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12

Reprinted from the American Journal of Physiology.
VOL. XX. — JANUARY 1, 1908. — NO. IV.

HYDROLYSIS OF AMANDIN FROM THE ALMOND.[1]

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THE chief part of the protein substance of the almond (*Prunus amygdalus var. dulcis*) consists of a globulin to which the name amandin has been given. An investigation of the proteins of the almond made in this laboratory[2] gave no evidence of the presence of more than one globulin in this seed. Amandin is characterized by its high content of nitrogen, about 19 per cent, and by the large proportion of nitrogen which it yields as ammonia on hydrolysis.

In preparing the amandin which was used for this hydrolysis we received the assistance of Mr. I. F. Harris, to whom we wish to express our thanks.

The almonds were freed from their outer coating by immersing them for an instant in hot water and from the greater part of their oil by pressure. The remainder of the oil was removed by petroleum benzine and the meal ground to a fine powder.

The oil-free meal was then extracted with one tenth saturated ammonium sulphate solution, the extract filtered clear, and enough crystals of ammonium sulphate dissolved in it to bring its concentration up to four tenths saturation. The precipitate thus produced was filtered out, dissolved in dilute sodium chloride brine, and the solution, after filtering perfectly clear, was dialyzed for several days. The amandin was thus precipitated for the most part as a somewhat gummy and coherent mass. This, when washed with water and with alcohol, dehydrated with absolute alcohol and dried over sulphuric acid, formed a snow-white powder.

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

[2] OSBORNE and CAMPBELL: Journal American Chemical Society, 1896, xviii, p. 609.

Five hundred grams of this amandin, equal to 457.2 gm. moisture 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 on the water bath until solution was nearly effected. The hydrolysis was then made complete by boiling the solution in the oil bath for fourteen and a half hours.

A preliminary removal of glutaminic acid, performed in the usual manner, yielded 83.64 gm. of the hydrochloride, or 67 gm. of the free acid. The filtrate from glutaminic acid hydrochloride was then concentrated under reduced pressure very sharply, and the residue esterified with alcohol and dry hydrochloric acid gas according to the directions of Emil Fischer.

After liberating the free esters from the hydrochlorides and shaking out with ether, the aqueous layer was freed from inorganic salts and the esterification repeated.

The combined esters were then distilled under diminished pressure with the following result:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	80°	0.8 mm.	14.32 gm.
II	92°	0.8 "	19.94 "
III	100°	0.70 "	53.05 "
IV	134°	0.70 "	50.79 "
V	150°	0.70 "	50.14 "
VI	200°	0.70 "	42.89 "
	Total		231.13 gm.

The undistilled residue weighed 68 gm.

Fraction I. — This fraction yielded 1.10 gm. of the hydrochloride of glycocoll ethyl ester. The melting-point was 144°.

Chlorine, 0.2866 gm. subst., gave 0.2925 gm. AgCl.
Calculated for $C_4H_{10}O_2NCl$ = Cl 25.45 per cent.
Found . . . . . = Cl 25.23 " "

The filtrate was added to the glycocoll filtrate from Fraction II.

Fraction II. — The esters were saponified with boiling water, the solutions evaporated to dryness under reduced pressure, and the residue extracted with boiling alcohol to remove the proline.

The insoluble portion was re-esterified and the glycocoll brought to separation as the hydrochloride of the ethyl ester. The yield

was 3.30 gm. By fractional crystallization of the free amino acids from water and from water and alcohol, there were further obtained from Fraction II 1.50 gm. of leucine, 6.45 gm. of alanine, 0.75 gm. of substance having the percentage composition of amino-valerianic acid and a fraction of perfectly definite appearance, which on analysis gave results agreeing closely for a mixture of equal parts of leucine and amino-valerianic acid.

Carbon and hydrogen, 0.1221 gm. subst., gave 0.2368 gm. CO_2 and 0.1087 gm. H_2O.

Calculated for equal molecules of leucine and amino-valerianic acid = C 53.12 ; H 9.66 per cent.

Found . . . . = C 52.89 ; H 9.88 per cent.

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

Carbon and hydrogen, 0.1542 gm. subst., gave 0.2294 gm. CO_2 and 0.1163 gm. H_2O.

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

Found . . . . = C 40.57 ; H 8.37 " "

The amino-valerianic acid was analyzed as follows:

Carbon and hydrogen, 0.1027 gm. subst., gave 0.1931 gm. CO_2 and 0.0901 gm. H_2O.

