compounds and enols), C≡C, C=N (oximes and an imine), or C=S bonds, benzenes, furans, pyrroles, and thiophenes. Adduct radicals containing sulfur were detected by their ESR spectra only in the case of certain olefinic compounds and oximes. The absence of detectable ESR lines for the other compounds can be the result of either slow reaction of the sulfide radical or broad lines for the sulfur containing radicals. From the fact that some sulfur containing radicals were observed it seems that slow reaction is the more likely reason. Values of the pK for the dissociation of sulfhydryl proton in three radicals of the type $HSCH_2\dot{C}XY$ were determined and found to be about 1.5–2 units lower than in the corresponding thiol.

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Protonation Reactions at Carbon Sites in the Anion Radicals of Certain Unsaturated Compounds and Aromatic Amino Acids[1]

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(Received October 9, 1974)

Publication costs assisted by the Petroleum Research Fund and the U.S. Atomic Energy Commission

The protonation reactions at carbon sites of the anion radicals of certain unsaturated compounds as well as aromatic amino acids and their analogs have been investigated in alkaline and neutral aqueous glasses by electron spin resonance spectroscopy. For the compounds acrylic acid, 3,3-dimethylacrylic acid, crotonic acid, acrylamide, and acrylonitrile protonation or deuteration is found to occur β to the electronegative group (R_3) to form an α-carbon radical: $R_1R_2C{=}CHR_3^- + H_2O \rightarrow R_1R_2CH{-}\dot{C}HR_3 + OH^-$. The anions were found to be stabilized when R_1 (or R_2) was a carboxyl (fumaric) or methyl (crotonic and 3,3-dimethylacrylic acid) group. The anions of acetylenedicarboxylic acid and hydrogen cyanide were also found to protonate in these aqueous glasses. The anions of four aromatic amino acids were found to protonate at carbon sites on the aromatic ring. Analogs of these amino acids which contain the aromatic ring were found to protonate at positions equivalent to those found in the amino acids. Molecular orbital calculations of the spin density and free valency show that the magnitude of these parameters can be correlated with the site of protonation and perhaps the tendency to protonate.

Introduction

Evidence has been presented in several investigations that anion radicals of compounds with unsaturated hydrocarbon linkages undergo irreversible protonation reactions at carbon sites. These investigations have been of ions in aqueous solutions or in the solid state.[2-7] For example, the acrylic acid anion has been investigated in aqueous solution by ESR[2] and pulse radiolysis techniques,[4] as well as by ESR in glassy matrices.[5-7] These results show that the anion which is initially formed by electron attachment subsequently protonates (reaction 1).

$$CH_2{=}CH{-}CO_2^{2-} + H_2O \longrightarrow CH_3{-}\dot{C}H{-}CO_2^- + OH^- \quad (1)$$

This type of reaction is of interest to radiation chemistry of certain biochemical systems. For instance, in γ-irradiated DNA it is now considered likely that one of the radicals formed results from protonation at a carbon site on the anion of thymine.[8] Studies of other DNA bases[9] as well as aromatic amino acids[10] suggest protonation at carbon sites in these cases as well.

In this study we have investigated a series of model compounds to determine the type of unsaturated molecules which readily protonate in aqueous glasses. We have also studied a number of aromatic amino acids and their analogs to determine if protonation reactions occur and at which sites they occur. We briefly consider possible theoretical reasons for the particular site of protonation.

TABLE I: Radicals Formed by Electron Attachment to Molecules with Localized Multiple Bonds

