Electron Spin Resonance Study of Radicals Produced in Irradiated Aqueous Solutions of Amines and Amino Acids[1]

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The radicals produced by reaction of hydroxyl radicals with some amines, amino acids, and related compounds in aqueous solutions have been studied by esr. Irradiation with high-energy electrons was carried out directly in the esr cavity. In most cases aminoalkyl radicals were formed by hydrogen abstraction from the carbon bearing the amino group. The radicals $\dot{C}H_2NH_2$, $\dot{C}H_2NHCH_3$, and $\dot{C}H_2N(CH_3)_2$ produced from mono-, di-, and trimethylamine have been observed in the pH range 7–13.5. In acid solutions no radicals with the expected structure $R\dot{C}HNH_3^+$ have been observed. In the cases of di- and trimethylammonium ions secondary reactions leading to radicals of the type $R_2\dot{N}H^+$ take place. The radical found in acid solutions of glycine is best described by the structure $NH_2\dot{C}HCOOH$ as supported by experiments in D_2O. In alkaline solutions of all amino acids radicals of the type $H_2N\dot{C}RCO_2^-$ were observed. In the case of α-aminoisobutyric acid a different radical, probably of type $R\dot{N}H$, was also observed at pH 13 and low concentration of solute. The results are consistent with a mechanism involving the reaction $RCH_2NH_3^+ + H$ or $OH \rightarrow R\dot{C}HNH_3^+ + H_2$ or H_2O in acid solution and the two competing reactions $RCH_2NH_2 + OH \rightarrow R\dot{C}HNH_2 + H_2O$ and $RCH_2NH_2 + OH \rightarrow RCH_2\dot{N}H + H_2O$ in alkaline solution. The latter reaction must be followed by a fast secondary step, $RCH_2\dot{N}H + RCH_2NH_2 \rightarrow RCH_2NH_2 + R\dot{C}HNH_2$, which causes the disappearance of $RCH_2\dot{N}H$ in most cases.

Introduction

The radicals produced from amines and amino acids have been studied under various conditions. Both γ radiolysis[2-6] and photolysis[4,7] of single crystals[2-5] and polycrystalline and glassy samples[6-8] have been used for the formation of radicals which were subsequently studied by esr. Direct, *in situ* esr observation has been employed to study the radicals produced from such materials by the Ti^{3+}–H_2O_2 method[9-13] in aqueous solutions. Aqueous solutions of amines[14,15] and amino acids[16] have also been investigated by the pulse radiolysis technique utilizing kinetic spectrophotometry to follow the radicals produced initially by the electron pulse. Finally, product analysis of γ-irradiated solutions has been used to infer the mechanisms of formation and reaction of the amino acid radicals.[17] Although the latter two methods have provided a considerable amount of information, they lack the specificity of radical identification which is obtained by esr.

Esr studies of radicals in aqueous amino acid solutions have been carried out using Ti^{3+}–H_2O_2 to generate OH in acid solutions[10,12] and Ti^{3+}–EDTA–H_2O_2 in neutral and slightly alkaline solutions.[11,13] Generally it was found that in alkaline solutions hydrogen abstraction takes place from the position α to the amino group, whereas in acid solution the abstraction is mainly from positions further away from the ammonium group which strongly deactivates the adjacent position. In acid solutions of methylamine the radical $\cdot CH_2\overset{+}{N}H_3$ has been observed by pulse radiolysis[15] but could not be detected by esr.[10] A similar difference exists for acid solutions of glycine where the results of pulse radiolysis experiments suggest[16] the radical $^+H_3N\dot{C}H$-

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COOH while the esr spectrum has been attributed to the species $H_2N\dot{C}HCOOH$.[10,12]

Esr spectroscopy has been used in this laboratory for the study of a number of radicals during continuous *in situ* radiolysis of aqueous solutions.[18-21] Radiolytic generation of radicals was found to have the advantage, relative to other chemical or photochemical means, of permitting the study of reactions of e_{aq}^- and of applicability over the full pH range. We have recently reported[20] an esr study of the deamination of a number of amino acids by e_{aq}^-. A disadvantage of this technique compared to pulse radiolysis is noticeable in those cases where secondary reactions can take place to form radicals which are long-lived compared with the primary radicals. In these cases only the secondary radicals are observed. The two methods are thus complementary to each other. We intend in this study to examine the reactions of OH radicals with amines and amino acids in aqueous solutions, with special emphasis on those points which could not be fully resolved by pulse radiolysis.

It should be noted that in cases where comparison of results is possible the esr spectra obtained here are essentially identical with those obtained with the Ti^{3+}–H_2O_2 system. In these cases (namely, with the amino acids previously studied) we have, therefore, only extended the results to higher pH. In terms of signal intensity (*i.e.*, radical concentration) our method is somewhat inferior to the use of Ti^{3+}–H_2O_2 although our resolution is somewhat better. The results reported here for amines are all new and provide important reference points for discussing the several problems encountered in interpretation of the amino acids results.

Experimental Section

The amino acids used were of the purest grade commercially available from Calbiochem, Cyclo Chemical, and Baker. Gaseous amines were obtained from Matheson. All the inorganic materials were Baker Analyzed reagents. Water was doubly distilled[18] and in the second distillation the vapor was passed with oxygen through a silica tube at ~600°. The pH was adjusted using potassium hydroxide, perchloric acid, sodium phosphates, and sodium tetraborate. Solutions were deoxygenated by bubbling with nitrous oxide. At pH > 3, N_2O scavenges practically all the hydrated electrons to form OH radicals. At lower pH values the competing reaction of e_{aq}^- with H_3O^+ takes place to form H atoms. Both OH radicals and H atoms can abstract hydrogen from the organic solutes. At pH > 3, ~90% of the reacting radicals are OH, and at pH < 2, ~50% are OH. In most cases of hydrogen abstraction the same radical is expected to be formed by either OH or H attack.

The irradiation was carried out in the esr cavity as previously described.[18,19] A flat silica cell of 0.5-mm internal spacing was used, and during irradiation the solution was driven through the cell at a flow rate of 1 cm^3/sec. In most cases no effect of flow rate on the spectrum could be observed at rates between 0.5 and 3 cm^3/sec. The solution was cooled slightly, before entering the cell, and all measurements pertain to about 15°. The total electron beam current was ~8 μA and that collected at an electrode in the solution was ~1 μA. Second-derivative spectra were recorded by use of two modulation frequencies. This method helps to minimize the interference by the signal from the silica cell so that only the region from 3 to 10 G above the center of the radical spectrum is obscured.

Results and Discussion

The two most probable paths for reaction of OH radicals with the basic form of amines and amino acids are hydrogen abstraction from either the α-alkyl group or the amino group

$$RCH_2NH_2 + OH \rightarrow \begin{cases} R\dot{C}HNH_2 \\ \dot{R}CH_2NH \end{cases} + H_2O \qquad \begin{matrix} (1a) \\ (1b) \end{matrix}$$

The amino alkyl radical can also be formed indirectly by

$$RCH_2\dot{N}H + RCH_2NH_2 \longrightarrow RCH_2NH_2 + R\dot{C}HNH_2 \qquad (2)$$

Chemical analysis of irradiated aqueous solutions of amines and amino acids[17,22] shows that most products are formed from radicals of the type $R\dot{C}HNH_2$. There is, however, also some evidence from this source[22] for reaction 1b. The occurrence of both (1a) and (1b) is supported by recent pulse radiolysis experiments.[15,16] Reaction 2 is expected to be relatively more important in these esr experiments than in pulse radiolysis because in the present esr work radical lifetimes are much longer (~1 msec) so that reaction 2 can compete more effectively with radical–radical reactions.

