LIII. THE TRANSFORMATION OF GLUTAMINIC ACID INTO L-PYRROLIDONECARBOXYLIC ACID IN AQUEOUS SOLUTION.

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In another paper [1914] an account will be found of a new method for the estimation of aspartic and glutaminic acids in the hydrolytic products of proteins, depending upon the insolubility of their calcium salts in alcohol. In studying the influence of lime upon glutaminic acid in aqueous solution at higher temperatures it was found that the amino-nitrogen content had been considerably reduced. The experiments described below were carried out in order to investigate this matter. The study of the behaviour of the new product when treated in aqueous solution with lime and alcohol was also an object of the experiments.

The glutaminic acid hydrochloride used in this and the other experiments described in this paper was a recrystallised specimen obtained from a caseinogen hydrolysis. Its purity is shown by the following analysis :

	Found per cent.	Calculated per cent.
Carbon	32.61	32.69
Hydrogen	5.80	5.45
Amino-nitrogen ..	7.74	7.63

25 g. glutaminic acid hydrochloride were dissolved in 700 cc. water, 28 g. of pure calcium oxide (about three times the equivalent) were added, and the mixture left in the autoclave for 2.5—3 hours at 140—145°.

The excess of calcium oxide was filtered off, and the liquid was reduced to about 100 cc. at 45° and 15 mm. Rectified spirit (97 % alcohol) was then

added in small quantities at a time with shaking, until no further precipitate resulted. The precipitate was filtered off and washed with rectified spirit, and by extraction with cold water was separated into two fractions, one of which was difficultly soluble and the other easily soluble. These two fractions and the alcoholic filtrate were treated separately as follows :

Fraction *A*, the difficultly soluble portion ;

„ *B*, the easily soluble portion ;

„ *C*, the alcoholic filtrate.

The calcium was quantitatively removed from fractions *B* and *C* by means of oxalic acid, the alcohol having previously been evaporated from fraction *C*. The calcium was precipitated from fraction *A* as the sulphate by digesting with a slight excess of normal sulphuric acid.

Treatment of Fractions A and B. The solutions obtained from these two fractions were then treated separately as follows :

Very small amounts of hydrochloric acid were removed by means of silver sulphate solution. The excess of silver sulphate was decomposed with sulphuretted hydrogen, and the filtrates were freed from sulphuretted hydrogen by evaporation to half bulk on the water bath. The sulphuric acid was then quantitatively removed by means of baryta.

Separation of a substance soluble in cold glacial acetic acid. The filtrates from the barium sulphate were then evaporated to dryness, and dried in the water oven. The residues were extracted with cold glacial acetic acid by triturating in the evaporating dishes with a pestle. The insoluble portions were filtered off by suction and washed on the filter with cold glacial acetic acid. The acetic acid was evaporated slowly from the filtrates leaving gummy residues which gradually crystallised on standing a long time over potash. These, as well as the insoluble portions, were dried and weighed.

The following weights of dried substances were obtained :

			Insoluble in cold glacial acetic acid	Soluble in cold glacial acetic acid
Fraction <i>A</i>	..	..	6.76 g.	0.86 g.
„ <i>B</i>	..	..	1.8 g.	4.1 g.

The 6.76 g. portion of Fraction *A* when crystallised from water gave characteristic crystals of glutaminic acid, which melted at 213–214°. The 1.8 g. portion of Fraction *B* also gave the correct melting-point for glutaminic acid.

Treatment of the 4.1 g. portion of Fraction B.

The amino-nitrogen content of this acetic acid soluble portion was estimated by the nitrous acid method.

0.1405 g. gave 2.15 cc. nitrogen at 17° and 747 mm. equal to 0.87 per cent. amino-nitrogen.

This portion was again extracted with a small quantity of glacial acetic acid, filtered by suction, and washed on the filter with glacial acetic acid, the washings, however, were kept separate. The insoluble part when dried weighed 0.61 g.