Compounds	Radical	Matrix	Hyperfine splitting, G	Temp, °K
A. Anions				
3,3-Dimethylacrylic acid	$(CH_3)_2C{=}CHCO_2^-H$	$LiCl–D_2O$	14.6 (6 H)	77
	$(CH_3)_2C{=}CHCO_2^{2-}$	NaOD	14.1 (6 H)	77
Crotonic acid	$CH_3CH{=}CHCO_2^{2-}$	NaOD	15 (4 H)[a]	77
Acetylenedicarboxylic acid	$CO_2^-C{\equiv}CCO_2^{2-}$	NaOD	4 G wide singlet	77
Fumaric acid	$CO_2^-CH{=}CHCO_2^{2-}$	NaOD	8 (2 H)[a]	77
B. Protonated Radicals				
Acrylic acid	$CH_3\dot{C}HCO_2^-$	LiCl or NaOH	24 (4 H)[a,b]	77
Acrylamide	$CH_3\dot{C}HCONH_2$	LiCl or NaOH	25 (4 H)[a]	77
Acrylonitrile	$CH_3\dot{C}HC{\equiv}N$	LiCl or NaOH	24.5 (4 H)[a]	77
Crotonic acid	$CH_3CHD\dot{C}HCO_2$	NaOD	40 (1 H)	
			21 (1 H)	165
3,3-Dimethylacrylic acid	$(CH_3)_2CH\dot{C}HCO_2^-$	NaOH	27.5 (2 H)	160
		LiCl	24.5 (2 H)	160
	$(CH_3)_2CD\dot{C}HCO_2^-$	NaOD	22 (1 H)	160
		$LiCl–D_2O$	21 (1 H)	160
Acetylenedicarboxylic acid	$CO_2^-CH{=}\dot{C}CO_2^-$	LiCl or NaOH	57 (1 H)	77
	$CO_2^-CD{=}\dot{C}CO_2^-$	$LiCl–D_2O$ or NaOD	8.6 (1 D)	77
Hydrogen cyanide	$H_2C{=}N^{\bullet}$	LiCl	89 (2 H) $A_{\parallel}^N = 32$ $A_{\perp}^N < 3.5$	110

[a] Further anisotropic structure was observed in these radicals, however, only the approximate isotropic result is reported. [b] The anisotropic structure in the ESR spectrum of this radical was interpreted (see text).

Experimental Section

All reagents and solutes were obtained commercially and were used without further purification except for acrylic acid and histidine which were purified by distillation and recrystallization, respectively.

The experimental procedure has been explained in our previous work.[11,12] Both 8 *M* NaOH and 12 *M* LiCl glasses were employed in this study. Electrons were generated by photolysis of 10–20 m*M* $K_4Fe(CN)_6$. The concentration of solute was approximately 2 m*M* for most compounds. Higher concentrations of histidine (20 m*M*) were found to be necessary.

The anions formed were often found to protonate when exposed to white light. Thus filtered light (≥4300 Å) was used for photobleaching trapped electrons.

The *g* values and hyperfine splittings in this work were measured relative to potassium peroxylaminedisulfonate (A_N = 13.0G and *g* = 2.0056).

Results and Discussion

Molecules with Localized Double Bonds. Electron attachment to a number of compounds with localized double bonds in neutral or alkaline matrices resulted in stable anion radicals in several cases (see Table I). We discuss these radical anions below.

3,3-Dimethylacrylic Acid. Electron attachment to 3,3-dimethylacrylic acid on NaOD at 77°K gave the ESR spectrum in Figure 1A. This spectrum which shows a 14.1-G hyperfine splitting due to the six methyl protons is clearly that of the dianion radical. This splitting is very close to that found for the acrylic acid anion in a nonaqueous matrix.[6] Similar results were found in LiCl-D_2O (Table I). In H_2O matrices some protonation occurred even at 77°K, so that the anion could not be isolated from the protonated species.

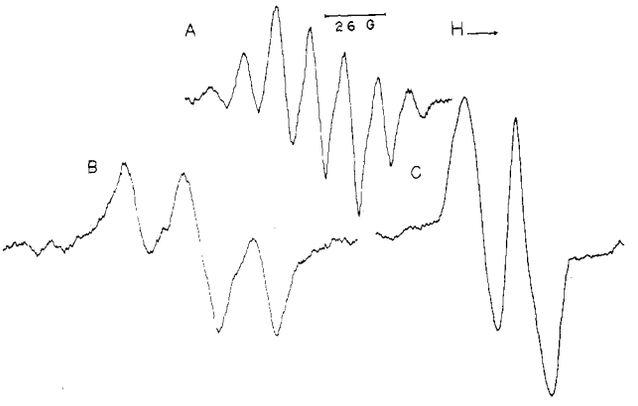


Figure 1. ESR spectra of radicals formed after electron attachment to 3,3-dimethylacrylic acid in alkaline glasses: (A) spectrum of the dianion at 77°K; (B) protonated radical, $(CH_3)_2CH\dot{C}HCO_2^-$, spectrum produced by photoprotonation of the dianion at 77°K in 8 *M* NaOH; (C) deuterated radical, $(CH_3)_2CD\dot{C}HCO_2^-$, spectrum produced by warming the dianion to 175°K in 8 *M* NaOD.

