In Situ Radiolysis Steady-State ESR Study of Carboxyalkyl Radical Trapping by 5,5-Dimethyl-1-pyrroline-*N*-oxide: Spin Adduct Structure and Stability

Hitoshi Taniguchi*,† and Keith P. Madden

Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556

Received: April 16, 1998

Short-lived carboxyalkyl radicals formed in the reaction of three mono- and two dicarboxylic acids with radiolytically produced hydroxyl radicals or hydrated electrons were trapped successfully with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) in dilute aqueous solution. The in situ radiolysis steady-state ESR spectra of the spin adducts were analyzed to determine accurate ESR parameters for these spin adducts in a uniform environment. DMPO spin adducts of the five carboxyalkyl radicals were identified for the first time. Parent radicals include carboxymethyl, dicarboxymethyl, 1,2-dicarboxyethyl, 1-amino-1-carboxymethyl (glycine radical), and 2-carboxyethyl radicals. ESR parameters for DMPO spin adducts of carboxyalkyl radicals are rather similar with nitrogen hyperfine coupling constants of approximately 16 G and with g values around 2.0054 except for glycine radical. Proton hyperfine coupling constants due to C_2-H vary between 21 and 25 G. Nitrogen and C_2-proton hyperfine coupling constants are smaller than those of alkyl radical adducts with larger g values. These changes are ascribed to the spread of the π-conjugation system to include the carboxyl groups. Moreover, the ESR spectrum of both the carboxymethyl and the 1,2-dicarboxyethyl radical is a superposition of spectra from adduct radicals with protonated and deprotonated carboxyl groups in the spin addend. The protonated radical adducts have small additional proton hyperfine couplings due to these carboxyl protons. These carboxyalkyl radicals exhibit intramolecular hydrogen bonding between the hydroxyl function and the aminoxyl oxygen in aqueous solution, which has a profound effect upon the stability of their DMPO spin adducts against spin adduct rearrangement and fragmentation. From carboxymethyl and dicarboxymethyl−DMPO adducts, $(CH_3)_2C(CH_2CH{=}CH_2)N(O\cdot)CH_2CO_2H$ and $(CH_3)_2C(CH_2CH{=}CH_2)N(O\cdot)CH(CO_2^-)CO_2H$ are proposed to form following the opening of the DMPO ring. A similar fragmentation reaction is proposed to occur from DMPO−dicarboxyhydroxymethyl adduct formed in γ-irradiated tartronic acid (hydroxymalonic acid) solution. DMPO spin adducts of glycine and 2-carboxyethyl radicals also show the small proton hyperfine splitting, revealing the intramolecular hydrogen bonding.

Introduction

In our preceding paper,[1] we have reported upon the structure, ESR parameters, and stability of DMPO spin adducts of σ, alkyl, and hydroxyalkyl radicals in dilute aqueous solution. Such information is necessary for kinetic studies of DMPO spin trapping, and in turn, kinetic information such as the reaction rate of spin adduct formation is indispensable for using the spin-trapping technique properly.

In the course of the study on DMPO spin adducts of hydroxyalkyl radicals, formation of strong intramolecular hydrogen bond was not observed clearly in our aqueous system, though it was revealed previously by Janzen et al.[2] in nonaqueous alcohol solutions. However, the interaction between the hydroxyl function and the aminoxyl oxygen could be seen in the asymmetrical ESR line shapes in the hydroxyethyl and hydroxypropyl spin adducts. The asymmetrical ESR spectra were observed when the addition of a prochiral parent radical to DMPO produced a spin adduct radical with two adjacent chiral centers. Hydrogen bonding of the hydroxyl substituent of the parent radical to the aminoxyl oxygen produces a population of two distinct spin adducts. The superposition of the two ESR spectra leads to the asymmetric line shapes.

Possibility of fragmentation following the ring opening was also discussed.[1] Such cleavage was reported only in OH radical adduct to phenyl-*N*-*tert*-butyl nitrone (PBN) by Kotake and Janzen[3] as far as we know.

In this study, we concentrate on the trapping of carboxyalkyl radicals that feature strong hydrogen bonding between the hydroxyl group of the spin addend carboxyl function and the aminoxyl oxygen, and a strongly electron-withdrawing effect of the spin addend on the DMPO ring. These two features enhance the polarization of the N_1-C_2 bond in the appropriate sense to produce spin adduct fragmentation. This tendency is significantly more pronounced than in the case of the hydroxyalkyl radical adducts to DMPO.

In this work, an in situ radiolysis steady-state ESR study of DMPO trapping is reported with five carboxyalkyl radicals from chloroacetate, 3-bromopropionate, malonic acid, succinic acid, and glycine. The spin adduct stability is also examined in the context of the above-mentioned intramolecular interactions.

Experimental Section

Experimental procedures were essentially the same as described in our preceding paper.[1] Sample solutions were prepared using reagent-grade water from a Millipore Milli-Q water system. DMPO was obtained from OMRF Spin Trap Source and used without further purification. ESR measurements were

† Permanent address: Department of Veterinary Radiology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan. Internet address: tanigu@po.cc.yamaguchi-u.ac.jp. FAX number: + 81-839-33-5908.

