γ-Radiolysis of disulfides in aqueous solution. II. D-Penicillamine disulfide[1]

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The γ-radiolysis of D-penicillamine disulfide (PenSSPen) in 3×10^{-4} *M* aqueous solution has been studied under aerated and deaerated conditions. G values were determined for the following products: penicillamine sulfinic acid ($PenSO_2H$), penicillaminic acid ($PenSO_3H$), β-hydroxyvaline (PenOH), 2-amino-3-methylbut-3-enoic acid ($HOOC \cdot CH(NH_2) \cdot C(CH_3)=CH_2$), penicillamine (PenSH), penicillamine disulfide-*S*-monoxide (PenS(O)SPen), valine (PenH), penicillamine trisulfide (PenSSSPen), and ammonia. The low yield of $PenSO_3H$ in aerated solution indicated that PenSOH did not react with oxygen or O_2^-. The trisulfide, which was obtained in high yield, was found to come mainly from the reactions:

$$PenSSPen + \cdot OH \rightarrow PenSSOH + Pen\cdot$$

$$PenSSOH + PenSH \rightarrow PenSSSPen + H_2O$$

Experiments with ·OH radicals produced chemically ($TiCl_3/H_2O_2$) and irradiations with cysteine or penicillamine present were used to confirm these reactions. These and the other reactions were tested with radical scavengers; formate and monochloroacetate ions and nitrous oxide.

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Introduction

In a previous communication (1) the γ-radiolysis of cystine in aqueous solution was reported and a mechanism proposed. Studies of cystine and related disulfides in aqueous solutions have been reported by several other workers recently (2–8) and the earlier literature has been reviewed (9).

The present paper reports a study of the radiolysis of D-penicillamine disulfide (2,2,5,5-tetramethylcystine) in dilute aqueous solutions. This compound was of particular interest for comparison with cystine since cysteine is a radioprotective compound whereas penicillamine is not radioprotective. Radiolysis of penicillamine disulfide has not been reported previously although the thiol, penicillamine, has been examined by several workers. Henriksen (10, 11) studied irradiated penicillamine in frozen aqueous solutions and in polycrystalline samples by electron spin resonance (e.s.r.) spectroscopy. Braams (12) obtained rate constants for reaction of the solvated electron with penicillamine using pulse radiolysis. It has also been investigated in solution by Armstrong and Humphreys (4) using e.s.r. spectroscopy. In this paper, the symbol "Pen" is used to represent $HOOCCH(NH_2)C(CH_3)_2S$— in structural formulae and hence PenSH is penicillamine and PenSSPen is the disulfide. The usual abbreviations, CySH and CySSCy, are used for cysteine and cystine respectively.

Experimental

A Technicon amino acid analyzer model NC-1 was employed with the modifications described previously (13). Columns of the following resins were used for analytical purposes: "Chromobeads" type A, 150 × 0.6 cm; "Chromobeads" type C2, 75 × 0.6 cm; Dowex 1-x8 (200–400 mesh) 150 × 0.6 cm. The nuclear magnetic resonance (n.m.r.) spectra were run on a Varian HA60I and infrared spectra on a Perkin–Elmer 221 G. Mass spectra and interpretations were performed by Morgan Schaffer Corp., Montreal.

D-Penicillamine (A grade) was purchased from Calbiochem and used for the preparation of the disulfide. Penicillaminic acid and β-hydroxyvaline were obtained from Calbiochem. Valine and other reference amino acids were purchased from Mann Research Labs. Nitrous oxide gas was supplied by Matheson, Coleman and Bell Ltd. and was purified by trap-to-trap distillation just before use. Monochloroacetic acid and sodium formate were Fisher certified reagents.

D-*Penicillamine Disulfide (PenSSPen)*

This compound was prepared by bubbling oxygen through an alkaline aqueous solution of penicillamine until a negative test was obtained with Ellman's reagent for thiols (14). The solution was neutralized, evaporated to dryness, and the product (crystallized from aqueous ethanol) had m.p. 198–199°.

Anal. Calcd. for $C_{10}H_{20}N_2O_4S_2$: C, 40.5; H, 6.7; N, 9.45; S, 21.6. Found: C, 40.3; H, 6.9; N, 9.5; S, 21.8.

