ON THE COMPLETE HYDROLYTIC DECOMPOSITION OF EGG-ALBUMIN AT 180° C.

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The fact that α lactic acid and CO_2 should originate in the manner I have described in the previous paper¹ is highly suggestive. If these two substances are evolved from muscle during its contraction and have for their antecedent a compound of pyruvic acid or its nitrile with prussic acid, the question at once presents itself—Can we, by combining pyruvic acid and its homologues, or their nitriles, with other substances produce a series of compounds of the same nature and constitution as are obtained by various processes from protein matter; can we, in fact, in this way synthesise a number of, or even all, the protein derivatives?

The task I have set before me is to prove that an affirmative answer must be given to the question; and I propose to do so by shewing that the synthesis of a very large number of compounds which are obtained from protein, or albuminous bodies, including among them Schutzenberger's leucéins (which I shall endeavour to prove are the imino-ketonic acids) and tyroleucine, as well as various pyridine and chinoline derivatives, can be effected by combining pyruvic acid and its homologues with prussic acid, with ammonia or with other substances, such as the fatty aldehydes, and then acting on these combinations in various ways.

I shall then be able to determine the composition of certain compounds or combinations of the above mentioned bodies, which,

1. See pp. 194 and 201.

according to Schutzenberger¹, result from the hydrolysis of albumin, viz. :—

The synthesis of tyrosine from oxy- or amido-benzaldehyde will then be discussed; and subsequently the synthesis of Schutzenberger's leucines, that is, of amino-caproic, -valeric, -butyric, -propionic and amino-acetic acids from the various aldehydes; and lastly the synthesis of the aspartic acid series from a similar source.

With the knowledge of the composition of these bodies we shall be able to determine precisely and completely what substances are furnished by the hydrolysis of albumin, as well as their respective amounts. Moreover, knowing the constitution and synthesis of each product we shall then, as I shall endeavour to show, be within measurable distance of determining the constitution and arriving at the synthesis of albumin itself.

ON THE SYNTHESIS OF PYRUVIC ACID, AND ON SOME PROTEIN DERIVATIVES WHICH CAN BE SYNTHESISED FROM IT

Synthesis of Pyruvic Acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{COOH}$).—This acid may be obtained by carefully acting upon acetyl-cyanide with HCl^3 and heating.

This method is applicable generally for the formation of the α ketonic acids from their nitriles. In the cold the amide is first formed.

The acid may also be regarded as a condensation product of

1. *Annales de Chemie et de Physique*, 5me sér., t. XVI, p. 398.
2. Claisen, *Shadwell*, Ber. XI, 620 u. 1563.

oxalic acid and acetic acid, and may be prepared in the following manner :—

To one molecule of di-ethyl-oxalate dissolved in four parts of absolute ether add one molecule of sodium, and then in small quantities at a time a little more than one molecule of acetic ether, taking care that the temperature of the mixture does not rise above the boiling point of the ether. The sodium is gradually dissolved, hydrogen being evolved, and the greater part of the somewhat dark coloured fluid solidifies, after a few hours, into a yellowish crystalline semi-solid mass, which, when drained from the liquid portion, is washed with ether. In this way the almost pure sodium compound of oxalacetic ether is obtained, as a thick white mass.¹

On adding dilute H_2SO_4 (10 per cent.) to the ester mixed with H_2O and ether, the sodium salt is resolved into

which warmed with dilute H_2SO_4 is converted into

SOME PROTEIN DERIVATIVES THE SYNTHESIS OF WHICH CAN BE EFFECTED FROM PYRUVIC ACID

The following substances have been obtained from protein :—

Leucéine (Schutzenberger)	...	...	...	$\text{C}_9\text{H}_5\text{NO}_2$
Gluco-protein	...	...	...	$\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4$
Alanine or α amino-propionic acid	...	...	...	$\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$
Picoline or methyl-pyridine	...	...	...	$\text{CH}_3 \cdot \text{C}_5\text{H}_4\text{N}$
Pyridine	...	...	...	$\text{C}_5\text{H}_5\text{N}$
Indol	...	...	...	$\text{C}_8\text{H}_7\text{N}$
Chinoline carboxylates	...	...	...	$\text{C}_6\text{H}_4 \begin{cases} \text{C} \cdot \text{COOH} - \text{CH} \\ \text{N} = \text{C} \cdot \text{R} \end{cases}$
and α R. Chinolines	...	...	...	$\text{C}_6\text{H}_4 \begin{cases} \text{CH} - \text{CH} \\ \text{N} = \text{C} \cdot \text{R} \end{cases}$

1. Wislicenus, *Annal. d. Chemie*, Bd. 246, s. 315-327.

I will now describe the methods by which, starting with pyruvic acid, these substances can be synthesised.

On the Synthesis of Schutzenberger's Leucéine ($C_3H_5NO_2$).—This, I suggest, is imino-pyruvic acid, $CH_3 \cdot C(NH) \cdot COOH$; the ammonium salt of which is precipitated when to a solution of pyruvic acid in ether or alcohol, alcoholic ammonia is added to exact neutralisation.¹

The ammonium salt is very soluble in water, and reduces Fehling's solution. On boiling it with water it is rapidly decomposed into CO_2 , NH_3 , uivitaminic acid ($C_9H_{13}NO_7$), and uivitaminic acid ($C_8H_7NO_4$) or picoline dicarboxylic acid, $(CH_3 \cdot C_5H_2N(COOH)_2)^2$

Synthesis of a Alanine ($CH_3 \cdot CH(NH_2) \cdot COOH$).—I have already described the method by which this substance can be synthesised from pyruvic nitrile (see page 203). The synthesis can also be effected directly from pyruvic acid by the following process:—

Take equal molecules of pyruvic acid and concentrated prussic acid and heat them in a sealed tube to 30° - 40° C., then add two molecules of alcoholic ammonia and heat the whole to 70° C. Wash the resulting crystals of amino-iso-succinamide with alcohol and then dissolve them in water and recrystallise.³

The following are the reactions:—

1. Beilstein's *Handbuch d. org. Chemie*, 3te Auf., Bd. 1, s. 587.

2. Bottinger, *Liebig's Annal.*, Bd. 188, s. 330; Bd. 208, s. 138.

3. Körner, Menozzi, *Gazetta chimica Italiana*, Vol. XVII, p. 426.

The crystals appear as large rhombic tablets which are soluble in twenty parts of cold, or three parts of boiling water, but are only slightly soluble in alcohol.

On boiling the amide with hydrochloric acid it is decomposed into CO_2 , NH_3 and alanine.

