The Reaction Between Wool and Phenyl *iso*Cyanate

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During some studies on the reactivity of the cystine linkages in wool, it was necessary to find methods for blocking the various polar side chains with minimum degradation of the fibre as a whole, and without introducing any coloration due to the reagent used. Various workers have found that the amino groups of proteins react readily with *isocyanates* in aqueous solution at pH 8 and 0° (Hopkins & Wormald, 1933, 1934*a, b*; Miller & Stanley, 1941). There have also been claims that *isocyanates* react with thiol and tyrosine hydroxyl groups (Fraenkel-Conrat, 1944; Miller & Stanley, 1941). This appeared to be an excellent method for the required purpose, although it was realized that reaction with wool would be more difficult than with the soluble proteins as penetration of the insoluble fibrous protein by the reagent would have to be accomplished.

Fraenkel-Conrat, Cooper & Olcott (1945) found that *isocyanates* reacted with proteins under anhydrous conditions at elevated temperatures. Besides reaction with the amino, thiol and phenolic groups, reaction with carboxylic, amide, guanidino and glyoxaline groups occurred.

The changes produced by reaction in aqueous media and under anhydrous conditions were of interest for the particular studies involved, so attempts were made to bring about reaction of wool with phenyl *isocyanate* under both sets of conditions. Very little success was achieved when using aqueous media, but by varying the conditions used by Fraenkel-Conrat *et al.* (1945), it has been found possible to obtain maximum reaction with wool, up to 44% of its weight of phenyl *isocyanate* being combined. The reaction has also been carried out on wools modified in various ways.

MATERIALS

The wool used was a 64's quality Merino wool from which the tip ends had been removed. The root ends were purified by extraction with ether followed by a large number of changes of distilled water. They were then allowed to condition at 65% relative humidity and 21°. For the experiments on load/extension characteristics the root ends of human hair fibres, purified as above, were used. Each fibre was examined microscopically before use to ascertain its freedom from medullation.

METHODS

Reaction in aqueous media. Treatment was carried out in 0.2M-Na₂HPO₄-KH₂PO₄ mixtures at pH 8. To reduce the rate of hydrolysis of the phenyl *isocyanate*, the reaction medium was cooled in ice to 0-5°. Agitation was effected by passing a stream of nitrogen through the reaction vessel. This was necessary because of the insolubility of the reagent in water. In some cases dioxan was added to the reaction medium and the *isocyanate* added in solution in dioxan. When using this technique, intermittent manual shaking was used. In general, the *isocyanate* was added over a period of some hours, 0.5-1.0 ml. being used for each g. of wool. When using single fibres, not more than 0.5 ml. reagent was used.

In all cases, aftertreatment consisted of rinsing the wool in ether, ethanol and water. The ether and ethanol served to free the wool from unchanged *isocyanate* and also *NN'*-diphenylurea which was invariably formed owing to hydrolysis of the *isocyanate* followed by reaction of the amine thus produced with another molecule of the *isocyanate*.

Reaction in anhydrous media. The procedure adopted was, in general, that used by Fraenkel-Conrat *et al.* (1945). The wool was dried for 4 hr. at 105° and then for at least 24 hr. over P₂O₅ *in vacuo*. A solution of the *isocyanate* in pyridine, which had been dried over BaO, was added and reaction allowed to proceed. Reaction was terminated by the addition of water, which caused the formation of a copious precipitate of diphenylurea. This was removed from the wool by rinsing in ethanol followed by extraction with ether in a Soxhlet apparatus. After thorough rinsing in water, the wool was allowed to condition at 65% relative humidity and 21°.

Methylation. Wool was subjected to nine successive treatments with dimethyl sulphate at room temp., each treatment being for 2 hr. 20 ml. of 0.2M phosphate (as above; initially at pH 6.5) and 0.2 ml. dimethyl sulphate were used for each g. of wool. After treatment, the wool was rinsed thoroughly in water and then allowed to condition.

Acetylation. Wool was boiled in acetic anhydride under reflux for 30 min. followed by prolonged rinsing with water to remove excess reagent. It was then allowed to condition.

Deamination. Two successive treatments with sodium nitrite and dilute acetic acid (Van Slyke's reagent) were given, using the conditions of Speakman & Stott (1934). The wool was then allowed to condition.