The acetic acid was slowly evaporated from this main filtrate by allowing the dish to remain on a warm water bath. When reduced to a small bulk the very slightly coloured gummy liquid was placed in a desiccator over strong potash solution. Large crystals separated on standing a long time, the whole of the gummy liquid eventually crystallising completely, the crystals all having the same appearance. These crystals were stirred with about 1 cc. cold glacial acetic acid, filtered by suction, and washed with a mere trace of acetic acid. The larger crystals were left on the filter in a colourless state. The object of this separation was to obtain a specimen completely devoid of light brown colour so that its specific rotatory power could be determined with accuracy. The weight of colourless crystals obtained was 0.47 g. A portion taken from this specimen commenced to soften at 142° and completely melted without colouration at 158°.

The filtrate from the colourless specimen again crystallised completely when treated in the manner already described, and portions were taken from this for analysis. The weights given below were constant at 100°.

The following results were obtained:

0.1449 g.; 0.2520 g. CO₂; 0.0748 g. H₂O; C = 47.43; H = 5.73 per cent.

0.1385 g. gave, by Dumas' method, 13.5 cc. nitrogen at 17° C. and 740 mm.; N = 11.00 per cent.

0.3023 dissolved in 24.76 g. glacial acetic acid gave 0.34° depression of the freezing-point by the cryoscopic method, giving a molecular weight of 140.

The colourless specimen was used for the following determinations:

0.3965 g. was dissolved in water and made up to 20 cc. at 20°. The amount of rotation obtained in a 200 mm. tube was - 0.45°: $[\alpha]_D^{20} = -11.35^\circ$.

The amino-nitrogen was determined in 7.05 cc. of the solution used for the rotation, equal to 0.1398 g. substance: 0.2 cc. nitrogen was obtained by the nitrous acid method.

5 cc. of the same solution, equal to 0.0991 g. substance required 1.5 cc. N/2 soda for neutralisation to delicate litmus paper. The calculated equivalent of N/2 soda is 1.53 cc. The carboxylic group was therefore exercising its full acidity.

On comparing the figures given in the following table, it will be seen that the substance was pyrrolidonecarboxylic acid. The calculated figures for pure glutaminic acid from which the substance was made are also shown side by side.

	Calculated for glutaminic acid per cent.	Found per cent.	Calculated for pyrrolidonecarb- oxylic acid per cent.
Carbon	40.83	47.43	46.51
Hydrogen	6.12	5.73	5.43
Nitrogen	9.52	11.00	10.85
Amino-nitrogen ..	9.52	traces	nil

The specific rotatory power -11.35° agrees very closely with that found by Abderhalden and Kautzsch [1910], who give figures for *l*-pyrrolidonecarboxylic acid ranging from -10.8° to -11.52° .

The substance was soluble in alcohol, strongly acid to litmus, and its melting-point agrees with that found by Menozzi and Appiani [1892] and also by Abderhalden and Kautzsch [1910] for *l*-pyrrolidonecarboxylic acid. The carbon is 0.9 per cent. too high, but all the other facts appeared to be so conclusive that a repetition of the combustion was thought to be unnecessary.

Treatment of Fraction C.

After filtering off the calcium oxalate, the hydrochloric acid was removed by means of silver sulphate solution. Excess of silver and sulphuric acid were then removed in the manner previously described. The liquid was evaporated to dryness, and the residue was dried and found to weigh 3 g. This weight would have been undoubtedly less if more water had been removed previously to precipitating the calcium salts with alcohol. This 3 g. was not extracted with cold glacial acetic, as a determination of its amino-nitrogen content appeared to give all the information required.

0.1315 g. gave by the nitrous acid method 4.8 cc. nitrogen at 15.5° and 761 mm. equal to 2.13 per cent. amino-nitrogen.

