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E.s.r. Study of Free Radicals in Irradiated Glycine[1]

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The e.s.r. spectra of the free radicals formed by irradiating glycine with X-rays have been studied. Five compounds were studied with systematic isotopic variation. These were glycine (NH_3^+–CH_2–COO^-), D_2-glycine (NH_3^+–CD_2–COO^-), N^{15}-glycine ($N^{15}H_3^+$–CH_2–COO^-), D_3-glycine (ND_3^+–CH_2–COO^-) and D_5-glycine (ND_3^+–CD_2–COO^-). Only those radicals which are relatively stable at room temperature were studied. The spectra are interpreted by the possible radical structures NH_4 and $\cdot CH_2$–COO^-.

Introduction

High energy irradiation of a number of organic compounds has been found to produce free radicals, whose structure may be investigated by electron spin resonance spectroscopy.

Electron spin resonance spectra of irradiated polycrystalline glycine[2–4] have been studied by a number of investigators. The single crystal spectra,[5] which show anisotropic hyperfine structure due to the dipole–dipole interaction between electron and nucleus, are extremely complex. Unequivocal interpretation of these spectra is considerably facilitated by studying spectra of various isotopic modifications of the compound under investigation.[6,7] In the present work, the compounds studied included the following modifications of glycine: (1) NH_3^+–CH_2–COO^- (glycine), (2) $N^{15}H_3^+$–CH_2–COO^- (N^{15}-glycine), (3) NH_3^+–CD_2–COO^- (D_2-glycine), (4) ND_3^+–CH_2–COO^- (D_3-glycine) and (5) ND_3^+–CD_2–COO^- (D_5-glycine).

Experimental

The compounds used were purchased commercially, except for D_3-glycine, which was made in the laboratory by exchanging glycine three times with D_2O under vacuum.[8] The isotopic content of the substituted positions of all compounds was at least 97.5% as determined by high resolution nuclear magnetic resonance. Crystals were grown by slow evaporation from aqueous solution; D_2O was used as solvent for the D_3 and D_5 compounds. The crystals used measured about 2–3 mm. along the longest edge.

Glycine is monoclinic, and the crystal space group is $P2_1/n$. The dimensions of the unit cell are $a = 5.10$ Å., $b = 11.97$ Å., $c = 5.46$ Å., $\beta = 111° 42'$.[9,10] The molecules are present in the crystal as zwitterions, and are held in double layers parallel to the ac plane by hydrogen bonds between the amine protons of one zwitterion and the carboxyl oxygen of four neighboring ions as is shown in Fig. 1. The positions of the hydrogen nuclei and the lengths of the N–H and C–H bonds have been determined accurately by Marsh.[10] The three N–H bonds are not quite equivalent—two are of equal lengths (0.85 Å.) and the third is somewhat longer (0.92 Å.). However, the frequency of libration of these hydrogen bonds is sufficiently rapid at room temperature that they act in an equivalent manner on the spin resonance spectrum.

The crystals were irradiated with a G.E. Maxitron 250 therapeutic X-ray unit; the total dosage was at least 10^6 rad. Irradiation at 90°K. resulted in the same spectra as irradiation at room temperature and subsequent cooling to 90°K. There was no evidence of additional radicals which were stable at low temperatures or which disappeared shortly after irradiation.

After irradiation the crystal was mounted on a quartz rod in a rotating shaft. The angle between the crystal axes and the magnetic field was known to within ±1° of arc. Spectra were obtained with a Varian V-4500 spectrometer using 100 kc. field modulation at both 9.5 and 9.1 Gc. Calibration was done with a Harvey–Wells gaussmeter using both the H^1 and Li^7 probes.

The crystals were oriented on a rod mounted in the cavity, and orientation with respect to a fiducial mark on the rod was determined by eye and checked by determining angles between the crystal faces in a two-circle goniometer. The direction of the field with respect to the crystal axes will be given by $\vec{Z} = \vec{H}/|\vec{H}| = (\alpha,\beta,\gamma)$ where α and β are the direction cosines of $\vec{Z}$ with the a- and b-axes of the crystal, and γ is the direction cosine of $\vec{Z}$ with respect to an axis perpendicular to a and b.

Results

In this investigation measurements have been made on both polycrystalline and single crystal samples. Results obtained for X-irradiated polycrystalline glycines are shown in Fig. 2. Glycine with normal isotopic content gives on X-irradiation the spectrum shown in Fig. 2a. This is the typical triplet previously reported by various investigators.[2–4] Figure 2b is the spectrum obtained when the α-protons are replaced by deuterium. The large triplet of the glycine spectrum is still present, but there are additional lines on either side of the central line. These are due to a second radical involving the α-deuterons which is superimposed on the triplet. This radical is a proton radical in the α-protonated compound, but its structure may be assumed to be hidden in the spectrum. Figure 2c is the spectrum obtained when the amine protons are replaced by deuterium. The entire spectrum has been compressed and radically altered, indicating that the over-all features of the spectrum are due to a radical in which amine protons cause the predominant hyperfine splitting. Further resolution of this spectrum could not be obtained by varying instrumental conditions. Figure 2d gives the spectrum obtained when all of the protons in glycine are substituted by deuterons. The spectrum is an incompletely resolved quintet which is compatible with the triplet shown in Fig. 2a. However, substitution of the α-protons with deuterium does not have precisely analogous effects in Fig. 2b and 2d, indicating that the triplet shown in 2a is a broadened composite of the actual radical spectra. Glycine samples in which N^{14} was replaced by N^{15} gave the triplet shown in Fig. 2a, and this spectrum is consequently not included in the figure.

