3. The ultraviolet absorption spectra of the flavone glucosiduronic acids and of their aglycones have been compared.

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The Amino Acid Composition of Wool

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When the present work was started early in 1953 no complete amino acid analysis of a single wool sample had been published, although Graham, Waitkoff & Hier (1949) had estimated fourteen amino acid constituents of a wool hydrolysate, chiefly by microbiological methods. While this paper was in preparation, however, our attention was drawn to a paper by Simmonds (1954), who has completed the amino acid analysis of an Australian 64's quality wool. The wool sample whose analysis is reported here was of the same quality, and it is of interest, therefore, to compare the two analyses. For a survey of amino acid analyses of wool, previously published in the literature, reference should be made to Simmonds (1954).

EXPERIMENTAL

Chromatographic separations and estimations of amino acids

All the columns used for starch chromatography were of 0·9 cm. diameter and 30 cm. length and were prepared according to the directions of Stein & Moore (1948) from starch manufactured by Gordon Slater Ltd., Manchester. Six different types of chromatograms were employed in the analysis of wool hydrolysates. Leucine, isoleucine, phenylalanine and methionine were separated on starch columns eluted with benzyl alcohol–*n*-butanol–water (1:1:0·280, by vol.). The volume of water in the mixture was smaller than that used by Stein & Moore (1948) because the columns were run at 18°. The columns were loaded with 3·5 mg. of

hydrolysate and 0·5 ml. fractions were collected for the estimations of leucine, phenylalanine and isoleucine, but for the accurate estimation of methionine, which occurs to the extent of 0·5% in the hydrolysate, 10 mg. loads were applied and 1·5 ml. fractions collected. Thiodiglycol was added to the eluting solvent for the methionine estimations. This chromatogram is illustrated in Fig. 1 and referred to hereafter as starch I. Valine and tyrosine were separated from 2·5 mg. of wool hydrolysate on starch columns eluted with 17% (v/v) aqueous 0·57 N-HCl in *n*-butanol; 0·5 ml. fractions were collected (Stein & Moore, 1948). The valine peak coincided with that of methionine, and the methionine contribution to the combined peak was deducted to give the
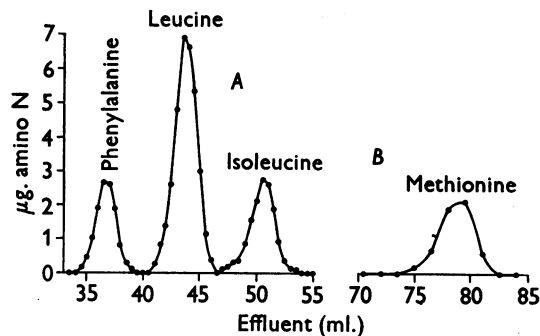

Fig. 1. Elution of amino acids from chromatogram starch I (Nin.) (see text). *A*, 3·5 mg. of wool hydrolysate for estimations of phenylalanine, leucine and isoleucine (0·5 ml. fractions); *B*, 10 mg. of wool hydrolysate for estimation of methionine only (1·5 ml. fractions).

valine content. The chromatogram is shown in Fig. 2 and referred to below as starch II. Tyrosine was also separated from 2 mg. of hydrolysate on a starch column eluted with 0·1N-HCl, and 0·5 ml. fractions were collected (Moore & Stein, 1949). This chromatogram is referred to as starch III. Columns loaded with 3 mg. of wool hydrolysate and eluted with n-propanol–0·5N-HCl (2:1, by vol.) were used for the separation of serine, glycine, arginine, lysine, histidine and cystine, 1 ml. fractions being collected (Moore & Stein, 1949). The arginine peak coincided with that of NH₃, but the latter was not estimated when the ⁶⁴Cu radiometric method of Blackburn & Robson (1953) was used to determine arginine. Although histidine was separated, it could not be accurately estimated in 3 mg. of hydrolysate. The chromatogram is shown in Fig. 3 and referred to later as starch IV. The amino acids separated on starch IV were also separated from 3 mg. of hydrolysate on a starch column eluted with n-butanol–n-propanol–0·1N-HCl (1:2:1, by vol.) until 82 ml. had been collected, when the solvent was changed to n-propanol–0·5N-HCl (2:1, by vol.) (Moore & Stein, 1949). 1 ml. fractions were collected. This chromatogram separates

