Amino Acid Sequences of Peptides from a Chymotryptic Digest of a Urea-Soluble Protein Fraction (U.S.3) from Oxidized Wool

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1. A chymotryptic digest of the protein fraction U.S.3 from oxidized wool was separated into 51 peptide fractions by chromatography on a column of cation-exchange resin. 2. The less acidic fractions were separated into their component peptides by a combination of cation-exchange-resin chromatography, paper chromatography and paper electrophoresis. 3. The amino acid sequences of 34 of these peptides were elucidated, and those of 14 others partially determined. 4. Overlaps between the tryptic and chymotryptic peptides from fraction U.S.3 have enabled ten extended amino acid sequences to be deduced, the longest containing 20 amino acid residues. 5. The relevance of the results to the structures of the helical and non-helical regions of wool is discussed.

Fraction U.S.3 is a polypeptide fraction extracted in 36% yield from oxidized wool by buffered urea solutions at neutral pH values (Corfield, 1963). Corfield, Fletcher & Robson (1967*a*) described the isolation and characterization of peptides from a tryptic digest of fraction U.S.3. These peptides fell into two groups with respect to their behaviour on columns of a cation-exchange resin. One group (T_u) consisted of peptides containing two or more cysteic acid residues; it was unretarded by the resin and attempts to obtain purified peptides from it were unsuccessful. The other group (T_r) consisted of the aggregate of peptides containing one or no cysteic acid residue that were retarded by the resin. It has been pointed out (Corfield, Fletcher & Robson, 1967*c*) that these two groups of peptides show striking resemblances in overall amino acid composition to certain fractions obtained from wool by other procedures. Further, Blackburn & Lee (1966) examined the peptides present in a tryptic digest of whole oxidized wool, and found that most of the peptides in the T_r group in this digest were also present in the T_r group obtained from fraction U.S.3. For these reasons, we have suggested that fraction U.S.3 is derived from a protein that constitutes the major part of the cortex of wool.

This paper describes the isolation and characterization of peptides from a chymotryptic digest of fraction U.S.3. With respect to their behaviour on resin columns, the chymotryptic peptides fall into two groups (C_u and C_r) corresponding to groups T_u and T_r and resembling them in overall amino acid composition. Overlaps between the sequences of tryptic and chymotryptic peptides

from groups T_r and C_r enable us to write some extended amino acid sequences from fraction U.S.3. None of these has a length exceeding 20 residues. The incompleteness of the results is almost certainly the principal factor determining this limit, but the possibility cannot entirely be dismissed that the average length of the helical sections is of this order, and that without information on the amino acid sequences in the regions of high sulphur content of fraction U.S.3 the probability of extending the sequences is small.

MATERIALS AND METHODS

Materials. Sources of enzymes and chemicals and the preparation of fraction U.S.3 were as given by Corfield *et al.* (1967*a*).

Digestion of fraction U.S.3 with chymotrypsin. Dry fraction U.S.3 (4.61 g.) was suspended in distilled water (200 ml.), stirred under N_2 at 37° and titrated to dissolution at pH 8.5 by the addition of 0.1 M-NaOH from an auto-titrator. α -Chymotrypsin (50 mg.) dissolved in water at pH 8.5 was added and the course of the digestion was followed by the uptake of alkali. After 140 min. the reaction was stopped by bringing the digest to pH 2.5 with 10 ml. of 98% formic acid. The extent of reaction was approx. 150% of that calculated from the tyrosine plus phenylalanine content of fraction U.S.3 (0.469 m-mole/g.). All the work described was done on a single digest.

Separation of peptides. The whole of the acidified digest was applied to a column (130 cm. $\times$ 7 cm.) of the sulphonic resin Zeo-Karb 225 (X2; 200 mesh) as used for fractionation of the tryptic digest of fraction U.S.3. The column was eluted with pyridine-formic acid and pyridine-acetic acid buffers according to the programme shown in Fig. 1. The

Fig. 1. Chromatography of a chymotryptic digest of fraction U.S.3 (4.6g.) on a column (130cm. $\times$ 7cm.) of Zeo-Karb 225 (X2) in pyridine-formic acid and pyridine-acetic acid buffers. The effluent fractions were combined as shown by the numbered bars above the chromatogram. Samples (1 ml.) of each effluent fraction (200 ml.) were taken for alkaline hydrolysis and colour development with ninhydrin. Buffers: A, 16.25 ml. of pyridine and 51 ml. of formic acid/l. (pH 2.8); B, 81.25 ml. of pyridine and 43.5 ml. of formic acid/l. (pH 4.3); C, 162.5 ml. of pyridine and 99 ml. of acetic acid/l. (pH 5.0); D, 162.5 ml. of pyridine and 4.5 ml. of acetic acid/l. (pH 6.5).

fractions were combined on the basis of analysis with ninhydrin to give, after removal of the solvent in a rotary evaporator and then freeze-drying, crude peptide fractions C1–C51.

Each fraction from C13 onwards was refractionated on a column (140cm. $\times$ 1.8cm.) of the same resin in equilibrium with a buffer of fixed composition (Table 1). The dry crude peptide mixture was shaken for 2 hr. with 10 ml. of the buffer, any appreciable residue was removed by centrifugation and the solution was applied to the column, which was maintained at 37°. Fractions (10 ml.) were collected at the rate of three/hr. Samples (0.1 ml.) were removed from each fraction for alkaline hydrolysis and reaction with ninhydrin. The fractions in the peaks corresponding to the peptide components were combined and the buffer was removed by freeze-drying. The fraction numbers at which the peptide fractions were eluted are given in Table 1. Fractions at this stage of the purification process were designated by Greek letters (e.g. C14 α , β or γ), or by R, denoting an insoluble residue.

Further purification of peptides from the chymotryptic digest was carried out by paper chromatography and high-voltage paper electrophoresis as described by Corfield *et al.* (1967a). Some of the peptides in the more acidic peptide subfractions (peptides C8 and C14), which did not migrate in butan-1-ol-acetic acid-water (4:1:5, by vol.), were separated in the one-phase solvent mixture propan-1-ol-water (2:3, v/v). R_F values and electrophoretic mobilities of peptides are given in Figs. 2 and 3. Letter A denotes peptides separated by chromatography in propan-1-ol-water, and letter B denotes peptides separated by chromatography in butan-1-ol-acetic acid-water (e.g. peptides C14 β A2 and C15 β B1). Peptides purified by high-voltage electrophoresis are designated by the letter

H for separations at pH 1.85 and by the letter K for separations at pH 6.5 (100 ml. of pyridine and 3.6 ml. of acetic acid/l.) and in most cases by a number indicating the order of the band measured from the anode end of the paper.

