Oxidation of Cyclic Dipeptide Radicals in Aqueous Solution: The Rapid Hydration of the Intermediate 1,6-Dihydropyrazine-2,5-diones (Cyclic Dehydrodipeptides). A Pulse-radiolysis Study

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The lifetimes of radiolytically generated 1,6-dihydropyrazine-2,5-diones (cyclic dehydrodipeptides) in aqueous solution have been studied by pulse radiolysis.

Hydroxyl radicals were allowed to react with the 2,5-dioxopiperazines glycine anhyride (**1**) and alanine anhydride (**2**). The resulting 3-yl radicals (**4**) and (**5**) have been oxidized by $IrCl_6^{2-}$ in a diffusion-controlled reaction [k(**4**) = 3.1 × 10^9; k(**5**) = 3.0 × 10^9 dm^3 mol^{-1} s^{-1}], which yielded the corresponding carbocations which rapidly deprotonate at N-4 (pK_a < 2) to form the short-lived 1,6-dihydropyrazine-2,5-diones (**6**) and (**7**).

At neutrality, spontanous addition of water to the –C=N– bond [k(**6**) = 640; k(**7**) = 5 ± 2 s^{-1}] prevents the isomerisation of (**6**) and (**7**) into their tautomeric 2,5-dihydroxypyrazines. In acidic and basic solutions a proton-catalysed [k(**6**) = 6.3 × 10^6; k(**7**) = 3.7 × 10^6 dm^3 mol^{-1} s^{-1}] and a base-catalysed [k(**6**) = 1.4 × 10^7; k(**7**) = 2.0 × 10^5 dm^3 mol^{-1} s^{-1}] addition of water is observed.

The 3-yl (**8**) (79%) and *N*-methyl (**9**) (21%) radicals resulting from H abstraction from sarcosine anhydride are also readily oxidized by $IrCl_6^{2-}$ (k = 3.6 × 10^9 dm^3 mol^{-1} s^{-1}). Blocking of N-1 and N-4 by methyl groups prevents deprotonation of the resulting carbocations (**12**) and (**14**) and their reaction with water can be monitored directly by pulsed-conductivity measurements. The positive charge on the exocyclic carbocation (**14**) is effectively stabilized by nitrogen and thus addition of water proceeds only slowly [k(**14**) = 150 s^{-1}]. However, in the case of the endocyclic carbocation (**12**), which is destabilized by the carbonyl group next to C-3, the reaction with water is fast [k(**12**) = 2.3 × 10^6 s^{-1}].

The instability of (**6**) towards attack by water (possibly attended by a general sensitivity towards nucleophiles), may be the reason why no successful synthesis of the tautomeric parent 2,5-dihydroxypyrazine has been reported so far.

Pulse radiolysis with optical or conductimetric detection frequently provides an effective tool for the study of the reactions of short-lived non-radical species after oxidative conversion of the radicals.[1–4]

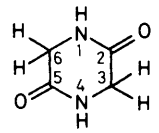
The oxidative conversion of peptides into acylimines has been proposed as a pathway to α-β-dehydropeptides in microbial peptides[5] and their possible significance for the biogenesis of peptide amides and α-keto acids has been pointed out.[6]

Dehydropeptides (acylimines) are regarded as common intermediates in the radiation-induced[7–13] or photochemical[14,15] oxidation of amino acids or peptides in aqueous solution. *N*-Acylimines derived from the peptide backbone are especially sensitive to hydrolysis and to our knowledge have not previously been directly observed. Acylimines were suggested as intermediates in the disproportionation reactions of amino acid or peptide derived radicals[10] and after oxidation of such radicals by transition-metal ions or their complexes [*e.g.* $Fe(CN)_6^{3-}$].[10,12] The addition of oxygen to radical sites next to NH followed by a base-induced elimination of $O_2^{-\bullet}$ also leads to such an intermediate.[9,11,13]

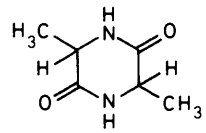
Furthermore, our interest in the dehydropeptides derived from cyclic dipeptides was stimulated because these heterocycles are the diketo-tautomers (1,6-dihydropyrazine-2,5-diones) of 2,5-dihydroxypyrazines [*cf.* (**17**)]. This is an interesting class of compound, the predominant tautomer of which, 5-hydroxypyrazin-2(1*H*)-one [*cf.* (**16**)], was found to undergo Diels–Alder reactions with electron-deficient or strained alkenes.[16,17] It was noted that 3,6-disubstituted 2,5-dihydroxypyrazines are relatively stable in alkaline solution but sensitive to acid, apparently because of hydrolytic fission of the pyrazine nucleus.[18] The monosubstituted 2,5-dihydroxy-3-phenylpyrazine was found to be unstable even in neutral solution.[19] Several attempts to prepare less highly substituted 2,5-dihydroxypyrazines have failed.[19,20] So far, no successful synthesis of the parent compound or of the monosubstituted derivatives has been reported, although the formation of 2,5-dihydroxypyrazine was observed in radiation-chemical[12,21] and photochemical[22] investigations.

The 1,6-dihydropyrazine-2,5-diones (**6**) and (**7**) derived from the cyclic dipeptides (2,5-dioxopiperazines) of glycine and alanine were suggested to be the precursors of their corresponding 2,5-dihydroxypyrazines after irradiation under vacuum and dissolution of the solids in alkaline solution.[21] Recently we suggested that the disproportionation of the cyclic peptide radicals (**4**) and (**5**) does not involve the abstraction of a nitrogen-bound hydrogen atom (*i.e.* the formation of 3,4-dehydropiperazines), but predominantly the abstraction of an H atom bound at C-6. This results in the formation of 5-hydroxypyrazin-2(1*H*)-ones (**16**; R = H, CH_3), which then isomerise to form the 2,5-dihydroxypyrazine anions or dianions in alkaline solution.[12]

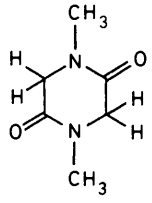
In this study we present further evidence that there is no isomerisation of the 1,6-dihydropyrazine-2,5-diones (**6**) and (**7**) derived from glycine anhydride (**1**) and alanine anhydride (**2**) to 2,5-dihydroxypyrazines [*cf.* (**17**)] in aqueous solution. A description of the characteristics of the rapid, spontaneous and acid–base-catalysed addition of water to the imine bond to yield the corresponding 3-hydroxy-2,5-dioxopiperazines (**10**) and (**11**) is given. For comparison the rate constants for the hydration of the carbocations derived from sarcosine anhydride



glycine anhydride
(1)



alanine anhydride
(2)



sarcosine anhydride
(3)

Table. Reaction rate constants for the oxidation of cyclic dipeptide radicals by $IrCl_6^{2-}$ (pH *ca.* 5.7), the hydration of the cyclic dehydropeptides, and the hydration of carbocations stabilized by nitrogen.