Assuming this substance to consist of a mixture of glutaminic acid and pyrrolidonecarboxylic acid, and calculating the amino-nitrogen as present

in glutaminic acid, the 3 g. contains 0.67 g. glutaminic acid and 2.33 g. pyrrolidonecarboxylic acid.

The weights of the two substances separated at the different stages are given in the following table :

		Insoluble in cold, glacial acetic acid	Soluble in cold, glacial acetic acid
Fraction <i>A</i>	..	6.76 g.	0.86 g.
„ <i>B</i>	..	2.41 „	3.49 „
„ <i>C</i>	..	0.67 „	2.33 „
Total ..	..	9.84 „	6.68 „

The 6.68 g. pyrrolidonecarboxylic acid corresponds to 7.61 g. glutaminic acid. 43—44 per cent. of the glutaminic acid originally taken had therefore been converted into pyrrolidonecarboxylic acid.

Transformation of glutaminic acid into l-pyrrolidonecarboxylic acid in aqueous solution in the presence of excess of calcium hydroxide, at 100°.

1 g. portions of glutaminic acid hydrochloride were mixed with 1.2 g. pure calcium oxide and 28 cc. water, *i.e.* in the same proportions as in the previous experiment. These mixtures were boiled in small flasks fitted with reflux condensers, on a sand bath, for different lengths of time. When boiling, the temperature recorded on a thermometer with its bulb immersed in the liquid was 100°. The liquids were cooled, and sufficient acetic acid added to dissolve the excess of calcium oxide. The solutions were then made up to 50 cc., and the amino-nitrogen determined in 10 cc. portions by the nitrous acid method.

A further similar mixture of the substances in the same proportion was kept at 40—45° for 8 hours, and the amino-nitrogen determined in the same way.

The results obtained are shown in the following table :

Weight of substance taken	Temperature	Time	Per cent. amino-nitrogen	Percentage transformed
0.9925 g.	100°	2.5 hrs.	6.45	16.66
0.9955 „	„	5 hrs.	6.17	20.29
0.9933 „	„	7.5—8 hrs.	5.91	23.64
0.9820 „	40—45°	8 hrs.	7.70	nil

The amino-nitrogen content of the glutaminic acid hydrochloride originally taken was 7.74 per cent. and the percentages shown had therefore been transformed.

The experiment was repeated using 0.16 g. of a specimen of free glutaminic acid (M.P. 213—214°), the other ingredients being in the same proportion,

viz., 0.24 g. calcium oxide and 5.6 cc. water. After 5 hours boiling, the amino-nitrogen present in the solution was determined. There resulted 20.7 cc. at 19° and 763 mm. equal to 7.47 per cent. amino-nitrogen. Pure glutaminic acid would have contained 9.52 per cent. ; 21.53 per cent. of the glutaminic acid had therefore been transformed.

In order to show that the same substance, viz., pyrrolidonecarboxylic acid, had been formed, the solutions left over from the amino-nitrogen determinations were combined. The calcium was removed by means of oxalic acid, and the hydrochloric acid by silver sulphate solution. The excess of silver and sulphuric acid were removed by means of sulphuretted hydrogen and baryta. The liquid was evaporated to dryness, the residue dried, and extracted with cold glacial acetic acid. The small amount of gummy liquid obtained by removing the acetic acid from the extract, crystallised very slowly when allowed to stand over potash. The crystals obtained were exactly similar in appearance to those isolated in the previous experiment. The whole of the preparation was thoroughly dried in a vacuum desiccator over calcium chloride, and the amino-nitrogen determined in a portion of the dry substance by the nitrous acid method. The following result was obtained :

0.1865 g. gave 3.45 cc. nitrogen at 16° and 766 mm., equal to 1.09 per cent. amino-nitrogen.

Effect of other bases.

It was thought, at this stage, that baryta would have the same effect as lime. On carrying out a similar experiment substituting for the lime its equivalent of baryta, however, all the glutaminic acid remained unchanged.

