where T_{1d}, T_{1sc}, and T_1 are the dipolar, scalar, and

$$\eta = \frac{0.5T_{1d}^{-1} - T_{1sc}^{-1}}{T_1^{-1}} \frac{\gamma_{^1H}}{\gamma_{^{15}N}} \quad (3)$$

total relaxation times, respectively, of the ^{15}N nucleus. The magnitude of T_{1d} should depend on the ^{15}N–H bond distance and the diffusional properties of the molecule, while T_{1sc}, in this instance, *i.e.*, in the absence of quadrupolar relaxation, should be related to the rate of chemical exchange[10a] and will be a maximum when $\omega_{^{15}NH}{}^2\tau_e{}^2 \simeq 1$. The ratio of the gyromagnetic ratios in eq 3 is −9.86, the minus sign resulting from the negative value of $\gamma_{^{15}N}$. As found earlier for ^{15}N studies with enriched ammonium chloride,[2] the ^{15}N nuclear Overhauser effect for glycine decreased with increasing pH; the values ranged from −4.85 in 12 *M* HCl (within experimental error of that expected for pure dipolar relaxation, *i.e.*, $T_{1d} = T_1$) to −2.9 at pH −0.3 and −2.15 for pH 6.4. The rather small change of η with pH indicates that $\omega_{^{15}NH}{}^2\tau_e{}^2 > 1$ in this pH range.

The values of $T_{1sc}{}^{-1}$ and $T_{1d}{}^{-1}$ can be determined if $T_1{}^{-1}$ and η are known, by using eq 3–6 on the assumption that the contribution from spin rotation and chemical-shift anisotropy are negligible.[18-20]

$$g = (1 - 0.2\,\eta)/3 \quad (4)$$

$$T_{1sc}{}^{-1} = gT_1{}^{-1} \quad (5)$$

$$T_{1d}{}^{-1} = T_1{}^{-1} - gT_1{}^{-1} \quad (6)$$

The ^{15}N relaxation times of glycine were determined by the time dependency of η,[20d] *i.e.*, the time constant for buildup of the Overhauser effect after applying the H_2 field. Here, s_t is the peak intensity measured at

$$\ln (s_\infty - s_t) = (t/T_1) + \ln (s_\infty - s_0) \quad (7)$$

time t after application of the H_2 field and s_∞ is the intensity recorded after waiting five times T_1. The slope of the plot of $\ln (s_\infty - s_t)$ *vs.* t yields $T_1{}^{-1}$ and the intercept gives the Overhauser enhancement ($1 + \eta = (s_\infty - s_0)/s_0$); the last has been termed the dynamic Overhauser effect (DO) as compared with the steady Overhauser effect (SO) measured by comparing the integrated intensities of the decoupled and undecoupled spectra directly. For glycine, the DO and SO effects were found to be similar for a given pH. The results are summarized in Table II. As anticipated, T_{1sc} decreases with increasing pH, that is, with increasing chemical exchange.

The relaxation of ^{15}N in glycine is markedly different from ^{15}N in $^{15}NH_4Cl$ under similar experimental conditions for which at pH −0.3 has been measured:[21] $T_{1sc}{}^{-1} = 0$, $T_1 = 38.3 \pm 1.5$; $T_{1d} = 58.1 \pm 2.2$; $T_{1sr} =$

(18) For an excellent review of both types of Overhauser studies see J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect—Chemical Applications," Academic Press, New York, N. Y., 1971; *cf.* also B. M. Fung, *J. Amer. Chem. Soc.*, 90, 219 (1968).

(19) The magnitude of η measured for glycine in the absence of exchange (*cf.* Table V) implies that contributions from spin rotation and chemical-shift anisotropy are small and can be neglected.[20a-d]

(20) For a discussion of these relaxation processes see (a) T. E. Burke and S. I. Chan, *J. Magn. Resonance*, 2, 120 (1970); (b) C. F. Schmidt and S. I. Chan, *ibid.*, 5, 151 (1971); (c) H. W. Spiess, D. Schweitzer, U. Haeberlen, and K. H. Hauss, *ibid.*, 5, 101 (1971); (d) K. F. Kuhlman and D. M. Grant, *J. Chem. Phys.*, 55, 2998 (1971).

(21) R. A. Cooper, unpublished results.

110 ± 4.1 sec; $\eta = -3.29$. The larger spin-rotation contribution and a smaller Overhauser effect for $^{15}NH_4Cl$ reflect its smaller spherical shape as compared to glycine. The large energy barrier (6.7 kcal/mol)[11,22] for internal rotation about the C–N bond in glycine precludes the importance of internal spin, while the significantly larger T_{1d} for ammonium chloride again reflects its smaller size (shorter τ_c).

