Photoinduced Electron Transfer, Decarboxylation, and Radical Fragmentation of Cysteine Derivatives: A Chemically Induced Dynamic Nuclear Polarization Study

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Abstract: The photoreactions of cysteine derivatives **I** with 4-carboxybenzophenone in D_2O were investigated by measurements of chemically induced dynamic nuclear polarization (CIDNP). The quenching mechanism is electron transfer from sulfur at every pH; even if the amino group of **I** is deprotonated, electron transfer from nitrogen does not participate. Decarboxylation of $\mathbf{I}^{\bullet+}$ to give α-aminoalkyl radicals $\mathbf{V}^{\bullet}$ occurs on the CIDNP time scale and causes strong cooperative effects. The decarboxylation rate is increased significantly by deprotonation of the amino function; this is due to product control. $\mathbf{V}^{\bullet}$ decays by two competing pathways. Fragmentation of the $C^{\beta}-S$ bond in $\mathbf{V}^{\bullet}$ yields vinylamine, which is hydrolyzed to acetaldehyde at pH ≲ 7.25, and thiyl radicals, which then attack the sensitizer to give combination products. Oxidation of $\mathbf{V}^{\bullet}$ by ground-state sensitizer leads to sulfur-containing aldehydes or other products, depending on pH. Relative rates of fragmentation and oxidation were determined from CIDNP signal intensities. From the temperature dependence of the polarizations, the activation energy of β-fragmentation was estimated to be 54 kJ mol^{-1}.

The photoreactions of sulfur-containing amino acids with 4-carboxybenzophenone have received some attention[1,2] because of the biological importance of these substrates and the model character of these reactions for the damage of cell components.[2a] It has been shown that the primary photochemical process is electron-transfer quenching of the excited triplet state of the sensitizer by the amino acid.[2a,b] While these molecules contain two possible donor sites, the thioether group and the amino function, the observation of dimeric radical cations $\rangle S\therefore\overset{+}{S}\langle$ has provided evidence in several cases that the electron is transferred from sulfur.[2a] One of the most important reactions of the radical cations is elimination of CO_2 to give α-aminoalkyl radicals, which are key intermediates with respect to the secondary chemistry in these systems.[1,2a,c,3] α-Aminoalkyl radicals are strongly reducing species,[3a,4] and their oxidation by surplus 4-carboxybenzophenone, which finally leads to aldehydes, provides one of their major decay pathways.[1,2c] Apart from decarboxylation of the sulfur-centered radical cations, deprotonation at C^{α} by the sensitizer anion to give an α-thioalkyl radical, which amounts to a net hydrogen abstraction, also plays some role.[2] The mechanisms of these photoreactions are therefore rather complex and involve several paramagnetic intermediates.

One of the most versatile methods for the study of complex radical reactions is the measurement of chemically induced dynamic nuclear polarizations (CIDNP).[5] CIDNP denotes the occurrence of anomalous NMR line intensities (enhanced absorption or emission) in the products of chemical reactions carried out in a magnetic field. This effect is caused by nuclear spin selective intersystem crossing in intermediate radical pairs. The high diagnostic value of CIDNP experiments draws on five sources: First, the diamagnetic reaction products are observed by high-resolution NMR, so their identification is often straightforward. Further advantages in this respect are that species with lifetimes exceeding a few tenths of a second are detectable with pulsed spectrometers and that the signal enhancement by the CIDNP effect mitigates the low sensitivity of NMR. Second, the polarization patterns, i.e., relative polarization intensities and phases of different protons, contain similar information as the EPR spectra of the radicals involved and therefore allow identification of the intermediates as well. Since the generation of spin polarizations is completed after the correlated life of the radical pairs, a few nanoseconds, CIDNP is sensitive to faster processes than conventional EPR spectroscopy, and it possesses an inherent clock. Third, the overall signal phases are related to the multiplicities of the reacting species. This information is often unaccessible by other methods. Fourth, geminate

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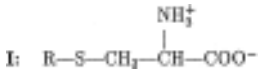
Table 1. Formulas, Abbreviations, and pK_{a2} of the Cysteine Derivatives Used



R	abbreviation	pK_{a2}[a]	pK_{a2}[b]
CH_3-	**Ia**	9.16 ± 0.08	8.80
CH_3-CH_2-	**Ib**	9.11 ± 0.04	8.65
$(CH_3)_3C-$	**Ic**	8.80 ± 0.10	*c*
$^-OOC-CH_2-$	**Id**	9.39 ± 0.06	8.89
$^-OOC-CH_2-CH_2-$	**Ie**	9.28 ± 0.06	9.08

[a] This work, in D_2O. [b] In H_2O (Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1974, 1989; Vol. 1, 6). [c] No literature data available.

processes of radical pairs and reactions of free radicals can be separated by their time dependence. Lastly, the polarizations can be employed as labels to trace the pathways from the intermediates to the products because the polarizations are created at an earlier stage than they are detected.

Despite these advantages, so far there seems to have been but a single CIDNP investigation of sensitized photoreactions of chemically unmodified sulfur-containing amino acids in which any polarizations could be detected, and in that study[6] (methionine sensitized by flavin) only extremely weak CIDNP signals of the starting material were observed and none of any reaction product. Methionine residues in a protein were also found to give rise to such polarizations.[7]

In this work, we use CIDNP experiments to study the photoreactions of five derivatives of cysteine with 4-carboxybenzophenone in aqueous solution. As we will show, electron transfer occurs from sulfur only, even in basic medium where the amino function is deprotonated. However, deprotonation of this group influences the overall reaction indirectly, by increasing the decarboxylation rate through stabilization of the resulting radical. Our CIDNP results further reveal that β-fragmentation of the α-aminoalkyl radicals competes with oxidation by surplus sensitizer and provides a major decay pathway of these intermediates. The polarization intensities allow us to determine the rates of fragmentation and oxidation.

Results and Discussion

Substrates and Protonation Equilibria. The amino acids employed, their abbreviations used in this work, and the pK_{a2} values of their cysteine groups are given in Table 1. pK_{a2} values were determined from the turning points in plots of the chemical shift of H^1 vs pH. Since D_2O was used as the solvent, the pK_{a2} values differ slightly from the values in H_2O, by 0.2−0.5 unit.

In almost all experiments of this work, pH was larger than 6.0, so the sensitizer 4-carboxybenzophenone was present in its anionic form;[8] the cysteine fragments of the amino acids were present in their zwitterionic forms at pH below pK_{a2} and in their anionic forms at higher pH. To simplify the nomenclature, we will omit the charges of the carboxy and amino functions. Thus, for instance, we denote the anion of 4-carboxybenzophenone by CB and the radical dianion of this compound by $CB^{\bullet -}$.

CIDNP Spectra below pH 7. Strong nuclear spin polarizations are observed in these systems upon irradiation, indicating the occurrence of radical pairs in the photoreactions. The appearance of the CIDNP spectra strongly depends on pH. The spectrum of **Ie** at pH 6.39, which is given in Figure 1, provides a typical example of the polarizations found below pH 7. Owing to the experimental technique employed (pseudo-steady-state measurements),[9] this spectrum, as well as all other spectra of this work, shows pure polarizations without background signals. Line intensities are undisturbed by nuclear spin relaxation in the diamagnetic reaction products including cross-relaxation.

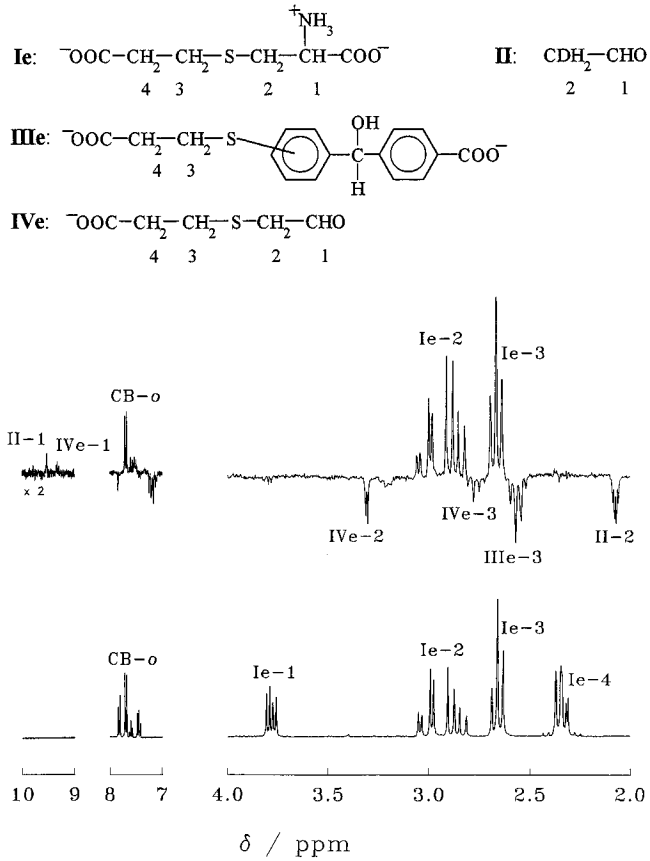


Figure 1. Top trace, background-free pseudo-steady-state CIDNP spectrum observed in the photoreaction of 4-carboxybenzophenone (CB) with *S*-(carboxyethyl)cysteine (**Ie**) in D_2O (pH 6.39) at room temperature. Bottom trace, NMR spectrum of the same system recorded in the dark. Only the spectral ranges of interest are shown. Experimental parameters: $[CB] = 8 \times 10^{-3}$ M, $[\mathbf{Ie}] = 2 \times 10^{-2}$ M. The assignment of the resonances of the products **Ie**, **II**, **IIIe**, and **IVe** refers to the structural formulas given at the top. The numbering of the protons has been chosen to emphasize their correspondence in the different products. CB-*o* denotes the signal of the *ortho* and *ortho′* protons of the sensitizer CB.

