compounds obtained from *cis*-$(NH_3)_2Pt(1\text{-}MeU)_2$, namely, in a face–face fashion with the filled d_{z^2} orbital of Pt "interacting" with the second metal. As shown for the mixed Pt_2Cu compound **2**, the steric bulk of the tmeda ligand causes a stronger tilting of the Cu coordination plane with respect to the Pt plane and a longer Pt–Cu separation as compared to similar compounds derived from *cis*-$(NH_3)_2Pt^{II}$, which is hardly surprising, however. With metal amine (amine = $(NH_3)_2$, en, dien) species, the interaction between CH_3 protons of tmeda and NH protons of the amine apparently becomes so unfavorable that a face–face orientation of the two metals is avoided. As a consequence, a face–back arrangement with monofunctional binding of the entering metal amine entity is realized that leads to short contacts between the metal and H5 of the bridging 1-MeU ring and large downfield shifts in the ^1H NMR spectra.

The proposed structure could be relevant with regard to C5–H activation of 1-MeU and subsequent covalent metal binding to C5. Such binding has been reported for both Pt[30] and Hg,[31] but the mechanism of formation of these organometallic compounds has not been established as yet.

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Supplementary Material Available: Tables of crystallographic and experimental details, anisotropic displacement parameters of heavy atoms, calculated positional parameters of H atoms, bond distances and angles, least-squares plane equations, and dihedral angles between planes (13 pages); listing of observed and calculated structure factors for **2** (50 pages). Ordering information is given on any current masthead page.

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Hydroxyl Radical Induced Decarboxylation and Deamination of 2-Methylalanine Catalyzed by Copper Ions

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Using the pulse radiolysis technique, the rate constants of the reactions of $^\bullet CH_2C(CH_3)(NH_3^+)COO^-$ (the radical derived via H-abstraction from 2-methylalanine) with Cu^{2+}_{aq} and Cu^+_{aq} to form unstable intermediates with a copper–carbon σ-bond have been determined to be (1.3 ± 0.3) × 10^7 and (1.3 ± 0.2) × 10^9 $M^{-1}\ s^{-1}$, respectively. In the case of cupric ions, the transient decomposes via a β-carboxyl elimination reaction yielding Cu^+_{aq}, CH_3COCH_3, and CO_2, whereas in the case of cuprous ions it decomposes via a β-amine elimination reaction yielding Cu^{2+}_{aq}, $CH_2{=}C(CH_3)COO^-$, and NH_3. A reaction mechanism for aliphatic radical induced amino acid damage catalyzed by copper ions in different oxidation states is suggested. This mechanism describes a plausible new pathway for biological damage induced by free radicals.

Introduction

Aliphatic free radicals react with many Cu^{II} and Cu^I complexes through a mechanism involving the formation of an organometallic intermediate with a metal–carbon σ-bond.[1-9]

$$Cu^{2+}_{aq} + R^\bullet \rightarrow (Cu^{III}{-}R)^{2+} \tag{1}$$

$$Cu^{+}_{aq} + R^\bullet \rightarrow (Cu^{II}{-}R)^{+} \tag{2}$$

The mechanisms of decomposition of these intermediates depend on the nature of the aliphatic radical and the nonparticipating ligands of the copper ion.

Until recently, most of the decomposition processes of $(Cu^{III}{-}R)^{2+}$ were described by

$$(Cu^{III}{-}R)^{2+} \rightarrow Cu^+ + R^+ \tag{3}$$

where R^+ is the oxidized form of the radical. We have recently demonstrated for the first time that Cu^{2+}_{aq} induces a β-carboxyl elimination reaction when it reacts with $^\bullet CH_2C(CH_3)_2COO^-$ via $[Cu^{III}{-}CH_2C(CH_3)_2COO^-]^{2+}$ as a transient.[7] $^\bullet CH_2C(CH_3)_2COO^-$ reacts also with Cu^+_{aq} to form a complex with a metal–carbon σ-bond, which decomposes both via homolysis and by a reaction with Cu^{2+}_{aq} to form $2Cu^+_{aq}$ and $(CH_3)_3CCOO^-$. We have also shown[9] that the complexes formed between $^\bullet CH_2C(CH_3)_2NH_3^+$ and Cu^+_{aq} and Cr^{2+}_{aq} decompose via β-elimination of NH_3 to yield 2-methylpropene, ammonia, and Cu^{2+}_{aq} or Cr^{3+}_{aq}. The rates of these β-elimination reactions were considerably slower than those of the analogous β-hydroxyl elimination reactions. It was suggested that the rates of the β-elimination processes are correlated to the C–O and C–N bond strength, which are cleaved in these reactions.[9]

In order to continue the research on the effects of different substituents on the β-elimination process of transients with a copper–carbon σ-bond, we have decided to investigate the mechanism of the decomposition of transients with a metal–carbon σ-bond containing both an amine and a carboxylate group at the β position to the carbon-centered free radical formed.

Free radicals are responsible for many deleterious effects in biological systems.[10] It has been well established that transition

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Table I. Dependence of the Observed Rates of Formation (k_f) and of the Two Consecutive Decays (k_d) of $[\mathrm{Cu^{III}{-}CH_2C(CH_3)(NH_3^+)COO^-}]^{2+}$ on Solution Composition[a]

