Electron Spin Resonance Study of Liquids during Photolysis. 21. Dipeptides[1,2]

David G. Doherty, Ralph Livingston,* and Henry Zeldes

Contribution from the Biology Division and Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830. *Received April 2, 1976*

Abstract: Aqueous solutions of dipeptides containing about 1% hydrogen peroxide have been photolyzed at room temperature and the resulting radicals studied by electron spin resonance. The peroxide gives ȮH which abstracts hydrogen from the peptide. Radicals are formed from L-prolylglycine and β-alanyl-L-proline by abstraction of hydrogen from the carbon located between the peptide nitrogen and carboxylate group. In the latter case the spectrum is affected by the presence of cis–trans isomers and dynamic phenomena. In both β-alanyl-L-valine and glycyl-L-valine the abstraction is almost entirely from the isopropyl side chain giving a mixture of radicals corresponding to loss of one or the other kind of hydrogen in the isopropyl group. Hydrogen is abstracted from the methyl group of β-alanylsarcosine to give a mixture of cis and trans forms of the radical. Glycyl-L-aspartate and β-alanyl-L-aspartate each give a mixture of two radicals. One of these is a direct result of hydrogen abstraction from the side chain while the other involves chemical modification of the side chain. Hyperfine couplings and g values have been measured and are discussed.

The most frequently reported radicals formed by ȮH attack on compounds containing a single peptide unit have been those resulting from abstraction of hydrogen from the carbon adjacent to the peptide nitrogen. A variety of studies using different methods have illustrated this behavior. Examples include the work of Garrison[3] and coworkers on the analysis of chemical products resulting from the γ radiolysis of aqueous solutions of simple peptides. A variety of compounds containing the peptide unit have also been studied by electron spin resonance (ESR). Typical is the formation of $CH_3CONH\dot{C}HCOOH$ from *N*-acetylglycine through ȮH attack.[4,5] There have also been extensive pulse radiolytic studies[6,7] of simple peptides in aqueous solution where optical absorption is used as the analytical method. The radicals formed by reaction with ȮH which give rise to the optical absorption have frequently been those produced by the above mode of reaction. In our earlier work[8] we studied the reaction of ȮH with dipeptides containing the simplest amino acids: glycine, L-alanine, and β-alanine. The peptides were in aqueous solutions containing a small amount, about 1%, of hydrogen peroxide which is a source of ȮH upon photolysis. The radicals are short lived, probably of the order of 1 ms, and were observed at steady-state concentration during continuous photolysis. All of the peptides reacted in the above manner with the modification that those containing β-alanine as the C-terminal residue gave a mixture of two radicals corresponding to hydrogen abstraction from both carbons located between the peptide nitrogen and the carboxylate group. We have now extended the previous study to dipeptides containing more complex amino acids. Included are amino acids with side chains which are susceptible regions for ȮH attack. As before, the peptides were synthesized by one of the authors (D.G.D.) and were studied as zwitterions in aqueous solution near room temperature.

Although reaction rate constants for the reaction of ȮH with some of the peptides reported here and for most of the amino acid constituents have been reported from pulse radiolytic studies,[7,9] the rate constants associated with specific sites in these substances generally have not been evaluated. Of special significance, however, was the analysis of chemical products formed in the γ radiolysis of aqueous solutions of amino acids which showed[3] that amino acids having progressively longer side chains showed progressively greater attack at the side chain. An ESR study[4] of valine has shown radicals from attack at the side chain. Although the behavior of the amino acids will be modified in the peptide, seeing radicals from side chain attack should not be surprising in the present work. The above considerations relate to the distribution of radicals formed. In these ESR experiments the relative strengths of spectra, apart from the usual considerations of line widths and multiplicities, will also depend on the radical lifetimes which have not yet been measured.

Experimental Section

The experimental arrangement was the same as used in the earlier study of peptides.[8] The solutions, typically a few grams of the peptide in 25 ml of water and containing 1% H_2O_2, were freed of dissolved oxygen by purging with helium and were photolyzed near room temperature as they slowly flowed through a flat silica cell positioned in the microwave cavity of the spectrometer. The uv source was a high-pressure mercury arc, Philips Type SP500W. The solutions were recirculated with a peristaltic pump and only contacted glass, silica, and Teflon. Estimated error limits of hyperfine couplings and g values are ±0.03 G and ±0.00004, respectively, unless otherwise stated.

Peptide synthesis was accomplished by activating the carbobenzoxy amino acid with either dicyclohexylcarbodiimide or carbonyldiimidazole and coupling it to the appropriate ester in methylene chloride, deesterifying the blocked peptide, and removing the carbobenzoxy group by catalytic hydrogenation as previously reported.[8] Where benzyl esters were used both groups were simultaneously removed in the hydrogenation process to yield the free peptide. Three new derivatives and two new peptides were isolated and analyzed and the structure was confirmed by NMR.[10] We were unable to confirm the rotation of $[\alpha]^{22}D$ −30.2° (*c* 0.5, H_2O) for β-alanyl-L-valine reported by Corbellini et al.,[11] although in addition to the above, we prepared the peptide by coupling carbobenzoxy-β-alanine via the acid chloride and azide to L-valine and L-valine methyl and benzyl esters, obtaining identical compounds in all instances. Analytical values are in Table I and for the new compounds are as follows.

***N*-Carbobenzoxy-β-alanyl-L-aspartic Acid Dibenzyl Ester:** yield 85%; mp 113–115 °C; $[\alpha]^{22}D$ +8.5° (*c* 2, HOAc). Anal. Calcd for $C_{28}H_{28}N_2O_7$ (504.52): C, 66.65; H, 5.59; N, 5.55. Found: C, 66.52; H, 5.65; N, 5.45.

β-Alanyl-L-aspartic Acid: yield 83%; mp 224–225 °C; $[\alpha]^{22}D$ +9° (*c* 2, H_2O). Anal. Calcd for $C_6H_{10}N_2O_5$ (190.16): C, 37.89; H, 5.30; N, 14.73. Found: C, 37.75; H, 5.42; N, 14.65.

β-Alanylsarcosine: overall yield 62%; mp 216–217 °C. Anal. Calcd for $C_6H_{12}N_2O_3$ (160.18): C, 44.99; H, 7.55; N, 17.49. Found: C, 44.80; H, 7.62; N, 17.40.