Apart from resonances of the starting material, the CIDNP spectrum of Figure 1 exhibits several polarized signals that are due to three new products, **II**, **IIIe**, and **IVe**. The structural formulas of these compounds are displayed in the figure. The connectivity of their spin systems was determined by combining CIDNP and double-resonance experiments. The same aldehyde, **II**, is formed with all five amino acids. Addition of acetaldehyde to the samples showed the chemical shifts of this compound and those of **II** to be identical; however, the splitting pattern revealed that **II** is in fact CDH_2CHO. **IIIe** and the corresponding products in the other systems are stable and were identified to be combination products of the sensitizer with RS−, where R is the respective substituent at sulfur in the cysteine. **IVe** and the analogous compounds with the other amino acids were very unstable and could not be detected in the reaction mixture directly after irradiation. Our characterization of these products as aldehydes $R-S-CH_2-CHO$ which are known to be formed

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Table 2. Chemical Shifts, Multiplet Patterns, and Polarization Phases of the Polarized Protons in the Products **III** and **IV**[a]

R	**III**−3	**IV**−1	**IV**−2	**IV**−3
CH_3-	1.88 (s, E)	9.28 (m, A)	3.24 (d, E)	2.29 (s, E)
CH_3-CH_2-	2.44 (q, E)	9.37 (m, A)	3.33 (d, E)	2.65 (q, E)
$(CH_3)_3C-$	*b*	9.35 (m, A)	3.41 (d, E)	*b*
$^-OOC-CH_2-$	3.04 (s, E)	9.34 (m, A)	3.30 (d, E)	3.22 (s, E)
$^-OOC-CH_2-CH_2-$	2.57 (t, E)	9.33 (m, A)	3.30 (d, E)	2.78 (t, E)

[a] For the general structures of **III** and **IV** and the numbering of the protons, see Figure 1. [b] Not applicable.

in these reactions,[1,2c] is based on the following evidence. From chemical shifts and multiplet patterns, two product moieties are identified. One is an $-S-CH_2-CHO$ fragment that is independent of the amino acid employed, the other is an RS− fragment like the one contained in the combination product **III**. That these two moieties belong to the same product is established by our observation that the relative polarizations of the protons in these two fragments are constant regardless of the experimental parameters, whereas, for instance, the ratio of these polarizations to the polarizations of **II** or **III** strongly depends on the sensitizer concentration (see below).

The NMR parameters of compounds **III** and **IV** are listed in Table 2. Lowering pH led to no changes in the relative intensities of the CIDNP signals. Experiments below pH ≈ 4.5, the pK_a value of the sensitizer,[10] were impracticable because the protonated form of CB is hardly soluble in water.

Identification of the Paramagnetic Intermediates. For the regenerated starting compound **Ie**, protons H^2 and H^3, which are connected to the carbon atoms adjacent to sulfur, are seen to be polarized with equal phase and intensity whereas the more remote protons H^1 and H^4 are unpolarized (Figure 1). This polarization pattern is in accordance with the spin density distribution in a sulfur-centered radical cation $\mathbf{I}^{\bullet+}$ (compare the data[11] for structurally similar radical cations). The basis of this reasoning is provided by the known fact that sign and magnitude of the polarization P_i of proton i in the products reflect sign and magnitude of the hyperfine coupling constant a_i of this proton in the paramagnetic intermediates; often, there is even direct proportionality between P_i and a_i.[5]

According to Kaptein's rule for CIDNP net effects,[12]

$$\Gamma_i = \mathrm{sgn}a_i \times \mathrm{sgn}\Delta g \times \mu \times \epsilon \qquad (1)$$

the polarization phase ($\Gamma_i = +1$, absorption; $\Gamma_i = -1$, emission) of proton i is determined by magnetic parameters of the intermediate radical pairs (sgna_i, sign of the hyperfine coupling constant of proton i; sgnΔg, sign of the difference of the g values of the two radicals of the pair, where the radical counted first contains proton i) and by the entry and exit channels of the pairs ($\mu = +1$, pair formation from triplet precursors; $\mu = -1$, pair formation from singlet precursors; $\epsilon = +1$, product formation from singlet radical pairs, which in our case means in a cage reaction; $\epsilon = -1$, product formation from triplet pairs, i.e., via radicals escaping from the cage). 4-Carboxybenzophenone reacts from its electronic triplet state, so μ is +1 in all our experiments. Sulfur-centered radical ions are known to possess very high g values ($g > 2.01$),[11b,13] whereas the g value of $CB^{\bullet-}$ cannot differ much from that of the benzophenone radical anion ($g = 2.0037$);[14] the hyperfine coupling constants of α-protons in a sulfur-centered radical cation are positive.[11b] The absorption signal observed for H^2 and H^3 of **Ie** is thus consistent with regeneration of the reactants by spin-allowed back electron-transfer of singlet pairs $\overline{\mathbf{I}^{\bullet+}CB^{\bullet-}}$.

The polarization pattern of the reaction products **II** and **IVe** is obviously different from that of the starting amino acid, showing opposite phases for vicinal protons. This pattern is compatible with CIDNP generation, at least to some degree, in alkyl radicals, where $\sigma-\pi$ spin polarization serves to induce a negative spin density at H^α and hyperconjugation a positive spin density at H^β.[15] From the known[1,2c] pathway of formation of aldehydes **IV** in these systems, via decarboxylation of the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ to give α-aminoalkyl radicals $R-S-CH_2-\dot{C}HNH_2$ ($\mathbf{V}^\bullet$) which are then oxidized by surplus CB, radical pairs containing $\mathbf{V}^\bullet$ appear as the most natural explanation for the observed polarization pattern. This mechanism has three implications. First, the other radical contained in the pairs must still be the radical anion $CB^{\bullet-}$ of 4-carboxybenzophenone because this is left unchanged by the decarboxylation of $\mathbf{I}^{\bullet+}$. Second, the aldehyde proton of **IV** must have been attached to the radical center in $\mathbf{V}^\bullet$; hence, for this proton a is negative. Third, the aldehydes **IV** are products of free radicals having escaped from the cage, so $\epsilon = -1$. Thus it would follow from eq 1 that the g value of $\mathbf{V}^\bullet$ is larger than that of $CB^{\bullet-}$. This is contrary to expectation based on the data for similar radicals (for instance, the g value of $CH_3\dot{C}HNEt_2$ is noticeably smaller than that of the benzophenone radical anion).[16] An explanation of this apparent discrepancy will be given below.

The magnitudes of the hyperfine coupling constants of α- and β-protons in α-aminoalkyl radicals are comparable, whereas more remote protons only possess extremely weak hyperfine coupling constants.[17] In contrast, the aldehyde proton H^1 of **IVe** is much more weakly polarized than the aliphatic protons H^2, and the polarizations of H^3 are found to be as strong as those of H^2. The polarization pattern observed therefore cannot be due solely to CIDNP generated in pairs $\overline{\mathbf{V}^\bullet CB^{\bullet-}}$. Rather there must be a significant contribution of the primary pairs $\overline{\mathbf{I}^{\bullet+}CB^{\bullet-}}$, in which polarizations of equal sign and magnitude are generated for H^2 and H^3; the emissive phase of these polarizations is in line with aldehyde formation by an escape reaction.

Radical pair type CIDNP means spin sorting, so polarizations of escape products must be accompanied by opposite polarizations of cage products. It seems strange that no resonances can be assigned to cage products of the radical pairs $\overline{\mathbf{V}^\bullet CB^{\bullet-}}$ (**III** is also an escape product; see next section). Two factors may provide an explanation for this anomaly. First, geminate recombination of $\overline{\mathbf{V}^\bullet CB^{\bullet-}}$ will occur at C^1 of $\mathbf{V}^\bullet$, so one expects merely a small influence on the chemical shift of H^2 and practically none on that of H^3; hence, the most strongly polarized signals of the cage products may be obscured by the educt resonances. Second, this recombination must yield a carbanion; owing to the reactivity of this intermediate, the polarizations might be distributed among several products and would thus be correspondingly weak.