Reaction	Scheme	k/dm^3 mol^{-1} s^{-1}	monitored at λ/nm
GA$^\bullet$ (**4**) + $IrCl_6^{2-}$	3	3.1×10^9	250/270
AA$^\bullet$ (**5**) + $IrCl_6^{2-}$	3	3.0×10^9	350/360
SA$^\bullet$ (**8**;**9**) + $IrCl_6^{2-}$	7,8	3.6×10^9	250/350/490
(**8**) + $IrCl_6^{2-}$	7	3.0×10^9	*b*
(**6**) + H_2O	5	640 s^{-1}	290
(**6**) + H_2O (H^+-catalysed)[a]	6	6.3×10^6	290
(**6**) + OH^-	5	1.4×10^7	290
(**7**) + H_2O	5	5 ± 2 s^{-1}	290
(**7**) + H_2O (H^+-catalysed)[a]	6	3.7×10^6	290
(**7**) + OH^-	5	2.0×10^5	290
(**12**) + H_2O	7	$2.3 \pm 0.5 \times 10^6$ s^{-1}	*b*
(**14**) + H_2O	8	150 s^{-1}	*c*

[a] The rate constant (as given in the Table) $k(H^+) = k_{6c}K_a^{-1}$, where K_a is the acid-dissociation constant; see text. [b] From pulsed-conductivity (DC) measurements (pH 4.0); see text. [c] From pulsed-conductivity (AC) measurements (pH 4.3); see text.

(**3**) were evaluated. The rate constants obtained in this study are summarized in the Table.

Results and Discussion

Radicals in the α-position to the peptide function can be conveniently produced by H-atom abstraction from the cyclic dipeptides (**1**) and (**2**) by OH radicals or H atoms generated in the radiolysis of N_2O-saturated aqueous solutions.[12,13,23] Under these conditions $G(^\bullet OH) = 0.56$ μmol J^{-1} and $G(H^\bullet) = 0.06$ μmol J^{-1} (Schemes 1 and 2).

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^\bullet OH, e_{aq}^-, H^\bullet, H^+, H_2O_2, H_2$$

Scheme 1.

$$e_{aq}^- + N_2O \longrightarrow {}^\bullet OH + N_2 + OH^-$$

Scheme 2.

In the case of glycine anhydride (**1**) and alanine anhydride (**2**) H abstraction at C-3 is quantitative, while in sarcosine anhydride (**3**) 20% H abstraction occurs at the activated methyl groups (Scheme 4).[12] The generation of radicals by H-atom abstraction from a solute and their subsequent one-electron oxidation was introduced as a method for carbocation production[24–27] with subsequent direct measurements of their lifetimes.[3,4] The cyclic-peptide radicals (**4** and **5**) are no longer easily oxidized by tetranitromethane but are still oxidized by $Fe(CN)_6^{3-}$ presumably by one-electron transfer.[12] The carbocations [mesomeric with the iminocations (**6a**) and (**7a**)] formed in this reaction readily deprotonate at N-4 to yield (1,6-dihydropyrazine-2,5-diones (**6**) and (**7**). Under the experimental conditions (≥1 mmol dm^{-3} cyclic dipeptide) the H abstraction from the solute is completed within a few microseconds (≤4 μs) and if the oxidation of the radical is fast compared with the lifetime of the dehydropeptide the addition of water to the imine bond can be studied.

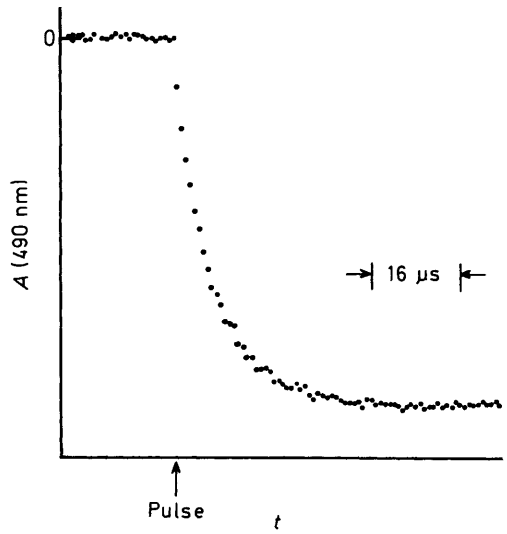
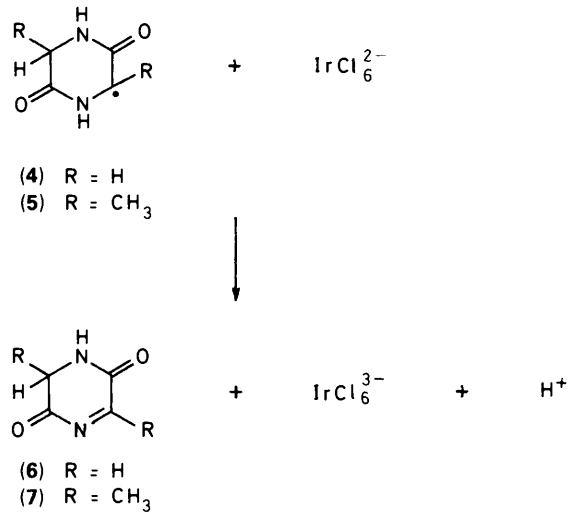


Figure 1. Change in absorbance at 490 nm of a N_2O-saturated sarcosine-anhydride solution (10^{-3} mol dm^{-3}; pH 5.7) irradiated with a 0.4 μs electron pulse of about 5 Gy in the presence of $IrCl_6^{2-}$ (3.8×10^{-5} mol dm^{-3}).

