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On Isolation and Amino Acid Composition of "β-Globulin" Extracted from the Seeds of Barley (*Hordeum vulgare*) *

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Experiments on the fractionation of the nitrogenous components extracted from barley seeds were begun by Osborne in 1895 [1]. This work was continued at the Carlsberg Laboratory in the years 1906–1916 by Schjerning [2] and later on by Bishop in England [3]. Bishop divided the nitrogenous components into four fractions:

1) a leucosin or albumin fraction,
2) an edestin or globulin fraction,
3) a hordein or alcohol soluble fraction, and
4) a glutelin or insoluble fraction.

During the second world war these English results have been critically examined by a group of investigators at "l'École de Brasserie de Nancy" in France under the leadership of Urion [4].

In the last few years Biserte and Scriban [5] from the University of Lille in France have made some amino acid analyses by paper chromatography on different fractions during the brewing process. But they have not tried to analyse the different native proteins in barley.

The present investigation is a continuation of a research program carried out by Quensel [6] at the Physico-chemical Institute in Upsala, Sweden, in the years 1936–1942.

Quensel restricted himself to investigations dealing with the globulins in barleyextracts, and he introduced ultracentrifugation and electrophoresis experiments as purity tests for the different fractions obtained after "salting

* A preliminary report on this work was given on the "7. nordiske kemikermøde, Helsingfors 21–25 august, 1950".

The final paper was presented at the "XIIth International Congress of Pure and Applied Chemistry, New York, September 10 to 13, 1951".

+ limb just before start.

+ limb 287 minutes after start.

Fig. 1. Electrophoresis diagram of β-globulin from barley.

Phosphate buffer, pH 7.05
Ionic strenght: 0.1
Potential gradient:
6.47 volt/cm
Mobility: $u = 3.5$ cm/voltsec.

out" by ammonium sulphate. He succeeded in fractionation of the globulin fraction in four components characterized by different sedimentation velocity and different electrophoretic mobility, and he named them α, β, γ, and δ-globulin after increasing molecular weight.

The molecular weights of these globulins were found to be respectively 26 000, about 100 000, 166 000 and about 300 000. Further progress along this line has been made in Sweden by Säverborn [7], Danielsson [7,8] and Sandegren [9].

EXPERIMENTAL

We have isolated β-globulin from a 1 molar sodium chloride extract of 2 000 g of finely ground barley grains of the type "Kenia" by adding ammonium sulphate to 15/100 of saturation. The precipitate has been purified by four purification cycles, each consisting of a resolution of the precipitate in 1 molar sodium chloride and a new precipitation by 15/100 of saturation with ammonium sulphate. The preparation is then dialysed and after a final precipitation is stored at $-10°$ C.

Quensel never succeeded in the isolation of pure β-globulin from barley. The preparations always were contaminated by α-globulin [6]. We have examined the purity of our preparation by the three following methods: diffusion, electrophoresis and ultracentrifugation.

The diffusion constant is found to be $D_A = 4.8_4$ when calculated by the "area method", and to be $D_M = 4.9_7 \cdot 10^{-7}$ cm^2/sec when calculated by the "second moment method". Identity of the two results would indicate homogenity of the preparation from the point of view of molecular weight.

The electrophoretic mobility is found to be $u = 3.5 \cdot 10^{-5}$ cm^2/volt sec at pH 7.05, and the peak is homogenous through the whole experiment.* See Fig. 1.

Quensel found the mobility $u = 3.6 \cdot 10^{-5}$ at pH 7.02 [6].

The sedimentation constant in 0,2 N NaCl is found to be 6.7 at 24° C.

* Apparatus placed at our disposal in the Carlsberg Laboratory by kind permission of Professor K. Linderstrøm-Lang.