As Figure 1 shows, the aromatic region of the CIDNP spectrum is dominated by a strong absorption signal, CB-*o*, for

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the *ortho* and *ortho′* protons of the sensitizer; the other protons are almost unpolarized. There are also some weaker new signals, which are most probably due to combination products but which we did not assign. The polarization pattern found for regenerated CB is in accordance with the intermediacy of radical anions $CB^{\bullet-}$ because *ortho* and *ortho′* are the only positions in these radicals for which one expects substantial proton hyperfine couplings. With the known sign of $a_{o,o'}$ (minus),[15] and the known multiplicitites of precursors and pairs undergoing back electron-transfer ($\mu = +1$, $\epsilon = +1$), the absorptive polarization of $H^{o,o'}$ indicates that the *g* value of $CB^{\bullet-}$ is smaller than the *g* value of the sulfur-containing radical, which is consistent with the above results obtained from the polarization patterns of the starting compound **Ie** and the cysteine-derived product moieties **II**−**IVe**.

The same behavior as for **Ie**, with respect to both educt and product polarizations, was also observed for the other amino acids.

Mechanism of Radical Fragmentation. The formation of acetaldehyde **II** on the one hand and of a combination product **IIIe** of the sensitizer with the cysteine substituent R on the other, which are both spin-polarized, implies fragmentation at sulfur of one of the intermediates. Given the structural formulas of **II** and **IIIe**,[18] it is natural to assume that the two products result from the same C−S cleavage step. That this is indeed the case is borne out by the observation that for all our systems the ratio of polarizations of protons **II**-2 and **III**-3 does not depend on experimental conditions such as sensitizer concentration or temperature, whereas, for instance, the ratio of polarizations of protons **II**-2 and **IV**-2 does (see below).

As Figure 1 shows, the polarization pattern of protons **II**-1, **II**-2, and **IIIe**-3 is identical with that of protons **IVe**-1, **IVe**-2, and **IVe**-3 (medium absorption, strong emission, strong emission, relative intensities being about 1:−8:−10 in both cases). Hence, the polarizations in **II** and **IIIe** must have the same origin as those of **IVe**, that is, they must stem from a superposition of CIDNP arising in the primary pairs $\overline{\mathbf{I}^{\bullet+}CB^{\bullet-}}$ and, to a smaller degree, of CIDNP arising in pairs $\overline{\mathbf{V}^{\bullet}CB^{\bullet-}}$; furthermore, **II** and **III** are escape products, as is **IV**. The same result was found for the other cysteine derivatives; there, however, the amounts of polarizations from $\overline{\mathbf{V}^{\bullet}CB^{\bullet-}}$ are somewhat larger than in the system **Ie**/CB.

The fact that the relative contribution of polarizations from pairs $\overline{\mathbf{V}^{\bullet}CB^{\bullet-}}$ to the total polarizations is the same for the fragmentation products and the products of oxidation of escaping $\mathbf{V}^{\bullet}$ by ground-state sensitizer shows that scission of the C−S bond does not occur in the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ but in the α-aminoalkyl radicals $\mathbf{V}^{\bullet}$. *β*-Thioalkyl radicals are indeed known to undergo cleavage of the bond between C^{β} and sulfur to give an olefin and a thiyl radical.[19] This reaction,

$$\mathrm{R{-}S{-}CH_2{-}\dot{C}HNH_2\ (V^{\bullet})} \longrightarrow \begin{cases} \mathrm{CH_2{=}CHNH_2\ (VI)} \longrightarrow \mathbf{II} \\ + \\ \mathrm{R{-}S^{\bullet}} \longrightarrow \mathbf{III} \end{cases} \quad (2)$$

provides a straightforward rationalization both of the products formed in our systems and of their polarizations. Thermodynamic considerations indicate that the thiyl radicals $R{-}S^{\bullet}$ should terminate preferentially by reaction with the aromatic rings of the sensitizer, which eventually leads to **III**, rather than by hydrogen abstraction from the solvent; owing to the small radical concentrations in our experiments, disulfide formation would not be expected to be significant. For the same reason, and because it is an escape product, **III** must stem from attack of $R{-}S^{\bullet}$ to ground-state sensitizer, not to $CB^{\bullet-}$. As shown in eq 2, the olefin resulting from $C^{\beta}{-}S$ cleavage of $\mathbf{V}^{\bullet}$ is an enamine (vinylamine), so hydrolysis of this compound is an obvious pathway to acetaldehyde. The exclusive incorporation of a single deuterium atom in the *β*-position of acetaldehyde supports the intermediacy of an enamine and thus cleavage of $\mathbf{V}^{\bullet}$ according to eq 2 because it is well known[20] that the first step of enamine hydrolysis is protonation at C^{β}, whereas this position is not involved in the later stages of this reaction. As will be shown below, in experiments at higher pH the enamine is also observed directly in the CIDNP spectra.

Rates of Radical Fragmentation. Cleavage and oxidation by ground-state CB should be competing reactions of the radicals $\mathbf{V}^{\bullet}$

$$\mathbf{II} + \mathbf{III} \quad \longleftarrow \overset{k_{frag}}{\longleftarrow} \quad \mathbf{V}^{\bullet} \quad \overset{k_{ox}[CB]}{\longrightarrow} \quad \longrightarrow \quad \mathbf{IV} \qquad (3)$$

(In eq 3, the primary products resulting from these two processes have been omitted for clarity.) It is therefore to be expected that relative rates of these reactions can be obtained from relative product yields. Since the aldehydes **IV** are rather unstable, such an analysis of the product distribution is not feasible with the usual methods.

However, the same information is accessible from the CIDNP spectra because polarization intensities are proportional to the amount of the respective product formed. The validity of this approach is based on the condition that the constant of proportionality is identical for the signals compared. This requirement is met under the following circumstances.

First, the polarizations considered must stem from the same source. This does not imply that the reaction may only proceed via a single radical pair; with more complex reaction mechanisms, as in our case, this is still fulfilled if each type of radical pairs makes the same relative contribution to these polarizations. The latter has already been shown in the preceding section; moreover, it seems to be ensured for our systems because pathways from the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ to one of the products **II**, **III**, or **IV** that bypass the α-aminoalkyl radicals $\mathbf{V}^{\bullet}$ are hardly conceivable from a chemical point of view. As long as this condition holds, the polarizations present in $\mathbf{V}^{\bullet}$ are simply partitioned between the products, and everything that influences concentrations and polarizations at a stage of the reaction up to and involving $\mathbf{V}^{\bullet}$, in particular such factors as the different efficiencies of CIDNP generation in the pairs $\overline{\mathbf{I}^{\bullet+}CB^{\bullet-}}$ and $\overline{\mathbf{V}^{\bullet}CB^{\bullet-}}$, affects the polarizations of all products in exactly the same degree. As a further consequence of the requirement that the polarizations evaluated have the same origin, only protons that were identical in the radicals should be compared, as, for instance, **II**-2 and **IV**-2 or **III**-3 and **IV**-3.

Second, errors due to nuclear spin relaxation must be avoided. With respect to the diamagnetic products, this poses no problems because of the experimental technique used (pseudo-steady-state CIDNP measurements),[9] but in our case the products of interest are formed via free radicals, and nuclear spin relaxation in these rather long-lived paramagnetic intermediates may not always be neglected. However, only *differences* of the relaxation rates count, so relaxation in $\mathbf{V}^{\bullet}$ is circumvented by considering protons that were identical in $\mathbf{V}^{\bullet}$, as already mentioned; the same holds for relaxation in free radicals $\mathbf{I}^{\bullet+}$. Even so, on the route to **III**

(18) As indicated in Figure 1, the structure of the combination products **III** is not yet known completely. However, this does not invalidate any of our mechanistic conclusions that follow.

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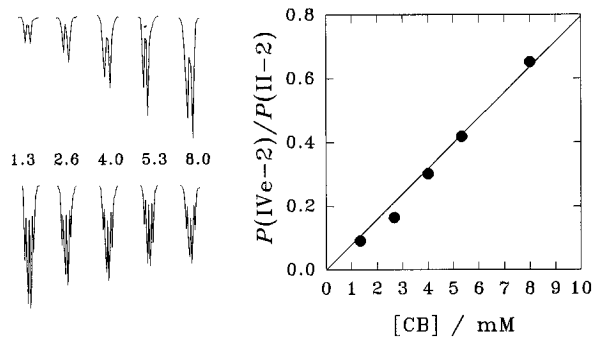


Figure 2. Left, dependence of the polarization intensities of the β-protons in the oxidation product **IVe** and the fragmentation product **II** (upper trace, CIDNP signal of **IVe**-2; lower trace, that of **II**-2) on the sensitizer concentration, [CB] (given between the traces; in 10^{-3} M). Right, ratio P(**IVe**-2)/P(**II**-2) of these polarizations as a function of [CB]. Polarizations P were determined by integration over the multiplets. Experimental parameters: [**Ie**] = 1×10^{-2} M, pH = 6.8, room temperature.

an additional paramagnetic species, the thiyl radical, is successor to **V**•, whereas after the stage of **V**• formation of both **II** and **IV** involves diamagnetic species only. Hence, comparison of the signals **II**-2 and **IV**-2 eliminates all effects of nuclear spin relaxation in the radicals, whereas comparison of the signals **III**-3 and **IV**-3 would be less reliable.