arginine and NH₃, and is referred to as starch V. On chromatograms starch IV and starch V the alanine and glutamic acid peaks coincide, and threonine and aspartic acid are only partially separated. Experiments with various buffered solvent mixtures showed that good separations of proline, NH₃, threonine, alanine and glutamic acid could be achieved by eluting starch columns with n-butanol–n-propanol–citrate buffer (1:2:1, by vol.). The buffer (pH 3) was a mixture of 0·1 M disodium hydrogen citrate and 0·1N-HCl (2:3, by vol.). 3 mg. of wool hydrolysate were applied and 1 ml. fractions collected. This chromatogram is shown in Fig. 4 and referred to as starch VI. No satisfactory means of completely separating aspartic acid from the other amino acids in wool hydrolysates on starch was found, and the aspartic acid contents were obtained by subtracting the estimated amount of threonine from the combined aspartic acid and threonine peaks obtained on starch IV and starch V.

Histidine, owing to its sparsity in wool hydrolysates, cannot be accurately determined by starch chromatography, as amounts of wool hydrolysate in excess of 3 mg. prevent its complete isolation from lysine and cystine. However, Dowex-50 columns 0·9 cm. in diameter and 15 cm. in length, prepared and eluted according to Moore & Stein (1951), enabled 19 mg. of wool hydrolysate to be fractionated with excellent resolution of the histidine and lysine peaks. This chromatogram is illustrated in Fig. 5 and referred to as Dowex-50.
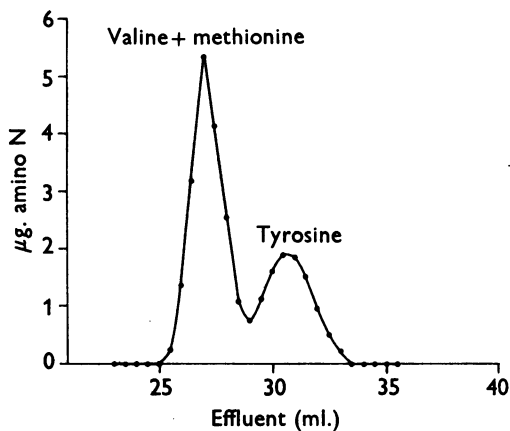

Fig. 2. Elution of valine, methionine and tyrosine from chromatogram starch II (Nin.) (see text). 2·5 mg. of wool hydrolysate; 0·5 ml. fractions.
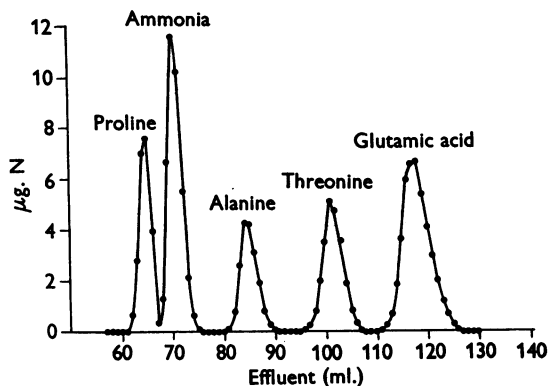
Fig. 4. Elution of proline, ammonia, alanine, threonine and glutamic acid from chromatogram starch VI (Nin.) (see text). 3 mg. of wool hydrolysate; 1 ml. fractions.

