

# **Hydrolysis of legumin from the pea / by Thomas B. Osborne and S.H. Clapp.**

## **Contributors**

Osborne, Thomas B. 1859-1929.  
Clapp, S.H.  
Royal College of Surgeons of England

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Wellcome Collection  
183 Euston Road  
London NW1 2BE UK  
T +44 (0)20 7611 8722  
E [library@wellcomecollection.org](mailto:library@wellcomecollection.org)  
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HYDROLYSIS OF LEGUMIN FROM THE PEA.<sup>1</sup>

BY THOMAS B. OSBORNE AND S. H. CLAPP.

*(From the Laboratory of the Connecticut Agricultural Experiment Station.)*

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The legumin used for this hydrolysis was prepared in the way described in the preceding paper.

Five hundred grams, equal to 449 grams of ash- and moisture-free legumin, were dissolved by warming in the water-bath with a mixture of 500 cc. of water and 500 cc. of hydrochloric acid of specific gravity 1.2. The solution was then boiled in a bath of oil for 15 hours.

The hydrolysis solution was then concentrated under reduced pressure to two-thirds of the original volume, saturated with hydrochloric acid gas and allowed to stand at 0°.

The glutaminic acid hydrochloride, freed from ammonia by boiling with baryta and recrystallized from strong hydrochloric acid, weighed 77.34 grams, which is equivalent to 61.95 grams of glutaminic acid. The free acid decomposed at 202°–203° with effervescence to a clear oil and gave the following analysis:

*Carbon and hydrogen:* 0.2272 gm. substance gave 0.3380 gm. CO<sub>2</sub> and 0.1283 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N: C = 40.81; H = 6.12 per cent.

Found.....C = 40.57; H = 6.27 " "

The filtrate from the hydrochloride of glutaminic acid was concentrated under reduced pressure to a thick syrup and esterified with alcohol and dry hydrochloric acid gas in the usual manner. The aqueous layer remaining after extracting the esters with ether, was then freed from inorganic salts and the esterification

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.



repeated. As considerable ester, soluble in ether, was obtained, the whole operation was again repeated, but this third treatment yielded only an insignificant quantity of ether-soluble ester.

The ether was then distilled off on the water-bath and the residue fractioned under diminished pressure with the following result:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	55°	10 mm.	33.82 gm.
II	85°	10 "	29.88 "
III { A	100°	10 "	27.06 "
		0.75 "	49.10 "
IV { A	140°	0.75 "	65.08 "
	200°	0.65 "	37.40 "
Total,			242.34 "

The undistilled residue weighed 107 grams.

*Fraction I.* (Temperature of bath up to 55°; pressure, 10 mm.; weight, 33.82 grams.) This fraction, which consisted largely of alcohol and ether, was evaporated to dryness with strong hydrochloric acid and the residue esterified with alcohol and dry hydrochloric acid gas. The glycocoll ester hydrochloride that separated on standing at 0°, weighed 0.56 gram. The filtrate was worked up conjointly with the glycocoll filtrate of Fraction II.

*Fraction II.* (Temperature of bath, up to 85°; pressure, 10 mm.; weight, 29.88 grams.) The ester of this fraction was saponified by boiling with water until the alkaline reaction had ceased. The dried amino-acids were freed from prolin with boiling absolute alcohol and re-esterified with dry alcohol and hydrochloride acid gas. The weight of glycocoll ester hydrochloride that separated at 0°, was 2.66 grams, equivalent to 1.43 gram of glycocoll. It crystallized from alcohol in the characteristic needles melting at 144°.

*Chlorine:* 0.3054 gm. substance gave 0.3174 gm. AgCl.

*Nitrogen:* 0.4008 gm. substance required 4.05 cc.  $\frac{5N}{7}$  HCl.

Calculated for  $C_4H_{10}O_2NCl$ : Cl = 25.45; N = 10.04 per cent.

Found..... Cl = 25.70; N = 10.11 " "

In the filtrate from the glycocoll the free amino-acids were regenerated and submitted to fractional crystallization. The less



soluble fractions yielded 4.23 grams of leucin, decomposing at  $298^{\circ}$ .

*Carbon and hydrogen:* 0.1077 gm. substance gave 0.2166 gm.  $\text{CO}_2$  and 0.0960 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_6\text{H}_{13}\text{O}_2\text{N}$ : C = 54.96; H = 9.92 per cent.

Found.....C = 54.85; H = 9.90 " "

The remainder of the fraction consisted mainly of alanin. Valin, if present at all, was in such small quantity that its isolation was not attempted.

The alanin weighed 9.34 grams.

*Carbon and hydrogen:* 0.1477 gm. substance gave 0.2184 gm.  $\text{CO}_2$  and 0.1067 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_2\text{N}$ : C = 40.45; H = 7.86 per cent.

Found.....C = 40.33; H = 8.02 " "

The substance decomposed at about  $290^{\circ}$ .

*Fraction III.* (A: Temperature of bath up to  $100^{\circ}$ ; pressure, 10 mm.; weight, 27.06 grams. B: Temperature of bath up to  $100^{\circ}$ ; pressure, 0.75 mm.; weight, 49.10 grams.)

