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HYDROLYSIS OF HORDEIN.[1]

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THE seeds of barley, like those of other cereals, contain a relatively considerable amount of protein soluble in alcohol of from 70 to 80 per cent by volume. An investigation of this seed, which was made some years ago in this laboratory, gave no evidence of the presence of more than one protein soluble in alcohol.[2] Extensive fractionations of this protein yielded products of the same composition and properties, and it was therefore proposed to call it hordein. The composition of hordein as shown by closely agreeing analyses of a large number of different preparations is: C 54.29; H 6.80; N 17.21; S 0.83; O 20.87 per cent.

The preparation of hordein used for this hydrolysis was made from freshly ground barley flour[3] by extracting with cold 75 per cent (by volume) alcohol. The alcoholic extract was filtered perfectly clear and concentrated to a thin syrup on a water bath at about 70°, under strongly reduced pressure. This concentrated solution was then poured into a large volume of distilled water containing much pure ice and, after washing with water, the precipitate was again dissolved in 75 per cent alcohol and its clear solution poured into several volumes of absolute alcohol. The resulting precipitate of hordein was digested with absolute alcohol until thoroughly dehydrated and the alcohol removed in the desiccator by sulphuric acid. A sample of the hordein thus prepared, after grinding fine and drying at 110°, was extracted for a long time with ether but yielded only traces of substance soluble therein.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Osborne: Journal American Chemical Society, 1895, xvii, p. 539.

[3] We are indebted to Dr. Frank Fuller of the Health Food Company of New York for this barley flour which he ground expressly for us. We wish here to express our thanks for his kind assistance.

Seven hundred grams of hordein, equal to 628.7 gm. ash and moisture free, were dissolved in a mixture of 700 c.c. of hydrochloric acid of specific gravity, 1.2 and 700 c.c. of water, by warming on the water bath for eight hours. The solution was then boiled for fifteen hours in a bath of oil and the hydrolysis completed.

After concentrating somewhat under reduced pressure the hydrolysis solution was saturated with hydrochloric acid gas and allowed to stand at 0°. The yield of glutaminic acid hydrochloride, after deducting for the ammonium chloride present, was 237.16 gm., equivalent to 189.73 gm. of glutaminic acid, which, with the 24.62 gm. isolated from Fractions V and VI of the esters, makes the total of glutaminic acid obtained in this hydrolysis 214.35 gm. or 34.07 per cent of the protein, while Osborne and Gilbert[1] found 36.35 per cent by their direct determination. The free acid decomposed at about 202°–203°.

Carbon and hydrogen, 0.2768 gm. subst., gave 0.4114 gm. CO_2 and 0.1547 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.82; H 6.12 per cent.
Found . . . . = C 40.53; H 6.21 " "

The filtrate from glutaminic acid hydrochloride was concentrated very sharply, under reduced pressure, and the very thick syrup esterified with alcohol and dry hydrochloric acid gas, by the method of Emil Fischer. The free esters were then liberated, shaken out, and dried in the usual manner. As a repetition of this process materially increases the yield of ester, the aqueous layer was freed from inorganic salts and the esterification repeated. By distillation under diminished pressure, the esters were divided into the following fractions:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	20 mm.	22.1 gm.
II	85°	9 "	38.4 "
III a	100°	9 "	37.1 "
III b	100°	2 "	53.4 "
III c	100°	0.48 "	46.7 "
IV	140°	0.33 "	63.5 "
V	200°	0.33 "	60.4 "
VI	210°	0.33 "	13.3 "
Total			334.9 gm.

The undistilled residue weighed 68 gm.

[1] OSBORNE and GILBERT: This journal, 1906, xv, p. 338.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	65°	20 mm.	22.1 gm.

From this fraction no glycocoll could be separated as the hydrochloride of the ethyl ester. It seemed to consist largely of leucine and alanine and was accordingly added to Fraction II.

Fraction.	Temp. of bath up to	Pressure.	Weight.
II	85°	9 mm.	38 4 gm.

The esters of this fraction were boiled with water until the alkaline reaction had ceased. After evaporating to dryness under strongly reduced pressure, the proline was extracted with boiling alcohol and the part remaining undissolved subjected to fractional crystallization from water and from water and alcohol. There were obtained from the less soluble part 4.75 gm. of leucine and 0.8 gm. of substance of perfectly homogeneous appearance, which on analysis gave figures agreeing best with a mixture of equal parts of leucine and valine.

Carbon and hydrogen, 0.1970 gm. subst., gave 0.3817 gm. CO_2 and 0.1716 gm. H_2O.

Calculated for equal molecules of leucine and valine

Found . . . . . { = C 53.12 ; H 9.66 per cent.
= C 52.84 ; H 9.67

The more soluble portion of this fraction was examined for glycocoll, but none could be isolated as the hydrochloride of the ethyl ester.

By systematic fractional crystallization there were obtained 2.72 gm. of alanine and 0.80 gm. of amino-valerianic acid. The alanine decomposed at about 290°.

Carbon and hydrogen, I, 0.1418 gm. subst., gave 0.2114 gm. CO_2 and 0.0972 gm. H_2O.
II, 0.1538 gm. subst., gave 0.2268 gm. CO_2 and 0.1094 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.45 ; H 7.86 per cent.