Most of the hyperfine structure is smeared out in the spectra of Fig. 2, but two conclusions concerning the free radical structure can be drawn from these spectra: (1) the predominant splitting is due to amine protons, since the greatest change on isotopic substitution occurs when these protons are replaced by deuterons, and (2) there is a second radical produced in which the α-protons predominate. Typical results of irradiation of single crystals of isotopically substituted glycines are shown in Fig. 3 at $Z = (0.972, 0, 0.236)$. The structure of the radical producing this spectrum has been interpreted by Ghosh and Whiffen[5] as NH_3^+–$CH\cdot$–COO^-, where the over-all splitting is a doublet due to the remaining α-proton; each component of this is split by three equivalent amine protons into a quartet, and each component of the quartet is in turn split into

(1) This investigation was supported in part by research grant RG-5144 from the Division of General Medical Sciences, Public Health Service, National Institutes of Health; and the United States Atomic Energy Commission.

(2) W. Gordy, W. B. Ard and H. Shields, *Proc. Natl. Acad. Sci. U. S.*, **41**, 983 (1955).

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(4) R. Servant, P. Augoyard and N. Ngoc Chau, *ibid.*, **249**, 751 (1959).

(5) D. K. Ghosh and D. H. Whiffen, *Mol. Phys.*, **2**, 285 (1959).

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

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

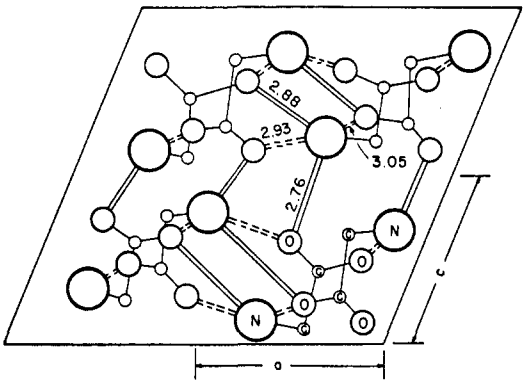


Fig. 1.—Double layer of zwitterions in glycine.

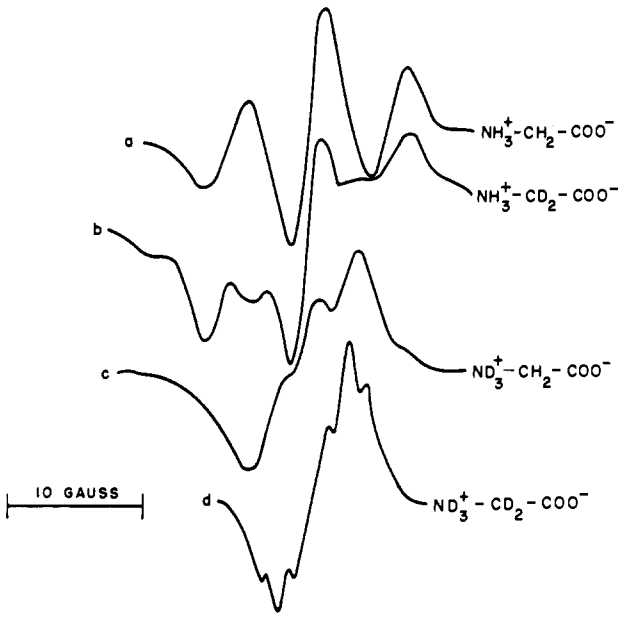


Fig. 2.—Spectra of X-irradiated polycrystalline glycines: a, glycine; b, D_2-glycine ($NH_3^+-CD_2-COO^-$); c, D_3-glycine ($ND_3^+-CH_2-COO^-$); d, D_5-glycine ($ND_3^+-CD_2-COO^-$).

a triplet by N^{14}. The outside triplets in Fig. 3a are the end components of the two quartets which have been split by N^{14}. The central portion of the spectrum of Fig. 3a has been explained by Ghosh and Whiffen as due to the radical $\cdot NH_2$.

Figure 3b gives results obtained with glycine crystals in which the α-protons have been replaced by deuterons. The only significant differences between the spectra of Fig. 3a and 3b are in the central portion of the spectrum; the end components of these spectra are the same. Hence the extreme portions of the spectrum cannot be explained by the radical $NH_3^+-CH\cdot-COO^-$ since a deuteron replacing a proton in the α-position would give a primary splitting into a triplet rather than a doublet. Moreover, the triplet would have total width about $^1/_3$ that of the proton doublet, and the overall width as well as the shape of the spectrum would be different.