The anion (LiCl) or dianion (NaOH) was found to protonate or deuterate upon warming to 160°K or photobleaching with unfiltered visible light. In H_2O matrices the protonated radical gave a spectrum consisting of a 24.5- to 27.5-G triplet due to two protons (Figure 1B). Of the two possible carbon sites available for protonation only protonation at the site β to the carboxyl as in reaction 2 could produce this spectrum.

$$(CH_3)_2C{=}CH{-}CO_2^{2-} + H_2O \longrightarrow OH^- + (CH_3)_2CH{-}\dot{C}H{-}CO_2^- \quad (2)$$

In D_2O matrices a 21- to 22-G doublet was observed as would be expected for the α proton in the radical, $(CH_3)_2CH{-}\dot{C}H{-}CO_2^-$ (Figure 1C). In contrast to our re-

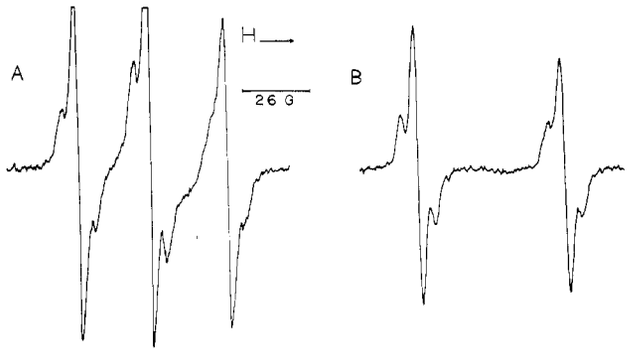


Figure 2. ESR spectra of radicals formed after electron attachment to acetylenedicarboxylic acid in 8 *M* NaOH: (A) spectrum at 77°K of the anion, protonated radical ($CO_2^-CH{=}\dot{C}CO_2^-$), and electron before photobleaching; (B) protonated radical spectrum at 110°K after removing the anion and electron by photobleaching. The structure on the major components in this spectrum is likely due to matrix interactions since it is not observed in D_2O matrices.

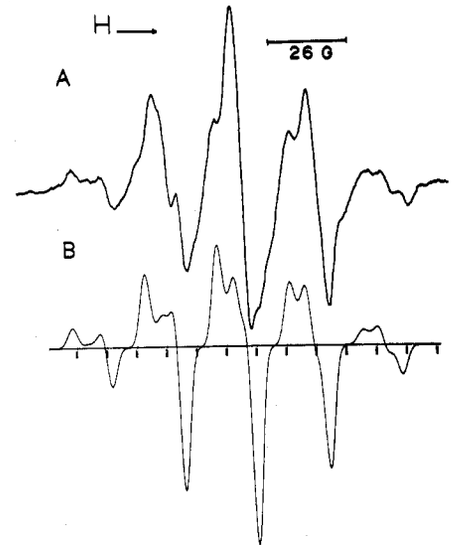


Figure 3. (A) ESR spectrum of the $CH_3\dot{C}HCO_2^-$ radical resulting from protonation at the β position in the acrylic acid anion at 77°K in 8 *M* NaOH. (B) Computer simulation of the anisotropic spectrum in A utilizing parameters described in the text.

sults Neilson and Symons[13] report that both H atom bombarded and γ-irradiated solid dimethylacrylic acid added hydrogen to the α carbon position. This may be a matrix effect or an inherent difference in the site of attack of the hydrogen atom.

An interesting concentration dependence was noted for the anion of 3,3-dimethylacrylic acid in LiCl–H_2O. Saturated solutions gave the anion without any protonation. Even warming did not result in complete protonation. This effect is considered to be due to agglomerates of the solute which hinder protonation by removing water from the reaction site.

