STUDIES ON OXIDIZED WOOL

I. A COMPARISON OF THE COMPLETENESS OF OXIDATION WITH PERACETIC AND PERFORMIC ACIDS

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Summary

The oxidation of wool with performic and peracetic acids has been compared by measuring the cysteic acid contents of hydrolysates of the oxidized wool. Whereas virtually complete oxidation takes place with performic acid, the oxidation with peracetic acid is incomplete. Performic acid can also further oxidize the partial oxidation products in wool treated with peracetic acid and it is concluded that performic acid is the more powerful oxidizing agent for wool. For the oxidation of the disulphide bonds prior to the extraction of proteins from wool, performic acid is therefore the better reagent.

I. INTRODUCTION

Performic acid has been extensively used for the oxidation of disulphide linkages in proteins following its successful application by Sanger (1949*a*) to the separation of the two peptide chains of insulin. From the results obtained with insulin (Sanger 1949*b*), lysozyme (Jollès-Thaureaux, Jollès, and Fromageot 1958; Thompson 1958), ribonuclease (Hirs 1956), papain (Kimmel, Thompson, and Smith 1955), chymotrypsin (Meedom 1956), and other proteins it is known that no splitting of peptide bonds takes place. Apart from the oxidation of cysteine and cystine residues to β-sulphoalanyl (cysteic acid) residues, the only other amino acids which are modified by the reagent are methionine and tryptophan, provided precautions are taken to avoid chlorination of tyrosine residues (Thompson 1954, Hirs 1956). Methionine is converted to the sulphone (Hirs 1956) and tryptophan takes up three atoms of oxygen (Toennies and Homiller 1942) and is converted to a product of unknown structure.

Aqueous peracetic acid was used for the oxidation of wool by Alexander, Hudson, and Fox (1950) because it is stable in water, has a specificity similar to performic acid, and dilute solutions dissolve very little wool. Performic acid is unstable; it is decomposed by water and in the presence of the large amount of formic acid necessary for its preparation considerable amounts of oxidized wool dissolve. For commercial treatment of wool fibres peracetic acid is therefore preferable. As a preliminary step in the isolation of wool proteins, however, this is not the case, since the results presented in this paper show that oxidation with peracetic acid is incomplete, whereas after performic acid oxidation virtually all the cysteine and cystine of wool may be detected as cysteic acid in hydrolysates of the oxidized wool.

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Alexander, Hudson, and Fox (1950) and Corfield, Robson, and Skinner (1958) studied the completeness of oxidation with peracetic acid by measuring the cystine content of hydrolysates after various periods of oxidation. This is an indirect method which can give erroneous results in that cystine may be oxidized to partial oxidation products which no longer analyse as cystine but which may be capable of further oxidation to β-sulphoalanyl residues.

In our experiments we have preferred analyses for cysteic acid in hydrolysates of oxidized wool for a true assessment of the completeness of oxidation.

As a preliminary step in the isolation of proteins from oxidized wool (O'Donnell and Thompson 1959) complete oxidation of the modified amino acid residues is desirable for complete splitting of disulphide cross-linkages and the formation of a stable derivative from cysteine and cystine residues.

II. Materials and Methods

Merino 64's wool in the form of solvent-degreased top was used. To remove residual oil the top was extracted four times with light petroleum, then with ethanol, and repeatedly with distilled water before being dried and conditioned at 63 per cent. R.H. and 20°C. The nitrogen (Kjeldahl) and sulphur (Zimmerman) contents of the dry wool were 16·9 and 3·26 per cent. respectively. Moisture contents were determined by overnight drying in an oven at 105°C. The ash content (800°C) was 0·16 per cent.

Performic acid reagent was prepared by mixing 1 vol. 30 per cent. hydrogen peroxide and 9 vol. 98–100 per cent. formic acid and allowing to stand 1–2 hr at room temperature before use (Toennies and Homiller 1942).

Peracetic acid (Laporte Chemicals) contained sulphuric acid catalyst. Titration by the methods of Swern (1953) showed the reagent to be 40 per cent. (w/v) peracetic acid with 6 per cent. hydrogen peroxide but no diacetyl peroxide was present.

