

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE 38, MASSACHUSETTS]

Raman Spectra of Carbon-Deuterated Glycine in Various Ionic Forms¹BY S. A. S. GHAZANFAR, DIRCK V. MYERS, AND JOHN T. EDSALL²

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Raman spectra are reported for C-deuterated glycine ($^+H_3N-CD_2-COO^-$) in the form of the dipolar ion dissolved in H_2O , and also for the conjugate cation in acid solution, and for the anion in alkaline solution. Similar studies are reported for completely deuterated glycine ($^+D_3N-CD_2-COO^-$) in D_2O in all three forms and for ordinary glycine ($^+H_3N-CH_2-COO^-$) in H_2O . The data were photoelectrically recorded; quantitative intensity measurements and semiquantitative depolarization values are reported. In confirmation of earlier photographic measurements from this laboratory by Takeda, *et al.*, it is found that the two C-H stretching frequencies of ordinary glycine at 2975 and 3010 cm^{-1} , which are sharp and quite distinct in solutions of the cation and dipolar ion, become fused into a single broad band in the anion, and the frequency shift decreases to about 2935 cm^{-1} . In C-deuterated glycine in H_2O the corresponding frequencies lie between 2100 and 2270 cm^{-1} ; there are, however, three distinct peaks instead of two. The downward displacement of the C-H stretching frequencies in the anion, as compared with the cation or dipolar ion, is similar to the displacement observed in ordinary glycine. Similar relations are observed for the C-D stretching frequencies in C-deuterated glycine in D_2O , but there is some overlap between one of the C-D vibrations and the N-D stretching frequency near 2200 cm^{-1} that is characteristic of the charged $-ND_3^+$ group. The frequencies characteristic of the carboxyl group are scarcely affected at all by ionization of the amino group, or by deuteration of either the amino group or the α -carbon atom.

The vibrational spectrum of glycine, the simplest of the amino acids, is of particular interest since it permits the study of the effects of ionization of both the amino and the carboxyl group, and of their interaction with the methylene group that separates the two ionizing groups. Several investigators have already studied the Raman and infrared spectra of glycine in solution, in its various ionic forms, both in ordinary water and in D_2O . The most recent of these studies, to our knowledge, are those of Takeda, *et al.*,³ in this laboratory, and of Tsuboi, *et al.*,⁴ in Tokyo. The latter paper proposes a detailed assignment of nearly all the vibrational frequencies of glycine, including some assignments for N-deuterated glycine (glycine- d_3 , $^+D_3N-CH_2COO^-$). These papers give references to the earlier literature on glycine and other amino acids.

Recently, C-deuterated glycine ($^+H_3N-CD_2-COO^-$) has become available in purified form. Comparison of its spectrum with that of ordinary glycine permits a more searching test of the assignment of certain frequencies. In particular we have examined the effect of ionization and deuteration of the amino group on the C-D stretching frequencies in the methylene group. As was first found by Garfinkel⁵ (see also ref. 3) the C-H stretching frequencies in ordinary glycine are displaced to lower values by 60 cm^{-1} or more, when the neighboring $-NH_3^+$ group loses its positive charge. Similar effects were observed in β -alanine⁶—although not in α -alanine—and were also found to be strongly present in several aliphatic diamines and the conjugate diammonium ions.⁶ Our present studies show that the effect of the $-NH_2$ or $-NH_3^+$ group in perturbing the C-D stretching vibrations is very similar to the previously observed effects on the C-H vibrations, although there are differences in detail.

Since this is apparently the first study of the Raman

spectrum of glycine with photoelectric recording, we have also reinvestigated the spectrum of solutions of ordinary glycine as anion, as dipolar ion, and as cation, to obtain quantitative intensity measurements and semiquantitative depolarization measurements. We have done the same for C-deuterated glycine in its different forms, both in H_2O and in D_2O solution.

Experimental Methods and Materials

We measured the spectra on a Cary Model 81 Raman spectrophotometer (Applied Physics Corp., Monrovia, Calif.) and determined intensities and depolarization factors as before.^{6,7} The intensities reported here were determined by measuring the areas rather than the heights of the Raman bands. C-Deuterated glycine ($^+H_3N-CD_2-COO^-$) was obtained from the Volk Radiochemical Co., Skokie, Ill. We examined it on a Beckman-Spinco amino acid analyzer and found it to move on the ion-exchange column at a rate indistinguishable from that of ordinary glycine, in the buffer system employed by Spackman, Stein, and Moore.⁸ A pH titration with alkali indicated that its pK value was very close to that of ordinary glycine. We used it for the study of Raman spectra without further purification. Conversion of the molecule to the anionic or cationic form and substitution of deuterium for hydrogen on the amino group were carried out as described by Takeda, *et al.*³

Ordinary glycine was obtained from the California Corp. for Biochemical Research and was recrystallized from hot water.

