

A Comparative Study of the Amino-acids, Contained in Various Silks and Mulberry Leaves.

By Ryugo Inouye,

with cooperation of Suehiko Iwaoka and

Katsu Hirasawa.

I.—INTRODUCTION.

SILK, admired from remote times as a most valuable textile fibre, belongs to a kind of skeletin as Krukenberg designated it, and is chiefly composed of amino-acids. It is produced by the silk-worm from mulberry leaves, taken as its only food. It is, therefore, interesting not merely from the standpoint of physiological chemistry to get some knowledge of the amino-acids both in silks and mulberry leaves, but also because of some valuable results bearing upon the feeding of the silkworm which will probably be derived from it.

Since several years ago, we have closely studied the topics and the following are some of the results of our investigations.

II.—PREVIOUS STUDIES ON THE CHEMISTRY OF SILK.

Before entering into an account of our investigations we wish to give a short historical note on silk-chemistry. For industrial purposes the chemical study of silk was made in comparatively early ages, as a knowledge of the chemical properties of silk was found to be of great benefit to silk bleaching and dyeing. Rigaut de St. Questan first studied the actions of different chemical reagents on silk in 1762; Marquer published his studies in silk dyeing in 1764, and Collomb investigated the effects of hot water on silk in 1785. The first chemical analysis of silk was accomplished by Road in 1801; then Mulder quantitatively determined the composition of raw silk in 1836, and his work was confirmed by Gerhardt in 1856. The actions of acids and alkalies on silk were studied by Durrwell, Waldenberger, Staedeler, Cramer, Weyl, Barreswill, Mulder, Vignon and Sisley, Richard, Obermayer, Pohl, Lidow, and Vogel. The actions of various salts on silk and also salts contained in it were investigated by Sobrero, Francézon, Guinon, and recently by Kitao and Yoshida. But the first knowledge

of the amino-acids was acquired by Hinterberger and Watenberger in 1853, who isolated tyrosine from the cleavage products of fibroin hydrolyzed by hydrochloric acid. Staedeler and Cramer obtained not only the same results by hydrolyzing silk, but glucocoll too. Sützenberger and Burgeois hydrolyzed fibroin with baryta-water by heating it under pressure and get, beside ammonia, oxalic acid, carbonic acid, acetic acid, and a mixture of amino-acids, which were known to consist of tyrosine, glycocoll, alanine, aminobutyric acid and an unsaturated acid, $C_4H_7NO_2$. Afterwards Weyl made a study of the cleavage products of silk and isolated aminopropionic acid from them, besides tyrosine and glycocoll, which were already known. He obtained three amino-acids in a pure state but could not find leucine. For further knowledge of the amino-acids in silk, we owe a great deal to E. Fisher and his collaborator, A. Skita. They employed the "Ester Method" for the separation of amino-acids of fibroin and sericine, and found not only glycocoll, alanine, and tyrosine, but also serine, phenylalanine, proline, and leucine, none of which had been obtained from silk. Abderhalden and his collaborators made great contributions to the amino-acids found in the various kinds of silk, while a little later U. Sudzuki, K. Yoshimura and the present author studied the amino-acids contained in Japanese wild silks. Here it should be added that E. Fisher and E. Abderhalden isolated glycy-l-tyrosine from silk in 1906.

III.—THE AMINO-ACIDS IN THE SILKS.

(a) Hydrolysis of the Silk of the Japanese Univoltine Species, "Kinjoh-matamukasi."

We took a number of cocoons for purpose of hydrolysis. Their total weight was 988 gm.; of which the silk-covers weighted 409 gm., and the pupae and skins casted, together 579 gm., that is, the silk covers were 41.4 per cent. of the total weight of the cocoons, and pupae and skin 58.6 per cent. The moisture of the silk-covers was 8.97 per cent., ash 0.802 per cent., and the total amount of nitrogen 18.25 per cent.

Hydrolysis by Concentrated Hydrochloric Acid.

300 gm. of the silk covers of the cocoons was boiled with concentrate hydrochloric acid under a reverted cooler for 6 hours, and then the mixture was filtered, but no residue was found. The filtrate was distilled under reduced pressure, until a syrup was obtained. It was then dissolved in absolute alcohol and the alcohol was evaporated under low pressure. The resulting syrup was again dissolved in absolute alcohol and saturated with dry gaseous hydrochloric acid. After leaving it 24 hours in a cool place, we got plenty of needle crystals, which were proved to be glycocoll-ethyl-ester hydrochloride. The

crystals were purified by recrystallization in hot alcohol. The filtrate of the glycocoll-ethyl-ester hydrochloride was then concentrated to a syrup from which the free esters of other amino-acids were isolated by the ester-method of E. Fisher.

For detecting the experimental losses we determined the nitrogen contents after each operation in course of the hydrolysis with the following result:

	Nitrogen of 100 gm. of the silk-covers of cocoons.
	In gm.
Total nitrogen	18.25
Hydrolysate	18.18
Insoluble residue	0.00
Glycocoll-ethyl-ester hydrochloride, separated.....	4.50
Filtrate of the glycocoll-ethyl-ester hydrochloride	12.34
Animal charcoal used for purification.....	1.05
Etherial solution of free amino-acid ester. (After setting amino-acid ester free with potassium car- bonate and caustic soda).....	3.18

The first distillation of amino-acide esters.

Fraction 1 (To 60°C on a water-bath, at 20 mm. pressure)	0.08
Fraction 2 (To 98°C, the boiling point at Uyeda, on a water-bath, at 15 mm. pressure)....	1.42
Fraction 3 (To 98°C on a water-bath, at 3 mm pressure)	0.25
Fraction 4 (To 200°C on an oil-bath, at 2 mm. pressure)	0.14
Distillation residue	1.28
Residue after the amino-acid-esters were extracted by ether. (A mixture of salts and the remaining amino-acid-esters)	8.75

As a comparatively large quantity of nitrogen still remained in the residue, when amino-acid esters had been liberated, it was neutralized with hydrochloric acid and the salts thus formed were filtered off. The same operations were repeated two or three times in order to separate the salts completely. Lastly the solution was saturated with dry gaseous hydrochloric acid, and the salts, thus formed, were filtered off. The operations were repeated until no more salts were to be separated; and then the solution was boiled for 5 hours under a reflux condenser. After decolorization with animal charcoal, the solution was concentrated *in vacuo* to a small volume and fractioned by the ester-method, as before.

The following shows the result of determination of nitrogen after each operation as in the former case.

	Nitrogen in 100 gm. of the silk-covers of cocoons.
	In gm.
The salts separated out.....	0.03
Animal charcoal used for purification.....	1.00
Solution decolorized by animal charcoal.....	7.62
Second ethereal solution of the free amino-acid-esters. (After the amino-acid-esters were set free by caustic soda, and potassium carbonate).....	4.35
The second distillation of amino-acid esters.	
Fraction 1 (To 98°C on a water-bath, at 15-18 mm. pressure)	0.89
Fraction 2 (To 98°C on a water-bath, at 15 mm. pressure)	0.93
Fraction 3 (To 98°C on a water-bath, at 3 mm. pressure)	0.11
Fraction 4 (To 180°C on an oil-bath, at 15 mm. pressure)	0.42
Distillation residue	1.30
Residue after extracting the amino-acid-esters by ether	3.13

The quantity of the amino-acid esters obtained by distillation, and that of the amino-acids separated by the hydrolysis of the esters were as follows.

The first distillation of the amino-acid esters.

Fractions	Heating equipment	Temperature	Pressure	Amino-acids esters	Amino-acids
1	Water-bath	To 60°C	20 mm.	29.02 gm.	0.46 gm.
2	"	To 98	15	15.72	13.72
3	"	"	3	3.29	1.47
4	Oil-bath	To 200	2	3.73	0.89
The distillation residue	—	—	—	8.85	—

The second distillation of the amino-acid esters.

Fractions	Heating equipments	Temperature		Pressure	Amino-acid esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water-bath	35-40°C	60°C	15-18 mm.	44.02 gm.	5.12 gm.
2	"	44-67	98	15	9.43	6.06
3	"	85	98	3-4	0.90	0.48
4	Oil-bath	115	180	15	9.21	2.57
The distillation residue	—	—	—	—	10.83	—

Each fraction thus obtained, was hydrolized by the ordinary method, and evaporated *in vacuo* to crystalize the amino-acids. From the amino-acids thus obtained, when impure, or when they are mixed together, copper salts were made and separated by means of fractional crystallization. The separation of tyrosine was made in another way, which will be described later on, and the distillation-residue was hydrolized with baryta-water for further isolation of amino-acids. The identification of the amino-acids obtained was made by nitrogen determination and copper estimation of their copper-salts, in ways which will be explained later. Thus we finally got the following results.

Amino-acids separated	First distillation	Second distillation	Total (In 100 gm. of silk covers of the cocoons)
Glycocoll	24.68 gm.	4.71 gm.	29.39 gm.
Alanine	10.38	6.34	16.72
Leucine	1.47	—	1.47
Aspartic acid	0.03	—	0.03
Glutamic acid	0.023	—	0.023
Proline,	0.05	0.056	0.106
Oxyproline	0.015	0.035	0.05
Serine	0.44	2.57	3.01
Tyrosine	—	—	4.72
Phenylalanine	0.09	0.55	0.64

Hydrolysis by 25 per cent Sulphuric Acid.

100 gm. of the silk covers of the same cocoons was heated in 300 cc. of 25 per cent sulphuric acid for sixteen hours. After that, the sulphuric acid was exactly neutralized with a concentrated solution of baryta water, and the precipitate formed was thoroughly filtered off. The filtrate was evaporated to a small volume for the crystallization of tyrosine, which was collected by filtration. The evaporation was repeated until no tyrosine separated out. The small quantity of tyrosine, which still remained in the mother liquor, was determined by the colorimetric method. The precipitate of barium sulphate, produced by neutralization, was repeatedly boiled in water until the precipitate gave no reaction with Millon's reagent, and the washing liquid was united with the filtrate of the barium sulphate and employed in the separation of tyrosine. At the end of every operation the nitrogen was determined, as in the former case, to see clearly the amount of losses which had occurred.

The result obtained was as follows:

	In 100 gm. of the silk-covers of cocoons.
	In gm.
Total nitrogen	18.25
Hydrolysate	17.88
Insoluble residue	0.35
Filtrate of barium sulphate	17.34
Precipitate of barium sulphate.....	0.34
Tyrosine separated	0.37
Animal charcoal used for discoloring	0.75
Filtrate of tyrosine.....	16.75

The tyrosine, thus separated out, amounted to 4.73 gm. from 100 gm. of the silk-covers of the cocoons.

(b) Hydrolysis of the Silk of the Polyvoltine Species of Formosa.

The weight of the cocoons taken for the analysis was 961 gm. of which the pupae and the chitinous skins casted, amounted to 564 gm., and silk to 397 gm., that is, the pupae and skins casted, were 58.7 per cent of the total cocoons in weight, and the silk 41.3 per cent. The silk contained 9.24 per cent of water, 1.46 per cent of ash, and 17.99 per cent of nitrogen.

The hydrolysis was performed in two ways, as in the former case,

Hydrolysis by Concentrated Hydrochloric Acid.