III. Experimental

(*a*) *Oxidation with Performic Acid*

Samples of wool (approximately 30 mg), equilibrated at 63 per cent. R.H. and 20°C, were cooled to 0°C in "Quickfit" stoppered test tubes. Performic acid reagent (1 ml) cooled to 0°C was added (i.e. at least 10 times the theoretical amount required to oxidize the known cysteine, cystine, methionine, and tryptophan content) and reaction continued at 0°C for 1–48 hr. Ice-cold water (10 ml) was then added and the mixture freeze-dried. The residue was hydrolysed under reflux with 2 ml 6N HCl (which had been twice distilled in glass) in an oil-bath at 138–140°C for 24 hr. The hydrolysate was diluted to 25 ml and 1-ml samples freeze-dried before analysis.

(*b*) *Oxidation with Peracetic Acid*

Peracetic acid (40 per cent. w/v) was diluted with the required solvent to give 1·6 per cent. or 5 per cent. peracetic acid (Alexander, Hudson, and Fox 1950). These authors studied only aqueous solutions of peracetic acid in which more hydrogen peroxide is formed on dilution due to hydrolysis of peracetic acid until equilibrium

is attained. We have also studied dilute solutions of peracetic acid in acetic acid and formic acid where equilibrium lies in the direction of less hydrogen peroxide.

In all experiments the liquor : wool ratio was 50 : 1.

Analytical small-scale experiments were carried out as described for performic acid. The residual fibres from larger-scale oxidations were analysed after washing the oxidized fibre free from reagent, drying, and conditioning. The oxidized fibres (approximately 40 mg) were hydrolysed under reflux as described above.

(c) *Analyses for Cysteic Acid*

Cysteic acid was separated from the other amino acids on 20-cm columns of sulphonated polystyrene resin ("Dowex-50 X8") equilibrated with 0·2N sodium citrate buffer at pH 3·1 (Moore and Stein 1954). The pH 3·1 buffer is preferable to the pH 3·42 buffer previously used (Kimmel, Thompson, and Smith 1955; Thompson 1956) since it gives a wider separation between cysteic acid and aspartic acid.

The accuracy of the analyses was improved by using an internal standard. The standard cysteic acid was passed through the column (3 ml of a 0·3 mM solution followed by 2 × 0·5 ml) and then 8 ml buffer. The freeze-dried sample was dissolved in buffer (usually 4 ml) and then 3 ml was added to the column, washed in with buffer (2 × 0·5 ml) and eluted with more buffer. A total of 35 tubes, containing equal-sized fractions of approximately 1 ml, were collected. To each tube was added one drop of 3·5N NaOH to bring the pH to 5, the tubes shaken, and analysed with 2 ml ninhydrin reagent according to the method of Moore and Stein (1948). By adding the cysteic acid sample in 3 ml buffer, the peak was spread over five tubes and enabled more accurate measurement and integration of the optical densities. Optical densities were measured in a Coleman Junior spectrophotometer at 570 mμ.

IV. Results

(a) *Sulphur Balance on Wool Samples*

The total sulphur of the wool top was 3·26 per cent. This is equivalent to 1019 μg-atoms S/g. Allowing 40 μg-atoms S/g for the methionine content of wool (Simmonds 1956), the total –S–S– and –SH groups of wool should contribute 979 μg-atoms S/g, assuming there are no unknown sulphur-containing compounds in wool. On analysis of a sample of wool top by polarographic methods on the intact fibre (Leach 1959), 465 ± 15 (95 per cent. confidence limits) μmoles cystine/g and 39 ± 3·3 (95 per cent. confidence limits) μmoles cysteine/g were found. This is equivalent to 969 ± 30 (95 per cent. confidence limits) μg-atoms S/g, in excellent agreement with the cysteine plus cystine content calculated from the total sulphur analysis.

Complete oxidation of the cysteine plus cystine of this wool should therefore give 969–979 μmoles cysteic acid/g.

(b) *Oxidation with Performic Acid*

Analyses at different times of oxidation showed that in 1 hr at 0°C 90 per cent. of the maximum yield of cysteic acid was obtained and that, after 4 hr, oxidation

was virtually complete. The precision of the analyses was not sufficient to detect any significant difference between the yields of cysteic acid after oxidation at 0°C for 6 hr, 16 hr, or longer. The shorter time of oxidation would reduce the possibility of secondary changes in other amino acid residues or peptide bonds. However, polarographic analyses (Leach 1959) have shown that although no cystine is detectable in the intact fibres in either case, there was 16–20 μmoles/g cystine in hydrolysates of wool oxidized for 5–6 hr but none in the hydrolysates of samples oxidized for 21 hr (see Table 6). For this reason we have used overnight periods (i.e. at least 16 hr) of oxidation before fractionation of the oxidized wool as described in Part II of the series (O'Donnell and Thompson 1959).

