STUDIES ON AMINO-ACID ANHYDRIDES, I. MOLECULAR COMPOUNDS OF DIKETOPIPERAZINE WITH COPPER HALIDES.

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Received May 14, 1928. Published June 28, 1928.

Recently one of the authors and Prof. Y. Shibata have published two papers on the study of absorption spectra of several diketopiperazines.(2) The motives of these works were to investigate the physical and chemical natures of these substances, the importance of which became to attract general attention in protein chemistry. In the present work, several behaviours of diketopiperazine on the formation of molecular compounds with some heavy metallic salts were studied. Such molecular compounds of amino-acids, polypeptides and amino-acid anhydrides with inorganic salts were first amply prepared by P. Pfeiffer and T. v. Modelski in 1912–1913.(3) Pfeiffer and Fr. Wittka(4) developed the studies and gave precise description of the molecular compounds of glycine anhydride with $CaCl_2$, $CaBr_2$, LiCl and LiBr,

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(2) Y. Shibata and T. Asahina, this journal, **1** (1926), 71; **2** (1927), 324.

(3) *Z. physiol. Chem.*, **81** (1912), 329; **85** (1913), 1.

(4) *Ber.*, **48** (1915), 1041 & 1289.

which had formerly been prepared by Pfeiffer and Modelski. Pfeiffer extended, with his co-laborators, those studies and showed various points of importance in producing the molecular compounds containing inorganic salts in the chemistry of protein and amino-acids.(1) As to the behaviours of diketopiperazine to organic matters, Powarnin and Tichomirow(2) described that glycine anhydride gave molecular compounds with poly-phenols. From their experimental results they suggested an *addition theory*, in opposition to the *peptisation theory*, on the chemism of tanning. Pfeiffer, with Olga Angern and Liu Wang, prepared several addition compounds of glycine anhydride and sarkosin anhydride with phenol derivatives, aromatic amines and some amino-acids and its derivatives.(3)

They also prepared the molecular compounds with phenol and amine derivatives of various azo-compounds, most of which are in actual use in dyeing. As the results of their so-called "Modellversuche" they proposed, on the theory of dyeing, a view that dyestuff-adsorption on the surface of wool and silk fibres is nothing but the formation of molecular compounds caused by the saturation of residual affinities of the molecules of dyestuffs and albuminous substances.(4) On the other hand the important rôle of salts of alkali-earth metals in the polymerisation of proteins, was elucidated by Prof. K. Shibata.(5)

The molecular compounds of diketopiperazines, mainly described up to the present time, by Pfeiffer may be classified in the following manner:

Mol. comp. with alkali and alkali-earth halides.

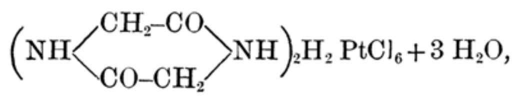
Mol. comp. with phenols, (prepared also by Powarnin and Tichomirow).

Mol. comp. with aromatic dyes (mainly azo-dyes).

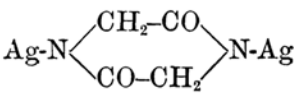
Mol. comp. with amino-acids.

Mol. comp. of enolic diketopiperazines with each other.(6)

One more class, also, can perhaps be added to i.e. molecular compounds with heavy metallic salts. Of such a type of compounds, the authors could find, in chemical literature, only those described by Th. Curtius and F. Göbel:(7)



$\left(NH\begin{matrix}CH_2-CO\\CO-CH_2\end{matrix}NH\right)_2H_2\,PtCl_6+3\,H_2O$,



$Ag\text{-}N\begin{matrix}CH_2-CO\\CO-CH_2\end{matrix}N\text{-}Ag$

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(1) P. Pfeiffer, "*Organische Molekürverbindungen*" (1927).

(2) *Chem. Zentr.*, (1924), III, 857.

(3) Pfeiffer, O. Angern and L. Wang, *Z. physiol. Chem.*, **143** (1925), 265.

(4) Pfeiffer and O. Angern, *Z. angew. Chem.*, **39** (1926), 253; Pfeiffer and L. Wang, *Z. angew. Chem.*, **40** (1927), 983.

(5) K. Shibata, *Acta phytochimica*, **2** (1924), 39 & 193.

(6) E. Abderhalden u. E. Schwab, *Z. physiol. Chem.*, **164** (1927), 278.

(7) *J. prakt. Chem.*, (2), **37** (1888), 178.

and [Glycine anhydride- copper],
but the latter two are salt-like compounds and not addition compounds.

Now, in this work, the present authors prepared the true molecular compounds of diketopiperazine with chloride and bromide of copper. Thus the readiness, with which glycine anhydride produces the molecular compounds with neutral heavy metallic salts, will be regarded due to the easy saturation of residual affinities, and the molecular compounds now obtained by the present authors, together with salt like compounds of Curtius and Göbel will probably be taken as "Modelle", as stated by Pfeiffer, for the compounds [protein- heavy metallic salts].

