Studies on Dehydroalanine Derivatives. I. Synthesis of N-Carboxy-dehydroalanine Anhydride

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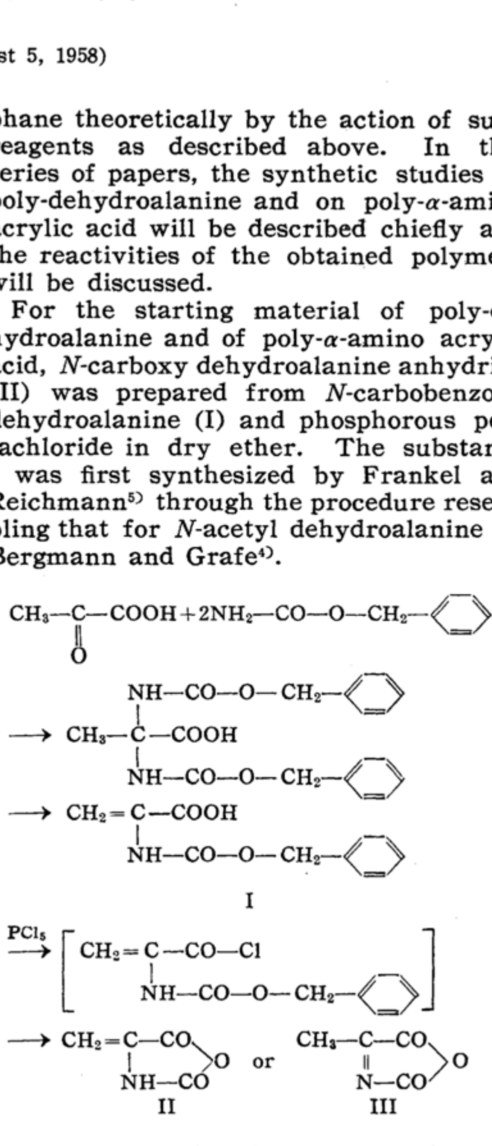
It is supposed that thermal or alkaline decomposition of serine, cysteine and peptides containing those amino acids proceed via dehydroalanine[1], and that dehydroalanine must play an important role as a transient intermediate in the course of enzymatic transformations of those substances[2]. If the synthesis of dehydroalanine were possible, this substance would be hydrolysed immediately into pyruvic acid and ammonia because of the presence of very unstable enamine structure in the molecule[3]. On the other hand, *N*-acyl derivatives of dehydroalanine are comparatively stable toward water, and actually some derivatives have been synthesized previously[4-6]. *N*-acetyl[4], *N*-carboethoxy, *N*-carbobenzoxy and *N*-formyl dehydroalanine[5] have been reported to be polymerized into *N*-acyl derivatives of poly-α-amino acrylic acid through a vinyl type polymerization. However, all efforts to prepare the poly-α-amino acrylic acid from these polymer derivatives remain unsuccessful because of the difficulty of the acid or alkaline hydrolysis of these polymers.

Each of the double bonds of suitable dehydroalanine derivatives is supposed to be reactive toward some reagents such as active hydrogen, hydrogen cyanide, hydrogen sulfide, indole and so on, and poly-dehydroalanine[7], if its synthesis is possible, may be converted into poly-alanine, poly-asparagine, poly-cysteine and poly-tryptophane theoretically by the action of such reagents as described above. In this series of papers, the synthetic studies on poly-dehydroalanine and on poly-α-amino acrylic acid will be described chiefly and the reactivities of the obtained polymers will be discussed.

For the starting material of poly-dehydroalanine and of poly-α-amino acrylic acid, *N*-carboxy dehydroalanine anhydride (II) was prepared from *N*-carbobenzoxy dehydroalanine (I) and phosphorous pentachloride in dry ether. The substance I was first synthesized by Frankel and Reichmann[5] through the procedure resembling that for *N*-acetyl dehydroalanine by Bergmann and Grafe[4].



The reagent, phosphorous pentachloride, and its reacted residuals scarcely affected the double bond of the substance II (or III) in the course of the reaction, but the reaction product crystallized very easily. After recrystallization from ethyl acetate and petroleum ether, colorless prisms with melting point of 111∼113° (decomp.) were obtained. This substance sublimed at 80° or higher temperature (0.1 mmHg) accompanied by partial polymerization.