Amino acid analysis of peptides and determination of amino acid sequences. The procedures and techniques were generally as described by Corfield *et al.* (1967a). For digestion with trypsin and subtilisin, the peptide was dissolved in 0.2 M-ammonium acetate buffer, pH 8.5 (0.2 ml.) and 10–30 μ l. of enzyme solution (1 mg./ml.) in the same buffer was added (Ambler, 1963). For digestion with Pronase, 0.2 M-ammonium acetate buffer, pH 6.5, was used. Digestion, at 37°, was for 16 hr. unless stated otherwise. Partial hydrolysis with 12 M-HCl (sp.gr. 1.18) at 37° was carried out for the times specified; partial hydrolysis with 0.03 M-HCl at 105° was carried out for 16 hr. In both partial and complete acid hydrolysis of small quantities of peptides, 0.1 M-thioglycolic acid (3 μ l./100 μ l.) was added to the acid used to prevent modification of tyrosine (Sanger & Thompson, 1963). The systematic use of phenol for this purpose (suggested by these authors as an alternative) has been found eventually to impair the performance of the ion-exchange resin columns of the amino acid analyser.

RESULTS

Amino acid compositions of fraction U.S.3 and various groups of peptides derived from it

About one-third of the material in the chymotryptic digest of fraction U.S.3 (designated C_u) runs as a peak unretarded by the Zeo-Karb 225 (X2) resin (Fig. 1, fractions C1–C3). The aggregate of peptides retarded by the resin (fractions C4–C44) is designated C_r. Equal portions of the fractions contributing to groups C_u and C_r were taken and combined for the determination of the overall amino acid compositions of these groups of peptides. The results are given in Table 2. The amino acid compositions of the unretarded (T_u) and retarded (T_r) groups of peptides from the tryptic digest of fraction U.S.3 (Corfield *et al.* 1967a) are also given.

Structures of peptides isolated from the chymotryptic digest of fraction U.S.3

The amino acid compositions of the peptides are given in Tables 3 and 4. In the sequences given below, the C-terminal residue is usually inferred from the specificity of chymotrypsin. Unless stated otherwise, values in parentheses refer to electrophoretic mobilities at pH 1.85 relative to alanine. Mobilities at pH 6.5 are relative to cysteic acid.

Peptide C8A6H1. The N-terminus was cysteic acid (DNS* method). Hydrolysis with concentrated hydrochloric acid at 37° for 16 hr. gave: Cya-(Glx,Leu₂,Pro) (0.04; 0.33 at pH 6.5); Ser-Phe

* Abbreviations (in amino acid sequences): Cya, cysteic acid; Mes, methionine sulphone; DNS, 1-dimethylamino-naphthalene-5-sulphonyl.

Table 1. *Rechromatography of peptides C13-C44*

Peptides were eluted from Zeo-Karb 225 resin (see the text) with the buffers shown and recovered from the fractions indicated.

Peptide fraction	Fraction nos. of subfractions				Pyridine-formic acid buffer	
	α	β	γ	δ	Pyridine concn. (M)	pH
C13	24-35	100-115	116-130	131-150	0.30	3.05
C14	29-43	82-90	98-106	107-115	0.40	3.25
C15	30-45	89-96	97-105	106-117	0.40	3.38
C16	37-50	109-120	121-131	132-143	0.40	3.38
C17	36-44	105-115	80-90	128-171	0.45	3.42
C18*	49-61	95-103	104-110	111-118	0.50	3.45
C19	66-76	130-140	147-158		0.50	3.45
C20	44-55	56-65	137-149	150-165	0.50	3.45
C21	45-57	58-72			0.50	3.45
C22	55-66	147-156	157-163		0.50	3.55
C23	48-58	59-68			0.50	3.55
C24	55-62	63-75			0.50	3.55
C25†	40-49	50-59	66-73	92-100	0.70	3.70
C26	43-56	60-73	102-116	117-129	0.70	3.70
C27	48-61	121-135			0.70	3.70
C28‡	53-57	58-64	65-76	129-137	0.70	3.80
C29	51-58	59-68	69-78	131-143	0.70	3.80
C30§	57-63	64-70	71-80	151-161	0.70	3.80
C31	159-169	170-177	178-188	189-197	0.70	3.80
C32	146-154				0.80	4.10
C33	56-65	66-75	76-85	144-154	0.80	4.13
C34	54-60	61-72	145-156		0.80	4.13
C35¶	50-55	56-65	66-73	121-130	0.80	4.13
C36	59-69	137-147			0.80	4.13
C39	80-87	92-104			0.80	4.13
C40	167-177				0.80	4.13
C44	148-167	195-215			0.80	4.13

* C18 ϵ : 119-130.

† C25 ϵ : 101-109; C25 ζ : 110-120.

‡ C28 ϵ : 138-150.

§ C30 ϵ : 162-171; C30 ζ : 172-182.

|| C33 ϵ : 155-165; C33 ζ : 166-175; C33 η : 176-185.

¶ C35 ϵ : 131-142.

(0.69) (DNS method). The probable sequence is therefore:

Cya-(Glx,Leu₂,Pro)-Ser-Phe

Peptide C13 γ B4. The *N*-terminus was serine (acrylonitrile method). Hydrolysis with concentrated hydrochloric acid at 37° for 72hr. gave: Ser-(Glx,Leu) (0.63) (acrylonitrile method); Glx-Leu (0.71) (acrylonitrile method); Ser (0.82).