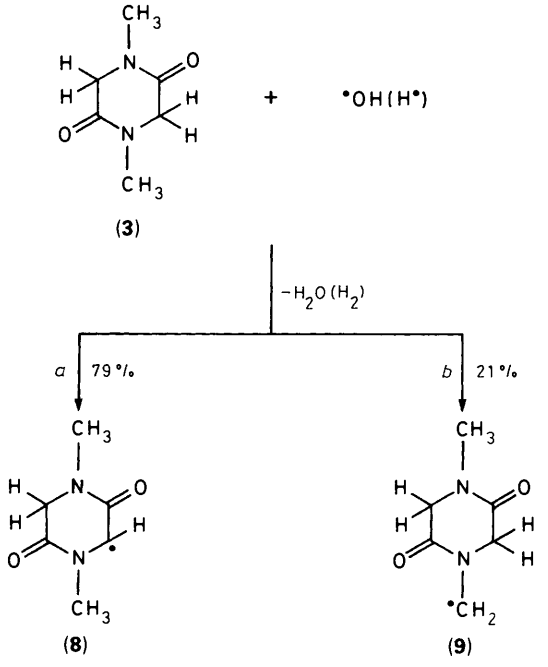
Oxidation of the Cyclic Peptide Radicals by $IrCl_6^{2-}$.—Though the radicals derived from glycine anhydride (**1**) and alanine anhydride (**2**) are readily oxidized by $Fe(CN)_6^{3-}$ {k[**4** + $Fe(CN)_6^{3-}$] = 1.0×10^8 dm^3 mol^{-1} s^{-1}; k[**5** + $Fe(CN)_6^{3-}$] = 8.0×10^8 dm^3 mol^{-1} s^{-1}},[12] the rate of oxidation (using moderately high concentrations of the oxidant) was found to be far too slow, to follow the reactions of the postulated intermediate dehydropiperazines. Using the more powerful oxidant[27] $IrCl_6^{2-}$ the oxidation is no longer rate determining. The rate constants for the oxidation of (**4**) and (**5**) (Scheme 3) and for the oxidation of the radicals (**8**) and (**9**) (Schemes 7 and 8); derived from sarcosine anhydride (**3**) (Scheme 4) were calculated from the observed first-order rate constants at different oxidant concentrations (3.8–9.2 × 10^{-5} mol dm^{-3}):

$$k[(\mathbf{4}) + IrCl_6^{2-}] = 3.1 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$k[(\mathbf{5}) + IrCl_6^{2-}] = 3.0 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$
$$k[(\mathbf{8}), (\mathbf{9}) + IrCl_6^{2-}] = 3.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}.$$

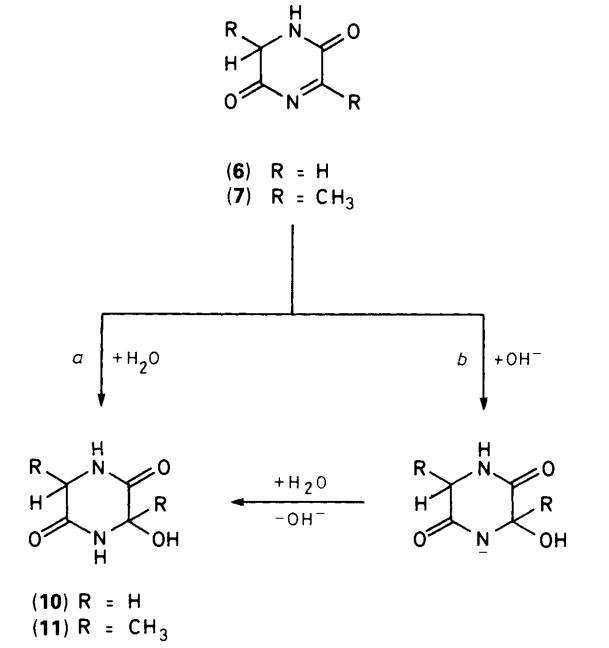
The yield of the reducing radicals [G(reducing radicals) = $G(IrCl_6^{2-}$ consumption)] was quantified by measuring the bleaching of the $IrCl_6^{2-}$ absorption at 490 nm after an electron pulse (Figure 1). The difference in extinction coefficients between $IrCl_6^{2-}$ [ε(490 nm) = 3 790 dm^3 mol^{-1} cm^{-1}] and $IrCl_6^{3-}$ [ε(490 nm) ≤ 10 dm^3 mol^{-1} cm^{-1}] is about 3 780 dm^3 mol^{-1} cm^{-1} (electron transfer). The same holds for another Ir^{III}



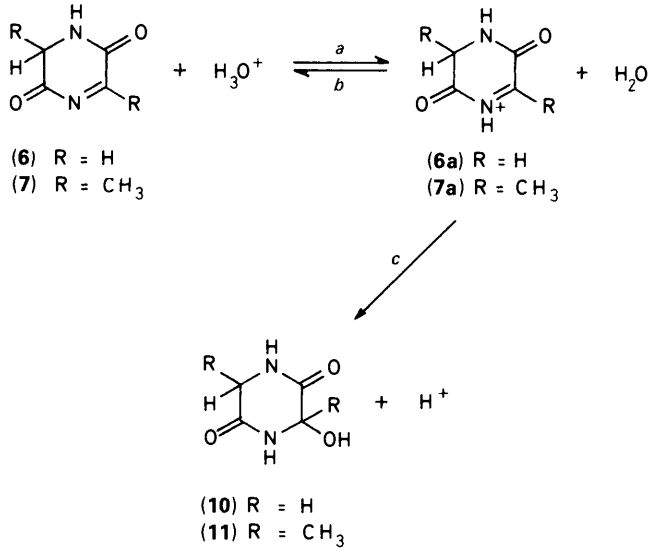
Scheme 3.



Scheme 4.



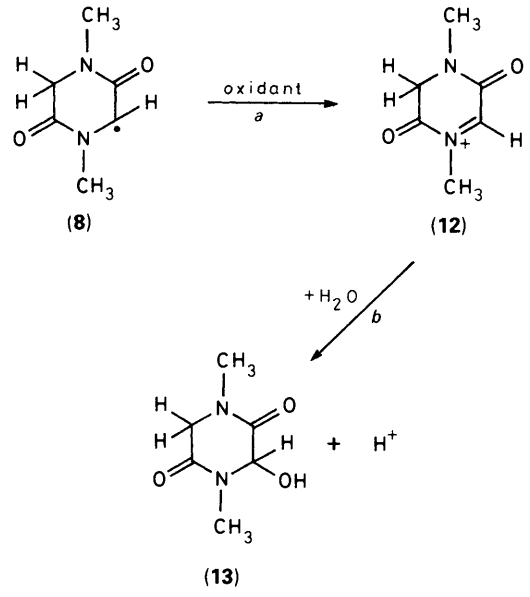
Scheme 5.