S1089-5639(98)01893-3 CCC: $15.00
Published on Web 07/31/1998

TABLE 1: ESR Parameters for DMPO Spin Adducts of Carboxyalkyl Radicals at Neutral pH

parent radical	*g* factor	*a*(N, NO)[a]	*a*(H, C_2−H)[a]	other hyperfine couplings[a]
CH_2CO_2H	$2.005\,41 \pm 2 \times 10^{-5}$	16.08 ± 0.02	22.78 ± 0.01	*a*(H, COOH) 1.68 ± 0.02
$CH_2CO_2^-$	$2.005\,40 \pm 2 \times 10^{-5}$	16.13 ± 0.02	22.83 ± 0.02	unresolved
$CH(CO_2^-)CO_2H$	$2.005\,47 \pm 1 \times 10^{-5}$	15.86 ± 0.03	23.22 ± 0.04	*a*(H, COOH) 0.69 ± 0.03
$CH(CO_2^-)CO_2H$[b]	$2.005\,65 \pm 1 \times 10^{-5}$	15.14 ± 0.02	4.03 ± 0.03	unresolved
$CH(CO_2^-)(CH_2CO_2H)$	$2.005\,41 \pm 1 \times 10^{-5}$	16.05 ± 0.02	20.95 ± 0.03	*a*(H, COOH) within line width
$CH(CO_2^-)(CH_2CO_2^-)$	$2.005\,43 \pm 1 \times 10^{-5}$	15.87 ± 0.02	25.27 ± 0.03	unresolved
$CH(CO_2H)NH_3^+$	$2.005\,53 \pm 1 \times 10^{-5}$	15.59 ± 0.01	22.43 ± 0.01	*a*(H, COOH) 0.69 ± 0.01
$CH_2CH_2CO_2H$	$2.005\,41 \pm 1 \times 10^{-5}$	16.13 ± 0.01	22.99 ± 0.02	*a*(H, COOH) 0.80 ± 0.02

[a] Hyperfine coupling constants in Gauss ($1\ G = 1 \times 10^{-1}$ mT). [b] ESR parameters for the rearranged spin adduct radical.

usually performed on freshly prepared sample solutions. The solutions were unbuffered for these steady-state studies. If necessary, the pH of sample solutions was adjusted using a small amount of dilute aqueous KOH (Fisher) solution or using equimolar KOH to sample acids, to observe ESR spectra in neutral region. Solution pH was measured using a Radiometer-Copenhagen model PHM84 meter.

The concentrations of parent compounds were 0.05−0.2 M, except for glycine (0.5 M). Most parent radicals were formed via reaction with hydroxyl radicals produced by water radiolysis. Carboxymethyl, dicarboxymethyl, 1,2-dicarboxyethyl, and 1-amino-1-carboxymethyl radicals were derived by hydrogen abstraction from sodium acetate (Fisher), malonic acid (Aldrich), succinic acid (Aldrich), and glycine (Fluka and Aldrich), respectively.

Reaction of the hydrated electron with chloroacetic acid or bromoacetic acid (Aldrich) and 2-bromo- and 3-bromopropionic acid (Aldrich) produced carboxymethyl, 1-carboxyethyl, and 2-carboxyethyl radicals, respectively. In these cases, 0.1 M *tert*-butanol (2-methyl-2-propanol) or sodium formate was usually added to the nitrogen (ultrahigh purity, Mittler)-deoxygenated solution to scavenge radiolytically produced hydroxyl radicals. Other carboxylic acids and related ester examined in this work are tartronic acid (hydroxymalonic acid, Aldrich), propionic acid (Aldrich), malic acid (Aldrich), glutaric acid (Aldrich), and *tert*-butyl formate (Aldrich).

The DMPO concentration used in these studies varied from 1 to 10 mM. Since the rate constants of most parent compounds with hydroxyl radicals are 10^8-10^9 L (mol s)$^{-1}$ [4,5] and that of DMPO is 2.8×10^9 L (mol s)$^{-1}$,[6] greater than 85% of the hydroxyl radicals react with the parent compounds. Except for the halogenated carboxylic acids mentioned above, the sample solutions were deoxygenated with nitrous oxide (U.S.P. grade, Mittler) to convert radiolytically produced hydrated electrons into hydroxyl radicals.

In situ radiolysis steady-state ESR spectra were recorded by irradiating flowing cooled aqueous solutions of the parent compounds and DMPO within the ESR cavity using a 2.8 MeV electron beam from a Van de Graaff accelerator. A 2.5 μA dc beam was used to produce a steady-state concentration of spin adduct radicals for field-modulation ESR spectra. When necessary to avoid secondary reactions, the beam current was decreased to 0.8 μA. The solution temperature was within the range 15−19 °C. The solution flow rate varied between 20 and 32 mL/min. The use of such a fast flow minimizes the intensity of spurious long-lived secondary radical products relative to the spin-adduct radicals.

X-band (9.2 GHz) in situ radiolysis ESR spectra of steady-state radical population were recorded in second-derivative presentation using magnetic field modulation and sequential phase-sensitive detection at 100 kHz and 200 Hz to determine the line positions for the parent and spin adduct radicals. Microwave power was sufficiently low such that power saturation effects were negligible. The ESR spectrometer and associated data acquisition system are described elsewhere.[7] An ESR spectrum of irradiated water itself was recorded each day and was subtracted from that day's DMPO-spin adduct ESR spectra to correct the baseline arising from the color-center signal in the irradiated quartz ESR flat cell.

The magnetic field was measured by NMR methods, with *g* factors measured with respect to that of the sulfite radical anion, $SO_3^{\bullet-}$ recently redetermined as $g = 2.003\,16$.[8] ESR line positions were measured using the Lorentzian/Gaussian fitting functions included in the IBM PC-based data analysis program ORIGIN. The line positions were analyzed to derive *g* factors and proton and nitrogen hyperfine couplings for parent and spin adduct radicals, taking second-order splitting into account.[9] The ESR parameters of spin adduct radicals are summarized in Table 1. Standard deviation of these parameters are mostly 1×10^{-5} in *g* factors and 0.02 G in hyperfine coupling constants.

Single-pulse electron irradiation with 2 μs duration and 200 mA intensity was also carried out to see the time profile of the decay of spin adducts of dicarboxymethyl radicals from malonic acid. Single-frequency field modulation and phase-sensitive detection at 25 kHz were used in this experiment.