TABLE 1

CYSTEIC ACID IN HYDROLYSATES OF WOOL OXIDIZED WITH PERFORMIC ACID AT 0°C

Time of Oxidation (hr)	Cysteic Acid Content* (μmoles/g wool)
7	971
24	938, 953
41	958, 989

* Each value is the result of a single analysis for a separate oxidation.

In Table 1 the results of cysteic acid determinations on samples of wool oxidized with performic acid are shown. The mean value of these analyses, and those for 24-hr periods of oxidation or longer with peracetic acid in formic acid (see Table 4) where oxidation is complete, is 965 ± 18 (95 per cent. confidence limits). This value, which has not been corrected for any losses during the oxidation, is in excellent agreement with the values calculated above from the sulphur content and the polarographic analyses for cysteine plus cystine. It is of interest that performic acid oxidation of cysteine, cystine, and other proteins (Schram, Moore, and Bigwood 1954; Schroeder *et al.* 1955; Hirs 1956; Hommes, Santema-Drinkwaard, and Huisman 1956; Thompson 1956; Wilcox, Cohen, and Tan 1957) has not always given quantitative conversion of cysteine plus cystine to cysteic acid and most authors have applied a correction factor to allow for this. However, it appears likely that recoveries of cysteic acid will vary with the technique of oxidation and the protein studied. For example, Bidmead and Ley (1958) have obtained yields of 96–103 per cent. of cysteic acid in the oxidation of cystine, and Li (1957) and Jirgensons and Ikenaka (1958) found all the –S–S– bonds in prolactin and human plasma albumin respectively were converted to cysteic acid residues.

With performic acid oxidation it is difficult to measure the weight of the freeze-dried product accurately. Freeze-drying is necessary because of the large

amounts of protein going into solution. Following a suggestion by Dr. J. M. Swan, it was found that if the performic acid reagent was saturated with sodium formate the solubility of the oxidized wool was repressed. Whereas 40 per cent. of the wool dissolves during oxidation for 24 hr at 0°C it was found that in the presence of sodium formate, less wool dissolved (weight increase 18 per cent. of the theoretical increase for full oxidation). The cysteic acid content of this residual oxidized wool, after washing with water, was 760 μmoles/g, considerably less than the values obtained in the absence of sodium formate (see Table 1). Hence oxidation has either been incomplete in the presence of sodium formate, or material rich in cysteic acid has dissolved during the isolation of the oxidized fibre.

TABLE 2

CHANGES IN WEIGHT FOR WOOL OXIDIZED WITH PERACETIC ACID

All oxidations carried out at 28°C except where indicated

Strength of Peracetic Acid and Solvent	Time of Oxidation (hr)	(A) Change in Dry Weight after Oxidation (%)	(B) Material* Dissolved during Isolation (%)	(A + B) Percentage Increase in Weight
1·6 per cent. in water	24 (room temp.)	+3·5	1·1	4·6
1·6 per cent. in acetic acid	24 (room temp.)	+4·2	—	—
5 per cent. in water	8	+2·8	1·5†	4·3
	24	−4·3	7·5†	3·2
	48	−10·0	13·0†	3·0
5 per cent. in acetic acid	8	+3·3	0·5†	3·8
	24	+2·4	1·4†	3·8
	48	+0·7	3·2†	3·9

* Estimated by Kjeldahl nitrogen determination assuming total nitrogen of dissolved material was the same as that of wool.

† It was not appreciated that loss of material may occur during washing with water and this was not measured. For 1·6 per cent. aqueous peracetic acid the losses were 0·8 per cent. in oxidation medium and 0·3 per cent. in washings.

(c) *Oxidation with Peracetic Acid*

The changes in weight of samples of wool oxidized in peracetic acid solutions are shown in Table 2. The theoretical increase in weight, assuming a weight increase of 48 (3 atoms oxygen) for each tryptophan and cysteine residue, 98 (2 × O_3H) for each cystine residue, and 32 (2 atoms oxygen) for each methionine residue, is 5·1 per cent. as shown in Table 3. The tryptophan content of wool is uncertain (Corfield and Robson 1955; Simmonds 1956) but its contribution to the weight increase is small compared to the total of the other amino acids oxidized.

