# 50. The Action of Sulphites on the Cystine Disulphide Linkages of Wool

3. The Subdivision of the Combined Cystine into Four Fractions Differing in their Reactivity Towards Sodium Bisulphite

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When wool is immersed in solutions of $NaHSO_3$ and then rinsed with water, some of the disulphide-S can be shown to have reacted as indicated in equation (1) [Elsworth & Phillips, 1938 *a, b*].

$$R_1.CH_2.S.S.CH_2.R_2 + M_2SO_3 \rightleftharpoons R_1.CH_2.SM + R_2.CH_2.S.SO_2OM. \tag{1}$$
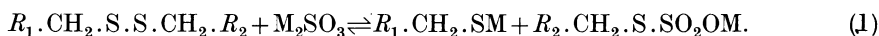
(R_1 and R_2, polypeptide chains.)

The optimum pH for the reaction is 5. Increasing the temperature, time of reaction or concentration of $NaHSO_3$ does not cause more than about one-third of the disulphide-S to react [Elsworth & Phillips, 1941]. The reaction is slowly reversed when the bisulphited wool is rinsed with cold water, but it is possible to free the treated wool from uncombined $NaHSO_3$ and to stop rinsing, when only small quantities of $NaHSO_3$, arising by the reversal of reaction (1), are removed from the wool.

In previous investigations, this procedure was followed because the presence of large quantities of uncombined $NaHSO_3$ in the treated, unrinsed wool would have interfered with the determination of SO_2 combined in *S*-cysteinesulphonate groups. The extent of the reaction can however be determined by hydrolysing the bisulphited wool with $5N$ HCl [Elsworth & Phillips, 1938 *a, b*] when two thiol groups (see equations 1 and 2) are found in the hydrolysate for each disulphide group which reacts with $NaHSO_3$.

$$R_2.CH_2.S.SO_2.OM \xrightarrow{\text{HCl}} R_2.CH_2.SH + MCl + H_2SO_4. \tag{2}$$
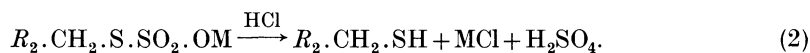
Experiment showed that unchanged disulphide-S in bisulphited wool is not reduced even when wool containing relatively large quantities of uncombined $NaHSO_3$ is hydrolysed (see also Lugg [1933]). The reaction between $NaHSO_3$ and wool has therefore been re-examined, the bisulphited wool being analysed before and after rinsing with water. It has been found that in addition to the disulphide-S which reacts with $NaHSO_3$ to give thiol and *S*-cysteinesulphonate groups which are relatively stable to water, another fraction of the disulphide-S reacts in a similar manner, but the thiol and *S*-cysteine-sulphonate groups produced revert to disulphide-S immediately the bisulphited wool is placed in water.

We have been able to show that 50% of the original cystine-S of the wool can be caused to react with $NaHSO_3$, and of this 50% somewhat less than half gives water-unstable thiol and *S*-cysteinesulphonate groups.

We have also found that when wool reacts with $NaHSO_3$, particularly above about $40°$, another fraction of the disulphide-S is converted into non-disulphide-S. There appears to be a limit to the amount of disulphide-S that can be easily transformed in this manner. Under the experimental conditions we have employed this fraction is rather more than half the original cystine-S which fails to give thiol and *S*-cysteinesulphonate groups.

EXPERIMENTAL

Material and methods

Unless otherwise stated, the wool used in this investigation was a virgin Cape wool of about 64's quality, cleaned without the use of soap by methods previously described [Elsworth & Phillips, 1941]. Before this cleansing, the tips were removed from the locks of wool, and during the cleansing discoloured and damaged fibres were removed by hand. Before analysis, samples of untreated and treated wools were conditioned by exposing them to a controlled atmosphere of 21° and 70 % R.H. until they attained constant weight. All analytical results are calculated on the weight of anhydrous wool taken for analysis. Determinations of total S were made by Barritt's modification [1934] of the Denis-Benedict method and thiol-S and disulphide-S by Shinohara's modification [1935 a, b] of Folin & Marenzi's method [1929]. Colorimetric determinations were made in a Spekker absorptiometer. Methionine-S was calculated from a determination of methionine by Baernstein's method [1936]. Using these methods of analysis, the wool contained 3·69 % total S, 3·25 % disulphide-S, 0·0 % thiol-S and 0·10 % methionine-S. Hence 0·34% of the S is unaccounted for and is called non-disulphide-S. This term is applied throughout this paper to S which is not returned as either disulphide-S, thiol-S or methionine-S.