This sample was chromatographically pure except for traces of the trisulfide (PenSSSPen) and was used for

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TABLE I

Elution times and color yields of products

Compound	Column	Elution time (min)	Color yield hw, 0.25 μE*	Notes
Glutamic	Dowex 1	200	15.3	Reference standard
$PenSO_2H$	Dowex 1	245	9.2	hw assumed equal to $PenSO_3H$
$PenSO_3H$	Dowex 1	300	9.2	
PenOH	Chromobeads A	180	9.3	
$\begin{matrix}COOH & CH_3\\ | & |\\ CH(NH_2)C{=}CH_2\end{matrix}$	Chromobeads A	250	12.1	Yield same as valine
PenSH	Chromobeads A	315	1.25	
PenS(O)SPen	Chromobeads A	370	7.5	Assumed equal to PenSSPen
PenH	Chromobeads A	460	12.1	
PenSSPen	Chromobeads A	590	7.5	
Norleucine	Chromobeads A	660	13.8	Reference standard
PenSSSPen	Chromobeads A	725	6.7	
NH_3	Chromobeads A	830	7.5	

*E = equivalent weight with respect to ninhydrin positive groups.

most of the radiation experiments. The nuclear magnetic resonance spectrum, run in trifluoroacetic acid, showed a broad peak at $\tau = 2.38$ due to the NH_3^+ and peaks at $\tau = 5.65$ (CH), $\tau = 8.21$ (CH_3), and $\tau = 8.38$ (CH_3).

Penicillamine Trisulfide (β,β′-divalyltrisulfide; PenSSSPen)

Preparation of this compound was attempted by the methods described by Fletcher and Robson for preparation of cystine trisulfide (CySSSCy) (15). The following preparation was the most successful. Sulfur (216 mg) was dissolved in chloroform (50 ml) and ethanol (200 ml) added. The solution was made alkaline by addition of concentrated ammonium hydroxide solution (5 ml) and penicillamine (1 g in 25 ml water) was added. The solution was stirred for 4 h and evaporated to dryness.

The product was purified by chromatography on a column of Dowex 50W-x8 cation-exchange resin using volatile buffers (16) and had m.p. 185° (corrected) after crystallization from aqueous ethanol; yield, 280 mg from half the starting material (40%).

Anal. Calcd. for $C_{10}H_{20}N_2O_4S_3$: C, 36.53; H, 6.09; N, 8.53; S, 29.26. Found: C, 36.34; H, 5.82; N, 8.44; S, 29.15.

The n.m.r. spectrum of the pure trisulfide was run in trifluoroacetic acid. It had peaks at $\tau = 2.40$ (NH_3^+) $\tau = 5.44$ ($\begin{matrix}|\\ -C-H\\ |\end{matrix}$) and methyl peaks at $\tau = 8.17$ and 8.41 with integrals in the ratio 3:1:3:3 as required by the assignment given. This spectrum is very similar to that of the disulfide except for slight shifts and a greater separation of the methyl peaks. The occurrence of two sharp methyl peaks in the spectrum shows that it is a linear trisulfide.

Irradiation and Analysis

Irradiations were performed using 25 ml samples in 25 ml round-bottom flasks as described previously. Thrice-distilled water was used for all solutions and the irradiation flasks were cleaned with hot chromic acid, rinsed thoroughly and steamed before use. A total dose of 10 000 rads at a dose rate of 800 rads/min was used except where otherwise stated. The irradiated solutions were analyzed as described previously (1). In order to measure ammonia yields, samples were pumped directly into a "Chromobeads" C2 column without freeze drying and then eluted in the usual way.

Order of Elution of Products and Color Yields

The elution times and color yields for the products and reference compounds are listed in Table I. These are for a flow rate of 30 ml of eluent per h. The Dowex 1 column was operated at room temperature and the "Chromobead" type A column was maintained at 60° throughout the run.

Isolation of Products

The products were separated by chromatography on a column of Dowex 50W-x8 cation-exchange resin using volatile buffer systems (16). A solution of penicillamine disulfide (2.4 g) in 4 l of water was irradiated with a total dose of 100 000 rads, concentrated, and chromatographed with acetic acid – pyridine buffers. A small portion of the effluent was drawn into the amino acid analyzer by the proportioning pump so as to provide a continuous record of the separation. Nine major peaks were observed, including unchanged disulfide and ammonia. Each product was purified by re-running it on the same column, operated at 45 °C, until chromatographically pure samples were obtained.