***N*-Carbobenzoxy-β-alanyl-L-valine Benzyl Ester:** yield 76%; mp 99–101 °C; $[\alpha]^{22}D$ −19.4° (*c* 2, EtOH). Anal. Calcd for $C_{23}H_{28}N_2O_5$ (412.47): C, 66.97; H, 6.84; N, 6.79. Found: C, 66.90; H, 6.95; N, 6.67.

***N*-Carbobenzoxy-β-alanyl-L-valine:** yield 75%; mp 138–140 °C; $[\alpha]^{22}D$ −23° (*c* 2, 0.5 N NaOH). Anal. Calcd for $C_{16}H_{22}N_2O_5$ (322.36): C, 59.61; H, 6.88; N, 8.69. Found: C, 59.70; H, 6.98; N, 8.59.

β-Alanyl-L-valine: yield 65%; mp 265–267 °C; $[\alpha]^{22}D$ −18.1° (*c*

Table 1. Physical Constants of Peptides and Derivatives

		Carbobenzoxy peptide ester		Carbobenzoxy peptide		Peptide	
		Mp, °C[a]	$[\alpha]^{22}D$, deg	Mp, °C	$[\alpha]^{22}D$, deg	Mp, °C	$[\alpha]^{22}D$, deg
Gly-L-Asp	Dibenzyl	86–87[c]	+9.5[d] (*c* 2, HOAc)			209–210	+12.5[e] (*c* 2, H_2O)
Gly-L-Val	Methyl	76–77[f]	−15.5 (*c* 2, EtOH)	Oil			−20.2[g] (*c* 2, H_2O)
L-Pro-Gly	Methyl	Oil		123–125[h]	−63 (*c* 2, MeOH)	239–240[i]	−20.5[i] (*c* 2, H_2O)
β-Ala-L-Asp	Dibenzyl	113–115[b]	+8.5 (*c* 2, HOAc)			224–225[b]	+9 (*c* 2, H_2O)
β-Ala-L-Pro	Methyl	Oil		94–95[j]	−45.1 (*c* 2, EtOH)	216–218[k]	−106[k] (*c* 1, H_2O)
β-Ala-L-Sar	Ethyl	Oil		Oil		216–217[b]	
β-Ala-L-Val	Methyl	76–77[l]	−14.5 (*c* 2, EtOH)	138–140	−23[b] (*c* 2, 0.5 N NaOH)	265–267	−18.1[m] (*c* 2, H_2O)
	Benzyl	99–101[b]	−19.4 (*c* 2, EtOH)				

[a] All melting points were taken in capillary tubes, uncorrected. [b] New compound. [c] A. Miller, A. Neidle, and H. Waelsch [*Arch. Biochem. Biophys.*, **56**, 11 (1955)] report mp 85–86 °C. [d] M. Lieflander [*Z. Physiol. Chem.*, **320**, 48 (1960)] reports $[\alpha]^{23}D$ +9.2° (*c* 2, HOAc). [e] Footnote *c*, $[\alpha]^{25}D$ +12° (*c* 5, H_2O). [f] P. G. Katsoyannis [*J. Am. Chem. Soc.*, **83**, 4053 (1961)] reports mp 78 °C, $[\alpha]^{28}D$ −15.5° (*c* 1.5, EtOH). [g] K. R. Rao, S. M. Birnbaum, R. B. Kingsley, and J. P. Greenstein report $[\alpha]^{25}D$ −19.7°; R. Camble, R. Garner, and G. T. Young [*J. Chem. Soc. C*, 1911 (1969)] report $[\alpha]^{20}D$ −19.7° (*c* 2, H_2O). [h] W. Grassman and E. Wünsch [*Chem. Ber.*, **91**, 449 (1958)] report mp 122–123 °C, $[\alpha]^{21}D$ −63.2° (*c* 5, MeOH); G. W. Anderson, J. E. Zimmerman, and F. M. Callahan [*J. Am. Chem. Soc.*, **86**, 1839 (1964)] report mp 124–125 °C. [i] H. N. Rydon and P. W. G. Smith [*J. Chem. Soc.*, 3642 (1956)] report mp 236 °C, $[\alpha]^{17}D$ −19.8° (*c* 4, H_2O); footnote *h*, $[\alpha]^{22}D$ −22.5° (*c* 2, H_2O). [j] H. T. Hanson and E. L. Smith [*J. Biol. Chem.*, **175**, 833 (1948)] report mp 91–93 °C. [k] Footnote *j*, mp 211 °C, $[\alpha]^{26}D$ −93.3° (*c* 1.5, H_2O). [l] K. Lloyd and G. T. Young [*J. Chem. Soc. C*, 2890 (1971)] report mp 62–65 °C, $[\alpha]^{20}D$ +5° (*c* 1, DMF). [m] Reference 5, mp 270 °C, $[\alpha]^{21}D$ −30.2° (*c* 0.5, H_2O).

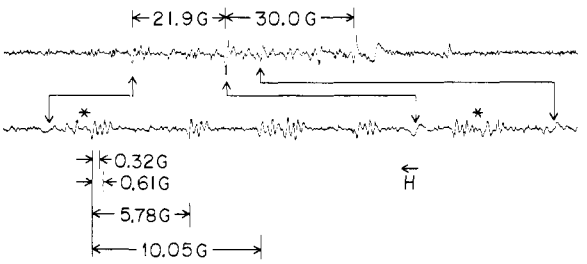

Figure 1. The spectrum from 3.3 g of β-alanyl-L-valine in 30 ml of water containing 1% H_2O_2 (pH 6.12). Top: Essentially the entire spectrum at low resolution with line spacings indicated that correspond to hyperfine couplings for one radical. Bottom: A portion of the spectrum at high resolution with line spacings indicated that correspond to parameters for a second radical. The region between the asterisks contains all of the components of the higher field 15-strength group; see text.

2, H_2O). Anal. Calcd for $C_8H_{16}N_2O_3$ (188.22): C, 51.05; H, 8.57; N, 14.88. Found: C, 51.11; H, 8.52; N, 14.76.