For comparison, persistent radicals from spin trapping reactions were produced by 1−2 min γ-irradiation of parent compounds-DMPO solutions within a Shepherd model 109 ^{60}Co irradiator, having a dose rate of 1.17×10^4 rad/min. Total radical concentrations were 73−146 μM L^{-1}.[10] These selected compounds for γ-irradiation are sodium acetate, malonic acid, tartronic acid, and glycine. Initial ESR spectra were usually acquired at least 2 min after sample irradiation. An IBM/Bruker ER 100 spectrometer was used to acquire X-band (9.8 GHz) ESR spectra of persistent radicals in second-derivative presentation by detecting the second harmonic of the 50 kHz modulation frequency. An alkaline aqueous solution of Fremy's salt (peroxylamine disulfonate dianion radical, Alfa) with a *g* factor of 2.005 54 and nitrogen hyperfine splitting of 13.01 G was used for the calibration of line positions measured using this spectrometer.[10]

Results and Discussion

Both α- and β-carboxyalkyl spin adducts to DMPO were definitively observed for the first time in this study. ESR parameters are summarized in Table 1. They are rather similar with nitrogen hyperfine coupling constants of approximately 16 G and with *g* values around 2.0054 except for glycine radical. Proton hyperfine coupling constants due to C_2−H vary between 21 and 25 G. Nitrogen and C_2-proton hyperfine coupling constants are smaller than those of alkyl radical adducts with larger *g* values.[1] These changes are ascribed to the spread of the π-conjugation system to include the carboxyl groups. The spin adduct radicals identified in this work were DMPO−carboxymethyl, DMPO−2-carboxyethyl, DMPO−dicarboxy-

methyl, DMPO−1,2-dicarboxyethyl, and DMPO−1-amino-1-carboxymethyl. All but the last two parent radicals are symmetric about the site of the unpaired electron and are expected to produce only one stereoisomeric form of the spin adduct. The other two parent radicals are asymmetric and could form a pair of spin adduct radicals exhibiting stereoisomerism if, as in the case of the hydroxyalkyl spin adduct radicals,[1] an intramolecular association between the aminoxyl function and the parent radical substituent can inhibit rotation about the C_2-N_1 bond to resolve the differential magnetic interactions of the two isomers. A complicating feature in the carboxyalkyl spin adduct is the pH-dependent protonation state of the parent radical substituent. These factors interact differently for each system studied as described below in detail.

(1) DMPO−Carboxymethyl Spin Adduct. The prototype carboxyalkyl spin adduct shows an ESR spectrum at neutral pH that is the superposition of the protonated and deprotonated spin adduct radicals. The *g* factor of the deprotonated species, 2.005 40, is the same, to within experimental error, as that of the protonated form, 2.005 41. These *g* factors are higher than those of the alkyl radical adducts,[1] indicating an increase in spin−orbit interaction in the carboxyalkyl spin adducts. The aminoxyl nitrogen splitting is 16.13 G in the deprotonated form, while it is 16.08 G in the protonated adduct. In both cases these are smaller than the value of a(H, C_2−H) observed in the DMPO alkyl adducts. These parameters are consistent with substantial π-orbital overlap between the aminoxyl fragment and the carboxyl function, a feature common to all the carboxyalkyl radicals examined in this study. The hyperfine coupling constants due to C_2−H are 22.78 and 22.83 G in protonated and deprotonated form, respectively. The magnitude of the coupling indicates that, as in the DMPO−alkyl spin adducts,[1] the equatorial alignment of the bulky parent radical substituent at C_2 positions the C_2−H fragment near parallel to the unpaired electron symmetry axis, resulting in efficient hyperconjugation. Unlike the alkyl radical adducts whose steric requirements dictate the extension of the alkyl chain outward into the equatorial plane, the attractive interaction between the aminoxyl and carboxyl functions facilitates the formation of the intramolecular cyclic structure. The C_2−H bond is therefore less constrained in the axial position, and ring motion then causes a smaller average proton splitting than in the case of the alkyl adducts.

In the DMPO−carboxymethyl radical adduct at neutral pH, the line shape has a triplet-like structure with an approximate line separation of 0.84 G. However, there was pH dependence with this splitting; shoulders started to appear on each peak at pH 6.9 and the splitting was observed at pH 9.1 and 10.4, but it was not observed at pH 4.9 and 11.2 in γ-irradiation experiments. In the intermediate pH region, two species having slightly different aminoxyl nitrogen hyperfine couplings overlapped. One spectrum reveals a 1.68 G coupling, while the other spectrum appears without this small splitting. Therefore, we could not assign this splitting to the methylenic protons of the carboxymethyl parent radical. We assign this composite spectrum to a superposition of the DMPO−CH_2CO_2H adduct with intramolecular hydrogen bonding, producing an additional small proton splitting, and the DMPO−$CH_2CO_2^-$ adduct. This small coupling is characteristic of the carboxyalkyl spin adducts to DMPO. OH adduct to DMPO was also observed at pH 4.9, 6.9, 9.1, and 10.4 and H adduct to DMPO was predominant at pH 11.2.

In DMPO−carboxyalkyl radical adducts with two carbons between C_2 and OH, stronger hydrogen bonding is expected to form a six-membered ring bridging an aminoxyl oxygen. The intensified interaction is demonstrated by this additional 1.68 G proton coupling in the ESR spectrum, owing to the hydroxyl proton hydrogen-bonded to the aminoxyl oxygen.