Because there will be considerable discussion of the dissociation constants of various amine radicals, it is useful to review the existing data. Pulse radiolysis studies of amino acids[16] and amines[15] utilizing the initial optical absorption of the transient have been carried out. These studies show a change in absorption centered at about pH 5 for solutions of $CH_3NH_3^+$ and attributed the change to the equilibrium $\dot{C}H_2NH_3^+ \rightleftarrows \dot{C}H_2NH_2 + H^+$. With glycine two partially compensating changes in absorption occur between pH 3 and

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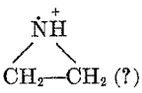
Table I: Structure and Coupling Constants of Radicals Produced in Irradiated Aqueous Solutions of Amines[a]

Amine	pH	Radical	g factor	a^N	a_{NH}^H	a_α^H	a_β^H	a_γ^H
Monomethylamine	7–13.5	$\dot{C}H_2NH_2$	2.00282	4.98	4.40 (2)	15.30 (2)		
Dimethylamine	7–13.5	$\dot{C}H_2NHCH_3$	2.00280	5.84	6.35 (1)	10.91 (2)		4.21 (3)
Trimethylamine	7–13.5	$\dot{C}H_2N(CH_3)_2$	2.00274	7.03		11.61 (2)		4.06 (6)
Dimethylamine	1		2.00360	19.22	21.90 (1)		33.56 (4)	
Trimethylamine	1	$CH_3-\overset{+}{N}H-CH_3$	2.00360	20.53	$\mp$28.28 (1)[b]		$\pm$28.56 (6)	

[a] Hyperfine constants are given in gauss and are accurate to $\pm$0.03 G. The g factors are measured relative to the peak from the silica cell and are accurate to about $\pm$0.00005. Second-order corrections have been made [R. W. Fessenden, *J. Chem. Phys.*, **37**, 747 (1962)]. The number of hydrogen atoms displaying the hyperfine constant is given in parentheses. [b] From the second-order pattern this hyperfine constant must have a sign opposite that of a_β^H.

6. These changes have been interpreted as arising from the two protonations of $H_2N\dot{C}HCO_2^-$. The change of the pK associated with the amino group in going from the parent compound to the radical seems to lie in the range 4–6 units. On the basis of these studies it appears that the radical in acid solutions of glycine is $^+H_3N\dot{C}HCO_2H$.

Amines, amino acids, and several related compounds have been irradiated in aqueous solutions saturated with nitrous oxide at different pH values, and the esr spectra were recorded during the irradiation. For every compound the known[23] or estimated rate constants for reactions with OH radicals and H atoms have been used to choose a concentration high enough to ensure scavenging of most of the primary radicals. In many cases several different concentrations were used to verify that full scavenging occurred. In certain compounds no spectra could be observed, *e.g.*, from isopropylamine, *tert*-butylamine, tetramethylammonium hydroxide, acetylalanine, and aminomalonamide. This result is thought to be due to the large number of splittings which divide the intensity among many lines and as a result reduce the line intensities to near or below the noise level. Another possible reason for the absence of lines from acid solutions is the chemical exchange of the protons of the ammonium group. As discussed below, this exchange can cause line broadening in radicals having the structure $>\dot{C}\overset{+}{N}H<$, and in fact no radical of this type has been observed in solution. Such radicals are expected to be formed in acid solutions of mono-, di-, and trimethylamine and some amino acids. In contrast, spectra were detected when the amino group was in a position further away from the unpaired electron or when the amino group was in the basic form.

Amines. The structure and coupling constants of the radicals observed in irradiated aqueous solutions of amines are summarized in Table I, and two representative spectra are presented in Figure 1. The esr spectra observed with neutral and alkaline solutions of mono-, di-, and trimethylamine could be assigned to the aminoalkyl radicals formed by hydrogen abstraction from a methyl group.

$$CH_3NR_2 + OH \longrightarrow \dot{C}H_2NR_2 + H_2O \qquad (3)$$

This assignment is based on the number of equivalent protons of each type and on the magnitudes of the hyperfine constants. The symmetry implicit in these groupings and the consistency of the hyperfine constants for these three radicals make it very probable that the spectra belong to the expected reaction products. These hyperfine constants parallel those found here and in previous work[11,13] for the radicals in neutral and basic solutions of amino acids. In particular the value of a_α^H is quite small (10–15 G) and a^N is $\sim$5 G. Both the small a_α^H and the large a_γ^H can be taken to indicate a relatively large spin density on the nitrogen and a consequent lowering of the carbon spin density. However, it seems unlikely that this spin density could be as low as 0.5 (using $Q_\alpha \cong 23$ G), and it is possible that the three bonds around the carbon are not in plane. The pronounced effect of substitution of the nitrogen in reducing a_α^H from 15.3 to 10.9 G seems also to support this idea. Such a large change seems unlikely for a planar radical site, but if the substitution were to change the departure of the radical from planarity, a much larger effect on a_α might occur. The greater electron donating power of $-NHCH_3$ as compared to $-NH_2$ would favor a more bent structure.

Because no change in the spectra of $\dot{C}H_2NH_2$, $\dot{C}H_2NHCH_3$, and $\dot{C}H_2N(CH_3)_2$ could be found between pH 7 and 13.5, it can be concluded that over this region the radicals are always in the basic form as a result of the lower pK of the radical as compared to that of the parent molecule.[15] The appearance of radicals of the type $\dot{C}H_2NR_2$ at pH 7 demonstrates that reactions 4 and 5 must occur.

$$CH_3\overset{+}{N}HR_2 + OH \longrightarrow \dot{C}H_2\overset{+}{N}HR_2 + H_2O \qquad (4)$$

(23) M. Anbar and P. Neta, *Int. J. Appl. Radiat. Isotopes*, **18**, 493 (1967).

