Pulse Radiolysis of Penicillamine in Aqueous Solution: the Thiyl Radical and the Disulphide Radical Anion

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Summary The RS· radical and the $RSSR^-$ radical anion of penicillamine have λ_{max} 330 nm, ϵ_{330} $1{\cdot}22 \times 10^3$ M^{-1} cm^{-1} and λ_{max} 450 nm, ϵ_{450} $7{\cdot}5 \times 10^3$ M^{-1} cm^{-1}, respectively, and K_1 for the equilibrium, $RS\cdot + RS^- \rightleftarrows RSSR^-$ is $2{\cdot}5 \times 10^2$ M^{-1} at pH 8.

ADAMS *et al.*[1] have shown that the thiyl radicals produced by pulse radiolysis of aqueous solutions of mercaptans combine with the sulphydryl anion to give a radical anion complex.

$$RS\cdot + RS^- \rightleftarrows RSSR^- \qquad (1)$$

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This complex has been observed with several compounds including cysteamine,[1] cysteine,[2] hydrogen sulphide,[3] and several alkyl mercaptans[4] The same transient was produced from disulphides by addition of solvated electrons [1]

$$\mathrm{RSSR} + e^-_{aq} \rightarrow \mathrm{RSSR}^- \qquad (2)$$

In the absence of RS^- the radical anion decays rapidly by the reverse of reaction 1 In both cases the thiyl radicals eventually combine to give disulphide

$$\mathrm{RS}\cdot + \mathrm{RS}\cdot \rightarrow \mathrm{RSSR} \qquad (3)$$

Adams *et al* noted a small difference between the spectrum of RSSR^- generated by reaction 1 and that produced by reaction 2 and suggested that the difference, higher absorption at about 300 nm, was due to the RS radical We have been investigating the transients produced by pulse radiolysis of penicillamine, $\mathrm{HO_2C\,CH(NH_2)\,C(CH_3)_2SH}$, in aqueous solution and have observed both the RS radical and the RSSR^- radical anion directly

Solutions were buffered with phosphate (8 mM) and were irradiated with 0 04—1 µs pulses of 35 Mev electrons Spectra were measured with a split light-beam system Aqueous potassium thiocyanate solution saturated with nitrous oxide was used for dosimetry,[5] $G\epsilon$ 4 22 × 10^4

The RSSR^- radical anion was produced from the disulphide by reaction 2 An argon-saturated solution of penicillamine disulphide (5 mM, pH 6 0), containing t-butyl alcohol (0 5 M) to remove OH radicals, was used The decay of the RSSR^- transient gives a good first-order plot with $k_{-1} = 1\ 5 \times 10^6\ \mathrm{s}^{-1}$ The extinction coefficient was determined by using short pulses (40 ns) and extrapolating the first-order plot to mid-pulse to obtain the initial concentration, and by assuming $G(\mathrm{RSSR}^-) = G(e^-_{aq}) = 2\ 7$ The spectrum of RSSR^- is shown in Figure 1, $\lambda_{\max}$ 450 nm, ϵ_{450} 7 5 × $10^3\ \mathrm{M^{-1}\,cm^{-1}}$ These values are similar to those reported by Adams *et al*[6] for cysteamine, $\lambda_{\max}$ 410 nm, ϵ_{410} 8 9 × $10^3\ \mathrm{M^{-1}\,cm^{-1}}$, and by Henglein and co-workers[4] for mercaptoethanol, $\lambda_{\max}$ 420 nm, ϵ_{420} 8 3 × $10^3\ \mathrm{M^{-1}\,cm^{-1}}$.

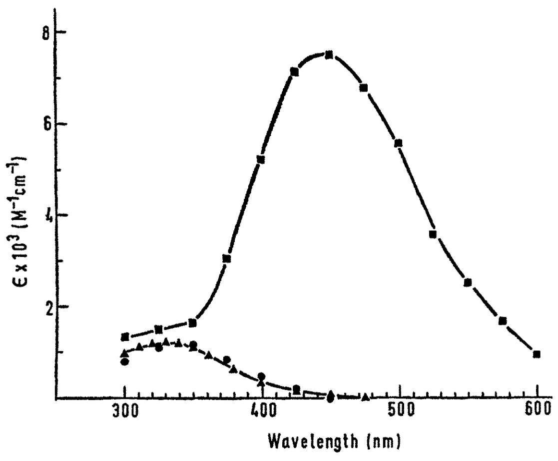

FIGURE 1 *Absorption spectra of the* RSSR^- *radical anion from penicillamine disulphide* (■), *the* RS *radical from penicillamine* (▲), *and the transient remaining after decay of the radical anion* (●).