Found . . . . = { I, C 40.66 ; H 7.61 per cent.
II, C 40.22 ; H 7.90 " "

The valine gave the following analysis:

Carbon and hydrogen, 0.1351 gm. subst., gave 0.2546 gm. CO_2 and 0.1165 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28 ; H 9.40 per cent.

Found . . . . = C 51.40 ; H 9.58 " "

Dissolved in 20 per cent hydrochloric acid $(a)\frac{20°}{D} = +26.95°$.

Fraction.	Temp. of bath up to	Pressure.	Weight.
III	100°	0.48 mm.	137.2 gm.

This fraction consisted mainly of the ester of *a* proline. It was saponified by boiling with water to the cessation of the alkaline reaction and the solution rapidly evaporated to dryness under strongly reduced pressure. The part remaining undissolved in absolute alcohol consisted almost wholly of leucine. The yield was 30.89 gm.

Carbon and hydrogen, 0.1946 gm. subst., gave 0.3930 gm. CO_2 and 0.1746 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . = C 55.08; H 9.97 " "

The substance decomposed at about 298°.

The alcohol solutions of the proline of Fractions II and III were united. After concentrating under reduced pressure and precipitating with ether, the substance was obtained as a white crystalline mass, which when carefully dried weighed 86.32 gm. On redissolving in absolute alcohol, the proline separated in the characteristic prisms, melting at 200°-205°.

Carbon and hydrogen, 0.2737 gm. subst., gave 0.5235 gm. CO_2 and 0.1981 gm. H_2O.

Calculated for $C_5H_9O_2N$ = C 52.18; H 7.83 per cent.
Found . . . . = C 52.16; H 8.04 " "

For the strict identification, the substance was converted into the copper-salt and by boiling the latter with alcohol, the lævo separated from the racemic.

The insoluble copper-salt of lævo-proline crystallized from water in the characteristic plates containing two molecules of water-of-crystallization.

Water, 0.3447 gm. subst. (air-dried), lost 0.0377 gm. H_2O at 110°.

Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2H_2O$ = H_2O 10.99 per cent.
Found . . . . . = H_2O 10.94 " "

Copper, 0.3026 gm. subst., gave 0.0817 gm. CuO.

Calculated for $C_{10}H_{16}O_4N_2Cu$ = Cu 21.81 per cent.
Found . . . . . = Cu 21.57 " "

The phenylhydantoine of the lævo-proline melted at 143°.

Carbon and hydrogen, 0.2668 gm. subst., gave 0.6547 gm. CO_2 and 0.1313 gm. H_2O.

Calculated for $C_{12}H_{12}O_2N_2$ = C 66.67 ; H 5.57 per cent.
Found . . . . . = C 66.92 ; H 5.47 " "

Fraction.	Temp. of bath up to	Pressure.	Weight.
IV	140°	0.33 mm.	63.5 gm.

From this fraction the ester of phenylalanine was removed with ether in the usual way. The weight of the hydrochloride of phenylalanine obtained was 16.36 gm.

The free acid decomposed at about 280° on rapid heating.

Carbon and hydrogen, 0.1489 gm. subst., gave 0.3583 gm. CO_2 and 0.0875 gm. H_2O.

Calculated for $C_9H_{11}O_2N$ = C 65.45 ; H 6.66 per cent.
Found . . . . = C 65.63 ; H 6.53 " "

The phenylisocyanate derivative melted at 177°–178° (uncorr.).

Carbon and hydrogen, 0.1126 gm. subst., gave 0.2796 gm. CO_2 and 0.0609 gm. H_2O.

Calculated for $C_{16}H_{16}O_3N_2$ = C 67.60 ; H 5.63 per cent.
Found . . . . . = C 67.72 ; H 6.01 " "

The aqueous layer was saponified by warming with an excess of baryta. After prolonged standing no barium aspartate had separated. The barium was accordingly removed with sulphuric acid and after concentration the solution was saturated with hydrochloric acid gas.

The precipitate that separated at 0° weighed 3.98 gm. and consisted of very nearly pure phenylalanine hydrochloride. The filtrate from phenylalanine was examined for aspartic acid; but efforts to isolate this substance, either as the copper or barium salt or in the form of the free acid, failed.

Fraction.	Temperature of bath up to	Pressure.	Weight.
V	200°	0.33 mm.	60.4 gm.
VI	210°	0.33 mm.	13.3 gm.

From this fraction there were isolated in the usual way 14.97 gm. of phenylalanine as the hydrochloride. The remainder of the fraction consisted mainly of glutaminic acid, which was isolated partly as the barium salt and partly in the form of the hydrochloride. The yield was 24.62 gm. of the free acid.

The substance decomposed at about 202°–203°.