Figure 3e is the spectrum at the same orientation of glycine in which N^{14} has been replaced by N^{15}. Broadening of the nitrogen hyperfine structure by quadrupole interaction is not present in this compound, and the hyperfine structure is clearer than in Fig. 3a and 3b, since the N^{14} triplets are replaced by doublets. Figures 3c and 3d are, respectively, spectra of totally deuterated glycine and amine-deuterated glycine at this orientation. As was the case for the polycrystalline compounds, these two spectra were very incompletely resolved at all orientations, and the only differences

Fig. 3.—Spectra of X-irradiated single crystals of glycine at Z = (0.972, 0, 0.236): a, glycine; b, D_2-glycine; c, D_5-glycine; d, D_3-glycine; e, N^{15}-glycine ($N^{15}H_3^+-CH_2-COO^-$).

between them are small ones in the central portion of the spectrum. The over-all width of the spectra of 3c and 3d is considerably less than that of 3a, b and e. In general, it will be shown that these spectra are entirely compatible with conclusions drawn from the spectra of amine protonated compounds.

The question now arises as to the structure of the radicals. It has been demonstrated that neither of the radicals proposed by Ghosh and Whiffen are consistent with all of the spectra obtained in the present study. There are two possible structures compatible with the spectra obtained. The first is an ammonium radical in which one proton has a different coupling to the nitrogen than the other three protons. This would give two overlapping quartets each component of which is split into an equal-intensity triplet by N^{14} or doublet by N^{15}, as in Ghosh and Whiffen's proposal. Such a reconstruction is shown for the spectrum of Fig. 3e (N^{15}-glycine) in Fig. 4b. This reconstruction and the reconstruction of Fig. 4c, as well as other reconstructed spectra, were made by assuming a gaussian line shape and plotting the sum of first derivatives of the indicated number of gaussians of the specified relative intensities with the spacing of the experimental spectrum. The second possibility is that the overlapping quartets of Fig. 4b are only apparent and that the spectrum is really the superposition of several spectra. As was mentioned above, Marsh[10] has reinvestigated the crystal structure of glycine and has found that the three amine protons are not equally bound to the nitrogen, but that one of these has a significantly different N–H bond distance from the other two. If it is assumed that the damaged species also possesses this non-equivalency, a second possible structure for the spectrum becomes evident. Such a possibility is shown in Fig. 4c, which is a reconstruction of the N^{15}-glycine spectrum on the basis of two overlapping pairs of triplets, rather than one overlapping

pair of quartets. Each pair of triplets results from a radical in which the predominant splitting is due to amine protons, but the two radicals are different.

Let us consider the first possibility of the two given above: that the spectrum of Fig. 4 is a doublet due to one singular *amine* proton, and the components of this doublet are in turn each split into a quartet of intensity ratio 1:3:3:1 by three equivalent amine protons, which would explain the glycine, N^{15}-glycine and D_2-glycine spectra. This gives a radical with the formula NH_4, and nine electrons on the nitrogen. The odd electron gives the spin resonance signal. It may be noted that the radical NH_4 was proposed about forty years ago as a constituent of ammonium amalgam.[11,12] More recently,[13] this radical has been suggested as a metastable intermediate in the mechanism of the exchange reaction of ammonia with deuterium.

It seemed extremely difficult to visualize a process which would result in the formation of a radical NH_4 which would be stable at room temperature, even in the hydrogen-bound form in which it would exist in glycine. A possible reaction scheme is analogous to that proposed for the formation of CH_5^+ in the reaction of methane with protons, and would involve a hydride ion transfer mechanism. We may then write NH_4 as $H_\sigma NH_3$, indicating the dissimilar protons as H_σ or H_N. The odd electron density would be predominantly in a σ-orbital, the σ-orbital being a linear combination of the H_σ 1s orbital and the N 3s orbital. The observed nitrogen hyperfine splitting agrees well with that calculated by assuming a nitrogen 3s orbital density. This may be done by comparison with the a_N given by Cole[14] for NH_3^+. Following the method of Cole for a_N (NH_3^+), we have

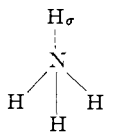
$$\frac{a_N(NH_4)}{a_N(NH_3^+)} = \frac{|\Psi_{3s}(N)|^2 J(NH_4)}{|\Psi_{2s}(N)|^2 J(NH_3^+)} \qquad (1)$$

where J is the $\sigma-\pi$ exchange integral. Using Hartree 2s and 3s wave functions,[15] we obtain

$$a_N(NH_4)/a_N(NH_3^+) = 0.083$$

This gives the value a_N (NH_4) = 5.53 mc. = 2.0 gauss for the nitrogen coupling constant in NH_4. The ratio of this coupling constant to the measured nitrogen hyperfine coupling constant is 2.0/2.9 = 0.69.