Reduction with mercaptoacetic acid. Wool was reduced by treatment at 35° for 18 hr. with 50 times its weight of 0.5M mercaptoacetic acid brought to pH 4.8 with NaOH (Patterson, Geiger, Mizell & Harris, 1941). The wool was then rinsed in water, ethanol and finally water, after which it was allowed to condition.

Acid-combining capacity. Wool was immersed in 0.001N-HCl for 24 hr. followed by successive rinsings with distilled water until the washings showed a pH of 5.2. It was then allowed to condition. Approx. 1 g. of wool so rinsed was immersed in 100 ml. of 0.1N-HCl and allowed to stand at 21° for 3 days, with periodical shaking. Portions of supernatant (25 ml.) were then withdrawn by pipette and titrated with 0.1N-NaOH. From the titres obtained the amount of acid combined was calculated and expressed as ml. of N-HCl combined/100 g. of dry wool.

Other methods. Load/extension curves and the work to stretch 30% were obtained according to the technique of Speakman (1947). Total nitrogen was determined by a micro-Kjeldahl procedure; thiol and disulphide S were estimated by the method of Shinohara (1935*a, b, c*) using a Spekker absorptiometer.

RESULTS

Treatment of wool with phenyl isocyanate in aqueous media

Various unsuccessful attempts were made to bring about reaction of wool with phenyl isocyanate in aqueous media at pH 8, using a wide variety of conditions. In order to minimize hydrolysis of the reagent, temperatures in the vicinity of 0° were necessary.

To detect small changes in the free amino-N content, much of the work was carried out on single fibres, using load/extension curves in distilled water and in 0.1N-HCl as a criterion of reaction, according to the technique of Speakman (1947). In the later work, dioxan was added to render the reagent soluble in the reaction medium (Creech & Jones, 1940, 1941*a, b*).

The results from this work were quite disappointing, as in only one instance was there any indication of reaction having occurred, and it was doubtful whether any real significance could be attached to the very small changes involved. This approach was therefore abandoned.

Treatment of wool with phenyl isocyanate in anhydrous media

Fraenkel-Conrat *et al.* (1945) successfully reacted dried cattle-hoof keratin with phenyl isocyanate dissolved in anhydrous pyridine, the treated protein containing only 15% of the number of acidic groups originally present, and only 11.5% of the basic groups. Treatment was for 2 days at 60°. As previous work suggested that pyridine under these conditions might cause some fibre degradation, initial experiments were carried out at 50° on dried wool, the reaction period being extended to 4 days. The extent to which reaction occurred was very slight, the acid-combining capacity being decreased from 79.9 to 76.5 ml. of N-HCl combined/100 g. dry wool. Speakman (1930) has shown that the interior of dry wool fibres is inaccessible to substances whose molecular size is greater than that of

n-propanol. In an attempt to increase the accessibility, wool was swollen in 98% formic acid and the acid then washed out with anhydrous pyridine, the latter replacing the former within the fibre, leaving it somewhat swollen. Experiments showed that, for a fibre with mean diam. 29.4 μ . in air, the mean diam. in formic acid was 41.4 μ . and after successive rinsings in pyridine it reached a steady value of 36.0 μ . 1 g. of wool was immersed, therefore, in 5 ml. formic acid, rinsed 5 times with separate portions of anhydrous pyridine and treated with 2 ml. phenyl isocyanate in 10 ml. anhydrous pyridine for 72 hr. at 70°. After treatment, reaction was terminated and the wool purified in the manner indicated under Methods. Table 1 shows the values for weight increase, nitrogen content and acid-combining capacity. Obviously reaction had occurred to a considerable extent. Subsequent work showed that the preliminary swelling of the fibre was unnecessary when treatment was carried out at 70°, reaction occurring equally well with unswollen anhydrous wool. Conditions were varied to find those which gave maximum reaction. Table 2 summarizes the values for weight increase, nitrogen content and acid-combining capacity for wools treated under various conditions.