The influence of the concentration of $NaHSO_3$

Weighed samples of the wool were immersed for 24 hr. at room temperature in fifty times their weight of $NaHSO_3$ solutions containing 1·0–16·0 % SO_2. Half of each sample (about 1 g.) was passed through squeeze rollers to remove adhering solution and then hydrolysed for 4 hr. with 5 N HCl (20 ml.), and the thiol-S of the hydrolysate determined. The other half of each sample was rinsed in two changes of water (200 ml. per g.) adjusted to pH 5 with a trace of acetate buffer. It was then hydrolysed and the thiol-S content of the hydrolysate determined. The results are plotted against the SO_2 concentrations of the $NaHSO_3$ solutions in which the two sets of wool samples were bisulphited in Fig. 1.

In order to show that the higher thiol-S contents of the hydrolysates of the unrinsed wools were not due to reduction of disulphide-S by uncombined $NaHSO_3$ during hydrolysis, a sample of wool (about 1 g.) which had been immersed for 24 hr. in a solution of $NaHSO_3$ containing 16 % SO_2 and then rinsed, as described above, was hydrolysed with 5 N HCl (20 ml.) containing 0·2 g. $NaHSO_3$. This quantity of $NaHSO_3$ would be present in the water retained by wool which had been immersed in a solution of $NaHSO_3$ containing 16 % SO_2 and then passed through squeeze rollers. The thiol-S content of the hydrolysate was 0·99 %, whereas when the bisulphited and rinsed wool was hydrolysed in 5 N HCl free from $NaHSO_3$, the hydrolysate contained 0·98 % thiol-S. The presence of uncombined $NaHSO_3$ in the unrinsed bisulphited wools was not therefore responsible for the larger amounts of thiol-S which their hydrolysates were found to contain. The curve relating the thiol-S contents of the hydrolysates of the rinsed wools to the SO_2 contents of the bisulphite solutions is very similar to the curve obtained by Elsworth & Phillips [1941] using a commercial woollen fabric and a virgin Australian 64's wool.
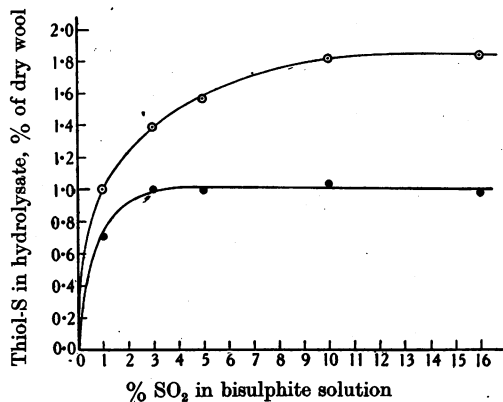

Fig. 1. ⊙—⊙ Unrinsed, bisulphited wool.
●—● Rinsed, bisulphited wool.

These wools contained respectively 3·60 and 3·42 % total S and 2·99 and 3·09 % disulphide-S. The maximum amount of their disulphide-S which reacted with NaHSO$_3$ to give water-stable thiol and S-cysteinesulphonate groups was 1·0 %, a figure identical with that given by the wool used in the present experiments. On the other hand, this wool reacted more quickly with the dilute NaHSO$_3$ solutions than did the wool of the commercial fabric.

The influence of the pH of the NaHSO$_3$

Elsworth & Phillips [1938 a] showed that wool reacts most readily with bisulphite to produce water-stable thiol and S-cysteinesulphonate groups at about pH 5; in solutions more acid than pH 3 or more alkaline than pH 6 far fewer disulphide groups reacted. We have repeated this experiment in order to see whether the production of water-unstable thiol and S-cysteinesulphonate groups is similarly influenced by the pH of the sulphite.