Third, none of the products may decompose significantly during the time needed for acquisition of its free induction decay. Even for **IV**, which is unstable, this appears to be fulfilled in our case because no manifestations (line broadening or dispersive signal components)[21] of such a fast secondary reaction could be observed.

To test whether eq 3 holds, e.g., whether the products **II** and **III** on the one hand and **IV** on the other are formed via a simple competition between fragmentation and oxidation of the α-aminoalkyl radicals **V**•, we varied the concentration of CB. An increase of [CB] should favor oxidation, hence formation of the sulfur-containing aldehyde **IV**, over fragmentation, which is formation of monodeuterated acetaldehyde **II** and combination product **III**. From the spectra at the left in Figure 2, which display the resonances of **IVe**-2 and **II**-2 as functions of the sensitizer concentration, this is seen to be qualitatively true. According to eq 3, the ratio of polarization intensities, P(**IV**-2)/P(**II**-2), of these two signals should be equal to k_{ox}[CB]/k_{frag}, so a plot of this polarization ratio vs [CB] should be linear, with vanishing intercept. This plot is given at the right in Figure 2. As is evident from it, the above assumption is also quantitatively true; furthermore, it shows the validity of the described evaluation procedure of relative CIDNP intensities.

The ratios k_{ox}/k_{frag} obtained for our cysteine derivatives are listed in Table 3. The rate constants k_{ox} for oxidation of **V**• by ground-state CB are known[2a] from pulse radiolysis experiments in which the rates of secondary formation of $CB^{\bullet -}$ were measured as functions of [CB]; they have also been compiled in the table. With these data, k_{frag} can be computed. As Table 3 shows, the values of k_{frag} are very similar for the amino acids employed, as are those of k_{ox}. Hence, the substituent R has no significant influence on the fragmentation of **V**• nor on the oxidation of this radical by CB.

Even in the absence of an electron acceptor such as CB, the lifetimes $1/k_{frag}$ of the radicals **V**• are seen to be about 70 ns only. This short life can fully rationalize why no oxidation of the α-aminoalkyl radical **Va**• by ground-state sensitizer was observed when the photoreaction of **Ia** was sensitized with 2×10^{-4} M *N*-(9-methylpurin-6-yl)pyridinium cation[22] or when pulse radiolysis experiments with **Ia** were performed in the presence of 2.5×10^{-5} M methylviologen.[4]

Table 3. Rate Constants of the Secondary Reactions of the Cysteine-Derived α-Aminoalkyl Radicals **V**• at Room Temperature (R is the substituent at sulfur)

R	k_{ox}[a] (10^9 M^{-1} s^{-1})	k_{ox}/k_{frag}[b] (M^{-1})	k_{frag} (10^7 s^{-1})
CH_3-	1.27	73	1.74
CH_3-CH_2-	1.08	84	1.28
$(CH_3)_3C-$		78	
$^-OOC-CH_2-$	0.78	68[c]	1.14
$^-OOC-CH_2-CH_2-$	0.96	79	1.21

[a] From ref 2a; estimated error ≤ 20%. [b] This work; ±10%. [c] Error ± 30% owing to low signal-to-noise ratio of the signals **II**-2 and **IVd**-2 caused by predominant reaction at the substituent R.

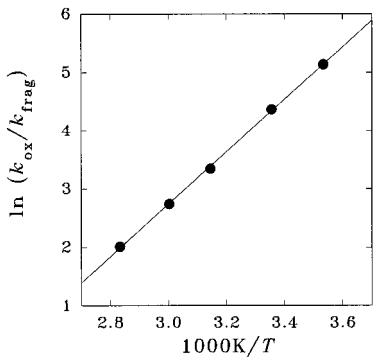


Figure 3. Plot according to eq 4 for compound **Ie**. For further explanation, see text.

Activation Energy of Radical Fragmentation. Assuming Arrhenius behavior, plots of $\ln k_{ox}/k_{frag}$ vs $1/T$ should be linear, with slopes that are determined by the differences of the activation energies E_a of fragmentation and oxidation of **V**• and intercepts that reflect the ratios of the preexponential factors, A, of both processes:

$$\ln \frac{k_{ox}}{k_{frag}} = \ln \frac{A_{ox}}{A_{frag}} + \frac{E_{a,frag} - E_{a,ox}}{R} \frac{1}{T} \qquad (4)$$

In Figure 3, such a plot is shown for the derivative **Ie**. From this graph, one obtains a value of 38.5 ± 0.7 kJ mol^{-1} for the difference $E_{a,frag} - E_{a,ox}$ and of about 1×10^{-5} M for the ratio A_{ox}/A_{frag}. The activation energies for oxidation of **V**• by CB are not known. However, calculation of the rate constant k_{diff} of a diffusion-controlled reaction by the Smoluchowski equation:

$$k_{diff} = \frac{8RT}{3\eta}\gamma \qquad (5)$$

modified by the Debye factor γ to take into account the Coulombic interactions between ions of charge z_1 and z_2,

$$\gamma = \frac{z_1 z_2 e^2}{4\pi\epsilon_0\epsilon_r dkT} \frac{1}{\exp[z_1 z_2 e^2/(4\pi\epsilon_0\epsilon_r dkT)] - 1} \qquad (6)$$

yields a value of about 3×10^9 M^{-1} s^{-1} for a reaction in water between two ions of charge −1 with an assumed encounter distance d of 4−5 Å. This is not much larger than the experimental value of k_{ox} in the system **Ie**/CB (compare Table 3); moreover, the Smoluchowski equation usually overestimates

(21) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon Press: Oxford, 1987; pp 211−215.

(22) Marciniak, B.; Hug, G. L.; Rozwadowski, J.; Bobrowski, K. *J. Am. Chem. Soc.* **1995**, *117*, 127−134.

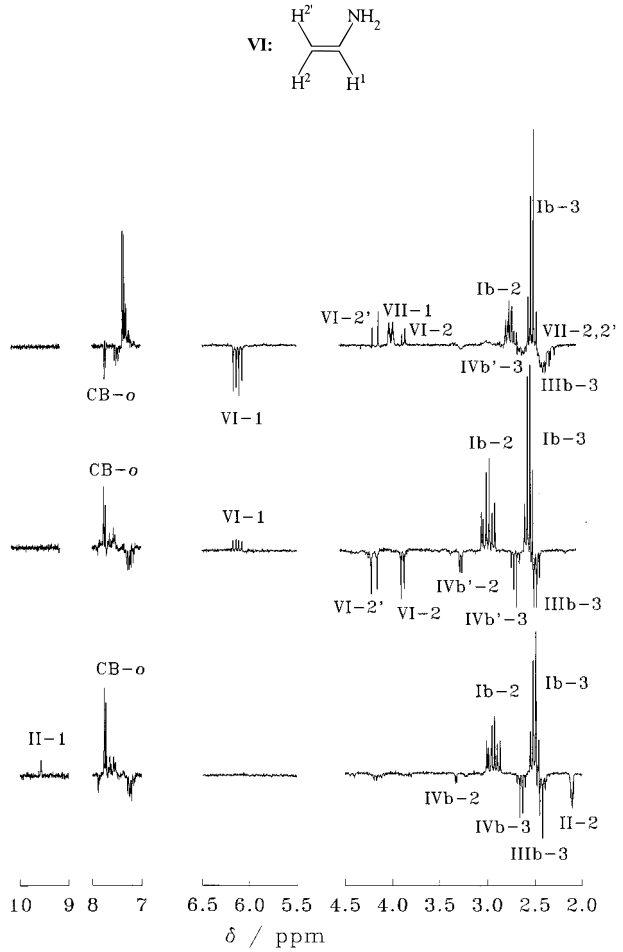


Figure 4. CIDNP spectra of the system 4-carboxybenzophenone/*S*-(ethyl)cysteine (CB/**Ib**) at different pH (bottom trace, pH = 6.29; center trace, pH = 7.63; top trace, pH = 11.43). For the assignment of the resonances of **II** and **VI**, see the formulas in Figure 1 and this figure, respectively. Signals **IV′** and **VII** are explained in the text. Experimental parameters: [CB] = 2×10^{-3} M, [**Ib**] = 2×10^{-2} M, room temperature.

k_{diff} slightly. Hence, we assume that the oxidation of $\mathbf{V}^{\bullet}$ by CB is practically diffusion controlled in this case and approximate $E_{a,ox}$ by the activation energy of the viscosity η of water, which is 15.6 kJ mol^{-1}.[24] Thus, we arrive at an activation energy of 54 kJ mol^{-1} for the fragmentation of $\mathbf{V}^{\bullet}$ according to eq 2, which may be uncertain by a few kilojoules per mole owing to the approximation involved.