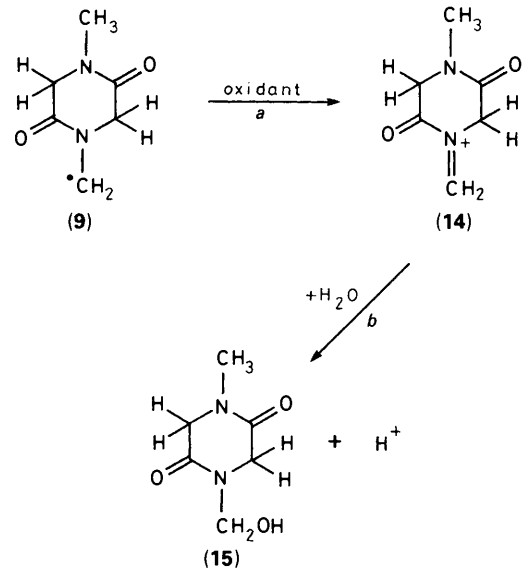
Scheme 6.

species not absorbing at this wavelength after reductive dechlorination.[27] G(reducing radicals) (accounting for the total reducing equivalents with *ca.* 10% contribution due the reaction of the H atom with $IrCl_6^{2-}$) is quantitative (0.58–0.62 μmol J^{-1} at pH *ca.* 5.4 and 11.4) in the case of the three investigated compounds. This was already reported previously for the glycine anhydride derived radicals when $G[Fe(CN)_6^{3-}$ consumption] was measured.[12] Notably, the full yield is obtained in the case of the sarcosine anhydride derived radicals as well, indicating that besides the 3-yl radicals (**8**) (79% of the initial radical yield[12]) the *N*-methyl radicals (**9**) (21%) also act as reductants (Schemes 7 and 8). This is confirmed by quantifying $G(H^+)$ in pulsed-conductivity (AC) measurements at pH 4.4 (see below).

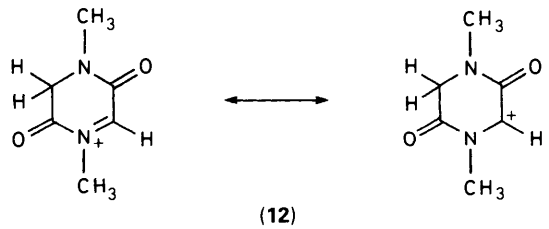
Absorption Spectra of the Intermediates.—Under the conditions used oxidation of the radicals by $IrCl_6^{2-}$ is fast (half-life ⩽ 6 μs), as is the proton loss at N-4 of the intermediate cations (**6a**) and (**7a**) at pH > 5 (Scheme 6*b*). The absorbance was measured when the oxidation of the glycine anhydride-derived radicals (**4**) was completed (pH 5.2; 45 μs after a 0.4 μs electron pulse of about 5 Gy) giving rise to a difference spectrum [Δ(1); full circles in Figure 2] due to the change in concentration of all absorbing species. The oxidation was followed by a second slower process (half-life = 1.1 ms), which is attributed to the decay of the dehydropiperazine (**6**). A similar (but somewhat faster) decay at pH 9.9 is shown in Figure 3. The resulting absorbance after the slower decay at pH 5.2 was measured 10.5 ms after the pulse [Δ(2); filled squares in Figure 2]. The difference between the two spectra [Δ(1) − Δ(2) = Δ(intermediate)] is the absorbance of the intermediate ($G \times \varepsilon$; filled triangles in Figure 2) and is thus attributed to the dehydropiperazine (**6**) [the final products (**10**) or (**11**) do not absorb above 250 nm].[12] The dehydropeptides do not show strong optical absorptions in the accessible wavelength region



Scheme 7.

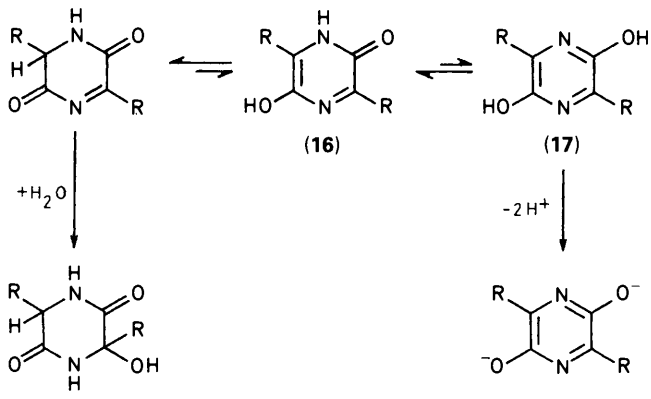


Scheme 8.



($\lambda > 250$ nm). However the absorption measured at 260 nm [$G\varepsilon$(260 nm) = 460 μmol J^{-1} dm^3 mol^{-1} cm^{-1}; pH 5.2; Figure 4] is comparable to the absorption which was reported for the intermediate isouracil [$G\varepsilon$(260 nm) ≈ 860 μmol J^{-1} dm^3 mol^{-1} cm^{-1}; pH 6.5] formed after oxidation of the 6-yl radicals derived by H abstraction from 5,6-dihydrouracil.[1]

The absorbance at 290 nm after completion of oxidation was found to be independent of pH in the range from pH 4–9. Below



Scheme 9.