Weight of glutaminic acid hydrochloride taken	Temperature	Time	Per cent. amino-nitrogen
0.9940 g.	100°	5 hrs.	7.63

The effect of substituting other bases for the lime was then ascertained. A pure specimen of the free glutaminic acid was used for this purpose, the amino-acid, water and base being in exactly the same proportion, *i.e.* 0.34 g. to 4 equivalents of the base made up to 12 cc. with water. These mixtures were boiled on a sandbath for 5 hours, under a reflux condenser and made up to 20 cc. with water. The amino-nitrogen was then determined in an aliquot portion. The following results were obtained :

Base	cc. taken from the 20 cc.	Nitrogen obtained, cc.	Temperature and pressure	Amino-nitrogen per cent.	Per cent. changed
Sr(OH) ₂	.. 9.9	27.85	20° 760 mm.	9.47	<i>nil</i>
NaOH	.. 9.75	27.7	21° 760 "	9.51	"
NH ₄ OH	.. 10.0	37.1	20.5° 760 "	10.4	"

According to van Slyke [1912] 38.9 per cent. of the nitrogen of the ammonia is liberated by nitrous acid in 5.5 minutes. Applying this correction for the ammonia found present in the solution after the five hours boiling the figure 10.4 was calculated. Although this result is only approximate it suffices to show that no change occurs in the presence of ammonia.

The outstanding difference between calcium hydroxide and the hydroxides of the other alkaline earths is one of solubility in water.

Thus calcium hydroxide is soluble only to the extent of 0.08 per cent. at 100°, while the other bases are very soluble at 100°. Of the three extra equivalents of lime in the 12 cc. solution only about 9 milligrams would be soluble. In the case of the baryta and strontia, however, all the excess would be dissolved. Lactam formation from a salt of glutaminic acid not hydrolytically dissociated appeared to be unlikely. It was therefore concluded that the excess of baryta, strontia and soda were preventing hydrolytic dissociation of the salts. In the case of the lime there was insufficient in solution to do this. The calcium salt of pyrrolidonecarboxylic acid also present would be dissociated, and an equilibrium obtained after 21 per cent. of the glutaminic acid had been transformed.

For this view to be tenable some of the glutaminic acid should be changed, if only one equivalent of baryta were used instead of four. The following experiment was therefore carried out to test this point.

A mixture of 0.17 g. glutaminic acid, 0.375 g. barium hydroxide (one equivalent) and 6 cc. water was boiled for 5 hours under a reflux condenser, and the whole of the solution used for the amino-nitrogen determination with the following results:

Nitrogen	Per cent. Amino-N.	Per cent. changed
24.3 cc. at 20.2° and 768.5 mm.	8.26	13.24

Transformation in aqueous solution.

Having thus established that hydrolysis of the salts of glutaminic acid, involving the presence of the free acid in the equilibrium mixture, appeared to be a condition essential to the formation of the lactam from those salts, there was no apparent reason why the change should not occur simply in aqueous solution, with no base present. This contention was supported by evidence obtained previously when making a preparation of the free acid from its hydrochloride. On this occasion 10 g. of the hydrochloride equal to 8 g. of the free acid were dissolved in water and to this solution heated in a flask on the water bath hot silver sulphate solution was added

until in slight excess. The excess was removed by sulphuretted hydrogen and the filtrate evaporated to half its bulk, on the water bath. Sufficient baryta was then added to the hot liquid to combine quantitatively with the sulphuric acid. After allowing to stand on the water bath until the barium sulphate could be easily filtered, the barium sulphate was removed, and the liquid evaporated on the water bath to crystallisation. The following fractions were obtained :

Crop 1	white	4.29 g.	M.P. 213—214°
" 2	"	1.49 "	" 198—199°
Mother liquor evap. to dryness and dried	light brown colour	1.55 "	Softening commenced at about 130°, all melted by 151°

The 10 g. glutaminic acid hydrochloride was taken from a very good specimen, the analysis of which is given on page 481.