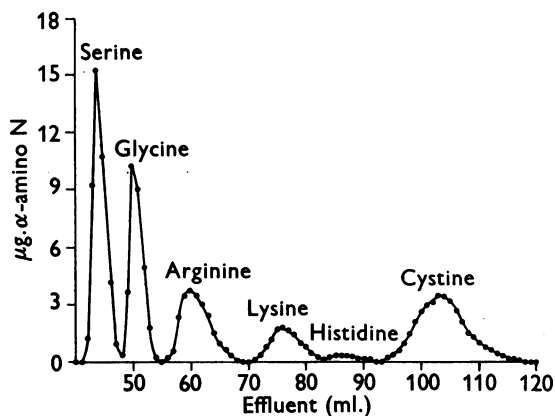Fig. 3. Elution of serine, glycine, arginine, lysine, histidine and cystine from chromatogram starch IV (⁶⁴Cu) (see text). 3 mg. of wool hydrolysate; 1 ml. fractions.
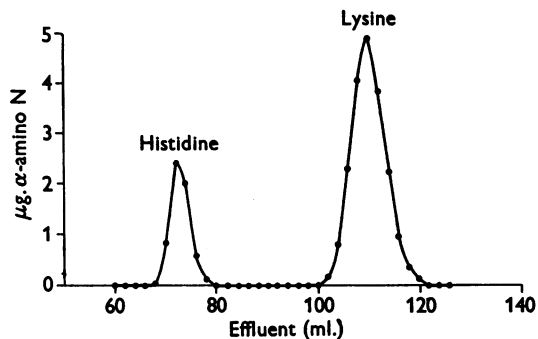

Fig. 5. Elution of histidine and lysine from chromatogram Dowex-50 (Nin.) (see text). 19 mg. of wool hydrolysate; 2 ml. fractions.

The recoveries of the amino acids from the seven different types of chromatogram, on separation from amino acid mixtures made to simulate a wool hydrolysate, were the same as those recorded by Moore & Stein, viz. $100 \pm 3 \%$ for all except glutamic acid and aspartic acid, which were recovered in 93 and 94 % yields, respectively. The recovery of aspartic acid was confirmed by starch chromatography with ^{14}C-labelled L-aspartic acid, when 94 % of the total activity was recovered. The only exception to the above was the $100 \pm 3 \%$ recovery of glutamic acid from starch VI, as there was no partial esterification of the acid in the buffered solvent.

Amino acid estimations were carried out in part by the photometric ninhydrin method of Moore & Stein (1948) and in part by the ^{64}Cu radiometric method of Blackburn & Robson (1953), as modified for use with starch chromatography by Corfield & Robson (1953). The latter method could not be used to estimate amino acids separated on starch I, as the solvent system prevented complete reaction between Cu^{2+} ions and amino acids and depressed the Cu^{2+} concentration over the copper phosphate suspension from 4×10^{-5}M to 2×10^{-6}M. Nor could it be used with fractions containing citrate, which forms complexes with Cu^{2+} ions. In all cases standard amino acid calibration curves were used, the data being obtained under reaction conditions identical with those for the column fractions. Calibration curves were repeated for each batch of ^{64}Cu because of changes in its specific activity, and also with different batches of ninhydrin reagent, which gave small variations in colour yields. All the amino acids used as standards were tested by filter-paper chromatography and elementary analysis, and shown to be free from amino acid impurities and from NH_3, sulphate and halide ions. The histidine and lysine standards were prepared from L-histidine monohydrochloride and L-lysine dihydrochloride respectively. In the presentation of the results, the chromatogram type has been stated with the abbreviated method of estimation in brackets, thus: starch IV (^{64}Cu); Dowex-50 (Nin.).

Other amino acid estimations

Tyrosine was estimated by the methods of Thomas (1944) and of Lugg (1937). Arginine was estimated by the method of Vickery (1940), glycine by the method of Alexander, Landwehr & Seligman (1945) and cystine by the method of Shinohara (1935). Serine and threonine were estimated by the method of Rees (1946).