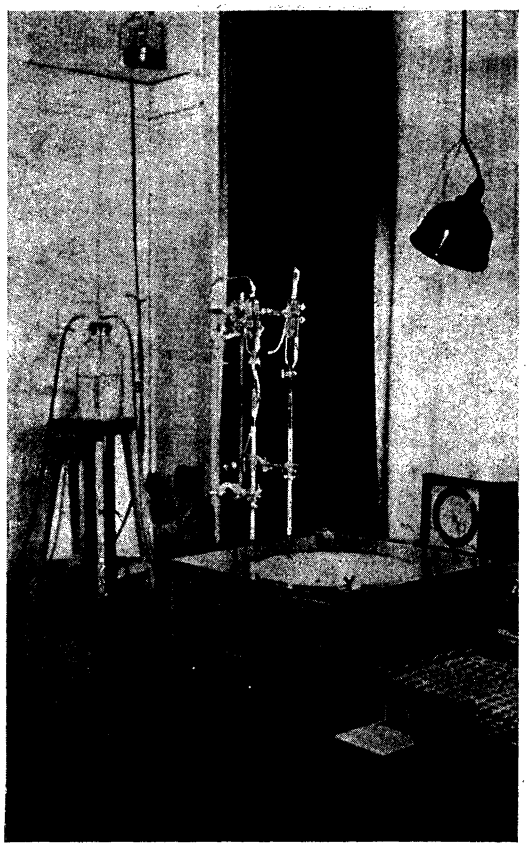

Fig. 2. Set-up for fraction collection from starch columns. A constant air pressure over the reservoirs for the solvents placed on top of the starch columns is maintained through a rubbertube connection to the lower one of the two leveling flasks. Seen to the left in the figure. The pressure corresponds to the difference in level between the two flasks. The fraction collector is constructed in the Carlsberg Laboratory by Professor K. Linderstrøm-Lang and M. Ottesen. For further details, see text.

It seemed to us that we had succeeded in isolating β-globulin from barley seeds in a practically pure state, and we decided to submit this preparation to amino acid analysis. We have chosen the method of Moore and Stein using starch chromatography [10].

The hydrolysis is performed in 6 *N* hydrochloric acid. It is boiled for 24 hours with reflux, and the hydrochloric acid is then removed by evaporation to dryness two or three times. The final residue (consisting of the amino acids) is dissolved in 2 ml of water and Kjeldahl-N determined on an aliquote by a micro method. From this it is calculated how many microliters we have to transfer to the column in order to have about two milligrams of the amino acids. This amount is directly transferred to the surface of the column by a Carlsberg micropipette. The starch column is placed over an automatic fraction collector shown in Fig. 2.

This machine has been constructed in the Chemical Department of the Carlsberg Laboratory. The movement of the circular test tube rack is electromagnetically controlled by an impulse from an electric clock that can be adjusted to give an impulse every five minutes or every multiple of five minutes. In our case it has been adjusted to give an impulse every thirty minutes which means that a new tube is placed under the starch column every half hour.

Fig. 3.

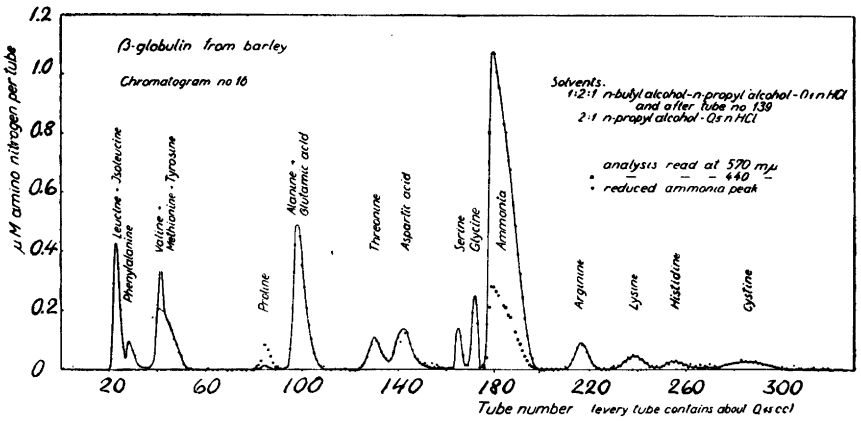

Table 1. Chromatographic analysis of hydrolysates of β-globulin from barley.
Solvents: 1 : 2 : 1 n-butyl alcohol — n-propyl alcohol — 0.1 N HCl and 2 : 1 propyl alcohol — 0.5 N HCl.