Seven products were obtained in quantities sufficient to allow examination.

Results

Identification of Products

Products 1 and 2 (penicillaminic and penicillamine sulfinic acids) both occurred in the first group. They were resolved by chromatography on a column of Dowex 1 anion-exchange resin using aqueous formic acid as eluent (17). The first product eluted was a strong acid which decolorized iodoplatinic acid. This result, together

with its chromatographic behavior, showed that it was penicillamine sulfinic acid, (valine-β-sulfinic acid), ($PenSO_2H$). The second product, also a strong acid, was found to co-chromatograph with commercial penicillaminic acid ($PenSO_3H$).

Product No. ***4*** contained sulfur but was not identified.

Anal. Found: S, 17.2.

Mass spectrometric analysis of the acid and of the ethyl ester indicated a molecular weight of 161 for the acid.

Product No. ***5*** *(β-Hydroxyvaline)*

The infrared spectrum of this product had a sharp peak at 3410 cm^{-1} indicating a hydroxy group. β-Hydroxyvaline appeared to be a likely product so it was synthesized as described by Mix (18) and compared with the isolated product. The infrared spectra (KBr) differed considerably. However, the two compounds co-chromatographed on paper and with the amino acid analyzer. Both gave identical n.m.r. spectra when run in trifluoroacetic acid. The infrared spectrum of the synthetic compound differed from the radiolysis product because the former was a racemic mixture whereas the latter was a pure optical isomer (19). The n.m.r. spectrum had a broad band at $\tau = 2.48$ (NH_3), a broad band at

$$\begin{array}{c} | \\ 5.65\ (-CH) \\ | \end{array}$$

which could be decoupled from the NH_3 to give a sharp peak, and two sharp peaks at $\tau = 8.28$ and $\tau = 8.50$ (2 CH_3 groups).

Product No. ***6*** *(2-Amino-3-methylbut-3-enoic Acid)*

This compound will be referred to as the "vinyl" derivative for convenience.

The infrared spectrum of this product indicated it was a neutral amino acid without other functional groups and analysis showed sulfur was absent. The mass spectrum of the ethyl ester showed that the compound was a vinyl derivative of valine: $CH_2{=}C(CH_3)CH(NH_2)COOH$. A weak n.m.r. spectrum was obtained (capillary sample tube) which was consistent with this structure: peaks; $\tau = 8.05$ (CH_3); $\tau = 5.1$ (broad, $-CH<$) $\tau = 4.62$ ($CH_2{=}$). Further evidence for the structure was obtained by catalytic hydrogenation. A mixture of the unknown and alanine in water was hydrogenated (H_2/Pt) and a portion of the solution examined using the amino acid analyzer before and after hydrogenation. The radiolysis product was reduced completely to valine whereas the alanine was unchanged.

Product No. ***8*** *(Penicillamine Trisulfide)*

The infrared spectrum of the product was very similar to that of penicillamine disulfide. The most noticeable difference was a sharp peak at 1695 cm^{-1} and another at 1270 cm^{-1} in the spectrum of the product. The n.m.r. spectrum was also very similar to penicillamine disulfide. The unknown had peaks at $\tau = 2.40$, 5.44, 8.17, 8.41, and 7.77. The peak at 7.77 was sharp and appeared to be due to acetic acid which had not evaporated during the freeze drying.

Anal. Calcd. for $C_{10}H_{20}N_2O_4S_3 \cdot CH_3CO_2H$: N, 7.22; S, 24.8. Found: N, 7.56; S, 23.54.

Penicillamine trisulfide (PenSSSPen) was synthesized and found to be identical with the radiolysis product as shown by infrared and n.m.r. spectra, and by chromatography. The synthetic sample also retained acetic acid when isolated by the same procedure.

Product No. ***9*** *(Valine)*

This product was shown to be identical to valine (PenH) by infrared analysis and chromatography.

Products not Isolated

The main product which was not isolated by the procedures described above was penicillamine. A peak was present in aerated and deaerated solutions which co-chromatographed with penicillamine but it was presumably oxidized to the disulfide during the preparative-scale chromatography and evaporations. The presence of penicillamine was confirmed using Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid)) and the yields were checked by this method (14, 20). The other significant peak which remained unaccounted for appeared on chromatograms between penicillamine and valine. PenS(O)SPen seemed a likely possibility for this compound and it was found to decompose if the solution was made alkaline. Disulfide-*S*-monoxides are unstable in solutions above pH 6 and decompose to yield sulfinic acid and disulfide.

