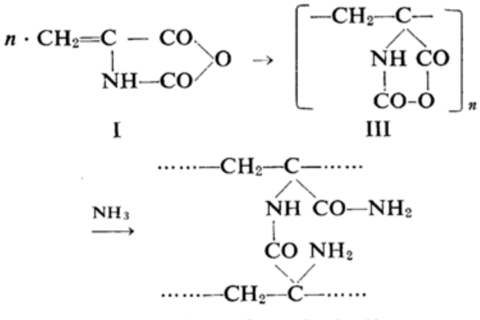
Studies on Dehydroalanine Derivatives. V. Radical Polymerization of N-Carboxy-dehydroalanine Anhydride and of N-Phthaloyl-dehydroalanine. Synthesis of a New Amphoteric Polymer

By Shumpei SAKAKIBARA

(Received July 13, 1960)

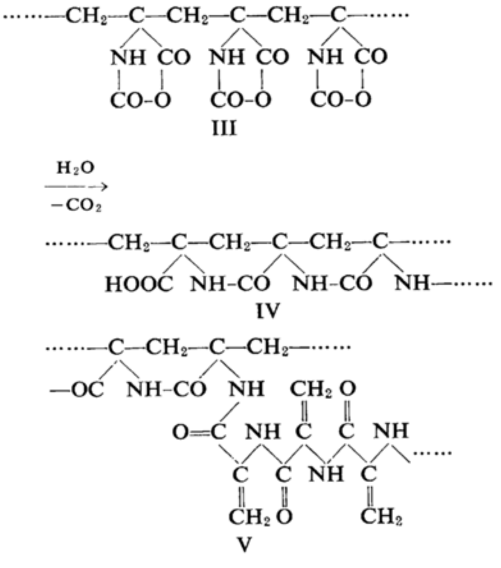
Dehydroalanine derivatives such as *N*-carboxy-dehydroalanine anhydride (I)¹⁾ and *N*-phthaloyl-dehydroalanine (II)²⁾ are considered as vinylidene derivatives. In the present communication, radical homo-polymerization and co-polymerization of compounds I and II are described.

As reported previously³⁾, compound I is readily polymerized to poly-dehydroalanine in pyridine at 100°C with the evolution of carbon dioxide. The polymer obtained is dark brown because of the presence of conjugated double bonds. On the other hand, it was found in this study, that, when this same compound I was heated at 50°C in a dry inert solvent such as ethyl acetate or acetone in the presence of a radical initiator, the solution changed to an opaque jelly within 2 hr. Since, no evolution of carbon dioxide was observed during the polymerization reaction, it was considered that active *N*-carboxy anhydride groups were still present in the polymer obtained. This suggestion was supported by the fact that, when the polymer obtained was dissolved in aqueous ammonia, the solution rapidly turned into a clear gel. Perhaps intermolecular cross-linkages are formed in the solution due to a secondary polymerization reaction of the remaining *N*-carboxy anhydride groups.



I III

However, separation of analytically pure poly-*N*-carboxy-α-amino acrylic acid anhydride (III) from the reaction mixture was unsuccessful because of the partial hydrolysis and secondary polymerization of the *N*-carboxy anhydride groups during the course of the purification of this compound. Actually, due to air humidity or impurities in the solvents used, analytical data of the polymers obtained from different experiments were between the theoretical values for formula III and those for the completely decarboxylated formula IV. Moreover, it was found that the nitrogen content of the compound gradually increased during storage over phosphorous pentoxide in a desiccator at room temperature. Since the *N*-carboxy anhydride groups should be in juxtaposition in polymer III, they may readily polymerize forming the poly-lactam IV.



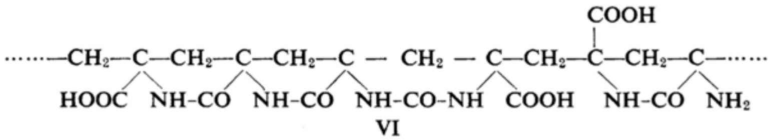
Structure V could scarcely be considered because polymerization of *N*-carboxy-dehydroalanine anhydride I to poly-dehydroalanine is very slow as described previously³⁾. This is also supported by the color; that is, the polymer obtained was white, not dark brown like poly-dehydroalanine.