Peptide C14 β A2. The *N*-terminus was threonine (DNS method). Hydrolysis with trypsin gave: (1) Cya-(Asp,Glx,Ser)-Lys (0.45) (acrylonitrile method); (2) Thr-Ala-(Ala,Glx,Val)-Lys (0.68) (Edman-DNS method); (3) Leu (0.77). Hydrolysis of tryptic peptide (2) with Pronase for 4hr. gave: (Ala,Thr) (0.81); Ala-Ala (0.94) (acrylonitrile method); (Glx,Val) (0.83); (Glx,Val)-Lys (1.40). The probable sequence is therefore:

Thr-Ala-Ala-(Glx,Val)-Lys-Cya-(Asp,Glx,Ser)-Lys-Leu

Hydrolysis with Pronase for 4hr. gave: Ser-(Glx,-Leu) (0.61) (DNS method); Ser-Glx (0.76) (DNS method); Ser-(Glx,Ser) (0.65) (DNS method). The probable sequence is therefore:

Ser-Ser-Glx-Leu

Peptide C14 δ A8H2. The *N*-terminus was alanine (DNS method). Hydrolysis with subtilisin gave: Ala-Leu (0.79) (acrylonitrile method); (Ala,Glx,-Leu) (0.30). The probable sequence is therefore:

Ala-Leu-Glx-(Glx,Gly,Ser)-Leu

Fig. 2. Separation of peptides by one-dimensional paper chromatography in propan-1-ol-water (2:3, v/v) (A) or butan-1-ol-acetic acid-water (4:1:5, by vol.) (B). The mixture of peptides (up to 6mg.), dissolved in water or 0.2M-ammonia (80–200μl.), was applied by an automatic streaking device as a line, 15cm. long, to a sheet of Whatman 3MM filter paper. The chromatograms were developed and the peptides located and eluted as described by Corfield *et al.* (1967a). Solid blocks denote peptide bands that give an intense stain with cadmium-ninhydrin reagent; open blocks denote bands giving stains of faint to medium intensity.

Fig. 3. Separation of peptides by paper electrophoresis (approx. 180v/cm.). For separations at pH1.85 the mixture of peptides was applied as an 8cm. line 20cm. from the anode end of a strip (100cm. × 15cm.) of Whatman 3MM paper. For separations at pH6.5 the load was applied at the middle of the strip. See also the legend to Fig. 2.

Peptide C15βB1. The *N*-terminus was Ala-Ser-Asx-Asx . . . (Edman-DNS method). Hydrolysis

with Pronase for 5hr. gave: (Ala,Asx,Ser) (0.50); (Asx,Ser) (0.75). Hydrolysis with concentrated hydrochloric acid at 37° for 48hr. gave (Asx₂,Phe,-Ser) (0.52); (Asx,Phe) (0.56); (Asx,Ser) (0.76); Ala. Hydrolysis with 0.03M-hydrochloric acid gave: (Ala,Ser) (0.85); Asx (0.60); Phe (0.62). The probable sequence is therefore:

Ala-Ser-Asx-Asx-Phe

Peptide C15βB2. The *N*-terminus was Ala-Ala-Asx-Asx . . . (Edman-DNS method). Hydrolysis with 0.03M-hydrochloric acid gave: Ala-Ala (0.90); (acrylonitrile method) Asx (0.60); Phe (0.60). The probable sequence is therefore:

Ala-Ala-Asx-Asx-Phe

Peptide C15βB3. The *N*-terminus was glycine (DNS method). The probable sequence is therefore:

Gly-Leu-Leu

Peptide C15γB8. No *N*-terminal amino acid was detected. Hydrolysis with trypsin gave: (Glx₂)-Lys (0.59); (Ile,Leu) (0.69). The probable sequence is therefore:

Glx-Glx-Lys-Ile-Leu

Peptide C15γB9. The *N*-terminus was Ile-Thr . . . (Edman-DNS method). Hydrolysis with concentrated hydrochloric acid for 24hr. gave: Ile-Glx (acrylonitrile method) (0.69); Thr-Leu (acrylonitrile method) (0.77). Hydrolysis with Pronase for 5hr. gave: Glx-Thr (DNS and acrylonitrile methods)

Table 2. *Amino acid compositions of peptide fractions from chymotryptic and tryptic digests of fraction U.S.3*

Results, expressed as the number of residues/1000 residues, are corrected for decomposition of amino acids during hydrolysis. C_u and T_u denote the groups of acidic peptides that are not retarded on Zeo-Karb 225 columns; C_r and T_r denote the aggregates of peptides that are retarded (see the text and Fig. 1). Values given for T_u and T_r are taken from Corfield *et al.* (1967a). Comparative values for γ -keratose and the helical fraction isolated from SCMKA by Pronase digestion are taken from the results of Corfield, Robson & Skinner (1958) and Crewther, Dobb, Dowling & Harrap (1967) respectively. C_r (calc.) is from the totals in Table 3. N.D., Not determined.

Amino acid	C _u	T _u	γ -Keratose	C _r	C _r (calc.)	T _r	Helical fraction
Ala	43	59	34	74	90	67	79
Arg	47	49	63	86	51	78	68
Asp	37	52	24	103	93	95	107
Cya	183	164	191	24	32	38	35
Glu	74	59	77	170	176	168	218
Gly	84	94	65	57	58	51	28
His	5	4	7	8	3	6	8
Ile	29	29	23	39	22	44	40
Leu	35	46	34	118	154	118	138
Lys	10	11	7	47	70	48	59
Mes	N.D.	4	N.D.	N.D.	3	8	2
Phe	12	16	15	31	35	29	19
Pro	145	114	130	4	10	21	11
Ser	131	139	141	84	83	77	66
Thr	86	88	107	52	45	49	36
Tyr	13	19	18	40	48	41	34
Val	63	54	55	56	26	65	53
Total	997	1001	996	993	999	1003	1000
Mean residue weight	111	110		114		115	

(0.73); Ile/Leu. The probable sequence is therefore:

Ile-Glx-Thr-Leu

Peptide C15δB5H2. The *N*-terminus was threonine (DNS method). The probable sequence is therefore:

Thr-(Leu,Pro)-Leu

Peptide C15δB5H3. The *N*-terminus was threonine (acrylonitrile method). The probable sequence is therefore:

Thr-Leu

Peptide C15δB5H4. The *N*-terminus was alanine (DNS and acrylonitrile methods). The probable sequence is therefore:

Ala-Leu-Leu

Peptide C16αB4. No *N*-terminus was detected. Hydrolysis with trypsin gave: (Asp,Cya,Glx,Ile)-Leu (0.05); Glx-Glx-Lys (0.90) (Edman-DNS method). The probable sequence is therefore:

Glx-Glx-Lys-(Asp,Cya,Glx,Ile)-Leu

Peptide C17αB4. The *N*-terminus was threonine (DNS method). Hydrolysis with trypsin gave: (1) Cya-Glx-(Asx,Ser)-Lys (0.36) (Edman-DNS method); (2) Cya-(Asx,Glx,Leu,Lys,Ser) (0.33);

(3) (Ala₂,Glx,Thr,Val)-Lys (0.67); (4) Leu (0.76). Hydrolysis of tryptic peptide (2) with concentrated hydrochloric acid at 37° for 48 hr. gave: (Asx,Cya,Glx) (0.04); (Leu,Lys,Ser) (1.17). Hydrolysis of tryptic peptide (3) with Pronase for 5 hr. gave: Ala-(Glx,Val) (0.50) (acrylonitrile method); (Ala,Glx) (0.80). The probable sequence is therefore:

