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## NATURE OF THE REACTION OF WOOL WITH ALKALI

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## ABSTRACT

The course of the reaction of the cystine in wool with alkali has been reinvestigated. The earlier findings that one sulfur atom is split from each molecule of cystine are confirmed. Of the residual noncystine sulfur in the alkali-treated wool, more than 25 percent has been accounted for as lanthionine. No significant amounts of sulfhydryl groups are in the treated wools. The results lead to the conclusion that the alkali cleavage of the disulfide group does not consist primarily in a hydrolytic rupture between the sulfur atoms with the formation of a sulfhydryl compound and a sulfenic acid, as postulated earlier; rather, they are more consistent with a mechanism recently advanced by Nicolet and Shinn, which involves a rupture between sulfur and carbon to yield dehydroalanine and a  $-\text{CH}_2-\text{S}-\text{SH}$  residue. An atom of sulfur is then eliminated from the latter and the sulfhydryl group thus formed reacts with dehydroalanine to form lanthionine.

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## I. INTRODUCTION

One of the most characteristic chemical properties of wool is the ease with which it is degraded in alkaline solutions. In earlier investigations [1, 2, 3]<sup>2</sup> it was shown that such degradation is closely associated with the lability toward alkalis of the disulfide groups in the cystine of the wool. A study of the course of the degradation revealed that during the early stages, a rapid splitting off of a portion of the sulfur occurred, the amount of sulfur being lost closely approaching 50 percent of the original total [2]. This behavior led to the conclusion that the first step in the alkali degradation of wool is a splitting of the disulfide group to yield one labile and one comparatively stable sulfur group. Such a conclusion appeared to agree well with a view that had been proposed by Schöberl and Eck [4] to explain the lability of sulfur in various disulfides. According to these investigators, the cleavage of disulfides by alkali consists in a hydrolytic rupture of the disulfide

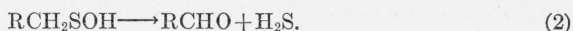
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<sup>2</sup> Figures in brackets indicate the literature references at the end of this paper.

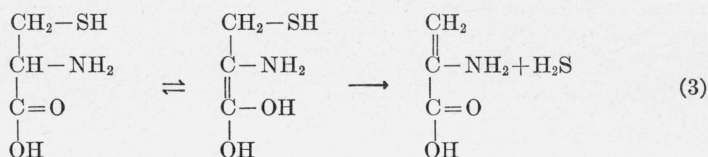
linkage with the formation of a sulfhydryl compound and a sulfenic acid according to the following scheme:



The sulfenic acid, being extremely reactive and unstable in alkaline solution, immediately loses  $H_2S$  to form an aldehyde as follows:



Another possibility given consideration at that time was that proposed by Nicolet [5], who postulated that the removal of sulfur from cystine by alkalis takes place as an elimination of hydrogen sulfide from an intermediate enolized form according to the following equation:

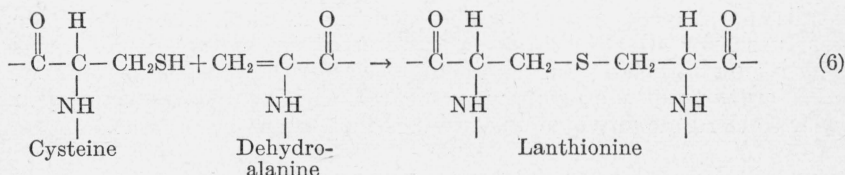
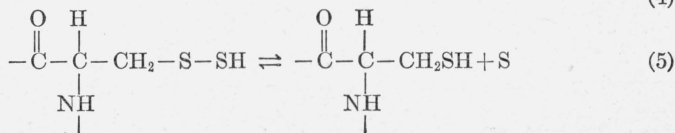
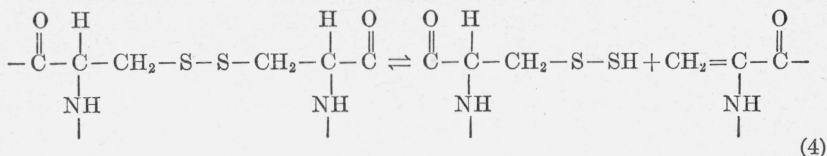


He further suggested that from cystine,  $H_2S_2$  would split out. Since this mechanism obviously failed to explain the loss of nearly half of the sulfur, little further attention was given to it.