If the structure III is more stable than

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[1] M. Bergmann and A. Miekeley, *Ann.*, **458**, 40 (1927); M. Bergmann and D. Delis, ibid., **458**, 76 (1927); B. H. Nicolet, *J. Biol. Chem.*, **95**, 389 (1932); B. H. Nicolet and L. A. Shinn, ibid., **140**, 685 (1941).

[2] C. V. Smythe, ibid., **142**, 387 (1942); E. Chargaff and D. B. Sprinson, ibid., **148**, 249 (1943); C. Fromageot, *Bull. soc. chim.*, **13**, 202 (1946).

[3] H. Scheibler and U. Scheibler, *Ber.*, **87**, 379 (1954).

[4] R. Adams, J. L. Johnson and B. Englund, *J. Am. Chem. Soc.*, **72**, 5080 (1950).

[5] M. Frankel and M. E. Reichmann. *J. Chem. Soc.*, **1952**, 289.

[6] T. Wieland, G. Ohnacker and W. Ziegler, *Ber.*, **90**, 194 (1957).

[7] This name represents the polypeptide of dehydroalanine in distinction from the vinyl-type polymer of dehydroalanine which is named as poly-α-amino acrylic acid in this series of papers.

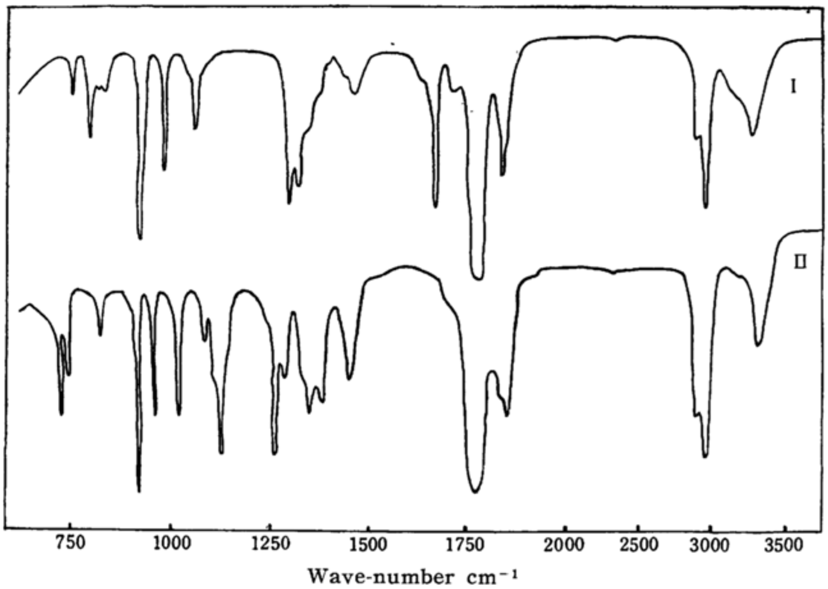
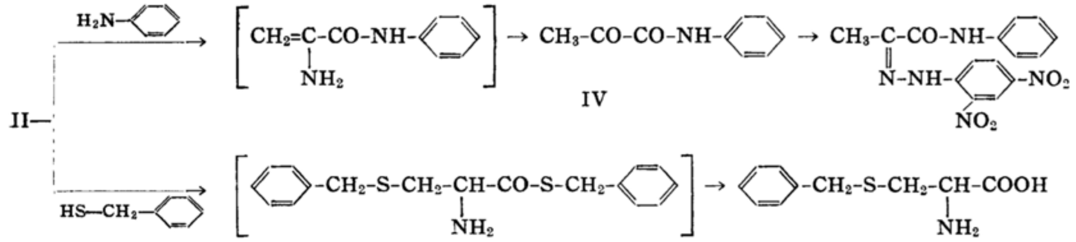


Fig. 1. Infrared spectra recorded on an Adam-Hilger H-80 infrared spectrophotometer: I, *N*-carboxy dehydroalanine anhydride; II, *N*-carboxy DL-alanine anhydride. Nujol suspension.

II, the position of the double bond in the molecule may shift from C=C to C=N in the course of the reaction. The infrared spectrum (Fig. 1) of the product, however, showed absorption bands which were all identical with those supposed for the structure II; that is, the absorption bands at 3050~3300 cm^{-1} indicated the presence of —NH— groups in the molecule, and the absorption bands at 1750~1900 cm^{-1} corresponding to carbonyl groups in a five-membered ring were identified completely as those of *N*-carboxy DL-alanine anhydride. The sharp band at 1675 cm^{-1}, which was not observed in the same region of the spectrum of *N*-carboxy DL-alanine anhydride, may be due to the presence of a double bond directly attached to the five-membered ring in the structure II. The absence of δN-H(amide II) band in both the spectra must be explained by the fact that both NH groups exist in five-membered rings of the respective *N*-carboxy anhydride.