Samples of wool were wetted with a dilute solution of sulphonated castor oil, rinsed in water and then soaked in a series of buffer solutions of increasing pH. They were then squeezed and immersed in a hundred times their weight of NaHSO$_3$ solutions of the same pH values as the buffer solutions in which they were soaked. These bisulphite solutions contained 3·87 % SO$_2$ and were adjusted to the required pH values by the addition of either 0·5 % sodium acetate or 0·5 % sodium borate. After 18 hr., the samples (each of about 0·5 g.) were removed from the bisulphite solutions, and passed through squeeze rollers. One-third of the samples was not rinsed. Another third was immersed, for a total period of $\frac{1}{2}$ hr., in three changes (each of 200 ml.) of buffer of the same pH as the bisulphite solutions in which they had been treated. The remaining third of the samples was also immersed in three changes of buffer, but the total time of immersion was 4 hr. instead of

Initial pH of sulphite solutions

Fig. 2. ⊙—⊙ Unrinsed, bisulphited wool. +—+ Rinsed, bisulphited wool. ×—× Labile fraction in unrinsed bisulphited wool (by difference).

$\frac{1}{2}$ hr. The thiol-S contents of the hydrolysates of the unrinsed bisulphited wools and of the bisulphited wools that had been rinsed for $\frac{1}{2}$ hr. are plotted in Fig. 2 against the initial pH values of the solutions in which they were bisulphited. The analyses of the hydrolysates of the bisulphited wools after 4 hr. immersion in buffers and the pH values of the bisulphite solutions in which they were treated are given in Table 1.
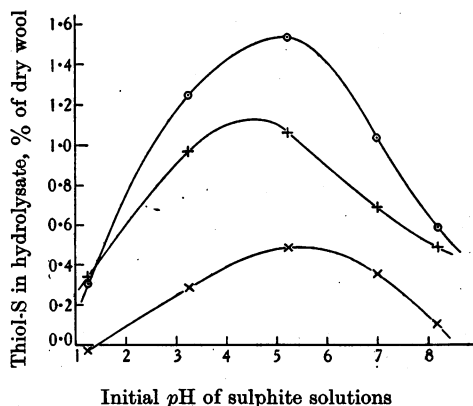
Table 1. *The sulphur distribution of the hydrolysates of wools bisulphited at different pH values and then immersed for 4 hr. in buffers of the same pH values*

No. of wool sample	pH of bisulphite		Disulphide-S %	Thiol-S %	Non-disulphide-S %
	Initial	Final			
1	1·22	1·12	2·82	0·34	0·43
2	3·26	3·10	2·50	0·64	0·45
3	5·22	5·06	2·56	0·56	0·47
4	6·98	6·83	2·78	0·47	0·34
5	8·18	8·07	2·88	0·36	0·36

Fig. 2 shows that the amounts of both the water-stable and water-labile thiol and S-cysteinesulphonate groups produced when wool is treated with NaHSO$_3$ are dependent on the pH of the sulphite solution. The optimum pH is 5 probably because at this pH

sulphite solutions contain the highest concentration of $NaHSO_3$. The curve relating to the water-stable groups is similar, although at a higher level, to that obtained by Elsworth & Phillips [1938 a]. From Fig. 1, the bisulphite solution used in these experiments (3·87 % SO_2) should have given, at pH 5, a wool containing 1·50 % of S as thiol and S-cysteine-sulphonate groups, and of these an amount equivalent to 0·49 % S would be water-labile. Actually a total of 1·54 % of S reacted of which 0·46 % gave water-labile thiol and S-cysteinesulphonate groups.

Rinsing the bisulphited wools for 4 hr. (Table 1) instead of $\frac{1}{2}$ hr., lowers the S present as water-stable thiol and S-cysteinesulphonate groups from 1·08 to 0·56 % in the wool bisulphited at pH 5, but has less effect on the wools bisulphited at higher or lower pH values. The non-disulphide-S of the original wool was 0·34 % and the non-disulphide-S increases slightly during treatment with $NaHSO_3$.

The formation of non-disulphide-S when wool is treated with solutions of $NaHSO_3$

Elsworth & Phillips [1941] showed that the action of $NaHSO_3$ on wool, particularly above 40°, caused the non-disulphide-S fraction to increase at the expense of the disulphide-S fraction. Analyses of various treated wools made during the present investigation suggested that the non-disulphide-S could increase to about 1·0 %, but it was difficult to produce wools containing more than this amount. Some of the analyses on which this conclusion is based are collected in Table 2.