The carbon, hydrogen and amino-nitrogen were then determined in the mother liquor portion of 1.55 g., with the following results :

0.1353 g. ; 0.2225 g. CO_2 ; 0.0707 g. H_2O .

0.1580 g. ; 7.4 cc. nitrogen at 19° and 764 mm. by the nitrous acid method.

	Calculated for glutaminic acid	Found	Calculated for pyrrolidone- carboxylic acid
Carbon	40.83	44.85	46.51
Hydrogen	6.12	5.81	5.43
Amino-nitrogen	9.52	2.71	nil

The 1.55 g. evidently consisted of a mixture of glutaminic acid and pyrrolidonecarboxylic acid, as a calculation based on the carbon content showed 29.2 per cent. of glutaminic acid present in the mixture, and on the amino-nitrogen content, 28.5 per cent.

It has already been shown that the carboxylic group of pyrrolidonecarboxylic acid exercises its full acidity when titrated with $\text{N}/2$ soda using delicate litmus paper. Osborne has shown that one of the carboxylic groups of glutaminic acid is neutralised by soda to delicate litmus paper. The amount of soda required to neutralise equal weights of these two acids should therefore be about the same.

The titration of a portion of the 1.55 g. gave a result which agreed with these facts.

As the glutaminic acid hydrochloride originally taken for this experiment gave very satisfactory figures on analysis, the conclusion arrived at was that this 1.1 g. pyrrolidonecarboxylic acid had been produced from glutaminic acid during the repeated heatings on the water bath when in aqueous solution.

As all the facts pointed to the change occurring in aqueous solution the following experiments were carried out.

Two portions of 0.34 g. taken from different specimens of glutaminic acid both melting at the correct temperature were each dissolved in 12 cc. water and boiled on a sand bath for 5 hours under a reflux condenser. The solutions were made up to 20 cc. and the amino-nitrogen determined in 10 cc. by the nitrous acid method.

The following results were obtained :

		Nitrogen cc.	Temperature and pressure	Amino-nitrogen per cent.	Per cent. changed
Specimen A	..	13.0	20° & 763 mm.	4.39	53.89
„ B	..	13.3	22.2° & 762 „	4.44	53.37

The amount of rotation was determined in a 200 mm. tube in the solution from specimen B before using it for the amino-nitrogen determination.

A reading of -0.01° was obtained, the liquid being practically inactive, $[\alpha]_D^{20} = -0.3^\circ$.

The specific rotatory power of the original glutaminic acid Specimen B was also determined and found to be $[\alpha]_D^{20} = +11.03^\circ$.

The specific rotatory power of *l*-pyrrolidonecarboxylic acid previously found was -11.35° . Abderhalden [1909] gives $+10.5^\circ$ for glutaminic acid in aqueous solution.

It is therefore evident that somewhere about half the glutaminic acid is converted into *l*-pyrrolidonecarboxylic acid by boiling the aqueous solution for 5 hours.

The next experiment was designed for the purpose of finding the effect of evaporating solutions containing glutaminic acid on the water bath. With this object in view 0.34 g. was dissolved in 150 cc. water and evaporated to dryness on the water bath. The time taken was 2.5 hours and the temperature of the liquid ranged from 81 to 83°.

When dry, the substance was dissolved in a little normal soda solution and made up to 20 cc. The amino-nitrogen was then determined in 10 cc. with the following results :

cc. Nitrogen	Temperature and pressure	Amino-nitrogen per cent.	Per cent. changed
27.2	18.6° & 761 mm.	9.23	3.1

The influence of temperature is therefore considerable. This gives an explanation for the fact that only 1.1 g. pyrrolidonecarboxylic acid appeared as the result of the repeated evaporations on the water bath, carried out in

the course of working up the 10 g. glutaminic acid hydrochloride already described.