In addition, the structure II was confirmed by the reactions with aniline and with benzylmercaptane. In the case of the former, pyruvic acid anilide IV was obtained in a yield of about 60%, and the structure IV was confirmed by the formation of the 2,4-dinitrophenyl hydrazone. In the case of the latter, *S*-benzyl cysteine formed was identified with an authentic sample by paper-chromatography. These results all supported the structure II for the product.

One of the characteristics of the substance II was its relative stability toward water and amines in comparison with ordinary *N*-carboxy anhydrides. For example, the substance II, which had been prepared about half a year ago and stored in a usual desiccator over phosphorous pentoxide without any precautions, indicated almost the same melting point as that of the original one in spite of the fact that almost all of drying agents have already become tarry. Moreover, the



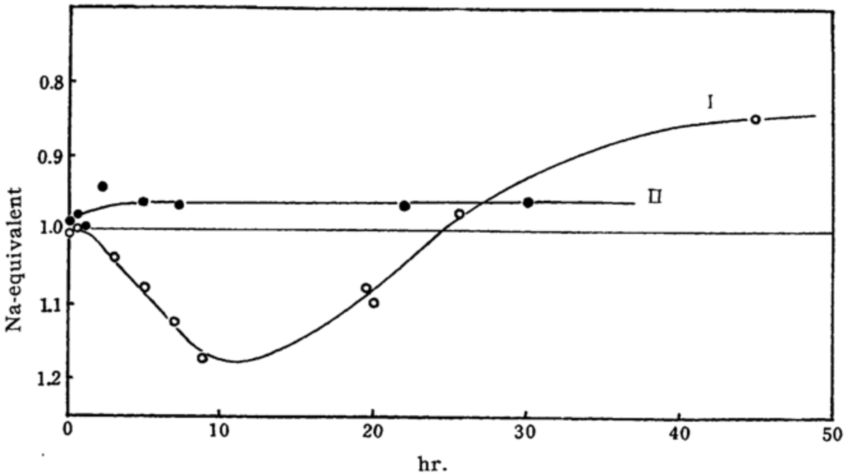
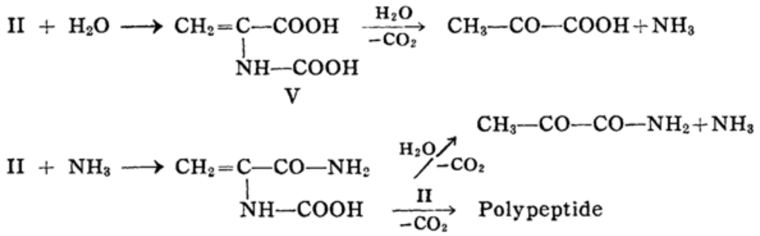


Fig. 2. Time—Na-equivalent curves of *N*-carboxy anhydrides at 30°±0.1° in dioxane containing an equal mole of water: I, *N*-carboxy dehydroalanine anhydride: II, *N*-carboxy DL-alanine anhydride.



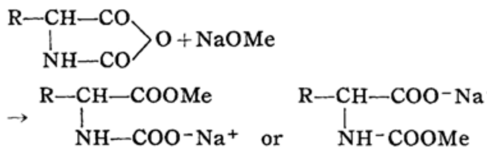
$$\mathrm{II} + H_2O \longrightarrow \underset{\underset{\mathrm{V}}{NH{-}COOH}}{CH_2{=}C{-}COOH} \xrightarrow[-CO_2]{H_2O} CH_3{-}CO{-}COOH + NH_3$$

$$\mathrm{II} + NH_3 \longrightarrow \underset{NH{-}COOH}{CH_2{=}C{-}CO{-}NH_2} \xrightarrow[-CO_2]{H_2O} CH_3{-}CO{-}CO{-}NH_2 + NH_3$$

$$\xrightarrow[-CO_2]{\mathrm{II}} \text{Polypeptide}$$

substance II immersed in water seemed to be rather stable below 30° and decomposed gradually under evolution of carbon dioxide at 35° or higher temperature.