Table 2. *Sulphur distribution of hydrolysates of bisulphited wools*

			Disulphide-S %	Thiol-S %	Non-disulphide-S %
1. Untreated wool			3·25	0·00	0·34
2. Wool exposed to 20% $NaHSO_3$ for 24 hr. at room temp.	(a)	Rinsed with 1% HCHO at pH 1·2	1·99	0·49	1·11
	(b)	Rinsed with 1% HCHO at pH 9·2	2·81	0·11	0·67
	(c)	Rinsed with water	1·93	0·92	0·74
3. Wool exposed to 20% $NaHSO_3$ for 72 hr. at room temp.	(a)	Rinsed with 1% HCHO (pH 5·6) at 70°	2·56	0·00	1·03
	(b)	Unrinsed	0·73	1·86	1·00
4. Wool boiled with 5% $NaHSO_3$ for $\frac{1}{2}$ hr.	(a)	Rinsed with water	1·82	0·76	1·01
	(b)	Rinsed with water (pH 9·0) for 2 hr.	1·72	0·81	0·97
	(c)	Unrinsed	0·67	1·76	1·10

The non-disulphide-S in the hydrolysate of the untreated wool was 0·34 % and in six out of eight experiments this increased to about 1·0 %, irrespective of the type of after-treatment. Rinsing with either alkaline or acid 1 % HCHO reverses the bisulphite reaction and does not produce more non-disulphide-S than does rinsing with water. Hence no combination occurs between the cystine-S and HCHO, similar to that which takes place when wool is treated with 1 % HCHO at 70° [Middlebrook & Phillips, 1942]. Bisulphiting at high temperatures (e.g. 100°, sample 4) is more effective in producing non-disulphide-S than is bisulphiting at room temperatures. Increasing the concentration of $NaHSO_3$ also favours non-disulphide-S formation, as is shown by comparing the non-disulphide-S contents of the wools treated with 6·15 % $NaHSO_3$ (Table 1) with those treated with 20 % $NaHSO_3$ (Table 2). It is also possible, as with samples 3 (b) and 4 (c) (Table 2), to obtain wools containing 1·8–1·9 % S which have reacted in the normal manner with $NaHSO_3$ and yet contain 1·0 % non-disulphide-S. Hence the fraction of the disulphide-S of the wool which passes to non-disulphide-S is not the same as the fractions which give water-stable and water-unstable thiol and S-cysteinesulphonate groups.

Sufficient HCl was added to the 5 % NaHSO$_3$ solution in which sample 4 was boiled to make it 5N with respect to HCl. It was then boiled for 4 hr. and on analysis was found to contain no disulphide-S and 0·29 % thiol-S, calculated on the weight of the original dry wool. To obtain the figures given in Table 2, 0·29 % thiol-S has been added to the thiol-S of the hydrolysate of the bisulphited wool. It was assumed that no wool dissolved from samples 2 and 3. In order to obtain more complete information on the solubility of wool in NaHSO$_3$ and on the factors involved in the conversion of disulphide-S into non-disulphide-S, two series of systematic experiments were made.

The solubility of wool in solutions of NaHSO ·

(a) *At room temperature.* Samples of the wool were immersed in one hundred times their weight of a series of solutions of NaHSO$_3$ containing increasing amounts of NaHSO$_3$ up to 26 %. After 10 days, the bisulphited wools were removed by filtration through sintered glass, and rinsed in distilled water over a period of several hours. The rather prolonged period of rinsing resulted in some conversion of the water-stable thiol and *S*-cysteinesulphonate groups into disulphide-S and consequently the thiol-S contents of the bisulphited wools are somewhat low.

Table 3. *Analyses of wool after immersion in solutions of NaHSO$_3$ for 10 days at room temperature**

No. of sample ...	1	2	3	4	5	6	7	8
Concentration of NaHSO$_3$, %	1·0	3·0	6·0	9·0	12·0	18·0	22·0	26·0
Loss in weight of wool, %	− 1·3	− 0·8	0·1	1·8	2·7	2·2	1·7	1·1
Disulphide-S of undissolved wool, %	2·22	2·22	2·34	—	2·04	1·93	1·98	2·04
Thiol-S of undissolved wool, %	0·80	0·74	0·60	—	0·77	0·83	0·81	0·88
Non-disulphide-S of undissolved wool, %	0·57	0·63	0·66	—	0·78	0·82	0·79	0·67

* Calculated as percentages of the weight of the untreated anhydrous wool.

Table 3 shows that the losses in weight are small and increase to a maximum in 12 % NaHSO$_3$ thereafter becoming smaller possibly because of the high salt concentration. The non-disulphide-S shows a similar variation with the concentration of NaHSO$_3$. There is very little difference between the amounts of water-stable thiol and *S*-cysteinesulphonate groups in the different wools which indicates that the same amount of S reacts in 1·0 % as in 26 % NaHSO$_3$ provided that the time of reaction is sufficiently prolonged.