The molecular compounds of glycine anhydride with other heavy metallic salts have also been prepared by us; and the results will soon be reported in this journal.

Experimentals.

1. *Glycine anhydride-Copper chloride.*

Glycine anhydride (0.5 gr.) was dissolved in 20 c.c. hot water and to this, a warm solution of 5 gr. copper chloride (hydrate) in 5 c.c. water was added, the mixture was at once cooled under the tap; minute crystals of light blue colour soon separated out which, after standing overnight in a ice-chest, were drained, washed with a small quantity of ethyl alcohol (50%), and pressed between sheets of filter paper. Yield 0.9 gr. To the filtrate, equal volume of alcohol was added and after standing overnight in a ice-chest, 0.1 gr. of the same (but a little larger) crystals were obtained from this. Total yield 1 gr. (with regard to the glycine anhydride used, 80% of the calculation for $C_4H_6O_2N_2 \cdot CuCl_2 \cdot 2\,H_2O$).

The compound has an aspect of a light blue rhombic needle; it is partly decomposed in water and alcohol. In the hot solvents the decomposition proceeds quicker and white crystals were obtained from the solution, which were identified, by microscopical examination (form, extinction, elongation) and also by the reaction proposed by Dr. Sasaki(1), to be the original glycine anhydride.

The glycine anhydride-copper chloride is insoluble and not or scarcely decomposed in acetone, benzene, ether and chloroform. On heating, the water of crystallisation is expelled at 110°C. and the compound turns reddish brown; on further heating it decomposes without melting.

It can only be recrystallised by dissolving in a warm concentrated solution of copper chloride and then cooling.

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(1) *Biochem. Z.*, **114** (1921), 63.

Analysis

1. (a) Nitrogen

Sample	vol. of N_2 (c.c.)	temp. (°C.)	press. (mm.)	% of N_2
5.437 mg.	0.464	13.8	758.2	9.93
3.561 〃	0.291	13.0	761.4	9.81
5.132 〃	0.434	12.5	762.4	9.95
6.284 〃	0.533	13.0	757.5	9.89
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2 \cdot 2\,H_2O$,				9.84

(b) Nitrogen (dried at 120°C. in vacuum)

Sample	vol. of N_2 (c.c.)	temp. (°C.)	press. (mm.)	% of N_2
6.035 mg.	0.586	13.0	749.3	11.24
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2$,				11.20

2. Copper

Sample	CuO	% of Cu
2.765 mg.	0.769 mg.	22.22
0.3520 gr.	0.0977 gr.	22.10
0.2086 〃	0.0573 〃	21.95
0.4033 〃	0.1132 〃	22.44
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2 \cdot 2\,H_2O$,		22.34

3. (a) Chlorine

Sample	AgCl	% of Cl_2
4.117 mg.	4.104 mg.	24.67
6.440 〃	6.395 〃	24.57
6.445 〃	6.413 〃	24.62
0.1743 gr.	0.1722 gr.	24.50
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2 \cdot 2\,H_2O$,		24.92

3. (b) Chlorine (dried at 120°C. in vac.)

Sample	AgCl	% of Cl_2
3.810 mg.	4.270 mg.	27.73
6.748 〃	7.510 〃	27.97
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2$,		28.53

4. Water of crystallisation

Sample	weight loss (at 120°C. in vac.)	% of H_2O
15.523 mg.	1.915 mg.	12.57
10.415 〃	1.305 〃	12.53
20.156 〃	2.553 〃	12.67
Calc. for $C_4H_6N_2O_2 \cdot CuCl_2 \cdot 2\,H_2O$,		12.65

2. *Glycine anhydride-Copper bromide.*

Glycine anhydride (1 gr.) was well pulverised and brought into a brown solution of copper bromide. (4.5 gr. $CuBr_2$ in 20 c.c. water). The copper bromide solution had previously been prepared by dissolving freshly precipitated copper oxide from a known quantity of copper vitriol, in a little excess of hydrobromic acid (d=1.206) and concentrating in a vacuum desiccator over conc. sulphuric acid.

Soon after the addition of the glycine anhydride to this solution, bright green monoclinic crystals (tabular along b (010)) separated out. After 2 days the crystals were collected, drained and pressed between sheets of filter-paper. Yield 3 gr. (with regard to glycine anhydride used, 90% of the calculation for $C_4H_6O_2N_2 \cdot CuBr_2 \cdot 2\,H_2O$)

The compound can easily be decomposed by water, acetone and alcohol, giving white powders, which were identified to be glycine anhydride by the crystal form and Sasaki's reaction. It is insoluble in other organic solvents the recrystallisation can be carried out by dissolving in copper bromide solution and then evaporating in the vaccum over conc. sulphuric acid.