Acetylenedicarboxylic Acid. The anion radical of acetylenedicarboxylic acid was observed at 77°K in alkaline matrices though it is overlapped with the protonated radical (Figure 2A). The anion was not observed in LiCl glasses under the same conditions. The singlet spectrum of the anion was distinguishable from the electron from its much narrower line width (4 G vs. 13 G). Complete protonation occurred upon photobleaching with unfiltered visible light or warming to 110°K (Figure 2B). The protonated radical (CO_2^-—CH=$\dot{C}$—CO_2^-) shows a 57-G splitting with some further structure in H_2O. Since this further structure was not observed for the protonated radical in NaOD (92% D) it is likely due to matrix interactions. The splittings for radicals in D_2O matrices (Table I) are very close to those predicted from those found in H_2O using the gyromagnetic ratio. The splittings found in this work are larger than those found in single crystal work (51–54 G)[3] or in aqueous solution at room temperature (50 G).[2] In both of these investigations the anion radicals were proposed as precursors to the protonated radicals; our results confirm this previous work.

Crotonic and Fumaric Acids. Both these compounds gave spectra expected for anion radicals in 8 *M* NaOD after electron attachment. The analysis of the spectra in terms of isotropic couplings is shown in Table I. Both spectra showed some anisotropic structure. Warming crotonic acid in NaOD resulted in an ESR spectrum which could only arise from deuteration at the β position (Table I). Fumaric acid did not protonate upon warming in NaOH to 180°K. The results for fumaric acid agree with those found in aqueous solution.[2,14]

Other compounds whose anion radicals did not protonate in these glasses were benzoic and muconic acids. Neta and Fessenden report similar results in aqueous solution.[2,29]

The anions of the other compounds in Table I were not stable at 77°K in any of the aqueous glasses employed in this study. The spectral parameters of protonated (deuterated) radicals are reported in Table I and discussed below briefly.

Acrylic Acid. The acrylic acid protonated radical ($CH_3\dot{C}HCO_2^-$) gave spectra which showed the anistotropic structure of the α proton in both LiCl and NaOH glasses (Figure 3A). In D_2O where deuteration occurred a well-resolved spectrum was found (Figure 4A). The protonated radical has been observed in several previous investigations of acrylic acid;[5,6] however, the anisotropic structure of the spectrum has not been interpreted. We have attempted such an interpretation and have employed a computer program which simulates anisotropic spectra to first order. The g and hyperfine tensors are assumed to be coaxial. From previous work on radicals of this type in single crystals as well as theory it is known that $A_{xx} > A_{zz} > A_{yy}$ and $g_{zz} > g_{xx} \simeq g_{yy}$. The z axis is perpendicular to the plane of the molecule. We have, therefore, employed these constraints on the variation of parameters for the α hydrogen. The best fit to experiment, shown in Figure 3B, yields A_{CH_3} = 24 G (assumed isotropic), the following parameters for the α proton A_{xx}^H = 38 G, A_{yy}^H = 11 G, A_{zz}^H = 20 G and $g_{xx} \simeq g_{yy}$ with $g_{xx} - g_{zz} = 0.0016$. As can be seen, the fit is best at the ends where the anisotropic structure of the α proton is free from the methyl group structure. Our anisotropic parameters are similar to, but about 3 G larger than, those found for the same radical in alanine single crystals at room temperature.[15]

The reconstruction shown in Figure 4B for the radical $CH_2D\dot{C}HCO_2^-$ assumes the parameters for the α proton, but a single isotropic (methyl) deuterium splitting of 3.7 G as well as two isotropic (methyl) protons at 24 G. Due to the fact the solution was not completely deuterated (92% D), the experimental spectrum was found to be overlapped with some of the protonated species. We estimated that about 20% of the radicals are protonated. The simulation, therefore, contains both deuterated (80%) and protonated

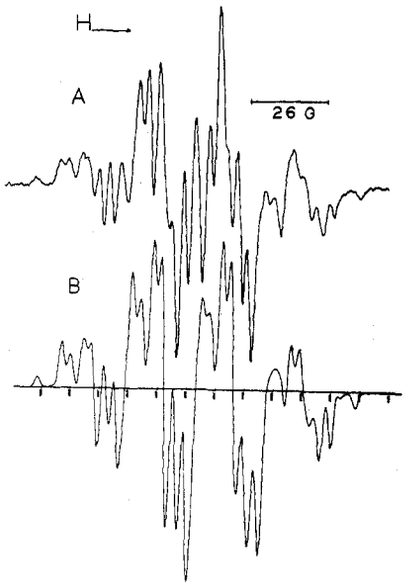


Figure 4. (A) ESR spectrum of the $CH_2D\dot{C}HCO_2^-$ and $CH_3\dot{C}HCO_2^-$ resulting from deuteration and protonation at the β position in acrylic acid anion at 77 K in 8 *M* NaOD (92% D). (B) Computer simulation of the anisotropic spectrum in A assuming 80% $CH_2D\dot{C}HCO_2^-$ and 20% $CH_3CHCO_2^-$. Parameters utilized in the reconstruction are described in the text.