The persistence of this coupling into the higher pH range is somewhat unexpected since the pK_a of acetic acid is 4.8, but the presence of the small coupling indicates the interaction of the zwitterionic canonical form of the DMPO−carboxyalkyl radical adduct is an important contributor to the radical structure. The negative charge contribution from the aminoxyl center and the carboxylate fragment interact in the same manner as in the salicylic acid radical, stabilizing the protonated radical beyond the pK_a of the carboxylic acid substituent itself. In addition, a carboxyl proton of acetic acid becomes more acidic in CH_2-CO_2H radical without the electron-donating character of a methyl group. The pK_a value was determined to be 4.5 in the radical by optical pulse radiolysis, down from 4.76 in the acid.[11] In a DMPO spin adduct, the electron-attracting character of an aminoxyl group might enhance the acidity to some extent. The initial structure in the dicarboxymethyl−DMPO spin adduct fragmentation scheme (see section 3 below) shows the favorable geometry for the interaction of the carboxymethyl spin addend group with the partial negative charge upon the aminoxyl oxygen atom.

At long times post-γ-irradiation, and in alkaline solution (at pH 9.1 and 11.2), a transformation occurs, yielding an aminoxyl species with appreciably different parameters than the starting spin adduct. The spectrum has the form of a triplet of triplets due to one aminoxyl nitrogen splitting (a(N, NO) = 16.4 G at pH 9.1 and 15.8 G at pH 11.2), and couplings to two equivalent protons (a(H, CH_2) = 5.8 G at pH 9.1 and 8.4 G at pH 11.2). These spectral parameters are quite different from the DMPO−hydrogen atom adduct but are reminiscent, if not closely, of the MNP−carboxymethyl spin adduct,[12] with g = 2.005 63, a(N, NO) = 16.15 G, and a(H, CH_2) = 8.41 G. The time course of this rearrangement reaction is shown in Figure 1, with the solution at pH 11.2. This new species must be the result of the opening of the DMPO ring, with subsequent adduct moiety migration to the aminoxyl center to produce $(CH_3)_2C(CH_2CH{=}CH_2)N(O\cdot)CH_2CO_2H$. Since the second spectrum from the top in Figure 1 shows sharp ESR lines under 10 times smaller modulation width than the others, the carboxyl group is probably deprotonated. An analogous fragmentation reaction occurs with greater facility in the DMPO−malonic acid radical adduct, where the rearrangement occurs quickly under milder conditions (see section 3 below for details).

(2) DMPO−2-Carboxyethyl Spin Adduct. This β-carboxyalkyl radical spin adduct serves as a complement to the DMPO−α-carboxyalkyl radical adduct, since the pK_a values of the parent compounds are different. The ESR spectrum recorded during continuous electron irradiation of a nitrogen-saturated, 0.1 M solution of 3-bromopropionate in the presence of 4.4 mM DMPO shows the dominant aminoxyl species in the DMPO−2-carboxyethyl adduct, with a smaller contribution from DMPO−OH and a trace of DMPO−H. The spectral parameters of DMPO−2-carboxyethyl are g = 2.005 41, a(N, NO) = 16.13 G, a(H, C_2−H) = 22.99 G, and a(H, COOH) = 0.80 G. The doublet structure of the individual lines is clear throughout the spectrum, consistent with the assignment of the DMPO−carboxymethyl spectrum. It should be noted that, for this adduct species, no rearrangement product was seen under the various experimental conditions employed, indicating that

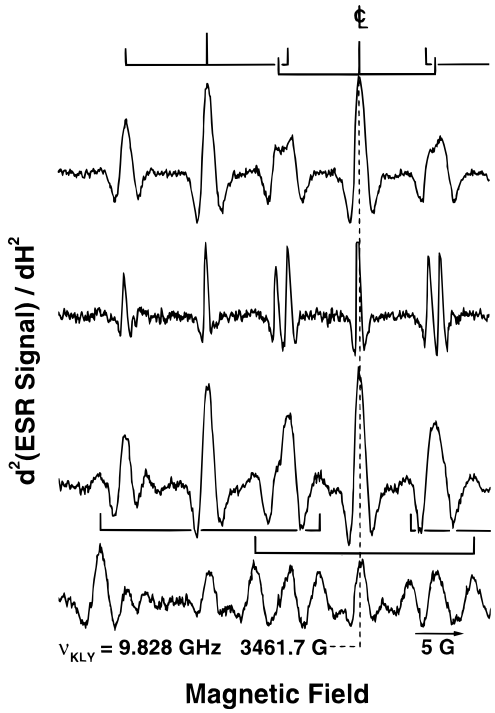


Figure 1. Change in ESR spectra of DMPO−carboxymethyl spin adduct with time after 1 min γ-irradiation of aqueous solutions containing 1 M sodium acetate, 0.4 M bromoacetate, and 10 mM DMPO at pH 11.2. The lower-field half of each ESR spectrum is reproduced. The dotted line shows the center of each spectrum. The intensity of ESR spectral lines for the spin adduct radical, DMPO-$CH_2CO_2^-$, decreased and that for the fragmentation radical, $(CH_3)_2C(CH_2CH{=}CH_2)$-$N(O\cdot)CH_2CO_2^-$, increased with time course from bottom to top after γ-irradiation. The second spectrum (from the top) was measured using 10 times smaller modulation width than the others to show the separation of each spectral line.

proximity of the carboxyl function to the C_2 position in the DMPO ring is an important factor in the rearrangement reaction. Though a small doublet ascribed to a hydrogen-bonded proton was observed, the triplet due to CH_2 protons in the addend was not observed, showing the electron-attracting character of an aminoxyl group.