The effect of acids upon the transformation.

Experiments were then carried out to show the effect of acids upon the transformation. 0.17 g. portions and the required amounts of the various acids were in each case made up to 6 cc. with water and the solutions boiled on the sand bath for 5 hours under reflux condensers. The amino-nitrogen was then determined in the whole of each solution. The results are given in the following table :

Acid	Ratio of the acid to glutaminic acid in equivalents	Percentage of acid in the solution	Amino-nitrogen obtained cc.	Temperature and pressure	Amino-nitrogen ; percentage of original substance	Per cent. changed
Acetic ..	4	4.6	10.5	19° & 760 mm.	3.55	62.7
Hydrochloric	2	1.38	22.3	22 & 768	7.51	21.12
Sulphuric ..	4	3.72	21.85	19.8 & 768	7.44	21.85
„ ..	2	1.86	16.9	18.5 & 771.5	5.82	38.87
„ ..	8	7.44	25.85	20.5 & 768.5	8.78	7.77

In calculating the number of equivalents in Column 2 of this table, glutaminic acid is considered as a mono-acid base, having regard to the function of its amino-group.

It will be noted that the inhibiting effect upon the lactam formation appears to correspond with the strength of the acid employed. Thus two equivalents of sulphuric acid have the same effect as one equivalent of hydrochloric acid. In the case of the acetic acid the liquid was boiled for 6 hours instead of 5 by mistake. At any rate, however, it may be said that weak acids such as acetic acid have no inhibiting effect. As glutaminic and aspartic acids occur together in proteins, the influence of aspartic acid was tested : 0.17 g. glutaminic and 0.17 g. aspartic acid were together dissolved in 12 cc. water and boiled for 5 hours as before. The amino-nitrogen estimation showed that 53.57 per cent. of change had taken place. It therefore appears that aspartic acid exercises no influence, as the same change was produced in water alone. It is, however, noteworthy that twice as much water *i.e.*, 12 cc. instead of 6 cc., was used for the treatment of this 0.17 g. glutaminic acid.

The figures obtained for the sulphuric acid show that as the amount of acid present in the solution increases, the inhibiting effect also increases, and it may be gathered that when the solution contains about 8 per cent. sulphuric acid, no change at all would occur. It appears reasonable to assume that the same result would be obtained with 3 per cent. hydrochloric acid present.

Reconversion of ring-nitrogen to the amino-form by means of strong hydrochloric acid.

In order to ascertain whether the reverse change, *i.e.*, *l*-pyrrolidone-carboxylic acid into glutaminic acid, occurs on using stronger solutions of a mineral acid, the following experiment was carried out :

0.3285 g. of *l*-pyrrolidonecarboxylic acid was boiled for 4 hours with three times the quantity of strong hydrochloric acid. The liquid was then made up to 20 cc. and the amino-nitrogen determined in 10 cc.

Nitrogen obtained, cc.	Temperature and pressure	Amino-nitrogen percentage of original substance	Per cent. changed
28.6	14.3° C. & 760 mm.	10.24	94.4

The original *l*-pyrrolidonecarboxylic acid contained practically no amino-nitrogen (0.14 g. gave 0.2 cc.). It is therefore obvious that strong hydrochloric acid hydrolyses it to glutaminic acid.

A further experiment, using 5 to 6 times the quantity of hydrochloric acid and boiling for 8 hours, gave a change of 92.2 per cent. of the pyrrolidone carboxylic acid into glutaminic acid. The time required for the complete change has not yet been determined.

The solutions became brown in colour as the boiling continued, and it therefore appears possible that traces of the pyrrolidonecarboxylic acid are decomposed. This colouration appears very frequently when dealing with solutions of this substance.

Abderhalden and Kautzsch [1910] have also shown that pyrrolidone-carboxylic acid is converted into glutaminic acid by heating with hydrochloric acid. They succeeded in obtaining glutaminic acid hydrochloride from the resulting solution.