300 gm. of the silk was boiled with 900 cc. of concentrated hydrochloric acid for six hours. In this case a small quantity of insoluble

residue remained after the completion of the hydrolysis. The filtrate from the residue was evaporated to a syrup *in vacuo* and when the water had been removed as possible, by repeated evaporation with absolute alcohol, dried gaseous hydrochloric acid was introduced into the solution. By this means glycine was separated as fine needle crystals, ethyl-ester hydrochloride. Other amino-acids were isolated by the ester-method and identified by analysis.

At the end of every operation in the course of the hydrolysis the nitrogen was, as in the former case, determined, the result being as follows:

	Nitrogen in 100 gm. of the silk-cover of cocoons, In gm.
Total nitrogen	17.99
Hydrolysate	17.93
Insoluble residue	0.004
Glycocoll-ethylester-hydrochloride	3.78
The filtrate of the glycocoll-ethyl-ester-hydrochloride.	13.99
First ethereal solution of the free amino-acid esters (after setting amino-acid esters in free state by neutralizing their chlorides with caustic soda and potassium carbonate)	3.62
The first distillation of the amino-acid esters.	
Fraction 1 (To 60°C on a water-bath, at 20 mm. pressure)	0.206
Fraction 2 (To 98°C on a water-bath, at 15 mm. pressure)	1.329
Fraction 3 (To 98°C on a water-bath, at 1-0.3 mm. pressure)	0.110
Fraction 4 (To 200°C on an oil-bath, at 1-0.3 mm. pressure)	0.142
Distillation residue	1.355
Residue, not extracted by ether	9.77

The last residue, after extracting the free amino-acids with ether, was neutralized with hydrochloric acid, and the salts thus produced were thoroughly filtered. From the filtrate, the amino-acid esters still remaining were again set free by the ester method and separated by fractional distillation. Nitrogen was determined at the end of every operation, as before; the results being as follows:

	Nitrogen in 100 gm. of the silk-covers of cocoons, In gm.
Salts produced by neutralization.....	0.272
Animal charcoal used for purification	1.250
Second ethereal solution of the amino-acid esters	

(after setting the esters free by caustic soda and potassium carbonate) 2.589

The second distillation of the amino-acid esters.

Fraction 1 (To 60°C on a water-bath, at 15-30 mm. pressure) 0.541
 Fraction 2 (To 98°C on a water-bath, at 15 mm. pressure) 0.645
 Fraction 3 (To 98°C on a water-bath, at 1-0.3 mm. pressure) 0.271
 Fraction 4 (To 200°C on an oil-bath, at 1-0.3 mm. pressure) 0.010
 Distillation residue 0.786
 Residue, not extracted by ether..... 5.868

The fractions and distillation residue were hydrolyzed by the usual methods, and then the following result was obtained.

The first distillation.

Fractions	Equipments for heating	Temperature		Pressure	Amino-acids esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water bath	37-43°C	42-60°C	15-30mm.	23.35 gm.	3.11 gm.
2	Water bath	55-62	60-68	15	6.12	3.79
3	Water bath	58-70	98	0.3-1	1.67	1.01
4	Oil bath	84	200	0.3-1	2.17	0.41
Distillation residue	—	—	—	—	5.88	—

The second distillation.

Fractions	Equipments for heating	Temperature		Pressure	Amino-acid esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water bath	37-43°C	42-60°C	15-30mm.	23.35 gm.	3.11 gm.
2	Water bath	55-62	60-98	15	6.12	3.79
3	Water bath	58-70	98	0.3-1	1.67	1.01
4	Oil bath	84	200	0.3-1	2.17	0.41
Distillation residue	—	—	—	—	5.88	—

The fractions thus obtained, were treated in the same way as before, for the separation of each amino-acid, and the distillation residue was hydrolyzed with baryta, which was then completely removed with sulphuric acid and employed for the same purpose as before.

The result summed up was as follows:

Amino-acids separated	First fractional distillation	Second fractional distillation	Total 100 gm. of the silk-covers of cocoons)
Glycocoll	20.77 gm.	3.42 gm.	24.19 gm.
Alanine	9.92	4.02	13.94
Leucine	0.30	0.30	0.60
Aspartic acid	present	0.41	0.41
Glutamic acid	present	—	present
Proline	0.001	0.008	0.009
Oxyproline	0.04	0.03	0.07
Tyrosine	—	—	3.89
Pheny alanine	0.59	0.25	0.84
Serine	0.33	—	0.33

Hydrolysis by Sulphuric Acid.

For the separation of tyrosine, 100 gm. of the cocoon silk was heated with 25 per cent sulphuric acid for 16 hours. When the boiling was over, the sulphuric acid was exactly neutralized with barium hydroxide, barium sulphate thus produced was filtered off, and the filtrate, together with the water used in washing out the precipitate of barium sulphate, was evaporated on a water bath, until tyrosine crystalized out. Then the mother liquor was allowed to stand for 24 hours and the tyrosine separated was filtered off. The filtrate was treated in the same way, until no more crystals of tyrosine could be obtained. The small quantity of tyrosine which still remained in the liquor, was determined by a colorimetric method. At the end of every operation nitrogen determination was performed to test the exactness of the analysis and the result was as follows:

	Nitrogen in 100 gm. of the cocoon silk.
Total nitrogen	In. gm. 17.99
Hydrolysate	17.31

Insoluble residue	0.63
Filtrate of barium sulphate	16.87
Barium sulphate	0.45
Tyrosine separated	0.30
Animal charcoal used for the purification.....	1.15
Filtrate of tyrosine	15.06

The total amount of the tyrosine thus separated from 100 gm. of the silk cover of cocoon was 3.80 gm.

The Identification of the Amino-acids Isolated from Both Silk.

Glycocoll, converted into ethylester hydrochloride was identified by its crystal form and melting point. In the first and second fractions it was obtained together with alanine; in this case glycine was separated from alanine by fractional crystallization, after it was changed into a copper salt, and identified by the determination of the copper. Alanine was identified by the estimation of the nitrogen. Proline was separated as a copper salt and identified by it; aspartic acid, serine, and leucine were respectively changed into copper salts and identified by determination of the copper.

1) Glycocoll:—0.455 gm. of glycocoll ethyl ester hydrochloride was taken for determination of nitrogen, and 0.46 gm. of nitrogen by Kjeldahl's method.

Theoretical number (as HCl, $\text{NH}_2\text{CH}_2\text{COOC}_2\text{H}_5$)	10.04 % N
Found	10.09 % N

2) Alanine:—0.1208 gm. of the alanine-copper gave 0.04 gm. of copper oxide.

Theoretical number (as $\text{Cu}(\text{C}_3\text{H}_6\text{NO}_2)_2$).....	26.54 % Cu
Found	26.51 % Cu

3) Leucine:—0.0203 gm. of the copper oxide was obtained from 0.0982 gm. of the leucine copper by burning.

Theoretical number (as $\text{Cu}(\text{C}_6\text{H}_{12}\text{NO}_2)_2$).....	19.66 % Cu
Found	20.70 % Cu

4) Aspartic acid:—0.486 gm. of the copper salt was ignited, and 0.1416 gm. of the copper oxide was obtained.

Theoretical number (as $\text{CuC}_4\text{H}_5\text{NO}_4 + 4\frac{1}{2}\text{H}_2\text{O}$)..	23.06 % Cu
Found	23.31 % Cu

5) Glutamic acid:—From 0.0522 gm. of the anhydrous copper salt, 0.0198 gm. of copper oxide was obtained.

Theoretical number (as $\text{CuC}_5\text{H}_7\text{NO}_4$).....	30.47 % Cu
Found	30.36 % Cu

6) Serine:—0.0406 gm. of the copper salt gave 0.0118 gm. of copper oxide.

Theoretical number (Cu(C ₃ H ₆ O ₃ N ₂)).....	23.40 % Cu
Found	23.36 % Cu

(c) Hydrolysis of the Cocoon Silk of Landybe.

Landybe, *Borocera Madagascariensis*, Boisduvael, is a wild silkworm, native of Madagascar, off the eastern coast of Africa. Its cocoon is grayish brown in colour and very rough in structure. Mr. Kijima, Japanese consul in Lyon, brought some from Madagascar and was so kind as to give them to me, when I was in Lyon.

The cocoons contained 9.68 per cent of water, and 6.77 per cent of ash. On boiling them in dilute sulphuric acid we found that 100 gm. of the cocoon silk gave 31.08 gm. of insoluble substance.

200 gm. of the cocoon silk, freed from impurities as completely as possible, was warmed in one litre of 25 per cent. sulphuric acid on a water-bath, until it was totally dissolved. The solution thus obtained was boiled for 16 hours, under a reflux condenser. The insoluble residue was then filtered off and washed with distilled water until the washing liquid gave no reaction of sulphuric acid.

The residue, washed and dried to the constant weight, was found to weigh 31.08 gm. for 100 gm. of the cocoons and contain 2.96 gm. of nitrogen. As much nitrogen still remained in the residue, it was again hydrolyzed with 25 per cent. sulphuric acid for twenty hours, and then filtered. The nitrogen in the filtrate and residue was determined; the result being as follows:

	In 100 gm. of cocoon-silks.
	Nitrogen in gm.
Total nitrogen in the first residue.....	2.96
First hydrolysate	0.91
Second residue	2.03
Second hydrolysate	1.05
Third residue	0.83

All the hydrolysates were put together, and the sulphuric acid was exactly neutralized with baryta, and the barium sulphate thus produced was filtered and boiled in water thoroughly to remove all nitrogen contained in the precipitate. The operation was repeated until the filtrate and precipitate gave no reaction with Millon's reagent. Even with these treatments, some nitrogen still remained in the precipitate of the barium sulphate.

The analytical result was as follows:

	In 100 gm. of cocoons.
After the first extraction.....	{ Extracted water..0.25 gm nitrogen
	{ Barium sulphate..1.14 " "
After the second extraction....	{ Extracted water..0.10 " "
	{ Barium sulphate..1.00 " "

These extracts were united with hydrolysate, and the whole liquid was evaporated under reduced pressure at 40° C for the crystallization of tyrosine. After evaporating to a small volume, the liquid was left to stand in a cold place for about 24 hours, and the tyrosine thus separated out was filtered. The operation was repeated until the mother liquor did not give Million's reaction at all. The filtrate of tyrosine was evaporated to dryness, absolute alcohol was added, and then dried hydrochloric acid gas was passed into the solution until it was saturated. In this way glycine was crystallized out as ethylester hydrochloride. After the complete separation of glycocoll, the mother liquor was further evaporated to a syrup and treated by the ester method for the separation of other amino-acids. The residue not extracted by ether, was dissolved by hydrochloric acid and salts produced by neutralization, were filtered, and the filtrate was evaporated under reduced pressure to a syrup, which was again treated with the ester method for obtaining the still remaining amino-acids. At the end of every operation in the course of the analysis, the nitrogen was determined for ascertaining the losses, and result was as follows:

	In 100 gm. of the cocoons silk.
	In gm.
Total nitrogen	15.80....
Hydrolysate	12.88
Insoluble residue	2.96
Barium sulphate precipitate	1.49
Filtrate of the barium sulphate	11.37
Tyrosine crystallized out	0.37
Mother liquor of tyrosine	10.43
Glycocoll separated (as ethylester hydrochloride)....	1.95
Mother liquor of glycocoll ethylester hydrochloride..	8.34
Etherial solution of free amino-acid esters (after setting the esters in free state by caustic soda and potas- sium carbonate)	0.68
The first distillation of amino acid esters.	
Fraction 1 (To 100°C on a water-bath, at 20 mm. pressure)	0.15
Fraction 2 (To 100°C on a water-bath, at 20 mm. pressure)	0.11
Fraction 3 (To 200°C on an oil bath, at 0.15 mm. pressure)	0.21
Distillation residue	0.16
Residue after extracting amino-acid esters with ether (mixture of salts and amino-acid esters not extracted by ether)	7.46
Sodium chloride produced by the neutralization of the solution of the above residue.....	0.10
Filtrate of sodium chloride	6.73

The second distillation of amino-acid esters.