Thr-(Ala₂,Glx,Val)-Lys-Cya-Glx-Asx-Ser-Lys-Leu

Peptide C17βB7. The *N*-terminus was alanine or serine (DNS method). Hydrolysis with concentrated hydrochloric acid at 37° for 48 hr. gave: Ser-(Gly,Ser) (0.75) (acrylonitrile method); Ser-Tyr (0.69); Ala. Hydrolysis with subtilisin gave: Ala-Ser (0.95) (acrylonitrile method); Gly-Ser-Tyr (0.61) (acrylonitrile method). The probable sequence is therefore:

Ala-Ser-Gly-Ser-Tyr

Peptide C18εB2. The *N*-terminus was glycine (acrylonitrile method). Hydrolysis with concentrated hydrochloric acid at 37° for 48 hr. gave: Ser-Gly (1.01) (acrylonitrile method); (Gly,Ser)-Phe (0.67). Hydrolysis with Pronase for 5 hr. gave: Gly-(Gly-Ser) (0.87) (acrylonitrile method); Phe (0.61). The probable sequence is therefore:

Gly-Ser-Gly-Phe

Table 3. *Composition of peptides from the chymotryptic digest of fraction U.S.3*

Results are given in nanomoles, and also (in parentheses) as interpretations to the nearest integer. Peptides marked with an asterisk were analysed either by high-voltage paper electrophoresis (Atfield & Morris, 1961) or by high-voltage paper electrophoresis followed by chromatography (Corfield & Simpson, 1965). All other peptides were analysed by automatic amino acid analysis (Cole, Corfield, Fletcher & Robson, 1966). For peptides omitted from this Table, because their sequences wholly or partially overlap those of others, see Table 4.

Peptide	Yield (μmoles)	Amino acid residues																Total	
		Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr		Val
C8AGH1	1.2			5(-)	15(1)	11(1)				25(2)			16(1)	13(1)	15(1)				7
C13YB4	2.3			1.9(-)		8.4(1)				7.9(1)					15(2)				4
C14SA2	1.0	40(2)		20(1)	17(1)	41(2)				21(1)	40(2)				14(1)	20(1)		16(1)	12
C14ASH2	1.0	24(1)		42(2)		40(2)	18(1)			43(2)					13(1)				7
*C15GB1	3.1	22(1)		14(2)									18(1)		20(1)				5
*C15GB2	5.1	15(2)											6.7(1)						5
C15GB3	0.6					3.1(-)	13(1)		3.4(-)	26(2)					2(-)				3
C15YB8	2.8					42(2)			18(1)	20(1)	21(1)					19(1)			5
C15YB9	3.3	2(-)				23(1)			20(1)	23(1)				43(1)		45(1)			4
*C15B5H2	0.1									91(2)						34(1)			4
*C15B5H3	0.3									37(1)									2
C15B5H4	2.5	19(1)								40(2)								3(-)	3
C16B4	1.7			24(1)	17(1)	78(3)			21(1)	25(1)	26(1)				6(-)		21(1)	6(-)	8
*C17B7	0.8	17(1)					19(1)								45(2)				5
*C18B2	1.4						14(2)								7.0(1)				4
*C18B4H2	0.1									51(2)			5.9(1)			8.1(1)			2
C19B3	0.4	49(2)				97(4)	9(-)	21(1)					53(1)		5(-)	52(1)		23(1)	10
C19YB1	2.9			9.3(-)		100(2)									5.7(-)	30(2)			4
C20B3H1	0.4	16(1)		13(1)		15(1)												14(1)	7
C20YB8	9.0	168(2)											76(1)						3
C20YB10	0.1									(1)									1
C20B4H1	0.2														18(1)	20(1)	16(1)		3
C20B4H2	0.2	21(1)				7.7(1)	7.7(1)			7.6(1)					26(1)		28(1)		3
C21B5	1.1			7.2(1)		53(1)	42(1)		84(2)	40(1)		8(-)			6.2(1)	7.0(1)	7.0(1)		6
C21R	2.3	35(1)		47(1)			76(1)								10(-)	54(1)	37(1)		9
C22B4	14.4																68(1)		2
C22YB2H	1.4		30(1)		27(1)		104(3)				13(1)		37(1)		63(2)	12(1)		33(1)	9
C24B4H3	1.4			21(2)		39(3)	13(1)						12(1)				3.4(-)		9
C25B1	4.6	37(2)	20(1)	24(1)	21(1)	23(1)				21(1)	25(1)				20(1)				10
*C25B2	2.0			23(1)		71(3)				48(2)	24(1)								7
C26B2	1.4			14(1)		3.4(-)				15(1)	23(2)				16(1)				5
C26B6H1	0.3	23(2)		9.5(1)	12(1)	37(3)	2.7(-)			22(2)	35(3)	11(1)			3.0(-)		11(1)		14
C26B6H2	0.2	4.2(-)	21(1)			90(4)				19(1)				16(1)	20(1)			3.9(-)	8
*C27B7	2.2					11(1)				23(2)	11(1)					11(1)			5
C28B1	1.3	35(1)		81(2)	47(1)					11(-)	76(2)						49(1)	40(1)	8
C28B4	6.1	10(1)	8.1(1)	6.7(1)		28(3)				10(1)					22(1)	6(-)			7
C29B2	0.8	30(1)	21(1)	25(1)	16(1)	68(3)				43(2)	38(2)				17(1)		14(1)		12
C30B2	2.2		13(1)		12(1)		17(1)		11(1)										6
C31B7	1.0																		3
*C31YB9	3.7		5.7(1)	15(2)						20(2)	12(1)								7
C33YB1H2	3.0	15(1)	22(2)	26(2)	60(4)				3.9(-)	14(2)			3.8(-)		15(1)		13(1)	14(2)	13
C34B4H1	1.5	12(1)	12(1)	21(2)						26(2)					12(1)		18(1)		8
C34B4H2	0.9					5.9(1)				6.1(1)	5.1(1)						5.1(1)		4
C35B3H	4.0	18(1)	16(1)	17(1)		34(2)									3.0(-)	15(1)	18(1)		7
*C35B4H2	1.0					5.9(1)				6.0(1)					13(2)				5
C35B6	2.3	25(2)	15(1)	13(1)		30(2)							12(1)			14(1)			8
*C36B5	1.5	42(1)								43(1)									2
C39B3	0.6	25(1)	24(1)		21(1)		51(2)						21(1)		48(2)			9.0(1)	8
C44B	22.0	10(1)				16(2)				16(2)	8.5(1)								7
Totals		28	16	27	10	51	18	1	6	46	20	1	11	3	25	14	15	8	300
Calc. totals		22	26	31	7	51	17	2	12	36	14	2	9	1	25	16	12	17	300

Table 4. *Peptides omitted from Table 3, because their sequences wholly or partially overlap those of others*

Compositions are given in nanomoles, and also (in parentheses) as interpretations to the nearest integer.

