

Reversible Crosslinking in Cellulose. I. Determination of Mercaptan and Disulfide in Modified Cotton

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Synopsis

Mercaptan and disulfide contents of modified cotton fabrics could be determined by application of Leach's polarographic method for wool, with minor modification.

It was found that mercaptocellulose prepared by the reaction of tosyl cellulose and potassium thiolacetate followed by alkaline hydrolysis contained considerable amounts of disulfide in addition to mercaptan. Iodometric titration of mercaptocellulose gave a higher value of mercaptan than that obtained by the polarographic method. The mercaptan and disulfide contents and methylene blue uptake of the iodine-treated samples indicate that some of the mercaptan groups further undergo oxidation to acidic groups as a result of the iodine treatment.

The spatial effect in the oxidation of mercapto groups in modified cotton is briefly discussed.

INTRODUCTION

The chemically reversible crosslinking in modified cotton fabrics has been of interest from both theoretical and practical viewpoints.¹⁻⁸ It is well known in the chemistry of wool that the disulfide crosslinks (cystine residues) can be ruptured by reduction and the resulting mercaptan groups (cysteine residues) reform the crosslinks by mild oxidation. Several modified cotton fabrics containing disulfide crosslinks have been prepared and their reactions have been studied by analogy with the reactions of wool fibers.^{1-6,9}

Schwenker et al. reported the preparation of 6-mercaptopcellulose from tosylated cotton and its oxidation to the corresponding cellulose disulfide.^{1,2} Reduction and reoxidation of the cellulose disulfide were also carried out. Changes in crease recovery of the fabric were generally consistent with the postulated reactions. A group at Southern Regional Research Laboratory showed that disulfide crosslinks were introduced into cotton by the reaction of dialdehyde cotton with acethydrazide disulfide.^{3,4} The crease recovery measurement suggested that the reduced form of this complex cellulose derivative was very susceptible to oxidation. Tesoro and his co-workers obtained a cellulose derivative containing mercaptan by treating

cotton with N-hydroxymethylacetylthioacetamide followed by hydrolysis.⁵ Oxidation of the product yielded the corresponding disulfide derivative. Repeated reduction-oxidation treatments were performed. The results suggested that the yield of the reaction decreased gradually as the reaction cycles were repeated.

It has been often assumed that the changes in crease recovery through reactions were solely dependent on the changes in number of crosslinks. However, this assumption has never been verified experimentally. In order to interpret changes in crease recovery resulting from oxidation of mercaptocellulose, the analysis of mercaptan and disulfide is very important. No satisfactory method of analysis was employed in the studies mentioned above. This paper will describe polarographic determination of mercaptan (SH) and disulfide (SS) in cellulose derivatives prepared from tosylated cotton. Some new aspects of the experiments made by Schwenker et al.^{1,2} will also be presented.

EXPERIMENTAL

Materials and Reagents

All samples were prepared from 140 × 70 bleached, scoured, and unmercerized cotton print cloth kindly provided by Fuji Spinning Co. Ltd., Japan.

Thiolacetic acid was prepared from acetic anhydride¹⁰ and neutralized with potassium carbonate. Potassium thiolacetate was recrystallized from ethanol.

Methylmercuric iodide was prepared from methyl iodide and recrystallized from methanol.¹¹

Mercaptocellulose (M). Cotton samples were treated with a 10% solution of tosyl chloride in pyridine (liquor ratio, 1:30) at 70°C for a desired time, washed with a pyridine-water mixture, extracted with acetone in a Soxhlet apparatus, and dried in air.

Three different procedures for the preparation of acetylthiocellulose from tosylated cotton are as follows: (a) Treatment with a 2.5% solution of potassium thiolacetate in dimethylformamide (liquor ratio, 1:100) at 75°C for 45 hr. (b) Treatment with a 2.5% solution of potassium thiolacetate in boiling methanol (liquor ratio, 1:100) for 48 hr. (c) Treatment with a saturated solution of potassium thiolacetate in boiling acetone (liquor ratio, 1:100) for 48 hr. The products were extracted with acetone in a Soxhlet apparatus, washed with water, and dried in air.

Mercaptocellulose was prepared by hydrolysis of acetylthiocellulose in 2% aqueous sodium hydroxide at room temperature for 1 hr. The products were washed with water free from oxygen and dried in air.