The NH_4 radical would thus have a structure analogous to that proposed by Gorin, *et al.*,[16] for CH_5^+ and by Higuchi[17] for CH_5, namely



in which the unpaired spin density, originally on the hydride ion, occupies the σ-orbital in question. The splitting due to H_σ would be expected to show some anisotropy except when rotated about the NH_σ bond axis. Rotation around the C–N bond of the undamaged molecule showed the NH_σ splitting to be isotropic within 2%, and to have a splitting of 57.8 gauss. The 2% anisotropy is well within the limits to which the crystal could be accurately positioned in this orientation. This implies that the NH_σ bond lies along the axis of the pyramid formed by the $-NH_3^+$ group.

(11) G. M. Smith, *J. Am. Chem. Soc.*, **26**, 844 (1904).
(12) G. Aronheim, *Z. physik. Chem.*, **97**, 95 (1921).
(13) L. Farkas and P. Harteck, *ibid.*, **B25**, 257 (1934).
(14) T. Cole, *J. Chem. Phys.*, **35**, 1169 (1961).
(15) D. R. Hartree, "The Calculation of Atomic Structures," John Wiley and Sons, Inc., New York, N. Y., 1957.
(16) E. Gorin, W. Kauzmann, J. Walter and H. Eyring, *J. Chem. Phys.*, **7**, 633 (1939).
(17) J. Higuchi, *ibid.*, **31**, 563 (1959).

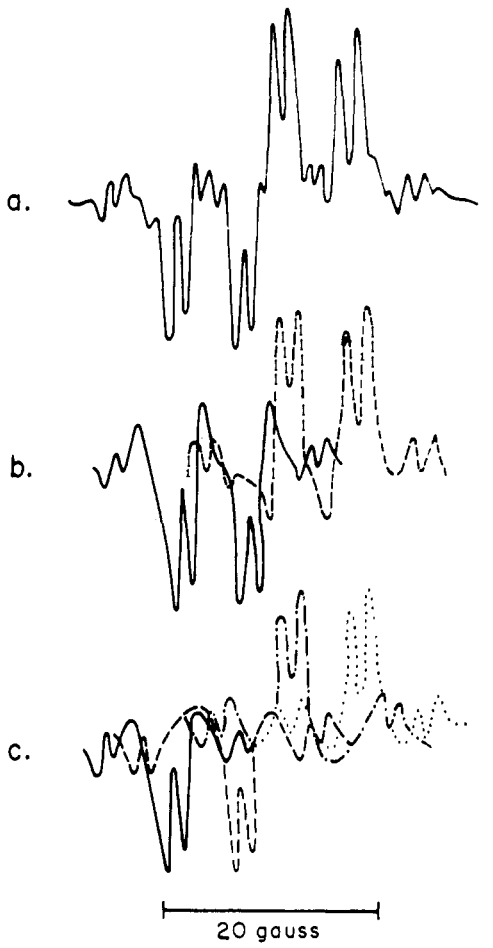


Fig. 4.—a, N^{15}-glycine spectrum at Z = (0.972, 0, 0.236); b, reconstruction of a by two overlapping quartets; c, reconstruction of a by two overlapping pairs of triplets.

Let us consider this value, T = 57.8 gauss, as the σ-orbital splitting. The components of this splitting along the x-, y- and z-axes would be

$$|T_x| = |T\alpha| = 13.0 \text{ gauss}$$
$$|T_y| = |T\beta| = 1.7 \text{ gauss}$$
$$|T_z| = |T\gamma| = 12.9 \text{ gauss}$$

where α, β, γ are the direction cosines of the C–N bond of the undamaged molecule with the x-, y- and z-axes. We have assumed that the NH_σ bond lies along this bond. Subtracting these values from the measured coupling constants, we obtain an anisotropic contribution to the coupling constants. These values are summarized in Table I.

TABLE I

COUPLING CONSTANTS FOR NH_4 (IN GAUSS)

Nucleus	A	B	C	a	a (Ghosh and Whiffen)
H_σ	52.0	2.3	41.1	31.8	26.8 ("H_C")
$H_1 = H_2 = H_3$	20.7	13.3	15.1	16.4	18.9 ("H_N")
N	2.7	4.3	1.8	2.9	3.5

H_σ along CN (or NH_σ) bond; T = 57.8.

The coupling constants were calculated on the assumption that the position of the three hydrogen bonds was not much altered from their position in the undamaged molecule, and that the NH_σ bond lies along the C–N bond of the undamaged molecule. Since the coupling constants were not calculated with reference to accurately determined canonical axes, their values may be somewhat in error. The σ-orbital spin density is given by the nitrogen 3s coupling ρ^σ = 0.69. The

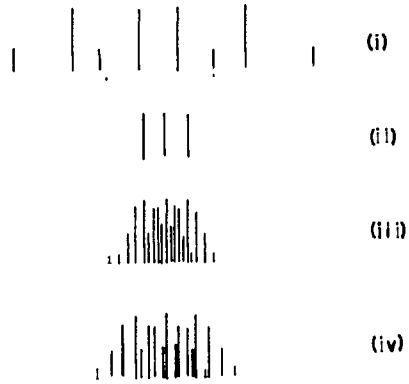


Fig. 5.—(i), Pair of quartets due to NH_4 (omitting N^{14} or N^{15} splittings); (ii), triplet due to $D\sigma$ of ND_4 at this orientation; (iii), spectrum of ND_4 with each component of triplet split into seven lines; (iv), spectrum of (iii) widened by 10% to conform with experimental results for D_3-glycine and D_5-glycine (see text).