(b) *At 70° for increasing periods of time.* The wool samples were immersed in twenty times their weight of a 12 % solution of NaHSO$_3$, maintained at 70°, for the times given in Table 4. Before analysis, the treated wools were squeezed and then rinsed several times with water, dried and conditioned. The thiol-S, disulphide-S and total N contents of the NaHSO$_3$ filtrates and washings from the treated wool were also determined and are given with the analyses of the treated wools in Table 4.

Table 4, line (v) shows that wool dissolves much more rapidly in solutions of sodium bisulphite at 70° than it does in similar solutions at room temperatures. Except after prolonged treatment, the dissolved wool contains almost equal amounts of thiol and disulphide-S (line ii(a)). The thiol-S in the hydrolysate of the undissolved wool tends to remain constant (line iii(a)). This is shown more clearly when the values given in line iii(a) are re-calculated on the basis of the weight of undissolved wool, instead of on the basis of the original weight of the anhydrous untreated wool. On the other hand, on either basis of calculation, the disulphide-S in the hydrolysate of the undissolved wool (line iii(b)) shows a progressive fall.

Table 4. *Analyses of undissolved bisulphited wool and wool dissolved by a 12°/₀*
solution of NaHSO₃ at 70°*

No. of sample ...	1	2	3	4	5
(i) Duration of treatment, hr.	19	44	70	162	260
(ii) Dissolved wool:					
(a) Thiol-S in hydrolysate, %	0·27	0·36	0·62	0·63	0·50
(b) Disulphide-S in hydrolysate, %	0·27	0·37	0·52	0·81	0·91
(c) Pyruvic acid in hydrolysate, %	—	0·24	0·35	0·69	—
(iii) Undissolved wool:					
(a) Thiol-S in hydrolysate, %	0·85	0·76	0·71	0·69	0·60
(b) Disulphide-S in hydrolysate, %	1·52	1·32	0·83	0·45	0·28
(c) Pyruvic acid in hydrolysate, %	0·21	—	—	0·17	0·18
(iv) Non-disulphide-S, % (by difference)†	0·68	0·78	0·91	1·02	1·30
(v) Loss in weight of wool, %	10·3	14·7	24·4	31·2	37·4
(vi) Total N of dissolved wool, %	2·19	2·76	4·39	5·79	6·60
(vii) Ratio total N to sum of thiol-S and disulphide-S in hydrolysate of dissolved wool	4·70	4·39	4·46	4·66	5·44
(viii) Ratio total N to sum of thiol-S, disulphide-S and non-disulphide-S in hydrolysate of undissolved wool	4·57	4·64	4·64	4·54	4·05

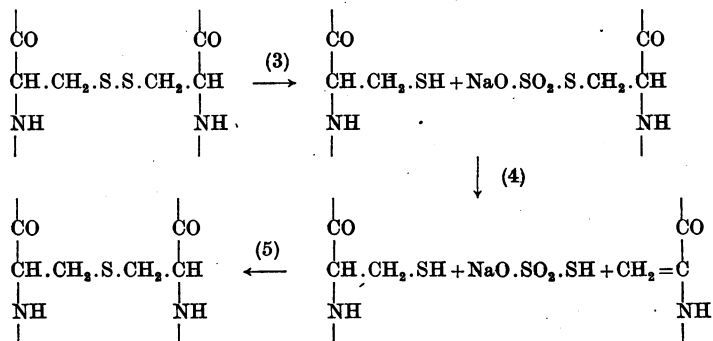* All results are calculated as percentages of the original weight of the anhydrous untreated wool.

† Total S of original wool (less methionine-S, 0·10%) minus sum of thiol-S and disulphide-S of dissolved and undissolved wool.

