Hydrolysis of excelsin / by Thomas B. Osborne and S.H. Clapp.

Reprinted from the American Journal of Physiology.
Vol. XIX. — June 1, 1907. — No. I.

HYDROLYSIS OF EXCELSIN.[1]

By THOMAS B. OSBORNE and S. H. CLAPP.
[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

THE greater part of the protein substance of the Brazil-nut (*Bertholletia excelsa*) consists of the globulin excelsin which may be obtained in beautiful hexagonal crystals when the protein separates slowly from solution. These crystals have no effect on polarized light, and, as Maschke[2] states, "undoubtedly belong to the regular system." The same form would result if an octahedron were cut parallel to two opposite faces. As it is possible to obtain a large quantity of this globulin in a perfectly homogeneous crystallized condition, and as excelsin is also precipitated by ammonium sulphate,[3] between comparatively narrow limits, the opportunity is presented of obtaining a protein preparation which offers a better guarantee of chemical individuality than do amorphous preparations of other proteins.

The results of this hydrolysis of excelsin, like that of crystallized oxyhæmoglobin of the horse blood made by Abderhalden,[4] shows that excelsin yields as many amino acids as most of the other chemically less well defined protein preparations.

In making our preparation of excelsin for this hydrolysis great care was taken to obtain a product which consisted wholly of perfectly formed crystals. In the preparation of this excelsin we received valuable assistance from Mr. I. F. Harris, for which we here wish to express our thanks.

The character of the material used for the hydrolysis is best shown by the following microphotographs, for which we thank Prof. E. T. Reichert, of the University of Pennsylvania.

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

[2] Maschke: Journal für praktische Chemie, 1858, lxxiv, p. 436.

[3] *Cf.* Osborne and Harris: This journal, 1905, xiii, p. 436.

[4] Abderhalden: Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 484.

The oil-free meal of the Brazil-nut was extracted with 3 per cent ammonium sulphate solution, heated to 50°, and the perfectly clear extract dialyzed until the greater part of the dissolved excelsin was deposited as crystals. These were then washed thoroughly with dilute sodium chloride solution and then with dilute alcohol which was gradually increased in strength up to absolute alcohol. The preparation was then dried over sulphuric acid.

Of the perfectly crystallized excelsin thus prepared 500 gm., equal to 450 gm. water and ash-free, were suspended in a mixture of 500 c.c. of water and 500 c.c. of hydrochloric acid of specific gravity 1.19, and warmed in a bath of boiling water for two and one-half hours. The heating was then continued in a bath of oil and the hydrolysis solution boiled for eighteen hours.

The esterification of the hydrochlorides of the amino acids and the liberation and extraction of the free esters were then executed in the manner often described. The dark-colored ether extracts were dried in the usual manner with potassium carbonate and anhydrous sodium sulphate.

The aqueous layer was freed from inorganic salts in the usual way, and the esterification repeated. As the yield of ester was considerable, the whole process was again repeated, but this last treatment yielded very little of ether-soluble ester. After distilling off the ether on the water-bath the esters were fractioned under diminished pressure as follows:

Fraction	Temp. of bath up to	Pressure	Weight
I	51°	10 mm.	43.55 gm.
II	90°	10 "	32.77 "
III	105°	0.47 "	91.65 "
IV	130°	0.40 "	59.57 "
V	170°	0.38 "	56.83 "
Total			284.37 gm.

The undistilled residue weighed .132 gm.

Fraction I. — This fraction consisted largely of alcohol and ether. It yielded, after esterification with alcohol and hydrochloric acid, 2.98 gm. of glycocoll ester hydrochloride, equivalent to 1.33 gm. of glycocoll. The melting-point was 144°.

Nitrogen, 0.4044 gm. subst., required 4.19 c.c. 5/7 N HCl.
Chlorine, 0.4158 gm. subst., gave 0.4265 gm. AgCl.