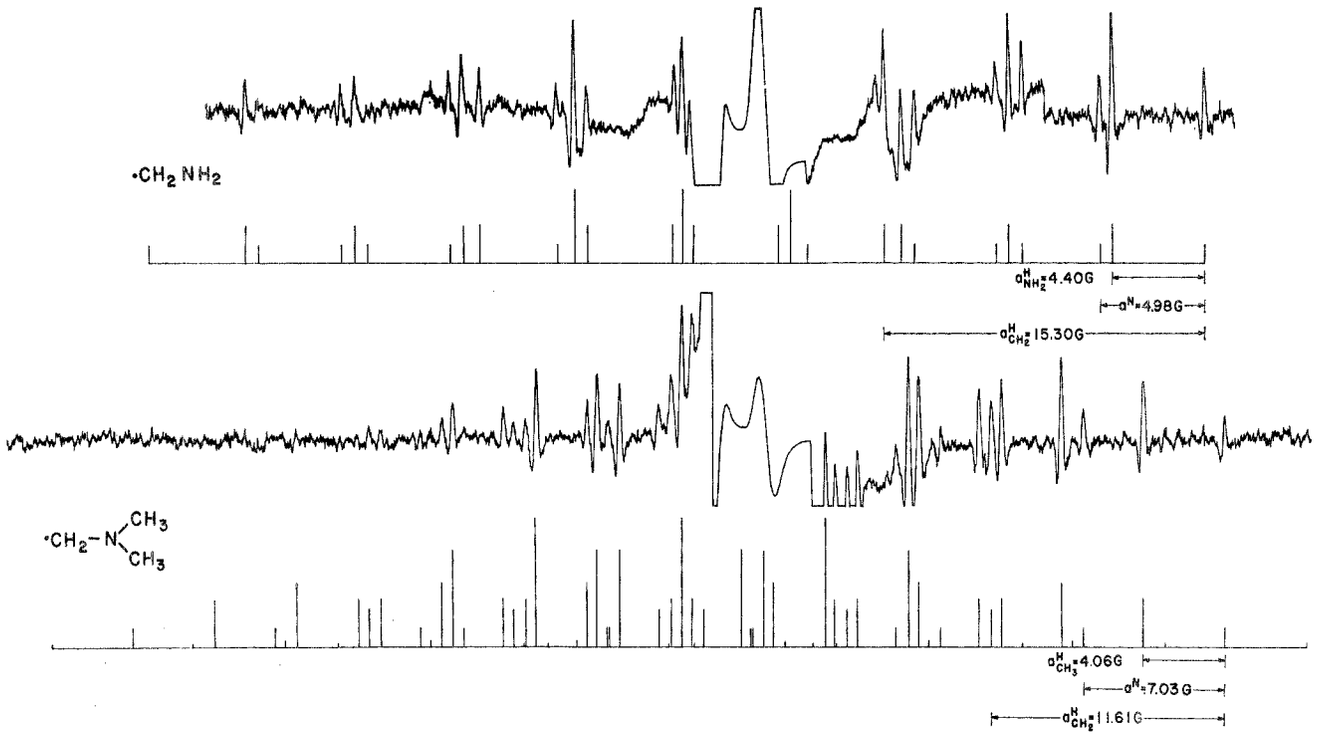


Figure 1. Second-derivative esr spectra of aqueous solutions of monomethylamine (top) and trimethylamine (bottom) saturated with N_2O at pH 12 during irradiation with 2.8-MeV electrons. Magnetic field increases to the right. The stick spectra show the relationship of the lines. The large signal from the silica cell is seen just above the center of the spectrum and is recorded at a gain 100 times less than the other portions. The low-field portion of the bottom spectrum was also recorded under conditions giving a somewhat higher signal-to-noise ratio, but no continuous scan of that is available. Both of these spectra show a pronounced intensity effect in that the high-field lines are considerably stronger than their low-field counterparts.

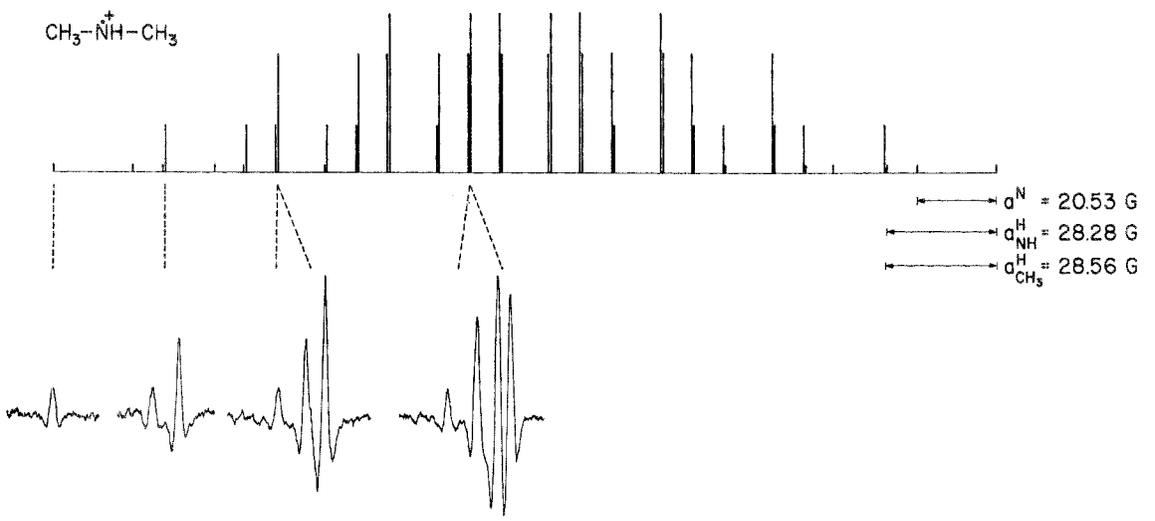


Figure 2. A schematic representation of the spectrum observed during radiolysis of a 0.1 M solution of trimethylamine at pH 1. Because of the large number of line groups, it is not possible to reproduce a continuous scan. Portions of the spectra with resolved second-order structure are shown. Similar line groups were observed at the other designated positions.

$$\dot{C}H_2\overset{+}{N}HR_2 \longrightarrow \dot{C}H_2NR_2 + H^+ \qquad (5)$$

Although the reaction of OH with methylamines in acid solutions must form aminoalkyl radicals (eq 4) the protonated forms of the radicals could not be observed (possibly as a result of the line broadening by chemical exchange of the $\overset{+}{N}H$ protons). Instead, the esr spectra observed in strongly acid solutions of dimethylamine and trimethylamine could be assigned to radicals produced by secondary reactions.

The spectra detected from irradiated acid solutions (pH 1) of di- and trimethylamine (~0.1 M) are different from those found in all other cases in that a large nitrogen splitting of about 20 G is evident (see Table I and Figure 2). These spectra clearly *cannot* be assigned to the radicals $\dot{C}H_2\overset{+}{N}H_2CH_3$ and $\dot{C}H_2\overset{+}{N}H(CH_3)_2$. The

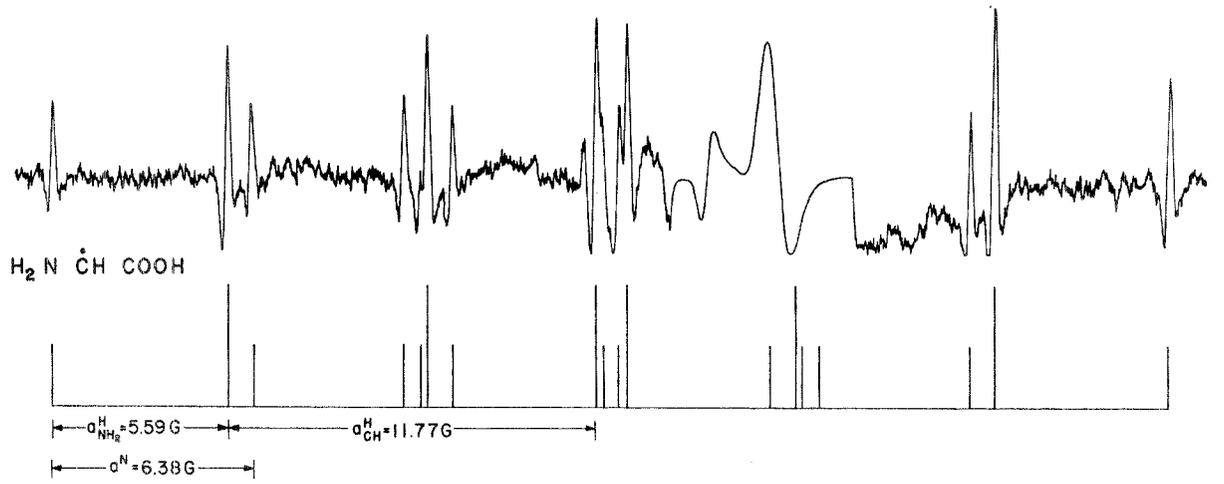


Figure 3. Esr spectrum of an aqueous solution of glycine (0.1 M) at pH 1 during irradiation. All lines are explained by the stick spectrum excepting one to the left of the center group.