Analysis

1. Nitrogen

Sample	vol. of N_2 (c.c.)	temp. (°C.)	press. (mm.)	% of N_2
15.070 mg.	0.971	15.5	765.5	7.53
		Calc. for $C_4H_6N_2O_2 \cdot CuBr_2 \cdot 2H_2O$,		7.50

2. Copper

Sample	CuO	% of Cu
0.2005 gr.	0.0424 gr.	16.89
0.2024 〃	0.0433 〃	17.09
Calc. for $C_4H_6N_2O_2 \cdot CuBr_2 \cdot 2\,H_2O$,		17.02

3. Bromine

Sample	AgBr	% of Br_2
10.915 mg.	10.830 mg.	42.23
6.715 〃	6.705 〃	42.50
Calc. for $C_4H_6N_2O_2 \cdot CuBr_2 \cdot 2\,H_2O$,		42.79

4. Water of crystallisation

Sample	weight loss (dried at 100–105°C. in vacuum)	% of H_2O
0.5244 gr.	0.0511 gr.	9.74
Calc. for $C_4H_6N_2O_2 \cdot CuBr_2 \cdot 2\,H_2O$,		9.67

In order to determine whether these molecular compounds are formed in the solution to any extent, the spectroscopical method proposed by Prof. Y. Shibata with Dr. T. Inoue and Dr. Y. Nakatsuka,(1) was here applied. The maximum end-absorption was found, in both cases, when the molar proportion of copper salt to the glycine anhydride was (7-8) : 1.

The degree of the compound formation seems to be quite small as the diagrams indicate, because the change of the wave lengths of the absorption end corresponding to the change of the molar proportion of the mixture was found to be rather small.

Fig. 1. Abscissa: The ratio of $\dfrac{\text{vol. (c.c.) of } \frac{1}{20} \text{ mol. aqueous } CuCl_2 \text{ soln.}}{\text{vol. (c.c.) of } \frac{1}{20} \text{ mol. aqueous } C_4H_6N_2O_2 \text{ soln.}}$

Fig. 2. Abscissa: The ratio of $\dfrac{\text{vol. (c.c.) of } \frac{1}{10} \text{ mol. aqueous } CuBr_2 \text{ soln.}}{\text{vol. (c.c.) of } \frac{1}{10} \text{ mol. aqueous } C_4H_6N_2O_2 \text{ soln.}}$

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(1) *Japanese J. of Chem.*, **1** (1922), 1.

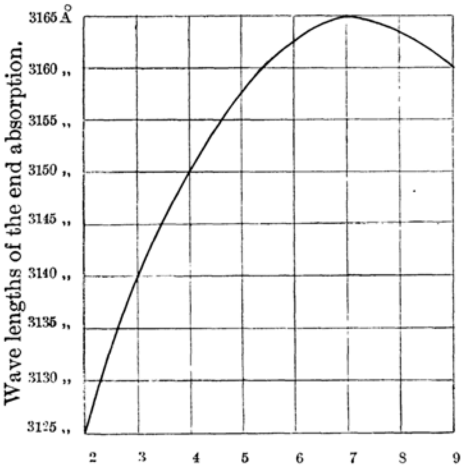

Fig. 1.

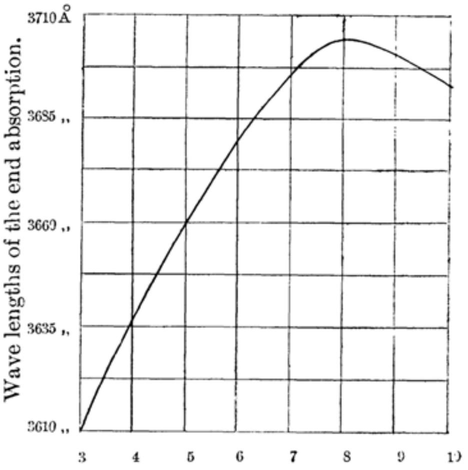

Fig. 2.

Thus the following equilibrium can be established,

$n\,CuCl_2(\text{or } Br_2) + \text{Glycine anhydride} \rightleftarrows (7\text{–}8)\,CuCl_2(Br_2)\cdot\text{Glycine anhydride}$
$\rightleftarrows CuCl_2(Br_2)\cdot\text{Glycine anhydride}\cdot 2\,H_2O$.

The authors are continuing the work in using other metallic salts and amino-acid anhydrides.

The best thanks of the authors are due to Prof. Y. Shibata and Prof. K. Shibata for the kindness of encouragement and guidance extended from time to time. The cost of the research was partly defrayed from the grant of the Imperial Academy for which they also express their hearty gratefullness.

Summary

1. Molecular compounds of diketopiperazine with copper chloride and bromide were prepared.

2. The formation of such compounds in aqueous solution with formulae other than those obtained in solid state was ascertained.

3. The meaning of these molecular compounds for the chemistry of protein was discussed.

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