$[\mathrm{Cu^{2+}_{aq}}]$, mM	pH	λ, nm	dose, krad	OD_0[b]	k_f, s^{-1}	OD_∞^1[c]	k_d^1, s^{-1}	OD_∞^2[d]	k_d^2, s^{-1}
0.5	3	300	1.0	0.080	1.1×10^4	0.057	46	0.006	0.13
1.0	3	300	0.5	0.042	2.2×10^4	0.031	36	0.004	0.14
1.0	3	300	1.0	0.082	2.1×10^4	0.051	42	0.004	0.15
1.0	3	300	1.5	0.124	2.6×10^4	0.093	46	0.005	0.10
1.0	4	310	1.0	0.089	2.3×10^4	0.072	17	0.001	0.08
1.0	2	300	1.0	0.063	2.2×10^4	0.033	20	0.003	0.12
1.0	1	300	1.0	0.031	1.1×10^4	0.014	5	0.003	0.25
1.0*	3	305	1.0	0.080	2.3×10^4	0.065	29	0.006	0.15
1.5	3	305	1.0	0.083	2.7×10^4	0.057	34	0.004	0.15
2.0	3	310	1.0	0.080	3.5×10^4	0.049	45	0.003	0.11
2.0	2.3	300	1.0	0.074	3.5×10^4	0.032	18	0.003	0.10

[a] All solutions were N_2O-saturated and contained 0.05 M 2-methylalanine except that indicated by an asterisk (*), which contained 0.1 M. The path length was 12.1 cm. [b] OD_0, the absorbance formed after the end of the pulse. [c] OD_∞^1, the absorbance formed after the end of the first decay reaction. [d] OD_∞^2, the absorbance formed after the end of the decomposition process.

metal ions and their complexes enhance the damage caused by these radicals.[11] However, little is known on the exact mechanism of the damage caused by the free radicals. Decomposition of complexes with a metal–carbon σ-bond, formed via the reaction of metal ion with aliphatic free radical, causes in many cases the cleavage of the aliphatic molecule. If similar reactions take place in biological systems, they may cause deleterious effects, especially if the decomposition process proceeds via a β-elimination reaction, as a formation of a double bond is a process that in most biological systems is irreversible. This study might shed light on the mechanism of deleterious free radicals in biological systems.

We have chosen to study the reaction of copper ions with the radical derived by H-abstraction of 2-methylalanine (2-amino-2-methylpropanoate), $(CH_3)_2C(NH_2)COO^-$. Only the carbon-centered free radical at the β position to the carboxylic group is formed by H-atom abstraction from 2-methylalanine at pH < 11 $^\bullet CH_2C(CH_3)(NH_3^+)COO^-$.[12,13] In this radical both the amine and the carboxylate groups are at the β position to the carbon atom with the unpaired electron. The aim of this study was to investigate the reaction of this radical with copper ions and to check which group will be eliminated via the decomposition of the transients formed. It is of interest to compare the results to those obtained when copper ions react with $^\bullet CH_2C(CH_3)_2OH$,[2,3,14] $^\bullet CH_2C(CH_3)_2NH_3^+$[9] and $\bullet CH_2C(CH_3)_2COO^-$[7] and to examine whether the substitution of a methyl group by carboxylic or amine groups on the carbon affects significantly the mechanism and the rate of the β-elimination reactions.

Experimental Section

Materials. All chemicals employed were of analytical grade. Solutions were prepared with distilled water which was further purified using a Milli-Q water purification system. Solutions containing cuprous ions were prepared by dissolving $Cu(CH_3CN)_4PF_6$ (Aldrich) in deaerated solutions. The pH was adjusted by adding $HClO_4$ before adding the cuprous salt.

Irradiation. Pulse radiolysis experiments were carried out with the Varian 7715 linear accelerator at the Hebrew University of Jerusalem. The pulse duration was 0.5–1.5 μs with a 200-mA current of 5-MeV electrons. Irradiations were carried out in a 4-cm Spectrosil cell using three light passes. A 150-W xenon arc produced the analyzing light. The detection system included a Bausch and Lomb grating double monochromator model D330/D331 Mk.II and an IP28 photomultiplier. The signal was transferred through a Sony/Tektronix 390AD programmable digitizer to a micro PDP-11/24 computer which operated the whole system. N_2O-saturated solutions containing 1 mM KSCN were used for dosimetry. The yield of $(SCN)_2^-$ was measured assuming $G((SCN)_2^-) = 6.1$ and $\epsilon_{475} = 7600\ M^{-1}\ cm^{-1}$. Trains of 1.5-μs pulses (2.5 ms between each pulse) have been delivered into small cylindrical glass bulbs (12-mL volume) filled with deaerated solutions and sealed with a rubber septum for products analysis. The total dose was in the range of 2–33 krad. In this case, the 0.01 M $FeSO_4$ in 0.8 N H_2SO_4 was used for determining the total concentration of the radicals formed.

Spectroscopic measurements were carried out using a Hewlett-Packard HP 8452A diode array spectrophotometer.

Analysis of Cu^+_{aq}. The initial concentration of cuprous ions and the change in their concentration after the irradiation were determined with the use of 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid (bathocuproinedisulfonic acid).[15] This compound is very soluble in water, and complete formation of Cu^I complex occurs over the pH range of 3–11. The cuprous complex formed is not oxidized by oxygen, and its molar absorptivity is 12 250 $M^{-1}\ cm^{-1}$ at 483 nm. The results show that the initial concentration of the cuprous ions was about 65% of the total copper. This may be due to contamination of the cuprous salt with cupric ions as indicated by the light blue color of the salt. The change in the concentration of Cu^+_{aq} after the irradiation was determined by adding 1 mL of deaerated solution containing 50 mM bathocuproinedisulfonic acid to 8-mL samples, to unirradiated blanks, and to irradiated samples.

Analysis of Carbon Dioxide. The yield of carbon dioxide was determined by gas chromatography using a Hewlett-Packard model 5890 instrument and a TCD detector. The gas samples were introduced through the rubber septa with gas-tight syringes and were separated on a 10 ft × $^1/_8$ in. stainless steel column packed with Poropak Q 80/100 mesh at 50 °C. Calibration curves were obtained in order to determine quantitatively the amount of CO_2 formed. This was done by irradiating N_2O-saturated solutions containing 1 mM $Co(NH_3)_6^{3+}$ and 0.1 M formate at pH 4.8. Under these conditions, all the primary radicals formed by the radiation are converted into CO_2^-, which subsequently reduces the metal to form CO_2.[16] Before the gas samples were taken, 0.1 mL of concentrated $HClO_4$ was injected into the bulb in order to shift the equilibrium $H^+ + CO_3^{2-} \rightleftharpoons CO_2 + H_2O$ toward CO_2.