In the reaction of propionic acid with hydroxyl radical and 2-bromopropionic acid with hydrated electron, only parent 1-carboxyethyl radical, $\cdot CH(CH_3)CO_2^-$ was identified at pH 8. ESR parameters agree well with those reported by Hewgill and Proudfoot.[13] The 2-carboxyethyl radical, $\cdot CH_2CH_2CO_2^-$, was not a major species under our conditions. Both were observed in approximately equal intensity in the $TiCl_3/H_2O_2$ oxidation system.[13]

From electron-irradiated aqueous *tert*-butyl formate solution, intense spectral lines of DMPO adduct were observed with ESR parameters, $g = 2.005\,39$, a(N, NO) = 15.71 G, and a(H, CH) = 18.71 G. Absolute line positions are almost the same as those of CO_2^- adduct,[1] suggesting that $\cdot CO_2C(CH_3)_3$ was not the trapped species here.

Another radical adduct, DMPO−$CH_2C(CH_3)_2OCO^-$, was also identified as a minor component. A proton hyperfine coupling due to C_2−H, 25.38 G, is the largest one in this study, showing favorable conformation around the N_1-C_2 bond for hyperconjugation. The g factor of 2.005 41 and nitrogen hyperfine coupling constant of 15.82 G are similar to those of structurally related 2-carboxyethyl radical adduct.

(3) DMPO−Dicarboxymethyl Spin Adduct. The α-dicarboxyalkyl radical adduct derived from malonic acid under fast flow conditions at neutral pH shows a single spin adduct, with $g = 2.005\,47$, a(N, NO) = 15.86 G, a(H, C_2−H) = 23.22 G, and a(H, COOH) = 0.69 G, consistent with the spectral characteristics of the DMPO−monocarboxyalkyl radicals. Under slow flow conditions, at pH 7.0 and above, a second aminoxyl radical signal grows in dramatically. The ESR parameters for this radical DMPO−dicarboxymethyl are $g = 2.005\,65$, a(N, NO) = 15.14 G, and a(H, CH) = 4.03 G. These two aminoxyl species are present in the ESR spectrum under continuous irradiation conditions as shown in Figure 2 with the two simulated spectra of species A (upper) and species B (lower).

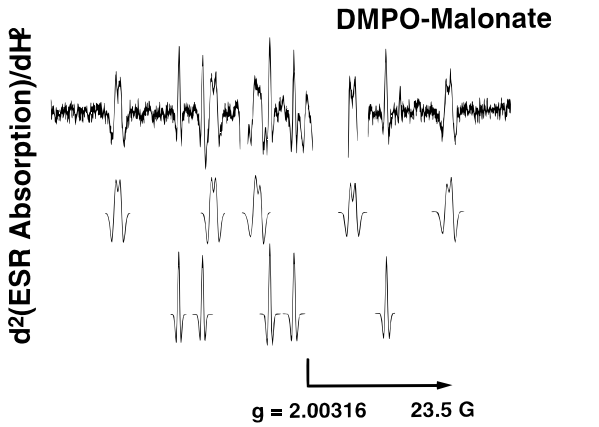


Figure 2. ESR spectra of aqueous solution containing 0.1 M malonic acid and 1 mM DMPO during continuous electron irradiation at pH 7.2. The simulated spectra below show the ESR line shape of two types of DMPO−dicarboxymethyl radical adducts with and without carboxyl proton splitting and intramolecular hydrogen bonding between carboxyl and aminoxyl groups. The spectral simulation was performed using the ESR parameters in Table 1 assuming a Lorentzian/Gaussian line shape.

From the time profile of the decay measured using single-pulse electron irradiation, species A is rather stable and species B decays faster with half-life of 0.8 min. If the same sample solution is irradiated in a ^{60}Co-γ source and then examined within 1 min postirradiation, only the ESR spectrum consisting of six doublets (species A) is seen. The transient spectrum (species B) has a larger g factor and a much smaller proton hyperfine coupling than is typical of other nitrone spin adducts. The detailed changes in the appearance of the ESR spectra at fixed flow rates and varying pH are shown in Figure 3.

The small proton splitting in the rearranged spin adduct radical is inconsistent with the geometric constraints of the DMPO ring system, so an open chain form of the aminoxyl radical must be involved. The most likely structure for such a radical would result from a transformation parallel to that observed for the DMPO−carboxymethyl adduct; this would result in an aminoxyl radical of the structure $R(CH_3)_2C-N(O\cdot)-CH(CO_2^-)_2$, where R is the alkyl fragment resulting from ring opening. The ESR spectrum of this species B is similar to that of the MNP spin adduct, $C(CH_3)_3-N(O\cdot)-CH(CO_2^-)$-R′, formed by the reaction of MNP and carboxyalkyl radicals R′. For example, a(N, NO) = 15.0 and a(H, CH) = 2.85 G for $R = CO_2^-$ in a mixed solvent of D_2O and *tert*-butanol (1:1).[14] The rearranged DMPO−dicarboxymethyl adduct is almost certain to have a different structure than a *tert*-butyl group adjacent to the aminoxyl center. This structural feature and the dilute aqueous environment could account for the increased coupling constants observed here.

The inference from the aforementioned considerations is that the electron-withdrawing nature of the dicarboxymethyl sub-