On the other hand by boiling the amide with one molecule of caustic baryta or other alkali until ammonia is no longer evolved we obtain barium amino-iso-succinate or barium iso-aspartate.¹

On treating the barium salt with an exact equivalent of H_2SO_4 to precipitate the baryta, and then evaporating the filtered solution *in vacuo* over H_2SO_4 we obtain prismatic crystals of iso-aspartic or amino-iso-succinic acid, $\text{CH}_3 \cdot \text{C}(\text{NH}_2) \cdot (\text{COOH})_2$.

On boiling this acid in water it is resolved into CO_2 and α alanine

On the Synthesis of Schutzenberger's Gluco-protein ($\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4$):— Dissolve one molecular equivalent of α alanine in a small quantity of water, then add to it one molecular equivalent of alcoholic ammonia. To one molecular equivalent of pyruvic acid dissolved in alcohol add, very slowly, two molecular equivalents of alcoholic ammonia avoiding a rise of temperature, when the ammonium imino-pyruvate will be formed. Pour into this the solution of alanine, adding, if necessary, a little water to form a clear solution. Evaporate *in vacuo*, when crystalline nodules will be formed consisting of small prisms radiating from a centre the composition of which after crystallisation is a compound of equal molecules of $\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ (alanine) and $\text{CH}_3 \cdot \text{C}(\text{NH}) \cdot \text{COOH}$ (imino-pyruvic acid), or $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_4$, the basic NH_3 having been dissipated.

1. Körner, Menozzi, *loc. cit.*, p. 429.

The analysis of the crystals by Mr. M. M. Pattison Muir, who kindly examined them for me, gave the following results :—

Experiment				Calculated for $C_8H_{12}N_2O_4$
C =	40.46	...	...	40.91
H =	7.26	...	...	6.82
N =	15.6	...	...	15.9
O =	36.68	...	...	36.37
<hr/>				<hr/>
100.00				100.00

Nenki (Ber. VII (2), 1593; VIII, 336; and X, 1032) and Kühne (Ber. VIII, 206) among the products of putrefying albumin.

*Synthesis of Indol.*¹—By adding pyruvic acid to a weak solution of phenyl-hydrazin in acetic acid, phenyl-hydrazin pyruvic acid is precipitated in a crystalline form. This is converted into the ester; the action of zinc chloride on which in an oil bath at 195° C. is so violent that only small portions at a time should be experimented with. Indol carboxylic acid and its ester are formed. By heating indol carboxylic acid to 230° it is decomposed into CO₂ and indol.

Synthesis of the Chinoline Carboxylates and the α R. Chinolines from Pyruvic Acid.—The chinoline carboxylates $C_nH_{2n-13}NO_2$, containing an alcohol radicle in the Py 2. position, are formed by heating pyruvic acid with the aldehydes and aniline in alcohol for four to five hours in a water bath;² the aniline being very slowly added during the process.

1. E. Fischer, *Annalen d. Chem.*, Bd. 236, s. 142.

2. Döbner, *Annalen d. Chemie*, Bd. 242, s. 270.

If the experiment is made at the ordinary temperature neutral bodies are formed

By heating the carboxylates, either by themselves or with soda-lime, they are resolved into α R. chinolines and CO_2

Synthesis of Picoline ($\text{CH}_3 \cdot \text{C}_5\text{H}_4\text{N}$).—On heating picoline dicarboxylic acid (obtained as above) to 274°C . it is resolved into CO_2 and mono-carboxylic acid¹

On fusing the calcium mono-carboxylate with soda-lime, it is converted into CO_2 and picoline²

Synthesis of Pyridine ($\text{C}_5\text{H}_5\text{N}$).—By oxidising 2-picoline with KMnO_4 it is converted into pyridine carboxylic acid³ :—

1. Bottinger, *Berichte*, Bd. 14, s. 67; Bd. 17, s. 92.
2. Beilstein's *Handb. d. org. Chemie*, Bd. 4, s. 166.
3. Weidell, *Berichte*, Bd. 12, s. 1992.

On heating this with alcoholic potash to 240° C. it is completely transformed into pyridine¹

Synthesis of Pyrrol ($\text{C}_4\text{H}_4(\text{NH})$).—On combining uvitaminic acid $\text{C}_9\text{H}_{13}\text{NO}_7$, obtained as above from ammonium imino-pyruvate, with baryta and subjecting the dry salt to the action of heat, a large amount of pyrrol distills over, together with NH_3 and other bases.²

ON THE SYNTHESIS OF ACETYL-ACETIC ACID AND OF CERTAIN PROTEIN DERIVATIVES WHICH CAN BE FORMED FROM IT

The next higher homologues in the pyruvic acid series are propionyl-formic acid, $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{COOH}$, and acetyl-acetic acid, $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOH}$. It is to the products derived from the latter that I will, at present, direct attention. This acid exists in the animal economy, being found in the urine of individuals suffering from diabetes, and is then generally accompanied by β oxybutyric acid, $\text{CH}_3 \cdot \text{CH}(\text{OH}) \cdot \text{CH}_2 \cdot \text{COOH}$, and acetone $\text{CO} \cdot (\text{CH}_3)_2$. I suggest that these substances are derivatives of the albuminous tissues.

Synthesis of Acetyl-Acetic Acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOH}$).—By acting upon acetic ether $\text{CH}_3 \cdot \text{CO}_2 \cdot \text{C}_2\text{H}_5$ with sodium alcoholate, sodium acetyl-acetic ether is produced.

On adding acetic acid to this and distilling at 130° C. acetyl-acetic acid $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOH}$ passes over.³

The free acid may also be obtained by mixing together 4.5 parts of the ethylic ester with 2.5 parts of KOH and 80 parts of H_2O letting the mixture stand for twenty-four hours, then acidulating with H_2SO_4 , and extracting the acid with ether.⁴

1. Beilstein's *Handb. d. Chemie*, 3te Aufl., Bd. IV, s. 141.

2. Beilstein, *loc. cit.*, Bd. I, s. 587.

3. Geuther, *Zeit. f. Chemie*, 1868, s. 652.

4. Ceresole, *Ber. XV*, p. 139, 1872.

Practically then, we may regard acetyl-acetic acid as the condensed product of two molecules of acetic acid.

On the Synthesis of Schutzenberger's Leucéine ($\text{C}_4\text{H}_7\text{NO}_2$).—This compound is, I suggest, imino-acetyl-acetic acid $\text{CH}_3 \cdot \text{C}(\text{NH}) \cdot \text{CH}_2 \cdot \text{COOH}$ known also under the name of amino-crotonic acid $\text{CH}_3 \cdot \text{C}(\text{NH}_2) : \text{CH} \cdot \text{COOH}$,¹ according to the view which is taken of the constitution of the compound.