Results and Discussion

Table I records the data for ordinary glycine in H_2O , as anion, dipolar ion, and cation. Table II gives the corresponding data for C-deuterated glycine in H_2O and Table III for C-deuterated glycine in D_2O , that is for glycine in which *all* hydrogen atoms have been replaced by deuterium. We have not studied ordinary glycine dissolved in D_2O , to give $^+D_3N-CH_2-COO^-$, and the conjugate anion and cation. Takeda, *et al.*,³ however, have previously studied such solutions; and we shall refer to their data in establishing certain correlations.

Before discussing the interpretation of these spectra we consider briefly the evidence from crystal structure concerning the glycine dipolar ion. Three crystal forms— α -glycine,^{9,10} β -glycine,¹¹ and γ -glycine¹²—

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(2) To whom correspondence concerning this paper and requests for reprints should be sent.

(3) M. Takeda, R. E. S. Izvazzo, D. Garfinkel, I. H. Scheinberg, and J. T. Edsall, *J. Am. Chem. Soc.*, **80**, 3813 (1958).

(4) M. Tsuboi, T. Onishi, I. Nakagawa, T. Shimanonchi, and S. Mizushima, *Spectrochim. Acta*, **13**, 253 (1958).

(5) D. Garfinkel, Ph.D. Thesis, Harvard University, 1955.

(6) S. A. S. Ghazanfar, J. T. Edsall, and D. V. Myers, *J. Am. Chem. Soc.* **86**, 559 (1964).

(7) E. L. Elson and J. T. Edsall, *Biochemistry*, **1**, 1 (1962).

(8) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.* **30**, 1120 (1958).

(9) G. Albrecht and R. B. Corey, *J. Am. Chem. Soc.*, **61**, 1087 (1939).

(10) R. E. Marsh, *Acta Cryst.*, **11**, 654 (1958).

(11) Y. Iitaka, *ibid.*, **13**, 35 (1960).

(12) Y. Iitaka, *ibid.*, **14**, 1 (1961).

TABLE I

| RAMAN SPECTRA OF ORDINARY GLYCINE IN WATER SOLUTION ^a | | | ⁺ H ₂ N-CH ₂ -COO ⁻ | | | ⁺ H ₂ N-CH ₂ -COOH | | |
|--|-------------------|--------|---|-----------|--------|---|-----------|--------|
| $\Delta\nu$ | Intensity | ρ | $\Delta\nu$ | Intensity | ρ | $\Delta\nu$ | Intensity | ρ |
| 507 | 8 | 0.53 | 502 | 8 | 0.60 | 494 | 5 | |
| 575 | 2 | (1) | 577 | 2 | 0.86 | | | |
| | | | 665 | 1 | 0.65 | 644 | 2 | 0.42 |
| 899 | 8 | 0.15 | 896 | 11 | 0.28 | 869 | 15 | 0.20 |
| 968 | 2 | 0.26 | 1027 | 4 | (1.0) | 1042 | 4 | 0.80 |
| 1100 | 4 | 0.24 | 1118 | 4 | 0.56 | 1123 | 3 | 0.51 |
| 1169 | 1 | (0.97) | | | | | | |
| 1315 | 5(s) ^b | | 1327 | 14 | 0.27 | 1308 | 3 | 0.63 |
| 1343 | 10 | 0.48 | | | | | | |
| 1407 | 28 | 0.53 | 1410 | 20 | 0.42 | | | |
| 1440 | Masked | | 1440 | 8 | 0.57 | 1433 | 7 | (1.0) |
| 1611 | 21 | 0.81 | 1615 | 19 | 0.71 | 1644 | 34 | 0.81 |
| | | | | | | 1744 | 19 | 0.29 |
| | | | 2879 | 1 | | | | |
| 2935 | 30 | 0.29 | 2968 | 19 | 0.18 | 2975 | 35 | 0.14 |
| | | | 3011 | 5 | 0.88 | 3011 | 13 | 0.74 |
| 3320 | 8 | 0.08 | | | | | | |
| 3385 | 4 | 1.0 | | | | | | |

^a $\Delta\nu$ is in wave numbers; ρ is the depolarization factor. Some abnormally high ρ -values are enclosed in parentheses. ^b The symbol s designates a shoulder on a peak.

pass from the crystal into aqueous solution, the C-N bond may rotate into a different orientation relative to the C-COO⁻ plane. The molecule could possess a plane of symmetry, with point group symmetry C_s, if the nitrogen were to lie in the plane of the -COO⁻ group or if the CCN plane were so oriented as to bisect the symmetrical COO⁻ group.¹³ However, since glycine does not assume either of these configurations with C_s symmetry in any of the three crystal forms, it seems probable that such configurations will not be favored in solution, although some molecules may exist, at any given moment, in one or the other of the C_s configurations. We therefore assume the molecule to lack symmetry elements. We note, however, that the data in Tables I, II, and III indicate a very wide range in the depolarization factors for different Raman bands. Vibrations that are symmetric with respect to the symmetry of individual groups—such as the symmetrical stretching frequencies of the CH₂ or the NH₂ group—are in general highly polarized; and certain other frequencies appear to be depolarized to the maximum possible extent.