Preparation and chromatogram no. / Constituent	N as per cent of Kjeldahl-N						Average	Standard deviation (in relative per cent)
	1st hydrolysate			2nd hydrolysate				
	7	11	16	17	22	23		
Leucine + isoleucine	11.20	10.41	10.45	10.15	9.52	9.06	10.13	± 3
Phenylalanine	2.55	2.68	3.11	2.99	2.37	2.28	2.66	± 5
Valine, methionine and tyrosine	13.60 ca	13.21 ca	14.03 ca	12.45 ca	10.52 ca	13.05 ca	12.81 ca	± 4
Proline	9.91	8.54	11.04	—	12.61	13.34	11.09	± 8
Glutamic acid and alanine	19.09 ca	16.29 ca	21.62 ca	18.85 ca	16.82 ca	17.75 ca	18.40 ca	± 4
Threonine	4.48	3.91	5.29	3.87	4.51	3.62	4.28	± 6
Aspartic acid	6.90	6.02	—	—	6.93	5.74	6.40	± 4
Serine	3.44	—	3.68	—	4.25	3.02	3.60	± 7
Glycine	5.07	—	5.71	—	6.87	4.04	5.42	± 11
Ammonia	17.1 ca	13.71	(13.71)	(13.71)	(13.71)	(13.71)	(13.71)	—
Arginine		5.37	5.26	—	7.31	—	5.98	± 11
Lysine	—	10.40 ca	3.05	—	2.51	—	2.78	± 10
Histidine	—		2.51	—	3.70	—	3.11	± 19
Cystine	—		9.09	—	5.89	—	7.49	± 21
						Sum	107.9 %	± 3 %

The different types of solvent mixtures specified by Moore and Stein [11] are used for separating the amino acids.

We first used *n*-butyl alcohol — *n*-propyl alcohol — 0.1 *N* hydrochloric acid in the proportion 1 : 2 : 1 followed by *n*-propyl alcohol — 0.5 *N* hydrochloric acid in the proportion 2 : 1 when the aspartic acid-peak has appeared. An example of the resulting effluent-concentration-curve is given in Fig. 3.

Fig. 4.

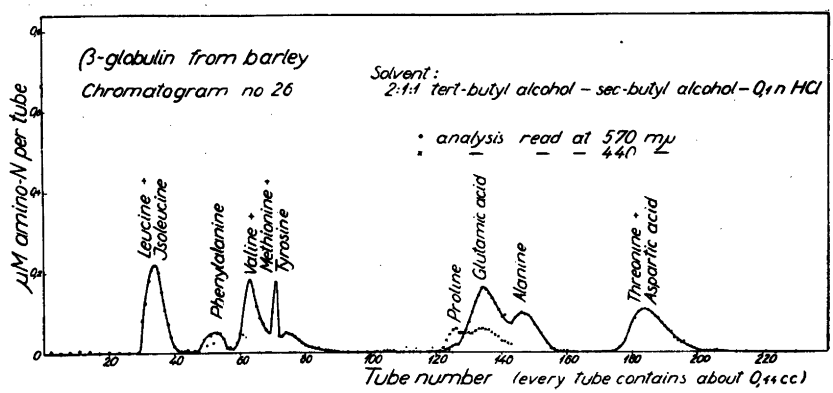

Table 2. Chromatographic analysis of hydrolysates of β-globulin from barley.