The ester of this acid may be prepared by mixing together, at the ordinary temperature, acetyl-acetic ether with strong aqueous ammonia, and shaking the mixture, from time to time, for several days.²

It may also be prepared by passing dry gaseous ammonia rapidly into pure acetyl-acetic ester;³ mixed with double its volume of ether. The yield being larger if ammonium nitrate is first mixed with the dry ester.

This imino- or amino-body crystallises in colourless thick monoclinic prisms which are not very soluble in water but readily soluble in ether, benzol and chloroform. Its melting point is 34°C . (Collie), 37° (Conrad). It is resolved into its constituents by dilute HCl , and by NaHO . The composition of this body $\text{C}_4\text{H}_7\text{NO}_2$ merely differs from that of amino-butyric acid $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ or $\text{C}_4\text{H}_9\text{NO}_2$, by two atoms of hydrogen.

Synthesis of β Oxy-Butyric Acid ($\beta \text{CH}_3 \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{COOH}$). If water is added to an alcoholic solution of imino-acetyl-acetic ester until it is slightly opaque and the mixture then placed in an ice bath

1. Conrad, Epstein, *Berichte*, B. 20, s. 3056.

2. Duisberg, *Liebig's Ann.*, Bd. 213, s. 166.

3. J. Norman Collie, *Liebig's Ann.*, Bd. 226, s. 294-301; and Conrad, Epstein, *Berichte*, Bd. 20, s. 3054.

combining β amino-crotonic ester, $\text{CH}_3 \cdot \text{C}(\text{NH}_2) : \text{CH} \cdot \text{CO}_2\text{C}_2\text{H}_5$, with paraldehyde and a little H_2SO_4 .¹ In the latter case $(\text{CH}_3 \cdot \text{CHO})_3$ we have

(ii) On treating this ester with nitrous acid $\text{H} \cdot \text{NO}_2$, it loses H_2 and is converted into

(iii) From this the mono-ethyl ester can be obtained by heating it with a molecular equivalent of alcoholic potash for some time in a flask, to which a reversible condenser is attached. The solution is then freed from alcohol by distillation and the residue dissolved in water, any unchanged diethyl ester is then extracted with ether or benzol, and the solution neutralised by adding a molecular equivalent of HCl . The solution is then evaporated to dryness in a water bath and the ester recrystallised from absolute alcohol.² The result is

1. Collie, *Liebig's Ann.*, Bd. 226, s. 314.

2. R. Michael, *Lieb. Ann.*, Bd. 225, s. 124.

If two molecules of alcoholic potash are employed CO_2 is disengaged and γ collidine or trimethyl-pyridine is left behind.

2 . 4 . 6 . trimethyl pyridine
or γ collidine.

If the mono-ethyl ester prepared as above is heated to 255°C . it is resolved into CO_2 and

which distils over.¹

On saponifying this with alcoholic potash and then adding sufficient HCl to convert the whole of the potash into KCl , evaporating the solution to dryness and treating the residue with absolute alcohol, on evaporation we obtain short needles of

Synthesis of Lutidine ($\text{C}_7\text{H}_9\text{N}$).—By the oxidation of one molecule of potassium collidine carboxylate with 2, 4, and 6 molecules of KMnO_4 we obtain respectively³ the potassium salts of

1. *Ibid.*, s. 131.

2. *Ibid.*, s. 134.

3. *Ibid.*, s. 136.

By fusing potassium lutidine dicarboxylate with lime we obtain¹

β Lutidine can also be obtained by mixing together 130 grammes aceto-acetic ester, 61 grammes aldehyde ammonia and 50 grammes of aldehyde² at 130° C., to form lutidine carboxylic ester.

From this lutidine carboxylate can be obtained, which fused with lime is resolved into CO_2 and 2,4 dimethyl pyridine or lutidine.

ON THE SYNTHESIS OF TYROLEUCINE ($\text{C}_7\text{H}_{11}\text{NO}_2$)

Perhaps the most important of Schutzenberger's experiments as elucidating the constitution of the leucéins is the one by which he succeeded in isolating tyroleucine $\text{C}_7\text{H}_{11}\text{NO}_2$ and caproic leucéine $\text{C}_6\text{H}_{11}\text{NO}_2$.

1. Engelmann, *Lieb. Ann.*, Bd. 231, s. 54.

2. Michael, *Ber.*, Bd. 18, s. 2022.

The following are the details of his experiment¹ :—

‘Ten kilogrammes of albumen were decomposed by baryta in a large autoclave, and the liquid having been freed successively from ammonia and from the excess of baryta by a current of carbonic acid and by sulphuric acid, it was then concentrated till it crystallised. I was able to isolate about 2 kilogrammes of crystalline deposit corresponding to the deposit (A).

‘From this considerable mass I was only able to procure—by repeated crystallisations carefully carried as far as possible—first, the substances mentioned already, tyrosine, leucine, butalanine, amido-butyric acid, crystalline compounds of the type $C_mH_{2m}N_2O_4$ ($m = 12$ and 10), and secondly, two new definite and crystallisable products, of which one, to which I give the name *Tyroleucine*, corresponds to the formula $C_7H_{11}NO_2$, and the other belonging to the type $C_nH_{2n-1}NO_2$; its composition is represented by the formula $C_6H_{11}NO_2$; it is a leucéine ($n = 6$), caproic leucéine. Tyroleucine belongs to the series $C_nH_{2n-3}NO_2$; it plays only a very secondary part in the composition of albumen, and appears in small quantities only in the products of its decomposition. With 100 grammes of substance, I completely overlooked its presence. By experimenting with 10 kilogrammes I was able to obtain from 60 to 70 grammes in a pure state. The existence of a definite and crystallised substance belonging to the type $C_nH_{2n-1}NO_2$ tends to prove that the compounds of the type $C_mH_{2m}N_2O_4$, which are so abundant, should be regarded as molecular combinations of leucines and of leucéines.

‘The following experiment, however, seems to shew that the compounds $C_mH_{2m}N_2O_4$ ($m = 10$ or 12) cannot always be separated, by successive crystallisations into leucines $C_nH_{2n+1}NO_2$ and leucéines $C_nH_{2n-1}NO_2$. A crystalline deposit in nodules or spherical masses radiating from a centre, gave the following result.

(a)	Substance	...	...	0.3495
	Carbonic acid	...	...	0.707
	Water	...	...	0.286

1. *Annales de Chimie et de Physique*, 5me série, t. XVI, pp. 345, 352.