TABLE II
RAMAN SPECTRA OF DEUTERATED GLYCINE IN H₂O

| ⁻ H ₂ N-CD ₂ -COO ⁻ in H ₂ O | | | ⁺ H ₂ N-CD ₂ -COO ⁻ in H ₂ O | | | ⁺ H ₂ N-CD ₂ -COOH in H ₂ O | | |
|---|-----------|--------|---|-----------|--------|---|-----------|--------|
| $\Delta\nu$ | Intensity | ρ | $\Delta\nu$ | Intensity | ρ | $\Delta\nu$ | Intensity | ρ |
| 498 | 6.2 | 0.49 | 499 | 5.3 | 0.57 | 493 | 4.9 | 0.48 |
| 666 | 0.3 | 0.63 | 659 | 0.8 | .85 | 640(b) | 1.3 | (1.03) |
| 819 | 0.7 | (1.56) | 824 | 1.4 | .77 | 709 | 0.4 | 1 |
| 872 | 3.4 | 0.21 | 872 | 3.9 | .24 | 840 | 4.9 | 0.21 |
| 933 | 2.7 | .45 | 912 | 4.2 | .32 | 918 | 4.2 | 0.48 |
| 1017 | 1.3 | .46 | 1041 | 1.9 | (1.12) | 1043 | 1.9 | (1) |
| 1073 | 2.5 | .74 | 1107 | 1.1 | (0.98) | 1113 | 1.1 | (0.94) |
| 1138 | 0.9 | .54 | 1199 | 4.2 | .42 | 1205(b) | 2.5 | .52 |
| 1266 | 1.3 | .79 | | | | | | |
| 1408 | 9.5 | .48 | 1404 | 10.0 | .32 | | | |
| 1602(b) | 9.0 | .42 | 1617(b) | 8.2 | (.89) | 1637(b) | 7.3 | .77 |
| | | | | | | 1741 | 6.0 | .30 |
| 2119 | 10.0 | .15 | 2161 | 6.2 | .19 | 2168 | 7.5 | .27 |
| 2142(s) | | | 2226(s) | | | 2238(s) | | |
| 2218 | 7.6 | .82 | 2260 | 6.0 | .61 | 2265 | 9.0 | .75 |
| 3320 | 8.7 | .15 | 2780(w) ^a | | | | | |
| 3390 | 3.4 | (1.36) | 2801(w) ^a | | | | | |
| 3407(s) | | | 2865(w) ^a | | | | | |

^a These lines are extremely weak and doubtful. See text. No comparable lines were observed in acid solution.

have been studied in detail. Although the packing of the molecules differs significantly in the different crystals, the dimensions and conformations of the individual molecules are quite similar in all of them. The C-C bond distance is approximately 1.525 Å., the C-N bond distance approximately 1.48 Å., and the CCN bond angle is close to tetrahedral (111-112°). The two C-O bond distances in the -COO⁻ group apparently differ slightly from one another in any one crystal form. Their mean values also differ slightly from one type of crystal to another. All the reported values lie in the range 1.237-1.265 Å. In spite of these differences, the two C-O bonds are so nearly alike that we may assume them to be equivalent with little error. We therefore take the -COO⁻ group to be symmetrical, with an internal symmetry C_{2v}. The C-C bond lies almost exactly in the plane of the COO⁻ group, but the nitrogen atom lies out of this plane, by 0.436 Å. in α -glycine, by 0.583 Å. in β -glycine, and by 0.309 Å. in γ -glycine. When the glycine molecules

C-H and C-D Stretching Frequencies.—The patterns of the C-H stretching frequencies in the various ionic forms of ordinary glycine, as found here by photoelectric recording, are in excellent agreement with the earlier photographic studies from this laboratory (see Takeda, *et al.*³). The results are shown on the right-hand side of Fig. 1. The dipolar ion and the cation both show the intense symmetric stretching frequency near 2975 cm.⁻¹ and the much weaker asymmetrical frequency near 3015. As Fig. 1 shows, there is a small shoulder at 2880 in the spectrum of the dipolar ion and a suggestion of a similar shoulder in the spectrum of the cation. When a proton is removed from the adjoining amino group the two major C-H stretching frequencies become merged in a single broad band

(13) We here neglect the orientation of the hydrogens in the -NH₂⁺ group as a factor involved in the total molecular symmetry. In either of the suggested C_s configurations, it is easy to see that one of the hydrogens could lie in the mirror plane and the other two symmetrically on each side of it. However, such refinements are scarcely worth pursuing in the present context.