Solvent: 2 : 1 : 1 tert.-butyl alcohol — sec.-butyl alcohol — 0.1 N HCl.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N			Average	Standard deviation (in relative %)
	1st hydrolysate	2nd hydrolysate			
	14	26	27		
Glutamic acid	10.46	11.92	12.70	11.69	± 5
Alanine	6.89	7.47	6.95	7.10	± 3

It is in all respects similar to the curve originally obtained by Moore and Stein [11] in experiments with a synthetic mixture containing 17 amino acids and ammonium chloride. The general appearance of the curve and the approximate position of the peaks are the same. There are no peaks in the figure which cannot be ascribed to the common amino acids. No evidence, therefore, has been obtained for the existence in β-globulin hydrolysates from barley of unsuspected amino nitrogen containing constituents. The abnormal height of the ammonia peak has to be ascribed to a content of ammonium sulphate in this hydrolysate. This preparation had not been submitted to dialysis after the last purification cycle. But the location of this abnormal high ammonia peak together with the position of the proline peak (it has a yellow colour) gives a confirmation of the correctness of the names assigned to the peaks in Fig. 3.

As a result of the principle in our fraction collector, that is working on a time basis, the ordinates in our effluent diagrams will not be in concentration units as in the diagrams of Moore and Stein [11] which were obtained by using a fraction collector working on a volume basis. In our diagrams the ordinates state the amount of amino nitrogen per tube.

As proposed by Moore and Stein we have chosen leucine as the reference substance, and in the calculations we have used the colour factors given by them. In Table 1 we have collected the results from 6 diagrams of the type just mentioned.

Fig. 5.

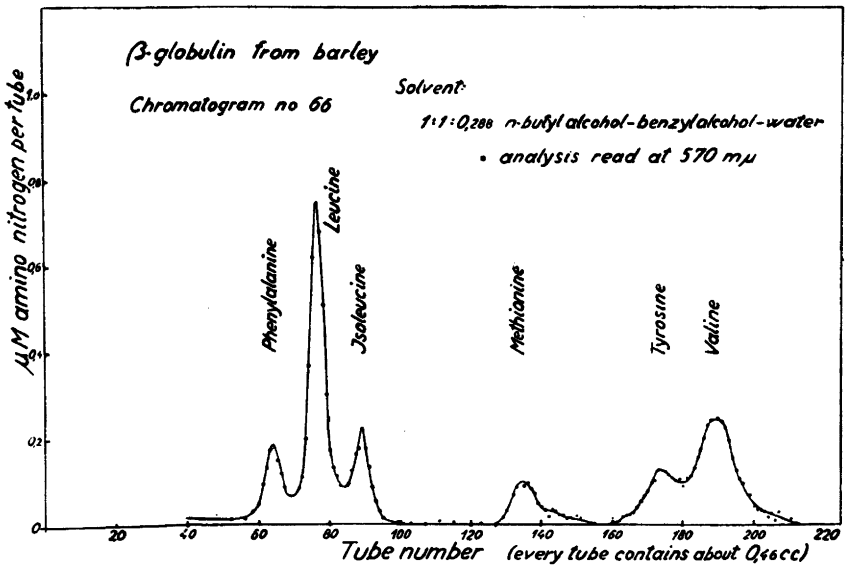

Table 3. Chromatographic analysis of hydrolysates of β-globulin from barley.
Solvent: 1 : 1 : 0.288 n-butyl alcohol — benzylalcohol — water.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N				Standard deviation (in relative %)
	2nd hydrolysate			Average	
	66	67	68		
Phenylalanine	3.34	2.89	3.16	3.13	± 4
Leucine	10.30	10.50	10.10	10.30	± 1
Isoleucine	3.29	4.50	4.16	3.98	± 9
Methionine	2.05	2.08	2.03	2.05	± 0.5
Tyrosine	4.18	4.35	4.72	4.42	± 4
Valine	7.82	8.14	7.49	7.82	± 2

They give the nitrogen content in every amino acid in per cent of the total Kjeldahl-nitrogen in the hydrolysate.