In no case was the theoretical weight increase observed, even after allowing for the material dissolved during isolation of the oxidized fibre. The increase in weight (Table 2) ranged from 60 to 91 per cent. of the theoretical value and was generally less than that expected from the cysteic acid contents given in Table 4. However, the assumption that the nitrogen content of the dissolved material was the same as that of wool may not be justified. Moreover, it was not appreciated that loss of material may occur during washing with water and this was not measured in most cases.

TABLE 3

CALCULATED INCREASE IN WEIGHT OF WOOL ON OXIDATION

Amino Acid	(A) Amount Oxidized (μmoles/g wool)	(B) Increase in Residue Weight on Oxidation (g)	Increase in Weight (g) for 100 g Wool ($A \times B \times 10^{-4}$)
Methionine	40*	32	0·13
Tryptophan	48†	48	0·23
Cysteine	39	48	0·19
Cystine	465	98	4·56
			Total 5·11

* Simmonds (1956). † Corfield and Robson (1955).

It is of interest to note that the moisture content of the oxidized wools in Table 4 was about 10·6 per cent. which is considerably less than that of the original wool, 12·4 per cent. after equilibration at 63 per cent. R. H. The increase in the number of charged groups ($-SO_3^-$) has not given an expected increase in water uptake.

Table 4 shows the cysteic acid contents of hydrolysates of the freeze-dried reaction mixtures from peracetic acid oxidations of wool, i.e. the whole of the oxidized wool was analysed. The residual oxidized wools, i.e. the washed fibres, have usually increased in weight during oxidation (Table 2) due to uptake of oxygen, but some material has been lost by solution in the oxidizing solution or wash water. The cysteic acid contents of these residual oxidized wools are given in Table 5.

With 1·6 per cent. aqueous peracetic acid, 0·8 per cent. of the total nitrogen of a wool sample was lost in the oxidizing solution and 0·3 per cent. was subsequently washed out by water. The yield of cysteic acid in the freeze-dried reaction mixture (Table 4) averages 88 per cent. of that obtainable with performic acid, and the residual fibre contains 75 per cent. (Table 5).

Our average value of 732 μmoles/g for the cysteic acid content of the residual oxidized fibre obtained with 1·6 per cent. aqueous peracetic acid is in excellent agreement with that of 729 μmoles/g calculated from the data of Corfield, Robson,

and Skinner (1958) who also used aqueous peracetic acid. These workers did not consider their value low since it corresponded well with the chromatographic estimates of cystine in their sample of wool. However, their chromatographic cystine value was low compared with that which they obtained by the Shinohara (1935) method. Using their Shinohara value their cystine value is equivalent to 1002 μmoles cysteic acid/g wool. This value would have given sufficient sulphur to account for all the sulphur in their sample apart from the methionine sulphur.

TABLE 4

CYSTEIC ACID IN HYDROLYSATES OF WOOL OXIDIZED WITH PERACETIC ACID

All oxidations carried out at room temperature except where indicated

Strength of Peracetic Acid and Solvent	Time of Oxidation (hr)	Cysteic Acid Content* (μmoles/g wool)
1·6 per cent. in water	24	819, 879 } 849
1·6 per cent. in acetic acid	24	672
	24	733
1·6 per cent. in formic acid	16	939
	24	981
5 per cent. in water	41	899
5 per cent. in acetic acid	24 (0°C)	707
	7	795
	24	894
	41	902
5 per cent. in formic acid	7 (0°C)	885
	24 (0°C)	947
	41	1007

* Each value is the result of a single analysis for a separate oxidation. Excess oxidizing reagent was removed by freeze-drying.

For wools analysed by polarographic methods on the intact fibre, Leach (1959) has found that the total sulphur is accounted for by the cysteine, cystine, and methionine (cf. Cuthbertson and Phillips 1945) and therefore no unknown sulphur compound is present. During hydrolysis some cystine and cysteine are destroyed, but, provided the time and temperature of colour development is carefully controlled, the experience in this Laboratory is that the estimates by the Shinohara (1935) and polarographic methods (Stricks, Kolthoff, and Tanaka 1954) on hydrolysates are in good agreement (Maclaren, Leach, and O'Donnell, unpublished data).