most likely radicals with such a splitting are of the type $R_3\dot{N}^+$. The species $\dot{N}H_3^+$ and $(CH_3)_3\dot{N}^+$ are known from work on irradiated solids and the hyperfine constants are $a^N = 19.5$, $a_{NH}^H = 25.9$[24] and $a^N = 18.0$, $a_{CH}^H = 26.7$ G,[25] respectively, for the two radicals. In the case of trimethylamine the spectrum consists of 24 line groups and well-resolved second-order structure is present. From the number of line groups there must be one nitrogen splitting (20.53 G) and seven approximately equal proton splittings of about 28 G. The second-order structure is typical of *six* equivalent protons, however. This situation can arise if there are six equivalent protons with a coupling constant of one sign and another of nearly the same magnitude but of opposite sign.[26] The radical $(CH_3)_3\dot{N}H^+$ seems uniquely to satisfy these requirements (a_α^H should be negative while a_β^H is positive). From the coupling constants for $^+\dot{N}H_3$ and $(CH_3)_3\dot{N}^+$ it is clear that such a chance equivalence of the magnitudes of the α and β constants would be possible.

The spectrum from solutions of dimethylamine also shows second-order structure (typical of four equivalent protons), and this spectrum can be fit to a high degree of accuracy by the hyperfine constants given in Table I. The previously developed computer program[27] was used for this purpose and using 36 of the total of 54 lines an rms deviation of 0.02 G was obtained. This deviation is about that expected from the accuracy of the line position measurements. The nitrogen hyperfine constant and g factor of this radical are very similar to those of the radical discussed immediately above so that the structure must be similar. To have four equivalent protons a structure of the type $RCH_2\dot{N}^+HCH_2R$ seems most probable and the lack of any further splittings suggests $\underline{CH_2\dot{N}^+HCH_2}$. The value of a_β^H is larger than for the methyl protons in the same position as is the case when either a cyclic or straight-chain radical is compared to ethyl radical.[28] The splitting for the (presumably) NH proton is somewhat smaller than in $(CH_3)_2\dot{N}H^+$ or $\dot{N}H_3^+$. It should be noted that the cyclic structure is isoelectronic to cyclopropyl radical which has the abnormally low a_α^H of 6.5 G. If the cyclic structure is in fact correct, the bonds at the radical site must be more nearly in a plane than is the case for cyclopropyl radical.[28] The lack of exchange of the NH^+ proton in both radicals is consistent with the results on $N_2H_4^+$.[29]

The radicals from di- and trimethylamine can hardly be the result of any simple reaction and some secondary process is necessary. In support of this contention a reduction in signal height with increased flow rate is observed. No reasonable mechanism has been found for the formation of either of the two radicals suggested to explain the spectrum from solutions of dimethylamine. In the case of trimethylamine, however, a rather tentative mechanism can be suggested. It has been noted that the radical $\cdot CH_2N^+H(CH_3)_2$ must be formed in acid solutions although its spectrum is not observed (probably because of its broadened esr lines). If this radical undergoes disproportionation the compound $CH_2{=}N^+(CH_3)_2$ should be formed as suggested for amino acids.[17] The double bond will be very reactive toward OH addition so that this intermediate product can compete with the unreactive parent ammonium

(24) T. Cole, *J. Chem. Phys.*, **35**, 1169 (1961).

(25) A. J. Tench, *ibid.*, **38**, 593 (1963).

(26) With the values given in Table I overlap of pairs of second-order patterns (from the six equivalent protons) will occur in such a way as to give essentially no new lines. (A partially resolved line of unit intensity should appear between the first and second lines of each group in Figure 2.) This is possible because the second-order patterns all have common splittings. Some attempts at synthesizing the line shapes have been made, and although exact agreement has not been obtained no serious discrepancies are evident at this time.

(27) R. W. Fessenden, *J. Mag. Res.*, **1**, 277 (1969).

(28) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).

(29) J. Q. Adams and J. R. Thomas, *ibid.*, **39**, 1904 (1963).

Table II: Structure and Coupling Constants of Radicals Produced in Irradiated Aqueous Solutions of Amino Acids[a]

Compd	pH	Radical	g factor	a^N	a_{NH}^{H1} a_{NH}^{H2}	a_α^H	a_β^H	a_γ^H	a_δ^H
Glycine	1	$NH_2\dot{C}HCOOH$	2.00340	6.38	5.59 (2)	11.77 (1)			
Glycine	1 (in D_2O)	$ND_2\dot{C}HCOOH$	2.00340	6.21	0.87 (2)[b]	12.00 (1)			
Glycine	7–13.5	$NH_2\dot{C}HCOO^-$	2.00340	6.11	3.38 2.87	13.76 (1)			
α-Alanine	11–13.5	$NH_2\dot{C}(CH_3)COO^-$	2.00334	5.07	1.93 <0.2		13.86 (3)		
β-Alanine	11–13	$NH_2\dot{C}HCH_2COO^-$	2.00277	6.27	3.91 (2)	15.60 (1)	17.65 (2)		
Iminodiacetic acid	11–13	$^-OOCCH_2N\dot{H}CHCOO^-$	2.00337	6.84	5.13 (1)	12.77 (1)		5.61 (2)	
Nitrilotriacetic acid	9–13	$(^-OOCCH_2)_2N\dot{C}HCOO^-$[c]	2.00340	6.96		11.64 (1)		3.8–4.0 (4)[d]	
α-Aminoisobutyric acid	5	$H_3{}^+N-\dot{C}(CH_3)_2-COO^-$	2.00255	3.51		22.24 (2)		0.44 (3)	
α-Aminoisobutyric acid	11–13	$NH_2-\dot{C}(CH_3)_2-COO^-$	2.00249	2.97		21.94 (2)		0.72 (3)	
α-Aminoisobutyric acid	13 (low concn only)	$H\dot{N}-C(CH_3)_2-COO^-$	2.00282	10.44	<0.2			1.28 (6)	
Acetylglycine	8	$CH_3CONH\dot{C}HCOO^-$	2.00333	0.51	1.32 (1)	17.33 (1)			2.74 (3)
Acetylglycine	13.5	$CH_2{=}CNH\dot{C}HCOO^-$	2.00333	0.44	<0.2	19.91 (1)			3.48 (2)
Acetamide	9	$\cdot CH_2CONH_2$	2.00293	1.76	2.12 2.53	21.36 (2)			
N-Methylacetamide	1–13	$CH_3CONH\dot{C}H_2$	2.00281	2.20	<0.2	19.05 (2)			4.07 (3)