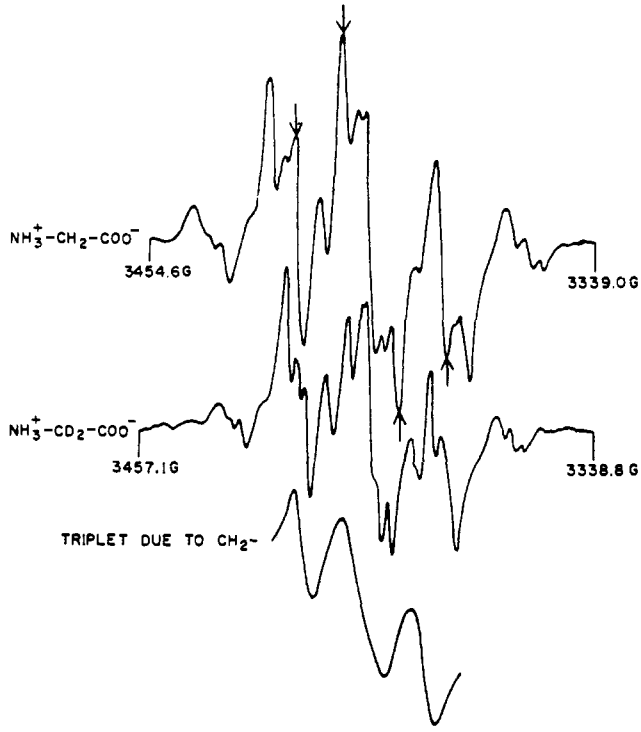


Fig. 6.—a, glycine at $Z = (1, 0, 0)$; b, D_2-glycine at $Z = (1, 0, 0)$; c, normalized difference between a and b, giving triplet due to $\cdot CH_2-COO^-$.

derivation of isotropic and anisotropic coupling is briefly summarized in Appendix I.

The spectra of the amine-deuterated compounds (Fig. 3c and 3d) may also be interpreted by ND_4, or $D_\sigma ND_3$. The spectrum in this case is an equal intensity triplet due to D_σ, each component of which is split into seven lines of intensity ratio 1:3:6:7:6:3:1. This spectrum is reproduced in Fig. 5, together with the $N^{15}H_4$ spectrum (excluding the N^{15} doublets). As is evident from Fig. 5 (iii), the spectrum corresponds well in outline to Fig. 3c and 3d, except that it is about 10 gauss too narrow. The splitting was calculated from the ratios of the magnetic moments. Now, since the pyramidal shape of the radical NH_4 is dependent on the hydrogen bonds of the amine protons, and since there is considerable expansion of the lattice when deuterons are substituted for protons,[18] the attendant increase in planarity of the three equivalent amine protons would give relatively more spin density in the

(18) A. R. Ubbelohde and K. J. Gallagher, *Acta Cryst.*, **8**, 71 (1955).

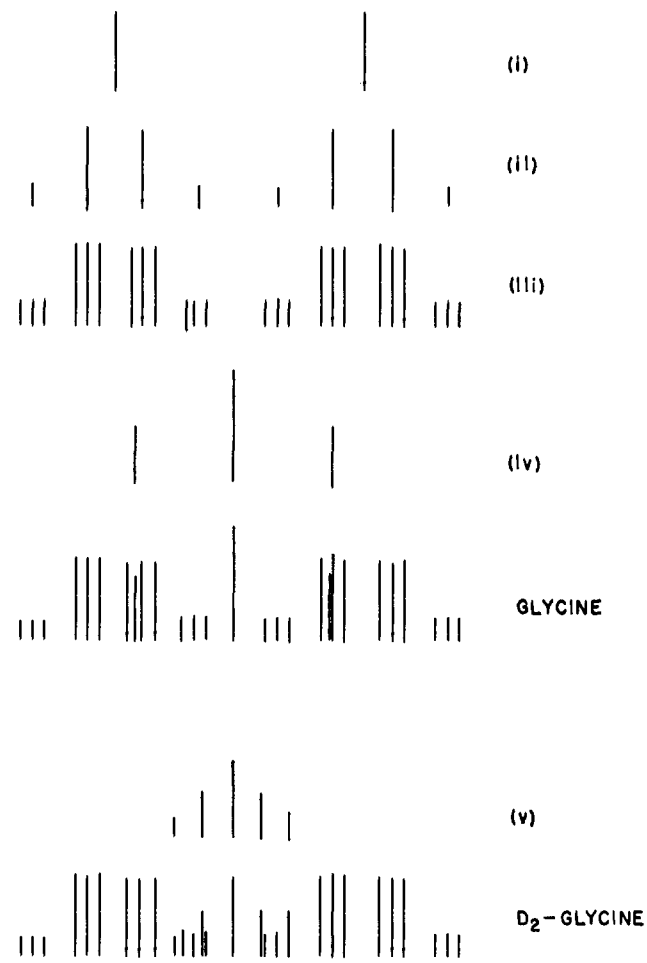


Fig. 7.—(i), Doublet due to $H\sigma$; (ii), quartets due to $H_1 = H_2 = H_3$; (iii), additional triplet splitting due to N^{14}; (iv), triplet due to $\cdot CH_2-COO^-$; (iii) and (iv), glycine at $Z = (1, 0, 0)$; (v), quintet due to $\cdot CD_2-COO^-$; (iii) and (v), D_2-glycine at $Z = (1, 0, 0)$.