‘These crystals, re-dissolved in water and purified by a fresh crystallisation, gave

	(b)	Substance	...	...	0.3105
		Carbonic acid	...	...	0.631
		Water	...	...	0.251
The percentage being					Calculated for
		<i>a</i>	<i>b</i>		$C_{12}H_{24}N_2O_4$
Carbon		55.1	55.42		55.38
Hydrogen		9.09	9.01		9.2

The composition remained unaltered, and we must therefore admit the existence of a body corresponding to the formula $C_{12}H_{24}N_2O_4$ ($m = 12$). This result has been frequently verified, not only as to the compound $C_{12}H_{24}N_2O_4$, but also for the lower homologues $C_{11}H_{22}N_2O_4$, $C_{10}H_{20}N_2O_4$, $C_9H_{18}N_2O_4$. In the experiment with 10 kilos of albumen, tyroleucine ($C_7H_{11}NO_2$) and caproic leucéine ($C_6H_{11}NO_2$) were extracted from the aqueous solution of the crystalline deposit (*A*) by first separating the greater part of the tyrosine and leucine, by means of fractional crystallisations. The mother-liquor, freed from traces of baryta by sulphuric acid, was decolourised by animal charcoal, and then again concentrated. A fairly copious deposit of spherical masses took place. From this I was able to obtain a considerable amount of two new bodies. This deposit was again treated with hot water, which dissolved it all except a residue of tyrosine; the dark liquid was decolourised by means of a little sub-acetate of lead, and filtered from the brown flocculi; the excess of lead was precipitated by sulphuretted hydrogen and the liquid filtered and decolourised by animal charcoal. This was twice concentrated and two crystalline products (*B*) and (*C*) in spherical masses or in granules were obtained.

‘The crystals (*B*) purified and recrystallised were composed of tyroleucine $C_7H_{11}NO_2$.

‘At a temperature of from 250° to 280° in an inert gaseous atmosphere tyroleucine is decomposed, furnishing a white sublimate, water, and carbonic acid, together with an oily volatile base having

the odour of horse-radish, which remains in the retort as an amorphous, transparent mass having a resinous fracture.

'An analysis of the chloroplatinate of the oily base shewed its composition to be $C_8H_{11}N$ which represents collidine or an isomeric substance. The vitreous residue on analysis furnished numbers corresponding with the formula $C_{14}H_{18}N_2O_2$. It appears from these results that tyroleucine heated above 250° is in part dehydrated and polymerised, while another portion is decomposed into carbonic acid, collidine and butalanine in accordance with the following equation :—

Dehydration takes place as in the following equation :—

Tyroleucine, therefore, must be regarded as a compound of butalanine (amino-valerianic acid) with a body whose composition is $C_9H_{11}NO_2$, differing from tyrosine by one atom of oxygen.

'An analysis of the crystals (C), obtained by concentrating the mother-liquor from which the tyroleucine had been obtained, shewed that it consisted of $C_6H_{11}NO_2$ or leucéine caproic.'

From the above extract it is evident that tyroleucine is composed of amino-valerianic acid $CH_3 \cdot (CH_2)_2 \cdot CH(NH_2) \cdot COOH$ and collidine carboxylate $C_9H_{11}NO_2$, or

which is obtained as shown on page 218 by heating collidine dicarboxylate, a compound which results from oxidation of hydrocollidine dicarboxylic ester, the latter being obtained by combining paraldehyde with the ester of β amino-crotonic acid.¹

1. Collie, *Liebig's Ann.*, Bd. 226, s. 314.

laevulinic acid or β acetyl-propionic acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$) and γ acetyl-butyric acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$), may also be obtained from sodium diacetic ester.

Synthesis of Imino-Laevalinic Acid.—By acting upon sodium-diacetic-ethyl ester with chlor-acetic ester, acetyl-succinic diethyl ester is produced.¹

Rash² prepared this by taking 52 grammes of acetyl-acetic ester, 9.2 grammes Na, 150 grammes $\text{C}_2\text{H}_5 \cdot \text{OH}$, and 125 grammes chlor-acetic ester.

On boiling the acetyl-succinic ester with twice its volume of dilute HCl, or with baryta water, it is resolved into CO_2 , $\text{C}_2\text{H}_5 \cdot \text{OH}$ and $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ (β acetyl-propionic acid) or laevulinic acid, together with a little acetic and succinic acids.

This combines with NH_3 to form $\text{CH}_3 \cdot \text{C}(\text{NH}) \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ (imino-laevulinic acid).

Synthesis of Imino-Acetyl Butyric Acid ($\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$).—By acting upon sodium diacetic ester with β iodo-propionic acid, acetyl-glutaric ester is produced.³

This boiled for eight to ten hours with one part of concentrated HCl and two parts of water is resolved into CO_2 , alcohol, and γ acetyl-butyric acid.⁴

1. Conrad, *Annalen*, Bd. 188, s. 218.

2. *Annalen*, B. 234, s. 36.

3. Wislicenus, Limpach, *An. d. Chemie*, p. 192, 128.

4. Wolff, *An. d. Ch.*, p. 216, 129.

Treated with a strong solution of alcoholic potash it is resolved into acetic acid and glutaric acid.

Combined with NH_3 acetyl-butyric acid forms $\text{CH}_3 \cdot \text{C}(\text{NH}) \cdot (\text{CH}_2)_3 \cdot \text{COOH}$ (imino-acetyl butyric acid).

SYNTHESIS OF SCHUTZENBERGER'S LEUCÉINES $\text{C}_5\text{H}_9\text{NO}_2$ and $\text{C}_6\text{H}_{11}\text{NO}_2$

Each of these bodies may be represented by two isomeric compounds. The leucéine $\text{C}_5\text{H}_9\text{NO}_2$ may be represented either by—

The leucéine $\text{C}_6\text{H}_{11}\text{NO}_2$ being represented by—

Each of these four leucéines being, I suggest, derivatives of protein.

The synthesis of the ester of the two amino-crotonic acids may be effected by passing NH_3 gas into an ethereal solution of methyl-crotonic and ethyl-crotonic ester respectively,¹ as in the formation of β amino-crotonic ester from acetyl-acetic ester.²

Or, the two esters may be formed from β amino-crotonic ester by acting upon it first by sodium ethylate and treating the resulting compound with CH_3I and $\text{C}_2\text{H}_5\text{I}$ respectively.

1. Conrad, Epstein, *Berichte*, Bd. 20, s. 3055 u. 3057.

2. See page 215.

Acting on the esters of these two amino-acids with dilute HCl they are converted into the corresponding crotonic esters and NH_4Cl ; and these acted upon by KHO are converted into the corresponding ketones.¹

I therefore venture to submit that these imino compounds of pyruvic acid and its homologues are the compounds which, in combination with the amino-fatty acids, Schutzenberger obtained by the hydrolytic decomposition of albumin—and to which he gave the name of leucéins, the combinations with the amino-fatty acids being named by him gluco-proteins.