[a] Hyperfine constants are given in G and are accurate to +0.03 G. The g factors are measured relative to the peak from the silica cell and are accurate to about ±0.00005. Second-order corrections have been made [R. W. Fessenden, *J. Chem. Phys.*, **37**, 747 (1962)]. The number of hydrogen atoms displaying the hyperfine constant is given in parentheses. [b] This value of a_{NH}^D when corrected by the ratio of proton to deuteron magnetic moments becomes 5.67 G in excellent agreement with the value in the proton containing radical. [c] Paul and Fischer[13] report a small additional splitting of 0.15 G at pH 9 and give the structure as $(^-OOCCH_2)_2N\dot{C}HCOOH$. We did not observe this splitting, but this result could be a consequence of proton exchange. No change in the spectrum was observed upon going to stronger base. [d] See Figure 6.

ion $CH_2{=}\dot{N}^+(CH_3)_2 + OH \rightarrow HOCH_2\dot{N}^+(CH_3)_2$. Because this species has both a hydroxyl group and a nitrogen on the same carbon some transformation such as $HOCH_2\dot{N}^+(CH_3)_2 \rightarrow OCH_2 + H\dot{N}^+(CH_3)_2$ could occur leading to the observed radical. The acid conditions (pH 1) might contribute by catalyzing the reaction.

Amino Acids. The spectrum obtained from an irradiated solution of glycine at pH 1 is presented in Figure 3; the coupling constants (Table II) are in good agreement with previous values.[10,12] With this compound no lines could be observed at pH 3. However, at pH 5–7 the alkaline solution spectrum was present with many additional lines. Because of the complexity of the spectrum and weakness of the line intensities, no successful analysis of the additional contribution was possible. In acid solutions (pH 1–3) of iminodiacetic and nitrilotriacetic acids no spectra could be observed, again possibly as a result of the exchange of protons of the ammonium group.

The radical produced by the reaction of OH with glycine in acid solution seems to be $NH_2\dot{C}HCOOH$ as concluded in several previous studies[10,12] and in the present work. The hyperfine constants for this species are similar to those for the radical $H_2N\dot{C}HCOO^-$ which is formed in basic solution, but the two NH protons appear equivalent and have a somewhat larger coupling constant (the two NH protons in $H_2N\dot{C}HCOO^-$ are not equivalent). Protonation of the carboxyl group could readily account for the difference. This result is not in agreement with the conclusions drawn from the pulse radiolysis experiments discussed earlier which suggest that the radical should exist in the form $^+H_3N\dot{C}HCO_2H$. The assignment of the esr spectrum observed at pH 1 to the radical $H_2N\dot{C}HCOOH$ would imply a $pK < 1$ and a change of the pK of the NH_3^+ group of more than 8 units from the parent compound. This disagreement is serious enough to require a search for alternative explanations.

One possibility is that the lines of $^+H_3N\dot{C}HCOOH$ are broadened by exchange of the $-NH_3^+$ protons and that the spectrum observed is from some other radical, possibly a product of secondary reactions. Reference to the hyperfine constants of Table II shows that it is possible for the CH_2 protons of a radical of the form $RCH_2NH\dot{C}HCOO^-$ to have a coupling constant of the size assigned to the NH_2 protons of $H_2N\dot{C}HCOOH$. To test if such a radical were responsible for the spectrum in acid solutions of glycine an experiment was performed with a D_2O solution to determine if the protons having the 5.59-G hyperfine constant would be exchanged. The spectrum obtained in this case did in fact, have the 5.59-G proton triplets replaced by 0.87-G quintets of intensity 1:2:3:2:1 as expected for two deuterium nuclei. This result shows that the protons in question are located on a nitrogen because of their ready exchange and further substantiates the interpretation of the spectrum in terms of the hyperfine constants. Based on the esr results alone there seems little doubt, therefore, that the radical detected in acid solutions of glycine is $H_2N\dot{C}HCOOH$.

It should be noted that in no case (neither from amines nor amino acids) have radicals of the form $H_3\overset{+}{N}\dot{C}R_2$ been observed in aqueous solution. No radicals were detected from methylamine and iminodiacetic and nitrilotriacetic acids and from di- and trimethylamines the only radicals found seemed clearly to be the result of secondary reactions. Similar results have been reported for other systems.[10,13] The possibility that spectra of radicals with an α-NH_3^+ group are undetectable because of line broadening caused by exchange of the NH_3^+ protons has been mentioned above. Here we would like to discuss this point more fully.

The hyperfine constants for radicals of the type $^+H_3N\dot{C}R_2$ as found in irradiated solids[2,3] are quite different from those for the basic forms, and depending on the kinetics of the proton exchange process this difference could lead to a large increase in line width. Proton exchange in alcohol radicals has been considered several times,[30,31] but in those cases the splitting by the exchangeable proton is small ($\sim$1 G). Furthermore the exchange is acid-catalyzed $\dot{R}OH + H^+ \rightleftharpoons \dot{R}OH_2^+$ and can be made very rapid in strong acid. In the case of a radical $\dot{R}NH_3^+$ the exchange reaction should be

$$\dot{R}NH_3^+ \underset{k'}{\overset{k}{\rightleftharpoons}} \dot{R}NH_2 + H^+$$

If the pK of the acid form is like that determined from the pulse work on methylamine, then the equilibrium will be to the left at pH 1 and the lifetime of the spin state of the radical $\dot{R}NH_3^+$ will be limited by the first-order dissociation. To estimate this rate we will use the relation $K = k/k'$ where K is the equilibrium constant. With the values $k' = 10^{10}$ and $K = 10^{-4}k$ becomes 10^6. To cause a line width increase to 1 G the first-order rate would have to be approximately 2×10^7 sec^{-1} (the equivalent of 1-G line width in radians sec^{-1}). With a lower pK and a higher value for k' such a value of k is possible. On the basic side of the equilibrium the lifetime of the spin state of $\dot{R}NH_2$ is limited by the protonation rate, k'. To avoid line broadening to 1 G the product $k'[H^+]$ must be less than 2×10^7, or at pH 1 k' must be less than 2×10^8 M^{-1} sec^{-1}. Again line broadening is possible. At this time it is not clear if such a line broadening is in fact occurring.

The structure and coupling constants of the radical produced in irradiated alkaline solutions of several other amino acids are summarized in Table II, and some representative spectra are presented in Figures 4–9. With all of the amino acids which give sufficiently

(30) H. Fischer, *Mol. Phys.*, 9, 149 (1965).

(31) H. Zeldes and R. Livingston, *J. Chem. Phys.*, 47, 1465 (1967).