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

Found . . . . = C 51.28 ; H 9.75 " "

The preparation of amino-valerianic acid seemed to be homogeneous under the microscope, but, owing to the small amount of substance at our disposal, we are unable to offer any real chemical evidence of the existence of this substance in the protein.

Fraction III. — This fraction was saponified and the proline extracted in the usual way. The part remaining undissolved in alcohol yielded 19.04 gm. of very nearly pure leucine.

Carbon and hydrogen, 0.4203 gm. subst., gave 0.4840 gm. CO_2 and 0.2154 gm. H_2O.

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

Found . . . . = C 54.93 ; H 9.95 " "

The substance decomposed at about 298°.

In the filtrate from leucine no definite substance could be isolated. The proline extracts of Fractions II and III were united.

The substance was converted to the copper salt, and the lævo separated from the racemic with boiling absolute alcohol. The yield of air-dry racemic proline copper was 2.61 gm., while of the amorphous copper salt of lævo-proline there were obtained 11.98 gm. dried at 110°. The racemic proline copper salt was analyzed as follows:

Water, 0.1163 gm. subst. (air-dried), lost 0.0124 gm. H_2O at 110°.
Calculated for $C_{10}H_{16}O_4N_2Cu \cdot 2\ H_2O$ = H_2O 10.99 per cent.
Found . . . . . = H_2O 10.66 " "
Copper, 0.1030 gm. subst., gave 0.0278 gm. CuO.
Calculated for $C_{10}H_{16}O_4N_2Cu$ = Cu 21.81 per cent.
Found . . . . . = Cu 21.56 " "

The lævo-proline was identified as the characteristic phenylhydantoine. The melting-point was 142°–143°.

Carbon and hydrogen, 0.1332 gm. subst., gave 0.3244 gm. CO_2 and 0.0672 gm. H_2O.
Calculated for $C_{12}H_{12}O_2N_2$ = C 66.67; H 5.57 per cent.
Found . . . . . = C 66.42; H 5.60 " "

Fraction IV. — The ester of phenylalanine was removed in the usual manner by shaking out with ether. The yield of phenylalanine hydrochloride was 2.94 gm. As from Fractions V and VI there were further obtained 11.35 gm. of the hydrochloride, the total yield of free phenylalanine from amandin was 11.71 gm., or 2.53 per cent. The substance was identified as the copper salt.

Copper, 0.1133 gm. subst., gave 0.0227 gm. CuO.
Calculated for $C_{18}H_{20}O_4N_2Cu$ = Cu 16.24 per cent.
Found . . . . . = Cu 16.01 " "

From Fraction IV there were further obtained 4.55 gm. of aspartic acid as the barium salt and 12.74 gm. of air-dry copper aspartate. From this fraction no glutaminic acid could be isolated as the hydrochloride.

Fraction V. — There were further obtained from this fraction 6.97 gm. of aspartic acid as the barium salt, 1.06 gm. of glutaminic acid hydrochloride, and 15.25 gm. of air-dry copper aspartate. The latter substance, crystallized in the tyrosine-like bundles of needles and dried in the air, gave the following analysis:

Copper, 0.1381 gm. subst., gave 0.0396 gm. CuO.
Nitrogen, 0.2100 gm. subst., required 1.12 c.c. 5/7 N—HCl.

Calculated for $C_4H_5O_4NCu\ 4\frac{1}{2}\ H_2O$ = Cu 23.07 ; N 5.08 per cent.
Found . . . . . = Cu 22.91 ; N 5.33 " "

The aspartic acid from the barium salt reddened but did not decompose at 300°.

Carbon and hydrogen, 0.2600 gm. subst., gave 0.3458 gm. CO_2 and 0.1292 gm. H_2O.

Calculated for $C_4H_7O_4N$ = C 36.09 ; H 5.26 per cent.
Found . . . . = C 36.26 ; H 5.51 " "

Fraction VI. — The aqueous layer of this fraction, remaining after the removal of phenylalanine ester with ether, was saponified with baryta in the usual way. After prolonged standing no barium aspartate had separated. The barium was accordingly removed and the concentrated solution separated 14.16 gm. of pure glutaminic acid. The decomposition point was at about 198°–199°.

Carbon and hydrogen, 0.1840 gm. subst., gave 0.2759 gm. CO_2 and 0.1064 gm. H_2O.