Comparable values of $E_{a,frag} - E_{a,ox}$ were also obtained for the other cysteine derivatives.

CIDNP Experiments in Basic Solution. Interesting changes of the product signals and of their polarization patterns occur when pH is raised. Figure 4 shows CIDNP spectra of **Ib** as examples.

Above pH ≈ 7.25, the signals from acetaldehyde **II** vanish, and a characteristic ABX pattern appears instead. From chemical shifts and coupling constants ($\delta(H^1) = 6.10$, $\delta(H^2) = 3.80$, $\delta(H^{2'}) = 4.10$; $^2J_{22'} \approx 0$ Hz, $^3J_{12} = 9.6$ Hz, $^3J_{12'} = 15.5$ Hz), the product giving rise to these signals was identified as vinylamine (**VI**), $CH_2{=}CHNH_2$; the spectral parameters listed are quite similar to those[16,25] of substituted vinylamines. There is ample precedent for formation of the latter compounds from α-aminoalkyl radicals produced in the sensitized photoreactions of tertiary aliphatic amines, although the mechanism is quite different in those instances, hydrogen abstraction being followed by reaction of the α-aminoalkyl radicals with surplus sensitizer.[16,25] Raising pH leaves unchanged the chemical shifts of **VI**. This shows that the amino group is not protonated for pH > 7.25, so the pK_a value of this group must be lower than about 7.

As can be seen in Figure 4, the polarization pattern of **VI** at pH ≈ 7.6 (absorption for H^1, emission for $H^{2,2'}$) is qualitatively and quantitatively identical with that of **II** at lower pH. This is consistent with formation of the aldehyde **II** by acid-catalyzed hydrolysis of **VI**. Moreover, the observation of **VI** provides direct evidence for the β-fragmentation of the radicals $\mathbf{V}^{\bullet}$ according to eq 2.

At the high magnetic field employed in our experiments, the polarizations of the protons in a particular product should be approximately proportional to the hyperfine coupling constants of these protons in the radicals weighted with their number.[5e] The ratio of hyperfine coupling constants of the α- and β-protons—i.e., those corresponding to H^1 and H^2 in $\mathbf{V}^{\bullet}$—in the model compound $CH_3CH_2\dot{C}HN(n\text{-}Pr)_2$ is −1:1.36,[17] so for **VI**, one would expect a polarization ratio $P(H^1):P(H^{2,2'})$ of about −1:2.7. The experimental ratio is larger than this (about −1:4 in Figure 4, center and bottom traces), thus again indicating that the polarizations of **VI** and its secondary product **II** partly arise in pairs $\overline{\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}}$ as well, which yield emissive polarizations for $H^{2,2'}$ but none for H^1. With **Ib** these polarizations are seen to stem predominantly from the pairs $\overline{\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}}$; this is also found for the other cysteine derivatives except **Ie**, for which polarizations from $\overline{\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}}$ have already been shown to prevail ($P(H^1):P(H^{2,2'}) \approx -1:8$; compare Figure 1). Common to all systems investigated is the unexpected sign of Δg of the pairs $\overline{\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}}$ under these experimental conditions that is obtained from the polarization phases with eq 1. Both this result and the behavior of **Ie** that is different from the other substrates with respect to the relative amounts of polarizations from the two radical pairs are related; these effects will be discussed below.

At high pH (top trace of Figure 4), the reaction still leads to the vinylamine **VI**, but under these circumstances **VI** displays the opposite polarization phases as at medium pH, namely, emission for H^1 and absorption for H^2. This change of phase with pH is paralleled by an inversion of the CIDNP signal CB-*o* due to the *ortho* and *ortho′* protons of the sensitizer. Both these observations can be rationalized with predominant generation at high pH of the polarizations of **VI** in the pairs $\overline{\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}}$, the polarization phases now indicating a "normal" *g*-value difference. On the basis of the hyperfine coupling constants, one would expect the absorption signals of $H^{2,2'}$ in **VI** to be larger by a factor of about 3 than the experimental intensities. This shows that at high pH there is still an emissive contribution from the pairs $\overline{\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}}$ to the polarizations of these protons.

In principle, the dependence of the polarization patterns of **VI** on pH might be caused by a change of the exit channel leading to this product. At pH ≳ 7, no signals from the aldehyde **IV** are detectable (see below), so determination of ϵ for **VI** by comparison of its polarization phases with those of **IV**, which is by necessity an escape product, is not possible any longer. However, ϵ can also be obtained from the phases of CIDNP

(23) As the pK_a value of α-aminoalkyl radicals is about 3.9,[4] the amino group of $\mathbf{V}^{\bullet}$ is deprotonated under our experimental conditions.

(24) Calculated from the values of η in Weast, R. C., Ed. *Handbook of Chemistry and Physics, Student Edition*; CRC Press: Boca Raton, FL, 1988; p F-19.

(25) (a) Roth, H. D.; Manion, M. L. *J. Am. Chem. Soc.* **1975**, *97*, 6886−6888. (b) Roth, H. D. In ref 5d, pp 53−61. (c) Goez, M.; Frisch, I. *J. Photochem. Photobiol. A* **1994**, *84*, 1−12.

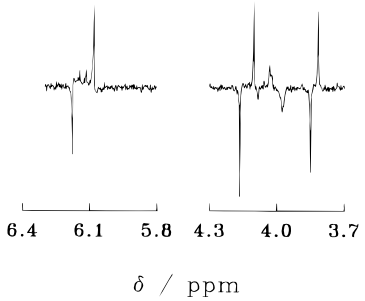


Figure 5. CIDNP multiplet effects in the system 4-carboxybenzophenone/*S*-(methyl)cysteine (CB/**Ia**) at pH 10.3. Other experimental parameters were as in Figure 4. Shown are the resonances **VI**-1 (left) and **VI**-2′, **VII**-1, and **VI**-2 (right). The structural formula of **VI** and the assignments are given in Figure 4. The spectrum was obtained by subtracting a set of free induction decays acquired with a flip angle φ, of 135° from a set acquired with $\varphi = 45°$.[26] For an explanation on why the two inner lines of **VI**-1 are missing, see ref 16, note 40.

multiplet effects, even if the sign of Δg is unknown. As $|\Delta g|$ for $\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}$ is estimated to be larger than 9×10^{-3}, whereas the value for $\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}$ should be about 7×10^{-4}, multiplet effects from the former pairs should be vanishingly small,[5] and no ambiguity is expected to arise from the participation of two radical pairs in the reaction. Kaptein's rule[12] for a multiplet effect of protons *i* and *j*:

$$\Gamma_{ij} = \mu \times \epsilon \times \mathrm{sgn}a_i \times \mathrm{sgn}a_j \times \mathrm{sgn}J_{ij} \times \sigma_{ij} \qquad (7)$$

connects the multiplet phase ($\Gamma_{ij} = +1$, E/A, i.e., emission at the low-field edge of the multiplet, absorption to high field; $\Gamma_{ij} = -1$, A/E) with the sign of the hyperfine coupling constants of both nuclei and the sign of the nuclear spin−spin coupling constant J_{ij} in the product. The parameter σ_{ij} takes into account whether both nuclei were contained in the same radical of the intermediate pairs ($\sigma_{ij} = +1$), or in different radicals ($\sigma_{ij} = -1$); μ and ϵ have the same meaning as in eq 1.

Net and multiplet effects were separated by their dependence[26] on the flip angle φ of the observation pulse. Two sets of spectra were acquired with φ being 45° and 135°, respectively. Subtracting the second set from the first yields pure multiplet effects, which are shown in Figure 5 for the compound **Ia**. The polarization pattern is seen to be an E/A multiplet. The signs of the hyperfine coupling constants in $\mathbf{V}^{\bullet}$ are known, as is μ; ^3J is positive.[27] In the present case, σ_{ij} must be +1; furthermore, this variable can be eliminated by a combined evaluation of net and multiplet effects,[28] which leads to the same result, namely, that **VI** is an escape product also at high pH.