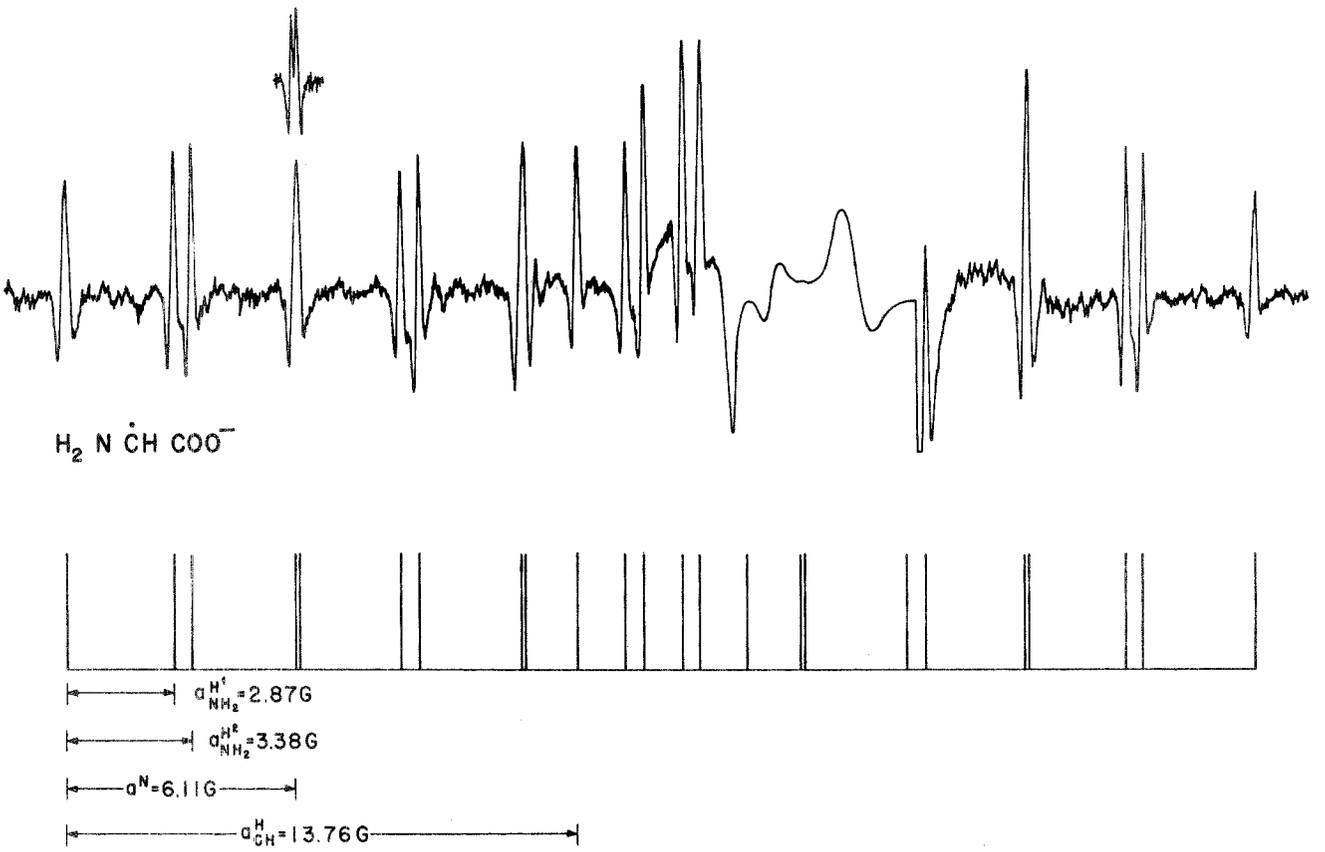


Figure 4. Esr spectrum of an aqueous solution of glycine (0.01 M) at pH 12 during irradiation. The fourth and fifth lines (of the stick spectrum) were resolved at a lower modulation as shown above the spectrum.

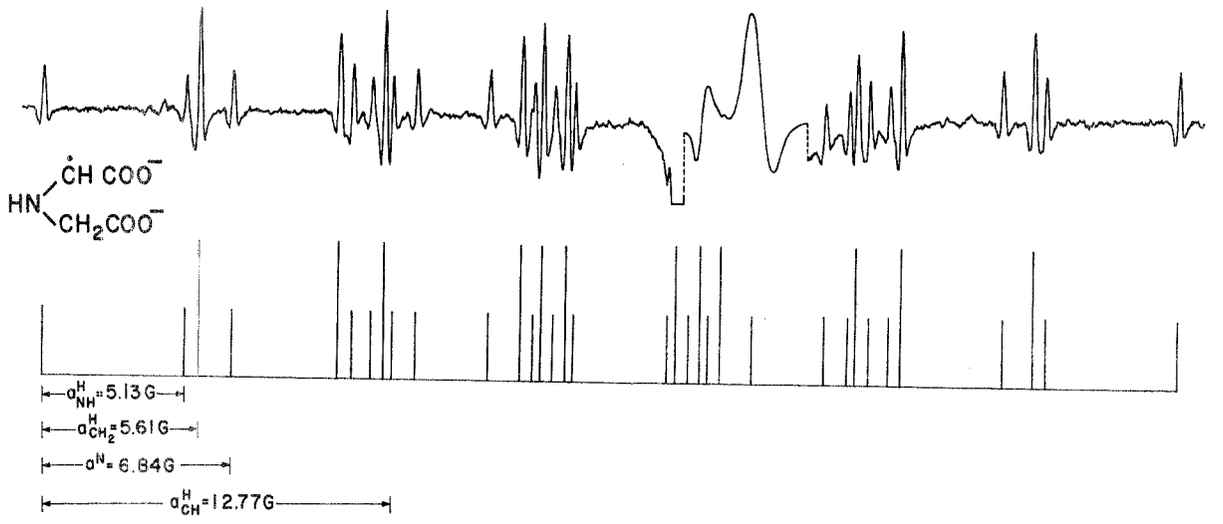


Figure 5. Esr spectrum of an aqueous solution of iminodiacetic acid (0.01 M) at pH 13 during irradiation.

intense spectra to be analyzed the aminoalkyl radical is observed. Furthermore, abstraction occurs to produce the α-aminoalkyl radical $R_2\dot{C}NH_2$ whenever a hydrogen α to the amino group exists. In the case of α-aminoisobutyric acid no such hydrogen is available and abstraction is at the β position producing $\dot{C}H_2C(NH_2)(CH_3)COO^-$. Some of these radicals (from glycine, α-alanine, nitrilotriacetic acid, and acetylglycine) were produced previously by the Ti^{3+}–EDTA–H_2O_2 method,[13] and the coupling constants reported here are in good agreement with those values. We have followed the assignments given by Paul and Fischer[13] for most of the amino acids and from the consistency of the values among the various radicals see no reason to question their assignments. All of the amino acids studied were also irradiated at pH > 13; in most cases no changes in the spectra were observed. However, a different spectrum than that found in neutral solution was ob-

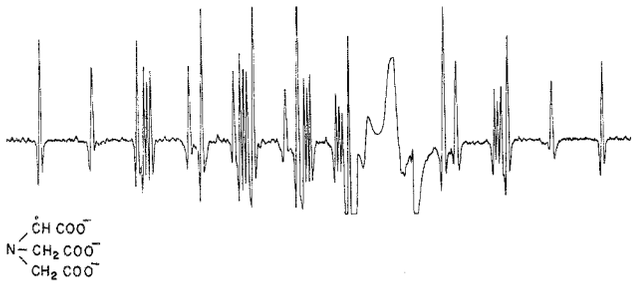


Figure 6. Esr spectrum of an aqueous solution of nitrilotriacetic acid (0.01 M) at pH 13 during irradiation. The varying peak heights reflect in part, varying line widths which are assumed to be a result of hindered internal rotation. No complete interpretation has been made. This spectrum is similar to that found by Paul and Fischer[13] but is better resolved.

served with strongly alkaline acetylglycine (see below).