(20%) radicals. The agreement for the end components is excellent and confirms that deuteration occurs at the same site as protonation.

Hydrogen Cyanide. The results of electron attachment to HCN in LiCl–H_2O at 77°K under acidic conditions is shown in Figure 5. This spectrum is assigned to the $CH_2{=}\dot{N}$ · radical. The hyperfine parameters reported in Table I are in good agreement with those found in aqueous solution by Behar and Fessenden[16] and those found in the solid state.[17,18] The acidic conditions used, of course, mean that attack by hydrogen atoms as well as by electrons may have occurred. The previous work in aqueous solution, however, showed both these intermediates result in the same radical.[16]

Acrylonitrile. The anion of acrylonitrile was found to immediately protonate at 77°K to form the $CH_3\dot{C}HCN$ radical. The ESR spectrum in H_2O glasses was an overall quintet with further resolution of the anisotropic structure of the α proton similar to that found in acrylic acid. Resolution of nitrogen splittings was not found. The splitting reported in Table I is the average separation between the major peaks in the spectrum.

It was first thought that the results for acrylonitrile would test the relative tendency of a carbon–carbon double bond and the cyano group to protonated after electron attachment. However, the spin density distribution (discussed later) in the anion places most of the unpaired electron in the double bond. This compound is, therefore, not a reasonable test of their relative potentials for protonation.

Acrylamide. The results for acrylamide are reported in Table I. Spectra of the protonated anion of acrylamide were similar to those found for acrylic acid and acrylonitrile, i.e., a 25-G quintet with some further resolution of the α proton anisotropy.

Seddon and Smith report results in γ-irradiated 8 *M* NaOH glasses containing acrylamide or acrylonitrile which are in agreement with those found here. A virtually identical spectrum with that found here for $CH_2D\dot{C}HCO_2^-$ was found by Seddon and Smith for $CH_2D\dot{C}HCOND_2$.[19] In view of the similarity in structure of the radicals this is reasonable; however, their interpretation included splittings arising from the amide group which are most likely a result of anisotropy in the α proton coupling and overlap of the spectrum due to the protonated radical.

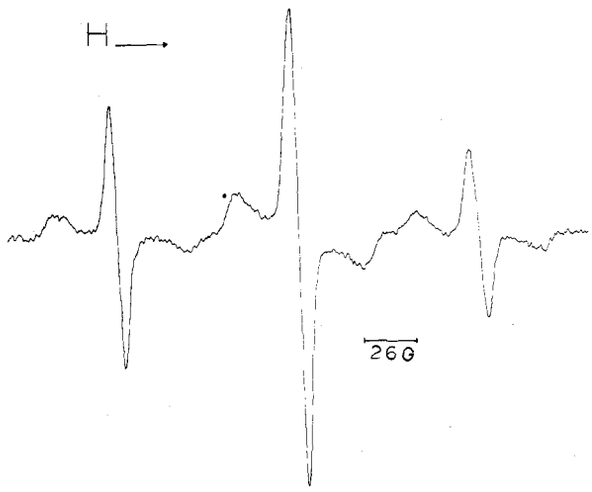


Figure 5. ESR spectrum of the H_2CN radical in 12 *M* LiCl (H_2O) at 110°K.

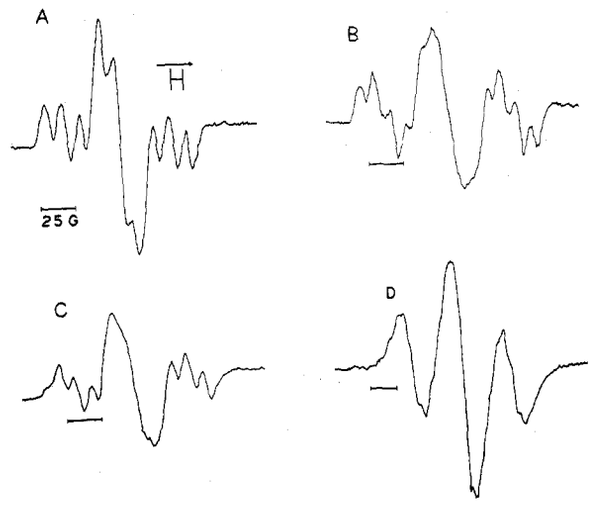
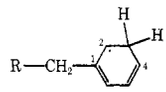
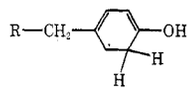
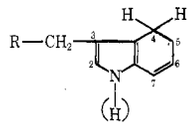
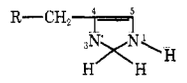