TABLE III
 RAMAN SPECTRA OF DEUTERATED GLYCINE IN D₂O^a

| D ₂ N-CD ₂ COO ⁻ —in D ₂ O— | | +D ₂ N-CD ₂ COO ⁻ in D ₂ O | | +D ₂ N-CD ₂ COOD in D ₂ O | | | |
|--|----------------|--|-----------------|--|-------------------|----------------|--------|
| $\Delta\nu$ | Inten- sity | $\Delta\nu$ | Inten- sity | ρ | $\Delta\nu$ | Inten- sity | ρ |
| 500 | 1 | 480 | 3b ^b | .. | 475 | 0.7 | 0.56 |
| .. | .. | 600 | .. | 0.34 | 625 | 0.2 | (.90) |
| .. | .. | 716 | w ^b | .14 | 767 | 4 | .67 |
| 815 | 2 | 812 | 3 | .66 | 810 | 2 | (.98) |
| 874 | 3 | 867 | 1.4 | .29 | .. | .. | .. |
| 956 | 3 | 950 | 2 | .36 | 953 | 5 | (>1) |
| .. | .. | .. | .. | .. | 1008 | 1 | 0.75 |
| 1067 | 2 | 1073 | 0.7 | (>1) | 1071 | 1 | (>1) |
| .. | .. | 1126 | 1.4 | 0.54 | 1123 | 2 | 0.66 |
| 1208 | 3 | 1200 | w | .88 | 1200 | 6 | 0.67 |
| 1408 | 10 | 1402 | 7 | .48 | 1367 | 3 | (>1) |
| 1580 | 3b | 1610 | 2b | .56 | .. | .. | .. |
| .. | .. | .. | .. | .. | 1731 | 8 | 0.40 |
| 2116 | 4 | 2129 | 5 | .50 | 2110 | 4 | .62 |
| 2163 | 3 | 2179 | 10 | .46 | 2183 | 10 | .45 |
| 2225 | 2 | .. | .. | .. | .. | .. | .. |
| .. | .. | 2266 | 4 | (>1) | 2268 | 1 | 0.66 |
| 2452 | 1 | .. | .. | .. | .. | .. | .. |
| 2520 ^c | b | 2510 | b | .. | 2520 ^c | b | .. |

^a Depolarization measurements were not made on the solution of the anion. ^b The symbol b denotes a broad Raman band and w denotes a very weak band. ^c The peak near 2520 arises primarily from D₂O.

which is displaced downward to 2935, 40 cm.⁻¹ below the symmetrical stretching frequency in the dipolar ion or the cation, and 75 cm.⁻¹ below the asymmetrical frequency. This band in the spectrum of the anion is strongly polarized ($\rho = 0.29$) which indicates that it represents predominantly a symmetrical stretching frequency. Presumably the asymmetrical vibration is also merged in the same band.

Figure 2 shows the C-D stretching frequencies of C-deuterated glycine in H₂O. The pattern of the spectra between 2100 and 2300 cm.⁻¹ shows a strong general resemblance to the similar data for ordinary glycine in the 2900-3050 range, as shown in Fig. 1. As with ordinary glycine the spectral pattern is much the same for the dipolar ion and for the cation, whereas the whole pattern is shifted to lower frequencies on removal of a proton from the amino group. There are, however, important differences from ordinary glycine. In the C-deuterated compound, all the different ionic forms appear to show three distinct frequencies between 2100 and 2300 cm.⁻¹. The middle one sometimes appears as a shoulder on one of the other bands, whereas in other cases it is clearly resolved from them (see Fig. 2). The highest and lowest of the three frequencies are displaced downward by 40-50 cm.⁻¹ when the amino group loses its positive charge. The shoulder of intermediate frequency undergoes a similar but much greater downward displacement, by 85-95 cm.⁻¹.

The interval between the two C-D stretching frequencies in the cation and dipolar ion—from about 2165 for the symmetrical to nearly 2265 for the asymmetrical frequency—is far greater than the interval between the corresponding frequencies (2975 and 3010) in ordinary glycine. Ordinary malonic acid and deuterated malonic acid¹⁴ differ in much the same way. In HOOC-CH₂-COOH the two stretching frequencies lie at 2956 and 3003, being therefore separated by 47 cm.⁻¹. In DOOC-CD₂-COOD the C-D stretching

