[Contribution from the Department of Chemistry, Polytechnic Institute of Brooklyn]

Relative Oxidizabilities of 2,4-Pentanedithiol, 2,5-Hexanedithiol, and Hydrolyzed Poly(vinyl thiolacetate)[1]

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Two new dithiols, 2,4-pentanedithiol and 2,5-hexanedithiol, were prepared to serve as low molecular weight analogs of hydrolyzed poly(vinyl thiolacetate). A kinetic study was made to determine the ease of oxidation of the hydrolyzed polymer and the two dithiols. The relative rates of oxidation were compared on the basis of the spectrophotometrically observed rate of reduction of a dye, sodium 2,6-dichlorobenzeneoneindophenol, occurring on reaction with these thiols. As a comparison, the relative rate of 2-mercaptoethanol was also determined. A new method for determining the mercaptan titer of hydrolyzed poly(vinyl thiolacetate) and its applicability to simple mercaptans is also described.

Because of the recognized critical importance of sulfhydryl oxidation-reduction reactions in vital biochemical systems, the detailed study of the chemistry of synthetic sulfhydryl polymers has become an important segment of current research. In particular, their oxidation-reduction properties are being measured and compared. The results promise to shed light on questions of the effects of steric and electrical factors on oxidizability and to point out practical possibilities of improving the effectiveness of chemical protection against physiological damage by radiation.[3]

The damaging effect of X-ray radiation is believed due to the oxidizing action of $\cdot O_2H$ and $\cdot OH$ radicals and hydrogen peroxide known to be produced by irradiation of water containing dissolved oxygen.[4] Sulfhydryl compounds such as cysteine and glutathione in relatively large amounts counteract this effect by detoxifying these radicals, most probably by a rapid reaction of these radicals with the thiol groups, a reaction of relatively low activation energy. Thus the efficiency of a sulfhydryl radiation prophylactic has been shown to be related in some way to its ease of oxidation.[5]

The activity of many enzymes has been shown to depend on the presence of sulfhydryl groups,[6] not all of which are of equal reactivity nor of equal importance for the proper functioning of the enzyme. The ease of oxidation of sulfhydryl groups has been shown to depend on the ease of dimerization of two initially formed thiyl radicals to form a disulfide bond. Resistance to oxidation of some of the sulfhydryl groups of the enzymes is thought to be due to the distance between sulfhydryl groups in the native protein which prevents the formation of a disulfide bond. Barron[7] has shown that for a series of dithiols, the greater the distance between the thiol groups the slower will be the oxidation. Overberger and Bonsignore[8] have shown that 2,4-di(*p*-mercaptophenyl)pentane oxidizes more than six times as fast as *p*-thiocresol in a system measured spectrophotometrically at $10^{-5}M$ concentration, explaining this by the fact that two thiol groups existing as part of the same molecule will be much more available than will single, monomeric thiol groups at the same over-all dilution.

Oxidation of polymer-borne sulfhydryl groups, however, might be expected to be hindered because of the decreased mobility and diffusibility of a thiyl radical attached to a polymeric chain. It was the purpose of this work to compare the oxidizability of polymer-borne sulfhydryl groups with model compounds in order to determine the extent of this effect.

It is well known[9] that addition polymerization does not proceed normally in the presence of thiol groups due to extensive chain transfer, so that the sulfhydryl function must be introduced after polymerization. Hydrolysis of the thiolacetate group has proved to be a convenient method for generating the free thiol group. Vinyl thiolacetate was known[10] and *p*-vinylphenyl thiolacetate has been prepared[11] so that the raw materials for practically any desired copolymer composition con-

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[1] This is the 22nd in a series of papers on new monomers and polymers. For the previous paper in this series see H. Ringsdorf and C. G. Overberger, *Makromol. Chem.*, **44**, 418 (1961).

[2] This paper comprises portions of dissertations submitted by J. J. Ferraro and F. W. Orttung in partial fulfillment of the requirements of the degrees of Doctor of Philosophy and Master of Science, respectively, in the Graduate School of the Polytechnic Institute of Brooklyn.