Estimation of tryptophan

Tryptophan has proved to be the most difficult of all the amino acids in wool to estimate, as it is wholly or partially destroyed during hydrolysis. The methods of Lugg (1937), Brand & Kassell (1939), Sullivan & Hess (1944) and Spies & Chambers (1948, 1949) have been used in these laboratories, but the tryptophan determinations were extremely erratic.

No tryptophan is present in acid hydrolysates of wool, and the amounts present in alkaline hydrolysates vary with hydrolytic conditions. The only satisfactory method for tryptophan estimation was found to be that of Moore & Stein (1949) on chromatograms of the type starch III, where the tryptophan peak appears after tyrosine. 1 g. (dry wt.) samples of wool were sealed in Pyrex glass bulbs with 6·5 g. of recrystallized $Sr(OH)_2$, $8H_2O$ and 10·8 ml. of water, and heated in an oven at 100° for periods varying from 2 to 52 hr.

Strontium was precipitated as $SrSO_4$ and removed by filtration, together with small amounts of SiO_2, and the filtrates were concentrated to 10 ml. Samples (0·19 ml.) of the hydrolysates were fractionated on starch III chromatograms. There was no interference with the tryptophan peak by slow-moving peptides in the partial hydrolysates, but when the 2 hr. hydrolysate was examined, a small peak, probably due to a peptide, appeared in front of the tryptophan. The relationship between the tryptophan found and the time of hydrolysis is shown in Fig. 6. The amount of tryptophan in wool was found by extrapolating the latter part of the curve back to zero time as shown.

Decomposition of the amino acids of wool during acid hydrolysis

Samples (approx. 0·2 g.) of glycine, DL-alanine, DL-valine, DL-leucine, DL-isoleucine, DL-phenylalanine, DL-serine, DL-proline, L-arginine, L-cystine, DL-threonine, L-tyrosine, L-histidine hydrochloride, L-lysine dihydrochloride, DL-glutamic acid, DL-aspartic acid and DL-methionine were refluxed separately for 24 hr. with 25 ml. of 5N-HCl. The HCl was removed by vacuum distillation and the residues were dissolved in 50 ml. of water. The NH_3 contents of suitable samples of these solutions were determined with the apparatus of Markham (1942), using a suspension of $Mg(OH)_2$ to liberate NH_3. Other samples were made alkaline and aerated to remove any NH_3, brought to pH 5 with HCl, and their amino acid contents estimated by the photometric ninhydrin method (Moore & Stein, 1948).

Total N and ammonia

The total N contents of conditioned wool samples were determined (Chibnall, Rees & Williams, 1943). Digestion was for 16 hr. and twelve determinations were made. For the determination of amide N about 1 g. of conditioned wool was refluxed with 50 ml. of 2N-HCl until the wool dissolved completely (usually 5 hr.). The solution was partly neutralized with 5 g. of KOH and diluted to 500 ml. 5 ml. of this solution were taken for estimation of NH_3 in the Markham apparatus as above. The NH_3 contents of 10 ml. samples of wool hydrolysates were likewise determined.
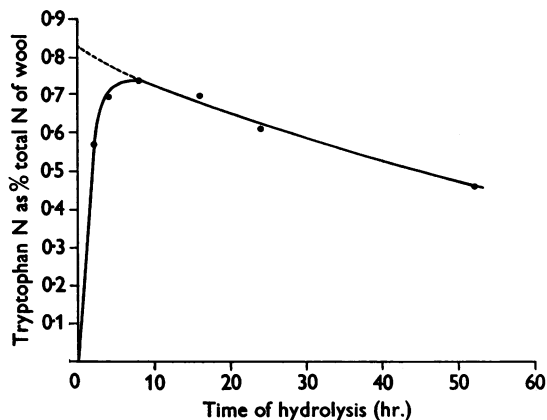

Fig. 6. Curve showing the amounts of tryptophan found after alkaline hydrolysis of wool for varying periods of time (see text).