Having made this advance we are now in a position to discuss the results which he obtained in a remarkable series of researches extending over several years. These results are published in *Comptes Rendus*, tome 80, p. 233; tome 81, p. 1108; tome 84, p. 124; tome 101, p. 1267; tome 102, p. 1289; tome 106, p. 1407; and tome 112, p. 198. The most important paper is contained in the *Annales de Chimie et de Physique*, 5me série, tome 16, p. 289, and gives the results of innumerable analyses made with extreme accuracy. From this paper I shall take the details which are necessary for my present purpose.

In his researches Schutzenberger employed white of egg coagulated by heat and with a slight excess of acetic acid. This was well washed with water, alcohol and ether—and dried at 140°C . Fifty or one hundred grammes of this mixed with water were treated with two to six times its weight of crystalline barium hydrate in a closed iron vessel, and heated to a temperature ranging from 100°C . to 250°C ., for periods varying from eight to one hundred and twenty hours (*loc. cit.*, p. 303). After the vessel had completely cooled down it was opened; generally there was entire absence of increased pressure or of unabsorbed gas. Sometimes if the temperature had been raised to near 200° and if a large proportion of baryta had been used, a certain

1. See page 223.

amount of hydrogen uncontaminated by carbon escaped from the digester. This Schutzenberger attributed to some secondary action of the baryta on the iron vessel.¹

The contents of the digester were now :—(i) Subjected to distillation and the amount of NH_3 determined. (ii) The insoluble deposit was separated from the liquid portion and the amount of barium carbonate and oxalate in the deposit was determined. (iii) The baryta contained in the filtered liquid was precipitated first as far as possible by passing a current of CO_2 through the solution kept at the boiling point for a considerable time,² then filtering off the precipitate and washing it : concentrating the filtrate and the washings and adding the *exact* quantity of H_2SO_4 necessary to precipitate the baryta still held in solution. (iv) The acid filtrate from these precipitates was then distilled and the amount of acetic acid contained in the distillate determined. In addition to acetic acid the distillate contained traces of formic acid, and an essential volatile oil consisting of pyrrol, etc. (v) The liquid was finally evaporated to dryness in a water bath. This dried residue was called by Schutzenberger the ‘*résidu fixe*,’ meaning thereby a mixture of substances which do not sublime nor volatilize at a temperature below 100°C .

Having advanced so far, Schutzenberger's next step was to endeavour from the analysis of albumin to construct such a molecular formula as might serve as a working hypothesis. Taking tyrosine as a basis, the percentage of which can be readily determined, as it crystallises readily and is only slightly soluble in cold water, he found as the result of numerous most carefully conducted experiments that the amount obtained from 100 grammes of albumin ranged between 2.5 and 3.5 grammes, the mean result being 3.4. Now it is improbable that tyrosine results from the combination of several molecules of albumin ; consequently, if we assume that one molecule

1. *Annales de Chimie et Physique*, 5me série, t. XVI, p. 296. Later on see page 230. I shall endeavour to shew that this hydrogen resulted from the action of the baryta on formic acid converting it into oxalic acid and hydrogen.

2. *Loc. cit.*, p. 298.

of tyrosine (= 181) is produced by the decomposition of one molecule of albumin, and that this contains 3·3 per cent. of tyrosine, we have as the molecular weight of albumin—

$$\frac{181 \times 100}{3.3} = 5484$$

On this ground alone Lieberkühn's formula $C_{72}H_{112}N_{18}O_{22}S$, of which the molecular weight is 1612, cannot be accepted as correct. Moreover, as Schutzenberger points out,¹ the proportion of nitrogen (15·8 per cent. in Lieberkühn's formula) is too small; the actual percentage being 16·6. Schutzenberger's analysis of albumin² gives the following results :—

Carbon	...	...	...	52·57
Hydrogen	...	...	...	7·16
Nitrogen	...	...	...	16·6
Oxygen	...	...	...	21·8
Sulphur	...	...	...	1·8
				<hr/>
				99·93

This agrees closely with those of

	Brittner ³ and Fleitman ⁴	
C	... 54·9	53·8
H	... 7·0	7·3
N	... 16·6	16·2
S	... 1·6	1·4

Based on this analysis he adopts as the formula for albumin, $C_{240}H_{387}N_{65}O_{75}S_3$ having a molecular weight of 5473, and the percentage composition of which is :—

Carbon	...	...	52·62
Hydrogen	...	...	7·07
Nitrogen	...	...	16·62
Oxygen	...	...	21·94
Sulphur	...	...	1·75
			<hr/>
			100·00

1. *Loc. cit.*, p. 383.

2. *Loc. cit.*, p. 384.

3. Beilstein, *Handb. d. Chemie*, 3te Aufl., Bd. IV, s. 1590.

4. *Watt's Dict.*, Vol. IV, p. 738.

The formula $C_{240}H_{397}N_{65}O_{75}S_3$, differing from the above by ten atoms of hydrogen, and having a molecular weight of 5483, corresponds more closely with Schutzenberger's analysis, and is the one I shall adopt. Its percentage composition is :—

Carbon ...	...	...	52.53
Hydrogen ...	...	...	7.24
Nitrogen ...	...	...	16.6
Oxygen ...	...	...	21.88
Sulphur ...	...	...	1.75
			<hr/>
			100.00

and I will endeavour by its aid to interpret the analytical results which Schutzenberger obtained.

Acting upon 100 grammes of albumin, in the manner previously described (see page 226), at a temperature of 180° C. with four or five parts of baryta he obtained

Nitrogen (in the form of NH_3)	...	...	4.03
Barium oxalate ...	...	...	17.6
Barium carbonate ...	...	...	11.0
Acetic acid ...	...	...	4.6—4.9

which, for a molecular weight of 5483, correspond very closely to

16 molecules of Ammonia		
4	„	Oxalic acid
3	„	Carbonic acid
and 4	„	Acetic acid

The 'résidu fixe' obtained under the same conditions after separating the baryta with CO_2 and then with H_2SO_4 and evaporating to dryness weighed 99.6 grammes, and on analysis yielded the following percentages very approximately¹

C	=	48.63
H	=	7.97
N	=	12.58
O	=	30.82
		<hr/>
		100.00

1. *Loc. cit.*, p. 385.

giving for the 'résidu fixe' the formula $C_{221}H_{435}N_{49}O_{105}$, the calculated percentage of which is—

C	=	48.95
H	=	8.03
N	=	12.01
O	=	31.01
		<hr/>
		100.00

Schutzenberger, moreover, determined that the decomposition of albumin by baryta under the conditions specified was one of hydrolysis, and that during the process one molecule of albumin at 180° C. combined with 60 molecules of water. With the results just given, the following equation represents the decomposition :—

the intermediate stage being :—

We have now to deal exclusively with the 'résidu fixe' $C_{221}H_{435}N_{49}O_{105}$. After trying by various methods to separate the constituents of this compound, Schutzenberger arrived at the conclusion that the only feasible plan was by fractional crystallisation from various neutral solvents such as water, alcohol, ether, etc. 'The different crystalline products can be distinguished, to a certain extent, by their form and appearance, especially when examined under the microscope. But, as we are well aware, the bodies of which the "résidu" is composed belong to small groups, each members of a homologous series, and frequently there is very little difference in the characters of the neighbouring terms of any single group. Moreover, two terms of the same group, or of two neighbouring groups, have a general tendency to crystallise together in their molecular proportions.