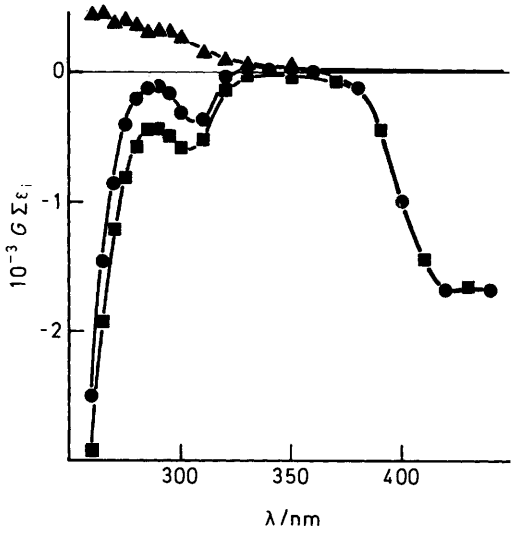


Figure 2. Difference spectra ($G\Sigma\varepsilon_i$/μmol J^{-1} dm^3 mol^{-1} cm^{-1}) of an N_2O-saturated glycine anhydride solution (10^{-3} mol dm^{-3}; pH 5.2) irradiated with a 0.4 μs electron pulse of about 5 Gy in the presence of $IrCl_6^{2-}$ (5 × 10^{-5} mol dm^{-3}). Filled circles: after completion of the oxidation [Δ(1); 45 μs after the pulse]. Filled squares: after the decay of the intermediate [Δ(2); 10.5 ms after the pulse]. Filled triangles: [Δ(1) − Δ(2)] = Δ(intermediate).

pH 4 the absorbance decreases; this could be due to the protonation of (**6**) at N-4, or because proton-catalysed water addition becomes too fast to monitor the spectroscopic properties of the transient properly. At pH *ca.* 9 the absorbance increases due to the deprotonation at N-1, but decreases again at pH > 10 because a rapid base-catalysed reaction sets in. From this measurement only a rough estimate of the acidic pK_a < 2 (protonation at N-4, Scheme 6*a*) and the basic pK_a ⩾ 11 for (**6**) (deprotonation at N-1) is possible.

Addition of H_2O *and* OH^- *to the* C=N *Double Bond.*—The pH dependence of the rate of hydration of the dehydropeptides (**6**) and (**7**) was monitored at 290 nm because of a favourable ratio of the chromophore loss of the dehydropeptides to the overall change in absorbance (see Figure 2). Due to the weak absorbance of (**6**) and (**7**) a dose of about 12–15 Gy per pulse was used which produced about 7–9 μmol (H^+) per pulse and made corrections of the pH for the unbuffered solutions necessary. The decay is found to follow first-order kinetics and the observed rates are dependent on the OH^- concentration. In alkaline solution the hydration rate constant is given by:

$$k_{obs} = k_{5a} + k_{5b}[OH^-]$$

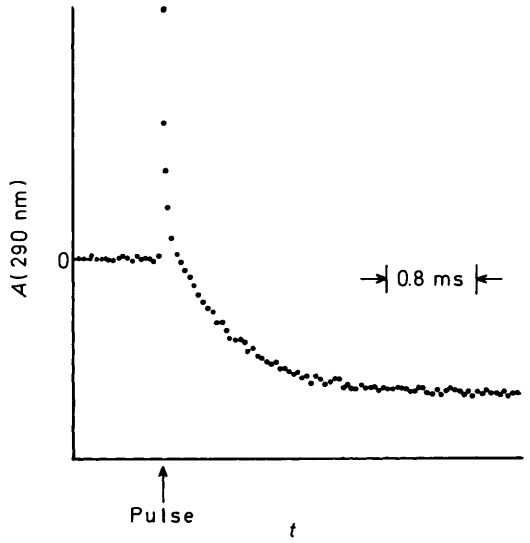


Figure 3. Change in absorbance at 290 nm of an N_2O-saturated glycine anhydride solution (10^{-3} mol dm^{-3}) irradiated with a 0.4 μs electron pulse of about 14 Gy in the presence of $IrCl_6^{2-}$ (4.6×10^{-5} mol dm^{-3}) at pH 9.9.

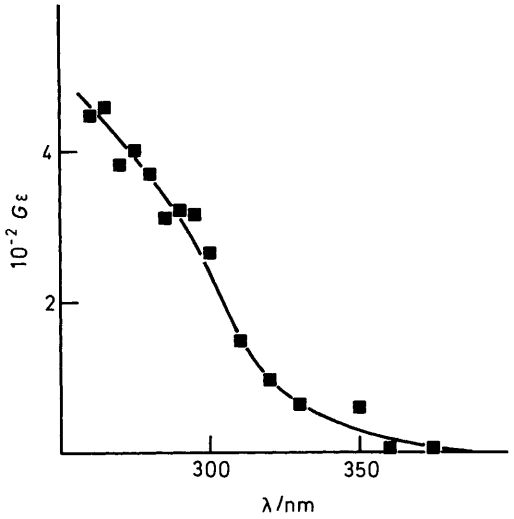


Figure 4. Transient absorption spectra [Δ(intermediate) = Δ(1) − Δ(2)/μmol J^{-1} dm^3 mol^{-1} cm^{-1}] assigned to the intermediate dehydropeptide derived from glycine anhydride (for conditions see Figure 2).

assuming that the rate of the proton-catalysed reaction and the rate of the back reaction (release of the poor leaving group OH^-) are negligible and that there is no change in the protonation state of the intermediate which alters the rate of the spontaneous or base-catalysed reaction. A plot of k_{obs} *vs.* [OH^-] is indeed linear (Figures 5 and 6, insets, full squares). From the intercepts $k(H_2O) = k_{5a} = 640$ s^{-1} and 5 ± 2 s^{-1} are obtained for the spontaneous addition of water to (**6**) and (**7**), respectively. From the slopes $k(OH^-) = k_{5b} = 1.4 \times 10^7$ dm^3 mol^{-1} s^{-1} and 2.0×10^5 dm^3 mol^{-1} s^{-1} are obtained for the base-catalysed hydration of (**6**) and (**7**), respectively.

Thus it seems that deprotonation at N-1 does not occur below pH 10.8 in the case of (**6**) and not below pH 11.5 in the case of (**7**). At higher pH, where the addition of OH^- becomes too fast to be monitored accurately, the slopes of log k_{obs} *vs.* pH appear to deviate from unity.