Although Schöberl's hypothesis was consistent with the loss of about half of the sulfur, it failed to explain a number of other observed facts. For example, his explanation calls for the production of sulfhydryl and aldehyde groups, whereas tests for each of these groups on hydrolysates of the alkali-treated wool were definitely negative. As pointed out earlier [2], there existed the possibility that recombination between the aldehydic and sulfhydryl groups may have occurred under the conditions of the hydrolysis. However, it was subsequently shown [6] that few if any free sulfhydryl groups are present in the alkali-treated wool even prior to hydrolysis. In addition, this hypothesis, when applied to wool, would result in the rupture of the disulfide cross-links, a process which would be expected to yield a material of very low strength and extremely high alkali-solubility [7]. Yet samples of wool, treated with alkali by methods described elsewhere, exhibited relatively high strengths and low alkali-solubilities (see table 1). In fact, the properties of the alkali-treated wools were such as to strongly suggest that if the disulfide cross-links are destroyed, they are in a measure replaced by new linkages.

Considerable support for the view that new linkages can replace ruptured ones was found in the recent work of Horn, Jones, and Ringel [8], who succeeded in isolating from alkali-treated wool a sulfur-containing amino acid having the formula  $(HOOC-CH(NH_2)-CH_2)_2S$ , which they named lanthionine. Since practically all of the sulfur in undegraded wool can be accounted for as cystine sulfur, it seems certain that this new amino acid is a product of the reaction of the cystine in wool with alkali. More recently Nicolet and Shinn [9] have offered an explanation for the formation of lanthionine,

postulating as a primary reaction a split between carbon and sulfur as follows:



This view has many advantages over the Schöberl hypothesis' since it not only explains the loss of half of the sulfur, but in addition it meets the requirements for linkage rebuilding and thus accounts for the relatively high strength and low alkali-solubility of alkali-treated wools. Finally, it offers an explanation for the absence of significant amounts of sulfhydryl and aldehyde groups in the wool hydrolysate.

On the basis of the new point of view resulting from the work of Horn, Jones, and Ringel, and that of Nicolet and Shinn, it appeared desirable to reinvestigate the course of the reaction of wool with alkalis.

## II. EXPERIMENTAL PROCEDURE

### 1. PREPARATION OF ALKALI-TREATED WOOLS

The reported yields of lanthionine from alkali-treated wools (about 5 percent [8]) are low. While it is recognized that the values obtained by isolation methods are always low, nevertheless it should be possible to form larger amounts of this amino acid in the alkali-treated material if the reaction proceeds as suggested. On the basis of earlier work [2], it appears that sodium sulfide, one of the products of the reaction of wool with alkali, could interfere with the formation of lanthionine in either of two ways. It might compete with cysteine in the reaction with dehydroalanine (eq 3), or it could split unreacted disulfide linkages and thus lower the amount of dehydroalanine that could be produced.

In order to overcome this difficulty, resort was had to a flow method, in which a fresh solution of alkali, either at 0° or 50° C was allowed to flow continuously over the wool. Thus the reaction products were constantly washed from the fibers. In addition, by working at these lower temperatures instead of at the boiling point, secondary reac-

tions would be kept at a minimum. At the lower temperature, 350 g of wool were placed in a 12-liter flask fitted with an inlet and an outlet tube. The flask was immersed in a water bath at 0° C, and 0.1 N NaOH, precooled to 0° C, was continuously flowed over the wool for 600 hours at a rate of about 2 liters per hr. In the experiment at 50° C, 133 g of wool was placed loosely in a cheesecloth bag, attached to a plunger, and agitated in a monel-metal cylinder that contained about 10 liters of 0.1 N NaOH solution at 50° C ± 2°. Fresh solution at the same temperature was continually siphoned into the bottom of the cylinder at a rate of about 12 liters per hour for 8 hr. The liquid in the cylinder was maintained at a constant level by removing the spent solution as rapidly as the fresh solution was introduced.