The aldehydes **IV** behave in a manner similar to **II**: Above pH ≈ 7, the signal **IV**-2 vanishes and is replaced by another signal, **IV**′-2. The latter is a doublet ($J = 5.2$ Hz) that is high-field shifted by about 0.1 ppm; it displays the same phase (emission) and the same relative intensity with respect to the educt signals as before. The signal that was due to **IV**-3 remains almost at the same position. The fate of the signal **IV**-1 cannot be determined owing to its extremely low intensity. The peaks **IV**′-2 and **IV**′-3 replacing the aldehyde signals at pH ≳ 7 exhibit the same dependence on the concentration of CB as do **IV**-2 and **IV**-3 at low pH. While so far we have not been able to identify the new product unambiguously, we explain these changes in the same way as for **II**, by the stopping of the reaction at the stage of an "earlier" diamagnetic product, and

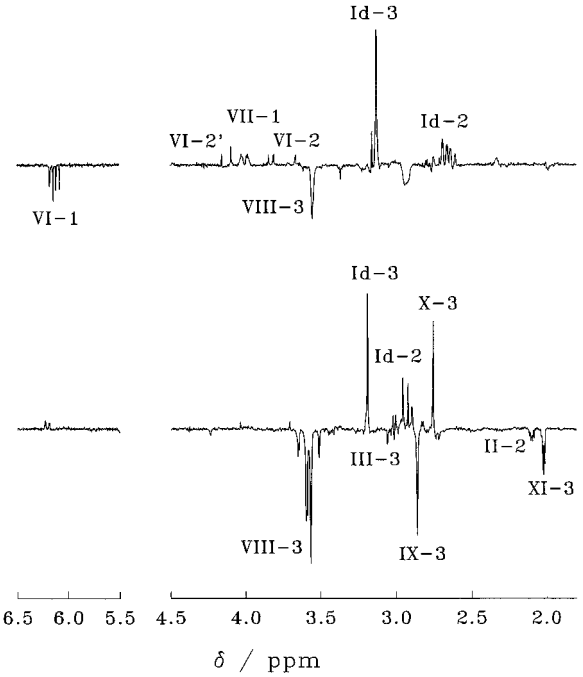


Figure 6. CIDNP spectra of the system 4-carboxybenzophenone/*S*-(carboxymethyl)cysteine (CB/**Id**) at different pH (bottom trace, pH = 6.33; top trace, pH = 11.11). Only the spectral regions of interest are shown. The signals **VIII**−**XI** are explained in the text. For the assignment of the other CIDNP signals, see preceding figures. Experimental parameters: [CB] = 2×10^{-3} M, [**Id**] = 2×10^{-2} M, room temperature.

we tentatively assign these signals to the imine (R−S−CH_2−CH═NH) formed by oxidation of $\mathbf{V}^{\bullet}$ by ground-state CB. When pH is raised further, **IV**′-2 decreases but does not change phase; for pH ≥ pK_{a2}, **IV**′-3 is buried under the educt signal **I**-2, which is shifted toward higher field by the deprotonation of the amino group.

The position and phase of the signals **III**-3 stemming from a combination product between CB and the thiyl radicals resulting from the fragmentation of $\mathbf{V}^{\bullet}$ are unaffected by pH. However, the intensity of these peaks decreases with increasing pH. The same is observed for the polarizations of the starting amino acid: Their polarization pattern (enhanced absorption for H^2 and H^3, no polarizations for the other protons) is independent of pH, but the signal intensities relative to those of the products are smaller at high pH.

Lastly, we mention for completeness that at pH ≥ pK_{a2} a further ABX pattern appears (**VII**-1, $\delta = 3.96$ ppm, absorption; **VII**-2,2′, $\delta = 2.30$ ppm, emission), which has not been identified yet. Its spectral parameters are identical in all systems investigated, so it must be attributed to a reaction of the cysteine moiety. From the phase of the multiplet effect (A/E, compare Figure 5), **VII** is seen to be a cage product. The fact that these signals are not observed below pH = pK_{a2}, nor other signals that can be related to them, leads us to infer that this product is due to a reaction of the deprotonated amino function.

CIDNP Spectra of *S*-(Carboxymethyl)cysteine (Id). The quencher **Id** shows a slightly more complicated behavior than the other four amino acids because its photoreactions also take place at the carboxymethyl substituent, not only at the cysteine function. This manifests itself by the occurrence (compare Figure 6, bottom trace) of four new CIDNP signals (**VIII**-3, **IX**-3, **X**-3, and **XI**-3) that are also found in the photoreactions of CB with compounds possessing an HOOC−CH_2−S− group but no cysteine moiety, as, for example, thiodiglycolic acid, $S(CH_2COOH)_2$. If the CIDNP spectrum of **Id** is shifted by

(26) Schäublin, S.; Höhener, A.; Ernst, R. R. *J. Magn. Reson.* **1974**, *13*, 196−216.
(27) Reference 15, p 67.
(28) Goez, M.; Frisch, I. *J. Am. Chem. Soc.* **1995**, *117*, 10486−10502.

about 0.05 ppm toward higher field such that the signal of the methylene protons of HOOC−CH_2−S− in **Id** coincides with that of the corresponding protons in thiodiglycolic acid, the signals **VIII**-3, **IX**-3, **X**-3, and **XI**-3 in both systems coincide as well; moreover, relative polarization intensities are very similar. All this clearly shows that **VIII**−**XI** are products of reactions at HOOC−CH_2−S−.

A detailed discussion of the new products is outside the scope of this work, which is focused on the reactions of the cysteine moiety, and will be given elsewhere.[29] Pertinent to the present investigation is, however, what intermediate these polarizations stem from. Time-resolved experiments show **VIII**, which gives rise to the strongest CIDNP signal in Figure 6, bottom trace, and also by far the largest of the four new signals, to be a cage product ($\epsilon = +1$). According to eq 1, the term $\mathrm{sgn}\Delta g \times \mathrm{sgn} a$ must thus be negative, which cannot be reconciled with generation of these polarizations in radical pairs $\mathbf{Id}^{\bullet+}\mathrm{CB}^{\bullet-}$ because the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ possess very high g values (i.e., $\Delta g > 0$) and positive hyperfine coupling constants for the protons of interest, H^3.[11b] However, radical cations of structure HOOC−CH_2−$X^{\bullet+}$−R, where X is a heteroatom such as N, O, or S, easily lose CO_2 to give α-substituted alkyl radicals $^{\bullet}CH_2$−X−R. This decarboxylation is rapid on the CIDNP time scale;[30] hence, it occurs within nanoseconds or even faster. The emissive polarization of **VIII**-3 is consistent with CIDNP generation in pairs $^{\bullet}CH_2$−S−R $CB^{\bullet-}$: The g value of the α-thioalkyl radical is certainly larger than that of $CB^{\bullet-}$ (for instance, $^{\bullet}CH_2SCH_3$ has[31] $g = 2.0049$), the hyperfine coupling constants of the protons at the radical center are negative,[15] and the product **VIII** contains a −CH_2−S group instead of the carboxymethyl group.[29]

At low pH (see Figure 6, bottom trace), the CIDNP spectrum of **Id** is dominated by the signal **VIII**-3. The polarizations of the starting amino acid—which were the strongest signals in the other systems—are noticeably smaller, and the polarizations of the products of reaction at the cysteine group are very weak. The polarization pattern of the educt **Id** (absorption for H^2 and H^3, no polarizations of H^1) is the same as in the other systems, indicating the intermediacy of sulfur-centered radical cations $\mathbf{Id}^{\bullet+}$. Likewise, the polarization pattern of **II** (absorption for H^1, emission for H^2) is identical with that observed with the other amino acids. These observations show that α-aminoalkyl radicals are indeed formed at pH ≈ 7, so decarboxylation at the cysteine moiety occurs, but decarboxylation of the carboxymethyl substituent prevails.

At pH above pK_{a2} (top trace of Figure 6), the picture is different. Under these circumstances, the emission signal **VIII**-3 is still prominent[32] and still the strongest of all the CIDNP signals that are caused by decarboxylation at HOOC−CH_2−S−. However, the most intense product signal in the CIDNP spectrum is now due to the vinylamine **VI**, the product of decarboxylation of the cysteine function. As the magnetic parameters of the intermediate α-thioalkyl and α-aminoalkyl radicals are constant within the pH range considered,[23] this change of relative polarization intensities with pH must be related to changes of relative product yields and, by the same token, to changes of relative decarboxylation rates. An effect of pH on decarboxylation of the *S*-carboxymethyl substituent seems hardly conceivable, since this group is deprotonated throughout the pH range investigated. Hence, it must be concluded that above pK_{a2} cysteine decarboxylation is significantly faster than below pK_{a2}.

Decarboxylation Pathway. Two explanations are conceivable for the increase of the decarboxylation rates of the cysteine moieties above pH ≥ pK_{a2}.

On the one hand, the deprotonated amino group stabilizes the resulting alkyl radicals much better than does -NH_3^+. AM1[33] calculations gave a difference in the heats of formation of $CH_3\dot{C}HNH_2$ and $CH_3CH_2NH_2$ of 81 kJ mol^{-1}; for $CH_3\dot{C}HNH_3^+$ and $CH_3CH_2NH_3^+$ this difference was computed to be 134 kJ mol^{-1}. Hence, deprotonation of the amino function should increase the driving force of decarboxylation by more than 50 kJ mol^{-1} and, unless the mechanism were different for the anionic and zwitterionic forms of our amino acids, should lower the activation barrier accordingly.