Fraction 1 (To 100°C on a water-bath, at 15 to 20 mm. pressure)	0.84
Fraction 2 (To 100°C on a water-bath, at 0.3 mm. pressure)	0.12
Fraction 3 (To 200°C on an oil bath, at 0.3 mm. pressure)	0.52
Distillation residue	0.62
The residue after extracting amino-acid esters with ether (the mixture of salts and amino-acid esters not extracted by ether)	4.01

The amino-acids finally separated were as follows:

	In 100 gm. of cocoon-silk.
	In gm.
Glycocoll	10.38
Alanine	3.70
Aspartic acid	0.75
Asparatic acid	0.75
Glutamic acid	2.08
Proline	0.33
Oxyproline	0.07
Tyrosine	4.37

The Identification of the Amino-acids Separated.

Glycocoll was identified by the crystal form and the melting point of its ethylester hydrochloride; alanine and leucine were by the determination of the specific rotation, the nitrogen contents, and the copper contained in the copper salts. Glutamic acid was determined by its hydrochloride.

1) Glycocoll:—0.2472 gm. of the substance required 18.22 cc. of 0.1 N H_2SO_4 for the determination of the nitrogen.

Theoretical number (as $C_4H_9NO_2$, HCl)	10.04 % N
Experimental number	10.33 % N

2) Alanine:—0.2765 gm. of the substance required 40 cc. of 0.1 N sulphuric acid for the determination of the nitrogen.

Theoretical number (as $C_3H_7NO_2$)	15.73 % N
Experimental number	16.07 % N

3) Leucine:—0.1638 gm. was taken for the nitrogen determination and 12.54 cc. of 0.1 N sulphuric acid was required for neutralization.

Theoretical number (as $C_6H_{13}NO_2$)	10.69 % N
Experimental number	10.73 % N

0.0676 gm. of the copper salt produced 0.0164 gm. of copper oxide by ignition.

Theoretical number (as $\text{Cu}(\text{C}_6\text{H}_{12}\text{NO}_2)_2$)..... 19.66 % Cu
 Experimental number 19.37 % Cu

4) Aspartic acid:—0.1098 gm. of the copper salt produced 0.0316 gm. of copper oxide by ignition.

Theoretical number (as $\text{CuC}_4\text{H}_5\text{NO}_4 + 4.5\text{H}_2\text{O}$)... 23.06 % Cu
 Experimental number 23.00 % Cu

5) Glutamic acid:—0.2154 gm. of the substance required 12.06 cc. of 0.1 N sulphuric acid for the determination of the nitrogen.

Theoretical number (as $\text{C}_5\text{H}_9\text{NO}_4$, HCl)..... 7.63 % N
 Experimental number 7.84 % N

6) Tyrosine:—0.2000 gm. of the substance required 11.17 cc. of 0.1 N H_2SO_4 for the determination of nitrogen.

Theoretical number (as $\text{C}_9\text{H}_{11}\text{NO}_3$)..... 7.74 % N
 Experimental number 7.81 % N

(d) The Total Hydrolysis of the Cocoon--silk of the Bivoltine Silk-worm, "Daiwanisiki" (Autumn Breed), and of the Sericine Separated from it.

The cocoons were raised at the Uyeda Sericultural College, Nagano-ken, in central Japan. After being gathered, they were dried in the usual way and preserved. For hydrolysis good cocoons were selected, and pupae, skin rejected, and other impurities were thoroughly removed, so that cocoon silk clean and pure, was employed for the analysis. They had the following general composition.

	In 100 gm. of cocoon-silk. In gm.	In 100 gm. of dry cocoon-silk. In gm.
Water	8.95	—
Ash	0.67	0.740
Nitrogen in various forms.		
Total nitrogen		18.191
Water soluble nitrogen		2.678
Nitrogen soluble in concentrated hydrochloric acid..		17.187
Of which, nitrogen precipitated by phospho- tungstic acid		1.303
Of which, nitrogen not precipitated by phospho- tungstic acid		15.494
Nitrogen insoluble in concentrated hydrochloric acid		1.075

300 gm. of cocoon silk was dissolved in about 900 cc. of concentrated hydrochloric acid and boiled for six hours under a reflux condenser. At the end of the operation the hydrolysate was diluted with

water and filtered off to separate the insoluble matter, which was thoroughly washed with water after filtration. The filtrate was evaporated under reduced pressure to dryness and the operation was repeated again and again, each time adding some absolute alcohol to remove moisture as far as possible. Finally dry gaseous hydrochloric acid was passed in to saturation. When it has been cooled for a long time, glycocoll crystallized as ethylester hydrochloride. The filtrate of the glycocoll ethylester hydrochloride was evaporated under reduced pressure to remove the hydrochloric acid and the remaining amino-acids were separated by E. Fisher's ester method. The residue, remaining after the extraction of amino-acid esters with ether, was dissolved again in hydrochloric acid, and the salts produced on dissolving, were filtered off and washed thoroughly with dilute alcohol. The filtrate and the washed liquid were put together, and again evaporated under reduced pressure to a small volume. Then the whole solution was boiled with concentrated hydrochloric acid under a reflux condenser for five hours and again evaporated in a vacuum till it was syrupy. The amino-acids, which still remained in the syrup, were separated by the ester method. At the end of each operation the nitrogen in the solution and the substance separated from it, was determined in order to know the amount of losses, and the result was as follows:

	Nitrogen in 1000 gm. of cocoons-silk.
	In gm.
Total nitrogen	18.191
Hydrolysate	17.704
Insoluble residue	—
Glycocoll ethylester hydrochloride	4.51
First ethereal solution of the amino-acid esters....	4.63
The first distillation of the amino-acid esters.	
Fraction 1 (To 60°C on a water-bath, at 18 mm. pressure)	0.75
Fraction 2 (To 98°C on a water-bath, at 16 mm. pressure)	2.02
Fraction 3 (To 98°C on a water-bath, at 0.4 mm. pressure)	0.19
Fraction 4 (To 200°C on an oil-bath, at 0.01 pressure)	0.36
Distillation residue	1.28
Residue after the amino-acid esters extracted with ether	6.23
Salts produced by the neutralization of potassium carbonate and soda with hydrochloric acid....	—
Second ethereal solution of the amino-acid esters..	4.03
The second distillation of the amino-acid esters.	
Fraction 1 (To 60°C on a water-bath, at 17 mm. pressure)	0.57

Fraction 2 (To 98°C on a water-bath, at 18 mm. pressure)	1.40
Fraction 3 (To 98°C on a water-bath, at 2 mm. pressure)	0.07
Fraction 4 (To 200°C on an oil-bath, at 0.1 mm. pressure)	0.05
Distillation residue	0.93

The residue after amino-acid esters have been extracted by ether 1.89

The fractions obtained by the distillation of the amino-acid esters were hydrolysed by the ordinary method and amino-acids were separated. The results of the fractional distillation were as follows:

The first distillation of the amino-acid ester.

Fractions	Heating equipments	Temperature.		Pressure	Amino-acid esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water bath	49°C	60°C	18 mm.	26.50 gm.	4.23 gm.
2	Water bath	83	98	16	16.80	11.53
3	Water bath	86	98	0.4	2.92	0.98
4	Oil bath	137	200	0.1	6.67	—
Distillation residue	—	—	—	—	10.85	—

The second distillation of amino-acid ester.

Fractions	Heating equipments	Temperature		Pressure	Amino-acid esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water bath	50°C	62°C	17 mm.	14.48 gm.	2.80 gm.
2	Water bath	93	98	18	12.12	7.72
3	Water bath	88	98	2	1.78	0.33
4	Oil bath	132	200	0.1	5.70	3.12
Distillation residue	—	—	—	—	7.71	—

From the mixture of the amino-acids obtained by distilling each fraction after hydrolysis, proline was extracted by absolute alcohol and other amino-acids were separated directly by fractional crystallization or by the same method after converting them into copper salts. The amino-acids thus isolated, were determined by analysis, finally the following amino-acids were obtained from 100 gm. of dry cocoon silk. Tyrosine was separated by another method, which will be described presently.

Hydrolysis by Sulphuric Acid.

50 gm. of the same cocoon-silk was digested with 200 cc. of 25% sulphuric acid for 16 hours under a reflux condenser, and then the sulphuric acid was exactly removed by adding concentrated solution of baryta. The filtrate of the barium sulphate thus produced was evaporated to a small volume and left till tyrosine crystallized. The filtrate of tyrosine was further evaporated in order to make the tyrosine, still remaining in the solution to separate. The operation was repeated till no more tyrosine could be obtained by crystallization, and the very small quantity of it, still in the mother liquor, was determined by a volumetric method. The barium sulphate, produced by the neutralization of sulphuric acid, was repeatedly boiled with distilled water to remove the tyrosine contained in the precipitate. The solution obtained by extraction was united to the tyrosine solution.

As described before, nitrogen was determined after each operation to know the amount of the loss, which might have taken place, and the result was as follows:

	In 100 gm. of dry cocoon silk.
	In gm.
Total nitrogen	18.191
Hydrolysate	17.301
Insoluble residue	0.622
Filtrate of barium sulphate	16.027
Precipitate of barium sulphate	0.601
Tyrosine separated	0.438
Animal charcoal used for decolorization	0.537

The amount of tyrosine separated from 100 gm. of dry cocoon silk was 5.68 gm.

Hydrolysis of Sericine Obtained from the Same Cocoon Silk.

General analysis.

The cocoon silk was made free from impurities and extracted first with alcohol and then with ether.

Separation of sericine:—Concerning the separation of sericine, only two or three investigations have been made. E. Cramer precipitated sericine by lead acetate from a water solution; S. Bondi evaporated a

sericine solution to a small volume and precipitated it by alcohol or acetic acid, while E. Fisher obtained it merely by evaporating the solution, got by treating raw silk with water at 118°C in an autoclave. The author extracted 889 gm. of the cocoon silk with water under two atmospheric pressures in an autoclave for 2 or 3 hours, and filtered it from the silk, which was thoroughly washed. The same operations were repeated until the extract gave no biuret reaction. The total solutions, thus obtained by extracting, were united together and evaporated on a water-bath to a syrup and dried in a desiccator. The following amount of sericine was obtained.

	In gm.
Sample	880
Sericine separated	213
In 100 gm. of the sample.....	24.2

The sericine thus obtained was preliminarily analysed, with the following result.