At the end of each treatment, the samples were removed from the alkali and washed with distilled water. A sodium nitroprusside test, used for the qualitative detection of sulfhydryl groups, was negative on both samples. Immediately after the washing treatment, small portions of the wools were treated with benzyl chloride [7] in order to combine any cysteine residues that might be present.

## 2. ANALYTICAL PROCEDURES

The cystine analyses were made by the Sullivan method [10], sulfur analyses by the oxygen-bomb method [11], alkali-solubilities by the method of Harris and Smith [12], and serine analyses by the method of Nicolet and Shinn [13].

## 3. ISOLATION OF LANTHIONINE

Lanthionine was isolated from the alkali-treated wool by a slight modification of the procedure described by Horn, Jones and Ringel [8]. The modification was to remove residual cystine from the residue containing the water-insoluble amino acids, and the method was carried out as follows. The residue was taken up in 150 ml of water and concentrated ammonium hydroxide was added dropwise until the residue was completely dissolved. One and one-half grams of sodium cyanide was then added to the solution, and the mixture was allowed to stand 1 hr, after which it was made acid to litmus by the addition of acetic acid. The solution was kept in a refrigerator for 48 hr, and the crystals which formed were filtered off. The filtrate was concentrated in a vacuum and allowed to stand in a refrigerator for 3 days and the crystals again collected. A third batch of crystals was obtained by again concentrating the filtrate. The crystalline precipitates were combined and further purified by suspension in 50 ml of water and by adding concentrated NH<sub>4</sub>OH dropwise until the solution was complete. A small amount of impurities was filtered off, and on acidifying the solution with acetic acid, a precipitate of wedge-shaped plates [8] was obtained. Analyses on the material were as follows:

		C	H	S
Calculated.....	%	34.59	5.81	15.41
Found.....	%	34.09	5.70	15.30

## III. RESULTS AND DISCUSSION

The effects of the treatments with alkali by the flow method are shown in table 1. Confirming earlier findings, it is seen that there is good agreement between the experimental values for the cystine contents of the treated wools and those calculated on the assumption that a molecule of cystine is destroyed for each atom of sulfur lost. That little, if any, free cysteine is in the alkali-treated wools is indicated by the values for the samples alkylated with benzyl chloride. This follows from the fact that in the Sullivan method both cystine and cysteine are determined, but not thioethers such as would be derived from the reaction of cysteine with benzyl chloride. Had any appreciable amounts of cysteine been present, the cystine values for those samples treated with benzyl chloride would have been much lower.

TABLE 1.—Effect on wool of treatment with alkali by the flow method

Sample	Cystine		Total sulfur	Alkali-solubility	Serine
	Found	Calculated <sup>1</sup>			
0.1 N NaOH flow for 600 hours at 0° C					
Untreated.....	11.3	%	3.50	10.5	9.5
Treated.....	4.4	3.1	2.40	5.0	9.4
Treated + C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl.....	4.2	-----	-----	-----	-----
0.1 N NaOH flow for 8 hours at 50° C					
Untreated.....	12.5	-----	3.50	-----	-----
Treated.....	2.7	1.1	1.98	-----	-----
Treated + C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl.....	2.5	-----	-----	-----	-----

<sup>1</sup> Calculated on the basis that each atom of sulfur lost by the wool represents the destruction of 1 molecule of cystine.

The question as to the fate of the residual noncystine sulfur in the treated wool arises. Earlier [2] it was suggested that a stable thio-compound was formed. Thus, for example, if the view of Schöberl were correct, a thioacetal could be formed. On the other hand, if lanthionine is the end product, the original mechanism postulated by Schöberl would not hold. As pointed out previously, however, the reported yields of lanthionine have been so low that there would be serious objections to the assumption that the alkali-degradation of the cystine in wool resulted principally in the formation of lanthionine as an end product.

