Synthesis of Cyclic Peptide. I.[1] *Preparation of Cyclo-di-β-alanyl from 1,4-Cyclohexanedione*

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Since the discovery of such naturally occurring cyclic polypeptides as gramicidines and tyrocidine having strong biological activities, synthetic methods of cyclic polypeptides have been studied. A method used for this purpose is that of cyclizing the corresponding linear peptide in dilute conditions. By this method some of the cyclic peptides having several amino acid residues were prepared successfully, though in some peptides, e.g. tri-α-peptide, cyclization was found difficult.

An alternative method to approach these peptides may be the expansion of a compound already having a cyclic structure. It is well known that cyclic ketone oxime rearranges to lactam by the Beckmann reaction in a good yield. Applying this principle to cyclic diketone compound, Tokura et al.[2] rearranged cyclododecane-1,6-dione dioxime with sulfur trioxide in liquid sulfur dioxide and obtained 1,7-diaza-6,12-dioxocyclododecane. In the same way, from 1,4-cyclohexanedione dioxime, a cyclic dipeptide of β-alanine should be produced. By heating di-*p*-tosylate of 1,4-cyclohexanedione dioxime and hydrolyzing the product, Knunyants and Fabrichnyi[3] obtained β-alanine, succinic acid and ethylenediamine. This result suggests the production of cyclo-di-β-alanyl as well as ethylenesuccinamide as the rearrangement products, and in fact Hall[4] has isolated the former from the rearrangement mixture. On the other hand, Mamlock[5] tried the same reaction using 1,4-cyclohexanedionedioxime monohydrochloride and polyphosphoric acid, but he only isolated 2-chloro-*p*-phenylenediamine as reaction product. Recently, Rothe[6] reported that 1,4-cyclohexanedione dioxime, treated with 8% oleum in a Beckmann rearrangement, gives only cyclo-di-β-alanyl, instead of the mixture of two cyclic amides.

We intended to re-examine these reactions in order to establish a route to obtain cyclo-di-β-alanyl. In the first place, a direct rearrangement was studied. According to Mamlock, 1,4-cyclohexanedione dioxime was treated with polyphosphoric acid at about 120°C for a short time, though the starting material was dioxime itself in order to avoid the complexity caused by chloride ion. The hydrolyzate of the reaction product was studied by paper-chromatography, but the spot corresponding to β-alanine or ethylenediamine could not be found, though the presence of a spot of lower R_f-value was recognized, which was proved to be due to *p*-phenylenediamine by paperchromatography. When such an acid as perchloric acid, sulfuric acid or trifluoroacetic acid, was used under similar conditions (50~100°C), similar results were also obtained. Treatment of the dioxime with polyphosphoric acid in the cold resulted in the mere recovery of the starting material.

Then the indirect method according to Knunyants and Fabrichnyi was examined secondly. Di-*p*-tosylate of the dioxime was heated in methanol at 100°C for 90 min. in an autoclave. In the hydrolyzate of reaction product, the presence of β-alanine and ethylenediamine was confirmed by paperchromatography, as they had observed, though no crystals corresponding to cyclo-di-β-alanyl were isolated after treatment of the syrup with Amberlite IRA-400 and CG-120.

Another reaction which will give the same results with the Beckmann rearrangement is the Schmidt reaction, as has been suggested by Rothe[7]. The latter will be more useful for our purpose, because milder conditions can be chosen. Thus, dry hydrogen chloride was passed into chloroform solution of 1,4-cyclohexanedione and hydrazoic acid at 0~5°C for about 2 hr. From the reaction mixture after removal of hydrogen chloride, a viscous syrup was obtained, containing many fine crystals, from which fine hexagonal plates or granules were isolated, which, after recrystallization

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1) Previous communication, *Pharm. Bull. Japan*, **9**, 834 (1961).

2) N. Tokura, R. Tada and K. Suzuki, This Bulletin, **32**, 654 (1959).

3) I. L. Knunyants and B. P. Fabrichnyi, *Doklady Akad. Nauk S. S. S. R.*, **68**, 701 (1949) : *Chem. Abstr.*, **44**, 1918 (1950).

