- Arnstein, H. R. V. & Crawhall, J. C. (1957). *Biochem. J.* **67**, 180.
- Arnstein, H. R. V. & Grant, P. T. (1954*a*). *Biochem. J.* **57**, 353.
- Arnstein, H. R. V. & Grant, P. T. (1954*b*). *Biochem. J.* **57**, 360.
- Bloch, K. & Clarke, H. T. (1938). *J. biol. Chem.* **125**, 275.
- Bovarnick, M. (1942). *J. biol. Chem.* **145**, 415.
- Cook, A. H. & Heilbron, I. M. (1949). In *The Chemistry of Penicillin*, p. 945. Ed. by Clarke, H. T., Johnson, J. R. & Robinson, Sir Robert. Princeton University Press.
- Demain, A. L. (1956). *Arch. Biochem. Biophys.* **64**, 74.
- du Vigneaud, V., Loring, H. S. & Craft, H. A. (1934). *J. biol. Chem.* **105**, 481.
- Friedmann, E. (1908). *Beitr. chem. Physiol. Path.* **11**, 177.
- Halliday, W. J. & Arnstein, H. R. V. (1956). *Biochem. J.* **64**, 380.
- Humphrey, J. H. & Lightbown, M. J. (1952). *J. gen. Microbiol.* **7**, 129.
- Jarvis, F. G. & Johnson, M. J. (1947). *J. Amer. chem. Soc.* **69**, 3010.
- Kurono, T. (1922). *Biochem. Z.* **134**, 434.
- Pfister, K., Leanza, W. J., Conbere, J. B., Becker, H. J., Matzuk, A. R. & Rogers, E. F. (1955). *J. Amer. chem. Soc.* **77**, 697.
- Popják, G. (1950). *Biochem. J.* **46**, 560.
- Ratner, S. & Clarke, H. T. (1937). *J. Amer. chem. Soc.* **59**, 200.
- Stevens, C. M., Inamine, E. & DeLong, C. W. (1956). *J. biol. Chem.* **219**, 405.
- Stevens, C. M., Vohra, P. & DeLong, C. W. (1954). *J. biol. Chem.* **211**, 297.
- Stevens, C. M., Vohra, P., Inamine, E. & Roholt, O. A. (1953). *J. biol. Chem.* **205**, 1001.
- Stevens, C. M., Vohra, P., Moore, J. E. & DeLong, C. W. (1954). *J. biol. Chem.* **210**, 713.
- Stone, R. W. & Farrell, M. A. (1946). *Science*, **104**, 445.
- Umbreit, W. W. (1955). In *A Symposium on Amino Acid Metabolism*, p. 48. Ed. by McElroy, W. D. & Glass, H. B. Baltimore: Johns Hopkins Press.
- Wood, J. L. & du Vigneaud, V. (1939). *J. biol. Chem.* **130**, 109.

The Amino Acid Compositions of Three Fractions from Oxidized Wool

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The complex histology of the wool fibre and its insolubility in all solvents are the main obstacles to the elucidation of its chemical structure. On histological grounds alone it is apparent that wool is not a homogeneous protein, and therefore the logical approach to the problem would be to isolate the different structural components and study them separately. Unfortunately, in our opinion, it is not possible to do this at present since it is by no means clear how many components there are in the wool fibre or how they can be separated in a pure state. Most of the chemical methods used in their isolation result in considerable degradation of material, and in some cases several components are inextricably mixed. Moreover, histologists differ about the exact sites in the fibre structure which the isolated components originally occupied, and there is some confusion in the naming of them. A good review of the histological findings has been given by Mercer (1953), and further reference should be made to the *Proceedings of the International Wool Textile Conference*.