* $6\text{H} \cdot \underset{\text{formic acid}}{\text{COOH}} + 2\text{SH}_2 + \text{S}$ being under the action of the BaH_4O_9 converted into $3(\overset{\text{oxalic acid}}{\text{(COOH)}_2}) + \text{S}_8 + 5\text{H}_2$

Intermediate products, therefore, are frequently obtained, adding considerably to the difficulty of arriving at a proper estimate.¹

The conclusions at which Schutzenberger arrived were based upon the results of upwards of five hundred analyses of the various products which he obtained from the 'résidu fixe.' In order to separate these products he found that in the first instance the best plan was to pass through the primary solution freed from acetic acid and filtered from the barium carbonate and oxalate a stream of carbonic acid for a considerable time, in order to precipitate the excess of baryta, and after filtering from the BaCO_3 to concentrate the solution, without precipitating the baryta which it still contains, with sulphuric acid. After a certain degree of concentration a crystalline pellicle forms on the surface and, on cooling, a copious crystalline mass (*A*) separated out, consisting of granules formed by the aggregation of crystals round a centre. Further concentration of the mother-liquor furnished additional crystals. Finally there remained a voluminous syrup (*B*) which required subsequent treatment to obtain its constituents in a crystallised form.

From the crystalline mass (*A*) crystals of tyrosine $\text{C}_9\text{H}_{11}\text{NO}_3$, and leucine $\text{C}_6\text{H}_{13}\text{NO}_2$, together with amino-valerianic acid $\text{C}_5\text{H}_{11}\text{NO}_2$, were obtained. These two latter were combined in various ways with the leucéins $\text{C}_6\text{H}_{11}\text{NO}_2$ and $\text{C}_5\text{H}_9\text{NO}_2$, forming the crystalline compounds—

The last fractional crystallisation from the mass (*A*) on analysis, furnished numbers which agree with those of amino-butyric acid, $\text{C}_4\text{H}_9\text{NO}_2$. Schutzenberger makes no mention here of the presence of amino-propionic acid $\text{C}_3\text{H}_7\text{NO}_2$. From analyses and statements, however, which will be found further on it is evident that it was

1. *Loc. cit.*, p. 332.

present, probably combined with $C_5H_{11}NO_2$ and so forming the compound $2 C_4H_9NO_2$.

Caproic leucéine, $C_6H_{11}NO_2$, crystallises in granules consisting of needles grouped round a centre, and is more soluble in water than leucine. It is soluble in boiling alcohol, and has a slightly sweet taste.¹

Amino-valerianic acid $C_5H_{11}NO_2$ is found in the primary crystallisations of tyrosine, tyroleucine, leucine and caproic leucéine, together with the intermediate products $C_mH_{2m}N_2O_4$ ($m = 12, 11$, and 10). It is more soluble in water than leucine, and is most readily obtained from the mother-liquor after the separation of the above products. It is therein associated with a considerable proportion of amido-butyric acid $C_4H_9NO_2$ and traces of alanine or amino-propionic acid, $C_3H_7NO_2$. This mother-liquor also contains the lower members of the series $C_mH_{2m}N_2O_4$ and $C_nH_{2n+1}NO_2$ ($m = 10-7$; $n = 5-4$).¹

For the extraction of amino-valerianic acid and the other substances referred to above, which are very soluble in water, the following process was adopted:—

The mother-liquor or uncrystallisable syrup (*B*) was first completely deprived of baryta by means of sulphuric acid and then evaporated to dryness. The residue was treated several times with boiling alcohol, which almost entirely dissolved it, and on cooling furnished a crystalline deposit. This deposit weighed about 3 or 4 per cent. of the albumin operated upon, and consisted of $C_9H_{20}N_2O_4$, crystallising from the alcoholic solution in tufts (houppes); this is a mixture of amino-butyric acids. $C_9H_{20}N_2O_4 = C_5H_{11}NO_2 + C_4H_9NO_2$.

amino- valerianic acid	amino- butyric acid
------------------------------	---------------------------

These two acids have a marked tendency to crystallise together in their molecular proportions, especially from an alcoholic solution. A large number of analyses were made of the crystalline deposits from

1. *Loc. cit.*, p. 352.

the alcoholic solutions of the dried residue of the mother-liquid (*B*) obtained in the manner above described. These analyses always indicated the presence of a compound of two allied substances. The separation of these substances, however, could be accomplished by using water as a solvent, in the following manner :—After dissolving the compound $C_9H_{20}N_2O_4$ [$= C_5H_{11}NO_2 + C_4H_9NO_2$] in water, the solution was decolourised with animal charcoal and then concentrated *in vacuo*, at a gentle heat (40° to 50° C.). Butalanine crystallised out during ebullition in the form of white leaflets resembling those of caprioic alanine, which, if present, was also deposited. The mother-liquid then, when highly concentrated, yielded crystals of amino-butyric acid.

Amino-valerianic acid closely resembles amino-caproic acid, or leucine, in appearance. It is almost equally volatile and more soluble in water, and under the microscope it appears as crystalline nodules, formed by short needles radiating from a centre and not in flat plates. It has a somewhat sweetish taste.

Amino-butyric acid is a comparatively large ingredient of the 'résidu fixe.' It crystallises, from a hot alcoholic solution, on cooling in small delicate pearly leaves resembling amino-caproic acid. It is almost insoluble in cold, but is more soluble in hot alcohol. It is readily soluble in water from which, when sufficiently concentrated, it crystallises in the form of nodules composed of needles grouped round a centre. It has a sweeter taste than amino-valerianic, or amido-caproic acid.

The extract obtained by boiling absolute alcohol furnished also the following products¹ :—

- (1) The leucines $C_5H_{11}NO_2$ (amino-valerianic acid), $C_4H_9NO_2$ (amino-butyric acid), $C_3H_7NO_2$ (amino-propionic acid).
- (2) The leucéins $C_5H_9NO_2$, $C_4H_7NO_2$, $C_3H_5NO_2$, and
- (3) Their intermediate products or combinations $C_{10}H_{20}N_2O_4$, $C_9H_{18}N_2O_4$, $C_8H_{16}N_2O_4$, $C_7H_{14}N_2O_4$.

1. *Loc. cit.*, pp. 359, 369.