Determination of Acetone in Solutions. The yield of acetone in the solutions was determined by the same gas chromatograph using a FID detector. Three-microliter liquid samples were taken and were separated on the same column as for the determination of CO_2 at 175 °C. Diethyl ether was used as an internal standard. Diethyl ether (0.5 mM) was added to all samples, and the ratio between the acetone and the ether peaks was determined and compared to those obtained when the ether was added to unirradiated samples containing known amounts of acetone.

Identification of Cu^I–2-Methylacrylate. The spectrum of the π-complex formed between Cu^+_{aq} and 2-methylacrylic acid ($CH_2{=}C(CH_3)COOH$) was measured in deaerated solutions at pH 3. This complex has a typical absorbance for a d–π* complex of Cu^+_{aq} and olefin in the near-UV with maxima at 250 and 310 nm.[2,17]

Production of the Aliphatic Radicals. The reaction sequence for radiation-induced production of aliphatic free radicals in N_2O-saturated solutions (N_2O = 0.022 M) containing 0.05–0.1 M 2-methylalanine is represented by eqs 4–8. The numbers in parentheses are G values which represent the number of molecules formed per 100 eV of energy absorbed in the solution.[18] It has been demonstrated using the ESR technique that

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$$H_2O \xrightarrow{\gamma} e_{aq}^- (2.75),\ OH^\bullet (2.75),\ H^\bullet (0.60),\ H_2 (0.45),\ H_2O_2 (0.75),\ H_3O^+ (2.75) \tag{4}$$

$$e_{aq}^- + N_2O \rightarrow N_2 + OH^- + OH^\bullet \qquad k_5 = 9.1 \times 10^9\ M^{-1}\ s^{-1}\ [19] \tag{5}$$

$$e_{aq}^- + H_3O^+ \rightarrow H^\bullet + H_2O \qquad k_6 = 2.2 \times 10^{10}\ M^{-1}\ s^{-1}\ [19] \tag{6}$$

$$H^\bullet + (CH_3)_2C(NH_3^+)COOH \rightarrow {}^\bullet CH_2C(CH_3)(NH_3^+)COOH + H_2 \qquad k_7 = 7 \times 10^4\ M^{-1}\ s^{-1}\ [19] \tag{7}$$

$$OH^\bullet + (CH_3)_2C(NH_3^+)COOH \rightarrow {}^\bullet CH_2C(CH_3)(NH_3^+)COOH + H_2O \tag{8}$$

the radical $^\bullet NHC(CH_3)_2COO^-$ is formed only at pH > 11.[12,13] We have determined the rate constant of reaction 8 using the competition kinetic method with thiocyanate as the competing substrate[16] to be $(1.2 \pm 0.1) \times 10^8$ M^{-1} s^{-1} at pH 3.

When the solutions contain also copper ions, the following reactions may take place:

$$e^-_{aq} + Cu^{2+}_{aq} \rightarrow Cu^+_{aq} \qquad k_9 = 3.5 \times 10^{10}\ M^{-1}\ s^{-1}\ [19] \tag{9}$$

$$OH^\bullet + Cu^{2+}_{aq} \rightarrow Cu^{3+}_{aq} + OH^- \qquad k_{10} = 3.1 \times 10^8\ M^{-1}\ s^{-1}\ [19] \tag{10}$$

$$H^\bullet + Cu^{2+}_{aq} \rightarrow Cu^+_{aq} + H^+ \qquad k_{11} \leq 1 \times 10^6\ M^{-1}\ s^{-1}\ [19] \tag{11}$$

$$H^\bullet + Cu^+_{aq} \rightarrow CuH^+_{aq} \qquad k_{12} = 5 \times 10^9\ M^{-1}\ s^{-1}\ [19] \tag{12}$$

$$OH^\bullet + Cu^+_{aq} \rightarrow OH^- + Cu^{2+}_{aq} \qquad k_{13} = 3 \times 10^9\ M^{-1}\ s^{-1}\ [20] \tag{13}$$

Thus, under the experimental conditions ([RH] ≥ 0.05 M, $[Cu^{2+}_{aq}] \leq$ 2 mM, $[Cu^+_{aq}] \leq$ 0.15 mM, pH 3), nearly all the primary free radicals formed by the radiation are converted into $^\bullet CH_2C(CH_3)(NH_3^+)COO^-$, which can subsequently react with the copper ions. A small part of the hydrated electrons may react via reaction 9 in some solutions, thus increasing slightly the initial concentration of Cu^+_{aq}. At pH <3 part of the hydrated electrons are converted into $H^\bullet$, which might react in some solutions via reaction 11. Thus clearly at pH ≥3 the main processes observed are due to the reaction of $^\bullet CH_2C(CH_3)(NH_3^+)COO^-$ with copper ions.

Results

Spectra and Kinetics. When N_2O-saturated solutions containing 0.5–2 mM $CuSO_4$ and 0.05–0.1 M $(CH_3)_2C(NH_2)COOH$ in the pH range 1–4 are pulse-irradiated, the formation and the decomposition of an intermediate are observed. The absorption spectrum of this intermediate is given in Figure 1a. The rate of the formation of the transient obeys a pseudo-first-order rate law, is linearly dependent on $[Cu^{2+}_{aq}]$, and independent of pH and [2-methylalanine] (Table I). The kinetics of the decomposition of this transient consist of two consecutive first-order reactions. The contribution of the first reaction to the overall decomposition process and its rate constant depend only on pH, whereas the rate constant of the second reaction is independent of $[Cu^{2+}_{aq}]$, $[(CH_3)_2C(NH_2)COOH]$, pH, and pulse intensity (Table I). The spectrum obtained 0.1 s after the end of the pulse, i.e., at the end of the first decay, is not identical to that obtained at the end of the formation process, 200 μs after the end of the pulse (Figure 1a,b).