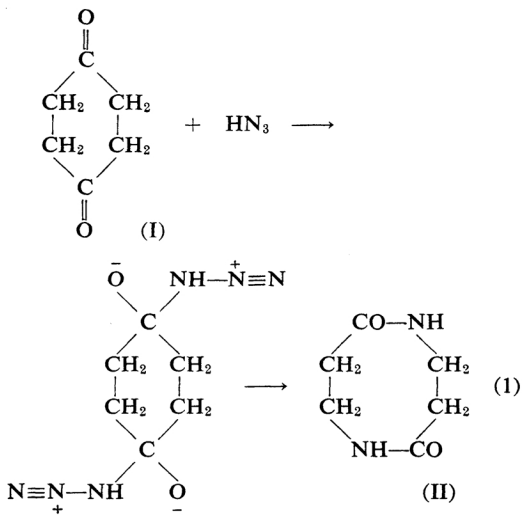
4) H. K. Hall, *J. Am. Chem. Soc.*, **80**, 6404 (1958).

5) L. Mamlock, *Bull. soc. chim. France*, **1956**, 1182.

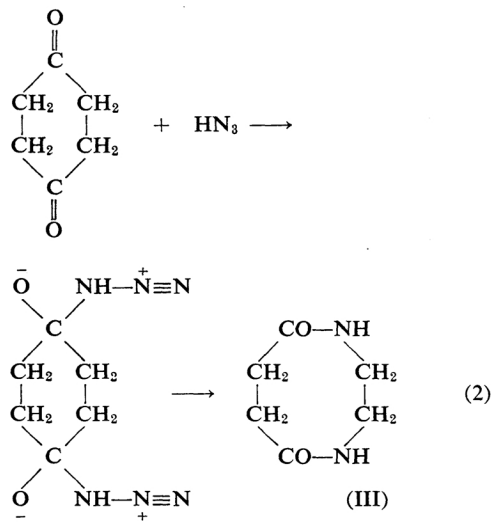
6) M. Rothe, *Acta chim. Akad. Sci. Hung.*, **18**, 449 (1949) ; *Chem. Abstr.*, **53**, 21693b (1959).

7) M. Rothe and J. Rothe, *Angew. Chem.*, **73**, 769 (1961) ; after the publication of our previous paper, M. Rothe repoted the experimental result of this reaction : *Chem. Ber.*, **95**, 783 (1962).

from ethanol, showed m. p. of 295~300°C. The elementary analyses and the molecular weight of this substance showed agreement with theoretical values for cyclo-di-β-alanyl. In Fig. 1 its infrared spectrum is shown. It was also confirmed that the paper-chromatogram of the hydrolyzate of this compound shows only one spot, which coincides completely with that of β-alanine itself. From these evidences, it is clear that the compound obtained here is the expected cyclo-di-β-alanyl, formed by the scheme:



But, the paper-chromatogram of the hydrolyzate of the crude syrupy reaction product shows in addition a different spot which was proved to be due to ethylenediamine, from that of the expected β-alanine. This shows that the reaction by the scheme 2 also occurs simultaneously, though the intermediate cyclic product III could not be isolated.



Experimental

Diethyl Succinate (IV).—Succinic acid was esterified with ethanol and sulfuric acid: b. p. 84~85°C/6 mmHg; b. p. 210~214°C.

Diethyl Succinosuccinate (V).—According to the method of Upenski[8], IV was condensed to V by sodium sand: m. p. 126~128°C (lit.[8] 126~127°C).

1, 4-Cyclohexanedione (I).—V was converted to I by the method of Vincent et al.[9]: b. p. 130~131°C/20 mmHg (lit.[9] b. p. 132°C/20 mmHg).

1, 4-Cyclohexanedione Dioxime (VI).—This was derived from I by usual method: m. p. 190~192°C (lit.[10] 192°C).

Attempted Beckmann Rearrangement of VI with Trifluoroacetic Acid.—A mixture of dioxime VI (1 g.) and trifluoroacetic acid (3 g.) was heated in a boiling water-bath for 30 min. After the reaction product was concentrated to dryness, the residue was boiled with 6 N hydrochloric acid for 8 hr. and paper-chromatogrammed. (Method: circular paper-chromatography; developer: *n*-BuOH: AcOH: H_2O=5 : 1 : 4; coloring agent: ninhydrin). Several spots were obtained but no one corresponding to β-alanine could be found. In this chromatogram, a spot having R_f value of ca. 0.29 was identified as that of *p*-phenylenediamine by comparison, after isolation, with an authentic specimen

The same reaction was tried by using polyphosphoric acid, perchloric acid or sulfuric acid, with similar results.