The introduction of a methyl group at C-3 (**7**) shows a pronounced substituent effect. The rate of the spontaneous addition of water is reduced by a factor of *ca.* 130, con-

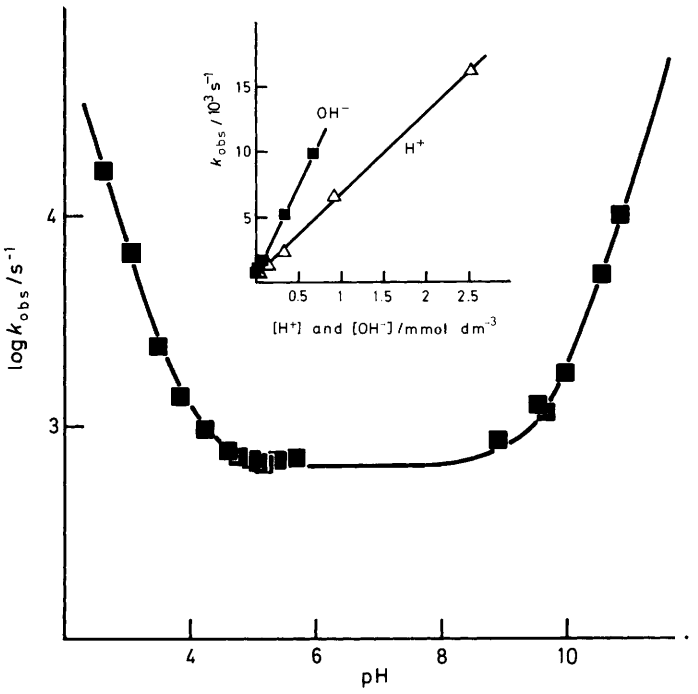


Figure 5. Plot of log k_{obs} *vs.* pH for the decay of the intermediate dehydropeptide (monitored at 290 nm) after oxidation of the glycine anhydride-derived radicals with $IrCl_6^{2-}$ [(4–8) $\times 10^{-5}$ mol dm^{-3}] after a 0.4 μs electron pulse of about 14 Gy. Solid line: calculated with the rate constants $k(H_2O)$, $k(OH^-)$, and $k(H^+)$ given in the text (see the Table). Inset: ■, Plot of k_{obs} *vs.* [OH^-]; △, Plot of k_{obs} *vs.* [H^+].

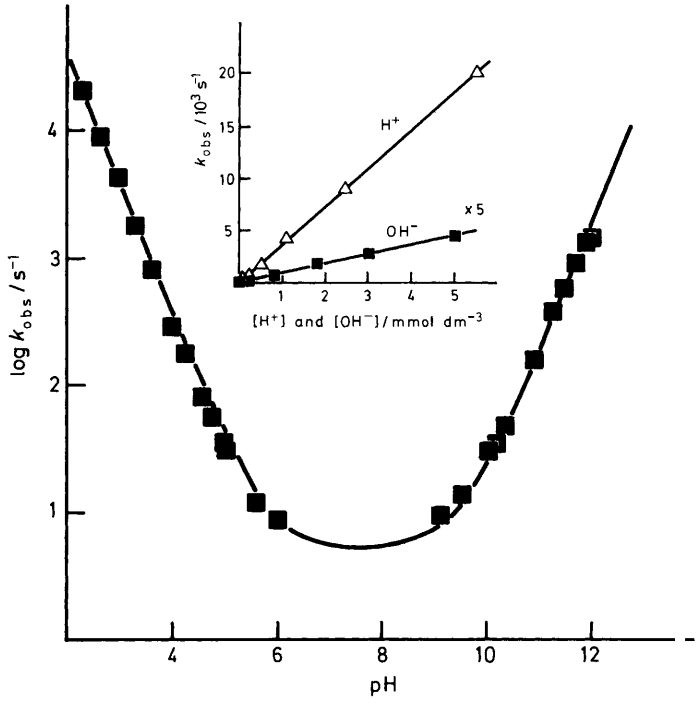


Figure 6. Plot of log k_{obs} *vs.* pH for the decay of the intermediate dehydropeptide (monitored at 290 nm) after oxidation of the alanine anhydride derived radicals with $IrCl_6^{2-}$ (conditions as in Figure 5). Solid line: calculated with the rate constants $k(H_2O)$, $k(OH^-)$, and $k(H^+)$ given in the text (see the Table). Inset: ■: Plot of ($5k_{obs}$) *vs.* [OH^-]; △: Plot of k_{obs} *vs.* [H^+].

comitantly the rate of the base-catalysed hydration decreases by a factor of 70. The electron-donating effect decreases the partial positive charge at C-3 of the polarized double bond making it

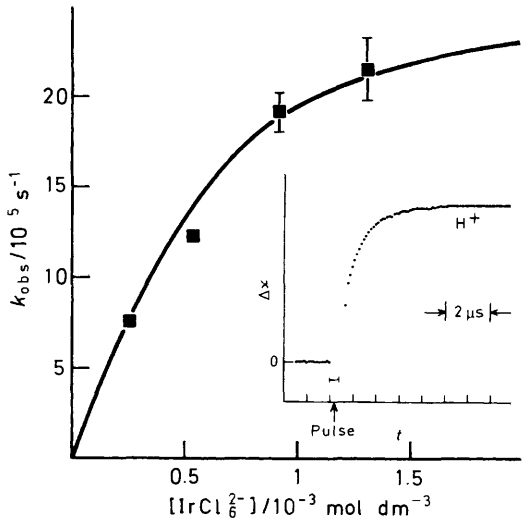


Figure 7. Plot of k_{obs} *vs.* $[IrCl_6^{2-}]$ for the conductivity build-up after oxidation and hydration of the sarcosine anhydride derived radicals after a 0.4 μs electron pulse of about 5 Gy at pH 4. Solid line: calculated with $[SA^{\bullet}] = 3 \times 10^{-6}$ mol dm^{-3}, k(oxidation) = 3.0×10^9 dm^3 mol^{-1} s^{-1}, and k(hydration) = 2.3×10^6 s^{-1}. Inset: Conductivity change due to formation of $IrCl_6^{3-}$ (oxidation) and proton release (hydration) ($[SA] = 5 \times 10^{-3}$ mol dm^{-3}; $[IrCl_6^{2-}] = 5.5 \times 10^{-4}$ mol dm^{-3}).

less attractive for nucleophilic attack. However in both cases the negative charge accepted from the nucleophile is effectively stabilized by the carbonyl group next to N-4.

Proton-catalysed Hydration.—In acidic solution the overall scheme may be written as in Scheme 6, involving attack of water on the imino cations (**6a**) and (**7a**), as inferred from the hydrolysis of Schiff bases,[28,29] leading to the following equation for the observed rate constant as long as the pH is considerably higher than the pK_a of the acylimine:

$$k_{obs} = k_{5a} + k_{6c}K_a^{-1}[H^+]$$

where K_a is the acid dissociation constant (Scheme 6) and k_{5a} the rate constant for the spontaneous addition of water. Plots of k_{obs} *vs.* $[H^+]$ give straight lines (Figures 5 and 6, insets, open triangles), giving rise to the same intercept for the spontaneous addition of water as determined above. From the slope $k(H^+) = k_{6c}K_a^{-1} = 6.3 \times 10^6$ and 3.7×10^6 dm^3 mol^{-1} s^{-1} are obtained for the proton-catalysed hydration of (**6**) and (**7**), respectively.