	In 100 gm. of sericine.
	In gm.
Water	1.08
Ash	1.28
Various forms of nitrogen.	

	* In 100 gm. of dry sericine.
	In gm.
Total nitrogen	17.50
Nitrogen dissolved by boiling the sericine with 25% sulphuric acid for 15 hours.....	14.31
In which, nitrogen not precipitated by phosphotungstic acid	2.21
In which, nitrogen not precipitated by phosphotungstic acid	12.10
Nitrogen not dissolved by boiling with 25% sulphuric acid for 15 hours.....	3.10

Total hydrolysis.

200 gm. of sericine was extracted with 95% alcohol for three hours, with the following result:

Matter soluble in alcohol.....	5.40
Matter insoluble in alcohol.....	194.60

The sericine, thus purified by alcohol, was hydrolysed with 25 per cent sulphuric acid under a reflux condenser for 15 hours, and the hydrolysate was filtered to separate insoluble residue. The latter was repeatedly washed with water until the washing liquid did not give Millon's reaction. The whole extracts were united to the hydrolysate and evaporated under reduced pressure to a small volume. Then sulphate thus produced was filtered off. The precipitate was boiled with water and washed, until the washing liquid did not give Millon's reaction. The extracts thus obtained, were united to the filtrate of barium sulphate, evaporated to a small volume, and decolorized with

blood charcoal. The liquid was again evaporated to a small volume and tyrosine was separated from it. The filtrate of tyrosine was evaporated again to a small volume for the further separation of tyrosine. The tyrosine, still remaining in the solution, was determined by the volumetric method. In this way 3.18 gm. of tyrosine was obtained from 100 gm. of sericine.

The solution from which tyrosine was separated, was evaporated to a syrup under reduced pressure, and treated repeatedly with absolute alcohol in order to remove water. The alcoholic solution was finally saturated with dry hydrochloric acid gas to crystallize glycocoll as ethyl-ester hydrochloride. By this means a small quantity of crystals was obtained, and the liquid was then evaporated in a vacuum to remove the excess of hydrochloric acid, and treated by the ester-method to separate amino-acids. After the first fractional distillation of amino-acid esters, the residue, not extracted by ether was dissolved by hydrochloric acid, and the salts produced by neutralization with acid, were filtered off. The filtrate was saturated with gaseous hydrochloric acid and the salts produced were again filtered. The liquid thus obtained was boiled under a reflux condenser for six hours and then evaporated to a syrup, which was employed again to separate amino-acids by the ester-method. The amino-acid esters thus obtained, were hydrolyzed by usual methods and amino-acids were isolated from each other. At the end of each operation the nitrogen was determined to know clearly the amount of loss which might have occurred, with the following result.

	Nitrogen in 100 gm. of anhydrous sericine.
	In gm.
Total nitrogen	17.50
Hydrolysate	14.34
Insoluble residue	3.16
Animal charcoal used for decolorization.....	0.24
Precipitate of barium sulphate	0.92
Filtrate of barium sulphate.....	12.34
Tyrosine separated by crystallization.....	0.25
First ethereal solution of free amino-acid esters.....	3.03
The First distillation of amino-acid esters.	
Fraction 1 (To 60°C on a water-bath, at 35 mm. pressure)	0.09
Fraction 2 (To 98°C on a water-bath, at 18 mm. pressure)	0.23
Fraction 3 (To 200°C on an oil-bath, at 0.01 mm. pressure)	1.00
Distillation residue	1.54
Residue after extracting amino-acid esters with ether..	9.25
Salts produced by neutralization the residue with hydrochloric acid	0.28
Solution of the amino-acid ester hydrochloride.....	8.82

Second ethereal solution of amino-acid esters..... 3.20

The second distillation of amino-acid esters.

Fraction 1 (To 60°C on a water-bath, at 18 mm. pressure) 0.09

Fraction 2 (To 98°C a water-bath, at 16 mm. pressure) 0.33

Fraction 3 (To 89°C on a water-bath, at 3 mm. pressure) 0.27

Fraction 4 (To 200°C on an oil-bath, at 0.01 mm. pressure) 1.06

Distillation residue 0.71

Residue after extracting amino-acid esters with ether.. 4.77

Each fraction obtained by distillation was hydrolyzed in the usual way and amino-acids crystallized, with the following result.

The first distillation of amino-acid esters.

Fractions	Heating equipments	Temperature		Pressure	Amino-acid esters	Amino-acids
		Inside of the flask	Outside of the flask			
1	Water-bath	45°C	60°C	35 mm.	45.1gm.	0.75 gm.
2	Water-bath	74	98	18	9.2	5.10
3	Water-bath	72	98	4	5.65	2.30
4	Oil-bath	—	200	0.1	20.00	10.90
Distillation residue	—	—	—	—	—	9.50

The second distillation of amino-acid esters.

Fractions	Heating equipments	Temperature		Pressure	Amino-acid esters	Amino-acids
		Inside	Outside			
1	Water-bath	42°C	60°C	17 mm.	30.60 gm.	1.5 gm.
2	Water-bath	77	98	16	10.96	5.50
3	Water-bath	83	98	2	7.60	2.65
4	Oil-bath	124	210	0.1	26.45	—
Distillation residue	—	—	—	—	—	9.65

The amino-acids thus obtained were separated from each other by fractional crystallization and the copper salts were also made from them for further separation. Each of the amino-acids was identified by analysis. Proline was separated from the other amino-acids by extracting each fraction with boiling alcohol and phenylalanine was extracted from the fourth fraction with ether, and crystallized as chloride. The amount of amino-acids obtained was as follows:

Amino-acids separated	The first and second distillation	In 100 gm. of anhydrous sericine	In 100 gm. of anhydrous sericine, extracted with absolute alcohol.
Glycocoll	7.64 gm.	3.86 gm.	3.93 gm.
Alanine	6.86	3.49	3.53
Leucine	0.78	0.39	0.4
Aspartic acid	7.73	3.91	3.97
Glutamic acid	1.6	0.81	0.82
Proline	0.67	0.35	0.35
Serine	11.66	5.89	5.99
Phenylalanine	0.96	0.49	0.49
Tyrosine	—	3.18	3.27

Identification of the Amino-acids Separated.

Glycocoll:—Its ethylester hydrochloride melted at 143°C. 0.1360 gm. of the copper salt gave 0.0470 gm. of copper oxide by burning.

Theoretical number (as $(\text{NH}_2\text{CH}_2\text{COO})_2\text{Cu} + \text{H}_2\text{O}$) 27.68 % Cu
 Experimental number 27.65 % Cu

Alanine:—0.3474 gm. of anhydrous alanine produced 0.5435 gm. of nitrogen by Kjeldahl's method.

Theoretical number (as $\text{C}_3\text{H}_7\text{O}_2\text{N}$) 15.73 % N
 Experimental number 15.64 % N

Leucine:—0.0456 gm. of the copper salt produced 0.0108 gm. of copper oxide by burning.

Theoretical number (as $(\text{C}_6\text{H}_{12}\text{NO}_2)_2\text{Cu}$) 19.66 % Cu
 Experimental number 18.95 % Cu

Aspartic acid:—From 0.0722 gm. of the copper salt we obtained 0.0210 gm. of copper oxide by burning.

Theoretical number (as $\text{Cu}(\text{C}_4\text{H}_7\text{NO}_4) + 4\frac{1}{2} \text{H}_2\text{O}$) 23.06 % Cu
 Experimental number 23.24 % Cu

Glutamic acid:—By burning 0.0942 gm. of the copper salt 0.0344 gm. of copper oxide was obtained.

Theoretical number (as $\text{Cu}(\text{C}_5\text{H}_7\text{NO}_4)$).....	30.47 % Cu
Experimental number	29.54 % Cu

Serine:—From 0.1449 gm. of the copper salt 0.0332 gm. of copper oxide was obtained.

Theoretical number (as $\text{Cu}(\text{C}_3\text{H}_6\text{O}_3\text{N})_2$).....	33.40 % Cu
Experimental number	22.91 % Cu

Besides the above, phenylalanine was determined by converting it into hydrochloride, and proline was separated by treating it with alcohol and determined by making a copper salt from it, but for analysis its quantity was too small. Tyrosine was determined by its crystal form and solubility in water.

IV.—THE AMINO-ACIDS IN THE MULBERRY LEAVES.

General Consideration.

As the mulberry leaves are practically the only food of the domesticated silkworm, it is obvious that silk is exclusively composed of the ingredients of mulberry leaves. And since silk consists, as has been stated above, principally of amino-acids, it may be thought that the amino-acids in the mulberry leaves play an important part in the formation of silk in the interior laboratory of the silk worm.

Concerning the nitrogenous compounds in the mulberry leaves, very little study has been made; the presence of adenine and aspartic acid in the leaves was ascertained by Z. Mimuroto and besides these compounds, choline and trigonerine were found in the leaves by S. Yoshimura and M. Kanai. Y. Koga made a study of the nitrogenous compounds in mulberry leaves and isolated guanine, adenine, hypoxanthine, glycocoll, alanine, leucine, valine, proline, and aspartic acid. Then E. Katayama hydrolyzed the protein matter of mulberry leaves and also the leaves themselves, and separated glycocoll, alanine, valine, leucine, proline, serine, aspartic acid, phenylalanine, guanine, adenine, hypoxanthine, histidine, lysine, choline, trigonerine, and tyrosine and determined them by analysis. My purpose was to hydrolyze young and mature leaves of two varieties of mulberry, "Rosow" and "Nedzumigaeshi," and to compare the amino-acids contained in them.

(a) The Amino-acids in Mature Leaves of "Rosow."

Preliminary Analysis.

Collection of Leaves.

On 30th August, 1913, the leaves we used were collected in the farm of the Uyeda Sericultural College at Uyeda, Nagano-ken, in

the middle Japan. Their fresh weight was 1859 gm.; they were dried and ground to fine powder for analysis.

Determination of Water in the Leaves.

The leaves were collected from the upper, middle and lower parts of a stem and immediately closed in weighing tubes separately. Thus they were protected from loss of water as far as possible, while they were being gathered.

	Water.
	%
The leaves in the upper part of a stem.....	77.17
The leaves in the upper part of a stem.....	77.32
The leaves in the middle part of a stem.....	69.89
The leaves in the lower part of a stem.....	65.84
Average	72.56

Summary of the General Analysis.

	In 100 gm. of the dry matter.
	In gm.
Part soluble in hot concentrated hydrochloric acid..	62.40
Part insoluble in hot concentrated hydrochloric acid..	37.60
Ash	11.21
Total nitrogen	3.79
Albuminous nitrogen	3.53
Water soluble nitrogen	0.93
Nitrogen soluble in hot concentrated hydrochloric acid.	3.049
Of the above nitrogen the part precipitated by phosphotungstic acid (with exception of ammonia nitrogen)	0.206
Part not precipitated by phosphotungstic acid	2.178
Ammonia nitrogen	0.665
Nitrogen insoluble in hot concentrated hydrochloric acid	0.56

Total Hydrolysis.