Results and Discussion

β-Alanyl-L-valine. The spectrum from β-alanyl-L-valine arises from a mixture of radicals with practically all of the lines at steady state coming from the two radicals formed by abstracting one or the other kind of hydrogen located on the isopropyl group. The spectrum from 3.3 g of the peptide in 30 ml of water containing 1% H_2O_2 (pH 6.12) is shown in Figure 1. The radical formed by abstraction of hydrogen from a CH_3 group gives a spectrum of six relatively broad lines some of which are indicated by connecting arrows in Figure 1. Parameters measured for this radical are given in Figure 2. For this set of measurements 2.35 g of the peptide was in 25 ml of water containing 1% H_2O_2 (pH 6.19). The bottom part of Figure 1 contains mostly lines from the radical formed by abstraction of the unique hydrogen of the isopropyl group. It is only a portion of the entire spectrum. There are six equivalent hydrogens from two methyl groups which give groups of lines having nominal intensities of 1-6-15-20-15-6-1. Most measurements were made on the two 15-strength groups of lines. The higher field group is illustrated in Figure 1 (bottom). The lines are very sharp and second-order splittings are resolved.[12] Every 15-strength line is split into three components of relative strengths 1-5-9. The 1-strength components are too weak to be seen in Figure 1. The smallest measured splitting of 0.32 G

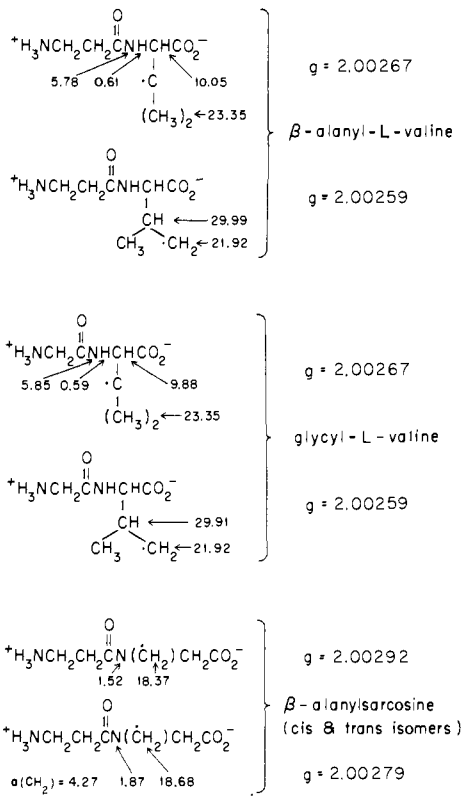

Figure 2. Formulas of radicals studied in photolyzed aqueous solutions containing the indicated dipeptide and hydrogen peroxide. Numbers with arrows are hyperfine coupling constants in gauss for the indicated nuclei.

is the spacing between the 5- and 9-strength components and agrees exactly with the computed second-order splitting.[12] The *g* value and hyperfine couplings for this radical are given in Figure 2. The measurements were made on the same solution used for Figure 1.

There were several additional lines present in the spectrum coming from neither of the above radicals. They were too weak for analysis, and their origin is unknown. They might have come from the radical formed by abstracting hydrogen from the carbon next to the peptide nitrogen, this being the radical

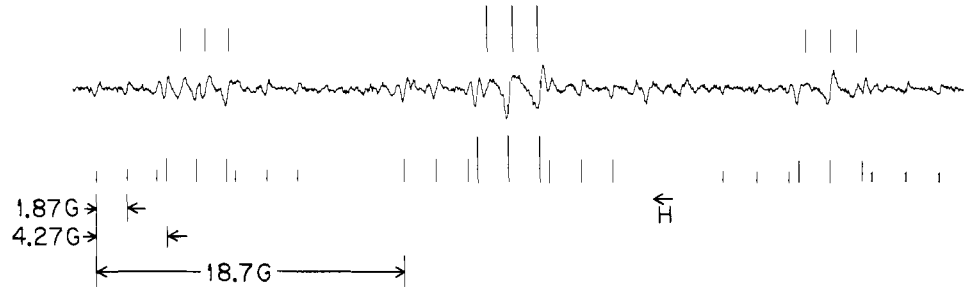

Figure 3. The spectrum from 1.8 g of β-alanylsarcosine in 25 ml of water containing 1% H_2O_2 (pH 5.20). Stick spectra indicate the locations of lines from two radicals that are cis and trans isomers.

expected if the behavior had been the same as found in the peptides studied earlier.[8]

Glycyl-L-valine. The appearance of the spectrum from glycyl-L-valine was practically identical with that from β-alanyl-L-valine illustrated in Figure 1, and the measured parameters given in Figure 2 show very little change. The measurements were made from solutions containing 2 g and also 3 g of the peptide in 25 ml of water containing 1% H_2O_2 (pH 5.60).

β-Alanylsarcosine. The spectrum from 1.8 g of β-alanylsarcosine in 25 ml of water containing 1% H_2O_2 (pH 5.20) is shown in Figure 3. The stick spectra indicate positions of lines that arise from a pair of radicals that are cis and trans isomers with parameters that are given in Figure 2. The stick pattern beneath the spectrum (Figure 3) includes splittings of 4.27 G that arise from a pair of equivalent hydrogens in a CH_2 group. There is ambiguity as to which CH_2 group of the peptide gives this splitting. The line width (full width between extremes of the derivative) for this radical is about 0.24 G. The line width for the other radical (stick pattern above the spectrum in Figure 3) is about 0.46 G. A small CH_2 coupling was not observed in the latter case. Moreover, the accuracies with which the parameters are known for this radical are substantially poorer than our usually quoted accuracy of ±0.03 G for hyperfine couplings. Besides the lines of the isomeric pair of radicals, there are a number of additional lines in the spectrum of unknown origin.