A double peak frequently appeared in the chromatograms corresponding to β-hydroxyvaline (PenOH). This may have been due to the presence of PenOOH which is probably the precursor of the PenOH.

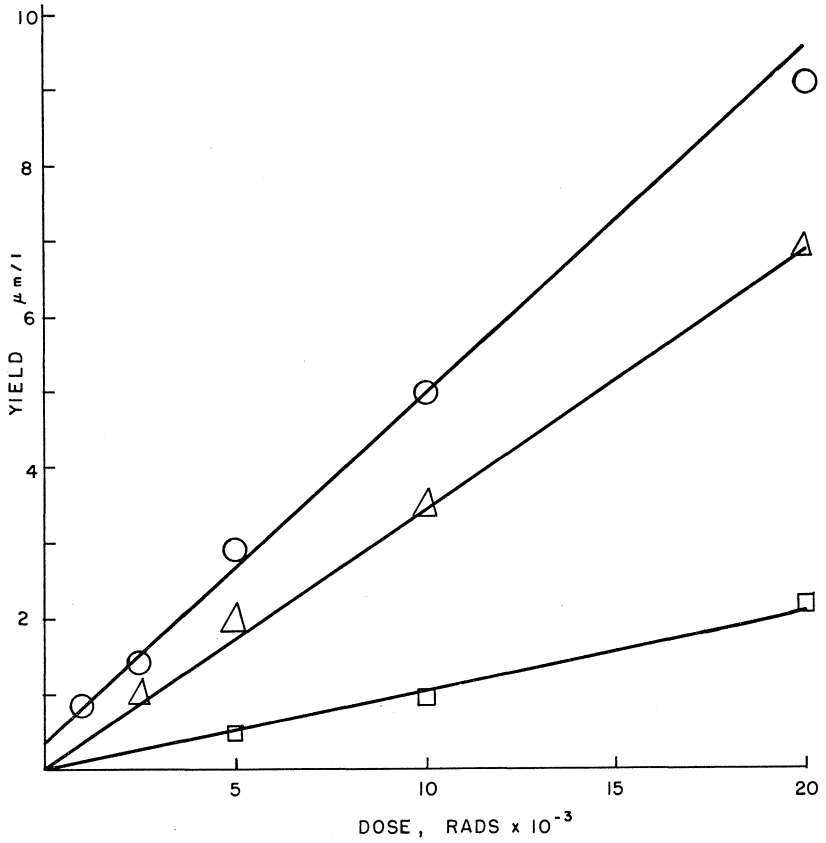


FIG. 1. Yield–dose plots for the major products of radiolysis of penicillamine disulfide in aerated solution: PenSSSPen (○); $PenSO_2H$ (△); $PenSO_3H$ (□).

TABLE II

Radiolysis of PenSSPen: G values and nitrogen balance for products after irradiation with 10 000 rads at ambient temperature

	Aerated			Deaerated		
Product	G value	Average deviation (±)	G(N)	G value	Average deviation (±)	G(N)
$PenSO_2H$	0.5	0.1	0.5	0.3	0.1	0.3
$PenSO_3H$	0.10	0.01	0.1	Trace	—	0.0
$PenSSO_3H$	0.05	—	0.05	0.05	—	0.05
PenOH	0.20	0.02	0.2	Trace	—	0.0
COOH CH₃ | | CH(NH₂)C=CH₂	0.11	0.01	0.11	0.33	0.08	0.33
PenSH	0.45	0.05	0.45	2.3	0.4	2.3
PenS(O)SPen	0.27	0.1	0.54	0.22	0.04	0.44
PenH	0.0	—	0.0	0.13	0.01	0.13
PenSSSPen	0.6	0.1	1.2	1.0	0.1	2.0
NH_3	1.5	—	1.5	0.75	—	0.75
TOTAL N			4.65			6.30
PenSSPen	−3.3	0.3	−6.6	−4.0	0.3	−8.0
RECOVERY			70%			80%