Total S, ash and moisture

The total S of a conditioned wool sample was determined by the Benedict-Denis method (Barritt, 1934 a). A weighed sample of conditioned wool was ashed to constant weight in a Pt crucible at 800°. To determine dry matter, a weighed sample of conditioned wool was heated to constant weight in an oven at 102°.

Wool sample and hydrolysis procedure

A quantity (6 lb.) of Australian Merino 64's quality virgin wool was 'tipped' by cutting off the top third of each lock of fibres to remove wool degraded by weathering. The root wool was extracted 6 times with the azeotropic mixture of benzene and methanol at 58·4°. After drying in air, each lock was washed separately in water at 45°, the bulk of the water removed in a centrifugal drier, and the wool air-dried for two days. Adhering vegetable matter was removed by combing the locks through a clean steel comb. The locks were drawn out by hand into slivers, gilled into one long sliver and chopped into pieces 1/32 in. in length on a special guillotine. After removing the last traces of grease by Soxhlet extraction with benzene–methanol, the wool was thoroughly stirred with distilled water (liquor to wool ratio, 10:1), and the water decanted.

The washing procedure was repeated 5 times and the wool finally separated on a large sintered glass filter. After drying in an oven at 40° to a water regain of about 8%, the sample was stored in stoppered bottles in the dark until required. Before use, a bottle was unstoppered and the wool conditioned for 1 week in a humidity room controlled at 15·5° and 65% relative humidity. Every time a sample was taken for analysis, a similar sample was removed for moisture-content determination.

With the exception of the hydrolysates prepared for tryptophan determinations, all hydrolysates were prepared by refluxing 1 g. (dry wt.) of wool for 24 hr. with 20 ml. of 5 N-HCl, removing HCl in vacuo and making the residue up to 10 ml. with water.

RESULTS

The results of all the amino acid analyses are recorded in Table 1. Where amino acids were estimated by both the radiometric method and the ninhydrin method, the results show good agreement. The colour yields obtained for all the amino acids by the ninhydrin method were in accord with those of Moore & Stein (1948, 1949, 1951) except in the solvent mixture n-butanol–benzyl alcohol–water (1:1:0·280, by vol.) in which the colour yields of phenylalanine, leucine and isoleucine were low by 9, 9 and 6% respectively. Three different samples of DL-leucine, three of DL-isoleucine and two of DL-phenylalanine, and recrystallized specimens of these amino acids, were examined, but no variation in colour yield greater than 0·5% was observed. Purification of the solvents used made no difference to the colour yields.

The results of experiments carried out to determine the amounts of decomposition of the amino acids of wool during hydrolysis are shown in

Table 2. Only serine, threonine, phenylalanine and arginine decomposed, and the arginine decomposition was insignificant. Threonine showed 8·3% decomposition when refluxed for 24 hr. with 5 N-HCl, but only 3·5% loss could be accounted for as ammonia, on the basis of one mole of ammonia formed for one mole of threonine decomposed. This is contrary to the findings of Rees (1946), but agrees with recent work by Phillips (1954), who found that threonine decomposed to the extent of 7–9% with the liberation of 3% of its nitrogen as ammonia, under hydrolytic conditions slightly different from those we employed.

The ash content of the wool sample was 0·18%, its total nitrogen content 16·35%, its amide nitrogen content 1·10% and the average ammonia-nitrogen content of its acid hydrolysates 1·40%.