Secondary formation of pyrrolidonecarboxylic acid in solutions of the products of the hydrolysis of proteins.

As *l*-pyrrolidonecarboxylic acid is hydrolysed to glutaminic acid by boiling with three times the quantity of hydrochloric acid for 4 hours, it is very difficult to conceive of its presence in the liquid arising from a 48 hours' hydrolysis of a protein with strong hydrochloric acid. Up to the present, no experiments similar to those described have been carried out with the inactive pyrrolidonecarboxylic acid, but it is a reasonable assumption that this also will be hydrolysed by strong hydrochloric acid. It has been shown in this paper how pyrrolidonecarboxylic acid may be formed

from glutaminic acid in aqueous solutions and even in solutions containing appreciable percentages of the mineral acids, as well as when solutions presumably containing a hydrolysed salt of glutaminic acid are heated. There are therefore several ways open for the secondary formation of pyrrolidonecarboxylic acid during the treatment of the products of protein hydrolysis. From these considerations it would therefore appear that *l*-pyrrolidonecarboxylic acid is a secondary substance when obtained from the products of the hydrolysis of proteins by hydrochloric acid.

The fact that an increased yield of glutaminic acid hydrochloride generally results when any solution of amino-acids containing glutaminic acid is boiled with strong hydrochloric acid before saturating with hydrochloric acid gas is very probably also accounted for by a re-transformation of pyrrolidonecarboxylic into glutaminic acid.

That even prolonged boiling with hydrochloric acid leaves glutaminic acid quite untouched, is shown by the following experiment :

0.2972 g. pure glutaminic acid was boiled for 48 hours with three times the quantity of hydrochloric acid in a small bulb to which a long tube condenser was sealed. The solution was made up to 20 cc. and the amino nitrogen determined in 9.65 cc. equal to 0.1434 g. glutaminic acid :

Nitrogen obtained, cc.	Temperature and pressure	Amino-nitrogen per cent.	Per cent. changed
23.6	19.5° C. & 761 mm.	9.49	<i>nil</i>

SUMMARY.

1. Glutaminic acid is converted into *l*-pyrrolidonecarboxylic acid, to a large extent when its aqueous solution is boiled for a length of time.

2. The change also occurs, but to a much smaller extent, at 81–83°, the temperature of a liquid evaporated in an open dish on the water bath.

3. The same change occurs on boiling an aqueous solution of a salt of glutaminic acid, but to a smaller extent than in an aqueous solution of the amino-acid.

No change takes place when excess of the base is present, probably because hydrolysis of the salt is prevented.

4. Very weak acids have no inhibiting effect upon the lactam formation. The mineral acids, however, do inhibit the change to an extent proportional to their strength and to the amount present. About 8 per cent. sulphuric

acid and about 3 per cent. hydrochloric acid present in the solution of glutaminic acid inhibit the change altogether.

5. The reverse change, viz., of *l*-pyrrolidonecarboxylic to glutaminic acid occurs on boiling with strong hydrochloric acid.

6. Glutaminic acid is unaffected by boiling with strong hydrochloric acid.

7. Pyrrolidonecarboxylic acid may be formed from glutaminic acid in many of the operations involving evaporation or heating when in aqueous solution, during the working up and separation of the products of protein hydrolysis. When found amongst these products, there appears to be no doubt as to its secondary nature.

8. *l*-Pyrrolidonecarboxylic acid is precipitated as the calcium salt by adding excess of alcohol to its aqueous solution previously made alkaline with calcium oxide; when present with glutaminic acid the two calcium compounds are precipitated together.

9. These two substances are also precipitated by adding basic lead acetate solution, and then excess of alcohol, to their aqueous solutions.

10. Glutaminic and *l*-pyrrolidonecarboxylic acids have been obtained in a state of purity by means of two successive extractions of their mixture with cold glacial acetic acid.

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