TABLE III

G values in the presence of radical scavengers

Scavenger	Concentration of scavenger	$PenSO_2H$	PenOH	Vinyl	PenSH	PenS(O)SPen	PenH	PenSSSPen
				Aerated				
No scavenger	0	0.49	0.19	0.11	0.46	0.22	0	0.6
Formate	10^{-4} *M*	0.31	0.20	0.14	0.4	0.36	0	0.4
	10^{-3} *M*	0.24	Trace	Trace	Trace	0.07	0	0.26
Chloroacetate	10^{-4} *M*	0.38	0.11	0.12	0.62	0.24	0	0.59
	10^{-3} *M*	0.24	0.07	0.10	0.3	—	0	0.23
Scavenger	Concentration of scavenger	$PenSO_2H$	PenOH	Vinyl	PenSH	PenS(O)SPen	PenH	PenSSSPen
				Deaerated				
No scavenger	0	0.3	Trace	0.33	2.3	0.22	0.13	1.0
Chloroacetate	10^{-4} *M*	0.50	Trace	0.23	2.3	0.17	0.09	0.77
	10^{-3} *M*	0.34	Trace	0.2	Trace	0.11	0.04	0.3
N_2O	2×10^{-3} *M*	0.30	Trace	0.28	1.7	0.24	0.08	1.35
	2×10^{-2} *M*	0.41	Trace	0.29	Trace	0.2	0.09	1.5

Yields of Radiolysis Products

The yields of the main products in aerated solutions were measured at various doses between 1 000 rads and 20 000 rads. The results are shown in Fig. 1. The yields measured were linear with dose up to 20 000 rads.

The yields of all products identified were measured or estimated after irradiation of PenSSPen with 10 000 rads. The results for both aerated and deaerated solutions are listed in Table II. The figures are average values from a number of experiments and the average deviation is given. Table II also gives the G values in terms of nitrogen atoms in order to estimate the product balance. The nitrogen was used rather than the sulfur since products were measured with ninhydrin which reacts with amino groups and ammonia.

Dose-Rate Effects

The effect of dose rate was examined in aerated solution in view of the fact that it had a remarkable effect on the yields of sulfinic and sulfonic acids from cystine (1). With penicillamine disulfide however, there was no measurable change in the yields of $PenSO_2H$ and $PenSO_3H$ when the dose rate was reduced from 800 rads/min to 10 rads/min. Disulfide concentration and total dose were kept constant at $3 \times 10^{-4}M$ and 10 000 rads respectively, while dose rates of 800, 600, 300, 100, and 10 rads/min were employed.

Effect of Radical Scavengers on Yields

The effect of radical scavengers on the yields was investigated using formate ions and monochloroacetate ions in aerated solution, and monochloroacetate ions and nitrous oxide in deaerated solutions (21). Formate ions react readily with ·H and ·OH radicals but relatively slowly with e^-_{aq}. Monochloroacetate ions react rapidly with e^-_{aq}. Nitrous oxide reacts with e^-_{aq} but gives rise to an ·OH radical. The results of these experiments are given in Table III.

Effect of CySH and PenSH

Experiments were carried out with CySH or PenSH present during the irradiation in order to evaluate the mechanism proposed for trisulfide formation. The mercaptans were present in low concentrations (10^{-6} and 10^{-5} *M*) so as to avoid significant competition with the disulfide ($3 \times 10^{-4}M$) for the primary ·OH and e^-_{aq} radicals. The results are presented in Table IV. The irradiations without mercaptans present were performed at about the same time and using the same solution of disulfide. (Small variations were frequently encountered between irradiations performed at different times.)

Discussion

Radiolysis of penicillamine disulfide differs from radiolysis of cystine mainly in the relative

TABLE IV

Effect of PenSH and CySH on G values of trisulfides

	Aerated					Deaerated	
		PenSH		CySH			
	No RSH	$10^{-6}M$	$10^{-5}M$	$10^{-6}M$	$10^{-5}M$	No RSH	CySH $10^{-5}M$
PenSH	0.53	0.64	0.67	0.62	0.86	3.3	3.5
CySSCy	—	—	—	0.02	0.24	—	0.06
CySSPen	—	—	—	0.02	0.21	—	0.31
CySSSCy	—	—	—	0	0	—	0
CySSSPen	—	—	—	0.06	0.22	—	0.08
PenSSSPen	0.60	0.58	0.68	0.52	0.41	1.00	0.80

amounts of the various products. The yields of sulfonic acid and trisulfide were significantly changed; the sulfonic acid yield was much lower and the trisulfide yield much higher (Table II). The yields reported previously (1) for cystine trisulfide were high by a factor of four due to an error in calculation. As before it was difficult to measure the loss of disulfide accurately at low dose and this may account for some of the discrepancy in the nitrogen balance in Table II. Also, some assumptions had to be made with respect to the color yields of certain products as indicated in Table I. Some minor products remained unidentified and these could have contributed significantly to the nitrogen balance if their color yields with ninhydrin were very low. The peak which was thought to be PenOOH was not included in the Table. It was usually similar to PenOH in color yield.