[3] Results to be reported have demonstrated that the hydrolyzed copolymers of *p*-vinylphenyl thiolacetate and methyl methacrylate are about as successful as a radiation prophylactic as any small molecule. However, as with all compounds tested to date, they are not effective if given to mice more than fifteen minutes before lethal irradiation.

[4] A. Hollaender, *Radiation Biol.*, Vol. I, McGraw-Hill, New York, 1954, pp. 256, 274, and 1008.

[5] D. G. Doherty, W. T. Butnett, Jr., and R. Shapiro, *Radiation Research*, **7**, 13 (1957).

[6] E. S. G. Barron, *Advances in Enzymology*, Vol. II, Interscience, New York, 1951, p. 219.

[7] E. S. G. Barron *et al.*, *Biochem. J.*, **41**, 62 (1947).

[8] C. G. Overberger and P. V. Bonsignore, *J. Am. Chem. Soc.*, **80**, 5431 (1958).

[9] C. E. Schildknect, *Polymer Processes*, Interscience, N. Y., 1956, p. 147.

[10] M. M. Brubaker, U. S. Patent **2,378,535** (1945).

[11] C. G. Overberger and A. Lebovits, *J. Am. Chem. Soc.*, **77**, 3675 (1955); **78**, 4792 (1956).

taining aliphatic or aromatic thiol groups are now available.

This work compares the oxidizability of the polymer-borne sulfhydryl group, obtained by hydrolyzing poly(vinyl thiolacetate), with two new dithiols, 2,4-pentanedithiol and 2,5-hexanedithiol.

EXPERIMENTAL

Oxidation Reactivity. This method was one devised by Basford and Huennekens[12] based on the spectrophotometric reduction of a dye, sodium 2,6-dichlorobenzeneoneindophenol. The method was modified for application to nonaqueous solvents of the type that would be expected to dissolve high molecular weight polymers. A sample of the dye (Eastman Kodak Co.) was purified to 99% purity by chromatography and was found to have a molar extinction coefficient of 4.71×10^4 at 643 mμ in air-free dimethyl formamide and of $3.05(-0.25) \times 10^4$ at 638 mμ in air-free acetonitrile. Dilution measurements showed that Beer's law was followed closely for both systems.

Procedure. The spectrophotometric procedure has been described previously.[8]

2,4-Pentanedithiol. A modified procedure of Hall and Reid[13] was found to be successful. 2,4-Dibromopentane, 172 g. (0.75 mole), and thiourea, 142 g. (1.87 moles), were mixed with 100 ml. of water and heated for 8 hr. on a boiling water bath at which time one phase formed. Sodium hydroxide, 150 g. (3.75 moles), in 200 ml. of water was added and an additional 8 hr. heating allowed. The crude yield, separated directly without extraction, was 86 g. (84%), which, after washing and drying, was distilled to provide 75 g. (73%) of pale yellow product, b.p. 67–68°/13 mm. Treatment with lithium aluminum hydride in ether and distillation gave an analytically pure water-white fraction, b.p. 67°/12 mm., n_D^{18} 1.5061.

Anal. Calcd. for $C_5H_{12}S_2$: C, 44.06, H, 8.88, S, 47.08. Found: C, 44.12, H, 8.85, S, 47.45.

2,5-Hexanedithiol. The same procedure as that described for the preparation of 2,4-pentanedithiol was used except that treatment with lithium aluminum hydride was not necessary. A colorless liquid was obtained in 73% yield, b.p. 87–88°/12 mm., n_D^{18} 1.5051.

Anal. Calcd. for $C_6H_{14}S_2$: C, 47.94, H, 9.40, S, 42.70. Found: C, 48.05, H, 9.32, S, 42.90.