Removal of Tosyl Groups From M. M was hydrolyzed with 3N sodium hydroxide at 50°C for 15 hr.

Reduction of M. Samples were treated with 1% methanol solution of

tri-*n*-butylphosphine containing 5% water at boiling temperature for 6 hr. The reduced samples were extracted with acetone or methanol in a Soxhlet apparatus for 5 hr. The mercaptan of the reduced samples was stable toward oxidation during extraction.

Iodine Oxidation of M. Iodine oxidation of M was carried out according to Schwenker et al.¹

Methylene Blue Uptake. Portions of a sample (250 mg) were treated with 0.4 mM methylene blue (liquor ratio, 1:100) at 30°C for 18 hr with shaking. The methylene blue adsorbed was determined colorimetrically.

Sulfur Analysis. Sulfur was determined by the standard oxygen combustion method with a Mitamura Micro Elementary Analyzer.

POLAROGRAPHIC DETERMINATION OF MERCAPTAN (SH) AND DISULFIDE (SS)

Reagents. The methylmercuric iodide solution of Leach for SH determination was prepared according to the literature.¹²

Methylmercuric iodide solution of modified composition was also prepared. Thus, 5×10^{-4} mole of methylmercuric iodide was dissolved in 1 liter of a Sørensen buffer of pH 7.3 containing 8 M urea, 0.5 M KCl, and 0.125 g gelatin with 3 ml dimethylformamide. This solution had some advantages over the TRIS buffer solution of Leach because of longer stock life, less loss of methylmercuric iodide during degassing with a stream of nitrogen, and slower oxygen reabsorption.

The methylmercuric iodide solution containing sodium sulfite for (SH + SS) determination was prepared according to Leach.¹²

Polarographic Determination of Methylmercuric Iodide. Polarographic measurements were made on Yokogawa Polarocorder Pol-12. Polarograms of the methylmercuric iodide solutions were recorded between -0.1 and -0.6 V to a mercury electrode at 20.0°C. It was necessary to remove dissolved oxygen by introducing a stream of nitrogen before the measurement when SH reagents were used. The diffusion current derived from the first wave of methylmercuric iodide was calculated from the polarogram by the standard method.

Determination of Uptakes of Methylmercuric Iodide. Portions (ca. 2 mm \times 2 mm) of a sample (15 to 25 mg) were placed in a stoppered glass tube and 10 or 15 ml of methylmercuric iodide solution was added by a pipette. The tube was shaken in a constant temperature bath for a given time. The portions of the sample were removed by decantation and the polarogram of the solution was recorded. The uptake of methylmercuric iodide was calculated from the following equation:

$$\text{Uptake of CH}_3\text{HgI } (\mu\text{moles/g sample}) = \frac{C \times V \times (I_d_s - I_d_t)}{W \times I_d_s}$$

where C is concentration of the stock solution ($\mu\text{M/liter}$), V is volume of the

reagent used (ml), W is weight of the sample used (mg), Id_s is diffusion current of the stock solution, and Id_t is diffusion current of the solution after being treated with the sample.

Recommended Procedure of SH and SS Determinations. The maximum and equilibrium uptake of methylmercuric iodide which corresponds to SH present should be obtained from at least three separate determinations of uptakes from the solution containing urea at room temperature at different periods (usually between one and five days).

(SH + SS) can be obtained from the maximum and equilibrium uptake of the methylmercuric iodide-sodium sulfite solution of Leach at 50°C.