ND_σ orbitals than in the NH case. This explains the excess width of the ND_4 spectrum qualitatively, though not quantitatively. It is unlikely that this anomalous width of the spectrum of the protonated compounds is due solely to the difference between electron dipole–proton dipole broadening and electron dipole–deuteron dipole broadening, since such a difference amounts to less than 0.01% of the total width. However, the anomalous widening may be due to a larger dipole–dipole interaction with hydrogen species of neighboring undamaged molecules which may be greater in the case of the amine-protonated species.

N^{14} splitting of the spectrum of ND_4 was not observed. This may be due to two factors: the splitting of the N^{14} triplet is only about half as large as that of the sextet due to three equivalent deuterons (3 and 6 gauss, respectively, in the orientation shown in Fig. 3) and the triplets are thus obscured in the total spectrum and the quadrupole interaction between N^{14} and D tends to broaden the lines still further.

The central portions of many of the spectra are complicated by a radical in which the splitting is due to methylene protons alone, which may be identified as $\cdot CH_2-COO^-$. The triplet which one would expect to see from this radical was not observed directly. However, the existence of the radical may be inferred both from the spectra of polycrystalline glycine and D_2-glycine, as has been discussed, and from the differences in the central portions of the glycine and D_2-glycine single crystal spectra. One such orientation

is shown in Fig. 6 in which the upper spectrum is of glycine and the lower of D_2-glycine at $Z = (1, 0, 0)$. If the lower spectrum is subtracted from the upper, a triplet results at the position of the lines indicated by arrows in the glycine spectrum, which may be attributed to the radical $\cdot CH_2–COO^-$. The triplet has an over-all width of 36.8 gauss (18.4 gauss between adjacent components).

Line replicas of the spectra of Fig. 6 are given in Fig. 7 in which (i), (ii), (iii) correspond to those portions of the spectra caused by NH_4: (iv) is the triplet of $\cdot CH_2–COO^-$ in the position and relative size of the structure at the bottom of Fig. 6. The sum of (iii) and (iv) in Fig. 7 is in good agreement with the glycine spectrum of Fig. 6; (v) in Fig. 7 is a reconstruction of the quintet which would result from $\cdot CD_2—COO^-$ at an arbitrary size. The summed spectrum of (iii) and (v) shows that this structure would quite likely be obscured in the center of the spectrum except for the central line.

Coupling constants for the $\cdot CH_2–COO^-$ radical are

$$|A| = 33.6 \text{ gauss}$$
$$|B| = 25.6 \text{ gauss}$$
$$|C| = 3.6 \text{ gauss}$$
$$a = \frac{1}{3}|A + B + C| = 21.0 \text{ gauss}$$
$$\rho = 21.0/23.9 = 0.88$$

These were calculated making the same assumption as above concerning the canonical axis. The measurements of the splitting were made by subtracting the D_2-glycine spectrum from the glycine spectrum at those orientations where the $\cdot CH_2–COO^-$ triplet was visible. This radical may be assumed to be a π-electron radical.

Some studies of the e.s.r. spectra have been done at temperatures ranging from $-125°$ to room temperature. These spectra are shown for N^{15}-glycine in Fig. 8. This compound was selected because the nitrogen hyperfine structure is better defined in the spectra than in the case of the N^{14}-compounds. The spectrum taken at the lowest observed temperature ($-125°$) shows a pair of overlapping triplets in place of overlapping quartets, indicating that rotation or libration of the amine protons is strongly inhibited at this temperature and the splitting due to the three equivalent amine protons is reduced to splitting by two amine protons. This type of effect has been observed in other e.s.r. studies at low temperatures.[19] As the temperature is increased, the triplets alter to quartets, and a totally different structure emerges in the center of the spectrum. The latter may be correlated the $\cdot CH_2–COO^-$ radical. The noticeable sharpening of the N^{15} hyperfine structure as the temperature is raised is as yet not fully understood.

Conclusion

There are two groups causing hyperfine splitting in the stable free radicals formed in irradiated glycine: NH_4 and $\cdot CH_2–COO^-$. Coupling constants have been measured for these, but more precise measurements are needed to determine the principal axes of the coupling tensors more accurately. Evidence from deuterium substitution in the methylene position precludes the existence of Ghosh and Whiffen's proposed radical $NH_3^+–CH\cdot–COO^-$.[5] There is good evidence that the C–N bond is ruptured, which agrees well with other work done on irradiated amino acids.[6] However, the concentration of free spin density in the amine group of the damaged radical seems to be unique in

(19) D. J. E. Ingram, "Free Radicals as Studied by ESR," Butterworths, London, 1958.