2500 gm. of the dry leaves ground to fine powder was boiled with three times its quantity of concentrated hydrochloric acid of specific gravity 1.17 under a reflux condenser for six hours. When it was decomposed and the biuret reaction has disappeared, the solution was diluted with water and filtered. The residue was washed with water, until the filtrate gave no reaction with a solution of silver nitrate. The residue was then dried and weighed. The filtrate and all washing liquids were put together and evaporated *in vacuo*. On the reduction of the volume by evaporation, much mineral salts, crystallized, and they were removed by filtering. Finally the solution was evaporated to dryness, absolute alcohol was added and the salts thus produced were filtered off. Then more absolute alcohol was added and saturated with dry gaseous hydrochloric acid of the purpose of separating glycooll as

ethylester hydrochloride. After allowing to stand for 24 hours with ice on seeding the solution with crystal of glycocoll-ethyl-ester hydrochloride, no separation of the compound was obtained. Then the solution was further concentrated to a syrup *in vacuo* at 40°C and from this syrup free esters were extracted with ether by the ester-method. The residue not extracted was dissolved in hydrochloric acid, and the solution was then evaporated to a small volume, and the salts produced were filtered off. The filtrate was treated with the same method for setting free the remaining esters. The operation was repeated, for extracting the still remaining esters. At the end of each operation the nitrogen was determined for ascertaining the amount of losses which might have taken place in the course of the analysis. The following was the result:

	Nitrogen in 100 gm, of the dried mulberry leaves.
	In gm.
Total nitrogen	3.79
Hydrolysate	3.16
Insoluble residue	0.58
Hydrolysate decolourized by animal charcoal.....	3.05
First animal charcoal	0.16
Second animal charcoal	0.22
Filtrate of the second animal charcoal	2.73
Filtrate of the inorganic salts produced.....	2.69
Inorganic salts produced	0.04

The first distillation.

The ethereal solution of the free amino-acid esters (after setting the esters in free state with caustic soda and potassium carbonate)	0.245
Fraction 1 (To 60°C on a water-bath, at 20-30 mm. pressure)	0.032
Fraction 2 (To 98.5°C on a water-bath, at 20-30 mm. pressure)	0.027
Fraction 3 (To 98.5°C on a water-bath, at 3-4 mm. pressure)	0.005
Fraction 4 (To 200°C on an oil-bath, at 3-4 mm. pressure)	0.044
Fraction 5 (To 240°C on an oil-bath, at 3-4 mm. pressure)	0.004
Distillation residue	0.055
Nitrogen in phenylalanine separated	0.77
The residue after extracting the esters with ether (mixture of the salts and the substances not extracted by ether)	2.32
Solution obtained by neutralizing potassium carbonate	

with hydrochloric acid and decolorized by animal charcoal 2.208
 Nitrogen absorbed by animal charcoal 0.09

The second distillation.

The ethereal solution of free amino-acid esters (After setting the esters in free state with caustic soda and potassium carbonate) 0.1116
 Fraction 1 (To 60°C on a water bath, at 15-20 mm. pressure) 0.011
 Fraction 2 (To 98°C on water-bath, at 15-20 mm. pressure) 0.037
 Fraction 3 (To 98°C on a water-bath, at 2-3 mm. pressure) 0.003
 Fraction 4 (To 200°C on an oil-bath, at 2-3 mm. pressure) 0.0098
 Distillation residue 0.032
 Residue after extracting the esters with ether 2.070
 Filtrate of the precipitate produced by phosphotungstic acid 2.000

The third distillation.

Ethereal solution of the amino-acid esters..... 0.051
 Fraction 1 (To 98°C on a water-bath, at 12-17 mm. pressure) 0.0134
 Fraction 2 (To 98°C on a water-bath, at 2 mm. pressure) 0.004
 Fraction 3 (To 200°C on an oil-bath, at 2 mm. pressure) 0.004
 Distillation residue 0.028
 Residue after extracting esters with ether..... 1.876

The results obtained by distilling amino-acid esters were as follows:
 follows:

The first distillation.

Fractions	Heating equipments	Temperatuer		Presure	Distillation time	Amino-acid esters (in 2500 gm.)
		Inside of the flask	Outside of the flask			
1	Water-bath	30°C	60°C	20-30mm.	1 hour	14.98 gm.
2	Water-bath	75-78	98	20-30	2	15.34
3	Water-bath	75-76	98	3-4	.5	16.94
4	Oil-bath	110-148	200	3-4	2	44.60
5	Oil-bath	150	240	3-4	0.5	1.80
Distillation residue	—	—	—	—	—	13.25

The second distillation.

Fractions	Heating equipments	Temperature		Pressure	Distillation time	Amino-acid esters (in 2500 gm.)
		Inside of the flask	Outside of the flask			
1	Water-bath	25-32°C	60°C	15-22mm.	2 hours 30 minutes	10.85 gm.
2	Water-bath	68	98	15-20	1 30	4.02
3	Water-bath	60	98	2-3	1 15	0.50
4	Oil-bath	148	200	2-3	2 —	3.86
Distillation residue	—	—	—	—	—	0.98

The third distillation.

Fractions	Heating equipments	Temperature		Pressure	Distillation time	Amino-acid esters (in 2500 gm.)
		Inside of the flask	Outside of the flask			
1	Water-bath	79°C	98°C	12-17mm.	2 hours	2.40 gm.
2	Water-bath	83	98	2	1	0.56
3	Oil-bath	120-133	200	2	1	1.24
Distillation residue	—	—	—	—	—	1.48

From the amino-acid esters thus fractionated, amino-acids were separated by hydrolysis and determined by analysis. The result was as follows:

	In 100 gm. of the dried leaves.
	gm.
Glycocoll	0.38
Alanine	2.1
Valine	0.647
Leucine	2.74
Aspartic acid	0.03
Glutamic acid	1.2
Phenylalanine	1.06
Proline	0.43
Oxyproline	0.023

Identification of the amino-acids separated.

Glycocoll:—Owing to the small quantity of the substance separated, it was difficult to identify it by analysis, but the crystal form and the melting point of the ethylester hydrochloride enabled me to determine with confidence that it was glycocoll.

Alanine:—0.1932 gm. of the copper salt gave 0.05116 gm. of copper by analysis.

Calculated (as $\text{Cu}(\text{C}_3\text{H}_6\text{NO}_2)_2$)	26.52 % Cu
Found	26.48 % Cu

Other properties peculiar to alanine—the sweet taste, solubility in water, and the crystal form,—gave great help in identification.

Valine:—0.2376 gm. of the substance gave 0.02846 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{CH}_3\text{CH}_2\text{CH}_2\text{CHNH}_2\text{COOH}$)	11.38 % N
Found	11.98 % N

0.2158 gm. of the substance gave 0.04644 gm. of copper.

Calculated (as $\text{Cu}(\text{C}_{10}\text{H}_{20}\text{N}_2\text{O}_4)$)	21.49 % Cu
Found	21.52 % Cu

Leucine:—0.1879 gm. of the copper salt gave 0.03698 gm. of copper.

Calculated (as $\text{Cu}(\text{C}_6\text{H}_{12}\text{NO}_2)_2$)	19.64 % Cu
Found	19.68 % Cu

From the peculiar bitter taste, the disinclination to dissolve in water, and the crystal forms it was also identified.

Aspartic acid:—The amount of the compound separated was too small to identify it by analysis, but the characteristic copper salt and the peculiar acid taste of the free acid conclusively proved that it was aspartic acid.

Glutamic acid:—0.1982 gm. of the substance produced 0.01873 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{C}_5\text{H}_9\text{NO}_4$)	9.50 % N
Found	9.50 % N

It was also identified by the melting point of the hydrochloride (190°C).

Proline:—This was also too small in quantity for analysis and therefore it was determined by its solubility in absolute alcohol and by the characteristic copper salt.

The Isolation of Tyrosine.

For the purpose of isolating tyrosine 200 gm. of the air dried mulberry leaves was boiled with 1000 cc. of 25 per cent sulphuric acid for 10 hours and then filtered, and the insoluble residue was thoroughly washed with water. The filtrate and washing liquid were united, and the sulphuric acid was removed with baryta. After filtering out the

barium sulphate thus produced, the solution was evaporated to a small volume, but no crystallization of tyrosine occurred, and the solution gave a very faint degree of Millon's reaction. The separation of tyrosine proved unsuccessful.

(b) The Amino-acids in the Young Leaves of "Rosow" Mulberry.

Preliminary analysis.

Collection of the leaves.

On the 26th, June, 1914, young leaves of the same mulberry trees from which the mature leaves had been gathered in 1913, were collected. Their total fresh weight was 13756 gm. and they were dried in the same method as before and submitted to analysis.

Determination of water.

Water was determined in the same way as before.

	%
Leaves in the lower part of the stem.....	77.997
Leaves in the middle part of the stem.....	78.090
Leaves in the Upper part of the stem.....	79.739
Average	78.608

The summary of the results.

	In 100 parts of dry matter. In gm.
Part soluble in concentrated hydrochloric acid....	61.26
Part insoluble in concentrated hydrochloric acid..	38.74
Ash	8.563
Total nitrogen	5.363
Albuminous nitrogen	4.556
Nitrogen soluble in water	1.55
Nitrogen soluble in concentrated hydrochloric acid..	4.396
Of which the part precipitated by phosphotungstic acid	1.748
The part not precipitated by phosphotungstic acid	2.64
Nitrogen insoluble in concentrated hydrochloric acid.	0.800

Total Hydrolysis.

1200 gm. of the finely powdered young leaves was boiled in 3600 cc. of concentrated hydrochloric acid ($d = 1.18$) under reflux cooler for six hours and then filtered. The filtrate thus obtained was evaporated to a syrup *in vacuo* and digested with absolute alcohol. The operation was repeated two or three times in order to remove water as far as possible. Finally it was dissolved in absolute alcohol and dry hydrochloric acid gas was passed into the solution to saturation for separating glycooll as ester-hydrochloride. After leaving it in a cold place for 24 hours no esterhydrochloride crystallized.

The ester-method was then applied for the separation of the remaining amino-acids. The distillation residue was treated with hydrochloric acid, the salts thus produced were filtered, and from the filtrate the still remaining amino-acids were separated by the same process.

At the end of each operation the nitrogen was determined as in the former cases to know the exact amount of losses on that had taken place in the course of the analysis. The result was as follows:

	In 100 gm. of the dry matter.
	In gm.
Total nitrogen in hydrolysate	4.396
Inorganic salts produced by hydrolysis.....	0.264
Filtrate of the salts	4.129

The first distillation of the amino-acid esters.

Etherial solution of free amino-acid esters (after having been set in a free state by caustic soda and potassium carbonate)	1.325
Fraction 1 (To 60°C on a water bath, at 15-20 mm. pressure)	0.058
Fraction 2 (To 98°C on a water-bath, at 10 mm. pressure)	0.392
Fraction 3 (To 98°C on water-bath, at 4 mm. pressure)	0.105
Fraction 4 (To 200°C on an oil-bath, at 0.5 mm. pressure)	0.196
Distillation residue	0.257
Residue not extracted by ether (mixture of insoluble matters in ether, caustic soda and potassium carbonate)	2.253

The second distillation of amino-acid esters.