Figure 6. ESR spectra of carbon protonated radicals formed after electron attachment to aromatic amino acids: (A) tryptophan radical spectrum at 160°K; (B) phenylalanine radical spectrum at 160°K; (C) tyrosine radical spectrum at 160°; (D) histidine radical spectrum at 77°K.

Aromatic Amino Acids. Electron attachment reactions with four aromatic amino acids were investigated. The ESR spectra immediately after electron attachment in alkaline and neutral glasses showed spectra indicative of protonation in all cases (except histidine in NaOH). The ESR spectra of the protonated anions of these amino acids (Figure 6) consist of a large triplet of 38–48 G between the components which are further split. The large splittings are due to the methylene protons produced by protonation on the aromatic ring. The further splittings are due to ring proton splittings. The assignment of specific sites of protonation from these results is not possible without recourse to theory. This is treated later. Table II gives the results of the analysis of the ESR spectra in both alkaline and neutral

TABLE II: Radicals Formed by Electron Attachment to Aromatic Amino Acids and Their Analogs

Compound	Suggested major radical[a]	Matrix	Hyperfine splittings, G: Methylene	Hyperfine splittings, G: Ring protons	Temp, °K
Phenylalanine		NaOH	48(2 H)	11(3 H)	77
		LiCl	47(2 H)	11(3 H)	160
Tyrosine		NaOH	42(2 H)	12(2? H)[b]	77
		LiCl	40(2 H)	12(2? H)	77
Tryptophan		NaOH	38(2 H)	12(2 H)	165
		LiCl	39(2 H)	12(2 H)	165
Histidine		LiCl	47(2 H)	7 (even no H)	77
Imidazole	*c*	LiCl	47(2 H)		77
Phenylacetic acid	*d*	NaOH	46(2 H)	11(? H)[b]	170
Phenol	*e*	NaOH	42(2 H)	11(2 H)	77

[a] R = $-CH(NH_3^+)CO_2^-$ in 12 *M* LiCl and $-CH(NH_2)CO_2^-$ in 8 *N* NaOH. [b] The ESR spectrum for this radical suggests more than one protonated radical is present. [c] This structure is analogous to that found for histidine. [d] This structure is analogous to that found for phenylalanine. [e] This structure is analogous to that found for tyrosine.

glasses. Below we discuss the extent of protonation in the various glasses.

For phenylalanine and histidine protonation in 12 *M* LiCl was nearly complete immediately after electron attachment, i.e., photolysis and photobleaching. Partial protonation was noted for tyrosine and tryptophan at this point. Upon warming to 165°K the ESR spectra (Figure 6) were essentially that of the protonated radicals. This is considered to be due to the loss of the other less stable radicals formed, i.e., anions and possible deaminated species, upon warming. The conversion of anion to protonated radical upon warming could not be proven owing to (1) an overall loss in signal intensity and (2) attack of hydrogen atoms which are produced by electron reaction with the matrix and mobilized on warming.

The results for histidine in NaOH showed no protonation. Only a weak singlet ESR spectrum indicative of the anion was found. All other aromatic amino acids showed partial protonation at 77°K in NaOH. The phenylalanine anion again protonated to the greatest extent. The fact that tryptophan, tyrosine, and phenylalanine protonate under both neutral and alkaline conditions is good evidence that the anion is the immediate precursor of the protonated radicals. For histidine the p*K* of the methylene proton in the protonated species could be such so as to be protonated in the LiCl glass but not in the NaOH glass. Alternatively, the anion of histidine in the neutral glass could react to form hydrogen atoms which then would attack the original or neighboring molecules. Further experiments utilizing different techniques will be necessary to distinguish between these hypothesis.