In view of the foregoing therefore most of the past and present research into the chemical structure of wool has been, and is being, directed towards the dissolution of the whole wool fibre and the isolation of large, homogeneous peptides from

the resultant solutions. Clearly such procedures are only second best, as the assignment of any given peptide to a histological component of the fibre cannot be free from doubt. Two main methods of dissolution have been used, and in both the essential preliminary degradative reaction has been the fission of the disulphide linkages. Gillespie & Lennox (1953) used alkaline thioglycollate solutions to reduce the disulphide linkages and to dissolve the wool, and Alexander & Hudson (1954) oxidized these bonds with peracetic acid and dissolved the oxidized wool in dilute ammonia solution. The latter method is analogous to that used by Sanger (1949) for the separation of the *A* and *B* chains of insulin, and has been adopted in the present work. Alexander & Hudson (1954) obtained three main fractions from oxidized wool which they named α -, β - and γ -keratoses. In our opinion it is doubtful whether the fractions are sufficiently well characterized to warrant the term 'keratoses', but we have retained this nomenclature because other workers have already made reports on their constitutions (Earland & Knight, 1955, 1956), their physical properties (Alexander, 1951; O'Donnell & Woods, 1955) and on the amounts of their *N*-terminal amino acids (Alexander & Smith, 1955). This paper describes the

preparation of the α -, β - and γ -keratases from an Australian Merino 64s quality wool (Corfield & Robson, 1955) and gives their amino acid compositions. The work is a necessary preliminary to a more detailed study of the chemical structure and physical properties of these fractions which is being undertaken in these laboratories.

EXPERIMENTAL

Oxidation of wool with peracetic acid. The following procedure was used to oxidize all the wool used in the investigation, amounts being varied proportionately as required; and the term 'oxidized wool' as used hereafter refers to wool treated in this way. An amount (1 g. dry wt.) of wool was stirred continuously for 24 hr. at 25° with 100 ml. of 1.6% (w/v) peracetic acid, filtered and washed with 1 l. of water. Total N determinations on the combined washings and filtrate showed that 1% of the total N of the wool dissolved. Cystine determinations, by the method of Shinohara (1935), showed that less than 1% of the disulphide linkages remained unoxidized.

Preparation of α -, β - and γ -keratases. Preliminary work showed that varying amounts of oxidized wool could be dissolved by solutions of NaH_2PO_4 , Na_2HPO_4 , Na_3PO_4 and NH_3 according to their concentrations and the time of extraction at room temperature, but in no experiment was the residue less than 10% of the original wool. Gradient extraction by 0.2M-phosphate solution over the range pH 4.5–11.5 resulted in the separation of two distinct fractions that dissolved in the pH ranges 4.5–7 and 9–11.5. The former represented 29% of the total N of the oxidized wool and was soluble at all pH values, whereas the latter furnished 56% of the total N and could be precipitated at pH 5. Similar yields of these fractions could be obtained by extracting oxidized wool at 20° for 24 hr. with 0.2M- Na_3PO_4 , acidifying to pH 5 with H_3PO_4 and estimating the total N in the resultant precipitate (α -keratase) and filtrate (γ -keratase). The difficulty in removing the phosphate from the soluble fraction, however, led us to abandon the procedure in favour of that outlined in Fig. 1. The 200 g. of oxidized wool used was prepared from the sample of Australian Merino 64s quality wool previously analysed by Corfield & Robson (1955).

The α - and γ -keratase preparations were obtained as dry, white powders, whereas examination of the β -keratase preparation showed that it retained certain fibre characteristics. The $\frac{1}{32}$ in. pieces of wool fibre resembled curved hollow tubes, indicating that most of the dissolved wool was derived from the wool cortex and that the β -keratase was probably mainly cuticular. Alexander & Earland (1950) in fact describe it as subcuticle membrane, but this statement has been challenged by Mercer (1953).

Hydrolysis procedure. All hydrolysates were prepared by refluxing approx. 0.4 g. of material with 20 ml. of 5.7N-HCl for 24 hr., removing HCl *in vacuo* and making the residues up to 10 ml. with water.

Chromatographic separations and estimations of amino acids. The basic amino acids were separated from 20 mg. of hydrolysate according to the directions of Moore & Stein (1951), and the other amino acids were separated from 5 mg. of hydrolysate by elution chromatography on 0.9 cm. $\times$ 100 cm. columns of the ion-exchange resin Zeo-Karb 225 (8 $\times$). Amino acid estimations on the eluent

fractions were made by the photometric ninhydrin procedure of Moore & Stein (1948).

Total nitrogen, total sulphur and ammonia. Total N contents of α -, β - and γ -keratases and of oxidized wool were determined (Chibnall, Rees & Williams, 1943). The NH_3 contents of the hydrolysates were determined as described previously (Corfield & Robson, 1955). The total S contents of the four samples were determined by the Benedict-Denis method (Barritt, 1934).