Etherial solution of free amino-acid esters (after they were set in free by caustic soda, and potassium carbonate)	0.741
Fraction 1 (To 60°C on a water-bath, at 15-20 mm. pressure)	0.021
Fraction 2 (To 98°C on a water-bath, at 10 mm. pressure)	0.20
Fraction 3 (To 98°C on a water-bath, at 4 mm. pressure)	0.032
Fraction 4 (To 200°C on an oil-bath, at 0.5 mm. pressure)	0.284
Distillation residue	0.19

The result of the first and second distillations was as follows:

The first distillation.

Fractions	Temperature		Pressure	Distillation time	Amino-acid esters	Amino-acids
	Inside of the flask	Outside of the flask				
1	45-48°C	60°C	15-20mm.	30'	19.5 gm.	3.20 gm.
2	88	98	10	1 h. —	54.75	33.76
3	85	98	4	40'	16.2	10.37
4	159	200	0.5	1 h. —	61.7	—
Distillation residue	—	—	—	—	39.4	—

The second distillation.

Fractions	Temperature		Pressure	Distillation time	Amino-acid esters	Amino-acids
	Inside of the flask	Outside of the flask				
1	47°C	60°C	15-20mm.	50'	9.93 gm.	1.81 gm.
2	85	98	10	50	24.74	17.92
3	76	98	4	30	7.06	2.79
4	158	200	0.5	1 h. 20	33.76	—
Distillation residue	—	—	—	—	20.11	—

From the fractions thus obtained, proline and phenylalanine were separated by the usual method and then other amino-acids were isolated by fractional crystallization and determined by analysis. The following amino-acids were finally obtained.

	In 1000 gm. of dried leaves.
	In gm.
Glycocoll	2.2
Alanine	12.86
Valine	19.92
Leucine	6.11
Proline	6.17
Oxproline	2.93
Phenylalanine	8.46
Serine	—

Aspartic acid	3.01
Glutamic acid	8.0
Tyrosine	—

Identification of the Amino-acids Separated.

Glycocoll:—0.3152 gm. of the substance gave 0.05882 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{CH}_2\text{NH}_2\text{COOH}$)	18.67 % N
Found	18.66 % N

0.1235 gm. of the copper salt gave 0.03416 gm. of copper by analysis.

Calculated (as $\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2)_2 + \text{H}_2\text{O}$)	27.68 % Cu
Found	27.66 % Cu

Alanine:—0.2784 gm. of the substance gave 0.0437 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{CH}_3\text{CHNH}_2\text{COOH}$)	15.73 % N
Found	15.70 % N

Valine:—0.2519 gm. of the substance gave 0.0402 gm. of nitrogen by Kjeldahl's method.

Calculated ($\text{C}_5\text{H}_{11}\text{O}_2\text{N}$)	11.96 % N
Found	11.98 % N

0.05146 gm. of copper was obtained from 0.2398 gm. of the copper salt by analysis.

Calculated (as $\text{Cu}(\text{C}_5\text{H}_{10}\text{NO}_2)_2$)	21.49 % Cu
Found	21.46 % Cu

Leucine:—0.2224 gm. of the substance gave 0.02379 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{C}_6\text{H}_{13}\text{O}_2\text{N}$)	10.65 % N
Found	10.70 % N

The peculiar bitter taste, the crystal form, and the disinclination to dissolve in water served also to identify it.

Aspartic acid:—0.2935 gm. of the substance gave 0.0308 gm. of the nitrogen by Kjeldahl's method.

Calculated (as $\text{C}_4\text{H}_7\text{NO}_4$)	10.53 % N
Found	10.49 % N

0.3125 gm. of the copper salt produced 0.0975 gm. of copper by combustion.

Calculated (as $\text{Cu}(\text{C}_4\text{H}_5\text{NO}_4) + \frac{1}{2}\text{H}_2\text{O}$)	31.22 % Cu
Found	31.20 % Cu

The peculiar acid taste and the disinclination of the copper salt to dissolve in water served also to identify it.

Glutamic acid:—0.2138 gm. of the substance gave 0.0204 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_5H_9NO_4$) 9.50 % N
 Found 9.56 % N

0.3125 gm. of the copper salt produced 0.09537 gm. of copper by analysis.

Calculated (as $Cu(C_5H_7NO_4)$) 30.47 % Cu
 Found 30.52 % Cu

Proline:—0.1396 gm. of the copper salt produced 0.03026 gm. of copper by combustion.

Calculated (as $Cu(C_5H_8O_2N)_2$) 21.79 % Cu
 Found 21.68 % Cu

Oxproline:—0.2324 gm. of the copper salt produced 0.0458 gm. of copper by combustion.

Calculated (as $Cu(C_5H_8O_3N)_2$) 19.64 % Cu
 Found 19.64 % Cu

(c) The Amino-acids in Mature Leaves of "Nedzumi-gaesi."

General Analysis.

Collection of the leaves.

The leaves were collected in the mulberry-field belonging to the Sericultural College at Uyeda, Nagano-ken on 5th September, 1913. The mulberry trees, from which the leaves were gathered, were three years old and they had been trained in "Negari-jitate." The leaves, the fresh weight of which was 22.936 gm., were at once exposed to the sun light and thoroughly dried. The dry leaves thus obtained were finely milled and kept in a bottle for analysis.

Determination of the Water Contained in the Leaves.

As in the former cases, the leaves were gathered from three—upper, middle, and lower—parts of a stem and closed instantly in three weighing tubes in order to prevent the loss of water as far as possible.

	Percentage of water.
Upper part	68.53
Middle part	65.34
Lower part	51.86
Lower part	60.27

Average 64.00

Summary of the general analysis.

	In 100 gm. of dry matter,
	In gm.
Ash	12.65
Nitrogen	3.72
Albuminous nitrogen	3.44
Nitrogen soluble in water	1.015
Of which the nitrogen precipitated by phosphotungstic acid	0.137
Nitrogen soluble in hydrochloric acid.....	2.999
Nitrogen insoluble in hydrochloric acid.....	0.625
Part soluble in boiling concentrated, hydrochloric acid	55.67
Part insoluble in concentrated, boiling hydrochloric acid	44.33

Total hydrolysis.

2500 gm. of finely powdered, air dried leaves were hydrolyzed as in the former cases, and the amino-acids were isolated by the esjer method. In the course of the analysis, too, nitrogen was determined at the end of every operation, in order to know the amount of losses, which might have taken place in the analysis. The result was as follows:

	In 100 gm. of dry leaves.
	In gm.
Hydrolysate	3.72
Nitrogen insoluble in concentrated hydrochloric acid..	0.62
Filtrate of the salts produced on evaporation of the hydrolysate	2.99
Salts filtered off	0.00
Etherial solution of the amino-acid esters (after setting the amino-acid free with potassium carbonate and caustic soda)	0.48

The first distillation of amino-acid esters.

Fraction 1 (To 60°C on a water-bath, at 10-15 mm. pressure)	0.04
Fraction 2 (To 98°C on a water-bath, at 10 mm. pressure)	0.16
Fraction 3 (To 98°C on a water-bath, at 2 mm. pressure)	0.07
Fraction 4 (To 200°C on an oil-bath, at 2 mm. pressure)	0.06
Distillation residue	0.06

Residue after extraction of the esters with ether (mixture of the salts with substances not extracted by ether)	2.502
Filtrate of the salts produced by neutralizing the residue with hydrochloric acid.....	2.182
Salts produced	0.35
Animal charcoal used for decoloring.....	0.04
Second ethereal solution of the amino-acid esters (after setting the amino-acids free with potassium carbonate and caustic soda).....	0.79
The second distillation of the amino-acid esters.	
Fraction 1 (To 60°C on a water-bath, at 35-40 mm. pressure)	0.0085
Fraction 2 (To 98°C on a water-bath, at 22-25 mm. pressure)	0.054
Fraction 3 (To 98°C on a water-bath, at 2 mm. pressure)	0.0875
Fraction 4 (To 119°C on an oil-bath, at 2 mm. pressure)	0.0826
Fraction 5 (To 210°C on an oil-bath, at 2 mm. pressure)	0.165
Distillation residue	0.205
Insoluble residue, when extracted by ether (mixture of the salts with substance not extracted by ether)	1.356

We got the following results on the first and second fractional distillation.

The first distillation.

Fractions	Temperature		Pressure	Distillation time	Amino-acid esters (in 2500 gm.)
	Inside of the flask	Outside of the flask			
1	30-55°C	40-60°C	10-15 mm.	1 hour	13.24 gm.
2	88	98	10	1 15'	33.86
3	87-88	98	2	1 30	17.55
4	143-170	200	2	1 30	41.60
Distillation residue	—	—	—	—	19.54
The substances crystallized on the wall of the distilling flask	—	—	—	—	1.3

The second distillation.

Fraction	Temperature		Pressure	Distillation time	Amino-acid esters (in 2500 gm.)
	Inside of the flask	Outside of the flask			
1	40°C	60°C	35-40 mm.	45'	42.12 gm.
2	50-63	98	22-25	45	25.77
3	81-82	98	2	40	25.02
4	86-88	110	2	40	61.51
5	140-150	210	2	1 hour 30	70.48
Distillation residue	—	—	—	—	70.72

The amino-acid esters thus separated were hydrolyzed by the usual methods and isolated each other by means of the fractional crystallization, with the following results:

	In 1000 gm. Of the dry matter,
	In gm.
Glycocoll	—
Alanine	6.68
Valine	16.00
Leucine	3.98
Aspartic acid	6.71
Glutamic acid	0.27
Phenylalanine	2.70
Serine	—
Proline	5.54
Oxproline	2.39
Tyrosine	—

Identification of the Amido-acids Separated.

Alanine:—0.2564 gm. of the substance gave 0.04019 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_3H_7O_2N$) 15.73 % N
 Found 15.65 % N

0.612 gm. of the copper salt produced 0.1572 gm. of copper by combustion.

Calculated (as $Cu(C_3H_6O_2N)_2$) 26.52 % Cu
 Found 25.68 % Cu

Valine:—0.3056 gm. of the substance gave 0.036 4 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_5H_{11}O_2N$)11.38 % N
 Found11.92 % N

0.1888 gm. of the copper salt produced 0.0421 gm. of copper by combustion.

Calculated (as $Cu(C_5H_{10}ON)_2$)21.49 % Cu
 Found22.29 % Cu

Leucine:—0.1793 gm. of the substance gave 0.0192 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_6H_{13}O_2N$)10.69 % N
 Found10.69 % N

0.1529 gm. of the copper salt produced 0.02998 gm. of copper by combustion.

Calculated (as $Cu(C_6H_{12}O_2N)_2$)19.64 % Cu
 Found19.70 % Cu

Aspartic acid:—0.2682 gm. of the substance gave 9.02811 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_4H_7NO_4$)10.53 % N
 Found10.48 % N

The characteristic acid taste and the crystal forms helped also to identify the substance.

Glutamic acid:—Owing to the small amount of the quantity separated, it could not be identified by analysis, but the crystal of the hydrochloride and the peculiar taste of the free acid enabled me to determine that it was glutamic acid.

Proline:—Proline was isolated and determined as copper salt. 0.2186 gm. of the copper salt produced 0.0588 gm. of copper oxide by combustion, from which 0.0471 gm. of copper was obtained.

Calculated (as $Cu(C_5H_8O_2N)_2$)21.79 % Cu
 Found21.54 % Cu

Oxyproline:—Oxyproline was also separated as copper salt. 0.2780 gm. of the salt produced 0.0688 gm. of copper oxide by combustion, from which 0.0551 gm. of copper was obtained.