Although our results with solutions of peracetic acid in water or acetic acid did not indicate quantitative conversion of cysteine plus cystine residues to cysteic

acid residues, quantitative oxidation was observed with formic acid as solvent. A solution of peracetic acid in formic acid will be converted to performic acid when equilibrium is reached, and a considerable amount of the oxidized wool dissolves in the excess formic acid. With 5 per cent. peracetic acid in formic acid at 0°C the rate of formation of performic acid is slower than at room temperature so that complete oxidation of wool has occurred in 24 hr but not in 7 hr (Table 4). This suggests that peracetic acid is a less powerful oxidizing agent than performic acid.

TABLE 5

CYSTEIC ACID CONTENTS OF HYDROLYSATES OF RESIDUAL OXIDIZED WOOL AFTER PERACETIC ACID OXIDATION AND WASHING WITH WATER

All oxidations carried out at 28°C except where indicated

Strength of Peracetic Acid and Solvent	Time of Oxidation (hr)	Cysteic Acid Content* (μmoles/g oxidized wool)
1·6 per cent. in water	24 (room temp.)	722, 742†
1·6 per cent. in acetic acid	24 (room temp.)	725
5 per cent. in water	8	791
	24	795, 772
	48	762
5 per cent. in acetic acid	8	812
	24	873
	48	828

* Each value is the result of a single analysis for a separate oxidation.

† Further oxidation of this preparation (duplicates 739, 745) with performic acid, at 0°C for 17 hr, increased the cysteic acid content to 838 μmoles/g of peracetic acid-oxidized wool (replicates 860, 820, 843, 827).

If the residual oxidized fibre obtained with 1·6 per cent. aqueous peracetic acid contains partial oxidation products of cystine, they should be converted by performic acid to cysteic acid. This was found to be the case, the yield of cysteic acid being increased from 742 to 838 μmoles/g. It is clear from these results that we cannot substantiate the claim of Alexander, Fox, and Hudson (1951) that the cystine that disappears on oxidation with 1·6 per cent. aqueous peracetic acid can be quantitatively accounted for as cysteic acid. The sulphur distribution in the present experiments can be satisfactorily accounted for as follows: the cysteic acid content for the freeze-dried reaction mixture (Table 4) minus the cysteic acid content of the residual peracetic acid-oxidized fibre (allowing for the increase in weight of 1 g wool to 1·035 g oxidized fibre) is $(849 - 742 \times 1{\cdot}035) = 81$ μmoles and represents the cysteic acid lost in the oxidizing solution. This value for the dissolved cysteic acid plus the cysteic acid content on the same residual fibre after further oxidation with performic acid ($838 \times 1{\cdot}035 = 867$) gives a total of 948

μmoles/g wool. This is in good agreement with the value obtained by direct performic acid oxidation of wool (965 μmoles/g) taking into account the fact that the 81 μmoles lost during isolation of the peracetic acid-oxidized fibre might also be increased by further oxidation with performic acid.

It should be emphasized that our results are based on hydrolysates of oxidized wool. Partial oxidation products of disulphides can undergo disproportionation reactions on hydrolysis (Lavine 1936) and cysteic acid may well be an end-product

TABLE 6

CYSTINE CONTENT OF HYDROLYSATES OF WOOLS OXIDIZED WITH PERACETIC AND PERFORMIC ACIDS

All oxidations carried out at 0°C except where indicated

Reagent	Time of Oxidation (hr)	Cystine Content of Hydrolysate* (μmoles/g oxidized wool)
1·6 per cent. aqueous peracetic acid	24 (room temp.)	27†
		34†
		32†
Performic acid	5	15
	5	15
	6	20†
	18	4†
	21	0

* Each value is the result for a separate oxidation and hydrolysis. In all analyses at least 250-mg samples of oxidized wool were hydrolysed and analysed by an adaptation of the method of Stricks, Kolthoff, and Tanaka (1954) by Dr. S. J. Leach as described by Human (1958).

† These values were for residual fibre samples from large-scale oxidations.

of some of these reactions. The cysteic acid content of hydrolysates may be higher than that of the intact fibre for partially oxidized wools.