Calculated for $C_4H_{10}O_2NCl$ = N 10.04; Cl 25.45 per cent.
Found . . . . . = N 10.36; Cl 25.34 " "

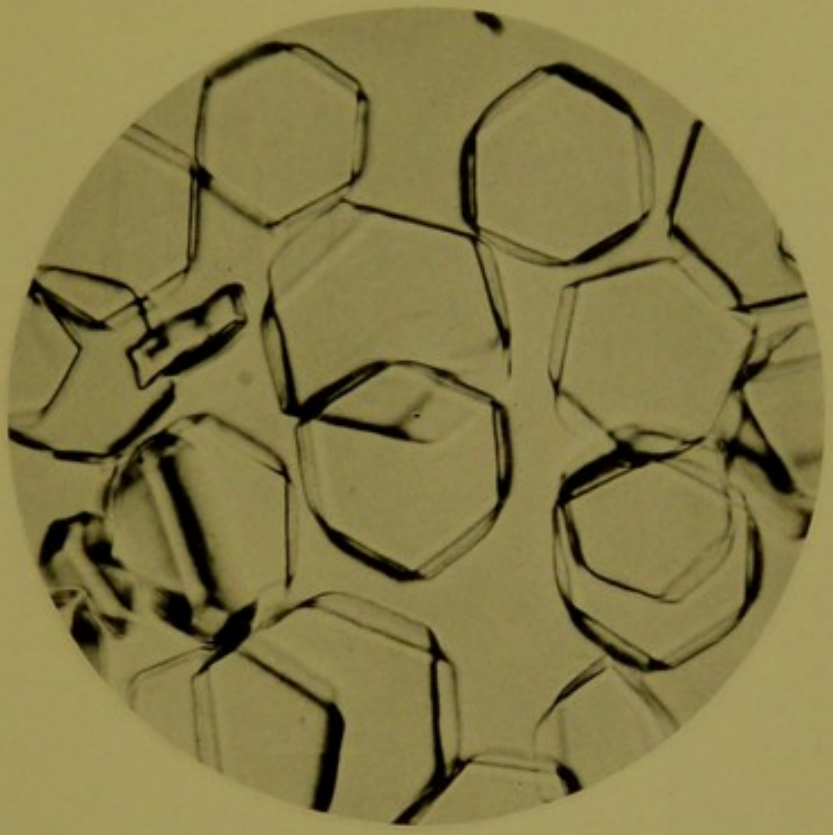

FIG. 1.

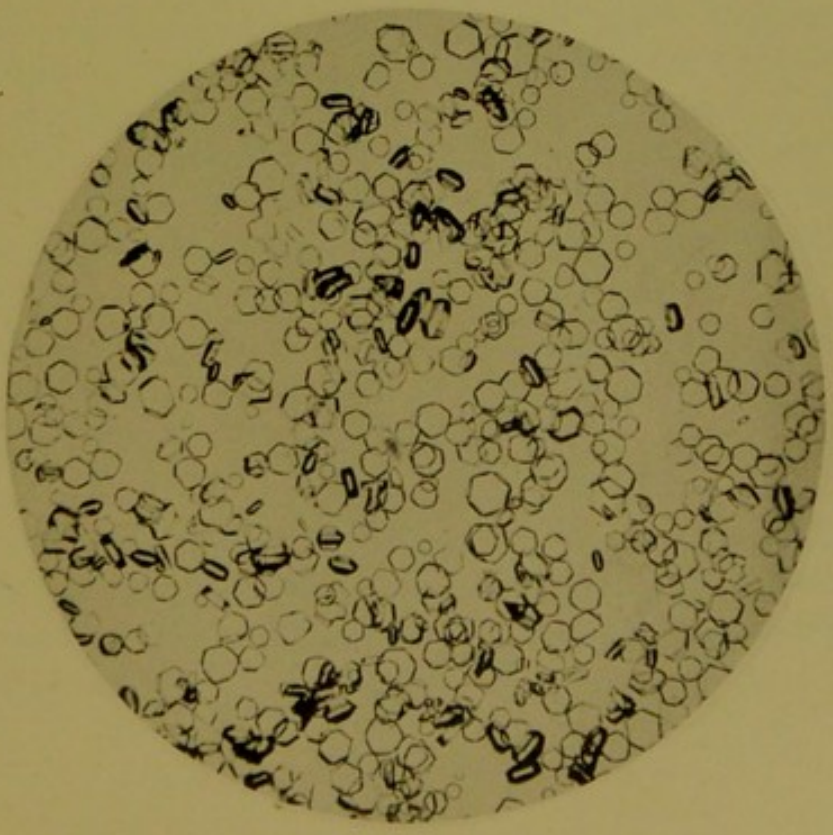

FIG. 2.