Calculated (as $Cu(C_5H_8O_3N)_2$)19.64 % Cu
 Found19.82 % Cu

(d) Amino-acids in the Young Leaves of "Nedzumi-gaesi."

General Analysis.

Collection of leaves.

On 19th May 1914 the leaves were collected in the mulberry

field belonging to our college from the same trees, from which the mature ones had been gathered in the previous year. The total weight of the leaves was 12584 gm. They were at once exposed to the sun light and after several days' drying they were finely milled and preserved for analysis.

Determination of water.

In exactly the same way as before, the fresh leaves were instantly closed in a weighing bottle on the farm in order to prevent any loss of water, and the following result was obtained.

	Percentage of water.
The leaves on upper part of the stem.....	80.36
The leaves on middle part of the stem.....	79.14
The leaves on middle part of the stem.....	79.44
The leaves on lower part of the stem.....	79.89
Mean	79.38

Summary of the results of general analysis.

	In 100 gm. of dry matter.
	In gm.
Ash	9.17
Total nitrogen	6.404
Nitrogen soluble in concentrated hydrochloric acid...	5.409
Albuminous nitrogen	0.9955
Nitrogen precipitated by phosphotungstic acid in the filtrate of the precipitates by copper hydroxide..	0.67
Nitrogen soluble in water	2.21
Nitrogen precipitated by phosphotungstic acid in the water extract	0.814
Nitrogen in the filtrate from the precipitate by phosphotungstic acid in the water extract	1.194

In 100 parts of the sample.

	In gm.
Parts soluble in hot concentrated hydrochloric acid..	64.25
Parts insoluble in hot concentrated hydrochloric acid..	64.25

Total hydrolysis.

200 gm. of finely powdered, air dried leaves was, as usual, boiled with concentrated hydrochloric acid ($d = 1.18$) under a reflux cooler for six hours. After having been completely hydrolyzed, the insoluble residue was filtered off and the filtrate was evaporated *in vacuo* at 40°C . to a syrup. For the purpose of removing the water contained in it, it was repeatedly evaporated with absolute alcohol. The large quantity of mineral salts which was separated out, was filtered off, absolute alcohol was again added and dry gaseous hydrochloric acid was passed through the solution until it was saturated. The whole

solution was then left in a cold place after the seeding of a small quantity of glycocoll-ethylester-hydrochloride. But we could not separate glycocoll by this method. The other amino-acids were then separated by the ester method. As in the former case nitrogen was determined at the end of every operation to know clearly the amount of losses which might have occurred in the analysis. The result was as follows:

	In 100 gm. of the dry matter.
Hydrolysate	In gm. 5.409
Mineral salts separated	0.500
Etherial solution of free amino-acids	1.579

The first fractional distillation.

Fraction 1 (To 60°C on a water-bath, at 20 mm. pressure)	0.048
Fraction 2 (To 99°C on a water-bath, at 20 mm. pressure)	0.363
Fraction 3 (To 99°C on a water-bath, at 6-7 mm. pressure)	0.023
Fraction 4 (To 180°C on an oil-bath, at 2-3 mm. pressure)	0.176
Fraction 5 (To 200°C on an oil-bath, at 0.1 mm. pressure)	0.116
Phenylalanine separated as hydrochloride.....	0.081
Distillation residue	0.242
Residue remaining after extracting amino-acid esters by ether (mixture of matter insoluble in ester, potassium carbonate, etc.)	3.247
Salts produced on neutralization of the residue with hydrochloric acid	0.165
Filtrate of the salts	3.075
Etherial solution of free amino-acid esters.....	0.869

The second fractional distillation.

Fraction 1 (To 60°C on a water-bath, at 15 mm. pressure)	0.049
Fraction 2 (To 98.5°C on a water-bath, at 10 mm. pressure)	0.282
Fraction 3 (To 98.5°C on a water-bath, at 3 mm. pressure)	0.030
Fraction 4 (To 200°C on an oil-bath, at 0.5 mm. pressure)	0.165
Distillation residue	0.30
Residue remaining after extracting amino-acid esters	

with ether (mixture of matter insoluble in ether, potassium carbonate. etc.) 2.00

On the first and second fractional distillation the next results were obtained.

The first fractional distillation.

Fractions	Temperature.		Pressure	Distillation hours	Amino-acid esters (in 2000 gm.)	Amino-acids (in 2000 gm.)
	Inside of the flask	Outside of the flask				
1	45°C	60°C	20 mm.	50'	61.85 gm.	6.24 gm.
2	88	99	20	1 h. 30	73.85	50.90
3	90	99	6-7	1 —	54.7	37.16
4	155	180	2-3	1 20	136.0	—
5	165-168	200	0.1	50	55.91	—
Distillation residue	—	—	—	—	65.33	—

The second fractional distillation.

Fractions	Temperature		Pressure	Distillation hours	Amino-acid esters (in 2000 gm.)	Amino-acids (in 2000 gm.)
	Inside of the flask	Outside of the flask				
1	48°C	60°C	15 mm.	4'	61.9 gm.	6.24 gm.
2	89	98.5	10	1 h. 20	57.91	38.28
3	88	98.5	3	45	11.3	2.00
4	168	200	0.5	1 —	61.3	—
Distillation residue	—	—	—	—	33.7	—

All the fractions were hydrolyzed by the usual methods. Proline was extracted with absolute alcohol and determined as copper salt. Phenylalanine was separated from the third and fourth fraction with ether, and appeared in crystals after changing into hydrochloride. Glycocoll, alanine, and valine were isolated by means of fractional crystallization from the first and second fraction after hydrolysis, and

valine, leucine, and aspartic acid also from the third and fourth fraction.

The amino-acids thus finally isolated were summarized as follows:

	In 1000 gm. of dry matter.
	In gm.
Glycocoll	1.79
Alanine	16.62
Valine	22.08
Leucine	17.02
Aspartic acid	31.70
Glutamic acid	0.97
Phenylalanine	12.20
Serine	—
Proline	7.55
Oxyproline	6.05
Tyrosine	—

Identification of the Amino-acids Separated.

Glycocoll:—From 0.1421 gm. of the copper salt. 0.0392 gm. of copper was obtained by analysis.

Calculated (as $\text{Cu}(\text{NH}_2\text{CH}_2\text{CO}_2)_2 + \text{H}_2\text{O}$).....27.68 % Cu
 Found27.63 % Cu

From 0.2304 gm. of anhydrous copper salt, 0.0693 gm. of copper was obtained by analysis.

Calculated (as $\text{Cu}(\text{NH}_2\text{CH}_2\text{COO})_2$)30.03 % Cu
 Found30.07 % Cu

Alanine:—0.2534 gm. of the substance gave 0.0397 gm. of the nitrogen by Kjeldahl's method.

Calculated (as $\text{CH}_3\text{CHNH}_2\text{COOH}$)15.73 % N
 Found15.69 % N

From 0.1896 gm. of the copper salt we obtained 0.502 gm. of copper by combustion.

Calculated (as $\text{Cu}(\text{C}_3\text{H}_6\text{NO}_2)_2$).....26.52 % Cu
 Found26.47 % Cu

Valine:—0.2715 gm. of the substance produced 0.03244 gm. of nitrogen by Kjeldahl's method.

Calculated (as $\text{C}_5\text{H}_{11}\text{O}_2\text{N}$)11.96 % N
 Found11.94 % N

0.2325 gm. of the copper salt gave 0.0609 gm. of copper by combustion:

Calculated (as $\text{Cu}(\text{C}_5\text{H}_{10}\text{NO}_2)_2$)21.49 % Cu
 Found21.46 % Cu

Kind of silks		In 100 gm. of the dry matter (in gm.)									
		Glyco- coll	Alanine	Leucine	Aspartic acid	Gluta- mic acid	Serine	Proline	Oxy- proline	Tyrosine	Phenyl- alanine
The name of silks	The state of silks										
√ Kinjoh-matta-mukasi	cocoon silk (true silk)	29.39	16.72	1.47	0.03	0.023	3.01	1.106	0.05	4.72	0.64
The polyvoltine species of Formosa	cocoon silk (true silk)	24.19	13.94	0.60	0.1	+	0.33	0.009	0.07	3.89	0.84
Landybe	cocoon silk (wild silk)	10.38	3.70	0.43	0.75	2.03	—	0.33	0.07	4.37	—
Ailanthus. 1)	cocoon silk (wild silk)	10.5	8.5	1.0	1.0	0.8	+	0.25	0.45	4.5	—
Italian silk fibroin 2)	Fibroin (true silk)	36.0	21.0	1.5	+	—	1.6	+	—	10.5	1.5
Italian rawsilk 3)	Raw silk (true silk)	33.5	20.0	0.75	1.0	0.25	1.9	0.8	—	9.0	1.2
Canton silk fibroine 4)	Fibroin (true silk)	37.5	23.5	1.5	0.75	—	1.5	1.0	—	9.8	1.6
Bengal silk 5)	Fibroin (true silk)	30.5	20.0	1.25	0.8	+	1.75	1.0	—	10.0	1.4
Japanese Haruko 6)	Fibroin (true silk)	35.0	22.6	0.7	1.0	0.07	0.7	0.7	—	9.7	1.3
New Chwang silk 7)	Fibroin	19.7	23.8	1.6	2.9	1.7	6.0	1.85	—	9.83	1.2
Tai-Tsao-Tsam 8)	Fibroin	25.2	18.2	0.9	2.1	2.0	1.2	1.0	—	7.8	1.0
Niet-ngo-stam 9)	Fibroin	24.0	18.5	1.2	2.0	3.0	1.5	1.2	—	7.8	1.0
Cheefoo-silk 10)	Fibroin	12.5	18.0	1.2	2.0	2.0	1.0	2.5	—	8.5	1.0
√ Japanese Saku-san 11)	Raw silk (wild silk)	5.7	4.8	1.2	1.0	+	?	+	—	1.4	—
Yamamai-silk 12)	Raw silk (wild silk)	6.3	7.2	1.3	1.0	0.6	?	+	—	2.0	+
Kuriwata-silk 13)	Fibroin (wild silk)	7.7	15.3	7.95	0.2	?	?	4.0	0.2	5.5	+
Indian tusser 14)	Fibroin (wild silk)	9.5	24.0	1.5	2.5	1.0	0.2	1.0	—	9.2	0.6
Schantung tusser 15)	Fibroin (wild silk)	14.5	22.0	1.8	1.0	1.75	1.0	2.5	—	9.7	1.0
Tailung-silk 16)	Raw silk (wild silk)	13.0	12.0	1.0	1.0	+	+	0.29	0.26	3.6	—
Daiwanishiki (the bivoltine silk worm)	cocoon silk (true silk)	25.42	22.58	0.62	0.11	+	2.77	0.32	—	5.68	0.25
Sericine from Italian silk 17)	Sericine (true silk)	0.1-0.2	5.0	—	—	—	6.6	—	—	5.0	—
Sericine from canton silk 18)	Sericine (true silk)	1.2	9.2	5.0	2.5	2.0	5.8	2.5	—	2.3	0.6
Sericine from European silk 19)	Sericine (true silk)	1.5	9.8	4.8	2.8	1.8	5.4	3.0	—	1.0	0.3
Sericine from Daiwanishiki	Sericine (true silk)	3.93	3.53	0.4	3.91	3.0	5.99	0.35	—	3.27	0.49

Leucine:—0.1982 gm. of the substance gave 0.0211 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_6H_{13}O_2N$)10.69 % N
Found10.65 % N

From 0.2512 gm. of the copper salt we obtained 0.04938 gm. of copper.