On the other hand, the deprotonated amino group is also a potential electron donor, so it would be natural to assume that for pH ≥ pK_{a2} a pathway via nitrogen-centered radical cations $\mathbf{I'}^{\bullet+}$, R−S−CH_2−$CH(N^{\bullet+}H_2)COO^-$, becomes accessible, i.e., that the first step of the reaction is electron transfer from nitrogen instead of sulfur. As the findings of the preceding section suggest, decarboxylation of $\mathbf{I'}^{\bullet+}$ might be considerably faster than decarboxylation at the cysteine moiety of the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$, both processes, though, leading to the same α-aminoalkyl radicals.

The polarization patterns again allow a clear-cut distinction between these two alternatives, increase of the decarboxylation rate by product control or an additional reaction channel. Aminium cations $\mathbf{I'}^{\bullet+}$ possess substantial hyperfine coupling constants of the α-protons (i.e., H^1), whereas the hyperfine coupling constants of these protons in the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ are negligible. Unless the decarboxylation of $\mathbf{I'}^{\bullet+}$ were very fast on the CIDNP time scale, polarizations from primary radical pairs $\mathbf{I'}^{\bullet+}\mathrm{CB}^{\bullet-}$ should thus be detectable in the regenerated starting amino acid. Experimentally, however, we did not observe polarizations of **I**-1 in any case, so either aminium cations $\mathbf{I'}^{\bullet+}$ are not formed in these reactions or they decarboxylate extremely rapidly as to prevent back electron-transfer after intersystem crossing of the pairs $\mathbf{I'}^{\bullet+}\mathrm{CB}^{\bullet-}$.

To test the latter hypothesis, we used alanine Ala, CH_3−$CHNH_2COOH$) as a model compound, which bears an obvious structural similarity to our cysteine derivatives but for which electron transfer is only possible from nitrogen. Part of the CIDNP spectrum obtained in the photoreaction of this amino acid with CB at pH 12 is displayed in Figure 7. As is seen in the figure, proton H^1 of Ala is polarized in absorption, but no polarizations can be detected for protons H^2. This is in accordance with the spin density distribution expected for the radical cation $Ala^{\bullet+}$. The polarization phase of H^1 is consistent with regeneration of the educts by in-cage back electron-transfer ($\mu = +1$, $\epsilon = +1$, $\Delta g > 0$, $a_{H^1} > 0$);[25] the fact that no CIDNP signals are observed for pH < pK_{a2} further corroborates electron-transfer quenching. Thus, nitrogen-centered radicals $\mathbf{I'}^{\bullet+}$ are formed in the alanine experiment and give rise to polarizations of H^1 in the substrate. No such polarizations are observed in the cysteine systems; hence, $\mathbf{I'}^{\bullet+}$ is not an intermediate in these reactions.[34] The increased decarboxylation rate at pH above

(29) Goez, M.; Rozwadowski, J.; Marciniak, B. Manuscript in preparation.

(30) Bowers, R. P.; McLauchlan, K. A.; Sealy, R. C. *J. Chem. Soc., Perkin Trans. 2* **1976**, 915−921.

(31) Gilbert, B. C. In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; Plenum Press: New York, 1990; pp 135−154.

(32) The fact that **VIII**-3 is an AB system at pH < pK_{a2} and a singlet at pH > pK_{a2} is due to an interaction between -COO^- of the 4-carboxybenzophenone moiety and -NH_3^+ of the cysteine function, which restricts rotation.[29]

(33) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

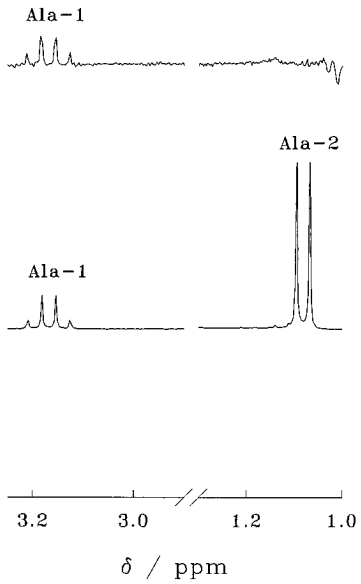


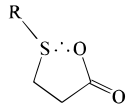
Figure 7. Top trace, part of the CIDNP spectrum of the system 4-carboxybenzophenone/alanine (CB/Ala) at pH 12.0. Bottom trace, the NMR spectrum of the same system in the dark. Only the resonances of the educt protons (left, Ala-1, $\delta = 3.17$ ppm; right, Ala-2, $\delta = 1.08$ ppm) are shown. Experimental parameters: [CB] $= 2 \times 10^{-3}$ M, [Ala] $= 1 \times 10^{-2}$ M, room temperature.

pK_{a2} must thus be due to the stabilizing influence of the deprotonated amino group on the alkyl radical produced by decarboxylation of $\mathbf{I}^{\bullet+}$.

Decarboxylation of $\mathbf{I}^{\bullet+}$ and CIDNP. Fast chemical transformations of radical pairs, RP1, into other radical pairs, RP2, affect the CIDNP signals if these reactions occur on a time scale $1/k$ of some 10^{-10}—10^{-6} s.[35] First, the polarizations in the products of RP1 decrease with increasing k because both the yields of these products decrease and the shorter life of the primary pairs reduces the probability of intersystem crossing by Zeeman and hyperfine interactions, i.e., interferes with the generation of nuclear spin polarizations in RP1. A manifestation of this effect is the low relative intensity of the polarizations of the starting amino acid that is observed in the photoreactions of **Id**.

Second, the products of RP2 exhibit polarizations from both radical pairs, their relative amounts depending on the magnetic parameters of RP1 and RP2 and on k. This effect is found for the decarboxylation products. From the relative contributions of RP1, it can be inferred that the decarboxylation of the carboxymethyl substituent in $\mathbf{Id}^{\bullet+}$ is much faster (almost no polarizations attributable to RP1) than decarboxylation of the cysteine moieties of the sulfur-centered radical cations $\mathbf{I}^{\bullet+}$ at pH $\leq$ pK_{a2} (significant polarizations from RP1). This is in line with expectation because in the former instance decarboxylation occurs at an α-position with respect to the heteroatom, so there is a direct interaction, whereas in the latter case decarboxylation occurs at C$^{\beta}$. It is known[36] that sulfur-centered radical cations bearing carboxy groups at C$^{\beta}$ are stabilized by formation of five-membered rings possessing a two-center three-electron bond between S and O (see Chart 1). It seems very likely that β-decarboxylation also proceeds via this intermediate. For completeness, we mention that for radical structures as shown in Chart 1 the spin density at H^{1} should be negligible, so pairs containing these intermediates would not give rise to polarization patterns that differ substantially from those caused by the open-chain cations $\mathbf{I}^{\bullet+}$.

Chart 1



Third, the polarizations of the products of RP2 are not simple superpositions of polarizations from RP1 and RP2. Rather, they can be described as arising in a radical pair with the combined properties of RP1 and RP2 weighted with the respective lifetimes. These so-called cooperative effects are well known, and examples have even been reported where neither RP1 nor RP2 on its own causes polarizations but in combination they do.[35b,c] The seemingly anomalous g-value difference of the pairs $\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}$ (RP2) at low pH can be explained in this way. In these reactions, Δg is expected to be very large and positive ($\approx 9 \times 10^{-3}$) for RP1, $\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}$, and smaller by more than a factor of 10 and negative ($\approx -7 \times 10^{-4}$) for RP2. Since the transformation RP1 → PR2 is not extremely fast on the CIDNP time scale under these experimental conditions, the positive contribution of RP1 to the effective value of Δg outweighs the negative contribution of RP2. With respect to the hyperfine coupling constants, the effect is smaller because the coupling constants have comparable magnitudes in both radical pairs. At high pH, the decarboxylation is significantly faster, so the influence of RP1 is correspondingly smaller, and the anomaly is not observed.

Likewise, the behavior of **Ie**, for which RP1 influences the relative polarizations of the products to a larger degree than in the case of the other amino acids although the hyperfine coupling constants should be almost identical, is rationalized by a lower rate of decarboxylation. The carboxyethyl group can effect a stabilization of the sulfur-centered radical cation in the same way as the cysteine substituent can (Chart 1), and **Ie** is the only one of our substrates that is capable of such an additional stabilization. It should be noted that this compound provides further evidence that decarboxylation is governed by product control: For $\mathbf{Ie}^{\bullet+}$, decarboxylation of the *S*-carboxyethyl group would be conceivable as well as decarboxylation of the cysteine moiety; however, the CIDNP spectra give no indication that the first pathway is taken. This is consistent with the fact that decarboxylation of the *S*-carboxyethyl substituent would yield a primary alkyl radical, $^{\bullet}\mathrm{CH}-\mathrm{CH}_2-\mathrm{S}-\mathrm{R}$, which is much more unstable than $\mathbf{Ve}^{\bullet}$.