Hydrolyzed poly(vinyl thiolacetate). Vinyl thiolacetate was prepared according to the modified procedure of Overberger.[14] Poly(vinyl thioacetate) was prepared according to Brubaker.[10] The polymer was hydrolyzed by heating under reflux in an atmosphere of prepurified nitrogen with 10% sodium hydroxide in water-alcohol until a clear yellow solution was obtained. Careful acidification with air-free 2*N* hydrochloric acid produced a gelatinous precipitate. While applying a steady stream of nitrogen, the precipitate was freed of liquids with the aid of a filter stick. Repeated washings with air-free water removed all salts. After washing with methanol to remove water, the hydrolyzed polymer was dried *in vacuo.* The polymer was then dissolved in air-free dimethyl formamide to give a clear solution with a purple hue. This solution was diluted and was used for the oxidation studies.

Mercaptan titer of hydrolyzed poly(vinyl thiolacetate). The mercaptan titer of the hydrolyzed polymer was determined by the nonaqueous potentiometric titration[15] of the hydrogen chloride released when an aliquot of the dimethyl formamide solution of the hydrolyzed polymer was added to a large excess of a pyridine solution of mercuric chloride. The hydrolyzed polymer is soluble in pyridine, but precipitated in the presence of mercuric chloride. This precipitate apparently did not interfere with the titration since consistent titer values were always obtained. Thus, titration values indicated 75% hydrolysis after 3 hr. heating under reflux and 92% hydrolysis after 13 hr. heating under reflux.

In general, 0.20–0.60 meq. of sulfhydryl compound is added to a solution of 2 g. of mercuric chloride in 50 ml. of reagent grade pyridine. The reaction is essentially complete in seconds. The hydrogen chloride is titrated directly with 0.05*N* base.

The applicability of this method to simple mercaptans is shown in Table I. In these examples, no precipitation occurred upon reaction with mercuric chloride.

RESULTS AND DISCUSSION

When quantitative decolorization rate determinations were attempted, it soon became evident that the oxidation-reduction reaction was not proceeding at direct molar equivalence. When mixed at the Basford and Huenneken[12] proportions, namely five moles of thiol for every six moles of oxidant, there was no perceptible decolorization of the system. Ratios of nearly ten thiol groups per dye molecule were found to be necessary for a reasonably measurable decolorization rate to occur; the rate became immeasurably fast at ratios of 20 to 1 or more. It was then found that in these nonaqueous systems slow but measurable dye decolorization at equimolar thiol/dye ratios occurred in the rigorous exclusion of air. Table II summarizes the results of this series of experiments. Thus, 2,5-hexanedithiol oxidizes 2.3 times as fast as 2-mercaptoethanol, while 2,4-pentanedithiol oxidizes 5.7 times as

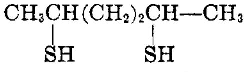
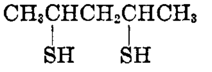
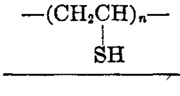
TABLE I

MERCAPTAN TITER OF SIMPLE THIOLS

Mercaptan	Meq.	%
n-Decylmercaptan	0.234	98.0
Thiophenol	0.328	98.0
Benzylmercaptan	0.378	94.0, 93.5
1,3-Propanedithiol	0.619	96.0
2,4-Pentanedithiol	0.690	99.0

TABLE II

OXIDIZABILITIES IN DIMETHYL FORMAMIDE, $10^{-5}M$ INDOPHENOL

Mercaptan	Obs. Rate × 10^{-5} μm./ml./min./—SH	Rel. Ox.
$HSCH_2CH_2OH$	1.7	1
 $CH_3\underset{SH}{CH}(CH_2)_2\underset{SH}{CH}—CH_3$	3.9	2.3
 $CH_3\underset{SH}{CH}CH_2\underset{SH}{CH}CH_3$	9.7	5.7
 $—(CH_2\underset{SH}{CH})_n—$	91.4	53.8

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(12) R. Basford and F. M. Huennekens, *J. Am. Chem. Soc.*, **77**, 3873 (1955).