Noteworthy features among the results are the N/S ratios of the dissolved and undissolved wools. In calculating these ratios, the methionine-S (0·10%) has been ignored, the total S of the original wool being taken as 3·59%. On this basis, the N/S ratio of the original wool (N, 16·5%) was 4·60. Further, it has been assumed that all the non-disulphide-S, originally present in the wool, and any formed during bisulphiting, remains in the undissolved wool. The N/S ratios of the dissolved and undissolved wools, after the different lengths of time of treatment (with the exception of sample 5 which was treated for very nearly 11 days) are then in fairly close agreement with the N/S ratio of the original wool. This suggests that when wool dissolves in NaHSO₃, the composition of the dissolved wool is similar to that of the original wool, although any cystine in the wool which is converted into non-disulphide-S remains in the undissolved wool. This conclusion is in agreement with previous investigations on the solvent action of sodium sulphide on wool. Marriott [1925] showed that the N/S ratios of hair before and after 26% had been dissolved by 0·1N Na₂S were closely similar. It is of interest to note that wool after treatment with Na₂S may contain non-disulphide-S in the form of lanthionine (Horn, Jones & Ringel [1941]; du Vigneaud & Brown [1941]), since Horn & Jones [1941] have shown that this acid [HOOC.CH(NH₂).CH₂.S.CH₂.CH(NH₂).COOH] can be produced in wool by boiling it for 1 hr. with 2% Na₂S.

During the first 70 hr. of treatment (Table 4, line iv), the non-disulphide-S increased to 0·91%. By an additional 98 hr. treatment, this value was raised to 1·02%. These results again suggest that the production of 1·0% of non-disulphide-S represents a definite stage in the transformation of the combined cystine of wool. It is evident however that this stage does not give a stable wool, since a further 98 hr. treatment caused the non-disulphide-S to increase to 1·30%.

On standing, S was deposited from the filtered NaHSO₃ solutions that had been in contact with wool for the longer periods. In addition, fairly copious precipitates of S were obtained, when such filtrates were acidified with HCl, suggesting that they contained sodium thiosulphate as well as free S. Theis & Richer [1941] have also observed

that thiosulphate is formed when hair is attacked by sulphites. A possible origin of this thiosulphate is the decomposition of S-cysteinesulphonate according to reaction (4).

$$
\begin{array}{llll}
| & | & | & | \\
\text{CO} & \text{CO} & \text{CO} & \text{CO} \\
| & | \quad (3) & | & | \\
\text{CH.CH}_2\text{.S.S.CH}_2\text{.CH} & \xrightarrow{\hspace{1cm}} & \text{CH.CH}_2\text{.SH} + \text{NaO.SO}_2\text{.S.CH}_2\text{.CH} \\
| & | & | & | \\
\text{NH} & \text{NH} & \text{NH} & \text{NH} \\
| & | & | & |
\end{array}
$$

$$\downarrow (4)$$

$$
\begin{array}{llll}
| & | & | & | \\
\text{CO} & \text{CO} & \text{CO} & \text{CO} \\
| & | \quad (5) & | & | \\
\text{CH.CH}_2\text{.S.CH}_2\text{.CH} & \xleftarrow{\hspace{1cm}} & \text{CH.CH}_2\text{.SH} + \text{NaO.SO}_2\text{.SH} + \text{CH}_2\text{=C} \\
| & | & | & | \\
\text{NH} & \text{NH} & \text{NH} & \text{NH} \\
| & | & | & |
\end{array}
$$

If such a decomposition occurred, the acid thiosulphate produced might be expected to decompose and yield free S. On the other hand, the wool when hydrolysed would be expected to yield cysteine equivalent to the S lost in this manner. Experimentally this is not observed.
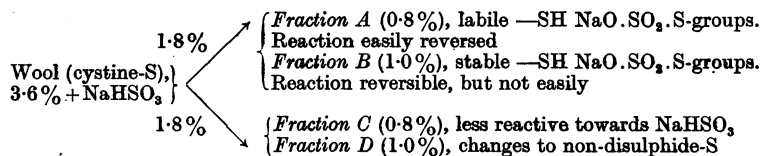
An additional consequence of decomposition according to reaction (4) would be the formation of combined α-aminoacrylic acid in the wool. When the wool was hydrolysed, this acid would be liberated and would decompose into pyruvic acid and ammonia. Using Lu's micro-method [1939] based on Case's method [1932], we have determined the pyruvic acid contents of the hydrolysates of some of the undissolved and dissolved wools. The pyruvic acid content of the hydrolysate of the untreated wool (4 hr. with $5N$ HCl, 20 ml. per g.) was 0·20 %, and the analyses given in Table 4, line iii (c) show that the pyruvic acid in the hydrolysate of the undissolved wool remains almost constant; a small increase is shown when the observed value is calculated on the actual weight of the undissolved wool. This small increase may have some significance as indicating that decomposition of S-cysteinesulphonate groups by reaction (4) occurs, but also suggests that by far the greater part of any α-aminoacrylic acid produced in the wool by such decomposition changes to a more stable compound. This more stable compound may be lanthionine, produced by the condensation of adjacent cysteine and α-aminoacrylic acid residues (reaction 5).