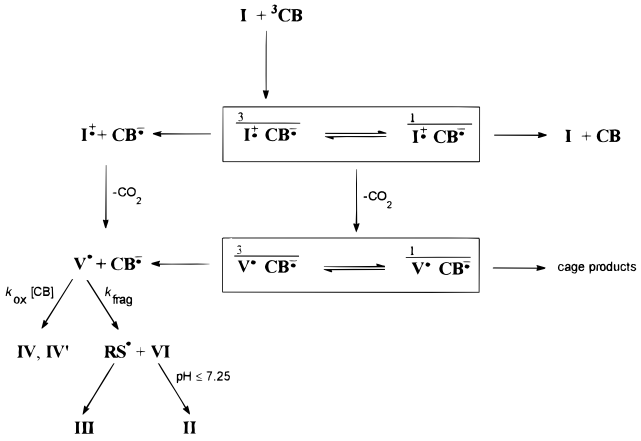
In principle, the rates of the processes RP1 → RP2 could be obtained from a quantitative evaluation of the CIDNP signal intensities.[35] Unfortunately, this is not very accurate at present

(34) A referee raised the question whether the observation of exclusive electron transfer from sulfur in our systems could be explained with electrochemical or gas-phase ionization data. As electrochemical oxidations of both amines and thioethers are irreversible, equilibrium potentials are inaccessible, and the measured peak potentials deviate from them by an amount depending on the rates of charge transfer and/or the rates of subsequent chemical reactions of the radical cations. While correlations within a class of compounds may thus still be permissible, comparison of such data for compounds from different classes is certainly problematic. With respect to gas-phase data, the ionization potentials (IPs) of simple model compounds, e.g., Et_2S and *i*-$PrNH_2$, 8.43 eV (Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17* (Suppl. 1)) and 8.63 eV (Aue, D. H.; Webb, H. M.; Bowers, M. I. *J. Am. Chem. Soc.* **1976**, *98*, 311−317), respectively, indeed seem to indicate a preference of electron transfer from sulfur. However, the difference of these values is comparable to the precision of the measurements and the changes of the IPs with the substituents at S or N. Hence, we are reluctant to draw definitive conclusions from the IPs.

(35) (a) Kaptein, R. *J. Am. Chem. Soc.* **1972**, *94*, 6262−6269. (b) den Hollander, J. A. *Chem. Phys.* **1975**, *10*, 167−184. (c) Kaptein, R. In ref 5d, pp 257−266. (d) den Hollander, J. A.; Kaptein, R. *Chem. Phys. Lett.* **1976**, *41*, 257−263.

(36) Marciniak, B.; Bobrowski, K.; Hug, G. L.; Rozwadowski, J. *J. Phys. Chem.* **1994**, *98*, 4854−4860.

Scheme 1



owing to the unavailable magnetic parameters of the radical pairs involved. However, the CIDNP effects allow an order-of-magnitude estimation of the decarboxylation rates. Decarboxylation of the *S*-carboxymethyl substituent in $\mathbf{Id}^{\bullet+}$ is fast on the CIDNP time scale but not as fast as to suppress all polarizations of the starting amino acid, so it must proceed on a time scale slightly shorter than about 1 ns. In contrast, at pH $<$ pK_{a2}, decarboxylation of the cysteine moieties in our sulfur-centered radical cations is fairly slow on the CIDNP time scale, as evidenced by the strong cooperative effects; on the other hand, an upper limit is given by the life of the resulting α-aminoalkyl radicals, about 50−70 ns. Hence, this decarboxylation must occur on a time scale of some 10 ns.[37,38] Cysteine decarboxylation in basic medium falls in between these extremes. The ability of this reaction to compete successfully with decarboxylation of the *S*-carboxymethyl group in $\mathbf{Id}^{\bullet+}$ indicates that it proceeds on a time scale of about 1 ns.

Reaction Mechanism. All our experimental observations are in accordance with the mechanism displayed in Scheme 1. Quenching of electronically excited CB by electron transfer is the first step of the reaction, as already inferred from other experiments.[2a,b] Electron transfer occurs from sulfur at every pH; electron transfer from nitrogen can be ruled out. After intersystem crossing of the resulting triplet radical pairs $\mathbf{I}^{\bullet+}\mathrm{CB}^{\bullet-}$, spin-allowed back electron-transfer regenerates the starting materials, which bear polarizations from the primary pairs.

The sulfur-centered radicals $\mathbf{I}^{\bullet+}$ of the amino acid undergo decarboxylation of the cysteine moieties to give α-aminoalkyl radicals $\mathbf{V}^{\bullet}$; these processes mostly[37] occur within the correlated life of the radical pairs ($k \approx 10^8$ s^{-1}) and become significantly faster ($k \approx 10^9$ s^{-1}) when the amino group is deprotonated.

Lastly, two competitive pathways of deactivation are open to the α-aminoalkyl radicals. One is fragmentation of the $C^{\beta}-S$ bond to give vinylamine **VI**, which is hydrolyzed to acetaldehyde **II** below pH ≈ 7.25, and a thiyl radical, which then forms a combination product **III** with the sensitizer. The other is oxidation by surplus ground-state sensitizer, which ultimately leads to a sulfur-containing aldehyde **IV** or another product, depending on pH. Relative rates of these two pathways were determined by evaluating CIDNP intensities at different sensitizer concentrations; by experiments at variable temperature, the activation energy of fragmentation could be estimated.

(37) As a consequence, decarboxylation may also take place to some extent in free radicals $\mathbf{I}^{\bullet+}$, which then act as random phase precursors to the pairs $\mathbf{V}^{\bullet}\mathrm{CB}^{\bullet-}$.

(38) Two entirely different effects could be responsible for the influence of pH on the observed gross rate of the reaction $\mathbf{I}^{\bullet+} \rightarrow \mathbf{V}^{\bullet}$. On the one hand, the actual *decarboxylation* step might simply be faster if the amino function of $\mathbf{I}^{\bullet+}$ is deprotonated. On the other hand, as $\mathrm{CB}^{\bullet-}$ can act as a base within the radical pair,[16] the overall decarboxylation rate at pH $<$ pK_{a2} might be limited by *deprotonation* of $-NH_3^+$ by $\mathrm{CB}^{\bullet-}$, i.e., within the cage. In a similar system, decarboxylation has been shown to depend on such a deprotonation step.[3d] On the basis of our CIDNP data, we cannot distinguish between these alternatives. We are indebted to a referee for drawing our attention to this point.

Conclusions

Owing to the unique features of CIDNP spectroscopy, it was possible to obtain some new insight into the mechanism of photooxidations of cysteine derivatives in this work.

On the one hand, the polarization patterns, which can be regarded as frozen signatures of short-lived intermediates that may be difficult to observe directly, provided unambiguous evidence that the first step of these reactions is electron transfer from sulfur, even under conditions where electron transfer from nitrogen could also occur. An order of the rates of the decarboxylation processes that limit the life of the resulting sulfur-centered radical cations could be established, and estimates of the rate constants could be given, because the correlated life of the radical pairs fixes a temporal window.

On the other hand, the analytical potential of the detection method, the signal enhancement by the CIDNP effect, and the possibility to observe "early" diamagnetic products allowed determination of relative rates of fragmentation and oxidation of the α-aminoalkyl radicals $\mathbf{V}^{\bullet}$ produced by decarboxylation of the radical cations. While this would in principle also be feasible by analysis of the final products once the decay pathways of $\mathbf{V}^{\bullet}$ are known, the advantages of CIDNP spectroscopy are that it affords precisely the latter information alongside with the kinetic data and thus enables one to judge the validity of the obtained kinetic parameters.

Experimental Section

Chemical Substances and Sample Preparation. All amino acids and the sensitizer CB were obtained commercially in the highest purity available (>99%) and used as received. Solutions of the reactants in D_2O were deoxygenated by bubbling purified nitrogen through them; then the NMR tubes were sealed. pH values were measured with a glass electrode.

CIDNP Measurements. The experiments were performed on a Bruker WM-250 NMR spectrometer. For data acquisition and experimental control, an 80486-based multitasking workstation equipped with a Keithley AD converter and a home-made programable pulse generator were employed. An excimer laser (XeCl, $\lambda = 308$ nm) that was triggered by the pulse generator served as light source. Samples were illuminated from the side and within the active volume only; for this, a modified probe[39] was used. A few millijoules were absorbed in the samples per flash, as determined actinometrically. The pulse sequences for the pseudo-steady-state CIDNP measurements have been described previously.[9] This technique allows virtual elimination of background signals and yields CIDNP signals that are undistorted by nuclear spin relaxation in the diamagnetic reaction products.

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JA9536678

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