Indirect Beckmann Rearrangement Using Di-*p*-tosylate of VI as an Intermediate.—Dioxime VI in pyridine was added to *p*-tosylchloride in pyridine at −8°C and the mixture was allowed to stand at 0°C overnight[3]. The di-*p*-tosylate was obtained as precipitate by diluting the solution with water. A solution of ditosylate in methanol was heated in an autoclave at 100°C for 90 min. After removal of the solvent, the residue was dissolved in water and passed through Amberlite IRA-400 and CG-120. Viscous syrup was obtained by concentrating the eluate. Crystallization from ethanol did not succeed. From the paper-chromatogram of the hydrolyzate of this syrup, spots corresponding to β-alanine and ethylenediamine were confirmed by the comparison with authentic specimens.

Schmidt Reaction of I.—To a chloroform solution of I (2 g.) and hydrazoic acid (0.6 N chloroform solution, 60 ml.), dry hydrogen chloride was bubbled at 0~5°C for about 2 hr., when a syrupy material containg fine needles was formed. After removal of chloroform, the residual syrup was dissolved in water and treated with silver acetate to remove chloride ion and then hydrogen sulfide to remove excess silver ion. Resulting aqueous solution was concentrated to dryness in vacuo. Syrupy material containing fine crystals was obtained. Paper-chromatogram of the hydrolyzate of this syrup showed the presence of spots corresponding to β-alanine and ethylenediamine. The

8) A. E. Upenski and I. Turin, *Chem. Zentr.*, III, 754 (1923); C. R. Hauser and B. E. Hudson, Jr., "Organic Reactions", Vol. I, 283 (1946).
9) J. R. Vincent, A. F. Thompson, Jr. and L. I. Smith, *J. Org. Chem.*, 3, 606 (1937).
10) A. Baeyer and W. A. Noyes, *Ber.*, 22, 2170 (1889).

syrup was dissolved in hot ethanol and cooled gradually, then hexagonal plates or granules melting at 295~300°C (lit. 298°C[4], 297~299°C[7]) were obtained. Yield was 27%.

Found: C, 50.91; H, 7.39; N, 19.69. Calcd. for $C_6H_{10}N_2O_2$: C, 50.69; H, 7.09; N, 19.71%.

Molecular weight (measured by Schwyzer's method[11]; solvent: CF_3COOH; control: diketopiperazine). Found: 127. Calcd. for $C_6H_{10}N_2O_2$: 142.

Paper-chromatogram of the hydrolyzate of this crystal with hydrogen chloride afforded only one spot corresponding to that of β-alanine.

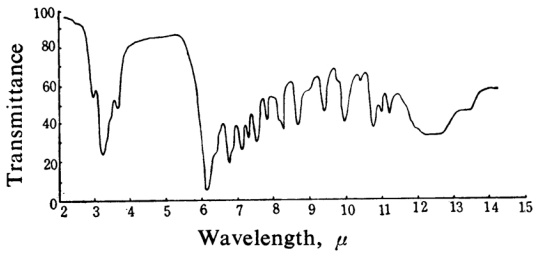


Fig. 1. Infrared absorption spectrum of cyclo-di-β-alanyl.

11) R. Schwyzer, B. Iselin et al., *Helv. Chim. Acta*, **39**, 872 (1956).

Infrared Absorption Spectrum. — The spectrum was recorded with a Perkin-Elmer Infracord, model 137. Phase: KBr disk. The result was shown in Fig. 1.

Summary

1) By the direct Beckmann rearrangement of 1,4-cyclohexanedione dioxime by polyphosphoric acid, trifluoroacetic acid, perchloric acid or sulfuric acid at about 100°C, no expected cyclo-di-β-alanyl but *p*-phenylenediamine was obtained.

2) By indirect Beckmann rearrangement using di-*p*-tosylate of 1,4-cyclohexanedione dioxime as an intermediate, the rearrangement occurs certainly though the isolation of the cyclic dipeptide was not successful.

3) By the Schmidt reaction of 1,4-cyclohexanedione, the expected rearrangement proceeded smoothly and from the reaction products cyclo-di-β-alanyl was easily isolated.

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