RESULTS

Amino acid analyses of the α -, β - and γ -keratase preparations are given in Table 1. The results are the means of triplicate determinations, and no corrections have been applied for amino acids destroyed during hydrolysis or chromatography. There were no unidentified peaks on the chromatograms, but the cystine and methionine peaks were missing; the former was presumably oxidized to cysteic acid and the latter to methionine sulphone. Cysteic acid was estimated during chromatography, but methionine sulphone did not emerge as a separate peak. However, methionine is only present to the extent of 0.56% in wool, and its omission cannot materially affect the overall analysis. No tryptophan determinations were made. Since the relation between the total S of proteins and their estimated content of S-containing amino acids is always of interest, the total S contents of all the preparations were determined.

In addition to these results, Table 1 contains the incomplete amino acid analyses of α -keratase and γ -keratase fractions prepared from an oxidized sample of Australian Merinos 56s quality wool tops. The γ -keratase preparation was isolated by the procedure outlined in Fig. 1, and the α -keratase by the second phosphate-isolation method described above in the preliminary experimental work. The results are given to only two significant figures as they represent only one analysis of each keratase, and the glycine and alanine peaks were not resolved sufficiently well for determinations of their contents to be made. On the assumption that the percentage yields of α -, β - and γ -keratases from oxidized wool are approx. 60, 10 and 30 respectively as claimed by Alexander & Hudson (1954) and substantiated by the present work, the figures in Table 2 have been derived from the results given in Table 1. This procedure serves as a check on the overall analysis.

DISCUSSION

The results presented in Table 1 show several interesting features. In the first place, the amino acid composition of oxidized wool is in close agreement with that of the virgin wool (Corfield & Robson, 1955) from which it was prepared, and for

Table 1. *Amino acid analyses of hydrolysates of oxidized wool, α -, β - and γ -keratoses*

Amino acid	Total N (%)						Australian Merino 64s quality wool (Corfield & Robson, 1955)
	γ -Keratose from wool tops (see text)	α -Keratose from wool tops (see text)	Fractions from oxidized wool			Oxidized wool before fractionation	
			γ -Keratose	α -Keratose	β -Keratose		
Alanine	—	—	2.58	4.83	4.84	3.99	4.12
Ammonia	—	—	11.05	10.25	8.61	9.34	—
Arginine	21.0	20.0	19.0	20.8	17.7	19.2	19.1
Aspartic acid	1.6	6.8	1.79	6.25	4.75	4.56	4.38
Cysteic acid	19.0	3.5	14.5	3.72	4.40	6.50	—
Glutamic acid	6.7	12.0	5.87	10.9	7.32	8.50	8.48
Glycine	—	—	4.97	5.16	6.53	6.09	6.29
Histidine	1.6	1.6	1.57	1.24	2.64	1.68	1.91
Isoleucine	2.3	2.7	2.14	2.49	2.85	2.34	2.44
Leucine	2.3	7.8	2.55	7.30	6.17	5.78	5.85
Lysine	1.3	4.8	1.03	4.60	6.43	4.02	3.92
Phenylalanine	0.9	1.9	1.15	1.94	2.35	1.90	2.07
Proline	11.0	2.1	9.85	2.69	4.57	4.89	5.05
Serine	11.5	6.8	9.70	6.70	7.99	7.50	7.87
Threonine	7.9	3.6	7.46	3.45	4.40	4.66	4.70
Tyrosine	1.4	2.7	1.41	2.44	2.25	2.37	2.62
Valine	5.0	3.9	4.15	3.98	4.41	3.97	4.16
Total	—	—	100.77	98.74	98.21	97.29	—
Total N (%)	13.2	15.9	13.5	16.0	15.6	15.7	16.3
Total S (%)	—	—	5.84	1.88	2.13	3.12	3.38

Fig. 1. Preparation of α -, β - and γ -keratoses from oxidized wool.

only three amino acids do the analyses differ by more than 5%. The amounts of histidine, phenylalanine and tyrosine are respectively 14, 6 and 11% less in oxidized wool than in virgin wool. The use of peracetic and performic acids to oxidize the cystine of proteins to cysteic acid has been reported partially to destroy other amino acids according to the conditions of reaction (Toennies & Homiller, 1942; Hirs, 1954). With particular reference to wool, Simmonds (1955) has shown that performic acid oxidation of a keratin derivative extracted from wool caused significant losses of serine, threonine, tyrosine, phenylalanine and histidine, and it is notable that the last three of these suffered partial loss during the peracetic acid oxidation of wool in the present work. It should also be borne in mind that 1% of the total nitrogen of virgin wool went into solution during this process.