In previous investigations of amino acids with aliphatic side chains in aqueous glasses we found that in a neutral glass deamination of the zwitterion readily occurs at 77°K.[11,20] The results of this investigation suggest that electron attachment and protonation at carbon sites in aqueous media can effectively compete with this deamination step in aromatic amino acids. Recent pulse radiolysis results of Mittal and Hayon confirm this conclusion.[21] Their results suggest that electron reaction with phenylalanine yields 60% of the ring protonated radical and 40% deaminated radical.

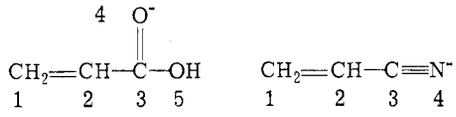
Analogs of the Aromatic Amino Acids. Since the aromatic ring is a site of attack in aromatic amino acids, several compounds which contain only the aromatic ring were investigated. The results of electron attachment to imidazole, phenol, and phenylacetic acid are reported in Table II. The extent of protonation was only partial for phenol and phenylacetic acid. Since warming did not induce more protonation, the protonation of the anion may have been induced by light during photolysis or photobleaching. Imidazole anion completely protonated in LiCl but showed no protonation in NaOH. As would be expected from the similar structures, the results for the analogs are nearly identical with those found for the aromatic amino acids. Experiments with indole, the analog of tryptophan, were unsuccessful due to its very low solubility in 8 *N* NaOH or 12 *M* LiCl.

Lamotte and Gloux have investigated γ-irradiated imidazole.[22] They identify the stable radical at room temperature as the same as that found here. They report the two methylene proton splittings differ in magnitude by 5 G, but their average value is 47 G as is found here.

Assignment of Sites of Protonation. MO Calculations and Further Experiments. Although the sites of protonation are unequivocally determined by analysis of the ESR spectra for the radicals from the localized bond compounds, in the case of the aromatic amino acids more information is needed. McLachlan MO calculations have been performed to aid in the assignment of the site of protona-

TABLE III: McLachan Spin Density (ρ) and HMO Free Valency (F) Calculations for Anion Radicals

Position	Acrylic Acid		Acrylonitrile		Phenylalanine		Histidine		Tryptophan	
	ρ	F	ρ	F	ρ	F	ρ	F	ρ	F
1	0.55	1.12[a]	0.54	1.15[a]	−0.07	0.40	0.14		0.03	
2	0.25	0.58	0.37	0.68	0.29	0.65[c]	0.60	1.04[b]	0.29	0.74[c]
3	0.10	0.23	0.02	0.46	0.29	0.65[b]	0.21		0.04	0.47
4	0.09		0.08		0.07	0.40	0.10	0.42	0.28	0.63[b]
5	0.01						0.15	0.66[c]	0.04	0.43
6									0.17	0.53
7									0.21	0.55[g]
8									0.04	0.18
9									0.08	0.18[d,f]



d, e

d

[a] This site is unequivocally determined by experiment to be the site of protonation. For acrylic acid ref 2, 5, and 6 have shown this as well. [b] Comparison of spin densities calculated for the protonated radicals with experiment suggest these sites are the likely sites of protonation. [c] A possible site which is somewhat less likely as the protonation site. Sites b and c could not be distinguished in our spectra. [d] See Table II for the numbering scheme. [e] The MO calculations for tyrosine gave very similar results to those of phenylalanine. [f] The MO calculations for tryptophan are for the structure with the nitrogen at position 1 protonated. [g] Possible protonation site from MO calculations which experiments with 7-methyltryptophan show to be unlikely as the site of protonation in tryptophan.

tion as well as to gain an understanding of the protonation process. Parameters have been employed which have been successful in predicting spin densities in other heterocyclic ion radicals.[11,23] Parameters for the ring methylene groups were those of Levy.[24]

Calculations were performed for all the possible protonated radicals of each aromatic amino acid. Those sites labeled b in Table III are those considered most likely to be sites of protonation from a comparison of experiment with the theoretical calculation. Those labeled c are possible sites which cannot be eliminated by the calculation alone. For phenylalanine and tyrosine these additional sites arise because the methylene protons in the amino acid side chain could orient so as to produce a single proton splitting of about the proper splitting. The ESR spectra could consist of both the radicals at sites b and c since they could not be distinguished experimentally.