Figures 5 and 6 show a plot of log k_{obs} *vs.* pH for the whole pH range examined. The solid lines are calculated from the rate constants $k(H_2O)$, $k(OH^-)$, and $k(H^+)$ according to:

$$\log k_{obs} = \log(k_{5a} + k_{5b}[OH^-] + k_{6c}K_a^{-1}[H^+])$$

Approaching the acidic pK_a of the acylimines, k_{obs} is expected to become independent of $[H^+]$ reaching a plateau value. The slopes of log k_{obs} *vs.* pH in the acidic region of Figures 5 and 6 are still unity at pH 2.6 or 2.3, respectively, the most acidic solutions studied. From this it is concluded that the pK_a values of (**6**) and (**7**) must be well below two, which is in agreement with the decrease in absorbance at 290 nm described above (see also below).

Lifetimes of Carbocations Stabilized by One Nitrogen Atom.—Trialkoxymethyl- and α-dialkoxyalkyl-carbocations were found to be sufficiently stabilized by their heteroatoms to measure their lifetimes in aqueous solution and structure–activity relations have been derived.[3,4] The lifetime of the monoalkoxy carbocation derived from the oxidation of the 2-isopropoxypropan-2-yl radical however was found to be too short to permit its study ($k \geqslant 2 \times 10^7$ s^{-1}).[3]

An estimate of the rate constant of water addition to the protonated *N*-acylimine can be obtained when the rate-determining protonation equilibrium is by-passed by blocking N-1 and N-4 with methyl groups. The sarcosine anhydride derived radicals formed by H abstraction from the ring carbon atoms (**8**) (79%) and from the *N*-methyl group (**9**) (21%) (Scheme 4),[12] are rapidly oxidized by $IrCl_6^{2-}$ leading to the formation of cations (**12**) and (**14**) (Schemes 7 and 8). These imino cations[30] can be regarded as stabilized carbocations [*cf.* (**12**)] in view of the attack of the nucleophile at the carbon atom as electrophilic centre. The rate of hydration can be monitored by measuring the change in conductivity due to the formation of a proton (Schemes 7 and 8; inset to Figure 7), while $IrCl_6^{2-}$ is converted into the more conducting[3] $IrCl_6^{3-}$. The rate constant of the oxidation–hydration process was monitored at pH 4 to make the neutralisation reaction after the electron pulse fast enough (half-life *ca.* 0.05 μs) in order to exclude interference with the build-up of conductivity due to hydration. At low concentrations of $IrCl_6^{2-}$ the rate of the conductivity build-up was found to depend on the oxidant concentration, thus oxidation is rate determining. On increasing the oxidant concentration the slope of k_{obs} *vs.* $[IrCl_6^{2-}]$ decreases though a plateau was not attained below an oxidant concentration of 1.3×10^{-3} mol dm^{-3} (Figure 7).

The curve in Figure 7 was computed with a modified program based on the Gear integration method,[31] with rate constants of 3.0×10^9 dm^3 mol^{-1} s^{-1} for the oxidation and 2.3×10^6 s^{-1} for the addition of water to the carbocations. When a higher rate constant for the oxidation step was used in the computation [*e.g.* 3.6×10^9 dm^3 mol^{-1} s^{-1}, the overall rate constant for the oxidation of both radicals (**8**) and (**9**) determined in the optical measurements] the curve became steeper and the fit to the observed rate constants became less satisfactory. Apparently the *N*-methyl radical (**9**) is a stronger reductant than radical (**8**) and its rate constant of oxidation is greater than 3×10^9 dm^3 mol^{-1} s^{-1}, whereas the rate constant for the oxidation of (**8**) is quite similar to the rate constants for the oxidation of (**4**) and (**5**) (3.1×10^9 and 3.0×10^9 dm^3 mol^{-1} s^{-1}, respectively). Thus, the rate of the observed conductivity build-up is mainly due to the oxidation of (**8**) and the hydration of its successor (**12**) (79% of the radical yield which includes the H atom from Scheme 1).

This is confirmed by quantifying the conductivity build-up, which is due to the hydration of (**12**) [$\lambda°(H^+) = 325$ cm^2 Ω^{-1} mol^{-1}, 20 °C] and the full yield for the oxidation[3] [$\lambda°(IrCl_6^{3-}) - \lambda°(IrCl_6^{2-}) = 100$ cm^2 Ω^{-1} mol^{-1}] including the reaction of the H atom with $IrCl_6^{2-}$. From this G(**12**) + $G(H^{\bullet}) = G$(**8**) + $G(H^{\bullet}) = 0.48$ μmol J^{-1} was obtained by pulsed DC (15 μs after the pulse; pH 4.0) and 0.52 μmol J^{-1} by pulsed AC measurements (60 μs after the pulse; pH 4.3). This is in good agreement with the value we obtained by gas chromatography in a product study [G(**8**) = 0.49 μmol J^{-1} (79%)],[12] assuming that in the absence of another effective scavenger the H atoms ($G = 0.06$ μmol J^{-1}) do predominantly abstract ring-bound hydrogen atoms from sarcosine anhydride (**3**) and thus contribute to the yield of (**8**). Thus, it is concluded that the endocyclic carbocation (**12**) rapidly adds water with $k(H_2O)$ *ca.* 2.3×10^6 s^{-1}.

The fast conductivity build-up in the AC experiment was followed by a much slower second build-up ($G = 0.13$ μmol J^{-1}) which is assigned to the hydration of the exocyclic carbocation (**14**) [$k(H_2O) = 150$ s^{-1}]. The combined yield of these two pathways accounts for the full radical yield.

The (dimethylamino)methyl radical was treated with oxygen to yield $O_2^{-\bullet}$ and the dimethylimonium ion, either by electron transfer or *via* a short-lived peroxyl radical (Scheme 10).[2]

$$(CH_3)_2NCH_2^{\bullet} + O_2 \longrightarrow (CH_3)_2N^+{=}CH_2 + O_2^{-\bullet}$$

Scheme 10.