The differences in amino acid compositions of the three keratoses are quite striking. γ -Keratose contains much larger amounts of cysteic acid, proline, serine and threonine, and much smaller

amounts of alanine, aspartic acid, glutamic acid, leucine, lysine, phenylalanine and tyrosine than does oxidized wool, whereas the reverse is true of α -keratose. It is perhaps surprising to find that β -keratose has an amino acid composition roughly similar to that of wool since it is said to be a mixture of cortical-cell membranes and resistant cuticle material (Mercer, 1953) comprising only 10% by weight of wool. However, its phenylalanine, glycine, lysine, valine and histidine contents are the greatest of all four preparations, whereas its arginine content is the least. A rough check on the detailed analyses given in Table 1 is furnished by the results in Table 2, which show that the composition of oxidized wool can be quantitatively accounted for by summing the amino acid compositions of the three keratoses in the proportions stated, with the exceptions of the glycine, histidine, lysine, phenylalanine and tyrosine totals, which are all low. No satisfactory explanation can be offered for these discrepancies.

The total nitrogen and total sulphur contents of the three keratoses are compared in Table 3 with the results of previous workers. There is fair agreement for the total nitrogen contents of α - and β -keratoses, but the figures for the total sulphur contents show wide differences. The total sulphur contents of β - and γ -keratoses, as determined by Alexander & Smith (1955), agree well with those found in the present work, but their figure for α -keratose is much higher. Earland & Knight (1955) claim that α - and β -keratoses contain much more sulphur than was found either in the present work or in that of Alexander & Smith. It is considered that their figures are probably too high, for if the total sulphur content of γ -keratose is taken as 6%, the total sulphur content of their wool would be 3.8%, which is much greater than is usually found in wool. When considering the total sulphur contents of the α -, β - and γ -keratoses and of oxidized wool obtained in the present work, it is interesting to note that their cysteic acid contents account for only 72.3, 73.6, 76.8 and 74.5% of them respectively. These results are in accord with the finding of Corfield & Robson (1955) that the cystine content of wool could account for only 80% of its total sulphur.

Table 2. *Amino acid composition of a mixture of 60% α -keratose, 10% β -keratose and 30% γ -keratose compared with that of oxidized wool*

Amino acid	Nitrogen (g.) from 600 g. of α -keratose + 100 g. of β -keratose + 300 g. of γ -keratose	Nitrogen (g.) from 1 kg. of oxidized wool
Alanine	6.44	6.26
Ammonia	15.7	14.6
Arginine	30.4	30.1
Aspartic acid	7.46	7.16
Cysteic acid	10.1	10.2
Glutamic acid	14.0	13.4
Glycine	8.01	9.56
Histidine	2.24	2.64
Isoleucine	3.70	3.67
Leucine	9.00	9.08
Lysine	5.83	6.30
Phenylalanine	2.67	2.98
Proline	7.27	7.68
Serine	11.6	11.8
Threonine	7.02	7.32
Tyrosine	3.26	3.72
Valine	6.19	6.23
Total N (%)	15.2	15.7
Total S (%)	3.09	3.12

Table 3. *Total nitrogen and total sulphur analyses of α -, β - and γ -keratoses by various authors*

Material	Total N (%)			Total S (%)		
	Earland & Knight (1955)	Alexander & Smith (1955)	Present work	Earland & Knight (1955)	Alexander & Smith (1955)	Present work
α -Keratose	16.11	15.95	16.0	2.90	2.5	1.88
β -Keratose	15.94	15.40	15.6	2.72	2.2	2.13
γ -Keratose	—	—	13.5	—	6.13	5.84

Finally, it is interesting to compare the amino acid analyses of the α - and γ -keratases prepared from virgin wool of 64s quality with those of the corresponding wool fractions obtained from wool tops of 56s quality. Reference to Table 1 shows that the agreement between the two sets of results is quite close, and it would appear that the two keratases can in fact be characterized by their amino acid compositions. Also, with α -keratase, neither a change in the degradation procedure adopted for its preparation nor a change in the quality of the wool used has a marked effect on its amino acid composition.