When N_2O-saturated solutions containing 0.05–0.15 mM Cu^+_{aq}, $(3–100) \times 10^{-5}$ M $CuSO_4$, and 0.05 M 2-methylalanine in the pH range 1–3 are pulse-irradiated, the formation and the decomposition of a different transient than that obtained in the absence of cuprous ions is observed. The transient formed in the presence of cuprous ions absorbs at 355 ± 5 nm with $\epsilon = 2100 \pm 200$ M^{-1} cm^{-1} (Figure 1b). The rate of the formation of this transient obeys a pseudo-first-order law, is linearly dependent on $[Cu^+_{aq}]$, and depends slightly on $[Cu^{2+}_{aq}]$ and pH (Table II). The kinetics of the decomposition of this transient consist of two consecutive first-order reactions. These two reactions are observed below 310 nm (Figure 2a). The rate constants of these reactions were determined to be 2.7 ± 0.8 s^{-1} and 0.15 ± 0.05 s^{-1}, respectively (Table II). The rate constant of the last reaction was found to be the same as that of the second decomposition process observed only in the presence of cupric ions (Tables I and II). The contribution of the two consecutive reactions to the overall decomposition process depends on the concentration of copper ions (Table II). The absorption spectrum observed after the end of the first decomposition process (Figure 3) is the same as that in Figure 1b, which is obtained just in the presence of cupric ions. Above 310 nm the absorbance of the complex with the Cu^{II}–carbon σ-bond exceeds that of the complex with the Cu^{III}–carbon σ-bond (Figure 1), while the rates of the two consecutive first-order reactions do not differ by more than one order of magnitude. Therefore, above 310 nm the two reactions are unseparable, and only one process is observed which fits better to a second-order reaction than to a first-order reaction (Figure 2b), in spite of the fact that it is neither of them, but a complex decay.

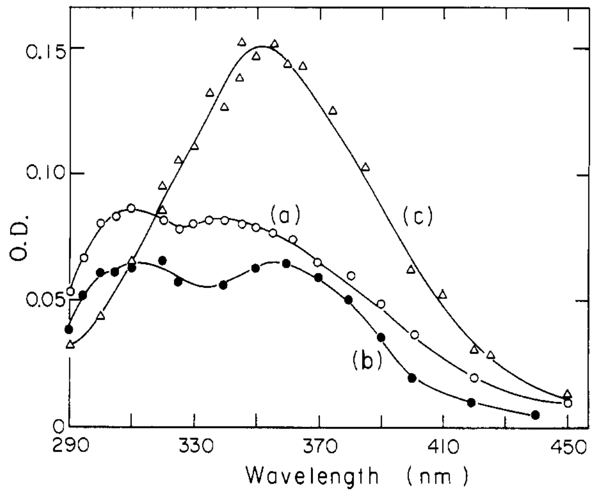

Figure 1. Absorption spectra of irradiated N_2O-saturated solutions at pH 3 containing 0.05 M 2-methylalanine and (a) 1 mM Cu^{2+}_{aq} measured 200 μs after the end of the pulse; (b) 1 mM Cu^{2+}_{aq} measured 0.1 s after the end of the pulse. (c) 0.1 mM Cu^+_{aq} and 30 μM Cu^{2+}_{aq} measured 40 μs after the end of the pulse. The optical path length was 12.1 cm, and the dose was 1 krad.

Product Analysis. N_2O-saturated solutions containing 0.5–2 mM $CuSO_4$ and 0.05 M 2-methylalanine at pH 3 and 6 were pulse-irradiated with only one pulse of 2 krad. The concentration of Cu^+_{aq} was determined within 20 s after the irradiation, and the results indicate that $G(Cu^+) = 5.6 \pm 0.6$. The yield of CO_2 and CH_3COCH_3 could not be determined with only one pulse of radiation by gas chromatography, and a total dose of 8–33 krad (5–20 pulses) was used. It was found that under all above-mentioned experimental conditions $G(CO_2) \approx G(CH_3COCH_3) = 2.0 \pm 0.5$.

N_2O-saturated solutions containing 0.1 mM Cu^+_{aq}, $(3–200) \times 10^{-5}$ M $CuSO_4$, and 0.05 M 2-methylalanine at pH 3 were pulse irradiated with a total dose of 8–33 krad. The change in the concentration of Cu^+_{aq} and the yield of CO_2 depend on $[Cu^{2+}_{aq}]$ (Table III).

As can be seen in Figure 4, in the presence of cuprous ions the absorption does not decay to zero at the end of the decomposition process. It does in the presence of cupric ions alone (Table I). The absorption spectrum of the final products differed from that of the transients formed. We have measured the spectrum of a pulse-irradiated N_2O-saturated solution containing 0.1 mM Cu^+_{aq}, 30 μM Cu^{2+}_{aq}, and 0.05 M 2-methylalanine at pH 3 by removing the 4-cm cell to the diode array spectrophotometer immediately after the radiation. The spectrum obtained was identical to that formed when the cuprous salt was added to deaerated 2-methylacrylic acid at pH 3. We also measured the absorbance around 300 nm in the presence of cupric ions and found out that it decreased with increasing $[Cu^{2+}_{aq}]$. Below 300 nm, we could not measure the absorbance as in the presence of Cu^{2+}_{aq} the absorption of the solution is too high.