Polymer III was soluble in aqueous alkaline solution accompanied with decarboxylation reaction, but not in inert solvents. When the polymer was hydrolyzed with aqueous N potassium hydroxide at 100°C, the greater part of the hydrolyzed polymer was precipitated on acidification of the medium. As shown from

---

1) S. Sakakibara, This Bulletin, **32**, 13 (1959).
2) S. Sakakibara, ibid., **34**, 171 (1961).
3) S. Sakakibara, ibid., **32**, 814 (1959).

the pH-titration curve of the hydrolyzed polymer (Fig. 1), there were few amino groups and these were far exceeded by the number of carboxyl groups. Perhaps this is due to ureide formation as well as lactam formation, as described below (formula VI).



$$\begin{array}{c} \cdots\cdots\text{—CH}_2\text{—C—CH}_2\text{—C—CH}_2\text{—C — CH}_2\text{ — C—CH}_2\text{—}\overset{\text{COOH}}{\text{C}}\text{—CH}_2\text{—C—}\cdots\cdots \\ \text{HOOC}\quad\text{NH-CO}\quad\text{NH-CO}\quad\text{NH-CO-NH}\quad\text{COOH}\quad\text{NH-CO}\quad\text{NH}_2 \end{array}$$

VI

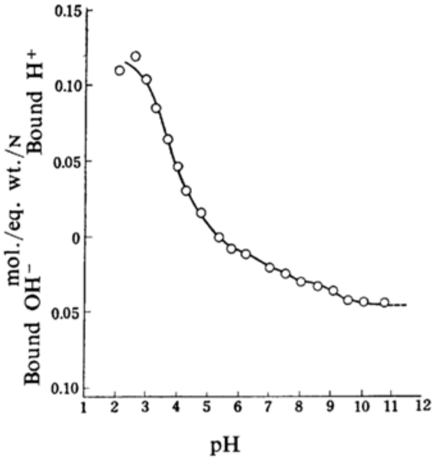
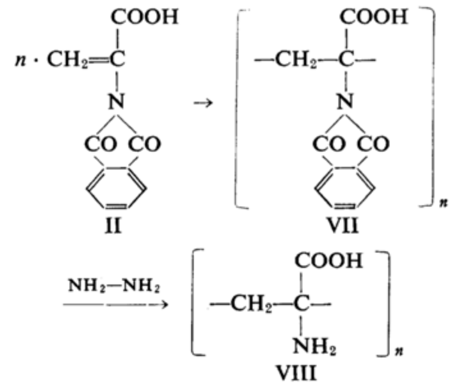
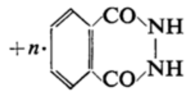


Fig. 1. pH-Titration curve of compound VI.

Frank[4] and Smets et al.[5] indicated that the equilibrium between a five-membered cyclic lactam and its open amino acid, in a polymer chain, tends to the former. Such a ureide-ring may be more stable against hydrolysis than a lactam.

Radical polymerizability of α-phthalimido acrylic acid (II) was rather weak. When the monomer was heated in methanol at 60°C for about 200 hr. in the presence of azobisisobutylonitrile, a polymer (VII) was separated as a methanol-insoluble oil. The polymer was finally obtained as white powder, and it was soluble in dimethylformamide, and in hot acetic acid.





After hydrazinolysis, poly-α-amino acrylic acid (VIII) was obtained in poor yield. To molecular weight of the starting polymer may not have been very high. The poly-α-amino acrylic acid obtained was soluble in water. However, elementary analysis and pH-titration (Fig. 2) indicated that the content of ionizable groups was lower, than the calculated values, for the same reason as explained above.