To account for the analytical values it is obvious that the 1 per cent. of nitrogen lost during isolation of the oxidized fibre (and containing 81 μmoles cysteic acid) using aqueous 1·6 per cent. peracetic acid constitutes a sulphur-rich fraction. This sulphur-rich fraction was not obtained when 1·6 per cent. solutions of peracetic acid in acetic acid were used. For higher concentrations of peracetic acid in acetic acid there was always considerably less material dissolved during isolation of the oxidized fibre (Table 2) than was the case with the corresponding aqueous solutions. These results suggest that with the aqueous solutions containing hydrogen peroxide some random splitting of peptide bonds has occurred to give more low molecular weight material. The increased proportion of dialysable material in γ-keratose

prepared using 1·6 per cent. aqueous peracetic acid compared with γ-keratose obtained from performic acid-oxidized wool (O'Donnell and Thompson 1959) supports this conclusion.

(*d*) *Cystine Contents of Wool Oxidized with Performic and Peracetic Acids*

The oxidized wools studied here contained no cystine in the intact fibre as measured by the method of Leach (1959). However, the presence of partial oxidation products leads to the formation of cystine on hydrolysis (Lavine 1936). Polarographic analyses (Stricks, Kolthoff, and Tanaka 1954) for cystine in hydrolysates of oxidized wools gave the values shown in Table 6. Oxidation is only complete after 21 hr of reaction with performic acid when no cystine could be detected.

V. Discussion

From the data presented in this paper it is apparent that performic acid is a more powerful oxidizing agent than peracetic acid. Oxidized wool was prepared with 1·6 per cent. aqueous peracetic acid under conditions reported by Alexander, Hudson, and Fox (1950) and Corfield, Robson, and Skinner (1958) to lead to complete loss of cystine. We found that this material had a cysteic acid content which was increased by 13 per cent. on further oxidation with performic acid. This difference cannot be attributed to different rates of diffusion of the reagents into the interior of the fibre since polarographic experiments, according to the method of Leach (1959), on the intact fibre have shown that within 1 hr of oxidation with aqueous peracetic acid very little cystine is detectable. In 98–100 per cent. formic acid, which swells wool fibres 50 per cent. more than in aqueous solutions (Speakman 1933), peracetic acid will not completely oxidize the cystine in 7 hr at 0°C, although reaction is complete in 24 hr. With performic acid in formic acid the reaction is virtually complete in 4 hr at 0°C.

Supporting evidence for the presence of partial oxidation products of cystine in peracetic acid-oxidized wool has been presented by Maclaren, Leach, and O'Donnell (unpublished data). They found that, even after short treatments with peracetic acid, almost complete loss of cystine was evident on polarographic examination of the intact fibre. Treatment of the oxidized wool with alkali gave a 200–300 per cent. increase (compared with that of the intact oxidized fibre) in the cystine content, due to hydrolytic oxidation and reduction of the partial oxidation products (Lavine 1936), while hydrolysis with acid gave a 300–400 per cent. increase in apparent cystine content.

The structure of the partial oxidation products is not known but could include some of the following modifications of the –S–S– bond (Lavine, Toennies, and Wagner 1934; Harris and Smith 1937): –SO–S–, $-SO_2-S-$, $-SO_2-SO-$, $-SO_2-SO_2-$, –SOH, $-SO_2H$. The increase in weight on oxidation suggests that the more highly oxidized modifications, e.g. $-SO_2H$, are present in the oxidized wools studied here. Moreover, the proportions of α-, β-, and γ-keratoses isolated from wool oxidized either with performic acid or with 1·6 per cent. aqueous peracetic acid are almost identical (O'Donnell and Thompson 1959) suggesting the cross-linking sulphur–sulphur bonds are broken in both cases.

Hydrolysis of most oxidized wools reformed some cystine (Table 6) as measured by the polarographic method. This does not agree with the results of Alexander, Hudson, and Fox (1950) and Corfield, Robson, and Skinner (1958) who used the Shinohara (1935) method and found virtually no cystine in the hydrolysates.

For the isolation of proteins from oxidized wool performic acid is a better oxidizing agent than peracetic acid since oxidation of the modified amino acid residues is complete. Our results suggest that there is also less random hydrolysis of peptide bonds. Moreover, it is known from studies of the action of the enzyme leucine aminopeptidase, which is specific for L-amino acid residues, on the separate chains of oxidized insulin and other oxidized proteins (Hill and Smith 1957), that performic acid oxidation does not cause racemization.

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