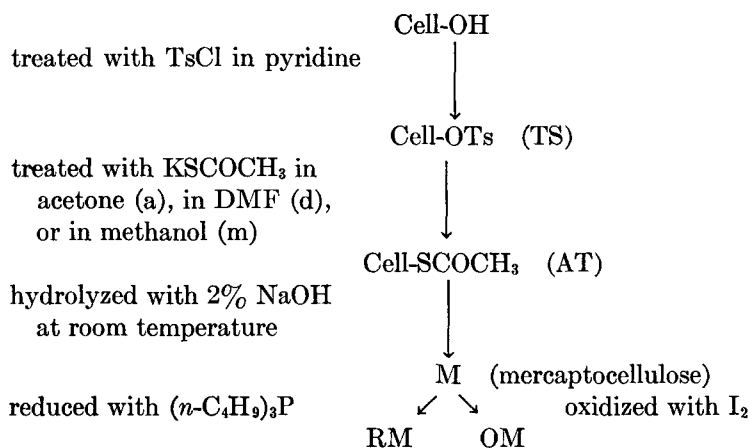
RESULTS AND DISCUSSION

A number of methods have been proposed for the determination of mercaptan (SH) and disulfide (SS) in intact wool. In the study of cotton derivatives, Tesoro et al.⁵ adapted the method of Burley and Horden¹³ utilizing the reaction of SH with *N*-ethylmaleinimide and the method of Weigmann and Rebenfeld¹⁴ utilizing the reduction of SS with 1,3-dithiothreitol for SH and SS determinations, respectively. They⁵ concluded that neither of the two analyses was satisfactory because a significant portion of either SH or SS remained inaccessible to the corresponding reagent.

The most widely used method for SH and SS determinations for intact wool seems to be the polarographic analysis published by Leach.¹² This method is based on the specific reaction of methylmercuric iodide with SH. Concentrations of methylmercuric iodide solution before and after the reaction with wool are determined polarographically, from which the amount of methylmercuric iodide consumed is calculated. The equilibrium and maximum uptake of methylmercuric iodide is considered to be the amount of SH present. Because of the small molecular size of the reagent to permit rapid diffusion into fibers and of the well-defined monofunctional stoichiometry of the reaction, this reagent is advantageous over other mercurial reagents such as the chloromercuri derivative of an azo dye¹⁵ which is sometimes used for SH determination.

According to Leach, the SS content is also obtained by the polarographic determination of methylmercuric iodide. When the reaction is carried out under alkaline conditions in the presence of sodium sulfite, sulfitolysis of disulfide bonds occurs and one mole of SS yields one mole of SH. The latter eventually reacts with methylmercuric iodide along with the mercaptan present originally. Thus, the equilibrium uptake of methylmercuric iodide under alkaline conditions in the presence of sodium sulfite corresponds to the sum of SH and SS.

In the present work the method of Leach was applied for the determination of SH and SS in modified cotton fabrics. The sample, primarily used in experiments for the establishment of an analytical method, was mercapto-cellulose (M) made by hydrolysis of acetylthiocellulose (AT).¹ M contains



Examples of sample designation: Sulfur contents of typical TS, 1.26% (1), 2.26% (2), 3.12% (3), and 3.40% (4). TS₁, TS of 1.26% sulfur content; AT_{a1}, AT prepared by the reaction of TS₁ with KSCOCH₃ in acetone; M_{a1}, M prepared by hydrolysis of AT_{a1}.

Fig. 1. Reaction scheme and sample designation.

not only SH but also SS, as will be discussed later. The reaction scheme and the sample designation are shown in Figure 1.

Leach carried out the reaction of wool with methylmercuric iodide in TRIS buffer of pH 7 at 20°C for the determination of SH. Figure 2 shows the reaction of M with methylmercuric iodide under similar conditions.

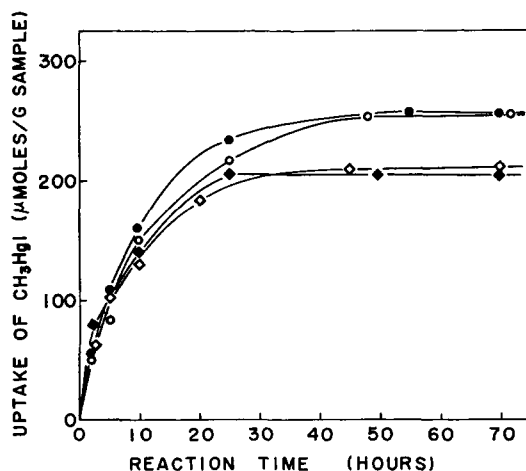


Fig. 2. Reaction of M with CH₃HgI under different reaction conditions. M_{a1}: In TRIS buffer (pH 7.2) at 20°C (◆); In Sørensen buffer (pH 7.3) containing 8 M urea at 30°C (◇). M_{a4}: In TRIS buffer (pH 7.2) at 20°C (●); In Sørensen buffer (pH 7.3) containing 8 M urea at 30°C (○).

