SPECTROSCOPICAL STUDY OF AMINO-ACID ANHYDRIDES.

I. ON THE CONSTITUTIONS OF SOME SIMPLE AMINO-ACID ANHYDRIDES.

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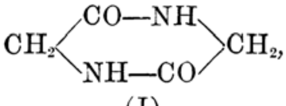
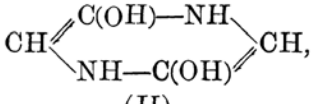
In the chemistry of protein substances, when it especially concerns to their constitutions, the well known polypeptide theory of Emil Fischer is now going to be replaced by the polymerisation theory of amino-acid anhydrides, as the results of the recent investigations of various biochemists (Trönsegaard, Abderhalden, Bergmann, Karrer, Keita Shibata etc.)

It is, therefore, obvious that the future studies of protein substances and their decomposition products will necessarily be attempted from the point of view of this new theory. In this reason we have undertaken the present investigation with the intention of determining the constitutions of various amino-acid anhydrides, which will be obtained at the end products of depolymerisation of proteins, in applying the spectroscopic method.

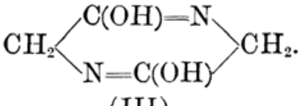
As for the constitution of diketopiperazine, which is regarded as the simplest amino-acid anhydride, it may be formulated as follows:



(I)



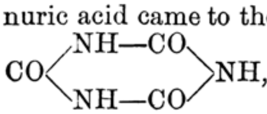
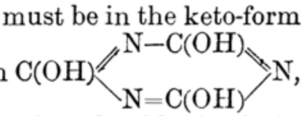
(II)



(III)

But it has not yet been confirmed which of these three formulae is most probably attributed to the substance, or whether it may take two or even three forms at the same time, or not.

In examining these formulae on the spectrochemical standpoint, we can at once find that each of them contains either of well known chromophore groups: $:C=C:$, $:C=N-$ or $:C=O$; among these, however, it is often noted that the carbonyl group, when two of them occur in the para-position in a saturated haxa-atomic ring, shows no selective absorption in any region of spectrum.

J. N. Hartley(1) observing no absorption band in the spectrogram of cyanuric acid came to the conclusion that this substance must be in the keto-form , instead of being in the enol-form , which is analogously constructed as benzene, and therefore should selectively absorb in ultraviolet.

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(1) Hartley *J. Chem. Soc.*, 39 (1881), 153; 41 (1882), 48; *Proc. Chem. Soc.* (1889), 46.

Martinet(1) also states in his book on the chromophoric nature of $C=N-$ group, in describing as follows: "Lorsque le chromophore $C=N$ se trouve dans un cycle, il est, comme dans une chaîne ouverte, plus favorable à la coloration qu'une double liason entre atomes de carbone." It is clear that by "coloration" he means any selective absorption.

Now, the spectrogram of some simple diketopiperazines, such as glycine anhydride, alanine anhydride and sarcosine anhydride, which were studied by us, showing no absorption band in any part of spectrum region, it may be concluded from the reasons just mentioned, that these diketopiperazines exist only in keto-form (I) in aqueous solution.

The diketopiperazines used in the present study were mainly prepared by heating the corresponding amino-acids in glycerol according to the method given by Balbiano(2) and also by Maillard.(3)

Experimental.

Glycin Anhydride. Glycine was prepared according to a method given by Kraut(4); the substance, which was purified by five times recrystallisations from water, is remarkably discoloured, when heated, at 226° and begins to melt at 232°, evolving brownish vapour. Glycine (2–6 gr.) was then well mixed with 1–6 times of glycerol, which was thoroughly dehydrated, and the mixture was kept in temperatures of 160–180° for 4–10 hours in an oil bath. On cooling the darkbrownish viscous liquid thus formed was treated with ethyl alcohol and the produced precipitates were filtered. This dark coloured mass was washed with water and from the wash liquid diketopiperazine was obtained and purified by recrystallisations from water.

When a sufficient quantity of glycerol was not used, the yield of diketopiperazine thus produced should remarkably be diminished, giving rather larger quantity of polymerised anhydride. In order to obtain a good yield of diketopiperazine the quantity of glycerol employed must be at least five times of that of glycine.