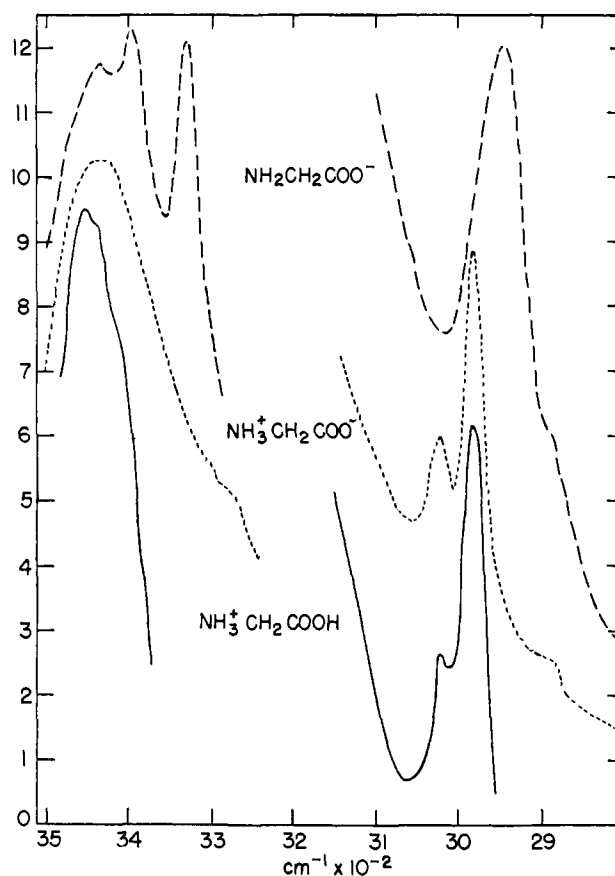


Fig. 1.—Raman spectra of ordinary glycine in H₂O, between 2800 and 3500 cm.⁻¹. Ordinate indicates relative intensity, from the spectra as recorded. The curves start from different base lines for the cation (bottom curve), dipolar ion (middle curve), and anion (top curve), so as to avoid overlapping. The curves on the left-hand side of the figure have also been displaced downward, relative to those on the right, so as to lie at a comparable level. These bands, with a peak in the region just above 3400 cm.⁻¹, arise purely from H₂O in the solutions of the cation and dipolar ion. In the solution of the anion (top left-hand curve) the N-H stretching frequencies are superimposed on the water band.

frequencies lie at 2159 and 2258, practically 100 cm.⁻¹ apart.

The major feature of the spectrum, however, is that the C-D stretching frequencies are displaced downward on the loss of a proton from the adjoining amino group, just as the C-H stretching frequencies are displaced in ordinary glycine.

The pattern of the C-D stretching frequencies for C-deuterated glycine in D₂O is shown in Fig. 3 (see also Table III). There are three clearly distinct frequencies between 2100 and 2300 cm.⁻¹ in all the different ionic forms of the completely deuterated glycine molecule, one near 2020, one near 2180, and one at 2225 in the anion and 2266-2268 in the dipolar ion and cation. The most intense band is that near 2180. When the deuterated amino group loses its positive charge the highest of the three frequencies, near 2267, undergoes a marked downward displacement, by about 40 cm.⁻¹. The other two are not much shifted. Since the solvent is D₂O, all of these bands appear as side peaks on the ascending limb of the D₂O band. The pattern for the dipolar ion is shown in Fig. 3.

The previous work from this laboratory³ on ordinary glycine dissolved in D₂O has shown clearly the presence

(14) J. T. Edsall, *J. Chem. Phys.*, **5**, 508 (1937).

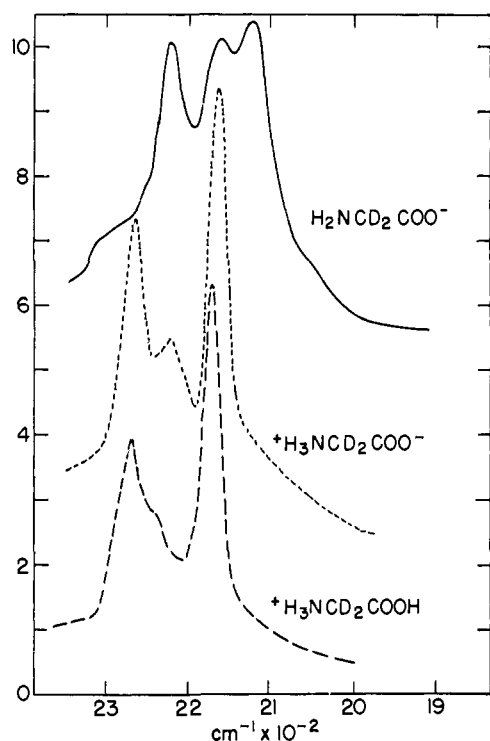


Fig. 2.—Raman spectra for C-deuterated glycine, as cation, dipolar ion, and anion, between 2000 and 2400 cm^{-1} . Compare with the curves on the right-hand side of Fig. 1.

of an N-D stretching frequency at 2200 cm^{-1} associated with the positively charged $-\text{ND}_3^+$ group. It is probable that this N-D vibration makes a major contribution to the peak near 2180 discussed above. In the anion this frequency is shifted downward by about 20 cm^{-1} and is relatively much weaker than in the other two ionic forms. This loss of intensity would favor the proposed assignment since the N-D stretching frequencies for the *uncharged* amino group lie quite outside of this region, above 2400 cm^{-1} (see below). The band near 2180 in the dipolar ion and cation, therefore, appears to be composite. It presumably arises from two nearly superimposed frequencies, one being a C-D and the other an N-D stretching mode. The peak at 2160 in the anion almost certainly arises from the C-D vibration only.

Only the highest of the C-D stretching frequencies undergoes a marked downward displacement when the deuterated amino group loses its positive charge. However, if we assign the lowest of the observed frequencies (2120 ± 10) in the cation and dipolar ion) to the symmetrical C-D stretching frequency it appears that this vibration is hardly shifted at all when the amino group loses its positive charge. The depolarization values for the frequency near 2120 are also higher than those observed for the symmetrical stretching of C-H or C-D bonds as listed in Tables I and II. These features are somewhat puzzling and raise some doubts as to the suggested assignment of this frequency.