C17 α B4 (3 μ moles): Ala 29(2), Asp 15(1), Cya 10(1), Glu 33(2), Leu 13(1), Lys 26(2), Ser 16(1), Thr 14(1), Val 12(1); cf. C14 β A2.

C25 β B4 (0.8 μ mole): Asp 8.3(1), Glu 20(2), Leu 8.5(1), Lys 8.0(1); cf. C25 ϵ B2.

C26 δ B3H4 (0.4 μ mole): Asp 7.3(1), Leu 9.2(1), Lys 9.1(1), Ser 7.8(1); cf. C26 α B2.

C40 α B2 (0.9 μ mole): Ala 4.3(-), Arg 18(1), Glu 39(2), Leu 22(1), Lys 19(1), Val 19(1); cf. C44 β .

Peptide C18 ϵ B4H2. The *N*-terminus was threonine (acrylonitrile method). The probable sequence is therefore:

Thr-Phe

Peptide C19 β B3. The *N*-terminus was Ala-Leu-Glx . . . (Edman-DNS method). Hydrolysis with subtilisin gave: Ala-(Glx₂,Leu,Val) (0.42) (acrylonitrile method); Ala-Leu (0.81) (acrylonitrile method); (Glx₂,Val) (0.61); (Glx-Leu) (0.69); (Ala-,Glx,His) (1.10). Hydrolysis with concentrated hydrochloric acid at 37° for 72hr. gave: Ala-(Glx,Leu) (0.61) (acrylonitrile method); Leu-Glx (0.65) (acrylonitrile method); Ala-Leu (0.83) (acrylonitrile method); (Glx₂,Leu,Val) (0.50); (Glx-,His) (1.22); (Ala,Glx,His) (1.14). The probable sequence is therefore:

Ala-Leu-Glx-(Ala,Glx₃,His,Val)-Leu

Peptide C19 γ B1. The *N*-terminus was threonine (acrylonitrile method). Hydrolysis with concentrated hydrochloric acid at 37° for 48hr. gave: Thr-Gly (0.86) (acrylonitrile method); Gly-Phe (0.92) (acrylonitrile method). The probable sequence is therefore:

Thr-Gly-Gly-Phe

Peptide C20 γ B8. The *N*-terminus was alanine (acrylonitrile and DNS methods). The probable sequence is therefore:

Ala-Ala-Phe

Peptide C20 γ B10. This was the free amino acid leucine.

Peptide C20 δ B4H1. The *N*-terminus was serine (acrylonitrile method). The probable sequence is therefore:

Ser-Thr-Tyr

Peptide C20 δ B4H2. The *N*-terminus was alanine (acrylonitrile method). The probable sequence is therefore:

Ala-Ser-Tyr

Peptide C21R. The *N*-terminus was Gly-Leu . . . (Edman-DNS method). Hydrolysis with pepsin [peptide dissolved in 98% formic acid (50 μ l.) and water (350 μ l.), and pepsin solution (1mg./ml.; 30 μ l.) added] for 72hr. gave: Gly-Leu-Asx (0.60) (Edman-DNS method); Ile-(Ala,Glx,Ile,Thr)-Tyr (0.39) (DNS method). Hydrolysis with concentrated hydrochloric acid at 37° for 72hr. gave: (Asx,Glx,Gly,Ile/Leu₂) (0.40); Asx-(Glx,Ile/Leu) (0.53) (DNS method); Thr-Tyr (0.61) (acrylonitrile method). The probable sequence is therefore:

Gly-Leu-Asx-Ile-Glx-(Ala,Ile)-Thr-Tyr

Peptide C22 β B4. The *N*-terminus was glycine (acrylonitrile method). The probable sequence is therefore:

Gly-Tyr

Peptide C22 γ B2H. The *N*-terminus was Gly-Ser . . . (Edman-DNS method). Hydrolysis with trypsin for 20hr. gave: (1) Ser-Val-(Cya,Gly₂)-Phe (0.12) (Edman-DNS method); (2) Gly-Ser-Arg (1.22) (acrylonitrile method). Hydrolysis of tryptic peptide (1) with concentrated hydrochloric acid at 37° for 24hr. gave: Ser-(Cya,Gly,Val) (0.12) (acrylonitrile method); Gly-Phe (0.77). The probable sequence is therefore:

Gly-Ser-Arg-Ser-Val-(Cya,Gly)-Gly-Phe

Peptide C24 β H4K3. The *N*-terminus was glutamic acid or glutamine (DNS method). Hydrolysis with subtilisin gave: (Asx₂,Glx,Gly,Lys) (0.28); (Glx,Phe) (0.60); Glx-(Thr,Tyr) (0.44) (acrylonitrile method). Hydrolysis with 0.03M-hydrochloric acid gave: (Glx,Phe,Thr,Tyr) (0.60); (Glx,Thr,Tyr) (0.50). Hydrolysis with concentrated hydrochloric acid at 37° for 48hr. gave: (Asx,Glx,Phe) (0.57); (Thr,Tyr) (0.72). This peptide was not split by trypsin or pepsin. The probable sequence is therefore:

Glx-(Asx,Glx,Gly,Lys)-Asx-Phe-Glx-Thr-Tyr

Peptide C25 β B1. The *N*-terminus was cysteine acid (DNS method). Hydrolysis with trypsin for 19hr. gave: (1) Cya-Ala-Lys (0.61) (DNS and acrylonitrile methods); (2) Leu (0.76); (3) Ser-(Ala,Asx,Glx)-Arg (0.82) (DNS method). Hydrolysis of tryptic peptide (3) with concentrated hydrochloric acid at 37° for 48hr. gave: Ser-Glx (0.75) (DNS method); (Asp-Glx,Ser) (0.61); Ala-Arg (1.27). The probable sequence is therefore:

Cya-Ala-Lys-Ser-Glx-Asx-Ala-Arg-Leu

Peptide C25 β B4. No *N*-terminal amino acid was detected. Hydrolysis with trypsin gave: leucine (0.75); no other peptide was detected. Hydrolysis with subtilisin gave: Asx-(Leu,Lys) (1.18) (DNS

method); (Leu,Lys) (1.30); no glutamic acid-containing peptides were detected. The probable sequence is therefore:

Glx-Glx-Asx-Lys-Leu

Peptide C25εB2. The *N*-terminus was Leu-Glx-Glx-Glx . . . (Edman-DNS method). Hydrolysis with subtilisin gave: Asx-(Leu,Lys) (1.15) (acrylonitrile method); (Leu,Lys) (1.27). Hydrolysis with concentrated hydrochloric acid at 37° for 72 hr. gave: Leu-(Glx₂₋₃) (0.44) (acrylonitrile method); (Asx,Leu,Lys) (1.10); Lys-Leu (1.28) (acrylonitrile method). This peptide was not split by trypsin or pepsin. The probable sequence is therefore:

Leu-Glx-Glx-Glx-Asx-Lys-Leu

Peptide C26αB2. The *N*-terminus was Lys-Asx . . . (Edman-DNS and acrylonitrile methods). Hydrolysis with concentrated hydrochloric acid at 37° for 72 hr. gave: (Leu,Lys,Ser) (1.20); Lys-Leu (1.30) (acrylonitrile method). Hydrolysis with subtilisin gave: (Asx,Ser) (0.27); Lys-Leu (1.31). The probable sequence is therefore:

Lys-Asx-Ser-Lys-Leu

Peptide C26δB6H1. No *N*-terminal amino acid was detected. Hydrolysis with trypsin gave: Glx-(Ala₂,Asx,Cya,Glx,Leu₂,Lys₂,Mes)-Lys (0.40) (DNS and acrylonitrile methods); Glx-Tyr (0.53) (acrylonitrile method); Ala-Lys (1.44) (acrylonitrile method). The probable sequence is therefore:

Glx-(Ala₂,Asx,Cya,Glx,Leu₂,Lys₂,Mes)-Lys-Glx-Tyr

Peptide C26δB6H2. The *N*-terminus was glutamic acid or glutamine (DNS method). Hydrolysis

Peptide C28δB1. The *N*-terminus was Lys-Lys-Asx . . . (Edman-DNS method). Hydrolysis with trypsin gave: Asx-(Ala,Asx,Cya,Val)-Tyr (0.06) (DNS and acrylonitrile methods); Lys-Lys (1.75). Hydrolysis with concentrated hydrochloric acid at 37° for 72 hr. gave: Lys-(Asx,Lys) (1.68) (acrylonitrile method); (Asx₂,Lys,Val) (1.19); (Asx₂,Cya,Lys,Val) (0.69); (Asx,Val) (0.53) [This peptide (*R_F* 0.65) was separated from free tyrosine (*R_F* 0.30) in the solvent butan-1-ol-acetic acid-water (4:1:5, by vol.)]. Hydrolysis with 0.03M-hydrochloric acid gave: (Ala,Cya,Tyr) (0.07); Asx (0.62); Val (0.85); Lys-Lys was not observed (probably lost). The probable sequence is therefore:

Lys-Lys-Asx-Val-Asx-Cya-Ala-Tyr

Peptide C28εB4. The *N*-terminus was glutamic acid or glutamine (DNS method). Hydrolysis with subtilisin gave: (1) Ala-(Glx,Leu) (0.57) (DNS and acrylonitrile methods); (2) (Asx,Arg,Glx₂) (0.81); (3) (Asx,Glx₂) (0.37). Hydrolysis of subtilisin peptide (2) with 0.03M-hydrochloric acid gave: Asx (0.61); Glx (0.68); (Arg,Glx) (1.12; 0.0 at pH 6.5). Hydrolysis with concentrated hydrochloric acid at 37° for 72 hr. gave: Glx-Leu (0.73) (acrylonitrile method); Glx-Arg (1.20) (acrylonitrile method); Ala,Arg,Glx₂) (0.96). The probable sequence is therefore:

Glx-Asx-Glx-Arg-Ala-Glx-Leu

Peptide C29αB2. No *N*-terminus was detected. Hydrolysis with trypsin gave: (Cya,Leu)-Lys

(0.03); (Ala,Asx,Glx₂)-Arg (0.61); (Leu,Ser) (0.75); Glx-Lys (0.90). The probable sequence is therefore:

[(Cya,Leu)-Lys,Glx-Lys,(Ala,Asx,Glx₂)-Arg]-Ser-Leu

with trypsin gave: (Glx₃₋₄,Leu,Pro,Ser) (0.34); Glx-Arg (1.14) (acrylonitrile method). The probable sequence is therefore:

Glx-Arg-(Glx₂₋₃,Pro,Ser)-Leu

Peptide C27βB7. The *N*-terminus was Leu-Glx . . . (Edman-DNS method). Hydrolysis with trypsin gave: (Glx,Leu,Thr)-Lys [*R_{Leu}* in butan-1-ol-acetic acid-water (4:1:5, by vol.) 0.83]; Leu. Hydrolysis with concentrated hydrochloric acid at 37° for 48 hr. gave: Leu-Glx (0.69) (acrylonitrile method); Thr-(Leu,Lys) (1.10) (acrylonitrile method); Thr-Lys (1.31) (acrylonitrile method). The probable sequence is therefore:

Leu-Glx-Thr-Lys-Leu

Peptide C30εB2. The *N*-terminus was arginine (DNS method). Hydrolysis with trypsin gave: (Cya,Gly,Ile,Ser)-Tyr (0.05); Arg (1.30). Hydrolysis with subtilisin gave: (Cya,Tyr) (0.05); (Arg,Gly,Ile,Ser) (1.02). Hydrolysis with concentrated hydrochloric acid at 37° for 72 hr. gave: (Gly,Ile) (0.86); Arg-(Gly,Ile) (1.19) (acrylonitrile method); Arg-Gly (1.45) (acrylonitrile method); (Cya,Ser,Tyr) (0.56 at pH 6.5); (Cya,Ser) (0.83 at pH 6.5); (Cya,Tyr) (0.69 at pH 6.5). The probable sequence is therefore:

Arg-Gly-Ile-Ser-Cya-Tyr

Peptide C31αB7. The *N*-terminus was lysine

(acrylonitrile method). The probable sequence is therefore:

Lys-Leu-Leu

Peptide C31γB9. The *N*-terminus was Val-Asx-Leu-Asx . . . (Edman-DNS method). Hydrolysis with trypsin gave: (Leu,Val) (0.75); (Asx₂,Leu,Val)-Arg (0.83). Hydrolysis with concentrated hydrochloric acid at 37° for 48hr. gave: (Asx₂,Leu,Val) (0.53); Val-Leu (0.75) (acrylonitrile method); Val-(Arg,Asx₂,Leu) (0.85) (acrylonitrile method); Arg-(Leu,Val) (1.00) (acrylonitrile method). The probable sequence is therefore:

Val-Asx-Leu-Asx-Arg-Val-Leu

Peptide C33ηB1H2. The *N*-terminus was Ala-Glx . . . (Edman-DNS method). Hydrolysis with trypsin gave: (Asx,Glx₂,Tyr) (0.26); Ser-(Asx,Glx,-Leu)-Arg (0.80) (acrylonitrile method); Ala-(Glx,-Leu)-Arg (0.89) (acrylonitrile method). Hydrolysis with 0.03M-hydrochloric acid gave: Ala-(Arg,-Glx₂,Leu,Ser) (0.87) (acrylonitrile method); Asx (0.61); (Glx₂,Tyr) (0.68). The probable sequence is therefore:

Ala-Glx-Leu-Arg-Ser-Glx-(Asx,Leu)-Arg-Asx-Glx-Glx-Tyr

Peptide C34αB4H1. The *N*-terminus was leucine (DNS method). Hydrolysis with trypsin gave: (1) (Ala,Leu,Ser)-Tyr (0.40); (2) (Ala,Ser)-Tyr (0.49); (3) Leu-(Arg,Asx₂) (0.91) (acrylonitrile method). Hydrolysis of tryptic peptide (1) with concentrated hydrochloric acid at 37° for 24hr. gave: Ser-Tyr (0.67); (Ala,Leu,Ser) (0.84); (Ala,Ser) (0.95). The probable sequence is therefore:

Leu-Asx-Asx-Arg-Leu-Ala-Ser-Tyr

Peptide C34αB4H2. The *N*-terminus was leucine (acrylonitrile method). The probable sequence is therefore:

Leu-(Glx,Lys)-Tyr

Peptide C35βB3H. The *N*-terminus was Asx-Glx . . . (Edman-DNS method). Hydrolysis with concentrated hydrochloric acid at 37° for 72hr. gave: Asx-Glx (0.65) (DNS and acrylonitrile methods); (Ala,Arg,Thr) (1.07); Thr-Arg (1.25) (acrylonitrile method); Glx-Tyr (0.47). Hydrolysis with Pronase gave: Asx-(Glx,Thr) (0.60) (DNS method); Asx-Glx (0.68) (acrylonitrile method); Ala-Glx (0.77) (DNS and acrylonitrile methods); (Arg,Glx,Thr) (1.19); Tyr (0.50). The probable sequence is therefore:

Asx-Glx-Thr-Arg-Ala-Glx-Tyr

Peptide C35εB6. The *N*-terminus was Arg-Ala . . . (Edman-DNS method). Hydrolysis with trypsin

gave: (Ala₂,Asx,Glx₂,Thr)-Phe (0.24); Arg (1.30). Hydrolysis with Pronase gave: (Ala,Asx,Glx₂) (0.62); Asx-Glx (0.72) (acrylonitrile method); Ala-Glx (0.81) (acrylonitrile method); (Ala,Thr) (0.88); (Ala,Arg,Thr) (1.04). Hydrolysis with 0.03M-hydrochloric acid gave: Glx-Phe (0.57). The probable sequence is therefore:

Arg-Ala-Thr-Ala-Glx-Asx-Glx-Phe

Peptide C39αB5. The *N*-terminus was arginine (DNS method). The probable sequence is therefore:

Arg-Leu

This peptide gave a very weak ninhydrin reaction.

Peptide C39βB3. The *N*-terminus was not determined. Hydrolysis with trypsin gave: (1) (Ala,-Cya,Gly₂,Ser)-Arg (0.37); (2) Ser-Phe (0.68). Hydrolysis of tryptic peptide (1) with Pronase gave: Ala-(Cya,Gly,Ser) (0.03) (acrylonitrile method); Gly-Arg (1.43). Hydrolysis of tryptic peptide (1) with concentrated hydrochloric acid at 37° for 48hr. gave: Ser-Cya (0.04) (acrylonitrile method);

Ala-Gly (1.03) (acrylonitrile method); Gly-Arg (1.41). The probable sequence is therefore:

Ala-Gly-Ser-Cya-Gly-Arg-Ser-Phe

Peptide C40αB2. The *N*-terminus was glutamic acid or glutamine (DNS method). Hydrolysis with trypsin gave: (1) Glx-Leu (0.75) (DNS and acrylonitrile methods); (2) (Arg,Glx,Lys,Val) (1.25). Hydrolysis of tryptic peptide (2) with Pronase gave: Glx-Lys (1.28) (acrylonitrile method); Arg (1.33); Val (0.78). Hydrolysis of the original peptide with Pronase gave: (Glx,Leu (0.72); (Arg,Val) (1.22); (Arg,Glx) (1.14). The probable sequence is therefore:

Glx-Lys-Val-Arg-Glx-Leu

Peptide C44β. The *N*-terminus was Leu-Glx . . . (Edman-DNS method). Hydrolysis with trypsin gave: (Glx,Leu) (0.69); Leu-Glx-Lys (1.02) (DNS method); Val-Arg (1.23). Hydrolysis with Pronase gave: (Glx,Leu,Lys) (0.99); (Arg,Glx,Val) (1.16); Leu (0.77). Hydrolysis with concentrated hydrochloric acid at 37° for 72hr. gave: (Glx,Leu) (0.70); Lys-(Arg,Val) (1.59) (acrylonitrile method). The probable sequence is therefore:

Leu-Glx-Lys-Val-Arg-Glx-Leu

DISCUSSION

In the analysis of the tryptic peptides from fraction U.S.3, the number of residues of each

amino acid occurring in peptides isolated in yields exceeding a certain arbitrary value was compared with that expected from the overall amino acid composition of group T_r peptides (Table 5 of Corfield *et al.* 1967a), and the agreement of the two sets of results was taken as evidence that the selection of peptides contributing to the major component of fraction U.S.3 was well founded. For peptides from the chymotryptic digest, there was no such clear division into 'major' and 'minor' peptides. The complexity of the digest necessitated additional purification steps with consequent loss of material, and we have therefore listed in Tables 3 and 4 all the peptides isolated, irrespective of yield. The number of residues of each amino acid (omitting from the totals certain peptides that duplicate parts of the sequences of longer peptides) does nevertheless agree reasonably well with the expected values calculated from the overall amino acid composition of group C_r peptides when it is considered that the total number of residues involved (300) is considerably smaller than the corresponding total (425) for the tryptic peptides. The incomplete analysis of the chymotryptic digest precludes an estimate of the molecular weight of the main component of fraction U.S.3 analogous to that obtained from the tryptic digest. The incomplete splitting at leucine residues by chymotrypsin is exemplified by several of the peptide sequences found, and is probably the main reason for the increased complexity of the chymotryptic digest.