The reaction proceeded very slowly and the uptake of methylmercuric iodide attained equilibrium virtually in more than one day. If the maximum and equilibrium uptake is assumed as the amount of SH in accessible regions, good first-order rate plots are obtained, as indicated in Figure 3, for the reaction of SH with excess methylmercuric iodide.

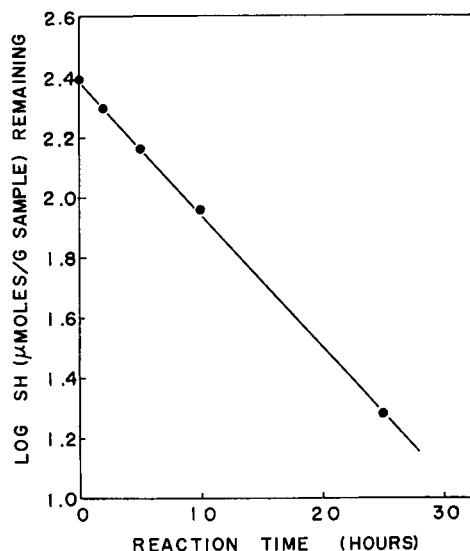


Fig. 3. First-order rate plots for the reaction of SH in M_{44} with CH_3HgI in TRIS buffer (pH 7.2) at 20°C .

In order to examine whether SH inaccessible to methylmercuric iodide is present, the reaction was repeated under swelling conditions using a Sørensen buffer of pH 7.3 containing 8 M urea. No difference was observed between the maximum uptakes of methylmercuric iodide under conditions with and without urea, as seen in Figure 2. To verify that the reaction in 8 M urea proceeds without any unspecified reaction, untreated and reduced wool samples were allowed to react with methylmercuric iodide in the urea

TABLE I
Equilibrium Uptake of CH_3HgI by Different Methods

Sample	Equilibrium uptake of CH_3HgI ($\mu\text{moles/g}$)		
	Leach's method	Modified method	
		30°C	50°C
Mercaptocellulose	203	213	—
	260	257	—
	—	188	190
	—	291	291
Wool	14	16	—
Reduced wool	201	216	—

solution. The maximum uptakes of methylmercuric iodide observed agreed well with the SH contents of the samples determined by the method of Leach. The results are listed in Table I. As the methylmercuric iodide solution in 8 *M* urea appeared to have some advantages, as described in the experimental section, this solution was used mostly in the present work rather than that of Leach.

Figure 2 also indicates that the urea did not have much influence on the rate of the reaction of methylmercuric iodide with SH. It was attempted to carry out the reaction at elevated temperature. The reaction proceeded a little faster at 50°C. The equilibrium uptake of methylmercuric iodide at 50°C was equal to that measured at 30°C. No methylmercuric iodide was taken up by dithiodipropionic acid in urea solution at 50°C. This suggests that no hydrolytic fission of disulfide bonds occurs at 50°C. However, when wool was treated with methylmercuric iodide in 8 *M* urea at 50°C, the uptake of methylmercuric iodide increased over the maximum uptake of methylmercuric iodide at 30°C.

Leach also observed that an unspecified reaction of wool with methylmercuric iodide took place at elevated temperature.¹² These results are illustrated in Figure 4. It was decided to carry out the reaction with the

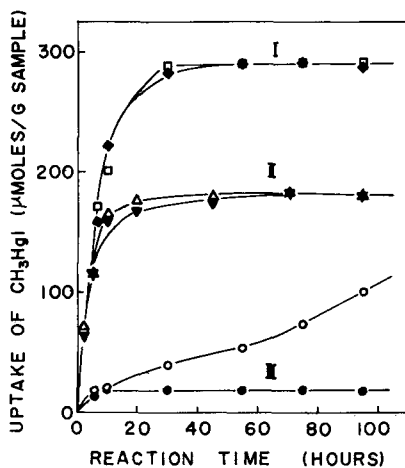


Fig. 4. Effect of temperature on the reaction of M and wool with CH_3HgI in Sørensen buffer (pH 7.3) containing 8 *M* urea. M (I and II): Treated at 30°C (◆, ▼); treated at 50°C (□, △). Wool (III): Treated at 30°C (●); treated at 50°C (○).

methylmercuric iodide solution containing urea at 30°C as standard condition (often cited as the neutral condition) for the determination of SH in modified cotton.