Stretching Frequencies of the Amino Group.—The two stretching vibrations of the uncharged amino ($-\text{NH}_2$) group at 3320 (symmetrical) and 3380 cm^{-1} (asymmetrical) are virtually identical in position, relative intensity, and depolarization factors, whether the adjoining methylene group is a $-\text{CH}_2$ or a $-\text{CD}_2$

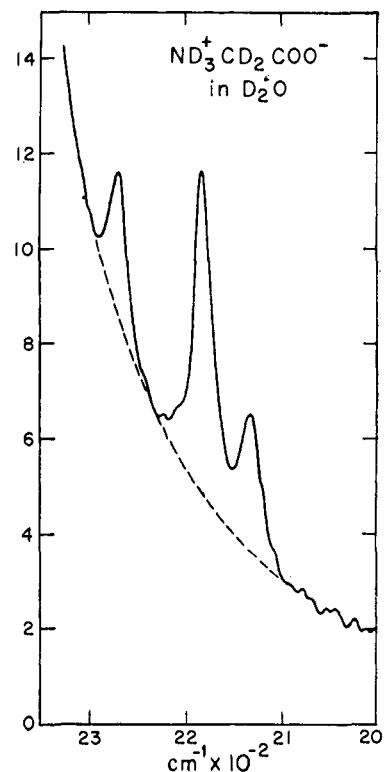


Fig. 3.—Raman spectrum of completely deuterated glycine in D_2O , between 2000 and 2300 cm^{-1} . The peaks due to C-D and N-D stretching emerge from the low frequency side of the D_2O band. The dotted line indicates the assumed base line, which is employed in estimating the areas under the peaks for intensity measurements. Small oscillations at lower right indicate the noise level of the background in some of the regions where peaks due to Raman bands are absent.

group (see Tables I and II and the left-hand side of Fig. 1). The corresponding frequencies for the $-\text{ND}_2$ group, with a $-\text{CH}_2$ group adjoining it, are 2434 and 2504 cm^{-1} (see ref. 3). The values for the $-\text{ND}_2$ group with, a $-\text{CD}_2^-$ group adjoining it, are slightly higher, 2542 and 2520 cm^{-1} according to Table III. This difference may not be significant; we are here comparing the earlier photographic data on one compound with the present measurements, photoelectrically recorded, on the other compound. Moreover, the peaks of these Raman frequencies are superimposed on the water (D_2O) band, and this makes their exact position slightly uncertain.

We have sought to observe the stretching frequencies of the $-\text{NH}_3^+$ group in $^+\text{H}_3\text{N}-\text{CD}_2\text{COO}^-$ and $^+\text{H}_3\text{N}-\text{CD}_2-\text{COOH}$. From earlier evidence,^{3,15} we expect these N-D frequencies to lie in the range 2750–3000 cm^{-1} . The absence, in C-deuterated glycine, of the C-H stretching frequencies in the same range should make the detection of the N-H vibrations much easier. Nevertheless, we have experienced great difficulties in detecting such vibrations. In one experiment we recorded very small peaks at 2780, 2800, and 2865 cm^{-1} in solutions of $^+\text{H}_3\text{NCD}_2\text{COO}^-$ (see Table II), but they were extremely weak and were observed only when the recording system was run at very high sensitivity, so that the noise level was considerable. In later runs we were unable to reproduce these findings, so we must regard the existence of these frequencies

(15) J. T. Edsall and H. Scheinberg, *J. Chem. Phys.*, **8**, 520 (1940).

as highly dubious. We never observed similar frequencies in the cation ${}^+\text{H}_3\text{NCD}_2\text{COOH}$. On the other hand, as we have pointed out above, Takeda, *et al.*,³ observed a well-defined N-D stretching frequency at 2200 cm.^{-1} both in the dipolar ion ${}^+\text{D}_3\text{NCH}_2\text{COO}^-$ and in the corresponding cation. They also observed similar frequencies in N-deuterated α - and β -alanine. The corresponding N-H frequency would be expected to lie above 2800 cm.^{-1} , probably above 2900 ; we can at present offer no explanation of the absence of such a frequency in the observed spectrum.

The C-O Stretching Frequencies and the N-H and N-D Deformation Frequencies.—The C=O stretching frequency of the un-ionized carboxyl group in the glycine cation remains essentially unchanged, regardless of the extent to which the hydrogen atoms in ordinary glycine have been replaced by deuterium. There is a very slight downward displacement from 1744 in ordinary glycine (Table I) to 1733 in completely deuterated glycine (Table III). This difference is probably just beyond the range of experimental error.

The symmetrical stretching frequency of the ionized carboxyl group, near 1400 , is even more completely independent of the deuteration of the methylene and amino groups and of the ionization of the latter group. The six values listed for this very intense frequency in Tables I, II, and III, all lie between 1402 and 1410 . These values appear to be identical within the limits of experimental error.