SUMMARY

1. An Australian Merino 64s quality wool has been oxidized with peracetic acid and its amino acid composition determined. The analyses is in excellent agreement with that of virgin wool (Corfield & Robson, 1955), except that cystine is converted into cysteic acid and that small losses of histidine, tyrosine and phenylalanine occurred during oxidation.

2. α -, β - and γ -Keratases were prepared from oxidized wool and their amino acid compositions determined.

3. Only 75% of the total sulphur of oxidized wool and of the three keratases could be accounted for by their respective cysteic acid contents.

4. The total nitrogen and total sulphur of the three keratases have been compared with the amounts determined by other workers. For total sulphur the agreement is not good.

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REFERENCES

- Alexander, P. (1951). *Kolloidzsch.* **122**, 8.
 Alexander, P. & Earland, C. (1950). *Text. Res. (J.)*, **20**, 298.
 Alexander, P. & Hudson, R. F. (1954). *Wool: Its Chemistry and Physics*, pp. 354-6. London: Chapman and Hall, Ltd.
 Alexander, P. & Smith, L. F. (1955). *Proceedings of the International Wool Textile Research Conference*, vol. B, p. 56. Melbourne: Commonwealth Scientific and Industrial Research Organization.
 Barritt, J. (1934). *J. Soc. chem. Ind., Lond.*, **53**, 291T.
 Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
 Corfield, M. C. & Robson, A. (1955). *Biochem. J.* **59**, 62.
 Earland, C. & Knight, C. S. (1955). *Biochim. biophys. Acta*, **17**, 457.
 Earland, C. & Knight, C. S. (1956). *Biochim. biophys. Acta*, **22**, 405.
 Gillespie, J. M. & Lennox, F. G. (1953). *Biochim. biophys. Acta*, **12**, 481.
 Hirs, C. H. W. (1954). *Fed. Proc.* **13**, 230.
 Mercer, E. H. (1953). *Text. Res. (J.)*, **23**, 388.
 Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
 Moore, S. & Stein, W. H. (1951). *J. biol. Chem.* **192**, 663.
 O'Donnell, I. J. & Woods, E. F. (1955). *Proceedings of the International Wool Textile Research Conference*, vol. B, p. 71. Melbourne: Commonwealth Scientific and Industrial Research Organization.
 Sanger, F. (1949). *Biochem. J.* **44**, 126.
 Shinohara, K. (1935). *J. biol. Chem.* **109**, 665.
 Simmonds, D. H. (1955). *Aust. J. biol. Sci.* **8**, 114.
 Toennies, G. & Homiller, R. P. (1942). *J. Amer. chem. Soc.* **64**, 3054.

The Identification of Two Lipid Components in Liver which enable *Penicillium notatum* Extracts to Hydrolyse Lecithin

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Fairbairn (1948) reported that aqueous dialysed extracts of the mould *Penicillium notatum* possessed an active phospholipase B which attacked the lysophosphoglycerides but had no action on the intact phosphoglycerides. The same preparation has been used as the basis of an enzymic method for the preparation of glycerylphosphorylcholine (Uziel & Hanahan, 1956) and here again no activity was found towards intact lecithin. Dawson (1957) attempted to detect lysolecithin in the crude lipids extracted from liver with a similar enzyme preparation from *P. notatum*, but found that,

although the mould extract did not attack purified ovoidlecithin, there was a rapid and almost quantitative release of the fatty acids from the lecithin present in the crude lipids extracted from liver. This activity was not caused by some difference between the ovoidlecithin and its counterpart in the liver lipids, since when purified the latter lost its susceptibility to attack by the *P. notatum* extracts. It became apparent that there was an additional factor in the crude lipid extract which was responsible for the *P. notatum* phospholipase B preparation attacking the liver lecithin. This paper