In the method of Leach for the determination of (SH + SS) of wool, the reaction of wool with methylmercuric iodide was performed in an ammonium buffer of pH 9.3 containing 0.2 *M* sodium sulfite and 8 *M* urea at 20°C. M was allowed to react with methylmercuric iodide under the

conditions of Leach.¹² The maximum and equilibrium uptake of methylmercuric iodide was considerably higher than that previously determined under the neutral conditions, indicating the presence of SS in M.

As shown in Figure 5, the reaction with methylmercuric iodide under alkaline conditions in the presence of sulfite proceeds much more slowly. The reaction carried out at 50°C is also shown in Figure 5. The results

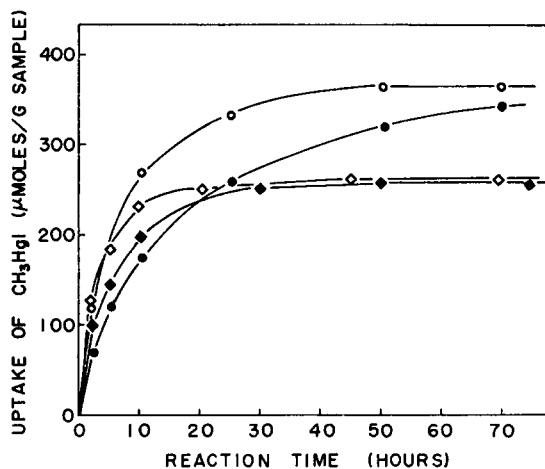


Fig. 5. Effect of temperature on the reaction of M with CH_3HgI in ammonium buffer (pH 9.3) containing 8 M urea and 0.2 M Na_2SO_3 . M_{Al} : Treated at 20°C (●); treated at 50°C (○). M_{At} : Treated at 20°C (◆); treated at 50°C (◇).

indicate that the reaction proceeded faster at the elevated temperature and a reasonable equilibrium uptake was obtained in a shorter time. The reagent solution was kept at 50°C for a long time but no loss of methylmercuric iodide was observed. Table II compares the equilibrium uptakes

TABLE II
Equilibrium Uptake of CH_3HgI in the Presence of Na_2SO_3
at Different Temperatures

Sample	Equilibrium uptake of CH_3HgI ($\mu\text{moles/g}$)		
	20°C	30°C	50°C
Mercapto cellulose	245	—	260
	—	217	218
	—	274	280
Wool	443	—	445

of various samples, including wool, determined at different temperatures. Experiments mentioned hereafter for (SH + SS) determination were done with the Leach's reagent at 50°C (often cited as the alkaline condition). The time to reach equilibrium varied with the samples.

It was confirmed that cotton cellulose does not adsorb any methylmercuric iodide under either neutral or alkaline conditions. M may contain tosyl and acetylthio groups in addition to SS. The effect of these residues was investigated. Tosylated cotton was found not to adsorb any methylmercuric iodide under either conditions. On the other hand, acetylthiocellulose (AT) reacted very slowly with methylmercuric iodide under neutral conditions, as shown in Figure 6. The consumption of

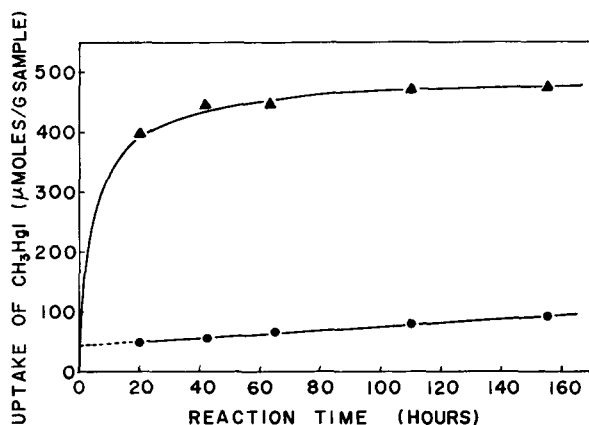


Fig. 6. Reaction of AT_{ds} with CH₃HgI. Reaction conditions: in Sørensen buffer (pH 7.3) at 30°C (●); in ammonium buffer (pH 9.3) with Na₂SO₃ at 50°C (▲). The corresponding M_{ds} contained 325 μmoles/g SH and 112 μmoles/g SS.

methylmercuric iodide by AT was far less than that of the corresponding M. The gradual uptake of methylmercuric iodide by AT was considered to be due to slow hydrolysis of acetylthio groups to SH under neutral conditions. Linear extrapolation of the plots of the uptake did not point zero and this could mean that AT already contained a small amount of SH before the alkaline hydrolysis treatment.