Calculated (as $Cu(C_6H_{12}NO_2)_2$)19.64 % Cu
Found19.66 % Cu

Aspartic acid:—0.3126 gm. of the substance produced 0.03298 gm. of nitrogen by Kjeldahl's method.

Calculated (as $C_4H_7NO_4$)10.53 % N
Found10.55 % N

The peculiar acid taste and the crystal form also helped us in the identification.

Glutamic acid:—Owing to the scarcity of the substance separated, we could not determine glutamic acid by analysis, but its peculiar taste, the crystal form of the hydrochloride, and its melting points enabled me to determine with conviction that it was glutamic acid.

Oxyproline:—0.2325 gm. of the copper salt gave 0.0458 gm. of copper by combustion.

Calculated (as $Cu(C_5H_8O_3N)_2$)19.64 % Cu
Found19.71 % Cu

Proline:—0.1892 gm. of the copper salt gave 0.04106 gm. of copper by analysis.

Calculated (as $Cu(C_5H_8O_2N)_2$)21.79 % Cu
Found21.70 % Cu

V.—SUMMARY AND COMPARISON OF THE

RESULTS.

We sum up here the results obtained concerning the silks, described above, together with those which have been studied by other investigators.

(1) E. Abderhalden and R. Inouye (see Bibliography), (2) E. Fischer and A. Skita (do.) (3) E. Abderhalden and G. Roose (do.), (4) E. Abderhalden and L. Behrend (do.), (5) E. Abderhalden and J. Sington (do.), (6) E. Abderhalden and A. Suwa (do.), (7) E. Abderhalden and Rilliet (do.), (8) E. Abderhalden and J. Schmid (do.), (9) E. Abderhalden and Brossa (do.), (10) E. Abderhalden and E. Welde (do.), (11) U. Sudzuki, K. Yoshimura, and R. Inouye (do.), (12) U. Sudzuki, K. Yoshimura, and R. Inouye (do.), (13) U. Sudzuki, K. Yoshimura, and R. Inouye (do.), (14) E. Abderhalden and W. Spack (do.), (15) E. Abderhalden and C. Brahm (do.), (16) E. Abderhalden and R. Inouye (do.), (17) E. Fischer and A. Skita (do.), (18) E. Abderhalden and W. W. Worms (do.), (19) F. W. Strauch (do.)

From the above results, it may be concluded that silk, both true and wild, chiefly consists of glycocoll, alanine, and tyrosine; and the other amino-acids are very much less in quantity. And although almost the same kinds of amino-acids are found in both kinds of silk, their percentages are widely different from each other.

Dr. Sington says in his work that more glycocoll is contained in true silk than wild, but we find that, not only glycocoll, but alanine and tyrosine also exist in large quantities in the former. On the other hand, more aspartic and glutamic acids are found in wild silk than in true. These relations between the quantities of constituent amino acids exist not only in true and wild silks, but among true silks themselves. Thus, for example, "Kinjomatamukasi," which is far better in quality than the polyvoltine species of Formosa as we have already studied, contains more glycocoll, alanine, and tyrosine than the latter, although both are true silks; as it may be known from the results given above.

It may further be added here that the silks of univoltine and bivoltine species have nearly the same chemical constitutions.

Sericine has a different chemical composition from fibroin, that is, the former contains comparatively a small quantity of glycocoll and alanine, but more serine, compared with the latter. It is hardly conceivable that sericine is made from fibroin only by the action of water, as some authority believes.

The sericine, separated from the cocoon silk of bivoltine species, is somewhat different in its chemical constitution from that of univoltine ones, so far as one may know from investigations. Thus more glycocoll is contained in the former than the latter, while the reverse is the case with alanine and leucine, but both in univoltine and bivoltine species serine exists in large quantity than any other amino-acids. That the sericine of the bivoltine species is more soluble in boiling

water than that of the univoltine ones, is probably due to these differences in the chemical composition.

As for the mulberry leaves, we summarize the results of the analysis as follows:

General analysis.

	"Rosow "		"Nedzumigaesi "	
	Mature leaves	Young leaves	Mature leaves	Young leaves
Water	72.58 %	78.608 %	64.00 %	76.38 %
Part soluble in hot concentrated hydrochloric acid	62.40	61.26	55.67	64.25
Part insoluble in hot concentrated hydrochloric acid	37.60	38.74	44.33	35.75
Ash	11.21	8.56	12.65	9.17
Total nitrogen	3.79	5.36	3.72	6.40
Albuminous nitrogen	3.53	4.56	3.44	4.44
Nitrogen soluble in con. HCl	3.049	4.40	2.99	5.41
Of which, nitrogen precipitated by phosphotungstic acid	0.206	1.748	—	—
nitrogen not precipitated by phosphotungstic acid	2.178	2.64	—	—
Nitrogen insoluble in con. HCl	0.56	0.80	0.62	0.99
Nitrogen soluble in water	0.93	1.55	1.015	2.21
Of which, nitrogen precipitated by phosphotungstic acid	—	—	0.137	0.81
nitrogen not precipitated by phosphotungstic acid	—	—	0.878	1.19

Total hydrolysis.

(In gm. in 1000 gm. of dry matter)

	"Rosow "		"Nedzumigaesi "	
	Mature leaves	Young leaves	Mature leaves	Young leaves
Glycocoll	0.38	2.2	0.0	1.80
Alanine	2.1	12.86	6.68	16.62
Valine	0.65	19.92	16.00	22.08
Leucine	2.77	6.11	3.98	17.02
Aspartic acid	0.03	3.01	6.71	31.70
Glutamic acid	1.2	8.00	0.27	0.97
Phenylalanine	1.06	8.46	2.70	12.20
Proline	0.43	6.17	5.54	7.55
Oxyproline	0.023	2.93	2.39	6.05
Tyrosine	—	—	—	—

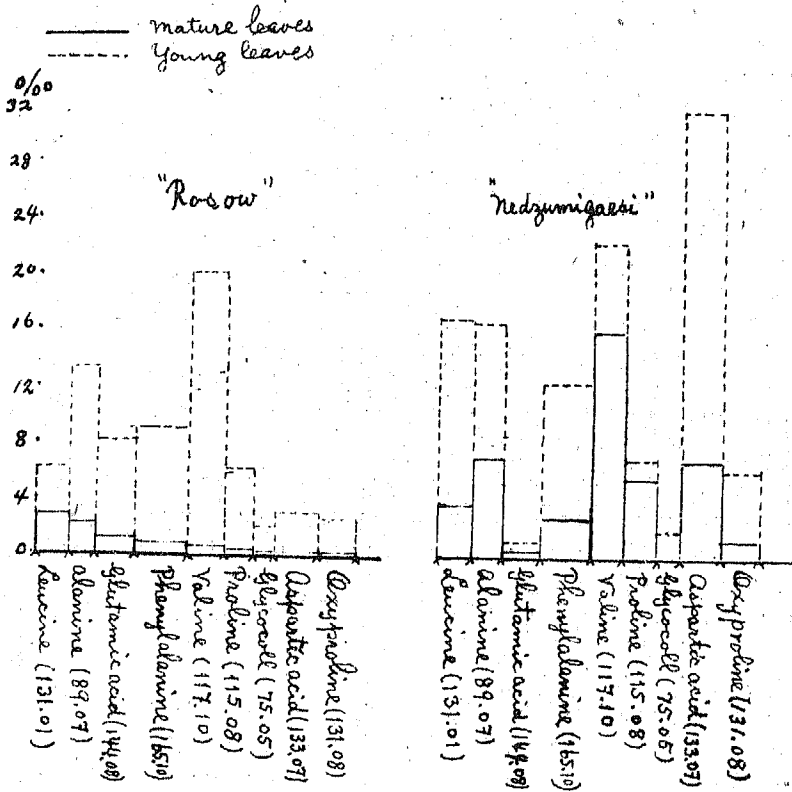
From the above result, it may be deduced that so far as we know, the amino-acids found in mulberry leaves vary in amount, according to the age of the leaves and the variety of the mulberry trees. In general mature leaves contain a small quantity of amino-acids when compared to young ones and this is specially the case with glycocoll, alanine, valine, aspartic acid, glutamic acid and phenyl-alanine. We may suppose it is on account of such a great difference between the amino-acids contained in the young and mature leaves, that the different chemical constitution exist between them and otherwise certain amino-acids may be found in free in the young leaves. Then if we study the differences between the two varieties of the mulberry trees, we may find an interesting fact.

In the variety "Rosow," aspartic acid is found in a comparatively small quantity and glutamic acid large, in both mature and young leaves, but with the variety "Nedzumigaesi" just the reverse is the case. Moreover it has aspartic acid in a very large quantity, especially in young leaves. From this fact it may be thought that in the latter variety aspartic acid plays the same part which glutamic acid does in the former, because those two acids stand very near in the chemical constitution.

More glycocoll is contained in "Rosow" than in "Nedzumigaesi," but the reverse is the case with alanine. Valine is found in greater quantities in both mature and young leaves of "Nedzumigaesi" than in those of "Rosow." Comparatively much leucine is contained in the young leaves of "Nedzumigaesi," and much phenyl-alanine is found in young leaves of both varieties, but much less in mature leaves. In regard to proline, 1-proline is generally more in both varieties than oxyproline, and "Nedzumigaesi" contains generally much more proline than "Rosow." It should be noted here that tyrosine and serine which are almost always found in silk, could not be separated from either mulberry leaves. The following diagrams will serve to show clearly the relations between the quantities of the amino-acids in both varieties.

From the above it may be concluded that the amino-acids in the mulberry leaves are very different in quantity according to the varieties and the stages of the growth.

When the results now obtained are compared with those of the silks, it will be at once clear what great differences there exist between the amino-acids which they contain. In silk, as I have already stated, glycocoll, alanine and tyrosine are the dominant constitutions, but with



the mulberry leaves that is not the case. Although glycocoll and alanine are found in them, they—especially the former—are very small in quantity. Tyrosine is so scarce in mulberry leaves, that it has hardly been separated from them. On the other hand, valine, which is almost absent in silk, is contained in mulberry leaves in a comparatively large quantity. Aspartic acid, leucine, phenylalanine, and proline are also predominant amino-acids in mulberry leaves, while they are very scarce in silk. As mulberry leaves are the only food of domestic

silkworms, striking chemical changes are evidently caused by them in forming silk out of the mulberry leaves,—very probably, tyrosine is synthesized from phenylalanine, and glycocholl and alanine by cleaving other amino-acids.

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上田蠶絲專門學校
歐文學術報告
第壹卷 第二號



THE
BULLETIN
OF THE
UYEDA SILK TECHNICAL COLLEGE.

Vol. I. No. 2.



September 10th, Taisho XV (1926).

UYEDA, JAPAN.



大正十五年九月十日