These results show that dry wool is capable of reacting to a considerable extent with phenyl isocyanate dissolved in anhydrous pyridine, the number of acid-binding groups being decreased to as low as 6% of their original value (Table 1). The solution/wool ratio was increased to 20 in series 2 because 10 ml. of solution was only just sufficient to cover 1 g. wool and slight losses of pyridine by evaporation tended to leave the upper portion of the wool uncovered. Despite the fact that the concentration of reagent with respect to the solution is thus halved, 2 ml. reagent/g. of wool is still sufficient to give maximum reaction.

As seen in Table 2, series 3, the use of still higher temperatures leads to much more rapid reaction, the greatest increase in weight occurring in 2-3 hr. However, the maximum extent to which reaction occurs is somewhat less than that from prolonged treatment at a lower temperature. Furthermore, when the time of treatment at 97° is increased beyond 3 hr., the weight increase becomes progressively smaller.

Table 1. *Effect of treatment of pre-swollen wool with phenyl isocyanate under anhydrous conditions on the weight, acid-combining capacity and nitrogen content*

	Untreated	Treated
Weight increase (%)	—	39
Acid-combining capacity (ml. N-HCl/100 g. original dry wool)	84.3	5.1
Total N (% of original conditioned wt. of wool)	13.8	19.4

Table 2. *Effect of treatment of dry wool with phenyl isocyanate under anhydrous conditions on the weight, acid-combining capacity and total-N content*

Series	Reaction conditions				Weight increase (%)	Total N as % of conditioned wt. of original wool	Acid-combining capacity (ml. N-HCl/100 g. of original dry wool)
	Pyridine (ml./g. wool)	Phenyl isocyanate (ml./g. wool)	Time (hr.)	Temp. (°)			
Untreated	—	—	—	—	—	13.8	84.3
(1) Varying time of treatment.	8	2	24	70	18	15.9	47.5
Solution/wool ratio, 10 (v/w)	8	2	48	70	32	17.7	43.8
	8	2	73	70	42	19.9	23.8
	8	2	97	70	44	19.5	16.1
(2) Varying concentration of isocyanate. Solution/wool ratio, 20 (v/w)	19.5	0.5	72	70	9	15.5	66.0
	19.0	1.0	72	70	21	17.6	53.6
	18.0	2.0	72	70	39	19.7	27.4
	16.0	4.0	72	70	32	18.3	19.9
(3) Varying time at elevated temperature; treatment carried out in boiling-water bath. Solution/wool ratio, 20 (v/w)	18.0	2.0	0.5	97	8	14.9	74.9
	18.0	2.0	1	97	10	15.0	57.4
	18.0	2.0	2	97	30	17.8	43.3
	18.0	2.0	3	97	30	17.8	33.9
	18.0	2.0	4.5	97	26	—	21.5
	18.0	2.0	7	97	20	—	30.7
	18.0	2.0	24	97	15	—	23.8

When boiling solutions of isocyanate in pyridine are used, there is evidence of reaction occurring in 5–10 min., but after 30 min. marked degradation of the wool was obvious, its colour becoming brownish and its handle very harsh.

It was concluded that for the reaction between dry wool and phenyl isocyanate in anhydrous pyridine, maximum reaction with minimum degradation is obtained by using longer times of treatment at lower temperatures. In all subsequent work, therefore, treatment was carried out at 70° for 72 hr.

Treatment of modified wools

In an attempt to obtain an indication of the degree to which different groups participate in the reaction, wool was modified in various ways and then treated with phenyl isocyanate under conditions found to give maximum reaction.

Methylation of wool with dimethyl sulphate at pH 6–7 leads to esterification of the carboxyl groups, some *N*-methylation and also some *O*-methylation of enolized peptide groups (Blackburn, Carter & Phillips, 1941).

Acetylation of proteins has been performed with ketene and with acetic anhydride, although use of the latter has been restricted largely to insulin. In the case of wool, acetylation will decrease the combining capacity for acid dyes. For this purpose the reaction is carried out by heating wool for 4 hr. at 60° in a mixture of acetic anhydride, acetic acid, sulphuric acid and pyridine (Munz & Haynn, 1922). Acetylation has been found to decrease in number the free amino groups and also phenolic groups of proteins. Lindley & Phillips (1947) acetylated wool in boiling acetic anhydride for

30 min. and suggested that this treatment modified groups other than the free amino groups. It was expected, therefore, that acetylation under these conditions would modify the reactivity of wool towards phenyl isocyanate.