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Table II. Dependence of the Rate of Formation (k_f) and the Two Consecutive Observed Rates of the Decay (k_q) of $[Cu^{II}-CH_2C(CH_3)(NH_3^+)COO^-]^+$ on Solution Composition[a]

$[Cu^+_{aq}]$, M	$[Cu^{2+}_{aq}]$, M	λ, nm	pH	OD_o[b]	k_f, s^{-1}	$OD_\infty{}^1$[c]	$k_d{}^1$, s^{-1}	$OD_\infty{}^2$[d]	$k_d{}^2$, s^{-1}
5×10^{-5}	3×10^{-5}	295	3	0.032		0.064	3.5	0.016	0.09
5×10^{-5}	3×10^{-5}	375	3	0.011	7.2×10^4				
5×10^{-5}	5×10^{-4}	300	3	0.040		0.056	2.7	0.013	0.11
9×10^{-5}	5×10^{-5}	300	3	0.040		0.054	2.8	0.028	0.17
9×10^{-5}	5×10^{-5}	375	3	0.115	1.3×10^5				
9×10^{-5}	3×10^{-4}	305	3	0.040		0.052	2.7	0.018	0.13
9×10^{-5}	5×10^{-4}	305	3	0.042		0.050	2.9	0.017	0.17
9×10^{-5}	1×10^{-3}	305	3	0.045		0.053	1.8	0.013	0.13
9×10^{-5}	5×10^{-5}	300	2	0.038	8.3×10^4	0.075	2.5	0.029	0.16
9×10^{-5}	5×10^{-5}	300	1	0.025	4.1×10^4	0.030		0.000	0.20
1.5×10^{-4}	7×10^{-5}	375	3	0.115	2.2×10^5				

[a] Solutions were N_2O-saturated and contained 0.05 M 2-methylalanine. The dose was 1 krad, and the path length was 12.1 cm. [b] OD_o, the absorbance formed after the end of the pulse. [c] $OD_\infty{}^1$, the absorbance formed after the end of the first decay reaction. [d] $OD_\infty{}^2$, the absorbance formed after the end of the decomposition process.

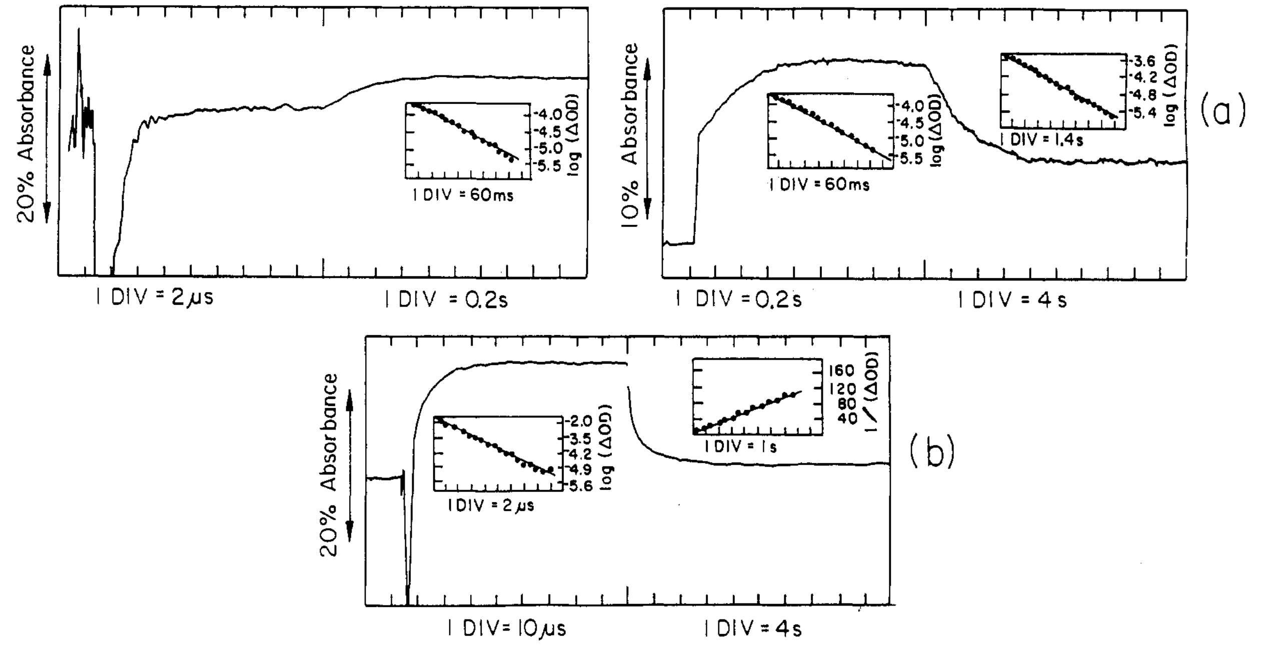


Figure 2. Kinetics plots obtained in pulse-irradiated N_2O-saturated solution containing 0.1 mM Cu^+_{aq}, 30 μM Cu^{2+}_{aq}, and 0.05 M 2-methylalanine at pH 3. (a) 300 nm; (b) 350 nm. The inset contains fits to first- and second-order reactions. The optical path length was 12.1 cm, and the dose was 1 krad.

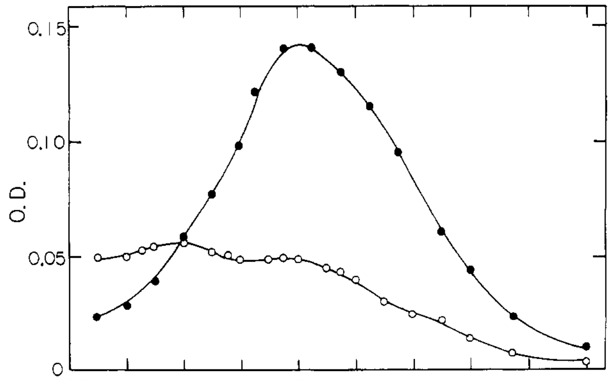


Figure 3. Absorption spectra of irradiated N_2O-saturated solutions at pH 3 containing 0.05 M 2-methylalanine, 30 μM Cu^{2+}_{aq}, and 0.1 mM Cu^+_{aq} measured 40 μs (●) and 1.6 s (○) after the end of the pulse. The optical path length was 12.1 cm, and the dose was 1 krad.