The results obtained here are consistent with the overall mechanism given above, but because of the effect of reaction 2 there is little evidence of reaction 1b. In order to demonstrate the occurrence of reaction 1b it is necessary to choose a compound with a relatively slow reaction 2. It is reasonable to assume that the rate of reaction 2 would parallel that of (1a) and also be effected by the activating amino group. Compounds having no CH bonds in a position α to the amino group would be less reactive. For this reason α-aminoisobutyric acid and t-butylamine were chosen to demonstrate this effect after the experiments with glycine and alanine at low concentrations failed to show the RṄH radicals. Unfortunately, the spectrum obtained with t-butylamine did not have strong enough lines to be analyzed (see above). With solutions containing only low concentrations of α-aminoisobutyric acid a second spectrum was observed in addition to that of $\dot{C}H_2C(NH_2)(CH_3)COO^-$ (Figure 7). As well as can be determined this radical has six equivalent protons with a hyperfine constant of 1.28 G and a nitrogen with a^N = 10.44 G. The size of the proton splitting seems clearly to indicate that these nuclei are in a γ or comparably distant position and the size of the nitrogen splitting suggests a new type of radical. The only radical with this configuration which can be produced without considerable rearrangement is $HṄC(CH_3)_2COO^-$ or its dissociated form $^-ṄC(CH_3)_2COO^-$. These species are also suggested by the chemical arguments given above. The latter radical must be considered because of the absence of a splitting in the spectrum by the NH proton. This radical, however, is isoelectronic to an alkoxy radical and might be expected to have a large g-factor anisotropy and broad lines even in solution.[32] The form $HṄC(CH_3)_2COO^-$ should show an extra splitting from the NH proton, but it is quite possible that rapid exchange of this proton could average this splitting to zero. In this connection it should be mentioned that the splitting by the OH proton of $(CH_3)_2\dot{C}OH$ is averaged out[19] at a pH of about 10, two units below the pK of this radical. It does not seem possible at this time to estimate the pK of the radical $HṄC(CH_3)_2COO^-$ and so to choose on this basis between these two alternatives. The fact that a nitrogen centered radical is observed only at millimolar concentrations demonstrates the importance of reaction 2 in our experiments.

Relatively little is known about this type of nitrogen centered radical. Recently Danen and Kensler[33] have reported solution spectra of $R_2Ṅ$ radicals formed photolytically. These possess nitrogen splittings of 14 G consistent with our assignment here. It should be noted that the radicals $R_2C{=}Ṅ$ also have similar nitrogen splittings.[21] Species with the structure RṄH have been invoked to explain the esr spectra observed from photolyzed frozen amines[7] and nitrogen hyperfine constants of ~30 G rather than the 10 G seen here were required to explain the spectra. This apparent discrepancy can be explained by reference to the full hyperfine tensor for nitrogen in the radical $H_2C{=}Ṅ$. This radical has an isotropic hyperfine constant in the solid[34] of ~9.5 G, but the splitting in the powder spectrum corresponds to the parallel value of the hyperfine tensor, namely 34.4 G. It is reasonable to assume a similar anistropy for radicals of the type RṄH. Thus the isotropic hyperfine constant of 10.44 G assigned here to the radical $HṄC(CH_3)_2COO^-$ and the ~30-G splitting found for RṄH in the solid are both of the magnitude one should expect.

Amides. Experiments were performed with acetamide and N-methylacetamide because the hyperfine constants of the radicals $\dot{C}H_2CONH_2$ and $CH_3CONH\dot{C}H_2$ (Table II) were considered useful in deciding what radical is formed from acetylglycine. The radical from acetamide has been observed previously,[35] and our hyperfine constants as given in Table II are in good agreement. The radical from N-methylacetamide could be either $CH_3CONH\dot{C}H_2$ as indicated or $\dot{C}H_2CONHCH_3$. Chemical arguments favor the former in that the hydrogens of the CH_3 group on nitrogen are more activated. Recent pulse radiolysis experiments[36] support this view. A comparison of hyperfine constants of other radicals derived from amides[13,35] also leads to this choice. The absence of a splitting from the NH proton does not seem disturbing because of the small size and variable nature of this type of splitting (the radical from α-alanine shows only one splitting).

(32) M. C. R. Symons, *J. Amer. Chem. Soc.*, 91, 5924 (1969).
(33) W. C. Danen and T. T. Kensler, *ibid.*, 92, 5235 (1970).
(34) J. A. Brivati, K. D. J. Root, M. C. R. Symons, and D. J. A. Tinling, *J. Chem. Soc. A*, 1942 (1969).
(35) R. Livingston and H. Zeldes, *J. Chem. Phys.*, 47, 4173 (1967).
(36) E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, *J. Amer. Chem. Soc.*, 92, 3898 (1970).