To these products, together with the two higher members of the series $C_{12}H_{24}N_2O_4$ and $C_{11}H_{22}N_2O_4$, Schutzenberger gave the name of gluco-proteins.

After completely exhausting the 'résidu fixe' with boiling absolute alcohol there still remains, as stated on page 231, a certain quantity of matter, amounting to about 3 or 4 per cent. of the albumin, which is readily soluble in water, and has a somewhat sweet taste. The aqueous solution after concentration becomes after a time a mass of crystalline grains. Its analysis corresponds to the formula $C_7H_{14}N_2O_4$.

These, then, are some of the substances (among many others) which, starting with pyruvic acid and its three higher homologues, can be synthesised in the laboratory. Their compounds with NH_3 , viz. :—

- (i) $CH_3 \cdot C(NH) \cdot COOH$ (imino-pyruvic acid); (ii) $CH_3 \cdot C(NH) \cdot CH_2 \cdot COOH$ (imino-acetyl-acetic acid); or $CH_3 \cdot C(NH_2) : CH \cdot COOH$ (amino-crotonic acid); (iii) $CH_3 \cdot C(NH) \cdot CH(CH_3) \cdot COOH$ (imino-methyl-acetyl-acetic acid); or, $CH_3 \cdot C(NH_2) : C(CH_3) \cdot COOH$ (amino-methyl-crotonic acid); and (iv) $CH_3 \cdot C(NH) \cdot CH(C_2H_5) \cdot COOH$ (imino-ethyl-acetyl-acetic acid); or, $CH_3 \cdot C(NH_2) : C(C_2H_5) \cdot COOH$ (amino-ethyl-crotonic acid),

have the same ultimate composition as the four compounds which Schutzenberger obtained from albumin and to which he gave the name of leucéins—a knowledge of the constitution of which was, in his opinion, the only thing wanting to solve the problem as to the general structure of proteid matter. He consequently endeavoured in various ways, but unsuccessfully, to effect their synthesis.

Those which he was able to isolate presented the following characters¹:—they crystallise with difficulty, or not at all, and are deliquescent; they combine with baryta from which they are not completely disengaged by CO_2 ; they are not precipitated by mercuric nitrate. Though uncrystallisable themselves, they form crystalline compounds with the amido-derivatives of the fatty acids $C_nH_{2n+1}NO_2$.²

1. *Comptes Rendus*, t. LXXX, p. 238.

2. *Loc. cit.*, t. CI, p. 1267.

They differ, as he pointed out, from the leucines, amino-crotonic, amino-valerianic acid, etc., to which the general formula $C_nH_{2n+1}NO_2$ may be given, by containing two atoms less of hydrogen than the corresponding leucine, and may be represented by the general formula $C_pH_{2p-1}NO_2$. When combined we have—

These characters correspond with the salts of pyruvic acid and its homologues. The salts of pyruvic acid if prepared at the ordinary temperature can be obtained in a crystalline form; but if these aqueous solutions are heated to the boiling point, their character is changed and, on evaporation, gummy uncrystallisable salts remain behind. If an aqueous solution of the acid itself is evaporated by heat, a syrupy non-volatile acid is left behind, which when heated with HCl to 100° C. is resolved into CO_2 and pyrotartaric acid.¹

Acetyl-acetic acid is a viscid fluid mixable with water, with a strong acid reaction. Its barium salt is amorphous and very soluble in water.

Methyl-acetyl-acetic acid or methyl-crotonic acid is a thick fluid mixable with water, which when heated is resolved into CO_2 and

Ethyl-crotonic acid possesses similar properties.

Having obtained from the 'résidu fixe' the various crystalline bodies to which I have referred, Schutzenberger then proceeds to resolve this latter into certain constituents. He arrives at the con-

1. Clermont, *Ber.*, Bd. 6, s. 72.

2. Beilstein, *Handb. d. Chemie*, 3te Aufl., B. IV, s. 601.

clusion, based on reasons which are fully set forth in his paper,¹ that the 'résidu fixe,' $C_{221}H_{435}N_{49}O_{105}$ is made up of the compounds :—

$C_9H_{11}NO_3$	...	...	tyrosine
+ 3 $C_9H_{16}N_2O_6$	...	...	mean of the strong amido-acids
+ 3 $C_9H_{20}N_2O_4$	...	...	leucines
+ 2 $C_7H_{18}N_2O_4$	...	...	gluco-proteins
+ 16 $C_9H_{18}N_2O_4$	...	...	gluco-proteins
<hr/>			
$C_{221}H_{435}N_{49}O_{105}$	...	...	<i>résidu fixe</i>

or in other words, when albumin is digested with five or six times its weight of barium hydrate for forty-eight hours, at a temperature of 180° C., it combines with 60 molecules of water and undergoes the following decomposition.²

I shall now endeavour to resolve these terms into their respective constituents. The last term in the equation $16 C_9H_{18}N_2O_4$ is the one which presents the greatest difficulty in this respect for its formula may be satisfied by an indefinite number of combinations. The following considerations, however, furnish us with material assistance in arriving at a satisfactory solution.

In the first place we know that with regard to a large number of organic bodies, condensation of three molecules takes place forming compounds much more stable than those consisting of one molecule only: such compounds, for instance, as the cyanides, the cyanates, the cyanamides, the aldehydes, etc. It is consequently not an unwarrantable assumption that in such a body as albumin this ternary condensation or triple combination takes place. As a working hypothesis, at all events, it is useful, and we will assume that the constituents of $16 C_9H_{18}N_2O_4$ exist in it either as single molecules or as compounds of three molecules or as multiples of three.

Secondly, we will assume that in this compound the leucines

1. See *Annales de Chimie*, 5me série, t. XVI, pp. 394-399.

2. *Loc. cit.*, pp. 385 and 398.

$C_nH_{2n+1}NO_2$ are combined with the corresponding leucéins $C_nH_{2n-1}NO_2$ forming the compounds $C_{2n}H_{4n}N_2O_4$.