The last four columns give the results from a hydrolysate containing extra ammonia (2. hydrolysate); the first two columns result from a hydrolysate without extra ammonia (1. hydrolysate). In column 7 the average of the results is given, and in the last column the standard deviation in relative per cent for these results is stated.

The data from this type of diagrams have to be combined with results obtained by chromatograms using other solvent mixtures to give a more complete picture of the hydrolysate. This will now be described.

Fig. 6.

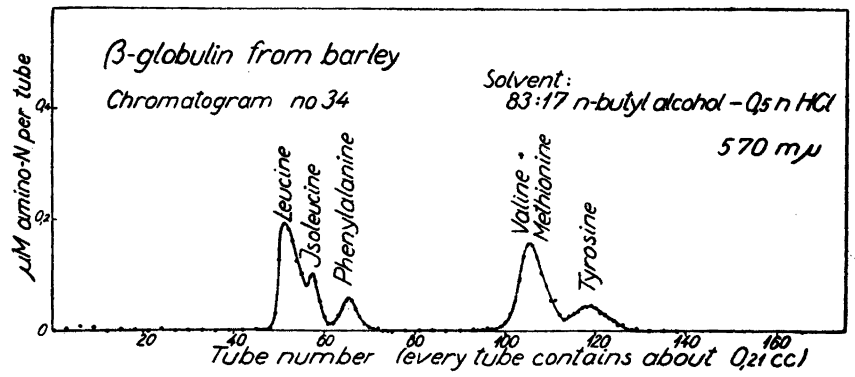

Table 4. Chromatographic analysis of hydrolysates of β-globulin from barley.

Solvent: 83 : 17 n-butyl alcohol — 0.5 *N* HCl.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N				Standard deviation (in relative %)
	1st hydrolysate	2nd hydrolysate		Average	
	35	34	36		
Leucine	8.27	7.70	8.07	8.01	± 2
Isoleucine	2.95	2.08	1.81	2.28	± 25
Phenylalanine	3.04	2.49	2.35	2.63	± 8
Valine and methionine	8.65	8.61	8.72	8.66	± 0.04
Tyrosine	5.16	3.69	4.81	4.55	± 10

Glutamic acid and alanine have been separated by tert-butyl alcohol — sec-butyl alcohol — 0.1 *N* hydrochloric acid in the proportion 2 : 1 : 1 *Cf.* [11].

Fig. 4 shows a diagram of this type. Although the glutamic acid and the alanine peaks are not fully separated in our diagrams, an estimation of the amount of the two components can be made. Of course, the contribution from the proline peak, not separated from the glutamic acid by this solvent has to be subtracted in the calculations.

The results from three chromatograms of this type are collected in Table 2.

This table is arranged as the previous table. In column 4 the mean values are given, and in the last column the standard deviation in relative per cent for these results is shown.

For the separation of the six fast moving amino acids on the 1 : 2 : 1-diagram, that is: leucine, isoleucine, phenylalanine, valine, methionine and tyrosine, Stein and Moore [12]

propose *n*-butyl alcohol — benzyl alcohol — water, mixed in the proportions 1 : 1 : 0.288, as the solvent most able to separate all of them. Fig. 5 gives an example of our result with this solvent. The results from three chromatograms of this type are collected in Table 3.

For the separation of these six amino acids we have also tried to use another solvent proposed by Moore and Stein [12]. It is a mixture of *n*-butyl alcohol — 0.5 *N* hydrochloric acid in the proportions 83 : 17. When used on our hydrolysates it gives a curve as shown in Fig. 6. It has only one drawback: it cannot separate valine and methionine. The results from three chromatograms of this type are collected in Table 4.

When we summarize the results from these four types of solvent mixtures represented by the values in Table 1 to Table 4, we have found the values shown in the first column of Table 5.