DISCUSSION

The estimations of serine and threonine by the method of Rees (1946), of tyrosine by the methods of Lugg (1937) and Thomas (1944), and of arginine by the method of Vickery (1940) were in good agreement with the chromatographic estimations, but glycine estimated according to Alexander et al. (1945) gave a value 15% lower than the chromatographic figure. In this case the latter is considered to be nearer the true value, since complete separation of glycine from the hydrolysate removes possible sources of interference with its estimation. The chromatographic estimation of cystine was 20% lower than the amount found by the method of Shinohara (1935), and if the latter estimate is correct the 3·38% sulphur content of the wool sample can be wholly accounted for as methionine sulphur and cystine sulphur. This is in accord with similar analyses made in these laboratories and elsewhere (Rimington, 1929; Barritt, 1934 b; Bailey, 1937; Cuthbertson & Phillips, 1945). On the other hand, Stein & Moore (1949) found that the cystine contents of hydrolysates of β-lactoglobulin and bovine serum albumin were 10% lower than cystine determined by other methods, and the figure for cystine in wool hydrolysates found by Simmonds (1954) using ion-exchange resin chromatography (Moore & Stein, 1951) was 38% lower than the amount he found by Shinohara's method. Stein & Moore (1949) stated that their cystine results may be low because of the differences in times of hydrolysis of the two samples. However, in our experience with wool hydrolysates, the Shinohara method gives the same cystine value for times of hydrolysis from 4 to 24 hr. Another method for cystine estimation as, for example, an isotope-dilution method, would furnish independent analyses and help to resolve the problem. Such a method is being investigated in our laboratories at

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Table 1. *Amino acid analyses of wool hydrolysates*

Amino acid	Hydrolysate	Method of separation and estimation	No. of estimations	Amino acid N (as % total N)	Average amino acid N (as % total N)	Amino acid/100 g. wool
Alanine	A	Starch VI (Nin.)	3	4·05±0·04		
	B	Starch VI (Nin.)	3	4·20±0·04	4·12	4·3
Arginine	A	Starch V (Nin.)	2	19·4±0·4		
	B	Starch V (Nin.)	2	18·8±0·2		
	C	Starch IV (⁶⁴Cu)	1	19·2	19·1	9·8
	D	Starch IV (⁶⁴Cu)	1	19·6		
	F	Vickery (1940)	2	21·2±0·2		
	G	Vickery (1940)	2	20·1±0·1	20·6	10·4
Aspartic acid	A	Starch V (Nin.)	2	4·15±0·07		
	A	Starch V (⁶⁴Cu)	1	4·51		
	B	Starch V (Nin.)	2	4·54±0·19	4·38	6·8
	B	Starch V (⁶⁴Cu)	1	4·35		
Cystine	C	Starch IV (⁶⁴Cu)	1	7·26		
	E	Starch IV (⁶⁴Cu)	1	6·73		
	D	Starch IV (⁶⁴Cu)	1	7·50		
	E	Starch IV (Nin.)	1	7·67	7·30	10·3
	A	Starch IV (Nin.)	2	7·52±0·02		
	A	Starch V (Nin.)	1	6·83		
	A, B, H, I, J, K	Shinohara (1935)	6	8·73±0·04	8·73	12·3
Glutamic acid	A	Starch VI (Nin.)	3	8·53±0·17		
	B	Starch VI (Nin.)	3	8·44±0·16	8·48	14·5
Glycine	C	Starch IV (⁶⁴Cu)	1	6·14		
	D	Starch IV (⁶⁴Cu)	1	6·61		
	A	Starch IV (Nin.)	2	6·55±0·39		
	B	Starch IV (Nin.)	1	6·35	6·29	5·5
	A	Starch V (Nin.)	2	5·97±0·12		
	B	Starch V (Nin.)	2	6·28±0·15		
	D	Alexander *et al.* (1945)	6	5·48±0·05		
	E	Alexander *et al.* (1945)	5	5·43±0·08	5·45	4·8
	C	Alexander *et al.* (1945)	6	5·45±0·10		
Histidine	A	Dowex-50 (Nin.)	1	1·87		
	B	Dowex-50 (Nin.)	3	1·93±0·01	1·91	1·2
Isoleucine	E	Starch I (Nin.)	1	2·50		
	D	Starch I (Nin.)	3	2·40±0·06	2·44	3·7
	C	Starch I (Nin.)	2	2·48±0·06		
Leucine	E	Starch I (Nin.)	1	5·90		
	D	Starch I (Nin.)	3	5·86±0·06	5·85	8·9
	C	Starch I (Nin.)	2	5·80±0·16		
Lysine	B	Starch V (Nin.)	2	3·74±0·04		
	C	Starch IV (⁶⁴Cu)	1	4·32		
	D	Starch IV (⁶⁴Cu)	1	3·67		
	E	Starch IV (⁶⁴Cu)	1	3·91		
	E	Starch IV (Nin.)	1	3·86		
	A	Starch IV (Nin.)	2	3·70±0·06	3·92	3·3
	B	Starch IV (Nin.)	1	3·77		
	A	Starch V (Nin.)	1	4·26		
	A	Dowex-50 (Nin.)	1	4·05		
	B	Dowex-50 (Nin.)	1	4·21		
Methionine	D	Starch I (Nin.)	1	0·314		
	A	Starch I (Nin.)	3	0·320±0·008	0·32	0·56
	B	Starch I (Nin.)	2	0·318±0·018		
Phenylalanine	E	Starch I (Nin.)	1	2·20		
	D	Starch I (Nin.)	3	2·02±0·04	2·07	4·0
	C	Starch I (Nin.)	2	2·11±0·02		
Proline	A	Starch VI (Nin.)	3	4·96±0·03		
	B	Starch VI (Nin.)	1	5·04		
	A	Starch V (⁶⁴Cu)	1	5·18		
	B	Starch V (⁶⁴Cu)	1	5·35	5·05	6·8
	A	Starch V (Nin.)	2	5·03±0·15		
	B	Starch V (Nin.)	2	4·98±0·15		

