CXIII. THE ACTION OF SULPHITES ON THE CYSTINE DISULPHIDE LINKAGES IN WOOL

I. THE INFLUENCE OF pH VALUE ON THE REACTION

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RIMINGTON [1929, 1, 2; 1930] and Barritt & Rimington [1931], using the methods of Sullivan [1929], Folin & Looney [1922] and Folin & Marenzi [1929], found that the cystine-S of animal hair and various wools was identical with the total S. Subsequently Barritt [1934] found that 2.4–4.8 % of the S of wool was present as methionine, and Bailey [1937, 1], using the methods of Lugg [1932; 1933], concluded that the remainder of the total S of wool and kemp could be accounted for as cystine.

It has been suggested that, owing to structural factors, all the cystine-S of keratin (hair, wool) may not be equally reactive or accessible. Marriott [1925] found that only 25 % of the total S of hair is readily attacked by cold dilute sodium hydroxide. Further, Astbury & Woods [1936] have drawn attention to similarities in the structure and elastic properties of myosin, containing 0.7–1.0 % of cystine [Bailey, 1937, 2], and hair with its high and variable cystine content. These authors have therefore raised the question whether only part of the S in hair is so disposed as to influence the structure and elastic properties of the keratin, the remainder being “vulcanizing” S imparting solely greater chemical resistance.

Analyses made in these laboratories on wool that has been “bleached” with sulphur dioxide and with solutions of sulphites have tended to indicate that, under certain conditions, such reagents may react only with a certain proportion of the disulphide linkages in wool. A quantitative study is therefore being made of the reaction between wool and these reagents to see whether all the disulphide groups in wool are equally reactive. The present investigation is concerned mainly with the nature of the reaction between wool and sulphite solutions with different pH values.

Clarke [1932] has shown that when cystine reacts with sulphites, salts of cysteine and *S*-cysteinesulphonic acid are produced:

The salts of *S*-cysteinesulphonic acid decompose in two ways: with acids they react as in (2) giving cysteine and a bisulphate, whereas with alkalis they react to give a bisulphite (or sulphite) and “substances which reduce the Folin-Looney phosphotungstic acid reagent.” It is probable that the initial stage of the reaction with alkali takes place, as in (3).

Whether the cystine disulphide groups in wool undergo similar reactions has not been proved hitherto, although Speakman [1936] has suggested that the 30% contraction in the length of wool fibres which occurs when they are boiled for 30 min. with 5% NaHSO_3 becomes possible because not only the salt linkages but also the disulphide linkages between adjacent polypeptide chains are broken and thiol and *S*-cysteinesulphonate side-chains are formed. We have found that the presence of thiol groups, produced as in (1), can be detected by means of Feigl's reagent [1934] (sodium azide in 0.1 *N* iodine) in wools that have been treated with NaHSO_3 . Further when such wools are hydrolysed with 5 *N* HCl , the hydrolysates contain cysteine which arises presumably by reactions (1) and (2), whilst when they are boiled with dilute H_3PO_4 , SO_2 is liberated, owing to the breakdown of *S*-cysteinesulphonate side-chains as in reaction (3). Analytical methods based on these reactions have been used in the present investigation to determine the extent of the reaction between the disulphide linkages of wool and solutions of sulphite under specified conditions.

EXPERIMENTAL

Treatment of wool with buffered solutions of sulphite. The wool used was in the form of a loosely woven thin fabric. During the course of manufacture, the wool of this fabric had been scoured, carded, carbonized (acidified with H_2SO_4 and then dried) and rescourd after neutralization. During such processing the wool may have undergone some chemical change and for this reason may not have been an entirely satisfactory experimental material. Large quantities of wool were, however, required for the present exploratory experiments and the fabric used was known to have been manufactured from sound wools and to have received careful processing to minimize chemical attack. The S content of the wool was normal (3.52%), and after purification (see below) its titration curve (Fig. 3) was closely similar to that obtained by Speakman & McMahon [1937] for the unexposed root portions from stables of virgin New Zealand Romney wool.

Portions (45 g.) of the fabric were wetted-out and immersed for 17 hr. in a series of buffered sulphite solutions each of 2 l. prepared as follows. Concentrated solutions of Na_2SO_3 containing 60 g. of SO_2 were brought to the desired pH by addition of H_2SO_4 or NaOH , made up to 1000 ml., and added to 1000 ml. of the universal buffer solution of Prideaux [1916] adjusted to the desired pH with 0.2 *N* NaOH . The final concentration of SO_2 in each solution was thus 3.0%. The equilibrium pH values of the solutions were determined with a glass electrode. On removal from the buffered sulphite solutions, the fabrics were passed through a wringer, rinsed in water to remove buffer salts and again passed through a wringer. After drying at room temperature, they were placed in a room maintained at 21° and 70% relative humidity for 2 days. They were then cut up into small pieces, their moisture contents determined by Barritt & Kings' method [1927] and samples weighed out for the analyses.