As before, the nitrogen content in every amino acid is given in per cent of the total Kjeldahl-nitrogen in the hydrolysate.

The last column gives the standard deviations in relative per cent for these values. For the amino acids leucine, isoleucine, phenylalanine, valine, and methionine the standard deviation seems to be only 3—5 %.

But for the amino acids emerging later, the standard deviation in relative per cent is about ± 10 and slowly increases up to ± 20 % for the last amino acids, mostly because these values are rather small and are only based on two determinations. *

The sum of the nitrogen values in the first column exceeds 100 per cent. This cannot be correct, and it must be due to some systematic incorrectness in the calculations we have made for arriving at these values. Possibly our leucine standard is not quite pure, so that our values will all be too high. Work is proceeding to clear up this question. In column 2 we have the same values as in column 1, but now they are all expressed in per cent of the nitrogen determinated in the *chromatogram*.

In accordance with the estimation of Rees [14] we have assumed a decomposition of threonine and serine during acid hydrolysis for 24 hours. Consequently the values for these two amino acids have been corrected upwards 5 and 10 per cent respectively, and the ammonia value is correspondingly corrected downwards. These corrections have been introduced in the values given in column 3.

The values in column 3 constitute at present our final result of the composition of a hydrolysate from β-globulin from barley. It is expressed as the nitrogen contents of the different amino acids. For facilitating the comparison of these values with tables giving the amino acid composition for proteins in other units, we have also calculated our values as:

a) g amino acid residue per 100 g of protein and
b) g amino acid per 100 g of protein.

These values are given in column 4 and 5 respectively.

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* Recently Moore and Stein [13] have published a method where an ion exchange column (Dowex 50) has been used instead of a starch column and where buffers are used for elution; there the sequence of some of the amino acids is also reversed. This will give much better values for the last (basic) amino acids than found here. Work is in progress to utilize this new technique.

Table 5. The amino acid composition of β-globulin from barley.

Constituent	N as % of Kjeldahl-N	N as % of total chromatographic N		Values in column 3 calculated as:		Standard deviation (in relative %)
		values uncorrected	corrected for assumed destruction	g amino acid residue per 100 g protein	g amino acid per 100 g protein	
	1	2	3	4 z)	5 æ)	6
Leucine	8.01	7.3	7.3	9.5	11.0	± 3
Isoleucine	2.28	2.1	2.1	2.7	3.2	± 3
Phenylalanine	2.80	2.5	2.5	4.2	4.7	± 5
Methionine	2.05	1.9	1.9	2.9	3.2	± 3
Valine	7.82	7.1	7.1	8.1	9.5	± 3
Tyrosine	4.48	4.1	4.1	7.7	8.5	± 9
Proline	11.09	10.1	10.1	11.2	13.3	± 8
Glutamic acid	11.69	10.6	10.6	15.7	17.8	± 5
Alanine	7.10	6.4	6.4	5.2	6.5	± 3
Threonine	4.28	3.9	4.3 x)	5.0	5.9	± 6
Aspartic acid	6.40	5.8	5.8	7.6	8.8	± 4
Serine	3.60	3.3	3.6 x)	3.6	4.3	± 7
Glycine	5.42	4.9	4.9	3.2	4.2	± 11
Amide-NH_3	13.71	12.5	11.8 x)	− 0.14	2.5	—
Arginine	5.98	5.4	5.4	2.4	2.7	± 11
Lysine	2.78	2.5	2.5	1.8	2.1	± 10
Histidine	3.11	2.8	2.8	1.5	1.7	± 19
Cystine + cysteine	7.49	6.8	6.8	8.0	9.4	± 21
Tryptophan y)		destroyed by hydrolysis				
Total	110.09%	100.0%	100.0%	100.16%	119.3%	

x) The average threonine and serine values have been raised by 5 % and 10 %, respectively, in accordance with the estimates of Rees (13) for decomposition of these amino acids during hydrolysis. The value for amide-NH_3 has been correspondingly reduced.