Nicolet & Shinn [1941] claim to have produced combined α-aminoacrylic acid in silk by the alkaline dehydration of combined serine and to have linked the methylene groups with benzyl mercaptan. It is interesting to note that the hydrolysate of the dissolved wool shows a much more pronounced increase in pyruvic acid content with increase in the duration of the bisulphiting (Table 4, line ii (c)). A possible explanation may be that when the S-cysteinesulphonate groups in the disorganized polypeptide chains of the dissolved wool decompose, they give combined acrylic acid residues which are less certain to find conveniently situated cysteine residues with which to combine, than are the α-aminoacrylic acid residues produced in the undisturbed polypeptide chains of the undissolved wool. A more detailed investigation of wool containing the non-disulphide-S produced by bisulphiting is now being made.

DISCUSSION

As the result of the present investigation, it is now possible to give a more definite picture of the variation in the reactivity of the combined cystine of wool towards NaHSO₃. Assuming that the total S of a 'perfect' wool can be entirely accounted for as the sum of the cystine-disulphide-S and the methionine-S, and that any non-disulphide-S found in wool has arisen from cystine-S, then the virgin Cape wool used in the experiments

described originally contained 3·6 % cystine-S and by reaction with NaHSO$_3$, this cystine-S has been subdivided into four fractions as in the following scheme.

Wool (cystine-S),}
3·6 % + NaHSO$_3$ }

1·8 %
{ *Fraction A* (0·8 %), labile —SH NaO.SO$_2$.S-groups.
{ Reaction easily reversed
{ *Fraction B* (1·0 %), stable —SH NaO.SO$_2$.S-groups.
{ Reaction reversible, but not easily

1·8 %
{ *Fraction C* (0·8 %), less reactive towards NaHSO$_3$
{ *Fraction D* (1·0 %), changes to non-disulphide-S

Whilst, under the experimental conditions which we have used, only half the disulphide-S of wool will react to give thiol and *S*-cysteinesulphonate groups, i.e. fractions (A+B) = fractions (C+D), our analytical results suggest that fractions A and C are each somewhat smaller than fractions B and D. There are however so many possible sources of small errors in our analyses that it remains conceivable that all the four fractions are equal. In addition, NaHSO$_3$ may not differentiate sharply between the reactivities of the different fractions.

The chief assumptions made in this scheme concern fraction D which passes to non-disulphide-S. We have included in this fraction the non-disulphide-S returned by our methods of analysis as being present in the untreated wool. We have thus assumed that a fraction of the disulphide-S is more readily converted into non-disulphide-S by a variety of agencies, such as light or alkali, than are the remaining fractions. Some evidence has been found which suggests that a fraction of the disulphide-S of wool is labile to alkalis. Elsworth & Phillips [1941] have shown, for example, that when a wool was boiled with a buffer of *p*H 8·5 for ½ hr., the non-disulphide-S increased from 0·51 to 1·06 %, but the wool lost only 0·04 % S. When this wool was boiled with a more alkaline buffer (*p*H 8·9), the non-disulphide-S remained unchanged (1·01 %), but the wool lost 0·31 % S. These results suggest that at *p*H 8–9 it is possible to convert 1·0 % of the S of the wool into non-disulphide-S and that this is to some degree a limiting value. This fraction of the S may therefore correspond to fraction D which is converted into non-disulphide-S by NaHSO$_3$. This is an aspect of the work which will form the subject of another investigation.

We have also shown [Middlebrook & Phillips, 1942] that the disulphide-S of wool can be reduced from 3·25 to 1·81 % by warming the wool with 1 % HCHO at 70° and *p*H 5·6. Wool, after treatment in this manner, does not react with NaHSO$_3$ to give water-stable thiol and *S*-cysteinesulphonate groups. Part of the disulphide-S that has reacted with HCHO probably belongs therefore to fraction B. Some other fraction of the disulphide-S is also involved in this reaction, since the wool lost 1·34 % disulphide-S. This other fraction may be fraction D which may have changed to non-disulphide-S. Evidence supporting this conclusion was obtained by distilling a sample of formaldehyde-treated wool with HCl. The distillate was found to contain 0·70 % HCHO by Bowes & Pleass's method [1939], whilst the hydrolysate was free from thiol-S but contained 2·56 % disulphide-S. Hence 1·03 % (3·59–2·56) of the wool-S was in the form of non-disulphide-S and this agrees in amount with the total quantity which, it is assumed, can arise from fraction D. Quantitative agreement with our proposed scheme of subdivision is not however good. Assuming that during the treatment with formaldehyde, the remainder of fraction D is converted into non-disulphide-S and fraction B reacts with HCHO to give thiazolidine-4-carboxylic acid, as suggested by Middlebrook & Phillips [1942], then we should have expected the disulphide-S to fall to 1·59 % instead of only to 1·81 % as was observed.