The hydrolysis is expected to proceed faster at higher pH. The uptake of methylmercuric iodide by AT increased more rapidly under alkaline conditions, as shown in Figure 6. An equilibrium was attained in about six days. The equilibrium uptake was between the (SH + SS) and the (SH + 2SS) values of the corresponding M. This could indicate that either AT contained some amount of SS as well or that the hydrolysis of AT at pH 9.3 was incomplete. These results demonstrate that acetylthio groups lead to errors in SH and (SH + SS) determinations.

AT was treated with 2% sodium hydroxide at room temperature for different periods and both apparent SH and (SH + SS) values were determined polarographically. The results shown in Figure 7 indicate that the hydrolysis was completed in a short time. M was prepared by the hydrolysis of AT with 2% sodium hydroxide at room temperature for 2 hr; therefore, it should not contain any acetylthio groups.

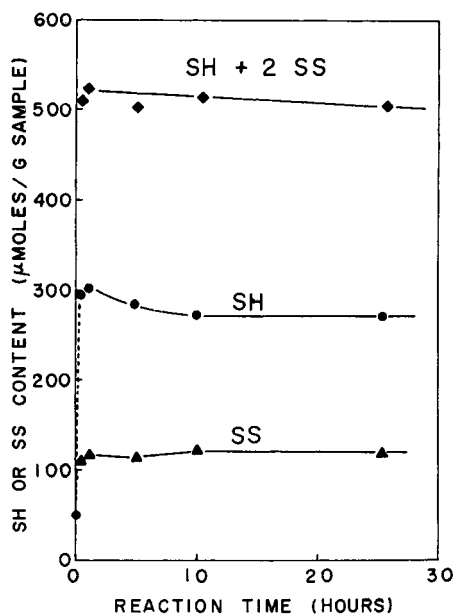


Fig. 7. Hydrolysis of AT_{d3} with 2% NaOH at room temperature.

The sulfur content of M determined by the conventional oxygen combustion method was compared with the sulfur content calculated from the SH and SS contents determined polarographically. As shown in Table III, the actual sulfur contents are much higher than the calculated values. This was supposed to indicate the presence of residual tosyl groups in M. Attempts to prepare AT free from tosyl residues were unsuccessful. The alkaline hydrolysis of M under vigorous conditions was found to eliminate tosyl residues. The sample lost some of its sulfur, and the sulfur content found agreed well with that calculated from SH and SS values. This provides another support for the proposed polarographic analysis of SH and SS. The results are summarized in Table III.

If the (SH + 2SS) value of M calculated from polarographic determination of SH and SS changes little by reduction of M, the analytical method

TABLE III
Treatment of M with 3N NaOH at 50°C for 15 Hours

Sample ^a	SH content (μmoles/g)	SS content (μmoles/g)	Sulfur content (%)		Sulfur content after NaOH treatment (%)
			found	calc'd ^b	
M_{d1}'	170	34	1.04	0.76	0.60
M_{d2}'	235	104	2.31	1.42	1.34
M_{d4}	305	142	3.25	1.88	1.76

^a M_{d1}' and M_{d2}' are second preparations of M_{d1} and M_{d2} , respectively.

^b Calculated from SH and SS contents.

employed must be **self-consistent**. The reducing reagent for this purpose should reduce the SS stoichiometrically under neutral conditions to avoid SS fission. The reagent should be easily removed from the treated fabrics. Thioglycolic acid,¹ sodium hydrosulfite,¹ sodium borohydride,^{1,3,4} and tetrakis(hydroxymethyl)phosphonium chloride⁵ were used by other investigators to reduce SS in cotton derivatives. However, none of these fulfills the above requirements. Tri-*n*-butylphosphine was recently reported to reduce cystine residues in wool quantitatively and specifically under mild conditions.¹⁶ M was reduced with tri-*n*-butylphosphine, and SH and SS contents were determined. The results summarized in Table IV