L-Prolylglycine. Figure 4 shows spectra obtained from 2.3 g of L-prolylglycine in 25 ml of H_2O containing 1% H_2O_2 (pH 5.83) and from 2.2 g of the peptide in 25 ml of D_2O containing 1% H_2O_2 (added as 98% H_2O_2). It is quite clear from the couplings that hydrogen is abstracted from carbon adjacent to the peptide nitrogen which is the behavior systematically observed in the simple peptides described earlier.[8] The assignment of couplings is given in Figure 5. The lines of Figure 4 are quite broad, 0.30 G in H_2O solution and 0.47 G in D_2O solution. The increase in width in going to D_2O is quite understandable. The 0.91-G coupling of the peptide hydrogen in H_2O solution is replaced by a much smaller deuterium coupling (calculated to be 0.14 G) which is unresolved in D_2O solution but which contributes to line width. The largest coupling (the α-hydrogen) and the g value are known with the estimated accuracy stated earlier, but the accuracy for the smallest couplings is somewhat poorer. In H_2O, for example, the lack of resolution left no choice but to take one hydrogen coupling to be equal to the nitrogen coupling and a second hydrogen coupling to be twice this value. The interpretation of the spectrum and assignment of couplings are corroborated by the measurements in D_2O. The small shift in the coupling for the α hydrogen (Figure 5) is typical of that previously seen in going from H_2O to D_2O as a solvent.[8]

β-Alanyl-L-proline. The spectrum from β-alanyl-L-proline, Figure 6, consists essentially of two widely spaced groups of hyperfine components. The appearance of the spectrum is

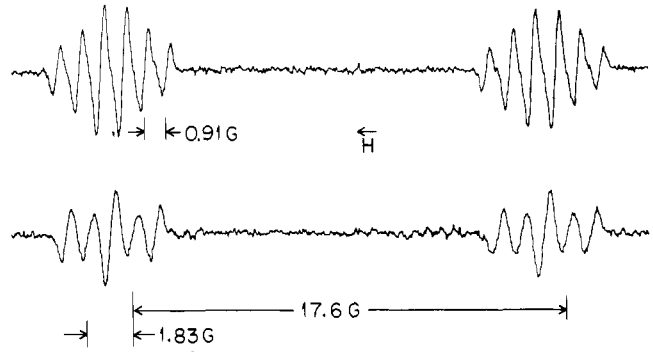

Figure 4. The spectrum (top) from 2.3 g of L-prolylglycine in 25 ml of H_2O containing 1% H_2O_2 (pH 5.83) and (bottom) from 2.2 g of the peptide in 25 ml of D_2O containing 1% H_2O_2.

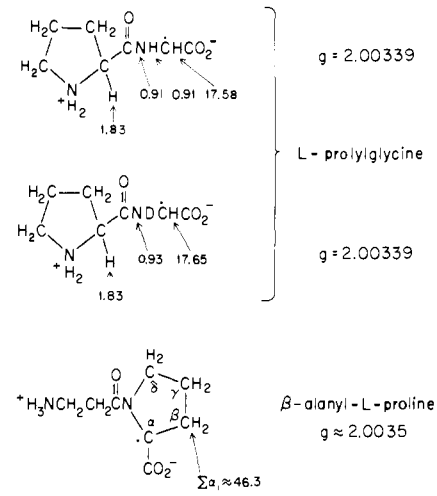

Figure 5. Formulas of radicals studied in photolyzed aqueous solutions containing the indicated dipeptide and hydrogen peroxide. Results for L-prolylglycine are given for H_2O (top) and D_2O solutions. Numbers with arrows are hyperfine coupling constants in gauss for the indicated nuclei.

unchanged using D_2O as a solvent. On close examination the two groups of lines are found not to be similar; there are differences in the number of components and their spacing. We interpret the spectrum as arising from primarily one radical which is present as geometrical isomers, the isomeric forms having slightly different g values and values for the various hyperfine coupling constants. As pointed out in later discussion, the presence of cis-trans isomers is not unexpected for peptides containing proline. The closely spaced components (about 0.5-G spacing) within a group of lines arise from weak hyperfine couplings of hydrogens and also, likely, nitrogen with there being a superposition of groups from the isomers which causes the two groups to be dissimilar. The spacing between

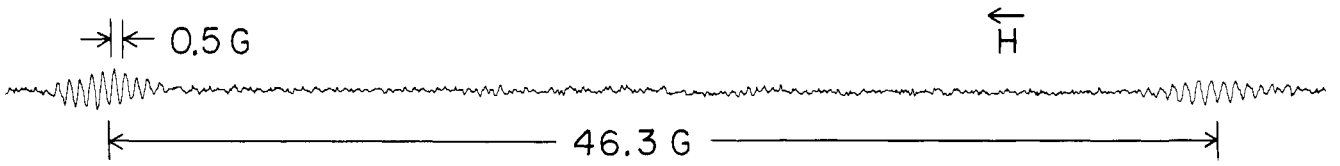

Figure 6. The spectrum from 2.0 g of β-alanyl-L-proline in 25 ml of water containing 1% H_2O_2 (pH 6.23).

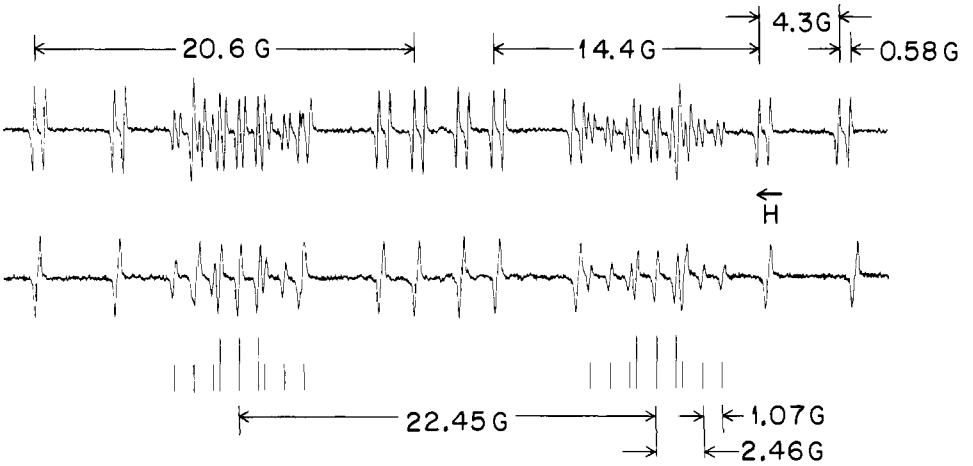

Figure 7. Spectra from aqueous solutions of the monolithium salt of β-alanyl-L-aspartic acid containing 1% H_2O_2. Top: 1.6 g of the peptide in 50 ml of H_2O titrated to a pH of 6.82 with 1 N LiOH. Bottom: 1.0 g of the peptide in 30 ml of D_2O titrated to a pH of 6.60 with 1 N LiOH (in D_2O).