This fraction consisted almost entirely of the esters of leucin and prolin. After saponifying with boiling water, the solutions were evaporated to dryness under reduced pressure and the prolin extracted with boiling alcohol. The part remaining undissolved yielded on fractional crystallization 31.65 grams of leucin.

*Carbon and hydrogen:* 0.1855 gm. substance gave 0.3742 gm.  $\text{CO}_2$  and 0.1670 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_6\text{H}_{13}\text{O}_2\text{N}$ : C = 54.96; H = 9.92 per cent.

Found.....C = 55.02; H = 10.00 " "

The substance decomposed at  $298^{\circ}$ .

The alcohol extracts containing the prolin of Fractions II and III were worked up conjointly. After separating from a small quantity of alcohol-insoluble substance by repeated evaporations to dryness under reduced pressure, the substance was converted into the copper salt and the laevo separated from the racemic prolin copper salt with boiling absolute alcohol.

The insoluble racemic salt was freed from copper with hydrogen sulphide and the filtrate from copper sulphide evaporated to dryness under reduced pressure. The residue proved entirely soluble in absolute alcohol. For identification the racemic prolin was



again converted to the copper salt and the latter recrystallized from water. The yield of the air-dried copper salt was 2.85 grams equivalent to 2.00 grams of prolin.

*Water:* 0.1278 gm. substance, dried in air, lost 0.0138 gm.  $H_2O$  at  $110^\circ$ .

*Copper:* 0.1134 gm. substance, dried at  $110^\circ$ , gave 0.0305 gm.  $CuO$ .

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

Found.....  $H_2O = 10.80$  " "

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

Found.....  $Cu = 21.49$  " "

The copper salt of the laevo-prolin, soluble in alcohol, weighed, when dried at  $100^\circ$ , 15.80 grams, equivalent to 12.47 grams of prolin.

For identification the phenylhydantoin was employed. It crystallized from water in the characteristic flat prisms, melting at  $143^\circ$ .

*Carbon and hydrogen:* 0.2241 gm. substance gave 0.5456 gm.  $CO_2$  and 0.1152 gm.  $H_2O$ .

Calculated for  $C_{12}H_{12}O_2N_2$ :  $C = 66.67$ ;  $H = 5.57$  per cent.

Found.....  $C = 66.40$ ;  $H = 5.71$  " "

*Fraction IV.* (A: Temperature of bath, up to  $140^\circ$ ; pressure, 0.75 mm., weight, 65.08 grams. B: Temperature of bath, up to  $200^\circ$ ; pressure, 0.65 mm.; weight, 37.40 grams.)

From this fraction the ester of phenylalanin was removed in the usual manner by shaking out with ether and the ether layer washed repeatedly with an equal volume of water. After saponification with strong hydrochloric acid, there were obtained 20.54 grams of the hydrochloride of phenylalanin equivalent to 16.82 grams of free phenylalanin. For identification the copper salt was employed.

*Copper:* 0.1422 gm. substance, dried at  $110^\circ$ , gave 0.0284 gm.  $CuO$ .

Calculated for  $C_{18}H_{20}O_4N_2Cu$ :  $Cu = 16.24$  per cent.

Found.....  $Cu = 15.96$  " "

The aqueous layer was saponified by warming with an excess of baryta and the racemized aspartic acid isolated as the barium salt. The yield was 13.43 grams.

*Carbon and hydrogen:* 0.2597 gm. substance gave 0.3451 gm.  $CO_2$  and 0.1279 gm.  $H_2O$ .

Calculated for  $C_4H_7O_4N$ :  $C = 36.09$ ;  $H = 5.26$  per cent.

Found.....  $C = 36.24$ ;  $H = 5.47$  " "



In the filtrate from the barium aspartate no glutaminic acid could be isolated as the hydrochloride. The remainder of the fraction consisted essentially of aspartic acid and serin and for the isolation of the former substance the copper salt was employed.<sup>1</sup> The weight of air-dry copper aspartate was 21.46 grams equivalent to 10.36 grams of aspartic acid.

*Copper:* 0.1317 gm. substance, dried in air, gave 0.0383 gm. CuO.

*Nitrogen:* 0.4428 gm. substance, dried in air required 2.33 cc.  $\frac{5N}{7}$  HCl.

Calculated for  $C_4H_5O_4NCu \cdot 4\frac{1}{2} H_2O$ : Cu = 23.07; N = 5.08 per cent.

Found ..... Cu = 23.23; N = 5.26 " "

The filtrate from the copper aspartate was freed from copper with hydrogen sulphide. By fractional crystallization there were obtained 2.45 grams of pure serin, which decomposed at about 240°.

*Carbon and hydrogen:* 0.2300 gm. substance gave 0.2909 gm. CO<sub>2</sub> and 0.1389 gm. H<sub>2</sub>O.

Calculated for  $C_3H_7O_3N$ : C = 34.29; H = 6.67 per cent.