The disulfide is attacked mainly at the sulfur atoms by e^-_{aq} and ·OH radicals with splitting of the sulfur–sulfur bond or a carbon–sulfur bond. Scission of the sulfur–sulfur bond probably occurs as follows:

[1] $\text{PenSSPen} + \cdot\text{OH} \rightarrow \text{PenSOH} + \text{PenS}\cdot$

[2] $\text{PenSSPen} + e^-_{aq} \rightarrow \text{PenS}^- + \text{PenS}\cdot$

Reaction [2] should occur mainly in deaerated solutions as the oxygen will scavenge the e^-_{aq} in aerated solutions. However, the solvated electron reacts rapidly with penicillamine ($k = 5.1 \times 10$ $M^{-1}\,s^{-1}$) (12), so it probably reacts rapidly with the disulfide also, allowing the latter to compete effectively with oxygen. (Solvated electrons react with cystine faster than with cysteine (12).) The PenS· radical has been detected in aqueous solutions by Armstrong and Humphreys using electron spin resonance spectroscopy (4). It was produced when ·OH radicals, from the $TiCl_3$ – H_2O_2 reaction, reacted with penicillamine in a flow system. Adams *et al.* (5) have suggested that the radical $RS\dot{S}R$ is an important intermediate in the radiolysis of cysteamine and cystamine. They observed a species with an absorption maximum at 4100 Å, obtained by ·OH oxidation and e^-_{aq} reduction of cysteamine and cystamine respectively. Since the $RS\dot{S}R$ decays to give the same products as reaction [2] it represents an intermediate in that reaction. With disulfides the radical anion is not produced by ·OH radicals so reaction [1] is satisfactory to explain the attack by ·OH at this position. A radical cation ($RS\dot{S}R^+$) has been suggested as an intermediate in the radiolysis of disulfides (7), but in the absence of any supporting evidence reaction [1] seems preferable since RS· is the only radical which has been identified in acidic solutions.

Sulfenic acids are unstable and usually decompose to sulfinic acid and disulfide. The following reactions are suggested for the decomposition of PenSOH in the irradiated solution:

[3] $2\,\text{PenSOH} \rightarrow \text{PenSO}_2\text{H} + \text{PenSH}$

[4] $\text{PenSOH} + \text{PenSSPen} \rightarrow \text{PenS(O)SPen} + \text{PenSH}$

[5] $\text{PenSOH} + \text{PenSH} \rightarrow \text{PenSSPen} + \text{H}_2\text{O}$

The sulfenic acid does not react with O_2 or O_2^- to form sulfonic acid as appeared to be the case with cysteine sulfenic acid. The PenS· radicals produced in reactions [1] and [2] may combine to give disulfide, or react with oxygen (in aerated solution) to produce sulfinic and sulfonic acids (1).

Rupture of the carbon–sulfur bond in the disulfide during radiolysis leads to products such

as trisulfide, valine, etc. In discussing the radiolysis of cystine (1) it was suggested that RS· radicals attacked the disulfide:

[6] $$RS\cdot + RSSR \rightarrow RSSSR + R\cdot$$

This seems unlikely in the light of results obtained with penicillamine disulfide. It appears that the carbon–sulfur bond is attacked by the ·OH radical giving an intermediate which reacts to yield trisulfide:

[7] $$RSSR + \cdot OH \rightarrow RSSOH + R\cdot$$

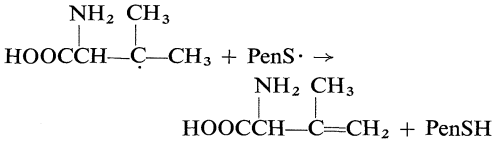
[8] $$RSSOH + RSH \rightarrow RSSSR + H_2O$$