the two groups of lines is 46.3 G. There is some uncertainty in this value because it is difficult to decide on what to take as corresponding points in the two groups, but this uncertainty is considerably less than 1 G. This splitting is larger than can arise from a single hydrogen in a π radical. The only reasonable interpretation we have found for this radical is that given in Figure 5. Hydrogen is abstracted from a carbon located between the peptide nitrogen and the carboxylate group, the identical point of attack found in the simplest peptides.[8] The splitting of 46.3 G is the sum of the coupling constants for the two β hydrogens. More centrally located groups of lines are not seen because of dynamic phenomena as discussed more fully later. The average coupling constant per β-hydrogen is about 23 G which is a very reasonable value. An approximate g value may be calculated. The value is 2.00355 without second-order corrections.[12] There will be a large correction from the β coupling constants; if the β-hydrogens are regarded as equivalent the corrected g value is 2.00350. In either case, the g value is reasonably close to that found for the simplest dipeptides[8] and for L-prolylglycine (Figure 5) which shows a similar site of hydrogen abstraction.

β-Alanyl-L-aspartate. The spectrum from aqueous β-alanyl-L-aspartic acid containing 1% H_2O_2 (1.6 g in 50 ml; pH 3.50) consists of clusters of moderately broad lines that are only partially resolved. An analysis was not made. Upon titrating to a pH in the vicinity of 6.5 (1 N LiOH), which gives the monolithium aspartate, the spectrum is greatly modified and consists of families of quite well-resolved sharp lines. Figure 7 shows spectra obtained in H_2O and D_2O. The top spectrum was obtained from 1.6 g of β-alanyl-L-aspartic acid in 50 ml of H_2O which was titrated to a pH of 6.82 with 1 N LiOH and to which was added 1% H_2O_2. In the bottom spectrum 1.0 g of the peptide was in 30 ml of D_2O which was adjusted to a pH of 6.60 with 1 N LiOH (in D_2O) and to which was added 1% H_2O_2 (as 98% H_2O_2). Two radicals are present. The stick spectrum at the bottom of the figure indicates the positions of the lines for one radical in D_2O solution. All lines in D_2O solution appear as closely spaced doublets in H_2O. Parameters for both radicals were measured with the solutions used for Figure 7, and these are given in Figure 8. The spectrum was also examined at a pH of 8.63. The appearance remained the same with the relative intensities of the two radicals unchanged. The identification of the radical formed by abstracting hydrogen from the aspartate side chain, **1**, is straightforward, but there is ambiguity in which way around to assign the two largest couplings. The choice given will be justified in later discussion. The assignment of the coupling of 0.58 G to the hydrogen on the peptide nitrogen is corroborated by the results in D_2O solution where doublets having this separation collapse to single lines (Figure 7). The computed deuterium coupling constant is 0.09 G which is unresolved with the spectrometer settings used to record Figure 7. The identity of the second radical, **2**, which is represented in D_2O solution by the stick spectrum of Figure 7 is not completely known. We have considerable confidence in the features of the structures and assignment of couplings given in Figure 8 which comes in part from a comparison of results with glycyl-L-aspartate. The assignment of the coupling of 0.32 G to the hydrogen on the peptide nitrogen is corroborated by the results in D_2O (Figure 7). The computed deuterium coupling constant is 0.05 G which is too small to be resolved. All observed hyperfine splittings are accounted for as shown in **2** of Figure 8; the group R cannot contain a hydrogen in a position β to the region of high spin density and is probably the original carboxylate of the peptide.

Glycyl-L-aspartate. The results for glycyl-L-aspartate are very similar to those for β-alanyl-L-aspartate. Spectra are shown in Figure 9 for solutions in H_2O and in D_2O. The solution for the top spectrum was 1.5 g of glycyl-L-aspartic acid in 25 ml of H_2O which was adjusted to a pH of 6.40 with 1 N LiOH and to which was added 1% H_2O_2. The bottom spectrum was with 1.7 g of the peptide in 25 ml of D_2O and with the pH adjusted to 6.60 and with 1% H_2O_2. The stick spectrum locates lines for one of the radicals, **4**. Measured parameters for both radicals are given in Figure 8. As before, the assignment of **3** is straightforward, and a comparison with **1** shows the most noteworthy difference to be the change in the coupling constant for one of the hydrogens from 13.60 G in **3** to 14.36 G in **1**. This

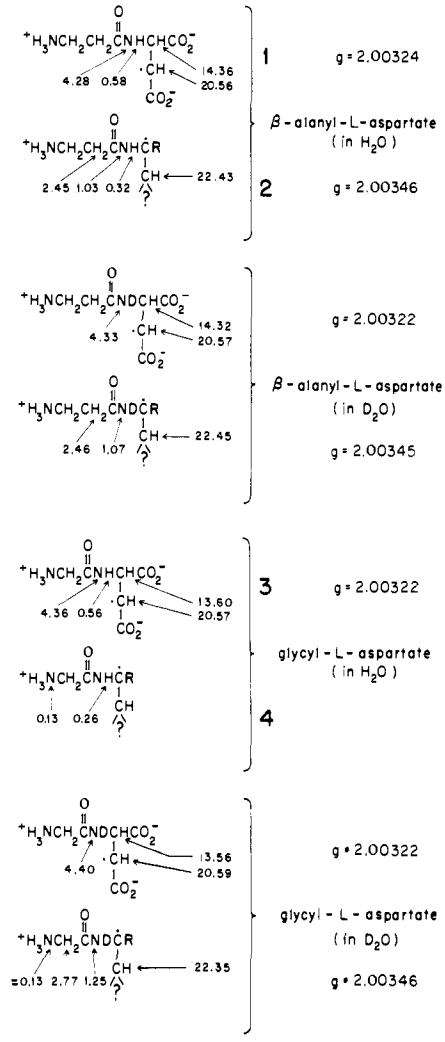

Figure 8. Formulas of radicals studied in photolyzed solutions containing the indicated aspartate and hydrogen peroxide in H_2O and in D_2O solution. Numbers with arrows are hyperfine coupling constants in gauss for the indicated nuclei. Radicals **2** and **4** and the corresponding radicals in D_2O are not completely identified.