The anhydride purified by repeated recrystallisations began to be discoloured at 262° and was completely decomposed at 295°. The nitrogen content was determined by Pregl's micro-Dumas method: 4.464 mg. of sample gave 0.956 c.c. of nitrogen at 20° under 765.9 mm. (Found: $N=24.7$. $C_4H_6O_2N_2$ requires $N=24.6\%$).

A 0.001 mol solution was spectroscopically examined using Adam-

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(1) Martinet, "Couleur et Constitution chimique," G. Doin, Paris, (1924), p. 74.

(2) Balbiano and Frasciati, *Ber.*, **33** (1900), 2323; **34** (1901), 1501.

(3) Maillard, *Compt rend.*, **153** (1911), 1078.

(4) Kraut, *Ann.*, **266** (1891), 292.

Hilger's quartz spectrograph. In this spectrogram, however, we could find no selective absorption all through the spectrum region, that is to say, the solutions of this substance with varying thicknesses from 100 to 1 mm. were quite transparent to extreme ultraviolet. A feebly alkaline solution of diketopiperazine of 0.001 mol concentration was also photographed in order to examin whether the substance change its constitution in alkaline medium or not. But it was also proved from its spectrogram that this was by no means the case.

The Anhydride of Alanine (C, C'-dimethyldiketopiperazine). 3.2 gr. of alanine was mixed with 6 c.c. of glycerol and the mixture was heated in an oil-bath at 170–180° for about 5 hours. Soon all crystals of alanine were dissolved, ammonia and carbon dioxide were evolved and the liquid turned slightly reddish brown. From this viscous liquid, crystals were separated out on cooling. After repeated recrystallisation from water, colourless fine crystals with silvery lustre were obtained. In this case polymerised anhydride was not produced. The crystals began to be discoloured at about 260° and melted, under slight decomposition, at 279°. Analysis of nitrogen : 2.575 mg. of sample gave 0.440 c.c. nitrogen at 19° under 766.2 mm. (Found : N= 19.8. $C_6H_{10}N_2O_2$ requires N=19.7%). A 0.001 mol solution of this anhydride (0.0142 gr. in 100 c.c. water) was examined spectroscopically, but no absorption bands were observed in its spectrogram.

Sarcosine Anhydride (N, N'-dimethyldiketopiperazine). 2 gr. of sarcosine was well mixed with 20 gr. glycerol and heated at 170–180°. Soon sarcosine was completely dissolved, methylamine vapour and carbon dioxide were evolved, and the liquid became brown in colour. After 9 hours heating the liquid was cooled, shaken with acetic ester two or three times, and, by evaporating off the ester, some crystals were obtained (0.5 gr.) After recrystallisation from absolute alcohol, it melted at 145.5–146.5°. Dry distillation of 4 gr. of sarcosine also gave 1 gr. of crystals in the distillate.⁽¹⁾ Its melting point was 144.5–145.5°. The melting point did not lower on mixing these two specimens, thus identity of these two products was confirmed. The analysis of nitrogen : 4.622 mg. of substance gave 0.794 c.c. of nitrogen at 18.5° under 757.2 mm. (Found : N=19.7. $C_6H_{10}N_2O_2$ requires N=19.7%). 0.1421 gr. of the anhydride was dissolved in 100 c.c. of water (0.01 mol solution) and was examined spectroscopically. In this case too, no selective absorption could be observed in its spectrogram.

Summary.

Some simple amino-acid anhydrides were prepared after Maillard's method and were examined spectroscopically. So far as the present investi-

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(1) F. Mylius, *Ber.*, 17 (1884), 287.

gation is concerned, it was proved that the solutions of these amino-acid anhydrides show no selective absorption. From this fact we came to the conclusion that these substances are considered to be only in keto-form in aqueous solution.

The experiment is going on with other amino-acids. We express herewith our best thanks to Prof. Keita Shibata's kind advices. The cost of this investigation was defrayed from the grant of the Imperial Academy, for which we wish to record our gratefulness.

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