Determination of the thiol content (—SH) of the sulphited wools. Samples (1.0 g.) of the wool from each fabric were hydrolysed by gently boiling under reflux with 20 ml. 5 *N* HCl for 4 hr. The hydrolysates were cooled, filtered through a sintered glass funnel, and the filtrate and washings made up to 100 ml. Their cysteine contents were determined by Shinohara's [1935, 1] modification of Folin & Marenzi's method [1929] except that in the present determinations the HCl in the 10 ml. of hydrolysate taken was neutralized by the addition of 10 ml. *N* NaOH before the hydrolysate was added to the buffer. This procedure introduced NaCl into the test solutions, but separate experiments showed that

the intensity of colour of the standard was not altered thereby. A $4 \times 10^{-4} M$ solution of cysteine hydrochloride (dried *in vacuo* over P_2O_5) in $0.2N$ HCl was used as a standard. It was standardized by Shinohara's method [1935, 2]. To the requisite volumes of buffer and N NaOH a sample (10 ml.) of each hydrolysate was added followed by 2 ml. of $0.01M$ $HgCl_2$ and the phosphotungstic acid reagent. In this solution the development of colour by the cysteine was inhibited by the $HgCl_2$ and the solution was used in the colorimetric measurements to compensate for the natural colour of the hydrolysates and also for the colour developed by the reduction of the phosphotungstic acid by any extraneous reducing substances present.

One aspect of the method employed to determine the thiol contents of the sulphited wools calls for further comment. The time (4 hr.) of hydrolysis of the wools with $5N$ HCl was short and was probably insufficient to hydrolyse the wool completely. Separate experiments showed however that increasing the time of hydrolysis did not alter the thiol contents of the hydrolysates. In view of the conclusion of Lugg [1933] that cysteine can be heated for 20 hr. in $5N$ HCl at 100° either alone or with urea and stable amino-acids without more than about 1% decomposition, it is unlikely that the thiol contents of the hydrolysates would have been greater had the time of hydrolysis been shorter.

From the cysteine contents, the thiol ($-SH$) contents of the sulphited wools were calculated as percentages of S present as thiol, and are plotted against the pH values of the sulphite solutions in which the wools were immersed in Fig. 1. This curve has not been corrected for the thiol-S content of the untreated wool which was found to be not $>0.06\%$; accurate determination being difficult because of the small amount present.

Fig. 1.

It was found that the thiol content of the hydrolysate from untreated wool could not be increased by the addition of small quantities of Na_2SO_3 during the hydrolysis. The small amounts of uncombined sulphite that the samples of wool taken for analysis may have contained did not therefore vitiate the analyses by causing reduction of disulphide groupings during the hydrolysis. This is in agreement with Lugg's finding [1932] that cystine in strongly acid solution is not reduced by SO_2 .

From Fig. 1 it will be seen that wool reacts most readily with sulphite solutions with pH values near to 5; in solutions more acid than pH 3 or more alkaline than pH 6 far fewer thiol groups are produced. The percentage of thiol-S found in the hydrolysate of the wool treated with sulphite solutions with pH values between 4 and 6 corresponds to about 1/7 of the total S in the wool. This proportion is surprisingly low considering that in these experiments quantities of wool containing about 1.7 g. of disulphide-S were treated with solutions containing nearly 120 g. of Na_2SO_3 for 17 hr.

The determination of the S-cysteinesulphonate groups in sulphited wool. It was found that the amount of SO_2 liberated from sulphited wool when it is boiled with acids depends on the acidity of the solution employed. For example, a sulphited wool (2 g.) gave 0.33 % SO_2 slowly when distilled with water (500 ml.), 0.39 % slowly when distilled with dilute H_3PO_4 (1 ml. acid, sp.gr. 1.75 to 500 ml. water), but only 0.09 % and very quickly when distilled with HCl (10 ml. acid, sp.gr. 1.16 to 500 ml. water). It was thus evident that in the more acid solutions the S-cysteinesulphonate groupings tended to decompose according to reaction (2) whereas in the less acid solutions, they decomposed according to reaction (3). Subsequent more detailed investigations which will be communicated later have shown that H_3PO_4 at the concentration used liberates the largest quantity of SO_2 from sulphited wool. The various sulphited wools were therefore distilled for 15 min. with acid of this concentration the distillate being passed into water to which 0.1 N iodine was added as required. The results obtained are recorded graphically in Fig. 2, the percentages of SO_2 liberated from the wools being plotted against the pH values of the sulphite solutions in which the wools were treated.

Fig. 2.

Comparison with Fig. 1 shows that the thiol contents of the treated fabrics run parallel with the amounts of SO_2 recovered from them by distillation: both curves reaching a maximum at about pH 5. From the quantitative standpoint, assuming that each disulphide link gives one thiol grouping and one S-cysteinesulphonate grouping, and that in the thiol determination the S-cysteinesulphonate grouping is returned as thiol, a fabric with an apparent thiol-S content of, say 1 %, should yield 1 % of SO_2 (the weight of 2S atoms being the same as the weight of the SO_2 molecule). Actually, the wool treated with sulphite at

pH 3 was returned as containing 0.46 % of thiol-S and gave 0.36 % of SO_2 , so that some agreement with theory is indicated. It is also noteworthy that the two wools treated in sulphite solutions of pH 6.6, which on analysis were found to have thiol-S contents falling above and below the curve in Fig. 1, were also found to yield percentages of SO_2 falling above and below the curve in Fig. 2: increase or decrease in the number of thiol groups is therefore accompanied by an increase or decrease in the number of *S*-cysteinesulphonate groups.