In the region between 1580 and 1640 three different modes of vibration may contribute to the observed spectra: (1) in H_2O solutions the bending frequency of the water molecule near 1630 is always present; (2) likewise in H_2O solutions one of the deformation frequencies of the ${}^-\text{NH}_3^+$ group is present; Tsuboi, *et al.*,⁴ assign a value near 1600 cm.^{-1} to a degenerate deformation frequency of the NH_3^+ group; (3) in solutions of the anion and the dipolar ion, in either H_2O or D_2O , the asymmetrical stretching frequency of the COO^- group always appears in the same region. This COO^- vibration appears, uncomplicated by the other two mentioned above, in the D_2O solutions of C-deuterated glycine. It lies at 1580 in the anion and at 1610 cm.^{-1} in the dipolar ion (Table III), and thus appears to be somewhat sensitive to the state of charge on the amino group. Takeda, *et al.*,³ have recorded similar differences between the dipolar ion and the anion of glycine and α -alanine when dissolved in D_2O .

The deformation frequency of the NH_3^+ group near 1600 cm.^{-1} would be expected to undergo a displacement to approximately 1200 for the ND_3^+ group. Tsuboi, *et al.*,⁴ assign a value near 1275 to the degenerate deformation frequency of the ND_3^+ group and one at 1170 to the symmetrical deformation. Edsall and Scheinberg¹⁵ observed a Raman band close to 1200 cm.^{-1} in the CH_3ND_3^+ ion and also in deuterated urea and the deuterated hydrazinium ion. We must note also that D_2O itself gives a Raman band in the same region. Similarly, we find a band close to 1200 cm.^{-1} in the cation and dipolar ion in solutions of deuterated glycine in D_2O (Table III). However, there is a Raman band at almost the same position, although with a much lower ρ -value, in solutions of the cation and dipolar ion of C-deuterated glycine in

H_2O , in which of course no ND_3^+ group exists. This band near 1200 may therefore be associated with some vibration of the CD_2 group since nothing analogous to it appears in ordinary glycine.

The Methylene Bending Frequency.—In deuterated malonic acid¹⁴ the symmetrical deformation frequency of the CD_2 group appears as a strong band at 1050 cm.^{-1} . This obviously corresponds to the CH_2 bending frequency near 1410 in ordinary malonic acid. The assignment of the corresponding frequency for deuterated glycine is not nearly so clear. In ordinary glycine, the CH_2 bending frequency is clearly the band near 1440 cm.^{-1} . For deuterated glycine in H_2O (Table II) a depolarized band near 1040 is apparent in the cation and dipolar ion. In the anion, however, there is no line in this region; instead there is one of lower frequency at 1017 and one somewhat higher at 1073 , the latter being depolarized. For deuterated glycine in D_2O (Table III) there is no band near 1040 in any of the different ionic forms, although a line near 1070 is clearly apparent in all; it has a high ρ -value and is apparently depolarized. Conceivably this may correspond to the CD_2 bending frequency but it appears odd that this frequency should be shifted to higher values on deuteration of the amino group, which is what the data of Tables II and III appear to suggest. We do not feel justified in drawing any final conclusions on this point.

Ordinary glycine shows an intense and highly polarized band near 900 in the anion and dipolar ion, which is lowered to 870 in the cation. This was termed the "sensitive frequency" by Edsall.¹⁶ It is assigned by Tsuboi, *et al.*,⁴ as a CCN symmetrical stretching vibration. When the amino group of ordinary glycine is deuterated⁴ the frequency shift is displaced downward to about 840 in the anion and dipolar ion and to 775 in the cation. When the α -carbon is completely deuterated but the amino group is not, the corresponding vibration appears to be that found at 872 in the anion and dipolar ion and 840 in the cation (Table II). For C-deuterated glycine in D_2O the identification of the corresponding vibration is more doubtful. It may be the Raman band which appears near 815 in the anion and dipolar ion and at 767 in the cation (Table III). However, the high ρ -values for this frequency make this particular assignment dubious, since the "sensitive" frequency in Tables I and II shows $\rho = 0.20$.

The band at 867 in the dipolar ion is much more strongly polarized but it is also of low intensity. It seems in any case improbable that this frequency should have a value as high as 867 in the completely deuterated molecule.

There are a number of Raman bands recorded in Tables II and III for which we can at present make no assignment with any confidence. However, we note that the strong band near 500 cm.^{-1} appears in approximately the same position, in all forms of glycine, regardless of the state of ionization or the extent of deuteration. It is the first frequency listed in all the columns of Tables I, II, and III. Tsuboi, *et al.*,⁴ attribute it to a rocking motion of the carboxyl group. As a first approximation the position of this band appears to be independent of either ionization or

¹⁶ J. T. Edsall, *J. Chem. Phys.*, **4**, 1 (1936).

deuteration of the carboxyl group. This would suggest that it must correspond to a vibrational mode that does not involve the hydrogen atoms in any significant way, and the proposal of Tsuboi, *et al.*,⁴ might well be correct.