Carbon and hydrogen, 0.1933 gm. subst., gave 0.2878 gm. CO_2 and 0.1035 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81 ; H 6.12 per cent.
Found . . . . = C 40.61 ; H 5.94 " "

The filtrate from glutaminic acid hydrochloride yielded no definite substance. It did not seem to contain an appreciable quantity of aspartic acid, while neither here nor in the filtrate from phenylalanine hydrochloride of Fraction IV could preparations be obtained which gave evidence of the presence of serine. In the filtrates from glutaminic acid hydrochloride of Fractions V and VI, after removal of the chlorine and subsequent conversion to the copper-salts, there was obtained, after slow concentration over sulphuric acid, a small quantity of copper-salt crystallizing in needle prisms, very unlike copper aspartate and of much greater solubility in water and which was possibly the copper-salt of a new amino acid. Unfortunately the amount of this substance was too small to warrant further investigation.

Tyrosine.

A quantity of hordein equal to 246 gm. ash and moisture free, was boiled with three times its weight of sulphuric acid and six times its weight of water for eight hours, and after removing the sulphuric acid with an equivalent quantity of barium hydroxide the tyrosine was separated by concentration and cooling. The crude tyrosine was dissolved in 5 per cent sulphuric acid and the solution treated with phosphotungstic acid. After removing the phosphotungstate precipitate the solution was freed from phosphotungstic and sulphuric acids with barium hydroxide and from barium with an equivalent quantity of sulphuric acid and 4.14 gm. of tyrosine separated by crystallization equal to 1.67 per cent.

Nitrogen, 0.2333 gm. subst., required 1.81 c.c. 5/7 N—HCl.
Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.76 " "

The precipitate produced by phosphotungstic acid in the solution of the crude tyrosine was decomposed with baryta, and after removing the barium, the solution was concentrated and the precipitation with phosphotungstic acid repeated until the Millon's reaction had ceased.

The filtrate from barium phosphotungstate, freed from barium with sulphuric acid, separated sparingly very thin prisms of brilliant mother-of-pearl lustre, which proved identical with a substance recently isolated by us [1] by a similar method from gliadin, and which we feel justified in regarding as a dipeptide of proline and phenylalanine. When heated side by side with a pure preparation from gliadin, the substance decomposed simultaneously with the latter at about 249° (uncorr.), and when mixed the decomposition point was not lowered.

Histidine.

Fifty grams of hordein, equal to 44.91 gm. ash and moisture free, were hydrolyzed and the bases determined according to the method of Kossel and Patten. The solution of the histidine was made up to 500 c.c. and found to contain nitrogen equal to 0.5770 gm. of histidine or 1.28 per cent.

Nitrogen, 100 c.c. solution required 3.13 c.c. 5/7 N—HCl = 0.0313 gm. N = 0.1565 gm. N in 500 c.c. = 0.5770 gm. histidine = 1.28 per cent.

The histidine was converted into the dichloride which decomposed at 232°–233°.

Chlorine, 0.0770 gm. subst., gave 0.0963 gm. AgCl.
Calculated for $C_6H_{11}O_2N_3Cl_2$ = Cl 31.14 per cent.
Found . . . . . = Cl 30.92 " "

Arginine.

The solution of the arginine was made up to 1000 c.c. and found to contain nitrogen equal to 0.972 gm. arginine or 2.16 per cent.

Nitrogen, 100 c.c. solution required 2.9 c.c. 5/7 N — HCl = 0.0290 gm. N = 0.2900 gm. N in 1000 c.c. = 0.9 gm. arginine. Adding 0.072 gm. for solubility of arginine silver = 0.972 gm. = 2.16 per cent.

The arginine was converted into the copper nitrate double salt.

Water, 0.1069 gm. subst., air dry, lost 0.0099 gm. H_2O.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 \cdot 3\ H_2O$ = H_2O 9.16 per cent.
Found . . . . . = H_2O 9.26 " "
Copper, 0.0962 gm. subst., dried at 100°, gave 0.0143 gm. CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2$ = Cu 11.87 per cent.
Found . . . . . = Cu 11.87 " "

[1] Osborne and Clapp: This journal, 1907, xviii, p. 123.

Lysine.

No lysine picrate could be obtained by the usual method, which confirms the recent observation of Brown,[1] who likewise failed to detect lysine in this protein.

The results of this hydrolysis were as follows:

	Per cent.		Per cent.
Glycocoll	0.00	Cystine	undetermined
Alanine	0.43	Tyrosine	1.67
Valine	0.13	Oxyproline	undetermined
Leucine	5.67	Arginine	2.16
Proline	13.73	Histidine	1.28
Phenylalanine	5.03	Lysine	0.00
Aspartic acid	not isolated	Ammonia	4.87
Glutaminic acid	36.35	Tryptophane	present
Serine	not isolated	Total	71.32

This hydrolysis shows that hordein is characterized by marked differences in the proportion of its decomposition products when contrasted with the other proteins that have been thus far analyzed. Like the other alcohol soluble proteins, it yields no lysine, relatively little histidine and arginine, and much ammonia. The very large proportion of glutaminic acid is practically the same as that obtained from gliadin.

The most marked feature, however, is presented by the very large proportion of proline, which greatly exceeds that yet obtained from any other protein, being practically twice as much as the relatively large quantity yielded by gliadin.

[1] Brown: Transactions of the Guinness Research Laboratory, 1906, i, pt. ii, p. 229.