y) A chromatographic estimation of tryptophan in the unhydrolysed protein has been made. We have found 3.8 g per 100 g protein. For further details see text.

z) The values in this column have been found by inserting the corresponding N-values from the 3rd column into the following general expression:

$$\frac{\frac{N_i}{n_i}\,(M_i - M_{H_2O})\;100}{\frac{N_i}{n_i}\,(M_i - M_{H_2O})}$$

where the index i refers to the amino acid in question, M is the molecular weight and n is the number of nitrogen atoms per molecule. The value of amide-NH_3 residue will show a negative figure when calculated in this way, because $(M_{NH_3} - M_{H_2O})$ has a negative value.

æ) The values in this column have been found by inserting the corresponding values from the 4th column (they are called $A_{R,i}$) into the following general expression:

$$\frac{A_{R,i}\;M_i}{M_i - M_{H_2O}}$$

The determination of tryptophan is exclued from this chromatographic analysis, because tryptophan is destroyed in the acid hydrolysis. The Hopkins-Cole reaction for tryptophan is positive on the unhydrolysed protein, so β-globulin from barley contains tryptophan.

An approximate quantitative estimation of tryptophan has been made by the spectrophotometric method for determination of the relative amount of tyrosine and tryptophan in a protein (Goodwin and Morton)[15]. We have found that the molarity of tryptophan amounts to 40 per cent of that of tyrosine. In Table 5, column 6, we have found the tyrosine content to be 8.5 g/100 g protein. It means that the tryptophan content in β-globulin from barley is about 3.8 g/100 g protein.

DISCUSSION

We have been searching the literature for amino acid analyses earlier performed on β-globulin from barley, but have found none. The nearest we have found is the amino acid analysis on the globulin edestin isolated from hemp or flax seeds. A summary of the most reliable of these results is given by Cohn and Edsall[16] in their monograph on amino acids and proteins.

There is a very poor agreement between the results stated therein for the amino acid composition of edestin and the values for β-globulin from barley which we have found, but it has to be remembered that the analyses have been performed in quite different ways.

The differences are still so great that they emphasize the need for amino acid analysis to be performed on every separate globulin fraction isolated from the different seeds. Significant differences have to be expected.

Until now the name edestin often has been uncritically used as the name for the globulin fraction of a seed protein, sometimes without giving the name of the seed. Of course this is very confusing and also reduces the value of the results. The arbitrary use of α, β, γ, and δ as prefixes introduced by Quensel[6] for characterizing the different groups of seed globulins is an improvement, but it is only regarded as a temporary solution of the problem. When more is known about the amino acid composition and other important properties of the proteins it will be justified to revise the names and try to find a nomenclature taking these results into consideration. But at the present moment we have to specify in every single case the fraction we are dealing with.

These investigations constitute the first part of a planned research program comprising a systematic investigation of the different protein components in barley, since possible differences in their structure and composition would be of considerable interest in investigations of the brewing process.

SUMMARY

"β-Globulin" from barley grains has been isolated.

The degree of purity has been established by electrophoresis, diffusion and ultracentrifuge experiments. After hydrolysis both *qualitative* and *quantitative* amino acid determinations have been made by starch chromatography according to the method of Moore and Stein. The following amino acids have been found: leucine, isoleucine, phenylalanine, methionine, valine, tyrosine, proline, glutamic acid, alanine, threonine, aspartic acid, serine, glycine, arginine, lysine, histidine, and cystine together with ammonia. A spectrophotometric estimation of the tryptophan content is also given.

In the analytical part of these investigations I have been assisted by Mrs. Kirsten Nielsen and Miss L. Block-Andersen. My thanks are also due to the chief of the Research Laboratory Dr. Birger Trolle and to the chief of the Carlsberg Laboratory, Professor K. Linderstrøm-Lang for stimulating interest in this work.

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