The accurate determination of the true *S*-cysteinesulphonate contents of the wools was complicated by the difficulty of ensuring that the treated wools were free from uncombined sulphites. Prolonged rinsing of the treated wools with cold water caused both the *S*-cysteinesulphonate and the thiol groups to disappear. For example, a fabric treated at ordinary temperatures with a 3 % solution of SO_2 buffered at pH 4.7 for 17 hr., rinsed in water, passed through a wringer and then dried and conditioned, gave on analysis 0.57 % of thiol-S. After "purification" by extraction and repeated rinsing in water, it gave only 0.30 % of thiol-S. The rate of disappearance of both the *S*-cysteinesulphonate and the thiol groups was however very slow: during the "purification" referred to, the fabric was in contact with water for over a week.

Determination of titration curves. The "purified" sulphited wool containing 0.3 % of thiol-S, and a sample of the untreated wool which had been similarly purified, were extracted with alcohol and ether. Small quantities of each wool were allowed to stand for several days in distilled water, small quantities of NaOH or HCl being added in the later stages, so that the final pH value of the liquid in equilibrium with the fabric was in the neighbourhood of 5.0. The material was then dried, allowed to condition in the constant humidity room and the acid and alkali adsorption curves were determined. The ash content of the "purified" sulphited fabric was 0.06 % and that of the purified untreated fabric 0.10 %.

These data are plotted in Fig. 3, from which it will be seen that whilst the acid combining powers of the sulphited and untreated wools are identical, the

Fig. 3. $\triangle$ — $\triangle$ Untreated. + — + Treated with buffered sulphite solution at pH 4.7.

alkali-combining power of the sulphite-treated wool is greater than that of the untreated wool. The greatest difference between the alkali absorptions of the two wools occurs at pH 10 and corresponds to about 0.40 % of thiol. This is

somewhat larger than that found in the unhydrolysed wool, the discrepancy being probably due to the decomposition of *S*-cysteinesulphonate groupings leading to increased alkali absorption.

DISCUSSION

Very strong additional evidence having thus been obtained to support the view that the cystine disulphide linkages in wool are broken by sulphites, it becomes of interest to consider the mechanism of the reaction. The reaction between cystine and sulphite is generally assumed to be somewhat of the nature of a double decomposition (reaction 1), but this view of the reaction does not explain the influence of *pH* on the rate of interaction of wool and solutions of sulphites demonstrated by the experiments now described. A more probable mechanism for the reaction might be the union of the sulphenic acid arising by hydrolysis of the disulphide group with HSO_3^- :

The reversible hydrolysis of the disulphide bond has been shown to occur in acid solution: Smiles & Stewart [1921] showed that the primary products of the hydrolysis of *m*-dithiobenzoic acid are *m*-thiolbenzoic acid and the corresponding sulphenic acid. Cystine is most readily hydrolysed in alkaline solution although when the alkalinity of the solution is high, the sulphenic acid produced decomposes and H_2S is liberated [Schöberl, 1933; 1936]:

Increase in *pH* (to about 10) will therefore favour reaction (4), whilst the optimum conditions for reaction (5) will be those at which the $[\text{HSO}_3^-]$ is at a maximum. Examination of the neutralization curve of H_2SO_3 with NaOH shows that up to *pH* 5, HSO_3^- will predominate, but that at increasingly greater *pH* values these will be replaced by SO_3^{2-} , until at about *pH* 9 the $[\text{HSO}_3^-]$ will be practically zero. Increase in *pH* will thus inhibit reaction (5) so that the optimum conditions for the formation of *S*-cysteinesulphonate will be at *pH* < 9, determined by the relative magnitudes of the opposing effects of increase in *pH* value on the two reactions (4) and (5). The experimental results summarized in Figs. 1 and 2 support this conclusion. The disulphide groups in wool react most readily with sulphite in solutions more acid than *pH* 9, the greatest amount of combination taking place in solutions of *pH* 5 in which the $[\text{HSO}_3^-]$ is near its maximum.

The present results by themselves are insufficient to prove that certain disulphide groups are more reactive than others. Indeed, if the *S*-cysteinesulphonate groups arise as indicated in (5), the reaction may be reversible and thus set a limit to the number of disulphide groups that react. Further discussion is therefore deferred.

SUMMARY

1. It is shown that the disulphide linkages of wool react with sodium bisulphite solutions at ordinary temperatures and thiol and *S*-cysteinesulphonate side-chains are formed.

2. The *S*-cysteinesulphonate side-chains decompose when the sulphited wool is heated with water, either thiol groups and bisulphate, or sulphenic acid groups and bisulphite being produced, depending on the acidity of the water.

3. The presence of thiol groups in sulphited wool which has been brought to equilibrium with water at pH 5 increases the alkali-combining power of the wool.

4. The mechanism of the reaction between the disulphide linkages of wool and sodium bisulphite is discussed.

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