Discussion

Reaction of $^\bullet CH_2C(CH_3)(NH_3^+)COO^-$ with Cu^{2+}_{aq}. The product analysis of pulse-irradiated N_2O-saturated solutions containing $CuSO_4$ points out that within experimental error at pH ≥3 all the initial radicals formed by the radiation are converted into the aliphatic radicals, which subsequently reduce Cu^{2+}_{aq} to Cu^+_{aq}. The observed rate constant of the formation reaction depends linearly on $[Cu^{2+}_{aq}]$ (Table I), and therefore it is suggested that the first reaction observed is reaction 14 and $k_{14} = (1.3 \pm 0.3) \times 10^7$ M^{-1} s^{-1} is obtained.

Table III. Yields of Loss of Cu^+_{aq} and Formation of CO_2 as a Function of $[Cu^{2+}_{aq}]$[a]

$[Cu^{2+}_{aq}]$, M	$[Cu^+_{aq}]$, M	$G(-Cu^+_{aq})_{exp}$[b]	$G(CO_2)_{exp}$[b]	$G(CO_2)_{cal}$[c]
3×10^{-5}	1.0×10^{-4}	3.3	0.8	0.65
5×10^{-4}	1.0×10^{-4}	2.3	1.2	1.15
1×10^{-3}	1.4×10^{-4}	2.5	0.9	1.05
2×10^{-3}	1.1×10^{-4}	0.9	1.9	1.85

[a] N_2O-saturated solutions containing 0.05 M 2-methylalanine at pH 3 were pulse-irradiated by a train of five pulses with a total dose of 8 krad. [b] Accuracy ±25%. [c] Calculated according to $G(-Cu^+_{aq})_{exp} = G_i - 2G_{H_2O_2} - 2G(CO_2)_{cal}$ (see text).

$$Cu^{2+}_{aq} + {}^\bullet CH_2C(CH_3)(NH_3^+)COO^- \rightarrow [Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+} \quad (14)$$

At pH ≥3 the cupric ions are complexed by 2-methylalanine (the solutions have an intense blue-violet color), and it is assumed that reaction 14 is also valid for $L_nCu^{2+}_{aq}$, where L = 2-methylalanine (Table I).

The transient complex with the Cu^{III}–carbon σ-bond decomposes via two consecutive reactions. As the rate and the contribution

Table IV. Effect of Methyl, Amine, and Carboxylic Substituents on the Specific Rates (k_d) of the β-Elimination Processes from Transients with a Copper–Carbon σ-Bond

complex	k_d, s^{-1}	β-elimination of	ref
(a) $(Cu^{II}-CH_2CH_2OH)^+$	$3.2 \times 10^3 + 3.8 \times 10^7[H^+]$	H_2O	21
(b) $[Cu^{II}-CH_2CH(CH_3)OH]^+$	$1.5 \times 10^4 + 1.5 \times 10^8[H^+]$	H_2O	21
(c) $[Cu^{II}-CH_2C(CH_3)_3OH]^+$	$5.0 \times 10^4 + 8.6 \times 10^7[H^+]$	H_2O	14
(d) $[Cu^{II}-CH_2C(CH_3)_2NH_3]^{2+}$	7.0	NH_3	8
(e) $[Cu^{II}-CH_2C(CH_3)(NH_3^+)COO^-]^+$	2.7	NH_3	this work
(f) $[Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+}$	0.15	CO_2	this work
(g) $[Cu^{III}-CH_2C(CH_3)_2COO^-]^+$	0.03	CO_2	6

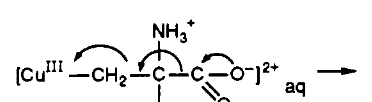
of the first decay reaction to the overall change in absorption depend on pH (Table I) and the spectrum obtained at the end of this reaction is not identical, though similar to that of $[Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+}$ (Figure 1), we assume that the first reaction is a ligand rearrangement process. The rate of this reaction seems to be too low to describe such a ligand rearrangement process as compared to analogous processes in $(L_mCu^{III}-R)^{2+}$,[8] but no other alternative interpretation can be suggested at this time.

After the formation of the second transient, the absorbance decays to zero, yielding Cu^+_{aq} as a final product. The product analysis also points out that carbon dioxide and acetone are formed with equal yields. $G(CO_2) \approx G(CH_3COCH_3) = 2 \pm 0.5$ are about 30% of $G(Cu^+_{aq}) = 5.6 \pm 0.6$. This difference may result from the fact that $G(Cu^+_{aq})$ was determined after one pulse of radiation was delivered to the sample, whereas $G(CO_2)$ and G(acetone) were determined after a train of pulses. As one of the products of the reaction of Cu^{2+}_{aq} and $^{\bullet}CH_2C(CH_3)(NH_3^+)COO^-$ is Cu^+_{aq}, the latter competes with Cu^{2+}_{aq} for the aliphatic radicals when the solutions are irradiated by a train of pulses forming different products. Indeed, in the case where it was determined that $G(CO_2) = G(CH_3COCH_3) = 2.0 \pm 0.5$, we measured $G(Cu^+_{aq}) = 0.4 \pm 0.1$. This value is lower than those determined for acetone and carbon dioxide as one has to remember that 2 equiv of Cu^+_{aq} are oxidized by 1 equiv of H_2O_2 which is formed during the irradiation with a yield of 0.75. It is therefore suggested that the decomposition reaction of $[Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+}$ is described by

$$[Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+} \longrightarrow Cu^+_{aq} + CO_2 + CH_2{=}C(CH_3)(NH_3^+) \quad (15)$$

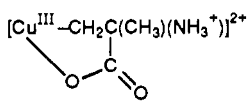
$$CH_2{=}C(CH_3)(NH_3^+) \xrightarrow{H_2O} NH_4^+ + CH_3COCH_3$$