Calculated for $C_5H_9O_4N$ = C 40.81 ; H 6.12 per cent.
Found . . . . = C 40.89 ; H 6.42 " "

There were further obtained from this fraction 3.4 gm. of glutaminic acid hydrochloride, while no copper aspartate could be obtained.

Tyrosine.

Fifty grams of amandin, moisture and ash free, were boiled with a mixture of 150 gm. sulphuric acid and 300 c.c. of water for ten hours. After removing the sulphuric acid with an equivalent quantity of baryta, the filtrate and washings were concentrated to small volume, and the crystalline substance that separated on standing was filtered out, washed well with water, and recrystallized. The tyrosine thus obtained in characteristic needles weighed 0.5635 gm. equal to 1.12 per cent.

The solution from which the tyrosine separated, together with the mother liquor from the recrystallization, was concentrated and a second separation obtained. This was dried and treated with

glacial acetic acid. All was dissolved, and no more tyrosine could be obtained from the hydrolysis solution.

Nitrogen, 0.5630 gm. subst., required 4.3 c.c. 5/7 N—HCl.
Calculated for $C_9H_{11}O_3N$ = N 7.73 per cent.
Found . . . . = N 7.64 " "

Histidine.

Fifty grams of moisture and ash free amandin were boiled for ten hours with a mixture of 150 gm. sulphuric acid and 300 c.c. of water, and the bases isolated according to the direction of Kossel and Patten. The solution of the histidine was made up to 300 c.c. and nitrogen determined in an aliquot part of it.

Nitrogen, 10 c.c. solution required 0.58 c.c. 5/7 N—HCl = 0.0058 gm. N = 0.1740 gm. N in 300 c.c. = 0.6438 gm. histidine, or 1.29 per cent.

Another determination, made with the same amount of amandin boiled for fourteen hours with the addition of 15 gm. sodium chloride,[3] gave a little higher result, namely, 1.87 per cent.

Nitrogen, 20 c.c. solution, required 1.69 c.c. 5/7 N—HCl = 0.0169 gm. N = 0.2535 gm. N in 300 c.c. = 0.9379 gm. histidine = 1.87 per cent.

The remainder of the histidine was converted into the dichloride, but, owing to a loss, its identity was not established.

Arginine.

The solution from this second hydrolysis, containing the arginine, was made up to 1000 c.c.

Nitrogen, 20 c.c. solution, required 3.68 c.c. 5/7 N—HCl = 0.0368 gm. N = 1.840 gm. N in 1000 c.c. = 5.9189 gm. arginine = 11.85 per cent.

The rest of the arginine was identified as the copper nitrate double salt.

Water, 0.2223 gm. subst., air dry, lost 0.0217 gm. H_2O at 110°.
Calculated for $C_{12}H_{28}O_4N_8Cu(NO_3)_2\ 3\ H_2O$ = H_2O 9.16 per cent.
Found . . . . . = H_2O 9.76 " "

[3] Cf. Hart, Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 348.

Copper, 0.1983 gm. subst. (dried at 110°), gave 0.0292 gm. CuO.

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

Found . . . . . = Cu 11.77 " "

Lysine.

The lysine in the two hydrolysis solutions was converted into the picrate, of which 0.5845 gm. was obtained in the solution to which the sodium chloride had been added and 0.8833 gm. in the solution without this salt. This is equivalent to 0.2277 gm. and 0.3441 gm. lysine respectively, or 0.46 and 0.70 per cent. of the amandin.

Nitrogen, 0.4260 gm. subst., required 7.9 c.c. 5/7 N—HCl (Kjeldahl-Jodlbauer).

Calculated for $C_6H_{14}O_2N_2 \cdot C_6H_3O_7N_3$ = N 18.67 per cent.

Found . . . . . = N 18.54 " "

The results of this hydrolysis were the following:

	Per cent.		Per cent.
Glycocoll	0.51	Serine	?
Alanine	1.40	Tyrosine	1.12
Valine	0.16	Arginine	11.85
Leucine	4.45	Histidine	1.58
Proline	2.44	Lysine	0.70
Phenylalanine	2.53	Ammonia	3.70
Aspartic acid	5.42	Tryptophane	present
Glutaminic acid	23.14[4]		59.00

[4] Osborne and Gilbert: This journal, 1906, xv, p. 350.