Thirdly, Schutzenberger¹ points out that the amount of the compound $C_{12}H_{24}N_2O_4$ obtained from one molecule of albumin, and consisting of $C_6H_{13}NO_2 + C_6H_{11}NO_2$, is about 15 to 16 per cent. ; 3 ($C_6H_{13}NO_2 + C_6H_{11}NO_2$) corresponds to 14 per cent. If we subtract this from 16 ($C_9H_{18}N_2O_4$), the remainder, he says, can only be resolved into 4 terms of C_5 and 22 of C_4 , that is, into 2 ($C_5H_{11}NO_2 + C_5H_9NO_2$) and 11 ($C_4H_9NO_2 + C_4H_7NO_2$).² In other words—

$$\begin{aligned} 16 (C_9H_{18}N_2O_4) &= 3 (C_6H_{13}NO_2 + C_6H_{11}NO_2) \\ &+ 2 (C_5H_{11}NO_2 + C_5H_9NO_2) \\ &+ 11 (C_4H_9NO_2 + C_4H_7NO_2) \end{aligned}$$

In making this calculation, however, Schutzenberger leaves out of consideration the presence of alanine $C_3H_7NO_2$ or $CH_3 \cdot CH(NH_2) \cdot COOH$ which, as he shews,³ is also contained in the 'résidu fixe'—'mais en petites quantités seulement.' One molecule of this combined with the corresponding leucéine $C_3H_5NO_2$ or $CH_3(NH)CH_2COOH$, forms the compound $C_3H_7NO_2 + C_3H_5NO_2 = C_6H_{12}N_2O_4$. Taking this into consideration, and that 2 ($C_4H_9NO_2 + C_4H_7NO_2$) may represent ($C_5H_{11}NO_2 + C_5H_9NO_2$) + ($C_3H_7NO_2 + C_3H_5NO_2$), the compound 16 ($C_9H_{18}N_2O_4$) or $C_{144}H_{288}N_{32}O_{64}$ may be represented by

$$\begin{array}{rcl} 3 (C_6H_{13}NO_2 + C_6H_{11}NO_2) & & = C_{36}H_{72}N_6O_{12} \\ + 3 (C_5H_{11}NO_2 + C_5H_9NO_2) & & = C_{30}H_{60}N_6O_{12} \\ + 9 (C_4H_9NO_2 + C_4H_7NO_2) & & = C_{72}H_{144}N_{18}O_{36} \\ + C_3H_7NO_2 + C_3H_5NO_2 & & = C_6H_{12}N_2O_4 \\ \hline & & C_{144}H_{288}N_{32}O_{64} \end{array}$$

which satisfies the assumption made above as to the ternary combinations of the molecules. It also satisfies the requirement that amido-butyric acid and its complement $C_4H_7NO_2$ are the dominant factors of the 'résidu fixe,' which is in conformity with Schutzenberger's analysis.⁴

1. *Loc. cit.*, p. 397.

2. *Loc. cit.*, p. 398.

3. *Loc. cit.*, p. 358. It is found in the portion of the 'résidu fixe' insoluble in alcohol.

4. *Loc. cit.*, p. 398.

respectively, with imino-ethyl-acetyl-acetic, imino-methyl-acetyl-acetic and imino-acetyl-acetic acids we have

Both glutamic and aspartic acids were obtained by Schutzenberger from the 'résidu fixe' and consequently must appear in the equation. Amino-malonic acid is not mentioned by him. The explanation I suggest is, that in the combination of amino-malonic acid and glutamic acid the compound would have the same molecular composition as aspartic acid, and as, according to Schutzenberger, the amido-acids of the same series show a great tendency to crystallise together (see p. 230), we can have $\text{C}_5\text{H}_9\text{NO}_4 + \text{C}_3\text{H}_5\text{NO}_4 = 2(\text{C}_4\text{H}_7\text{NO}_4)$

and in this way its presence might be overlooked.

Taking, then, the formula $\text{C}_{240}\text{H}_{397}\text{N}_{65}\text{O}_{75}\text{S}_3$ for the composition of albumin (differing from Schutzenberger's by H_{10}) we find that by the action of BaH_2O_2 at 180°C . it combines with 60 molecules of H_2O and is resolved first into—

and resolving (iii), (iv), (v) and (vi) into their components, the whole may be represented in the following tabular form:—

THE COMPLETE HYDROLYTIC DECOMPOSITION OF EGG ALBUMIN WITH BARIUM HYDRATE AT 180° C.

(i)	(ii)	(iii)	(iv)	(v)	(vi)
16 NH ₃ + 4 (COOH) ₂ oxalic acid + 3 CO ₂ + 4 CH ₃ · COOH acetic acid + S + 2 SH ₂ + H ₆	C ₈ H ₄ (OH) · CH ₂ · CH(NH ₂) COOH tyrosine	CH(NH ₂) · COOH CH ₂ · CH ₂ · COOH glutamic acid CH(NH ₂) · COOH CH ₂ · COOH aspartic acid CH(NH ₂) · COOH COOH amino-malonic acid	3 CH ₃ · (CH ₂) ₂ · CH(NH ₂) · COOH a amino-valerianic acid 3 CH ₃ · CH ₂ · CH(NH ₂) · COOH a amino-butyric acid	2 CH ₃ · CH(NH ₂) · COOH a amino-propionic acid	3 CH ₃ (CH ₂) ₃ · CH(NH ₂) · COOH a amino-caproic acid 3 CH ₃ · (CH ₂) ₂ · CH(NH ₂) · COOH a amino-valerianic acid 9 CH ₃ · CH ₂ · CH(NH ₂) · COOH a amino-butyric acid CH ₃ · CH(NH ₂) · COOH a amino-propionic acid
		CH ₃ · C(NH) · CH(C ₂ H ₅) · COOH imino-ethyl-acetyl-acetic acid CH ₃ · C(NH) · CH(CH ₃) · COOH imino-methyl-acetyl-acetic acid CH ₃ · C(NH) · CH ₂ · COOH imino-acetyl-acetic acid			3 CH ₃ · C(NH) · CH(C ₂ H ₅) · COOH imino-ethyl-acetyl-acetic acid 3 CH ₃ · C(NH) · CH(CH ₃) · COOH imino-methyl-acetyl-acetic acid 9 CH ₃ · C(NH) · CH ₂ · COOH imino-acetyl-acetic acid CH ₃ · C(NH) · COOH imino-pyruvic acid
C ₁₉ H ₁₉₂ N ₁₆ O ₃₀ S ₃ Schutzenberger's polynomial with H ₁₀ added	C ₉ H ₁₁ NO ₃ tyrosine	C ₂₇ H ₄₈ N ₆ O ₁₈ or 3 C ₉ H ₁₆ N ₂ O ₆ acids amidés forts	C ₂₇ H ₆₀ N ₆ O ₁₂ or 3 C ₉ H ₂₀ N ₂ O ₄ leucines	C ₁₄ H ₂₈ N ₄ O ₈ or 2 C ₇ H ₁₄ N ₂ O ₄ gluco-proteins	C ₁₄₄ H ₂₈₈ N ₉₆ O ₄₄ or 16 C ₉ H ₁₈ N ₃ O ₄ gluco-proteins
		C ₂₄₀ H ₅₁₇ N ₆₅ O ₁₃₅ S ₃ or C ₂₄₀ H ₃₉₇ N ₆₅ O ₁₃ S ₃ + 60 H ₂ O albumin	C ₂₂₁ H ₄₃₅ N ₄₉ O ₁₀₅ résidu fixe		