will be discussed later. Also, the results in D_2O corroborate the assignment of the 0.56 G coupling in **3** to the hydrogen on the peptide nitrogen. The spectrum for the second radical (indicated by the stick spectrum of Figure 9) is more complex than in β-alanyl-L-aspartate (compare with Figure 7). Extra very small splittings are present, and these come from hyperfine interaction with the terminal nitrogen of the glycine residue. Quantitative measurements were made in D_2O, and the results are given in Figure 8. With higher resolution than was used for Figure 9, the terminal nitrogen coupling which was not completely resolved was estimated as 0.13 G as given in Figure 8. Only the two smallest couplings of **4** were measured in H_2O. The remaining couplings and g value in H_2O should be essentially unchanged from those in D_2O. Again, the identity of **4** is not completely known. The nature of the main peptide chain including the indicated region of high electron spin density is consistent with earlier results[8] on simple peptides. For example, in glycylglycine and glycyl-L-alanine hydrogen is abstracted from the carbon adjacent to the peptide nitrogen leaving this carbon as the region of high spin density which is analogous to the present case. The coupling constants for the terminal nitrogen are 0.11 and 0.14 G, respectively, which are very similar to the value of 0.13 G in **4**. When β-alanine is the first residue, however, resolved couplings are not seen beyond the CH_2 group next to the carbonyl group as illustrated by earlier work[8] on β-alanylglycine and β-alanyl-L-alanine. These cases are analogous to the results for **2** found with β-alanyl-L-aspartate. If hydrogen had simply been abstracted from the carbon next to the peptide nitrogen in the aspartates the side chain would be unaltered from the initial aspartate, and a pair of strong hydrogen couplings would be seen. Instead, only a single strongly coupled hydrogen is present suggesting that the aspartate side chain has been chemically altered. More will be said later on this.

Additional Discussion

The α-coupling constant and g value for the radical from L-prolylglycine (Figure 5), 17.58 G and 2.00339, are virtually the same as those found[8] from glycylglycine, β-alanylglycine, and L-alanylglycine where the respective α-coupling constants are 17.53, 17.39, and 17.57 G and the g values are 2.00341, 2.00339, and 2.00339. The radical from L-prolylglycine should have a spin density of 0.72 on the α-carbon, the same as that on the three other peptide radicals.[8] Although the g values and α-coupling constants are virtually the same, there are variations in the coupling constants for the peptide nitrogen and attached hydrogen. As noted earlier,[8] these coupling constants are small, but generally a high value for one is associated with a low value for the other. L-Prolylglycine fits this trend. In the sequence L-prolylglycine, glycylglycine, and β-alanylglycine the nitrogen coupling for the radicals decreases from 0.91 to 0.70 to 0.56 G while the coupling constant for the attached hydrogen increases from 0.91 to 1.13 to 1.23 G.

In describing the results on the radical from β-alanyl-L-proline the presence of cis and trans isomers was given as the

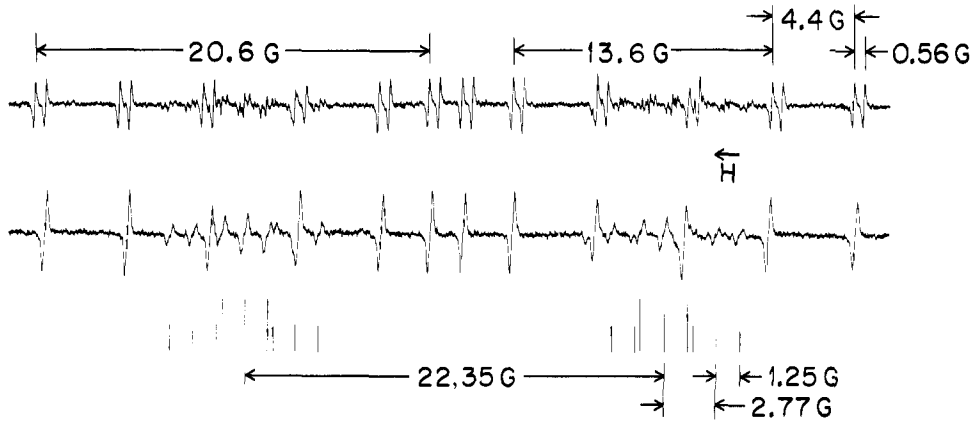

Figure 9. Spectra from aqueous solutions of the monolithium salt of glycyl-L-aspartic acid containing 1% H_2O_2. Top: 1.5 g of the peptide in 25 ml of H_2O titrated to a pH of 6.40 with 1 N LiOH. Bottom: 1.7 g of the peptide in 25 ml of D_2O titrated to a pH of 6.60 with 1 N LiOH (in D_2O).

reason why the two groups of lines in the spectrum (Figure 6) are not identical. Peptides containing proline frequently exhibit cis-trans isomerism. It is known from NMR studies,[13] for example, that glycylproline as the zwitterion in aqueous solution at a pH of 7 is present as an equal mixture of cis and trans forms. Moreover, unpublished NMR results[10] show that β-alanyl-L-proline is also present as cis and trans isomers. Isomers would also be expected for the radical formed by hydrogen abstraction. The approximate parameters given for the radical from β-alanyl-L-proline in Figure 5 should be regarded as an average for the two isomeric forms. It has been pointed out that the spacing of the two groups of lines in Figure 6, 46.3 G, is too large to be accounted for by a hyperfine interaction of a single hydrogen in a π radical. σ radicals may show very large hyperfine interactions, but their g values are usually smaller[14] than that of the free electron, 2.0023. Although the g value of the radical from β-alanyl-L-proline (Figure 5) is approximate, it is clearly very much larger than the free electron value; the radical is almost surely a π radical. The spacing of 46.3 G is interpreted as the sum of the coupling constants for the β-hydrogens indicated in Figure 5. There is an interesting mechanism for the dynamic process that likely causes the central lines of the spectrum for this radical to be unobservable. A number of structural studies[15] have been made on the proline ring in a variety of peptides. The ring atoms C^{β}, C^{α}, N, and C^{δ} (see Figure 5 for nomenclature) are nearly coplanar. C^{γ} in the peptide is displaced from this plane, either up or down with respect to the displacement of the carbon of the carboxylate group, so that the ring exists in one of two puckered conformations. The ring bond angles are essentially the same for the two conformations. Whereas the carbon of the carboxylate group is bent out of the plane of the ring, when the radical is formed by abstracting a hydrogen the hybridization of C^{α} becomes sp^2 with unpaired spin density occupying a π p orbital of this carbon. This means that in the radical the carbon of the carboxylate group should be in the plane of the proline ring, the barrier between the two puckered conformations should be greatly reduced, and it is proposed that C^{γ} rapidly wags back and forth between the two conformations. In a static puckered conformation the hyperfine coupling constants of the two β-hydrogens are assumed to be quite different. The spectrum for a single conformation would consist of the two outermost groups of lines as shown in Figure 6, but in addition there would be two more centrally located groups of lines. In the dynamic case, as C^{γ} wags, the hyperfine coupling constants of the two β-hydrogens essentially switch values, which has the effect of broadening the central groups of lines over a wide range of wagging frequencies but leaves the outermost groups of lines essentially unchanged.