As has been suggested in the case of $(CH_3)_3CCOOH$,[7] two alternative detailed mechanisms might be suggested for this reaction:

(I) 

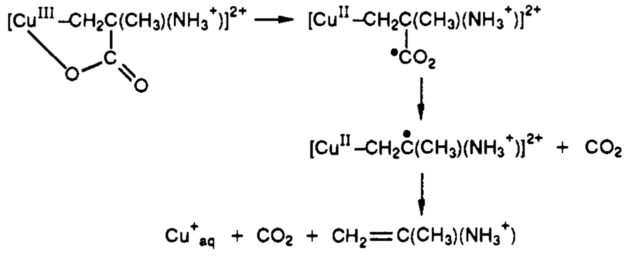
$$\longrightarrow Cu^+_{aq} + CO_2 + CH_2{=}C(CH_3)(NH_3^+) \quad (16a)$$

$$\text{(II)}\quad [Cu^{III}-CH_2C(CH_3)(NH_3^+)COO^-]^{2+} \longrightarrow$$



(16b)

followed by



Mechanism II was preferred in the case of $(CH_3)_3CCOOH$, as a cyclic intermediate is expected to stabilize the intermediate toward heterolysis. This explains why the half-life for the decomposition of the intermediates in both systems is considerably longer than, for example, those of $[Cu^{III}-CH_2C(CH_3)_2OH]^{2+}$ [14] and $(Cu^{III}-CH_2COOH)^{2+}$.[2,3] However, $k_{15} = 0.15 \pm 0.05$ s^{-1} is about 5 times higher than the rate constant of the β-carboxyl elimination in the case of $(CH_3)_3CCOOH$ and Cu^{2+}_{aq}.[7] Thus, substitution of the methyl group by the amine group does not have a major effect on the rate of the β-carboxyl elimination process.

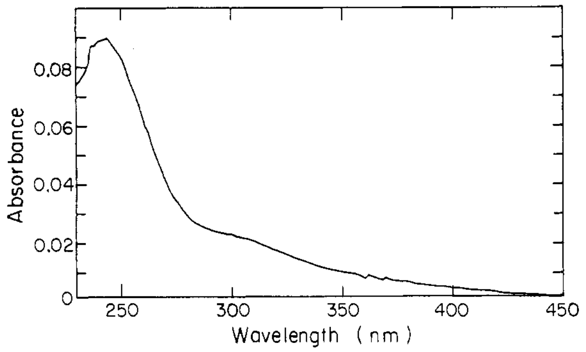


Figure 4. Absorption spectrum measured 30 s after the irradiation of N_2O-saturated solution containing 0.1 mM Cu^+_{aq}, 30 μM Cu^{2+}_{aq}, and 0.05 M 2-methylalanine at pH 3. The solution was irradiated with one pulse of 2 krad in a 4-cm cell. The cell was removed immediately after the irradiation to a diode array spectrophotometer.

Reaction of $^{\bullet}CH_2C(CH_3)(NH_3^+)COO^-$ with Cu^+_{aq}. The spectrum of the transient formed when cuprous ions are added to N_2O-saturated solutions containing 2-methylalanine ($\epsilon_{max}^{375} = 2100 \pm 200$ M^{-1} cm^{-1}) is similar to many other $(Cu^{II}-R)^+$ complexes.[2,4-9,14,20] Therefore, it is suggested that the first reaction observed in this system is reaction 17, where $k_{17} = (1.3 \pm 0.2) \times 10^9$ M^{-1} s^{-1} (Table II). Thus, the competition of reaction 14 with reaction 17 is insignificant under our experimental conditions.

$$Cu^+_{aq} + {}^{\bullet}CH_2C(CH_3)(NH_3^+)COO^- \rightarrow [Cu^{II}-CH_2C(CH_3)(NH_3^+)COO^-]^+ \quad (17)$$

The $(Cu^{II}-R)^+$ transient decomposes via two consecutive reactions. The spectrum of the species formed after the end of the first decomposition reaction is identical to that of $(Cu^{III}-R)^{2+}$ (most probably the one after the "ligand rearrangement" process), and indeed the rate of the second decomposition reaction is the same as that measured for the decomposition of $(Cu^{III}-R)^{2+}$ in the absence of cuprous ions. Moreover, CO_2 was found as one of the final products, and its yield increases with increasing $[Cu^{2+}_{aq}]$ (Table III). The spectrum measured at the end of the decomposition process (Figure 4) is identical with that of cuprous ion bound to 2-methylacrylic acid, indicating that 2-methylacrylic acid is also one of the final products. In agreement with the above results, it was found that the yield of oxidation of cuprous ions is significantly smaller than the yield of the aliphatic free radicals and $G(-Cu^+_{aq})$ decreases with increasing $[Cu^{2+}_{aq}]$ (Table III). These results point out that one of the routes of decomposition of $[Cu^{II}-CH_2C(CH_3)(NH_3^+)COO^-]^+$ is β-elimination of ammonia yielding Cu^{2+}_{aq}, 2-methylacrylate, and ammonia:

$$[Cu^{II}-CH_2C(CH_3)(NH_3^+)COO^-]^+ \rightarrow Cu^{2+}_{aq} + NH_3 + CH_2{=}(CH_3)COO^- \quad (18)$$

In addition, the overall mechanism of decomposition involves equilibrium 17 followed by reaction 14.

$$[\mathrm{Cu^{II}\text{–}CH_2C(CH_3)(NH_3^+)COO^-}]^+ \rightleftharpoons \mathrm{Cu^+_{aq}} + {}^{\bullet}\mathrm{CH_2C(CH_3)(NH_3^+)COO^-} \quad (-17)$$