TABLE IV
Reduction of M With Tri-*n*-butylphosphine

Sample ^a	SH content (μ moles/g)	SS content (μ moles/g)	SH + 2SS (μ moles/g)
M _{m1}	45	82	209
M _{m4}	93	126	345
M _{s2}	273	87	447
M _{d3'}	287	114	515
RM _{m1}	173	19	209
RM _{m4}	279	37	353
RM _{s2}	370	31	432
RM _{d3}	384	64	512

^a Letter R signifies reduced sample. M_{d3'} is second preparation of M_{d3}.

show that most SS cleaved to SH and there was no loss in (SH + 2SS). It is interesting to note that the reduced sample was stable in air. Cysteine residues in reduced wool are usually very unstable in air and form disulfide again.

Finally the polarographic determination of SH was compared with iodometry of SH by Schwenker et al.^{1,2} It was believed that the oxidation proceeded quantitatively as in the case of some simple mercaptans. It seems, however, that SH groups in side chains of polymeric material cannot always come close together to form disulfide. Frankel-Conrat¹⁷ reported that mercaptan in undenatured tobacco mosaic virus yielded sulfenyl iodide intermediate and this did not produce disulfide when treated with iodine. Mack et al.³ reported that the spatial arrangement of SH groups was important for the readiness of SH oxidation to SS. It seems that the oxidation of SH in a polymer network is slow and not stoichiometric. Iodine consumption of M was found to be surprisingly high and exceeded the theoretical value calculated from SS values before and after the iodine treatment. Tesoro et al.⁵ also reported that iodometry gave higher values than the theoretical ones. The increase in SS was comparatively small although a major portion of SH disappeared by the iodine treatment. Thus (SH + 2SS) values decreased considerably by the iodine oxidation. The elemental sulfur analysis showed that there was no loss in sulfur by the oxidation. It was found that iodine-treated samples (OM) adsorbed much

TABLE V
 Iodine Oxidation of M

Sample	SH content (μ moles/g)		SS content (μ moles/g)		Sulfur content (%)		Decrease in SH + 2SS (μ moles/g)	I ₂ consumed (μ eq/g)	Methylene blue uptake (μ moles/g)	
	before I ₂ ox.	after I ₂ ox.	before I ₂ ox.	after I ₂ ox.	before I ₂ ox.	after I ₂ ox.			before I ₂ ox.	after I ₂ ox.
M _{a1}	214	5	51	71	1.22	1.27	169	643	8.3	90
M _{a2}	284	37	104	139	2.47	2.34	177	918	9.9	77
M _{a3}	325	16	112	139	—	—	255	1100	6.3	—
M _{a4}	305	41	142	147	3.44	3.42	254	979	5.0	38

methylene blue. The data summarized in Table V suggest that most of SH groups in M undergoes oxidation to form acidic sulfur groups (probably sulfonic acid) rather than SS groups. Oxidation of SS to various intermediates and sulfonic acid was reported for wool.^{18,19} These would account for the high iodine consumption of OM.

It is postulated at present from these experiments that when AT is hydrolyzed in alkaline medium, some newly formed SH groups favorably arranged are readily oxidized by air to form SS, and the remaining SH groups unfavorably placed cannot form SS and are oxidized to other derivatives when being treated with iodine. More detailed work on this point will be described in a subsequent paper.

CONCLUSION

(1) The mercaptan and disulfide contents of modified cotton fabrics prepared from acetylthio cellulose can be determined by application of Leach's polarographic method, with minor modifications. Tosyl residues do not interfere in the analysis based on the reaction of mercaptan and methylmercuric iodide, but acetylthio residues will give a positive error because of slow hydrolysis of acetylthio groups into mercaptan groups. Actually, samples for analysis, which were prepared by hydrolysis under alkaline conditions, contained some residual tosyl groups but no acetylthio groups.

(2) Mercapto cellulose freshly prepared by the reaction of tosyl cellulose and potassium thiolacetate followed by alkaline hydrolysis contains considerable amounts of disulfide, in addition to mercaptan. Probably air oxidation takes place during the alkaline hydrolysis procedure.

(3) Iodometric titration of mercapto cellulose gives high values of mercaptan present in the sample. Mercaptan and disulfide contents and methylene blue uptake of the iodine-treated samples indicate that some mercaptan groups undergo oxidation further to acidic groups by the iodine treatment.

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