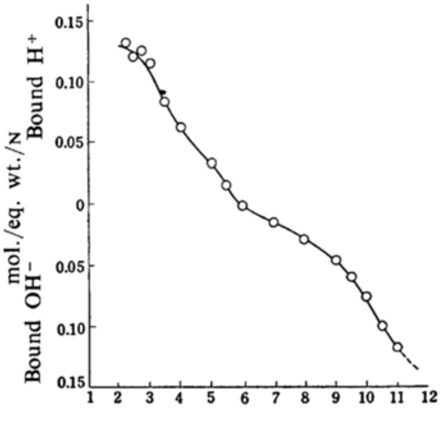


Fig. 2. pH-Titration curve of compound VIII.

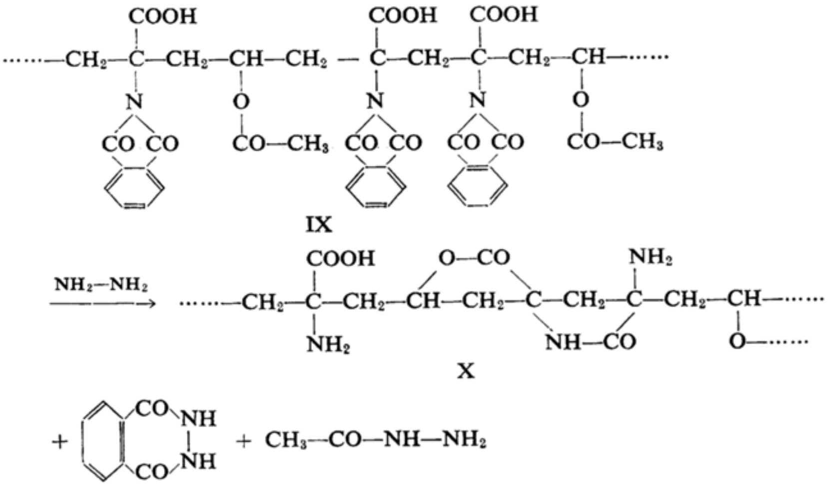
Copolymerization of monomer II with vinyl acetate (V. Ac.) in methanol gave a gelatinous precipitate (IX-a). From the mother liquor, a second fraction (IX-b) was precipitated on addition of ether. Elementary analysis indicated that polymer IX-a was about 8 : 5-co-polymer (II : V. Ac.) and polymer IX-b was about 1 : 5-co-polymer (II : V. Ac.). The former was only soluble in dimethyl formamide, but the latter was soluble in pyridine, glacial acetic acid, dimethyl formamide, *m*-cresol, chloroform, hot ethanol, a mixture of methanol and acetone, and of ethyl acetate and acetone. Polymer IX-b was sparingly soluble in methanol, ethyl acetate and acetone, and insoluble in

---

[4] H. P. Frank, *J. Polymer Sci.*, **12**, 570 (1954).

[5] M. Vrancken and G. Smets, ibid., **14**, 521 (1954); A. Conix, N. V. Mortsel and G. Smets, ibid., **15**, 221 (1955).

benzene, toluene and water. After hydrazinolysis of these copolymers, water soluble polyampholytes (X) were obtained. The pH-titration curves of these copolymers (Figs. 3 and 4) were of a typical polyampholyte-type, the structure of which was assumed to be as follows:



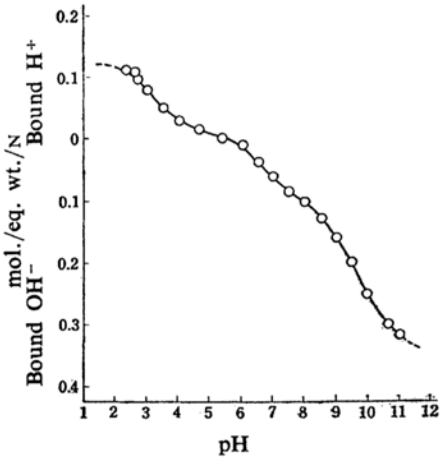


Fig. 3. pH-Titration curve of compound X-a.