Found ..... C = 34.49; H = 6.71 " "

*Residue after Distillation.* The residue that remained after distilling off the esters weighed 107 grams. It was dissolved in hot alcohol. The solution separated on cooling a precipitate weighing 3.32 grams. The filtrate was evaporated to dryness, the residue was taken up in water and saponified by warming with an excess of baryta. After removing the baryta with sulphuric acid, the solution was concentrated and saturated with hydrochloric acid gas. After prolonged standing at 0°, no hydrochloride of glutaminic acid could be made to separate, even at different concentrations of the solution.

#### TYROSIN.

Fifty grams of the legumin, equal to 44.89 grams ash- and moisture-free, were hydrolyzed with three parts, by weight, of sulphuric acid and six parts of water for eleven hours and, after removing the sulphuric acid with an equivalent quantity of barium hydroxide, the solution was concentrated until a crystal-

<sup>1</sup>Fischer and Dörpinghaus: *Zeitschr. f. physiol. Chem.*, xxxvi, p. 474, 1902.



line product separated. This was filtered out and the filtrate further concentrated and a second separation obtained. These were then recrystallized from hot water and the crude tyrosin which was thus obtained was dissolved in boiling water, decolorized with bone black and recrystallized. Dried at  $110^{\circ}$  this tyrosin weighed 0.7 gram, equal to 1.56 per cent.

*Nitrogen:* 0.3162 gm. substance required 2.40 cc.  $\frac{5N}{7}$  HCl.

Calculated for  $C_9H_{11}O_3N$ : N = 7.73 per cent.

Found ..... N = 7.59 " "

The filtrate and washings from the tyrosin were concentrated and histidin, arginin and lysin determined by the method of Kossel and Patten.<sup>1</sup>

#### HISTIDIN.

The solution of the histidin contained nitrogen equal to 1.0870 gram of histidin, equal to 2.42 per cent.

Histidin solution = 500 cc. 50 cc. required 5.90 cc.  $\frac{5N}{7}$  HCl = 0.2950 gm. N in 500 cc. = 1.087 gm. histidin or 2.41 per cent.

This histidin was identified as the dichloride. It decomposed at about  $232^{\circ}$ – $233^{\circ}$  and gave, on warming, the biuret reaction.

*Chlorine:* 0.1481 gm. substance gave 0.1854 gm. AgCl.

Calculated for  $C_6H_{11}O_2N_3Cl_2$ : Cl = 31.14 per cent.

Found ..... Cl = 30.95 " "

#### ARGININ.

The solution of the arginin contained nitrogen equal to 4.542 grams of arginin or 10.12 per cent.

Arginin solution = 1000 cc. 50 cc. required 7.2 cc.  $\frac{5N}{7}$  HCl = 1.44 gm. N in 1000 cc. = 4.47 gm. arginin. Adding 0.072 gm. for the solubility of the arginin silver = 4.542 gm. or 10.12 per cent.

The arginin was identified as the copper nitrate double salt.

*Water:* 0.1937 gm. substance air dry lost 0.0189 gm.  $H_2O$  at  $110^{\circ}$ .

*Copper:* 0.1019 gm. substance, dried at  $110^{\circ}$ , gave 0.0152 gm. CuO.

Calculated for  $C_{12}H_{28}O_4N_8Cu(NO_3)_2 \cdot 3H_2O$ :  $H_2O$  = 9.16 per cent.

Found .....  $H_2O$  = 9.76 " "

Calculated for  $C_{12}H_{28}O_4N_8Cu(NO_3)_2$ : Cu = 11.87 per cent.

Found ..... Cu = 11.92 " "

<sup>1</sup>. Kossel and Patten: *Zeitschr. f. physiol. Chem.*, xxxviii, p. 39, 1903.

## LYSIN.

The lysin was isolated and identified as picrate, of which 4.95 grams were obtained, equal to 4.29 per cent.

*Nitrogen:* 0.1232 gm. substance, dried at 100°, gave 20.6 cc. moist N<sub>2</sub> at 19.5° and 752.1 mm.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>2</sub>.C<sub>6</sub>H<sub>3</sub>O<sub>7</sub>N<sub>3</sub>: N = 18.70 per cent.

Found ..... N = 18.96 " "

## CYSTIN.

Owing to the very small amount of sulphur in legumin no attempt was made to separate cystin.

The results of this hydrolysis are given in the following table:

## HYDROLYSIS OF LEGUMIN FROM THE PEA.

Glycocoll.....	0.38	per cent.
Alanin.....	2.08	" "
Valin.....	not isolated	
Leucin.....	8.00	per cent.
Prolin.....	3.22	" "
Phenylalanin.....	3.75	" "
Aspartic acid.....	5.30	" "
Glutaminic acid.....	13.80	" "
Serin.....	0.53	" "
Cystin.....	not determined	
Tyrosin.....	1.55	per cent.
Arginin.....	10.12	" "
Lysin.....	4.29	" "
Histidin.....	2.42	" "
Ammonia.....	1.99	" "
Tryptophan .....	present	
Total.....	57.43	" "