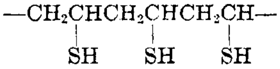
(13) W. P. Hall and E. E. Reid, *J. Am. Chem. Soc.*, **65**, 1466 (1943).

(14) C. G. Overberger, H. Biletch, and R. G. Nickerson, *J. Polymer Sci.*, **27**, 381 (1958).

(15) Potentiometric titration procedure of R. H. Condiff and P. C. Markinas, *Anal. Chem.*, **28**, 792 (1956) was used.

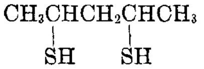
fast. These values indicate that 2,4-pentanedithiol is oxidized 2.5 times as fast as 2,5-hexanedithiol. It would seem then that dithiols, under these conditions ($10^{-5}M$) oxidize more rapidly than monomeric thiols as was found by Overberger and Bonsignore.[8] Furthermore, in a series of dithiols, ease of oxidation appears to increase as the distance between the two thiol groups decreases. These findings confirm the general conclusion of Barron.[7]

At the same thiol concentration, hydrolyzed poly(vinyl thiolacetate) is oxidized 53.8 times as fast as 2-mercaptoethanol and 9.4 times as fast as its model 2,4-pentanedithiol. These large differences can probably be explained by the fact that a particular thiol group in hydrolyzed poly(vinyl thiolacetate) is flanked by two thiol groups while in the model, a thiol group has only one nearest neighbor. This statistical difference apparently accounts for the large increase in the ease of oxidation. That no intermolecular oxidation had taken place in the polymer was shown when no precipitation occurred during the rate measurements.



$$-CH_2\underset{SH}{CH}CH_2\underset{SH}{CH}CH_2\underset{SH}{CH}-$$

hydrolyzed poly(vinyl thiolacetate)



$$CH_3\underset{SH}{CH}CH_2\underset{SH}{CH}CH_3$$

2,4-pentanedithiol

In order to compare the oxidizabilities of the previously mentioned thiols to those listed in Table III, 2,4-pentanedithiol was oxidized in pH 10 aqueous-ethanol borate buffer with indophenol. In this system 2,4-pentanedithiol was oxidized 20 times as fast as *p*-thiocresol while 2,4-di-(*p*-mercaptophenyl)-pentane was oxidized only 6.4 times as fast. Furthermore, since hydrolyzed poly(vinylphenyl thiolacetate) is oxidized 7.6 times as fast as *p*-thiocresol, comparative rates show that hydrolyzed poly(vinyl thiolacetate) is oxidized 24.7 times as fast as the corresponding phenyl polymer (Table IV). This decreased rate is probably due to the fact that the steric requirements of the phenyl group is sufficient to inhibit approach of a neighboring phenyl thiyl radical. We are continuing to investigate the general problem.

TABLE III

OXIDIZABILITIES[8] IN *p*H 10 AQUEOUS BORATE BUFFER, $10^{-5}M$ INDOPHENOL

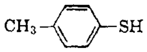
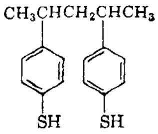
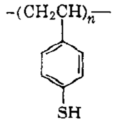
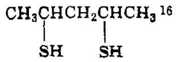
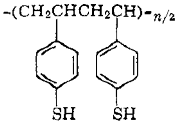
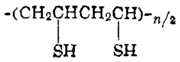
Mercaptan	Obs. Rate × 10^{-3} μm./ml./min./—SH	Rel. Ox.
	1.17	1
	7.51	6.4
	8.88	7.6
 $CH_3\underset{SH}{CH}CH_2\underset{SH}{CH}CH_3$ [16]	23.0	20

TABLE IV

OXIDIZABILITIES OF POLYMERS

Mercaptan	Rel. Ox.
	1
 $-(CH_2\underset{SH}{C}HCH_2\underset{SH}{C}H)-_{n/2}$	24.7

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[16] This work; *p*H 10 aqueous-ethanol borate buffer.