Valine has been studied[4] at a pH of 7 using H_2O_2 and the titanium redox system with a rapid mixing technique. Two radicals are formed as a result of abstraction of the two kinds of hydrogen in the isopropyl side chain. This result is similar to that observed for β-alanyl-L-valine and glycyl-L-valine (Figure 2), and the reported g values and hyperfine couplings for hydrogens in the isopropyl side chain are virtually the same as given in Figure 2. Consider the radical made by abstracting the unique hydrogen of the isopropyl group in each of these peptides (Figure 2). In both cases the methyl hydrogens have a coupling constant of 23.35 G. This value may be used as in earlier work on peptides[8] to compute the spin density on the central carbon. The radical should be planar about the central carbon with sp^2 hybridization and with the unpaired electron in a $2p_z$ orbital of the carbon. The methyl hydrogens are on a β-carbon with respect to the central carbon, and the β coupling constant, a_{β}^{H}, is given approximately by the relation:[16]

$$a_{\beta}^{H} = B_{\beta}^{H}(\cos^2\theta)\rho \qquad (1)$$

where ρ is the electron spin density for the unpaired electron in the $2p_z$ orbital, θ is the azimuthal angle between the axis of the p_z orbital and the bond with which the β-hydrogen is attached to the radical, and B_{β}^{H} is a constant, 58.6 G.[17] In the presence of motion an appropriate average of $\cos^2\theta$ must be taken; it has the value 0.5 for free rotation which is what would be expected for methyl groups. The value of the coupling of the methyl hydrogens with eq 1 leads to a spin density of 0.80 on the central carbon. If there were free rotation about the bond from the side chain to the main peptide chain the same hyperfine coupling would be expected for the hydrogen on the peptide chain. The experimental values, however, are about 10 G (10.05 G for β-alanyl-L-valine and 9.88 G for glycyl-L-valine). This means that the side chain is not freely rotating, and the preferred conformations are ones in which θ is large. Equation 1 (taking the angle to be time independent) gives solutions for θ of 62.5 and 117.5°.

Sarcosine has also been studied[4] by the rapid mixing technique using H_2O_2 and the titanium redox system (pH 10). Hydrogen abstraction takes place from the carbon between the nitrogen and the carboxylate group. Pulse radiolytic studies[6] have been carried out on glycylsarcosine, and the optical absorption has been assigned to a similar radical. In contrast, we find that with β-alanylsarcosine (Figures 2 and 3) the predominant radical observed results from abstraction of a methyl hydrogen to give a radical exhibiting cis-trans isomerism. Cis-trans isomers are known for peptides containing sarcosine. NMR studies[18] of glycylsarcosine in neutral solution show it to be a mixture of cis and trans isomers, and a similar unpublished study[10] on β-alanylsarcosine shows a mixture of isomers.

As pointed out earlier, the identification of **1** and **3** in Figure 8 for the aspartates is straightforward, but there is ambiguity as to which way to take the assignment of the two large coupling constants. A comparison may be made[19] with $\dot{C}H_2CO_2^-$ where the hyperfine coupling for hydrogen is 21.16 G and g = 2.00324. These parameters are very similar to those given in Figure 8, support the assignment of the strong hydrogen coupling, and indicate that there is very high spin density on the carbon of the aspartate side chain. If the aspartate side chain were freely rotating a coupling substantially larger than 20.5 G would be expected for the hydrogen on the main peptide chain. Instead, values of 14.36 G in **1** and 13.60 G in **3** were found which means that the aspartate side chain is not isotropically rotating, and a large value for θ in eq 1 is preferred. The average of $\cos^2\theta$ is somewhat different for the two cases causing the variation from 14.36 to 13.60 G. We would expect these coupling constants to be quite temperature dependent. Aspartic acid at a pH of 8 has been studied[4] using H_2O_2 and the titanium redox system. A similar radical is formed with a g value of 2.0032 and hydrogen hyperfine couplings with assignments consistent with **1** and **3** of 20.84 and 16.71 G. We have pointed out that we are quite confident of the nature of the main peptide chain and assignment of couplings for **2** and **4** in Figure 8, and R is probably the carboxylate group. Neither **2** nor **4** can be the radicals obtained by simply abstracting a hydrogen from the original peptides. Moreover, it appears that they are not the result of ȮH attack on a final, stable, diamagnetic product of photolysis, since the intensities of these radicals relative to **1** and **3**, respectively, did not change from the start of photolysis with fresh solutions to the end of photolysis several hours later even though the absolute intensities decreased considerably indicating substantial depletion of the solutions. One possibility is that **2** and **4** have **1** and **3**, respectively, as precursors.

Acknowledgment. We thank M. A. Turner for preparing starting materials used in the synthesis of the peptides.