Deamination of wool with nitrous acid has been investigated by Speakman & Stott (1934). Reaction occurs mainly with free amino groups, although there is some evidence for oxidation of cystine (Schmidt, 1929; Lough & Lewis, 1934; Cockburn, Drucker & Lindley, 1948). Cockburn *et al.* (1948) found that the amino nitrogen was substantially diminished in the first deamination; subsequent deaminations produced very little additional change.

Reduction of wool with mercaptoacetic acid can be carried out under a variety of conditions (Patterson *et al.* 1941). Those chosen for the present work converted some 65% of the disulphide-sulphur into thiol-sulphur. It was hoped that treatment of this wool with phenyl isocyanate would indicate whether reaction with thiol groups would occur under these conditions.

After wools had been modified in these various ways, they were treated with phenyl isocyanate (2 ml./g. wool) in anhydrous pyridine (18 ml./g. wool) at 70° for 72 hr. For purposes of comparison, parallel experiments were run with pyridine only, omitting the reagent. Table 3 shows the results obtained.

Properties of wool treated with phenyl isocyanate under anhydrous conditions

Whilst the properties of phenyl isocyanate-treated wool have not been investigated to any great extent, it is remarkable for its lack of chemical

Table 3. Reactivity of modified wools towards phenyl isocyanate under anhydrous conditions

Treatments	Weight increase (%)	Total N (% of conditioned wt. of original wool)	Acid-combining capacity (ml. N-HCl/100 g. original dry wool)	Thiol S (% of dry wt. of original wool)	Disulphide S (% of dry wt. of original wool)
1. Untreated	—	13.8	84.3	0.06	3.10
Untreated, then pyridine	-1	14.2	—	—	—
Untreated, then pyridine-phenyl isocyanate	39	19.7	27.4	—	—
2. Methylated	—	14.2	53.1	—	—
Methylated, then pyridine	-1	14.4	60.6	—	—
Methylated, then pyridine-phenyl isocyanate	35	18.5	13.3	—	—
3. Acetylated	—	13.4	21.8	—	—
Acetylated, then pyridine	-1	13.6	25.6	—	—
Acetylated, then pyridine-phenyl isocyanate	2	13.7	27.7	—	—
4. Deaminated	—	13.9	35.2	—	—
Deaminated, then pyridine	-2	14.1	36.7	—	—
Deaminated, then pyridine-phenyl isocyanate	30	17.8	16.4	—	—
5. Reduced	—	14.5	80.5	2.11	1.10
Reduced, then pyridine	-1	14.7	72.2	0.32	2.86
Reduced, then pyridine-phenyl isocyanate	32	18.4	24.5	0.50	1.33

reactivity, being resistant to attack by acids, alkalis and reducing agents. The following examples serve to illustrate the behaviour of treated wool:

(i) Untreated wool, when boiled in N hydrochloric acid (50 ml./g.), rinsed and dried, showed a loss in weight of 3 % after 15 min. and 28 % after 60 min. Phenyl isocyanate-treated wool under the same conditions showed a loss in weight of only 2 % after 60 min.

(ii) Untreated wool, when digested with 0.1N sodium hydroxide (50 ml./g.) at 50°, rinsed and dried, showed a loss in weight of 5 % after 30 min., 7 % after 60 min. and 11 % after 180 min. Phenyl isocyanate-treated wool under the same conditions showed no loss in weight after 180 min.

(iii) When untreated wool was boiled in a 30 % (w/v) solution of sodium bisulphite (100 ml./g.) for 30 min., the disulphide-sulphur content was decreased from 2.74 to 0.23 %. Phenyl isocyanate-treated wool under similar conditions showed a decrease in disulphide-sulphur from 2.69 to 2.22 %.

DISCUSSION

The preceding results show that, under anhydrous conditions, reaction between wool and phenyl isocyanate occurs to a considerable extent.

It can be calculated that, if all the free amino groups in wool react with phenyl isocyanate, the increase in weight would be of the order of 10 %. The fact that weight increases considerably in excess of this value are obtained is in agreement with the view of Fraenkel-Conrat *et al.* (1945) that, under these anhydrous conditions, a wide variety of the side-chain groups of proteins undergo reaction. Using the figures of Simmonds (1954) for the composition of 64's Merino wool, it is found that, if every side chain with a possibility of reacting with

isocyanate bound one molecule of the reagent, the wool would show a resultant weight increase of the order of 50 %. Since weight increases of 40–45 % are actually obtained, it appears that practically all such side chains do, in fact, react with phenyl isocyanate in anhydrous pyridine.