Corfield, Fletcher & Robson (1967b) summarized the reasons for suggesting that the protein from which fraction U.S.3 is derived constitutes a major proportion of the cortex of wool, and that it contains amino acid sequences of relatively high sulphur content and disordered structure linked to sequences that are predominantly α -helical. The regions of high sulphur content give rise to the γ -keratose fraction by peptide-bond fission when oxidized wool is extracted under alkaline conditions and subsequently fractionated. In the present work, the peptides of high cysteic acid content and net negative charge, liberated from the high-sulphur regions by enzymic attack, constitute the groups (T_u and C_u) that are not retarded by the resin. The aggregates of retarded peptides (T_r and C_r) exhibit a low sulphur content and a paucity of residues of amino acids known to destabilize α -helical segments in proteins. In Table 2 the amino acid compositions of the T_u and C_u groups and the T_r and C_r groups are compared respectively with that of γ -keratose (Corfield *et al.* 1958) and that of the α -helical material isolated by Pronase digestion of fraction SCMKA from reduced and carboxymethylated wool (Crewther & Harrap, 1965, 1967; Crewther *et al.* 1967). Although the T_r and C_r aggregates have a composition generally similar

to that of the helical material, they contain a higher proportion of the 'helicophobic' amino acids glycine, phenylalanine, serine, threonine and tyrosine, and a lower proportion of the 'helicophilic' amino acids glutamic acid and leucine (Prothero, 1968). This is not unexpected in view of the observation that digestion of fraction SCMKA with trypsin or chymotrypsin produces material of lower helical content than that obtained by digestion with Pronase (Crewther, Gillespie, Harrap, O'Donnell & Thompson, 1966).

Tentative overlaps between the amino acid sequences of tryptic and chymotryptic peptides from fraction U.S.3 are listed in Fig. 4. In two cases (peptides 1T/19 κ 2 and 1T/21 α 1) the sequence of a tryptic peptide has been modified on the basis of the sequence results from the chymotryptic digest, and in peptide 1T/19 ζ 2 we have inserted a glutamic acid or glutamine residue in accordance with the findings of Hosken, Moss, O'Donnell & Thompson (1968) (see also discussion in Corfield *et al.* 1967c). So long as the question of micro-heterogeneity of the major proteins of wool is still *sub judice* and the yields of peptides are not quantitative, only those sequences that actually overlap can be assumed to represent sequences present in a particular chain; in extensions to non-overlapping parts of the peptides variations in amino-acid sequence could occur. Sequence VIII in Fig. 4 may represent an N-terminal sequence, or non-specific splitting by one of the enzymes may have occurred.

This work has been done on a sample of wool heterogeneous in the sense that it was taken from a bale and not from an individual fleece. This can be criticized, but it may also be defended in an exploratory study on a material that, from a technological viewpoint, is always heterogeneous. In their evidence for sequence heterogeneity, Thompson & O'Donnell (1967) found no difference between wool from a single Merino fleece and wool from a flock of similar animals.

Recognized factors that might produce variations in the amino acid sequences of the protein chains in wool are summarized by Thompson & O'Donnell (1967). A possible example of such variation is exhibited by the two peptides C15 β B1 and C15 β B2, which have respectively the sequences Ala-Ser-Asx-Asx-Phe and Ala-Ala-Asx-Asx-Phe. The codon groups for alanine and serine differ by a single base. The two peptides may of course represent different and unrelated sections of polypeptide chain. The evidence for heterogeneity will obviously be stronger, the longer is the known sequence in which the altered residue is embedded. It is also apparent that factors as yet unknown that characterize differences in mechanism between the synthesis of fibrous and of globular proteins may introduce other sources of variability.

Fig. 4. Extended sequences from tryptic and chymotryptic peptides of fraction U.S.3. Peptide C25εB2 apparently contained three residues of Glx (see the text), but the structures of the remaining peptides and comparison with peptides N₉ and N₁₁ of Fell (1967) suggest that only two residues are present in this sequence.

Thompson & O'Donnell (1967) made a careful study of the fragments produced by cyanogen bromide cleavage of the major components (Components 7 and 8) extracted from reduced and carboxymethylated wool, and concluded that only a minor proportion of the macromolecular species present has an identical amino acid sequence. O'Donnell & Thompson (1968) found unambiguous evidence for the presence of two different but related *N*-terminal sequences in one of these components,

and there may be others (O'Donnell, 1968). However, the considerable number of amino acid residues that we have established in sequences has produced only one possible example of heterogeneity, that of the peptides C15βB1 and C15βB2 noted in the previous paragraph.

There is one other sequence in our results that has a bearing on the subject of heterogeneity: sequence VI in Fig. 4 comprising the peptides 1T/19ζ2, 1T/24ε4, 1T/24ζ3, C44β and C40αB2.

This sequence, which also occurs in Component 8 (Hosken *et al.* 1968; O'Donnell, 1969), contains a preponderance of amino acid residues that favour helix formation (Prothero, 1968), and thus probably originates in the helical regions of the structure. Of the contributing peptides, 1T/24 ϵ 4 and 1T/24 ζ 3 were isolated in yields (from 4.6 g. of fraction U.S.3) of 37 and 60 μ moles respectively (Table 4 of Corfield *et al.* 1967a) and C44 β in a yield of 22 μ moles. If the mean yield of these peptides is taken as 30 μ moles (1T/24 ζ 3, the dipeptide Val-Arg, could occur more than once in fraction U.S.3), then, by taking the molecular weight of fraction U.S.3 as 75 000, not less than 50% of fraction U.S.3 has its structure identical over at least this portion of the sequence. The yield of fraction U.S.3 from wool is 36%, and thus the corresponding value for wool is 18%. As the amount of helical material in wool is about 30% (60% of low-sulphur protein with a helix content of 50%) (Harrap, 1963; Bendit, 1968), our findings are consistent with the hypothesis that a substantial portion of the ordered material in wool has a unique structure.

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