Apparently fraction A does not react with HCHO at 70° and *p*H 5·6. If this conclusion is correct, it was thought possible that formaldehyde-treated wool would react with NaHSO$_3$ to give water-labile thiol and *S*-cysteinesulphonate groups. A formaldehyde-treated wool (disulphide-S, 1·81 %) was therefore immersed for 17 hr. at room temperature in fifty times its weight of 20 % NaHSO$_3$, and without rinsing with water was hydrolysed.

The hydrolysate did not contain thiol-S and contained only 1·07 % disulphide-S. By the dual treatment, fraction A was therefore converted into a form of non-disulphide-S, possibly by interaction with the HCHO.

Goddard & Michaelis. [1934; 1935] found that wool was dissolved by strongly alkaline solutions of thiolacetic acid owing to the complete reduction of the disulphide-S. Patterson, Geiger, Mizell & Harris [1941] have studied the reduction of the disulphide-S of wool with solutions of thiolacetic acid of low acidities and it is of interest to compare the extent of disulphide reduction which they obtained with our results. These workers reduced wool in buffer solutions of thiolacetic acid and coupled the thiol groups produced with p-chlorobenzyl chloride. The cystine contents of the chlorobenzylated wools were then determined by Sullivan & Hess's method [1930] using an improved technique [Harris & Smith, 1937], and the decrease in cystine content was taken as a measure of the extent to which the original disulphide-S of the wool had been reduced. The tips were not apparently removed from the wool used by Patterson et al. [1941] and it was part of a batch purified by Sookne & Harris [1937] by extraction with alcohol and ether followed by rinsing with water at 40°. The diameter of the fibres of this wool was 35μ, so that the 'quality' of the wool on the Bradford system was 46's: the diameter of the fibres of our wool was about 22μ. Unfortunately Patterson et al. [1941] do not give a complete analysis of the S of the wool, but their results show that it contained 12·2 % cystine corresponding to 3·26 % disulphide-S. They showed that a M solution of thiolacetic acid at pH 4·5 and 35° reduced the cystine content of the wool to 3·75 % (1·00 % disulphide-S) and a 2M solution gave very little more reduction under the same experimental conditions. Wools reduced in this manner thus contained 2·26 % of thiol-S and this appears to be a limiting value when the reduction takes place between pH 2 and 6. For example, Patterson et al. [1941] showed that the cystine content of the wool was reduced to just below 4·0 % in ¾ hr. by a M thiolacetate solution at pH 4·5 and 50° and no further reduction occurred when the reaction time was extended to 20 hr.

It thus appeared probable that thiolacetic acid reduced about 2·0 % disulphide-S under the conditions used by Patterson et al. [1941]. In order to find out whether the disulphide-S reduced belonged to fractions A and B, a sample of our wool was immersed for 18 hr. in one hundred times its weight of M thiolacetic acid containing 0·5 % NaAc, the pH having been adjusted to 4·8–5·0 by the addition of HCl. The treated wool was rinsed thoroughly with water and then conditioned. It then contained 1·33 % disulphide-S and 1·89 % thiol-S. It was bisulphited in one hundred times its weight of 20 % NaHSO$_3$ at room temperature for 22 hr. On analysis (without rinsing) it was found to be unchanged, since it contained 1·28 % disulphide-S and 1·99 % thiol-S. Fractions A and B that normally react with NaHSO$_3$ had therefore been reduced by the thiolacetic acid and consequently, under the experimental conditions employed, the wool was inert towards NaHSO$_3$.

SUMMARY

1. When wool reacts with NaHSO$_3$, 50 % of the cystine-S is converted into thiol and S-cysteinesulphonate groups, of which slightly more than half are stable to water, whilst the remainder are water-labile.

2. By the action of bisulphite, slightly more than half the disulphide-S which does not give thiol and S-cysteinesulphonate groups can be converted into non-disulphide-S.

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