According to this mechanism, assuming a steady state for the concentration of the aliphatic radical, rate eq 19 is obtained:

$$-\mathrm{d}[(\mathrm{Cu^{II}\text{–}R})^+]/\mathrm{d}t = k_{\mathrm{obs}}[(\mathrm{Cu^{II}\text{–}R})^+] \quad (19)$$

where

$$k_{\mathrm{obs}} = k_{18} + k_{-17}k_{14}[\mathrm{Cu^{2+}}]/(k_{17}[\mathrm{Cu^+}] + k_{14}[\mathrm{Cu^{2+}}])$$

The rate of the decomposition (k_d) of $(\mathrm{Cu^{II}\text{–}R})^+$ obeys a first-order rate law and at low concentrations of $\mathrm{Cu^{2+}_{aq}}$ $k_d{}^1$ approaches $k_{18} = 2.7 \pm 0.8$ s^{-1}. As $k_{14} = 1.3 \times 10^7$ M^{-1} s^{-1}, $k_{17} = 1.3 \times 10^9$ M^{-1} s^{-1} and under the experimental conditions $[\mathrm{Cu^{2+}_{aq}}]/[\mathrm{Cu^+_{aq}}] \leq 20$, $k_{\mathrm{obs}} = k_{18} + k_{-17}[\mathrm{Cu^{2+}}]/100[\mathrm{Cu^+}]$. Therefore, it is expected that $k_d{}^1$ would depend on $[\mathrm{Cu^{2+}_{aq}}]$ only if $k_{-17} \gg k_{18}$. As seen in Table II, $k_d{}^1$ is within experimental error independent of $[\mathrm{Cu^{2+}_{aq}}]$, indicating from the extreme conditions ($[\mathrm{Cu^{2+}_{aq}}]/[\mathrm{Cu^+_{aq}}] = 20$) that $k_{-17} < 30$ s^{-1}. This result is in agreement with the fact that the intercept of the line obtained by plotting the observed rate constant of the formation as a function of $[\mathrm{Cu^+_{aq}}]$ (Table II) is close to zero, indicating that equilibrium 17 is shifted strongly to the right. From the relative yields of β-carboxyl and β-amine eliminations (Table III), the change in $k_d{}^1$ under high and low concentrations of $\mathrm{Cu^{2+}_{aq}}$ cannot exceed 20%, which is within our experimental accuracy. These results point out that the rate of homolysis of $[\mathrm{Cu^{II}\text{–}CH_2C(CH_3)(NH_3^+)COO^-}]^+$ is lower than that of $[\mathrm{Cu^{II}\text{–}CH_2C(CH_3)_2COO^-}]$, which is in accord with the expectation that electron withdrawing substituents slow down the homolysis of the $\mathrm{Cu^{II}\text{–}C}$ σ-bond.

If one bears in mind that 1 mol of $\mathrm{Cu^+_{aq}}$ is oxidized by 2 mol of $\mathrm{H_2O_2}$ formed during the radiation of water, then, according to the suggested mechanism of the decomposition of $(\mathrm{Cu^{II}\text{–}R})^+$, $G(-\mathrm{Cu^+_{aq}}) = G_i - 2G_{\mathrm{H_2O_2}} - 2G(\mathrm{CO_2})$, where $G_i = G_e + G_{\mathrm{OH}} + G_{\mathrm{H}} = 6.1$. Indeed within experimental error $G(-\mathrm{Cu^+_{aq}})$ and $G(\mathrm{CO_2})$ measured obey this equation (Table III).

The rate constant of the β-elimination of ammonia from $[\mathrm{Cu^{II}\text{–}CH_2C(CH_3)_2NH_3^+}]^+$ was determined earlier to be 6.7 ± 1.5 s^{-1}, which is about 2.5 times higher than $k_{18} = 2.7 \pm 0.8$ s^{-1}. Thus, it is concluded that substitution of a methyl group at the β position to the radical by a carboxylic group does not affect significantly the rate of the deamination process.

Concluding Remarks

(i) The substitution of a methyl group by an amine group at the β position to the carbon-centered radical has only a small effect on the specific rate of the β-elimination of $\mathrm{CO_2}$ (Table IVf,g). This result is in accord with the suggested mechanism where an electron transfer occurs from $\mathrm{COO^-}$ to $\mathrm{Cu^{III}}$. However, this substitution affects the rate of homolysis and the rate of oxidation of the transient complex by $\mathrm{Cu^{2+}_{aq}}$ as expected for electron-withdrawing groups which stabilize the metal–carbon σ-bond, although in this case these groups are at the β position to the carbon-centered free radical.

(ii) When a methyl group is substituted by a carboxylic group at the β position to the carbon-centered radical, the rate of the β-deamination process decreases (Table IVd,e). This result is in accord with those obtained in the case of β-hydroxyl elimination, where electron-donating substituents increase the rate of the reaction (Table IVa–c).

(iii) This study may shed some light on the mechanism of the biological damage initiated by free radicals. Organic free radicals are usually formed via their reaction with $\mathrm{OH^{\bullet}}$ radicals, which can be formed in biological systems via ionizing radiation, via absorption of UV light, and via the Fenton-like reactions. The exact mechanism of the damage caused by the free radicals is not yet clear. This study demonstrates that copper ions react with carbon–centered free radicals to form transients with a copper–carbon σ-bond, which decompose via β-elimination processes causing the cleavage of the amino acid. As copper compounds are present in biological systems, this mechanism can be one of the pathways for the damage occurring in biological systems and might also explain the effect of copper compounds as radiosensitizers.[21–23]

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Registry No. ${}^{\bullet}\mathrm{CH_2C(CH_3)(NH_3^+)COO^-}$, 17456-64-3; $\mathrm{CH_3C(CH_3)(NH_3^+)COO^-}$, 62-57-7; $\mathrm{Cu^{2+}}$, 15158-11-9; $\mathrm{Cu^+}$, 17493-86-6; $\mathrm{CH_3COCH_3}$, 67-64-1; $\mathrm{CO_2}$, 630-08-0; $\mathrm{CH_2{=}C(CH_3)COO^-}$, 18358-13-9; $\mathrm{NH_3}$, 7664-41-7.

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