HELIOTYPE CO.

The filtrate from the glycocoll, after freeing from chlorine, yielded 1.75 gm. of alanine.

Fraction II. — This fraction was saponified with boiling water, evaporated to dryness under reduced pressure, and the proline extracted with boiling absolute alcohol. The part insoluble in alcohol, esterified in the usual way, yielded 2.59 gm. of glycocoll ester hydrochloride of melting-point 144°, equivalent to 1.39 gm. of glycocoll.

The filtrate from the glycocoll was freed from chlorine and submitted to a systematic fractional crystallization. There were obtained 3.23 gm. of substance having the percentage composition of amino-valerianic acid and 8.74 gm. of alanine.

Carbon and hydrogen, 0.1717 gm. subst., gave 0.3238 gm. CO_2 and 0.1470 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28; H 9.40 per cent.
Found . . . . = C 51.43; H 9.51 " "

The needles of the alanine decomposed at 290° and gave the following analysis:

Carbon and hydrogen, 0.1425 gm. subst., gave 0.2118 gm. CO_2 and 0.1033 gm. H_2O.

Calculated for $C_3H_7O_2N$ = C 40.45; H 7.86 per cent.
Found . . . . = C 40.54; H 8.05 " "

Fraction III. — This fraction was boiled with eight volumes of water for six hours, when the solution no longer reacted alkaline to litmus. The slightly colored solution was then evaporated to dryness under reduced pressure and the proline extracted with boiling absolute alcohol. From the part remaining undissolved there were obtained 39.15 gm. of leucine. The substance decomposed at about 298°.

Carbon and hydrogen, 0.1854 gm. subst., gave 0.3746 gm. CO_2 and 0.1647 gm. H_2O.

Calculated for $C_6H_{13}O_2N$ = C 54.96; H 9.92 per cent.
Found . . . . = C 55.10; H 9.87 " "

The substance in the filtrate appeared to consist, to a large extent, of amino-valerianic acid, but its isolation from this fraction was so difficult that we were able to obtain only 0.98 gm. of substance which appeared to be essentially homogeneous.

Carbon and hydrogen, 0.2155 gm. subst., gave 0.4032 gm. CO_2 and 0.1862 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28; H 9.40 per cent.
Found . . . . = C 51.03; H 9.60 " "

The remainder of the preparation of crude amino-valerianic acid weighed 2.57 gm.[1]

On recrystallizing from dilute alcohol it gave figures agreeing fairly well with the calculated.

Carbon and hydrogen, 0.1522 gm. subst., gave 0.2840 gm. CO_2 and 0.1286 gm. H_2O.

Calculated for $C_5H_{11}O_2N$ = C 51.28; H 9.40 per cent.
Found . . . . = C 50.88; H 9.38 " "

We were, however, unable to arrive at a preparation of perfectly homogeneous appearance either by fractional crystallization of the copper salt or by racemizing. It is probable that the isolation of this substance was rendered more than usually difficult by the presence, in this fraction, of a slight amount of aspartic ester, which, as is well known, is not converted smoothly to the free acid on boiling with water.[2]

The alcohol soluble portions of fractions II and III consisted essentially of proline, of which there were obtained 16.42 gm. For separation of the active from the racemized, the copper salt was employed and 3.70 gm. of air-dried racemic proline copper and 17.52 gm. of the lævo salt, dried at 110°, was obtained in the usual way.

Water, 0.3902 gm. subst., lost 0.0429 gm. H_2O at 110°.
Copper, 0.1564 gm. subst., gave 0.0379 gm. CuO.

Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2\ H_2O$ = H_2O 10.99; Cu 19.41 per cent.
Found . . . . . = H_2O 10.99; Cu 19.36 " "

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

Carbon and hydrogen, 0.2297 gm. subst., gave 0.5586 gm. CO_2 and 0.1174 gm. H_2O.

Calculated for $C_{12}H_{12}O_2N_2$ = C 66.67; H 5.56 per cent.
Found . . . . . = C 66.32; H 5.67 " "

[1] We have included this weight in calculating the percentage of this substance yielded by excelsin.

[2] FISCHER, E.: Berichte der deutschen chemischen Gesellschaft, 1901, xxxiv, p. 433.