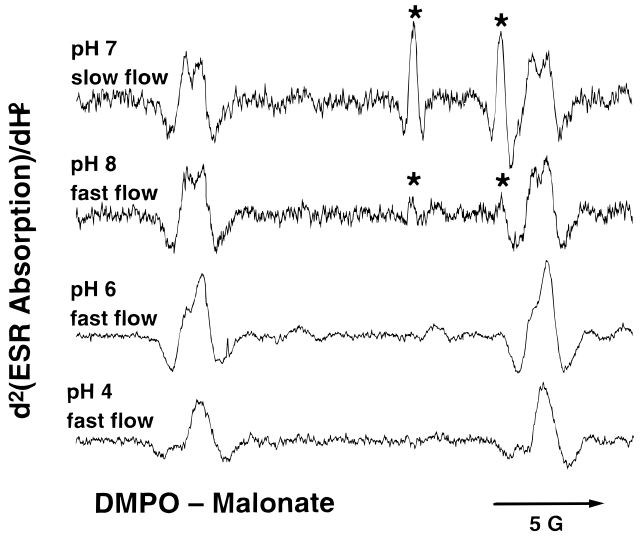
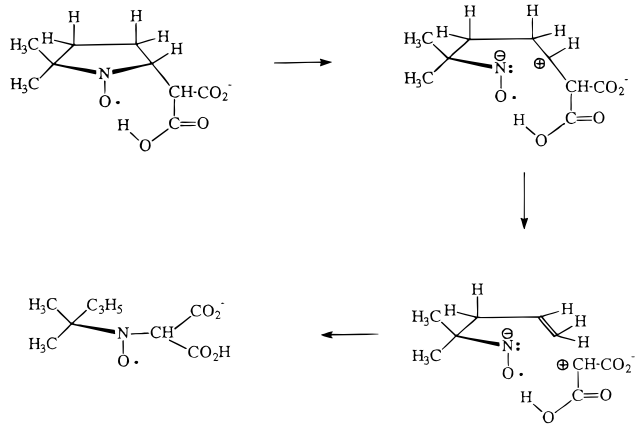


Figure 3. Partial ESR spectra of DMPO−$CH(CO_2^-)CO_2H$ radical adducts at pH 4.0−8.0. These spectra are from the region containing the $I_N = 0$ component on the low-field portion of the spectrum shown in Figure 2. Asterisks indicate lines belonging to the spin adduct radical fragmentation product (see text). These spectra were obtained by in situ radiolysis of N_2O-saturated aqueous solutions with 2 M malonic acid and 1.1 mM DMPO at pH 4.0 and pH 6.0 and with 1 M malonic acid and 1.1 mM DMPO at pH 7.0 and pH 8.0. For the lower three traces, the solution flow rate was increased over that used in Figure 2.

SCHEME 1



stituent combined with hydrogen bonding to the aminoxyl oxygen provides a channel for spin adduct fragmentation and rearrangement following the ring opening of DMPO spin adducts. Resultant aminoxyl radical seems less resistant to second-order termination than the initial spin adduct radical. Kotake and Janzen[3] showed a fragmentation pathway in the decay of the PBN−hydroxyl radical spin adduct, yielding an MNP anion radical and a benzaldehyde molecule. An analogous reaction could be catalyzed by the carboxyalkyl substituent (Scheme 1). The efficiency of such fragmentation reaction is expected to show a strong pH dependence. Aqueous solutions of 0.1−2 M malonic acid and 1.1−5 mM DMPO were adjusted to pH values of 1.0, 4.0, 6.0, 7.0, 8.0, or 10.5 and subjected to continuous electron irradiation. As shown in Figure 3, the fragmentation radical marked by asterisks in the ESR spectrum appears dramatically at pH 8.0 using fast flow. Its concentration increased at higher pH and at slower flow rate showing the slow fragmentation reaction from species A to B. On the other hand, the doublet due to a carboxyl proton appears first as shoulders at pH 6.0 then starts to be separated at pH 7.2 (see Figure 3), showing stronger hydrogen bonding. At lower pH, the carboxyl group must be protonated. However, the doublet due to a carboxyl proton was not observed here below pH 6. This discrepancy might be explained as follows; an aminoxyl group is surrounded by many protons at lower pH, which weaken the hydrogen bond between the aminoxyl oxygen and carboxyl proton so that the proton hyperfine splitting may vanish.

In order to pursue the pH effect further, we examined the pH dependence of ESR parameters for malonic acid radical itself. The hyperfine coupling constant of the α-proton, a(α-H), changed as follows with pH values in parentheses: 20.26 G (pH 2.0), 20.25 (2.1), 20.24 and 20.22 (4.3), 20.31 and 20.02 (5.8), 19.88 (7.2), 19.94 (10.5), and 19.94 (12.3). Other reported values are 20.29 G (pH 1.7) and 19.81 (6.9)[15] and 19.95 (12).[16] The value of a(α-H) in acidic form is supposed to be 20.32 G in 0.4 M H_2SO_4.[17] At pH 5.8, two forms existed almost equally. We thus reconfirmed the second pK_a of carboxyl proton in malonic acid radical is 5.7, the same as that of the parent acid, as obtained by optical pulse radiolysis measurements.[18] However, we could not determine the first pK_a because of line broadening in ESR spectra around pH 3. Simic et al. did not either, and they only cited the first pK_a of the acid as 2.86.[18] Therefore, there might be an appreciable increase of the pK_a in DMPO adduct, leading to the protonated form even at higher pH.

A similar fragmentation reaction leading to $(CH_3)_2C(CH_2CH{=}CH_2)N(O\cdot)C(OH)(CO_2^-)CO_2H$ might occur in γ-irradiation of tartronic acid (hydroxymalonic acid) and DMPO proceeding from a DMPO−dicarboxyhydroxymethyl adduct. The rearranged aminoxyl radical shows three broad lines with a(N, NO) of 16.5 G, which is similar to 16.70 G in MNP−$C(OH)(CH_3)_2$ adduct.[12] The line width of this species and that of the initial spin adduct radical were too broad to be directly observed in in situ radiolysis steady-state ESR experiments where hydroxyl and hydrogen adducts to DMPO were the major species observed.