For clarifying the hydrolytic reaction of the *N*-carboxy anhydride II, it was dissolved in dioxane containing an equal mole of water (against II) and the solution was allowed to react in a bath at 30° ±0.1°. Then, the solution was titrated with sodium methoxide at times according to the procedure of Berger et al.[8] and equivalent values obtained



$$\underset{NH{-}CO}{R{-}CH{-}CO}\!>\!O + NaOMe \longrightarrow \underset{NH{-}COO^-Na^+}{R{-}CH{-}COOMe} \text{ or } \underset{NH^-COOMe}{R{-}CH{-}COO^-Na^+}$$

(the residual amount of the *N*-carboxy anhydride plus acidic material formed, such as pyruvic acid) were plotted against time (curve I on Fig. 2). This curve I was compared with that of *N*-carboxy-DL-alanine anhydride (curve II) under the same condition. In this comparison, it was very interesting to find that the curve I fell down at first, then, recovered gradually and began to rise only after 30 hours, notwithstanding the fact that no practical change of titration values was observed within 30 hours in the case of the *N*-carboxy-DL-alanine anhydride. The reason for the increase in the titration values for the substance II beyond the original line should be explained by the formation of carbamic acid V by hydrolysis. Furthermore, this carbamic acid V would possibly be more stable than those from other ordinary *N*-carboxy amino acid anhydrides at room temperature, since the carbamic acid contains the conjugated double bond in the molecule. If the assumption is reasonable, the considerable stability of the *N*-carboxy anhydride II above mentioned should be explained by the retardation of the chain reactions for polypeptide-forming polymerization. On the other hand, *N*-carboxy-DL-alanine anhydride may be hydrolyzed gradually into carbamic acid of DL-alanine, which is decarboxylated immediately into DL-alanine; then, it will be possible to consider that the titration values

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[8] A. Berger, M. Sela and E. Katchalski, ***Anal. Chem.***, **25**, 1554 (1953).

of the solution should not change practically, since alanine formed is titrated by sodium methoxide in the same manner as *N*-carboxy anhydride under this condition. The upward tendency of the latter part of the titration curve I may also be explained by the gradual formation of the pyruvic acid amide or by the gradual polymerization which was initiated by the formed ammonia.

This material II will be expected to undergo the poly-peptide-forming polymerization and the vinyl type polymerization independently. The details of both cases will be published in later communications.

Experimental

***N*-Carbobenzoxy dehydroalanine (I).**—It was prepared from α,α-dicarbobenzoxyamido propionic acid[9] (m.p. 138.5°) according to the procedure of Frankel and Reichmann[5]. The yield of the low product with m.p. 107° was about 76∼83%. After successive recrystallizations from toluene containing a small amount of hydroquinone, colorless plates with m.p. 112∼113° (decomp. at 160°) were obtained. Frankel[5] reported m. p. 105° (decomp.) and Wieland[6] m. p. 119° (decomp.).

Anal. Found: C, 59.65; H, 4.50; N, 6.33; No. of double bond, 0.982[10]. Calcd. for $C_{11}H_{11}O_4N$: C, 59.72; H, 5.01; N, 6.33; No. of double bond, 1.00.

***N*-Carboxy dehydroalanine anhydride (II).**—A solution of 10 g. of I in 50 ml. of dry ether was cooled to 0° in an ice water bath and 10.5 g. of powdered phosphorous pentachloride was added in one portion. Then, the suspension was shaken vigorously with intermittent cooling in the bath for about 50 min. The supernatant was decanted quickly into a distillation flask and was concentrated to syrup under reduced pressure at 25∼30°. During the course of the treatment, the residual syrup crystallized gradually. After about 30 min., 50 ml. of petroleum ether was added to the residual crystalline mass and the mixture was cooled in an ice water bath for about one hr. Then, the crystalline mass was filtered off and washed with some petroleum ether. The crude crystals were recrystallized from ethyl acetate-petroleum ether after treatment with some active charcoal. The yield of the light yellow prisms with m.p. 105∼108° (decomp.) was 3.5 g. (70%). After recrystallization from the same solvents, the colorless prisms of 3.1 g. with m.p. 111° (decomp.) were obtained. After sublimation at 80∼90° (0.1 mmHg), this substance melted and decomposed at 111∼113°.

Anal. Found: C, 42.56; H, 2.60; N, 12.13. Calcd. for $C_4H_3O_3N$: C, 42.49; H, 2.67; N, 12.39%.