Fraction IV. — From this fraction the ester of phenylalanine was shaken out with ether and saponified by warming with strong hydrochloric acid. There were isolated 6.74 gm. of phenylalanine as the hydrochloride.

Carbon and hydrogen, 0.2331 gm. subst., gave 0.5576 gm. CO_2 and 0.1431 gm. H_2O.

Calculated for $C_9H_{11}O_2N$ = C 65.45 ; H 6.66 per cent.
Found . . . . = C 65.24 ; H 6.82 " "

The aqueous layer after saponifying with baryta yielded 7.77 gm. of aspartic acid as the barium salt.

Carbon and hydrogen, 0.2159 gm. subst., gave 0.2868 gm. CO_2 and 0.1119 gm. H_2O.

Nitrogen, 0.2947 gm. subst., required 3.15 c.c. 5/7 N HCl.

Calculated for $C_4H_7O_4N$ = C 36.09 ; H 5.26 ; N 10.53 per cent.
Found . . . . = C 36.22 ; H 5.75 ; N 10.68 " "

The filtrate from barium aspartate, freed from barium, was saturated with hydrochloric acid gas. It separated no glutaminic acid hydrochloride at 0°. After freeing from chlorine, the remainder of the aspartic acid was separated as the copper salt. There were obtained 11.75 gm. of pure copper-aspartate, equivalent to 5.68 gm. of aspartic acid.

Nitrogen, 0.4321 gm. subst., required 2.28 c.c. 5/7 N HCl.

Copper, 0.4192 gm. subst., gave 0.1222 gm. CuO.

Calculated for $C_4H_5O_4N\,Cu\,4\frac{1}{2}\,H_2O$ = Cu 23.07 ; N 5.08 per cent.
Found . . . . . = Cu 23.29 ; N 5.27 " "

In the filtrate from copper-aspartate nothing definite was isolated.

Fraction V. — This fraction was treated precisely as the foregoing. There were obtained 9.26 gm. of phenylalanine as the hydrochloride, 10.32 gm. of glutaminic acid as the barium salt and 9.41 gm. as the hydrochloride.

The glutaminic acid hydrochloride was converted to the free acid, which decomposed at 202°–203°.

Carbon and hydrogen, 0.2214 gm. subst., gave 0.3301 gm. CO_2 and 0.1265 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81 ; H 6.12 per cent.
Found . . . . = C 40.66 ; H 6.35 " "

The filtrate from glutaminic acid hydrochloride yielded further 8.03 gm. of pure copper-aspartate, equivalent to 3.88 gm. of aspartic acid.

Serine appeared to be present in the filtrate from the copper-aspartate, but it was not isolated in a state of purity.

The Residue after Distillation.

The residue remaining in the bulb after the distillation of the esters weighed 132 gm. It was dissolved in boiling alcohol and, after cooling, filtered from the insoluble (wt. = 4.70 gm.). The filtrate was evaporated to dryness and saponified by warming with excess of baryta for eight hours. There were obtained 23.25 gm. of glutaminic acid hydrochloride, equivalent to 18.62 gm. of glutaminic acid. The free acid decomposed at 202° – 203°.

Carbon and hydrogen, 0.2983 gm. subst., gave 0.4455 gm. CO_2 and 0.1739 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81; H 6.12 per cent.
Found . . . . = C 40.73; H 6.47 " "

This makes the total yield of glutaminic acid obtained by the ester method 38.32 gm. or 8.52 per cent of excelsin, which falls considerably below the 12.94 per cent obtained by Osborne and Gilbert[1] with the direct method.

Cystine.

Although excelsin contains about the same amount of total sulphur as gliadin, the amount of sulphur obtained as sulphide on boiling excelsin with strong sodium hydroxide solution is only one half that similarly obtained from gliadin.[2]

This smaller proportion of cystine may possibly explain our failure to isolate any of this substance from excelsin by the same process as that which readily yielded cystine when applied to gliadin.[3] Cystine could not be obtained also from the crude tyrosine by means of mercuric sulphate, as in the case of glutenin.

Tyrosine.