(4) DMPO−1,2-Dicarboxyethyl Spin Adduct. This dicarboxyalkyl radical is the first dicarboxylate in this study that is asymmetric owing to covalent bonding of the parent molecule. Since this adduct would be expected to hydrogen bond to the aminoxyl center in the same manner as the symmetric carboxylates, two different spin adduct ESR spectra are expected and found. The first has a g factor of 2.005 43, aminoxyl nitrogen splitting of 15.87 G, and proton hyperfine splitting of 25.27 G. Although the parent compound, succinic acid, is expected to be protonated at both carboxyl groups at neutral pH, no further splitting is observed in this radical. The pK_a values for succinic acid radical are assumed to be 3.3 and 4.3 but are still uncertain by optical pulse radiolysis measurements.[19] The increase in the pK_a value is again assumed in the DMPO adduct with a proton present in at least one carboxyl group. The second adduct has a g factor of 2.005 41 and an aminoxyl coupling of 16.05 G with a β-proton coupling of 20.95 G. The ESR spectral line width of this radical is noticeably larger than those of the first species, suggesting the carboxyl proton coupling is small enough to be unresolved in both species. No facile rearrangement chemistry was noted in this system, again underscoring the rather specific geometric requirements for the adduct fragmentation reaction to occur.

The parent free radical in this case is sufficiently unreactive that the choice of an optimum spin trap concentration is confounded by the tendency of the spin trap to react with the precursor radicals for the radiolysis of water. In Figure 4, we show the changes in the ESR spectrum as a function of the spin trap concentration. Clearly, there is a narrow window

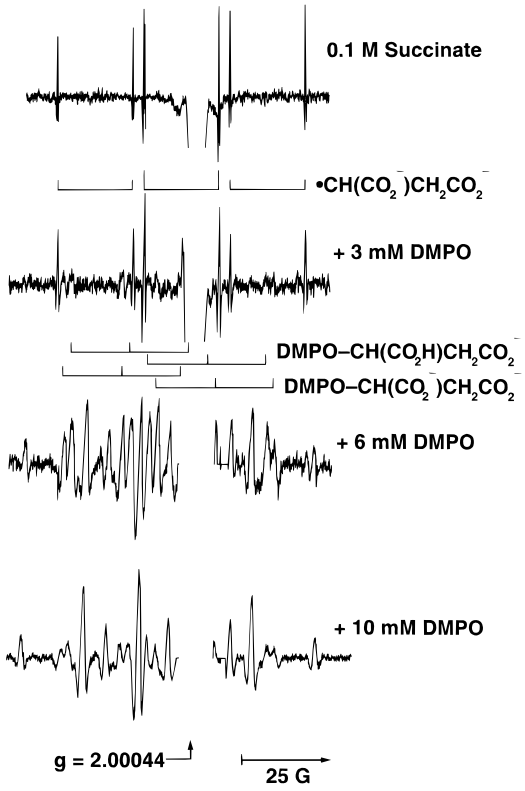


Figure 4. Changes in the ESR spectrum of DMPO−1,2-dicarboxyethyl spin adduct as a function of the spin trap concentration. Electron-irradiated aqueous solutions contained 0.1 M succinic acid and DMPO. The concentrations of DMPO were 0, 3, 6, and 10 mM, respectively, from the top. The pH value of the sample solutions was adjusted to 6.6. ESR spectral lines due to succinic acid radical $\cdot CH(CO_2^-)CH_2CO_2^-$ were observed in the top two traces. Those of DMPO-$CH(CO_2H)CH_2CO_2^-$ adduct (second stick figure from top) and the DMPO-$CH(CO_2^-)CH_2CO_2^-$ adduct (third stick figure from top) were observed with DMPO, but most clearly in 6 mM DMPO. In the lower two spectra hydrogen atom and/or hydrated electron adducts to DMPO were observed to be appreciable spectral components, in addition to a minor hydroxyl radical adduct. The *g* factor (2.000 44) indicator for the color-center in irradiated quartz is shown at the bottom of the figure.

where optimum scavenging of the carboxyalkyl radical occurs, while at higher DMPO concentrations, the hydroxyl radical, hydrogen atom, and hydrated electron are scavenged by the trap, perturbing the overall chemistry. This shows the advantage of knowledge of specific rate constants for spin trap reaction when designing spin-trapping experiments.

With malonic acid and succinic acid, parent radicals, $^-{}_2OC\dot{C}HCO_2^-$ and $^-{}_2OC\dot{C}HCH_2CO_2^-$ were also observed. The ESR parameters are in excellent agreement with literature values.[16]

From malic acid and glutaric acid, only parent radicals, $^-{}_2OC\dot{C}(O^-)CH_2CO_2^-$, $^-{}_2OCCH_2\dot{C}HCH_2CO_2^-$, and $^-{}_2OCCH_2CH_2\dot{C}HCO_2^-$ were identified with ESR parameters that correspond closely to those reported previously.[20,16,21]

(5) DMPO−Aminocarboxymethyl Spin Adduct. In the glycine spin-trapping experiments, it was found that for any reasonable concentration of DMPO added to the glycine solution the concentration of the hydroxyl radical spin adduct was relatively high, indicating the hydroxyl radical's low reaction rate constant with the zwitterionic form of this amino acid. The concentration used for glycine was comparatively high (0.5 M), and 3 mM DMPO was used for this study. The ESR parameters are $g = 2.005\,53$, an aminoxyl nitrogen coupling of 15.59 G, a proton hyperfine coupling constant of 22.43 G, and a carboxyl proton coupling constant of 0.69 G. The small proton hyperfine coupling reveals the intramolecular hydrogen bonding. Although this radical is asymmetric, it is interesting to note that only one ESR spectrum is observed in this system. The intramolecular association between a carboxyl proton and an aminoxyl oxygen might inhibit rotation around the C_2-N_1 bond and happen to show no difference in magnetic interaction among the expected diastereomers of this spin adduct.

From a γ-irradiated aqueous glycine solution at pH 11, an additional CO_2^--type spin adduct was identified with the following ESR parameters: $a_N = 15.4$ and $a_H = 18.4$ G. Asmus et al. found CH_2NH_2 radical via electron transfer and decarboxylation after OH addition to a free electron pair at the nitrogen of glycine in basic aqueous solution.[22] Armstrong et al. proposed the formation of $NH_2CH_2CO_2\cdot$ is inaccessible with OH from solution thermochemical considerations.[23] So far, no reaction scheme can be proposed to show the formation of CO_2^--type spin adduct from the irradiated glycine solution.