Table 1 (cont.)

Amino acid	Hydrolysate	Method of separation and estimation	No. of estimations	Amino acid N (as % total N)	Average amino acid N (as % total N)	Amino acid/100 g. wool
Serine	L	Rees (1946)	1	8·41	8·07	9·9
	M	Rees (1946)	5	8·00±0·12		
	C	Starch IV (^{64}Cu)	1	8·51		
	D	Starch IV (^{64}Cu)	1	7·97		
	A	Starch IV (Nin.)	2	8·14±0·06	7·87	9·7
	B	Starch IV (Nin.)	1	7·77		
	A	Starch V (Nin.)	2	7·51±0·06		
	B	Starch V (Nin.)	2	7·66±0·08		
Threonine	A	Starch VI (Nin.)	3	4·74±0·16	4·71	6·5
	B	Starch VI (Nin.)	3	4·69±0·04		
	L	Rees (1946)	2	4·55±0·05	4·56	6·3
	M	Rees (1946)	5	4·56±0·02		
Tryptophan	See text	Starch III (Nin.)	See text	—	0·82	0·94
Tyrosine	A	Starch III (^{64}Cu)	1	2·57		
	B	Starch III (^{64}Cu)	1	2·66	2·62	5·5
	A	Starch III (Nin.)	2	2·64±0·01		
	B	Starch III (Nin.)	2	2·62±0·09		
	N	Lugg (1937)	8	2·83±0·03	2·79	5·9
	O	Lugg (1937)	8	2·75±0·03		
	P	Thomas (1944)	3	2·63±0·01		
	Q	Thomas (1944)	2	2·65±0·01	2·64	5·6
	R	Thomas (1944)	2	2·63±0·03		
Valine	A	Starch II (^{64}Cu)	1	4·24		
	B	Starch II (^{64}Cu)	1	4·21		
	E	Starch II (Nin.)	3	4·20±0·04	4·16	5·7
	D	Starch II (Nin.)	3	4·08±0·06		
	C	Starch II (Nin.)	2	4·15±0·02		

Table 2. *Losses of amino acids during acid hydrolysis*

For further details see text.

Amino acid	Recovery (%)	Ammonia formed (N as % total N)
Serine	90·8	9·1
Threonine	91·7	3·5
Phenylalanine	97·6	0·5
Arginine	100	0·3

present. Although the chromatographic estimation of 10·3 g. cystine/100 g. wool cannot account for the total sulphur in wool we prefer to record it as being nearer the true figure than the Shinohara value.