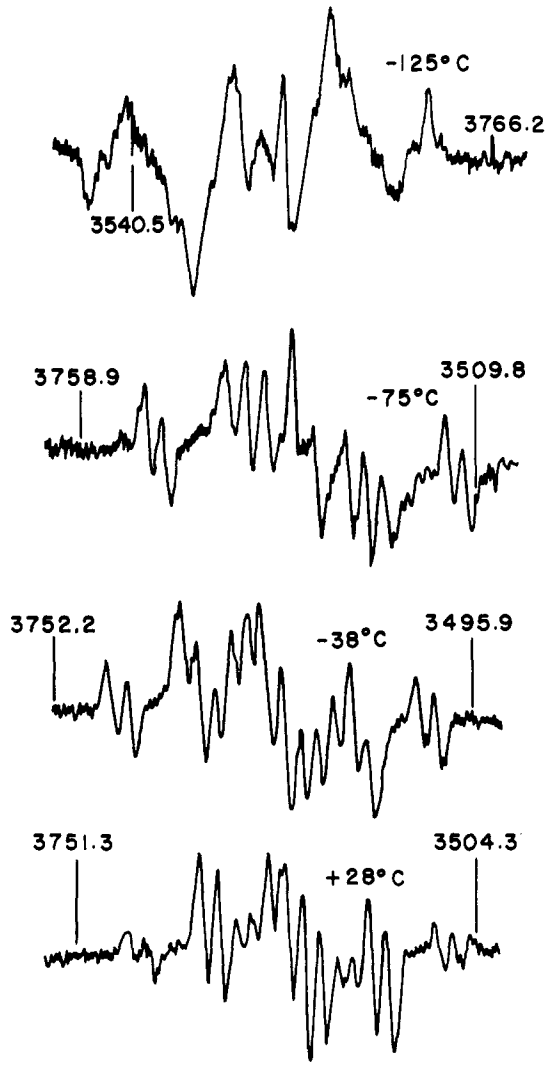


Fig. 8.—N^{15}-Glycine at temperatures from $-125°$ to room temperature.

glycine.[20] Due to the complexity of the spectra, no lines readily identifiable as second-order transitions[6,21] were observed.

Acknowledgments.—The authors wish to thank Dr. Timothy Merz of the Biology Department for performing the X-irradiations.

Appendix I

The spin Hamiltonian which may be used to interpret electron spin resonance spectra may be written

$$\mathcal{H} = \beta\vec{S}\cdot g\cdot\vec{H}_0 - \Sigma_j \left(\frac{\mu_j}{I_j}\right)\vec{I}\cdot\vec{H}_0 - \mathcal{H}_{hf} \qquad (1)$$

The first term in eq. 1 describes the interaction of the electron with the magnetic field, and the second is a sum over j magnetic nuclei of the interaction of these nuclei with the magnetic field. The last term describes the hyperfine splitting due to interaction of the electron spin with the nuclear spin. In the absence of any appreciable anisotropy in the g-factor of a radical ($\Delta g \leq 10^{-3}$)[17] the hyperfine interaction may be written

$$\mathcal{H}_{hf} = hAS_zI_z + hBS_xI_x + hCS_yI_y \qquad (2)$$

The coupling coefficients A, B, C arise from the sum of the anisotropic nuclear dipole–electron dipole interaction and the isotropic Fermi contact interaction and may be written as in eq. 3.[16]

(20) I. Miyagawa and W. Gordy, *J. Am. Chem. Soc.*, **83**, 1036 (1961).
(21) H. M. McConnell, C. Heller, T. Cole and R. W. Fessenden, *ibid.*, **82**, 766 (1960).

$$A = A_d + a$$
$$B = B_d + a \quad (3)$$
$$C = C_d + a$$

The isotropic coupling constant a is

$$a = \frac{g|\beta|g_N\beta_N}{h}\frac{8\pi}{3}\rho(\vec{r}_N) \quad (4)$$

where $\vec{r}_N$ is the vector position of the magnetic nucleus. The anisotropic coupling constants may be written[12]

$$A_d = \frac{-g|\beta|g_N\beta_N}{h}\rho(\vec{r})\frac{(1-3\cos^2\theta)}{|\vec{r}-\vec{r}_N|^3}\,dV$$
$$B_d = \frac{-g|\beta|g_N\beta_N}{h}\rho(\vec{r})\frac{(1-3\sin^2\theta\cos^2\varphi)}{|\vec{r}-\vec{r}_N|^3}\,dV \quad (5)$$
$$C_d^2 = \frac{-g|\beta|g_N\beta_N}{h}\rho(\vec{r})\frac{(1-3\sin^2\theta\sin^2\varphi)}{|\vec{r}-\vec{r}_N|^3}\,dV$$

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY 4, CALIF.]