Reaction of II with aniline.—*Formation of pyruvic acid anilide (IV).*—A solution of 1.13 g. of II in 20 ml. of ethyl acetate was cooled to −10° and 5 g. of aniline was added into the solution, then the mixture was allowed to react overnight in a refrigerator at 0∼4°. A small amount of yellow precipitate formed was filtered off and the mother liquor was washed successively with 0.5 N hydrochloric acid, water, 0.5% sodium bicarbonate solution and water, dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure. The residual syrup was transferred into a sublimation vessel with some ethyl acetate. The main product IV was then separated by sublimation at 100° (0.1 mmHg) from yellow by-products. The colorless needles with m.p. 103∼105° were obtained in the yield of 1.0 g. (60%); Nef[11] reported m.p. 104°.

Anal. Found: N, 8.65. Calcd. for $C_9H_9O_2N$: N, 8.60%.

2, 4-Dinitrophenyl hydrazone of IV.—To a solution of 50 mg. of IV in 5 ml. of ethanol was added a solution of 100 mg. of 2, 4-dinitrophenyl hydrazine in 5 ml. of 2 N hydrochloric acid, and the mixture was heated for a short time in a boiling water bath and cooled. The voluminous yellow crystals which formed were filtered off, washed with dil. hydrochloric acid and water, and dried. The yield of the yellow crystals was about quantitative and they melted at 245∼247° after recrystallizasion from glacial acetic acid.

Anal. Found: C, 52.48; H, 3.82; N, 21.2. Calcd. for $C_{15}H_{13}O_5N_5$: C, 52.49; H, 3.82; N, 20.4%.

Reaction of II with benzyl mercaptane.—A solution of 100 mg. of II and 500 mg. of benzyl mercaptane in 5 ml. of dry acetone was allowed to react overnight at room temperature. The mixture was concentrated to dryness and the residuals were hydrolyzed for one hr. with 6 N hydrochloric acid. The hydrolysate was extracted with ether and the aqueous layer was concentrated to dryness. The residuals were extracted with absolute ethanol and the extract was concentrated. From the residuals, *S*-benzyl cysteine was detected by paper-chromatography, which was identified by comparison with an authentic sample.

Reaction of II with water.—The substance II (350 mg.) was dissolved in 15.00 ml. of freshly distilled dry dioxane containing 56 mg. of water. The solution was divided into 14 test tubes so that each tube contains 1.00 ml. of the solution, and the tubes were sealed. Then, they were allowed to react in a bath at 30°±0.1° simultaneously. Each of the portions was opened successively at times and dry nitrogen was introduced for about one min. into the solution, which was then titrated with a methanol-benzene solution (ca. 0.3 N) of sodium methoxide using thymol blue as an indicator according to the procedure of Berger, et al.[8]. The sodium methoxide solution was standardized every 5 hr.

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[9] E. Martell and R. M. Herbst, *J. Org. Chem.*, **6**. 878 (1941).

[10] This value was determined by back-titration of the bromine using sodium thiosulfate after a sufficient amount of bromine in glacial acetic acid was added to the sample.

[11] J. U. Nef, *Ann.*, **270**, 299 (1892).

with purified benzoic acid. After about 8 hr. the reacting solution began to become turbid, and after about 20 hr. yellow precipitates began to form. (Curve I on Fig. 2)

Reaction of *N*-carboxy-DL-alanine anhydride with water.—*N*-Carboxy -DL-alanine anhydride[12] (m.p. 45°) (356 mg.) was subjected to the reaction under the same condition described above. After about 30 hr. the reacting solution remained clear (Curve II on Fig. 2). A suspension of 18.3 mg. of DL-alanine in 1 ml. of dimethyl formamide was titrated with a methanol-benzene solution (0.306 N) of sodium methoxide with an indicator of thymol blue, and 0655 ml. (0.975 equivalent for DL-alanine) of the solution was required for neutralization.

Summary

N-Carboxy dehydroalanine anhydride was first synthesized from carbobenzoxy dehydroalanine. The structure of the *N*-carboxy anhydride was confirmed by the analysis of its infrared spectrum and by the investigation of its chemical behavior. The hydrolytic reaction of the *N*-carboxy anhydride was compared with that of *N*-carboxy DL-alanine anhydride, and it was supposed that dehydroalanine carbamic acid might exist stably in dioxane at ordinary temperature.

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[12] J. L. Bailey, *J. Chem. Soc.*, **1950**, 3461.