Conclusions

Chemical exchange has profound effects on both the nmr line shapes and relaxation times of ^{15}N contained in glycine and, presumably, in other amino acids. Exchange rate data can be obtained from modulation of the scalar interaction as well as chemical-shift averaging. Although two mechanisms of line broadening could be distinguished with glycine, it is possible with other systems that line broadening may result from both processes acting simultaneously. One way of checking this would be to monitor the proton-decoupled ^{15}N signals, which will broaden only if chemical-shift averaging is taking place. The relaxation time for ^{15}N in glycine is comparable to those observed for ^{13}C, and this provides encouragement for application of the Fourier transform technique for studying the ^{15}N resonances of amino acids at the natural-abundance level.

Acknowledgments. We thank Professors S. I. Chan and D. M. Grant for many helpful discussions during the course of this work.

(22) R. G. C. McElroy, R. Y. Dong, M. M. Pintar, and W. F. Forbes, *J. Magn. Resonance*, **5**, 262 (1971).

Equilibrium and Kinetics of Glyconitrile Formation in Aqueous Solution

Gordon Schlesinger and Stanley L. Miller*

Contribution from the Department of Chemistry, University of California, San Diego, La Jolla, California 92037. *Received October 14, 1972*

Abstract: The equilibrium constant (K_G) for the dissociation of glyconitrile to formaldehyde and hydrogen cyanide in aqueous solution was measured relative to the known dissociation constant of lactonitrile. Over the temperature range 25–70° $\log K_G = 1.66 - 2184/T$ ($K_G = 2.1 \times 10^{-6}$ M at 25°). The enthalpy of dissociation was found by calorimetry to be +9.96 kcal mol^{-1}. The rate of addition of hydrogen cyanide to formaldehyde in dilute, acetate-buffered (pH 3.8–4.7) aqueous solution was measured between 25 and 45°. In this pH range the reaction is first order in both CN^- and unhydrated formaldehyde (at 25° $k_2 = 3.5 \times 10^5$ M^{-1} sec^{-1}, and $\Delta H^{\mp} = 5.02$ kcal mol^{-1}). No general acid or general base catalysis was observed. In aqueous solution at 25° the dissociation constant for mandelonitrile was found to be 5.2×10^{-3} M, and the second-order rate constant for the addition of CN^- to benzaldehyde was measured to be 1.13×10^2 M^{-1} sec^{-1}. The equilibrium and rate constants for each of the steps in the formation and dissociation of the cyanohydrins of formaldehyde, acetaldehyde, acetone, and benzaldehyde are evaluated. A plot of log K' (dissociation of the cyanohydrin to the unhydrated aldehyde) *vs.* $\Sigma\sigma^*$ gives a straight line, as do plots of log k_2 and log k_{-2} *vs.* $\Sigma\sigma^*$, except for mandelonitrile where the deviations are attributed to a resonance effect. The prebiotic significance of the reaction between formaldehyde and hydrogen cyanide is discussed.

The equilibrium formation of cyanohydrins from aldehydes or ketones and hydrogen cyanide is a reaction that has been extensively studied,[1] although there have been relatively few investigations of the kinetics. However, the cyanohydrin equilibrium with formaldehyde, the simplest aldehyde, has never been investigated, although the dissociation constant is known to be very small.[2] This reaction is of importance in prebiotic chemistry, since the synthesis of purines requires free hydrogen cyanide[3] and the synthesis of sugars requires free formaldehyde.[4] The value of this equilibrium constant is relevant to the question of whether both purines and sugars could have been synthesized at the same time on the primitive earth. We have therefore investigated the equilibrium and rates of glyconitrile formation, as well as the enthalpy of the reaction.

(1) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," 2nd ed, Cornell University Press, Ithaca, N. Y., 1969, pp 995–999, and references therein.

(2) W. J. Svirbely and J. F. Roth, *Anal. Chem.*, **26**, 1377 (1954).

(3) J. Oro and A. P. Kimball, *Arch. Biochem. Biophys.*, **94**, 217 (1961), **96**, 293 (1962); R. Sanchez, J. P. Ferris, and L. E. Orgel, *Science*, **153**, 72 (1966); *J. Mol. Biol.*, **30**, 223 (1967); **38**, 121 (1968).

(4) A. Butlerow, *Justus Liebigs Ann. Chem.*, **120**, 295 (1861); R. Mayer, K. Runge, and H. Dreschsel, *Z. Chem.*, **3**, 134 (1963); N. W. Gabel and C. Ponnamperuma, *Nature (London)*, **216**, 453 (1967); C. Reid and L. E. Orgel, *ibid.*, **216**, 455 (1967).

Experimental Section

Reagents. Hydrogen cyanide was prepared from sodium cyanide and sulfuric acid and stored as the pure solid at −78°. The required amount was transferred in a vacuum line into a flask containing deaerated water. Formaldehyde was prepared by distillation of a slurry of paraformaldehyde in dilute aqueous sulfuric acid. Both solutions were standardized by methods described below, and both were stored in a refrigerator. The other chemicals used in the experiments were reagent grade and used without further purification.

The acetic acid–sodium acetate buffers were prepared by mixing appropriate volumes of standardized acetic acid and sodium acetate solutions.

The acetaldehyde solution was prepared by adding a weighed amount of vacuum distilled acetaldehyde to an appropriate quantity