Under such anhydrous conditions the accessibility of the fibre to the reagent was not expected to be very great, and it was rather surprising, therefore, that reaction occurred so readily. Apparently, however, under these conditions, pyridine is capable of producing some swelling of the fibre. Measurements of fibre diameter showed that, for a fibre whose diameter in air was 29.4 μ , drying reduced this to 27.5 μ . On transferring the dry fibre to hot anhydrous pyridine, the diameter increased to 30.1 μ . This degree of swelling would probably be sufficient to allow access of the reagent. Furthermore, when wool was treated with phenyl isocyanate in anhydrous xylene (which would not have any affinity for the fibre) under conditions which gave maximum reaction when using pyridine as the solvent, there was no indication of reaction having occurred.

Confirmation of the participation of a wide variety of side chains in the reaction is given by the treatment of modified wools with phenyl isocyanate. The extent to which wool reacts is decreased slightly by methylation or deamination before treatment with isocyanate, indicating that carboxylic and amino groups take part in the reaction. The fact that the acid-combining capacity of methylated wool subsequently treated with isocyanate is much lower than that of isocyanate-treated wool for a slightly lower uptake of isocyanate suggests that the carboxyl groups do not react completely.

In the case of reduced wool, it is seen that, with pyridine alone, approximately 1.8% of thiol-sulphur is lost and the disulphide-sulphur increases by the same amount. Hence, under these conditions, most of the thiol groups are reoxidized to disulphide groups. When isocyanate is present, however, reaction of the thiol groups with the reagent occurs preferentially, as only 0.23% thiol-sulphur is reoxidized, the remainder reacting with the isocyanate. Hence, thiol groups in wool react readily with phenyl isocyanate under anhydrous conditions.

Acetylated wool is incapable of reacting with phenyl isocyanate under these conditions. From the results of the present work and that of Fraenkel-Conrat *et al.* (1945), a wide variety of groups in wool are capable of reacting with phenyl isocyanate and it is concluded, therefore, that acetylation with boiling acetic anhydride for 30 min. leads to extensive reaction with the same side chains—amino, phenolic, thiol, amide, guanidino, glyoxaline and carboxylic. It appears rather improbable that acetic anhydride would react with carboxyl groups. However, it has already been pointed out that reaction of these groups with phenyl isocyanate apparently does not proceed to completion and the method of experiment is not sufficiently precise for it to be inferred that carboxyl groups do, in fact, react during treatment with acetic anhydride.

Whilst temperatures greater than 70° lead to more rapid reaction, the amount of isocyanate bound to the wool is somewhat less (Table 2). Two possibilities suggest themselves. First, these more drastic conditions may lead to a general attack on the wool with consequent loss of protein. That such attack is not due to the pyridine alone is shown by the fact that dry wool, on treatment with anhydrous pyridine at 97° for 8 hr., did not show any loss in weight. Secondly, prolonged treatment at 97° may lead to decomposition of the substituent groups introduced initially. Support for the former suggestion is given by the experiments carried out using boiling solutions of isocyanate in pyridine, where there was evidence of reaction occurring in 5–10 min., but after 30 min. marked degradation of the wool was obvious.

SUMMARY

1. Difficulty was experienced in obtaining any reaction between wool and phenyl isocyanate in aqueous media.

2. Under anhydrous conditions considerable reaction occurs. Amino, carboxyl and thiol groups all react and probably phenolic, amide, guanidino and glyoxaline groups also undergo reaction.

3. The rate of reaction increases greatly with increasing temperature, but at higher temperatures, protein degradation occurs simultaneously.

4. Acetylation of wool with boiling acetic anhydride leads to reaction with a wide variety of side chains. All groups capable of reacting with phenyl isocyanate under anhydrous conditions probably react also with boiling acetic anhydride.

5. Wool treated with phenyl isocyanate exhibits a greatly reduced chemical reactivity, becoming more resistant to attack by acids, alkalis and reducing agents.

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