Concluding Remarks

Spin adducts with DMPO were identified for the first time from two aliphatic carboxylic acids, one amino acid, and two dicarboxylic acids. Parent radicals include carboxymethyl, dicarboxymethyl, 1,2-dicarboxyethyl, 1-amino-1-carboxymethyl (glycine radical), and 2-carboxyethyl radicals. In all spin adducts except deprotonated radical adducts, additional small proton hyperfine coupling constants around 0.7−1.7 G were observed. Both nitrogen and C_2-proton hyperfine coupling constants are smaller and *g* factors are larger than those of DMPO−alkyl radical adducts,[1] suggesting the spread of π-conjugation including carboxyl groups. However, ESR parameters did not change much from those of hydroxyalkyl radical adducts.[1] The DMPO spin adduct of 1,2-dicarboxyethyl radical showed an exceptionally large C_2-H proton hyperfine coupling constant 25.3 G, with a rather small nitrogen coupling constant 15.9 G. Bulky carboxyl substituents are considered to result in a steric conformation much more favorable to hyperconjugation.

One of the most interesting aspects in this study was to observe both protonated and deprotonated forms when trapping carboxymethyl and 1,2-dicarboxyethyl radicals from sodium acetate and succinic acid. One shows strong, specific intramolecular interaction between the hydroxyl function of the protonated carboxyl moiety and the aminoxyl oxygen, as evidenced by an additional small proton hyperfine splitting in the ESR spectrum of DMPO−carboxymethyl adduct. The geometry is favorable for a strong hydrogen bond to form. This interaction can have a profound effect upon the stability of the adduct radical. The DMPO−dicarboxymethyl adduct shows this clearly.

To our best knowledge, there is only one report in which stereoisomer radicals were observed in diastereomers in DMPO adducts of aliphatic alcohol radicals.[2] In our study, the rather bulky carboxyl group has a preference for two sterically preferred configurations, one for each diastereomer. The strong hydrogen bond, however, overwhelms steric effects, causing similar configurations and similar ESR spectra for both isomers.

The electron-withdrawing inductive effect of the dicarboxymethyl substituent leads to opening of the DMPO ring, producing an aminoxyl radical less resistant to second-order termination. A similar fragmentation mechanism is possible for DMPO adducts of carboxymethyl and dicarboxyhydroxymethyl radicals. The efficiency of this fragmentation reaction shows a strong pH dependence.

Acknowledgment. This work was supported by the Office of Basic Energy Sciences, United States Department of Energy. This is Contribution No. NDRL-4056 from the Notre Dame Radiation Laboratory.

References and Notes

(1) Taniguchi, H.; Madden, K. P. *Radiat. Res.*, submitted.
(2) Kotake, Y.; Kuwata, K.; Janzen, E. G. *J. Phys. Chem.* **1979**, *83*, 3024−3029.
(3) Kotake, Y.; Janzen, E. G. *J. Am. Chem. Soc.* **1991**, *113*, 9503−9506.
(4) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513−886.
(5) Logan, S. R. *J. Chem. Soc., Perkin Trans. 2* **1989**, 751−754.
(6) Madden, K. P.; Taniguchi, H. *J. Phys. Chem.* **1996**, *100*, 7511−7516.
(7) Madden, K. P.; McManus, H. J. D.; Fessenden, R. W. *Rev. Sci. Instrum.* **1994**, *65*, 49−57.
(8) Jeevarajan, A. S.; Fessenden, R. W. *J. Phys. Chem.* **1989**, *93*, 3511−3514.
(9) Fessenden, R. W. *J. Chem. Phys.* **1962**, *37*, 747−750.
(10) Madden, K. P.; Taniguchi, H. *J. Chem. Soc., Perkin Trans. 2* **1993**, 2095−2103.
(11) Neta, P.; Simic, M.; Hayon, E. *J. Phys. Chem.* **1969**, *73*, 4207−4213.
(12) Madden, K. P.; Taniguchi, H. *J. Am. Chem. Soc.* **1991**, *113*, 5541−5547.
(13) Hewgill, F. R.; Proudfoot, G. M. *Aust. J. Chem.* **1976**, *29*, 637−647.
(14) Bergene, R.; Minegishi, A.; Riesz, P. *Int. J. Radiat. Biol.* **1980**, *38*, 383−394.
(15) Maruthamuthu, P.; Taniguchi, H. *J. Phys. Chem.* **1977**, *81*, 1944−1948.
(16) Laroff, G. P.; Fessenden, R. W. *J. Chem. Phys.* **1971**, *55*, 5000−5008.
(17) Behar, D.; Samuni, A.; Fessenden, R. W. *J. Phys. Chem.* **1973**, *77*, 2055−2059.
(18) Simic, M.; Neta, P.; Hayon, E. *J. Phys. Chem.* **1969**, *73*, 4214−4219.
(19) Cabelli, D. E.; Bielski, B. H. J. *Z. Naturforsch.* **1985**, *40b*, 1731−1737.
(20) Dixon, W. T.; Norman, R. O. C.; Buley, A. L. *J. Chem. Soc.* **1964**, 3625−3634.
(21) Davies, M. J.; Gilbert, B. C.; Thomas, C. B.; Young, J. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1199−1204.
(22) Mönig, J.; Chapman, R.; Asmus, K.-D. *J. Phys. Chem.* **1985**, *89*, 3139−3144.
(23) Armstrong, D. A.; Rauk, A.; Yu, D. *J. Chem. Soc., Perkin Trans. 2* **1995**, 553−560.