In the present investigation, considerably higher yields of lanthionine were obtained. From a 94.7-g sample of wool that had been treated for 600 hr at 0° C, 1.47 g of lanthionine was obtained. Since, as seen from table 1, the cystine content of this sample had decreased 6.9 percent, a theoretical yield of 5.67 g of lanthionine might have been expected. The actual yield was thus 25.5 percent. Similarly, 2.2 g of a theoretical 7.7 g of lanthionine (28.8-percent yield) was obtained from the sample treated for 8 hr at 50° C. While these results ac-

count only for somewhat more than one-fourth of the sulfur which is no longer present as cystine, nevertheless, the amount is appreciable and, in view of the difficulties involved in the isolation technique, serious consideration must be given to the possibility that lanthionine may be a principal end product in the reaction of the cystine in wool with alkali.<sup>3</sup>

It should be pointed out that lanthionine might conceivably be formed by the reaction of cysteine with a dehydroalanine residue which might be formed from the reaction of the serine in wool with alkali [9]. That this was not the case is indicated by the data in the last column of table 1, which show that at 0° C the original content of serine has not been altered by the alkali treatment.

If, as the results of the present investigation indicate, an appreciable amount of sulfhydryl groups is not present in the alkali-treated wool, there still remains to be explained the apparent abnormal base-binding capacity of wool. It will be recalled that wool fails to reach equilibrium with alkaline solutions in titration experiments, a fact that was attributed [14] to the reaction of the base with the disulfide groups of the cystine. More specifically, on the assumption that the cleavage proceeds according to the Schöberl hypothesis, it was postulated that the "extra" base was bound by sulfhydryl groups (eq 1). Obviously, this postulate is no longer valid and a new explanation must be sought. The earlier titration experiments indicated that the apparent "extra" base bound was equivalent to the sulfur which split from the wool. In light of Nicolet's hypothesis (eq 4, 5, and 6), the phenomena could be accounted for if the sulfur which is split from the wool (eq 5) reacted with base in such a way as to consume 1 equivalent of base per sulfur atom. In order to test this possibility, 0.0109 g of sulfur was dissolved in 20 ml of ethyl alcohol<sup>4</sup> and the solution mixed with 20 ml of 0.1242 *N* sodium hydroxide. The solution was kept at 25° C and aliquots withdrawn at intervals, and titrated with standard hydrochloric acid. The results are given in table 2.

TABLE 2.—Reaction of sodium hydroxide with sulfur (0.0109 g) at 25° C

Time	Total NaOH present	NaOH consumed	NaOH consumed per <i>M-eq</i> of sulfur
<i>hr</i>	<i>M-eq</i>	<i>M-eq</i>	<i>M-eq</i>
0.....	2.48	.....	.....
1.....	2.32	0.16	0.50
20.....	2.19	.29	.87
48.....	2.18	.30	.89
115.....	2.18	.30	.89

It will be seen that nearly 1 equivalent of sodium hydroxide per atom of sulfur has been consumed. While the nature of reaction of sulfur with sodium hydroxide is not clear, nevertheless, the results are sufficiently close as to be able to account for the so-called "extra" base take-up in titration experiments. Finally, it may be pointed out that the results of these titration experiments, although constituting

<sup>3</sup> This suggests that the permanent set, of the type produced by treating wool fibers with alkali, may result in part from the formation of lanthionine. Such a possibility has already been pointed out by Speakman and Whewell [3], since they have suggested that —C—S—C— bonds might be formed under these conditions.

<sup>4</sup> Sulfur is not readily soluble in dilute NaOH. In the degradation of the cystine in wool, atomic sulfur is involved and would immediately go into solution.

only indirect evidence, may be considered as further support for the reaction proposed by Nicolet and Shinn.

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