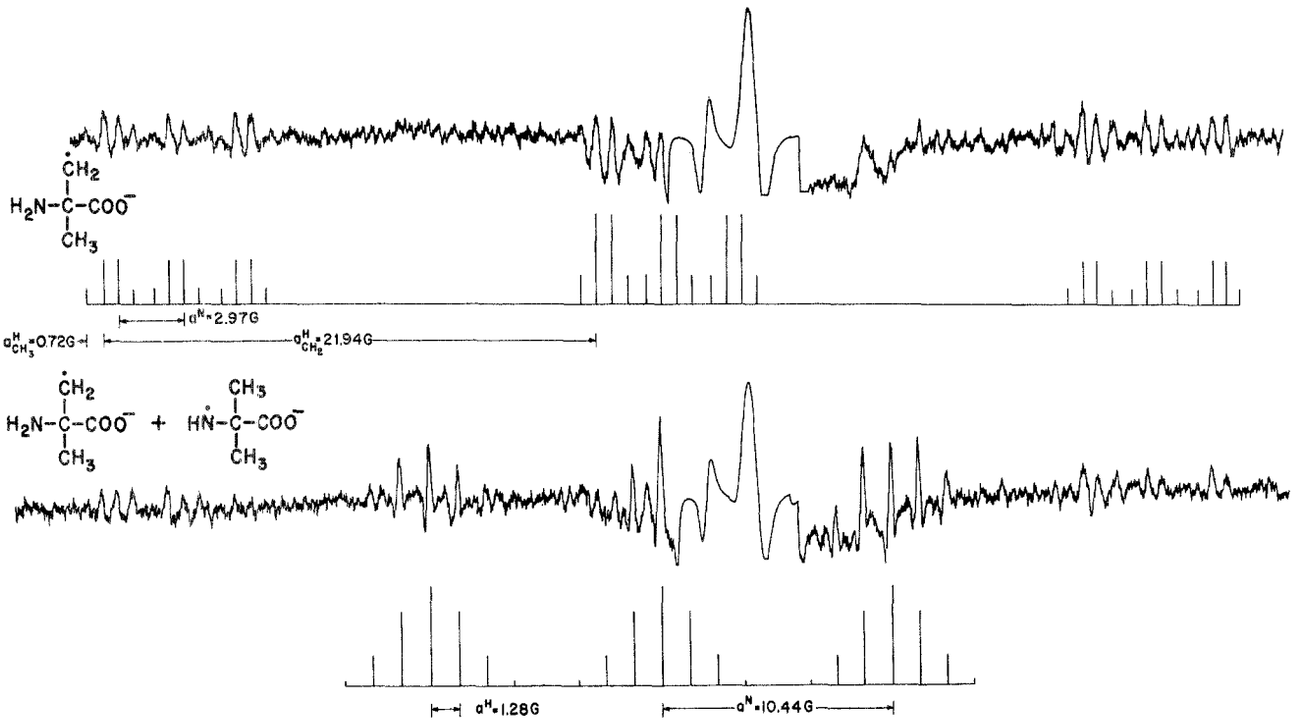


Figure 7. Esr spectra of aqueous solutions of α-aminoisobutyric acid (top, 0.1 M; bottom, 2.5×10^{-3} M) at pH 13.3. The stick spectrum at the top shows the relationship of the lines of the $\cdot CH_2C(NH_2)(CH_3)COO^-$ radical which is present at both concentrations. The stick spectrum at the bottom shows the lines of the $H\dot{N}C(CH_3)_2COO^-$ radical formed only at the low concentration.

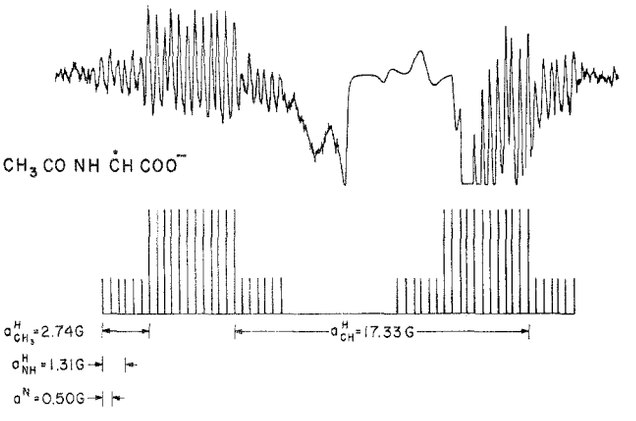


Figure 8. Esr spectrum of an aqueous solution of acetylglycine (0.01 M) at pH 8 during irradiation.

At pH 8 acetylglycine gives a radical with hyperfine constants similar to those of the radical from *N*-methylacetamide. The radical must be $CH_3CONH\dot{C}HCOO^-$. Paul and Fischer[13] have also detected this radical and give the same assignment. In stronger base (pH 13) a different spectrum is obtained (Figure 9). This spectrum has similar parameters to those found in the less alkaline solution (without the splitting by the NH proton) but is distinguished by having only a triplet splitting of ~3 G rather than the quartet from the terminal CH_3 group. At this time it is not clear whether this is a different form of the same radical or a new radical formed in some secondary reaction. Because no transformation of the starting material occurs in this pH range it is difficult to see why the species $CH_3CONH\dot{C}HCOO^-$ would *not* be formed. Therefore, either this radical undergoes some change or for some reason the lines become broad and unobservable while a new radical is formed. The first alternative seems the more reasonable. The species $CH_2{=}C(O^-)NH\dot{C}HCOO^-$ which is equivalent to dissociation of the OH proton from enol form $CH_2{=}C(OH)NH\dot{C}HCOO^-$ is suggested as at least consistent with the hyperfine constants.

Conclusion

The results obtained are consistent with the radical formation mechanism defined by reactions 1a, 1b, and 2. Because reaction 2 is usually fast, there is little direct evidence for reaction 1b in most cases. Only in the case of α-aminoisobutyric acid was a nitrogen centered radical detected which is believed to come from reaction 1b. In general the results obtained in very alkaline solutions of amines and amino acids are not different than those obtained in milder base (~pH 9) both in this work and previously.[13]

In agreement with previous results no radicals of the type $R_2\dot{C}NH_3^+$ were found in acid solutions of amino acids. This observation has been extended to solutions of the methylamines. The absence of such a spectrum cannot be explained by a lack of reactivity on the part of the acid form of the amine because secondary product

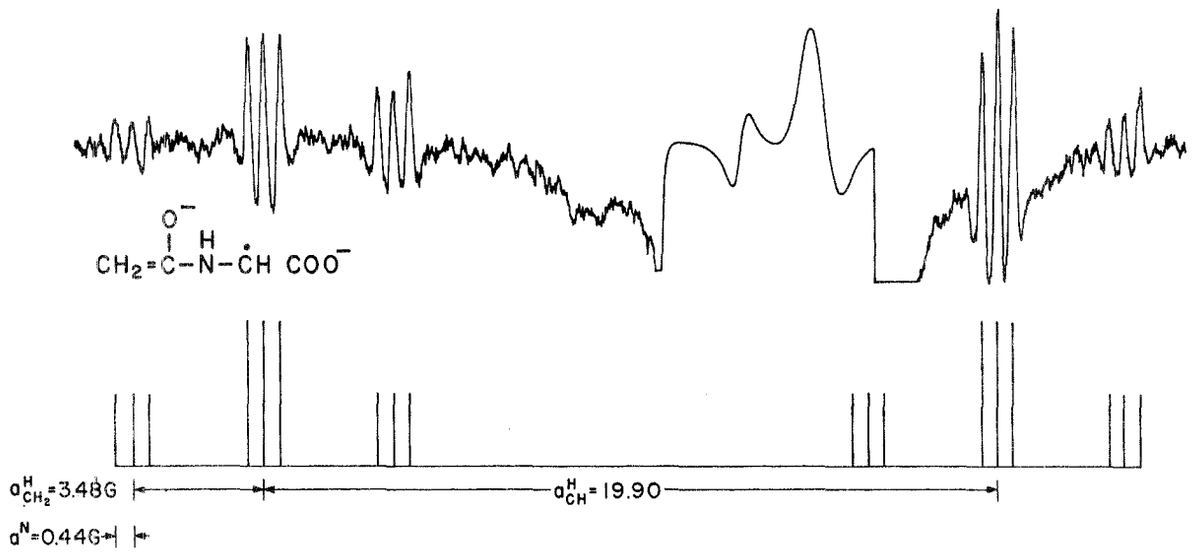


Figure 9. Esr spectrum of an aqueous solution of acetylglycine (0.01 *M*) at pH 13.5 during irradiation.

radicals were observed in the cases of di- and trimethylamine. It is possible that proton exchange in the radical $R_2\dot{C}NH_3^+$ broadens the lines of the esr spectrum making them unobservable. Finally, we are forced to agree with earlier workers that the esr spectrum observed in strongly acid solutions of glycine is best attributed to the radical $H_2N\dot{C}HCOOH$ although this assignment does not seem in agreement with the results of pulse radiolysis experiments. We believe this disagreement merits further investigation.

Acknowledgment. The authors wish to acknowledge several helpful discussions with H. Fischer.