References and Notes

(1) Research sponsored by the Energy Research and Development Administration under contract with Union Carbide Corporation.
(2) Part 20: H. Zeldes, R. Livingston, and J. S. Bernstein, *J. Magn. Reson.*, **21**, 9 (1976).
(3) W. M. Garrison, *Radiat. Res. Rev.*, **3**, 305 (1972).
(4) H. Paul and H. Fischer, *Ber. Bunsenges. Phys. Chem.*, **73**, 972 (1969).
(5) P. Neta and R. W. Fessenden, *J. Phys. Chem.*, **75**, 738 (1971).
(6) (a) M. Simic, P. Neta, and E. Hayon, *J. Am. Chem. Soc.*, **92**, 4763 (1970); (b) P. S. Rao and E. Hayon, *J. Phys. Chem.*, **79**, 109 (1975).
(7) G. Scholes, P. Shaw, R. L. Wilson, and M. Ebert, "Pulse Radiolysis", M. Ebert, J. P. Keene, A. J. Swallow, and J. H. Baxendale, Ed., Academic Press, New York and London, 1965, pp 151–170.
(8) R. Livingston, D. G. Doherty, and H. Zeldes, *J. Am. Chem. Soc.*, **97**, 3198 (1975).
(9) L. M. Dorfman and G. E. Adams, *Natl. Stand. Ref. Data Ser., Natl. Bur. Stand.* **No. 46**, 43–50 (1973).
(10) Results personally communicated by B. M. Benjamin.
(11) A. Corbellini, P. Maggioni, and P. G. Pietta, *Gazz. Chim. Ital.*, **97**, 1126 (1967).
(12) R. W. Fessenden, *J. Chem. Phys.*, **37**, 747 (1962).
(13) V. Madison and J. Schellman, *Biopolymers*, **9**, 511 (1970).
(14) T. Yonezawa, I. Noda, and T. Kawamura, *Bull. Chem. Soc. Jpn.*, **42**, 650 (1969).
(15) R. Balasubramanian, A. V. Lakshminarayanan, M. N. Sabesan, G. Tegoni, K. Venkatesan, and G. N. Ramachandran, *Int. J. Protein Res.*, **3**, 25 (1971).
(16) (a) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **32**, 1535 (1960); (b) E. W. Stone and A. H. Maki, *ibid.*, **37**, 1326 (1962).
(17) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).
(18) V. J. Morlino and R. B. Martin, *J. Am. Chem. Soc.*, **89**, 3107 (1967).
(19) R. Livingston, J. K. Dohrmann, and H. Zeldes, *J. Chem. Phys.*, **53**, 2448 (1970).

Strong Acid Chemistry. 4.[1] Direct Reduction of Alkyl Chlorides

Richard H. Schlosberg,* Michael Siskin,* William P. Kocsi, and Frederick J. Parker

Contribution from Exxon Research and Engineering Company, Corporate Research Laboratories, Linden, New Jersey 07036.

Received April 14, 1976

Abstract: The interaction of haloalkanes with such superacids as HF-TaF_5, HCl-$AlCl_3$, and HBr-$AlBr_3$ has been investigated. The outstanding feature of these reactions is the initial rapid conversion of the alkyl halide to the corresponding alkane in good yields via a hydride transfer mechanism. The impact of our results on interpretation of carbocation formation is discussed.

Almost a century ago, Friedel and Crafts[2] reported that "when a small amount of anhydrous aluminum chloride was added to amyl chloride an immediate vigorous evolution of gas was observed in the cold. The gas was composed of hydrogen chloride accompanied by gaseous hydrocarbons not absorbed by bromine." The precise nature of these saturated hydrocarbons has not been well understood. In the course of our continuing studies of hydrocarbon chemistry in strong acids we now report the observation of the direct reduction products of several alkyl chlorides.

Results and Discussion

While there exists an extensive literature on the behavior of alkyl halides in acidic and superacidic media, we believe this paper reports the first conversion of C_1–C_3 alkyl halides to the corresponding alkane. We have looked at the following systems in HF–TaF_5 at 40 °C: CH_3Cl, C_2H_5Cl, and i-C_3H_7Cl. The latter systems and n-C_3H_7Cl were also studied in HCl:$AlCl_3$ at 140 °C. In all cases the gas phase was analyzed and, where reaction occurred, the corresponding alkane was found to be the major product. Thus, for example, after 5 min at 140 °C in a 45-cm^3 Hastelloy C reactor charged with aluminum chloride and 2-chloropropane, the pressure was 500 psig of which 70.7% was propane by mass spectroscopic analysis. In other words, of the initial charge of isopropyl chloride, 34.2% of the carbon is converted to propane under these conditions. A comparable sample of 1-chloropropane under identical reaction conditions, of excess alkyl halide vs. Lewis acid, was converted after 5 min to the extent of 18% to propane. The results of these studies are summarized in Table I. In both of these cases the other major component in the gas phase was hydrogen chloride. After 15 min the relative amount of propane had decreased as a result of further acid-catalyzed polycondensation reactions of the propane itself.[3]

With 10:1 HF–TaF_5 at high Lewis acid:alkyl halide ratios, selectivity to the alkane is significantly lower, with hydrogen the only other major product in the gas phase. This indicates that, in this more acidic system, a major competing reaction is polycondensation of the alkyl halide similar to that previously described by Olah.[4]

Since the ratio of $HX:MX_n$ to alkyl halide could conceivably affect the effective acidity of the medium and, hence, the reaction mechanism, these reactions were also carried out under widely varying ratios. The results indicate that in AlX_3 systems the stoichiometry does not alter the primary pathway. In the more acidic 10:1 HF–TaF_5 system, however, the isopropyl cation in 2-chloropropane undergoes a shift in reaction mechanism at low acid:alkyl halide ratio to form the direct reduction product, propane. The more acidic methyl cation, on the other hand, undergoes only slow polycondensation (~25% in 1 h) reaction and no direct reduction at the low acid:alkyl halide ratio.

The major finding is the direct reduction of the haloalkane to the hydrocarbon. Equations 1, 2, and 3 present three alternatives which explain the observed results: i.e., alkane formation via dialkylhalonium ions, via haloalkyl carbenium ions, or via propyl carbenium ions (applies only to C_3 and larger systems).

via dialkylhalonium ions:

$$2R_2CHCl + MX_n \rightleftharpoons (R_2CH)_2Cl^+(MX_nCl)^-$$

$$(R_2CH)_2Cl^+(MX_nCl)^- + R_2CHCl \rightarrow R_2CH_2 + (R_2CHCl^+CR_2Cl)(MX_nCl)^- \quad (1)$$

via haloalkyl carbenium ions:

$$R_2CHCl + MX_n \rightleftharpoons R_2^+CH(MX_nCl)^-$$

$$R_2^+CH(MX_nCl)^- + R_2CHCl \rightarrow R_2CH_2 + R_2^+CCl(MX_nCl)^- \quad (2)$$