This carbocation is better stabilized than (**12**) and (**14**) (which do have destabilizing[30] carbonyl groups in 2- and 5-position of the ring). In this case the spontaneous addition of water was found to be too slow to be monitored by pulse radiolysis and the addition of OH^- to form (hydroxymethyl)dimethylamine did not reach the diffusion-controlled limit, the rate constant being only $k = 2.8 \times 10^8$ dm^3 mol^{-1} s^{-1}.[2] However the addition of OH^- to (**12**) (which is too fast to be monitored) could well be diffusion controlled.

The rate constant $k_{7b} = 2.3 \times 10^6$ s^{-1} for the hydration of (**12**) can serve as an estimate for the rate constant of water addition (k_{6c}) to the imino cation (**6a**) (though this might even react somewhat faster). By substitution of k_{6c} with k_{7b} in the equation: $k(H^+) = k_{6c}K_a^{-1} = 6.3 \times 10^6$ dm^3 mol^{-1} s^{-1} a pK_a value of about 0.4 for the protonation of (**6**) at N-4 is calculated.

Conclusions

The formation of dehydropeptides, produced as unstable intermediates in the oxidation of cyclic peptide radicals, can now be regarded as being well established. The mechanism for the rapid acid–base catalysed hydration of the intermediate formed after electron transfer to $IrCl_6^{2-}$ shows similarities with the formation of the carbinolamine intermediate in the hydrolysis of Schiff bases.[28,29] However, in contrast with most of these carbinolamines[28] the 3-hydroxy-2,5-dioxopiperazines (**10**) and (**11**), formed by the addition of water to the dehydropeptides, are sufficiently stable against subsequent hydrolysis to be determined quantitatively by gas chromatography and were isolated after irradiation on a preparative scale.[12]

In contrast with the 1,6-dihydropyrazine-2,5-diones (**6**) and (**7**) their tautomeric 5-hydroxypyrazin-2(1*H*)-ones (**16**) were found to isomerise and yield the corresponding 2,5-dihydroxypyrazine dianions in alkaline solution. These appeared to be stable on the time-scale of pulse radiolysis [(Scheme 9); R = H, CH_3].[12] Thus it seems possible that the kinetic instability of the parent (**17**; R = H) and monosubstituted 2,5-dihydroxypyrazines is mainly due to the fast addition of water (or probably other nucleophiles) to their diketo-tautomers. The polar C=N double bonds in these compounds are vulnerable to nucleophilic attack because of the effective charge delocalisation by the neighbouring carbonyl group. The introduction of a methyl group in the 3-position (**7**) slows down the spontaneous hydration by a factor of 130 and the half-life is increased from 1.1 ms to 0.14 s at room temperature and pH 7. In view of these results the kinetic stability against attack by water of the more highly substituted 2,5-dihydroxypyrazines appears to be mainly due to: (*i*) The shift of the keto–enol equilibria towards the enol form (see Scheme 9), (*ii*) the decrease in the rate of the spontaneous addition of water to the acylimine bond because of steric hindrance and/or negative (inductive) effects on the C-3 atom, and (*iii*) a decrease in the rate of the proton-catalysed addition of water to these dehydropeptides by shifting the protonation equilibria towards lower pH by introducing electron-withdrawing substituents.

The reported procedures to synthesise 2,5-dihydroxypyrazines do usually involve the addition of water and/or dilute acids in the course of the work-up,[18–20] which might spoil a successful synthesis of the unstable parent compound.

The examples for the hydration of carbocations stabilized by nitrogen do virtually cover the whole range of rates (stabilization) accessible by pulse radiolysis and might thus serve as basic data for more detailed investigations of related compounds of biological interest.

Experimental

Glycine anhydride (GA) (Aldrich) and *meso*-DL-alanine anhydride (AA) (Aldrich ABC) were recrystallized twice from triply distilled water. Sarcosine anhydride (SA) (Janssen), dimethyl sulphoxide, KSCN (Merck), K_2IrCl_6 (Ventron) and other chemicals of the highest purity available were used as received.

Cyclic dipeptide solutions (1×10^{-3} mol dm^{-3}) in triply distilled or reagent-grade Millipore Milli-Q water were bubbled with oxygen-free (Oxisorb, Messer-Griesheim) nitrous oxide for 30 min; the oxidant concentration was $3.8–9.2 \times 10^{-5}$ mol dm^{-3}. In the pulse-conductimetric (DC) experiments with sarcosine anhydride, where high concentrations of K_2IrCl_6 (2.6×10^{-4}–1.3×10^{-3} mol dm^{-3}) were used the concentration of the cyclic dipeptide was $(5–10) \times 10^{-3}$ mol dm^{-3}.

The pulse-radiolysis experiments were carried out at 20 ± 1 °C with a 2.8 MeV Van de Graaff electron accelerator with electron pulses of 0.4 μs duration. The applied doses to study the oxidation reactions were 4–5 Gy, the dose in the pulsed-conductivity (DC) measurements was approximately the same, thus producing radical concentrations of about 3×10^{-6} mol dm^{-3}. In order to study the hydration of the dehydropeptides derived from glycine anhydride and alanine anhydride a somewhat higher dose (12–15 Gy per pulse) was necessary because of the low extinction coefficients of the intermediates. In the pulsed-conductivity (AC) measurements a dose of about 5–18 Gy, a sarcosine anhydride concentration of 2×10^{-3} mol dm^{-3}, and an oxidant concentration of $(1–2.3) \times 10^{-4}$ mol dm^{-3} was used. The pulse radiolysis set-up has been described elsewhere.[3,32] For the optical measurements dosimetry was performed with N_2O-saturated 10^{-2} mol dm^{-3} KSCN solutions taking $G(SCN)_2^{-\bullet}] = 0.62$ μmol J^{-1} and ε(480 nm) = 7 600 dm^3 mol^{-1} cm^{-1}.[33] For pulse-conductimetric experiments 10^{-2} mol dm^{-3} dimethylsulphoxide solutions at pH 5 were used taking $G(H^+) = 0.62$ μmol J^{-1}.[3,34]

The pH was adjusted with $HClO_4$, H_2SO_4, or NaOH. Cyclic dipeptides are not stable in alkaline solutions.[35] To avoid hydrolysis in experiments at high pH, neutral solutions of the solutes were mixed with an alkaline solution just before reaching the pulse-radiolysis cell. The pH was measured in the effluent.

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