Since this paper was submitted we have seen the important paper of Suzuki, Shimanouchi, and Tsuboi,¹⁷ who studied the infrared spectra of crystals of $^+H_3NCH_2COO^-$, $^+D_3NCH_2COO^-$, and $^+D_3NCD_2COO^-$. They studied these molecules only in the isoelectric form and were not concerned, as we are here, with the effects of ionization. Their data and ours are generally in good agreement, especially if one allows for the fact that they studied the infrared spectra of crystals, whereas we have studied the Raman spectra of solutions. There is one rather strong vibrational frequency in the infrared spectra, which lies between 330 and 360 cm^{-1} , to which we have observed nothing corresponding in the Raman spectra. Suzuki, *et al.*,¹⁷ assign this to a CCN bending motion. They have calculated the frequencies for a seven-body model of isoelectric glycine with C_s symmetry, assuming a Urey-Bradley type of force field. The $-NH_3^+$ group was taken as a single atom in the model. Their assignments of observed frequencies to modes of vibration are much

(17) S. Suzuki, T. Shimanouchi, and M. Tsuboi, *Spectrochim. Acta*, **19**, 1195 (1963).

more detailed than ours; in general, our more limited attempts at such assignments are in harmony with theirs. They conclude that, in the compounds containing $-CD_2-$ groups, the skeletal stretching vibrations couple heavily with the CD_2 scissoring and wagging modes. Thus no one of the vibrations in the 800–1200 cm^{-1} region can be uniquely assigned to a CD_2 scissoring motion, a conclusion which gives a rational basis for our failure to identify such a vibrational mode in the Raman spectra.

Our studies on the effects of ionization do lead us to question their assignment of the 1334 cm^{-1} frequency in $^+H_3NCH_2COO^-$ to a CH_2 wagging motion. In the Raman spectrum of the cation $^+H_3NCH_2COOH$ this frequency lies at 1308 (Table I). It is relatively weak, with $\rho = 0.63$. In the dipolar ion the observed Raman band is at 1327; it is very intense, and ρ is only 0.27. This suggests that two different modes of vibration are involved, and that the one which is most intense and characteristic in the dipolar ion is somehow associated with the ionized carboxyl group. Apart from this one point, our conclusions appear to be almost entirely in harmony with those of Suzuki, *et al.*, insofar as the two investigations overlap.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY, ITHACA, NEW YORK]

The Influence of Hydrogen and Hydrophobic Bonds on the Stability of the Carboxylic Acid Dimers in Aqueous Solution¹

BY EUGENE E. SCHRIER,² MARCIA POTTLE, AND HAROLD A. SCHERAGA

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The temperature dependence of the molality quotient of dimerization for formic acid has been determined in a 3 *m* Na(Cl) ionic medium by potentiometric titrations at 10, 25, 40, and 55°. The enthalpy of dimerization, $\Delta H_D = 0 \pm 1$ kcal./mole, is considered to be equivalent to ΔH_D° , giving the strength of a typical hydrogen bond between polar groups in water solution. If it is assumed that cyclization is not a mechanism for the increasing stabilization of the carboxylic acid dimers with chain length, the increase of the dimerization constant may be attributed to the formation of pairwise hydrophobic bonds between the carboxylic acid side chains. Experimental values of the standard free energy of formation for methyl-methyl, ethyl-ethyl, propyl-propyl, and benzyl-benzyl hydrophobic bonds are in quantitative agreement with the values for the same side-chain interactions calculated from the theory of Némethy and Scheraga.³

Introduction

The contribution of hydrophobic bonds to the stability of protein conformations in aqueous solution has recently been considered by several investigators. Némethy and Scheraga³ developed a theoretical treatment of these interactions which predicts, among other things, the thermodynamic parameters for pairwise hydrophobic bond formation between nonpolar side chains. In order to verify and extend the conclusions of this theory, experimental results are required on an adequate model system. Dimers of small hydrocarbon molecules in aqueous solution would be the most satisfactory model but such dimers will not form because of the low solubility of hydrocarbons in aqueous

solution. This necessitates the use of model compounds containing polar groups.

The carboxylic acids represent a useful series of such model compounds. It has been shown by several groups of investigators⁴⁻⁸ that the carboxylic acids dimerize in aqueous solution. Rossotti and co-workers⁸ have carried out the most extensive series of measurements and have shown that, while higher oligomers form in solutions of the acids after acetic, dimerization is the major process occurring over most of the low concentration range for all the acids investigated.

(4) A. Katchalsky, H. Eisenberg, and S. Lifson, *J. Am. Chem. Soc.*, **73**, 5889 (1951).

(5) (a) M. Davies and D. M. L. Griffiths, *Z. physik. Chem. (Frankfurt)*, **2**, 353 (1954); (b) *ibid.*, **6**, 143 (1956).

(6) D. R. Cartwright and C. B. Monk, *J. Chem. Soc.*, 2500 (1955).

(7) G. R. Nash and C. B. Monk, *ibid.*, 4274 (1957).

(8) (a) D. L. Martin and F. J. C. Rossotti, *Proc. Chem. Soc.*, 60 (1959); (b) *ibid.*, 73 (1961); (c) K. Schlyter and D. L. Martin, *Kgl. Tek. Hogskol. Handl.*, No. 175 (1961); (d) J. D. E. Carson and F. J. C. Rossotti, in "Advances in the Chemistry of the Coordination Compounds." The Macmillan Co., New York, N. Y., 1961, p. 180.

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(2) National Institutes of Health Postdoctoral Fellow, 1962–1963.

(3) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).