One hundred gm. of excelsin, equal to 90.19 gm. water and ash-free excelsin, was boiled with 100 c.c. concentrated hydrochloric acid and 100 c.c. of water for six hours on the oil bath. After evaporating to a syrup and repeatedly evaporating with water under strongly

[1] Osborne and Gilbert: This journal, 1906, xv, p. 350.
[2] Osborne: Journal American Chemical Society, 1902, xxiv, p. 140.
[3] Osborne and Clapp: This journal, 1906, xvii, p. 231.

reduced pressure, the remainder of the hydrochloric acid was neutralized by an equivalent amount of sodium carbonate, as found by determining the amount of chlorine in an aliquot part of the solution. After concentrating to about 400 c.c. the substance that separated was filtered out and the filtrate further concentrated and a second separation obtained. These two separations were recrystallized from water and the product obtained dissolved in 5 per cent sulphuric acid and the solution treated with phosphotungstic acid. After removing the phosphotungstic precipitate the solution was freed from phosphotungstic and sulphuric acids and evaporated to dryness. The residue was extracted with boiling glacial acetic acid and the part that remained undissolved weighed 2.83 gm. equal to 3.03 per cent.

Nitrogen, 0.2930 gm. subst., dried at 100°, required 2.24 c.c. 5/7 N HCl.
Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.65 " "

Arginine.

Fifty gm. of excelsin, equal to 47.18 gm. water, and ash-free, were hydrolyzed, and the arginine determined, as directed by Kossel and Patten.[1] The solution of the arginine contained nitrogen equal to 7.49 gm. of arginine. Adding 0.072 gm. for the solubility of arginine silver in the solutions from which it was precipitated, we have 7.562 gm. arginine or 16.02 per cent.

10 c.c. solution required 4.83 c.c. 5/7 N HCl = 0.0483 gm. N, or 2.415 gm. in 500 c.c. = 7.49 gm. arginine.

The arginine was converted into the nitrate.

Nitrogen, 0.2945 gm. subst, dried over H_2SO_4, required 8.29 c.c. 5/7 N HCl.
Calculated for $C_6H_{14}O_2N_4 \cdot HNO_3 \cdot \frac{1}{2} H_2O$ = N 28.46 per cent.
Found . . . . . = N 28.18 " "

The arginine nitrate was then converted into arginine copper nitrate.

Water, 0.2848 gm. subst., lost 0.0264 gm. H_2O at 110°.
Copper, 0.0981 gm. subst., gave 0.0135 gm. CuO.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2 \cdot 3 H_2O$ = H_2O 9.16; CuO 10.79 per cent.
Found . . . . . = H_2O 9.27; CuO 11.00 per cent.

[1] Kossel and Patten: Zeitschrift für physiologische Chemie, 1903, xxxviii, p. 39.

Histidine.

The solution of the histidine, equal to 500 c.c. from 47.18 gm. of dry and ash-free excelsin contained nitrogen equal to 0.6945 gm. histidine or 1.47 per cent.

100 c.c. solution required 3.77 c.c. 5/7 N HCl = 0.0377 gm. N in 100 c.c., or 0.1885 gm. in 500 c.c. = 0.6945 gm. histidine.

This histidine was converted into the dichloride which crystallized in the rhombohedral crystals characteristic of this salt. These decomposed at about 233°.

Chlorine, 0.0761 gm. subst., gave 0.0947 gm. AgCl.

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

Found . . . . . = N 30.76 " "

Lysine.

The lysine picrate obtained from 47.18 gm. of excelsin by the method of Kossel and Patten weighed 1.9884 gm. equal to 0.7725 gm. lysine picrate or 1.64 per cent.

Nitrogen, 0.3000 gm. subst., dried at 110°, required 5.60 c.c. 5/7 N HCl (Kjeldahl-Jodlbauer).

Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$ = N 18.70 per cent.

Found . . . . . = N 18.66 " "

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

Hydrolysis of Excelsin.

	Per cent.		Per cent.
Glycocoll	0.60	Cystine	not found
Alanine	2.33	Oxyproline	" "
Amino-valerianic acid	1.51	Tyrosine	3.03
Leucine	8.70	Arginine	16.02
Proline	3.65	Histidine	1.47
Phenylalanine	3.55	Lysine	1.64
Aspartic acid	3.85	Ammonia	1.80
Glutaminic acid	12.94	Tryptophane	present
Serine	not found		
Total			61.09

No striking feature is shown by the hydrolysis of excelsin beyond the unusually large proportion of arginine that was found.