In deaerated solution the disulfide may also be attacked at the same site by e^-_{aq} or ·H radicals with formation of a hydrodisulfide (22) and hence, trisulfide:

[9] $$RSSR + e^-_{aq} \rightarrow RSS^- + R\cdot$$

[10] $$RSS^- + RSOH \rightarrow RSSSR + OH^-$$

The tertiary radical Pen· formed from penicillamine disulfide should be more stable than the primary radical Cy· formed in the case of cystine and this may be the reason for the larger yield of trisulfide from penicillamine disulfide. Reaction [7] appears to be more important than [9] since the yield of trisulfide was increased in deaerated solution by addition of nitrous oxide. The Pen· radical produced in reactions [7] and [9] (in the case of penicillamine disulfide) could react with oxygen in aerated solution to form, eventually, hydroxyvaline (PenOH). In deaerated solutions it may react with e^-_{aq} or ·H to give valine (PenH). In both aerated and deaerated solutions it may lose a hydrogen to yield the vinyl compound: e.g.

[11]


An alternative mechanism which was considered for formation of trisulfides was production of RSS· radicals by the primary species followed by combination with RS· radicals: e.g.

[12] $$RSSR + \cdot OH \rightarrow RSS\cdot + ROH$$

However, this reaction is unsatisfactory since the yield of ROH (hydroxyvaline) is always much lower than the yield of trisulfide. Also, since hydroxyvaline is produced only in aerated solution, it must be formed by a different mechanism. Similar arguments apply in the case of e^-_{aq} or ·H atoms reacting with the disulfide to form RSS· and valine.

The formation of trisulfides was also investigated by reacting ·OH radicals (produced chemically from $TiCl_3$ and H_2O_2) with disulfides in solution, using a flow system similar to that described by Armstrong and Humphreys (4). Good yields of trisulfides were produced when a disulfide (CySSCy or PenSSPen) was present in solution when the ·OH radicals were produced. If the disulfide was added after a short time (3 s) no trisulfide was obtained. When ·OH radicals were reacted with a disulfide and a mercaptan added after 3 s, the yield of trisulfide was not significantly changed. However, if the mercaptan of a different disulfide was used, a mixed trisulfide was also produced. Thus when PenSSPen was reacted with ·OH radicals followed by addition of CySH, both PenSSSPen and PenSSSCy were produced. But when PenSSPen was reacted with ·OH and CySSCy was added, PenSSSPen was obtained with only trace amounts of CySSSPen. Reactions [7] and [8] provide a reasonable explanation of these results.

The experiments with PenSH and CySH present during the radiolysis offer further support for reaction [8]. As shown in Table IV, the presence of PenSH had almost no effect on the yield of trisulfide in aerated solution. Thus, reaction [8] is either very fast or else it is the only important reaction of the PenSSOH intermediate. This explains why the dose–yield plot of the trisulfide is linear within the limits of experimental error. When CySH was present, there was a decrease in the yield of PenSSSPen together with a yield of CySSSPen of similar magnitude in aerated solutions, but little change in deaerated solutions. The high yield of PenSH from reaction [2] probably swamped the CySH added in deaerated solutions. Only a small amount of the CySH and PenSH reacted directly with the primary radical as shown by the low yield of CySSCy in deaerated solution. The mixed disulfide produced in deaerated solution probably came from CySH and PenSOH (analogous to reaction [5]).

The effect of radical scavengers on the yields provided some information on the reactions

suggested (Table III). In aerated solutions, formate (·OH scavenger) reduced the yields of $PenSO_2H$ and PenSSSPen significantly. In deaerated solutions, the yield of $PenSO_2H$ increased slightly in the presence of chloroacetate and N_2O (both e^-_{aq} scavengers). However, the most significant change was the increase in PenSSSPen with N_2O present in deaerated solutions. At the same time the yield of PenSH was reduced to a trace while other products were virtually unchanged. This is consistent with reactions [7] and [8] being the main source of trisulfide. PenSH is produced in reactions [2] [3], and [4] and it may also be an intermediate in others.

The mechanism outlined above is sufficient to explain the main features of the radiolysis of penicillamine disulfide. The significance of these results to the theories of radiation protection will be discussed in the following paper on radiolysis of cysteine–penicillamine mixed disulfide.

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