The results of the present amino acid analysis of wool are given in Table 3, together with the analyses by Simmonds (1954) and by Graham *et al.* (1949). The figures for serine, threonine and phenylalanine shown in Table 3 have been corrected by the factors 100/90·8, 100/91·7 and 100/97·6, respectively, in accordance with the losses of these amino acids in recovery experiments (Table 2). Whilst the correction factors are probably too low, because there is evidence, at least for serine, that amino acid residues decompose more rapidly when combined than in the free state (e.g. Corfield & Robson, 1953), the exact amounts of decomposition cannot be determined.

There is an excess of ammonia in wool hydrolysates over that due to amide hydrolysis, but this cannot confidently be used as a measure of the decomposition of threonine, serine and phenylalanine as there is some uncertainty in the amide ammonia estimation. It is certain, however, that the whole of the excess ammonia cannot be accounted for if the rates of decomposition of serine, threonine, phenylalanine and arginine are no greater during the hydrolysis of wool than under the conditions of the experiments in Table 2.

The agreement between our amino acid analyses and those of Simmonds (1954) is not so close as might have been expected in view of the fact that they were both obtained from samples of Australian 64's quality wool. In particular, his estimations of alanine, histidine, isoleucine, leucine, lysine, phenylalanine and valine are 20 % or more lower than our figures. The nitrogen balances of the two analyses are both close to 100 %, but that of Simmonds includes the nitrogen from two unidentified peaks he found on his chromatograms, one of which is probably due to hydroxylysine. This amino acid has been found previously in wool hydrolysates by Van Slyke, Hiller & McFadyen (1941) and its presence confirmed by Middlebrook (1949), but it comprises only 0·18 % of the wool. No unidentified peaks were found on starch chromatograms, but it is

Table 3. *Amino acid analyses of wool
by various authors*

N as % total N of wool

Amino acid	Simmonds (1954)	Present work	Graham, Waitkoff & Hier (1949)
Alanine	3·51	4·12	—
Amide N	7·46*	6·73	—
Arginine	20·3	19·1	21·1
Aspartic acid	4·24	4·38	4·7
Cystine	7·93 (4·95)	7·30	9·9
Glutamic acid	8·58	8·48	9·2
Glycine	5·80	6·29	—
Histidine	1·46	1·91	1·8
Isoleucine	1·97	2·44	3·0
Leucine	4·90	5·85	5·3
Lysine	3·25	3·92	3·9
Methionine	0·39	0·32	0·4
Phenylalanine	1·75	2·12†	2·1
Proline	5·33	5·05	6·1
Serine	7·25	8·66†	—
Threonine	4·61	5·12†	4·8
Tryptophan	1·73	0·82	—
Tyrosine	2·97	2·62	2·7
Valine	3·57	4·16	4·2
Unknown (1)	1·18	—	—
Unknown (2)	0·71	—	—
Total	98·89	99·39	79·2

* Uncorrected for decomposition of serine and threonine during hydrolysis.
† Corrected for loss during hydrolysis.

possible that an unknown, present in small amount, might have coincided with a known amino acid peak and thereby escaped detection. The total N and total S contents of the two wool samples are also different.

On the whole our analyses agree well with those of Graham *et al.*, the agreement for five amino acids being within ± 5 % and for ten within ± 10 %, with larger discrepancies in the estimations of proline, isoleucine, cystine and methionine. This last only occurs to the extent of 0·5–0·7 % in wool and is consequently difficult to determine accurately, and the large discrepancies between cystine estimations obtained by different methods have already been mentioned.

As Simmonds has pointed out, wool is almost certainly not a homogeneous protein, and differences in the amino acid composition of different wool samples are to be expected. This contention is supported by the analytical data shown in Table 3.

SUMMARY

1. The amino acid composition of a sample of Australian Merino 64's quality wool has been determined.
2. The results obtained have been compared with recent amino acid analyses of wool.

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