The MO calculations for tryptophan suggest three possible sites of protonation (2, 4, 7). To further aid in this assignment experiments were performed with 4-methyl- and 7-methyltryptophan in 8 N NaOH. Analysis of the ESR spectrum for 7-methyltryptophan gave two protons at 38 G split by four protons at 13 G. Since the splitting due to four protons indicates coupling to the methyl group at position 7, the site of protonation must be position 4. This is the only protonation site which produces the required spin density at position 7 after protonation. The spectrum for 4-methyltryptophan showed less protonation. However, the spectrum did show an overall triplet of ca. 40 G. This indicates protonation at one of the other sites (2 or 7) and suggests that methyl substitution can shift the site of protonation. Therefore these experiments cannot conclusively show the site of protonation in tryptophan itself. However, the results suggest that position 4 is the major site of protonation.

MO calculations for the precursors, the anions, were also performed. In Table III we report the spin density and free valency distributions for the anions of the aromatic amino acids as well as those of acrylic acid and acetonitrile.

For acrylic acid and acrylonitrile the site of protonation is the carbon site of highest spin density and free valency. This appears to hold true for the aromatic amino acids as well. The sites which are the likely sites of protonation are also the sites of high spin density and free valency. Our calculations of the charge density showed that this parameter did not appear to be as good an indicator as the free valency or spin density.

It would be of interest to be able to compare our assignments of the site of protonation to those found from less ambiguous methods such as ESR of single crystals. However, studies of γ-irradiated single crystals of aromatic amino acids[25,27] have reported H-addition radicals only in the case of histidine[25] and its analog imidazole.[22] In these cases, however, the H addition occurs at the position predicted by the free valency calculation. Liming and Gordy report radicals analogous to those found here in γ-irradiated polymeric aromatic amino acids.[28] They interpret their results in a similar manner to this work; however, they consider attack of hydrogen atoms instead of protonation of anions. Our results as well as those of Mittal and Hayon[21] suggest that protonation is the mechanism at least in aqueous media.

Conclusions

The results for the compounds in Table I show that the anions of compounds with structure $R_1R_2C{=}CHCO_2^-$ are stabilized when R_1 is a carboxyl or a methyl group. The stabilization of the carboxylic group was also found by Neta and Fessenden.[2,29] They attributed this to further delocalization of the electron over the carboxylic group. They considered the protonation to be related to localized double bond structure. However, we have shown that even aromatic ring structures can protonate, if the free valency is appropriately large. The stabilization caused by a methyl group is likely due to steric effects. The methyl group most likely tends to protect the carbon site from the approach of water. This same effect of methyl substitution has been observed by Hayon et al. in pulse radiolysis study of substituted acrylic acids as well.[4] The fact that the protonation reactions occur as readily in NaOH as in LiCl shows the

reaction is with H_2O not H_3O^+. This conclusion is also in agreement with previous work.[2]

Recent work by Fessenden and Chawla has shown that the acrylate dianion protonates at the β carbon more readily than the acrylic acid anion.[2b] Neither anion was stable at low temperature in our results, thus we were unable to observe this effect.

The experimental results for the aromatic amino acids show that some protonation on the ring structure occurs in each case. Examination of the free valency calculations for the anions of aromatic amino acids and other compounds indicate that protonation occurs at sites with high values of the free valency (about 0.63 or greater). The three anions which showed no carbon protonation in our studies, i.e., the anions of fumaric, benzoic, and muconic acids, have free valencies equal to or under 0.60.

As we mentioned initially, the anion of the DNA base thymine has been found to protonate in an alkaline glass at a carbon site (position 6). The free valency at this position is 0.77. The anions of the purine DNA bases, which have less of a tendency to protonate, have free valencies under 0.65.[30] However, cytosine and uracil which have similar free valencies to thymine have not been found to protonate.

Taken all together the facts suggest that the free valency is indicative but is not alone sufficient to predict whether or not rapid protonation at carbon sites will occur (for example, steric factors must be considered). Free valency calculations, however, seem able to predict the likely site or sites of protonation if the reaction does occur.

Acknowledgment. Acknowledgment is made to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. This research was also supported in part by the U.S. Atomic Energy Commission.

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(1) This research was supported by the U.S. Atomic Energy Commission and by the Petroleum Research Fund.
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