A 2 5 mM solution of penicillamine at pH 5 and saturated with nitrous oxide was pulse-irradiated and the spectrum obtained is shown in Figure 1 At pH 5 the mercapto-group is un-ionised so that the RS radical produced by

$$\cdot\mathrm{OH} + \mathrm{RSH} \rightarrow \mathrm{H_2O} + \mathrm{RS}\cdot \qquad (4)$$

should not be converted into RSSR^- the spectrum confirms this in that absorption at 450 nm is very low We attribute this spectrum with $\lambda_{\max}$ at 330 nm to RS By assuming $G(\mathrm{RS}) = G(\mathrm{OH}) = 5\ 95$[5] we determined ϵ_{330} 1 22 × $10^3\ \mathrm{M^{-1}\,cm^{-1}}$ and $k_3 = 2\ 6 \pm 0\ 25 \times 10^9\ \mathrm{M^{-1}\,s^{-1}}$ This value of ε is much higher than that reported recently by Jayson *et al*[7] for the RS radical from mercaptoethanol, $\lambda_{\max}$ *ca* 350 nm, $\epsilon_{\max}$ 0 13 × $10^3\ \mathrm{M^{-1}\,cm^{-1}}$ The RSSR^- produced from the disulphide of penicillamine decays to a more stable transient which has the spectrum shown in Figure 1 This spectrum matches very closely that assigned to RS, as expected if RSSR^- decays by the reverse of reaction 1

When penicillamine was irradiated in solutions with pH in the range 7—9, the resulting spectra had contributions from both RS and RSSR^- The ratio of these species should be given by the equilibrium expression

$$K_1 = \frac{[\mathrm{RSSR}^-]}{[\mathrm{RS}\cdot][\mathrm{RS}^-]}$$

The fraction of penicillamine in the form RS^- was determined as a function of pH by the method of Benesch and Benesch[8] $[\mathrm{RSSR}^-]$ was calculated from the maximum absorption at 450 nm and [RS] from the stoicheiometric relationship $G(\mathrm{OH}) = G(\mathrm{RS} + \mathrm{RSSR}^-)$ From these quantities the equilibrium constant K_1 was determined to be $2\ 5 \pm 0\ 1 \times 10^2\ \mathrm{M^{-1}}$ at pH 8 0, independent of concentration over the range studied, 1 to 10 mM The expected absorption spectra for solutions of penicillamine were calculated from K_1 and the spectra of RS and RSSR^- The calculated curves for several concentrations of penicillamine are shown in Figure 2, together with the experimental points obtained by pulse irradiation of the appropriate solutions The agreement between the two lends

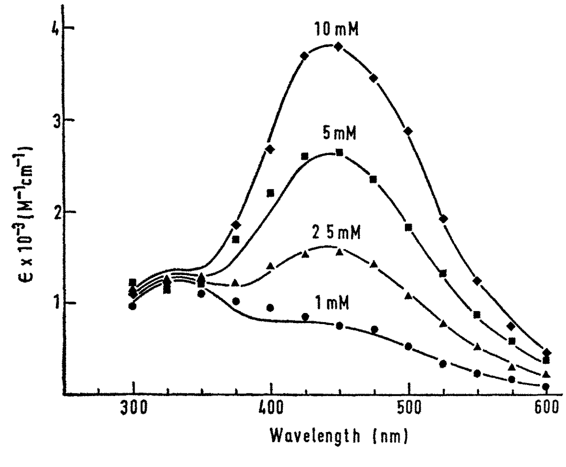

FIGURE 2 *Absorption spectra of pulse irradiated penicillamine solutions at pH* 8 0 *Solid lines are calculated curves, points were measured experimentally*

support to the values obtained for K_1 and the extinction coefficients. During these experiments it was observed that the absorption at 450 nm reached a maximum shortly after the pulse whereas the absorption at 330 nm was highest at the end of the pulse. This is consistent with RS· radicals being formed during the pulse, then reacting to give the $RSSR^-$ radical anion.

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