A Critical Re-evaluation of the Hammett Acidity Function at Moderate and High Acid Concentrations of Sulfuric Acid. New H_0 Values Based Solely on a Set of Primary Aniline Indicators

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A uniform, overlapping set of Hammett indicators, consisting solely of primary anilines, was established by the addition of eight new indicators, procured in this work. The pK values of two former indicators were re-evaluated. Employing the new pK values of indicators the Hammett acidity function was redetermined for sulfuric acid above 60%. The newly established H_0 values were found to be progressively more negative than those of the Paul and Long scale. The maximum deviation at the highest acidity was found to be 1.1 units.

Introduction

The demonstrated utility of the H_0 acidity function in aqueous solution and the potential applicability to mixed solvent systems has stimulated a great deal of interest in the correlation of reaction rates with H_0 and in drawing conclusions about mechanisms of acid-catalyzed reactions.[2] The success of such a treatment, however, is intrinsically dependent on the validity of the H_0 scale as well as upon the correctness of the reported H_0 values. The quantitative formulation of the H_0 function[3] (eq. 1) imposes the following conditions on the indicators which are employed for the

$$H_0 = \log a_{H^+}f_B/f_{BH^+} = pK_{BH^+} - \log [BH^+]/[B] \quad (1)$$

$$pK_{CH^+} - pK_{BH^+} = \log\frac{[CH^+]}{[C]} - \log\frac{[BH^+]}{[B]} - \log\frac{f_C f_{BH^+}}{f_{CH^+}f_B} \quad (2)$$

$$pK_{CH^+} - pK_{BH^+} = \log\frac{[CH^+]}{[C]} - \log\frac{[BH^+]}{[B]} \quad (3)$$

determination of the H_0 scale for any particular acid system: (i) that the pK values of the indicators be accurately and firmly established and (ii) that the indicators employed be valid H_0 indicators such that the relationship between the pK values of two consecutive indicators, described by eq. 2, can be reduced to that of eq. 3; *i.e.*, that the activity coefficient ratio of the free base to the protonated base be equal for both indicators, and hence that the H_0 scale determined be independent of the indicators employed for measurement. Any H_0 scale determined with a particular set of indicators will necessarily incorporate all the inadequacies of such a set.

The present set of indicators, which for the most part consists of the original indicators determined by Hammett and Deyrup,[4] does not comply with either requirement. The colorimetric method employed by Hammett and Deyrup was very inadequate for the measurement of accurate ionization ratios and for the detection of major medium shifts, most important for the determination of accurate pK values.[5] Examination of the original data reveals these shortcomings in the lack of parallelism and in non-linearity of the plotted ionization curves. The pK values of indicators employed at lower acidity regions have been, in part, re-examined by modern spectrophotometric techniques,[6,7] disclosing a number of failings in Hammett's original data. The indicators used at higher acidities have not been restudied.

The requirement that the indicators employed constitute a valid set has not been seriously examined. The present set of Hammett indicators includes compounds of widely differing chemical constitution. The activity coefficient behavior of these has not been studied, and in the past it has been assumed that eq. 3 adequately describes the protonation behavior of these compounds. This is not true in the light of recent studies on activity coefficient behavior of molecules, especially in more concentrated acid solutions, where the nature of the medium is changing more rapidly. Deno and Perizzolo[8] have shown that for neutral molecules examined, the activity coefficients remained reasonably constant with acid concentration, regardless of structure; the same does not obtain for charged species. The latter show a strong dependence of their activity coefficient behavior on acidity, differing with charge type. Other data[9-11] are available which demonstrate that even for uncharged molecules the activity coefficients may vary over a wide range with acidity.

Other factors also contribute to a potential breakdown of the equivalency of eq. 2 and 3. Taft[12] has shown that the H_0 scale can be demonstrated to be dependent on chemical structure of the indicators, even for chemically closely related compounds. The protonation behavior was found to be different for secondary and tertiary anilines from that for primary

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(2) See, for example, F. A. Long and M. A. Paul, *Chem. Rev.*, **57**, 935 (1957).

(3) For an excellent review of the H_0 acidity function see M. A. Paul and F. A. Long, *ibid.*, **57**, 1 (1957).

(4) L. P. Hammett and A. J. Deyrup, *J. Am. Chem. Soc.*, **54**, 2721 (1932).

(5) We have shown [D. S. Noyce and M. J. Jorgenson, *ibid.*, **84**, 4312 (1962)], for example, that when medium shifts are treated appropriately, the indicator benzylideneacetophenone gives ionization ratios which parallel those of primary anilines, when plotted against sulfuric acid concentration. Hammett's original data result in bad curvature; the previously assigned pK value was shown to be in error by 0.7 unit.

(6) E. Högfeldt and J. Bigeleisen, *ibid.*, **82**, 15 (1960).

(7) K. N. Bascombe and R. P. Bell, *J. Chem. Soc.*, 1096 (1959).

(8) N. C. Deno and C. Perizzolo, *ibid.*, **79**, 1345 (1957).

(9) L. P. Hammett and R. Chapman, *ibid.*, **56**, 1282 (1934).

(10) R. J. Gillespie and J. A. Leisten, *Quart. Rev.*, **8**, 40 (1954).

(11) F. A. Long and R. Bakula, private communication.

(12) R. W. Taft, Jr., *J. Am. Chem. Soc.*, **82**, 2965 (1960).