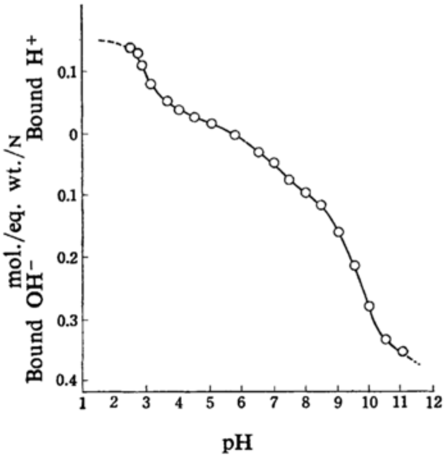


Fig. 4. pH-Titration curve of compound X-b.

If the sequence of co-monomer-segments was at random, five-membered lactam formation, as in poly-α-amino acrylic acid, should not occur. As shown in Figs. 3 and 4 however, the number of ionizable groups was about 30% of the theoretical value. The values increased inversely with the number of amino acid segments. In this cases, the number of amino groups was greater than that of carboxyl groups. Perhaps, amino acid segments may have been localized in the molecule. Moreover, partial intramolecular lactone formation may have occurred.

If copolymerization of small amounts of compounds I or II with vinyl monomers such as acrylonitrile were possible, these would improve the affinity for dyeing of the parent polymers. Moreover, these would be useful as key-substances for preparing graft-copolymer of vinyl- and amino acid-polymers.

Experimental[1]

Polymerization of I to III.—A solution of I (2 g.) and azobisisobutylonitrile (0.1 g.) in purified ethyl acetate (20 ml.) was heated at 50°C in a sealed tube for about 4 hr. The opaque jelly formed, was crushed up finely and became powdery on addition of dry ether. The powder formed III was collected centrifugally, washed with ether and dried over phosphorus pentoxide in a desiccator; colored gradually above 180°C, sintered at about 250°C, yield 40% (0.8 g.).

Found[2]: C, 44.5; H, 4.9; N, 15.8. Found[3]:

---

[1] All melting points were determined by using capillary tubes in a Kanechlole-bath, maximum temperature of which was 300°C.

[2] Analyzed immediately after preparation.

[3] Analyzed after storing the same sample for 10 days in a desiccator over phosphorus pentoxide.

C, 46.9; H, 5.07; N, 16.9. Found[*4]: C, 46.5; H, 4.1; N, 16.5. Calcd. for $C_4H_3O_3N$ (III): C, 42.5; H, 2.6; N, 12.4%. Calcd. for C_3H_3ON (IV): C, 52.2; H, 4.35; N, 20.3%.

Formation of VI from III.—The polymer obtained above (0.5 g.) was dissolved in aqueous N potassium hydroxide (20 ml.) and the solution was heated in a sealed tube at 105°C for about 20 hr. The hydrolyzate was dialyzed against distilled water for 2 days and then lyophilized. White spongy material (0.4 g.) was obtained, which was soluble in water at above pH 6. For analysis, this materials was dissolved in distilled water, and reprecipitated by adding hydrochloric acid. The precipitates formed were washed with water, ethanol and, ether and dried; colored gradually above 250°C without melting.

Found: C, 49.1; H, 5.1; N, 17.3. Calcd. for $C_3H_3ON\text{-}0.1\cdot CO_2\text{-}0.2\cdot H_2O$ (VI): C, 48.5; H, 4.4; N, 18.2%; equiv. wt./N=77.0.

Polymerization of II to VII.—A solution of II (4 g.) and azobisisobutylonitrile (0.6 g.) in methanol (10 ml.) was heated in a sealed tube at 60°C for about 200 hr. The polymer formed (VII) precipitated on addition of ether and washed with methanol and ether, and dried; sintered at 250°C and melted at 270°C, yield 55% (2.2 g.).

Found: C, 60.3; H, 4.2; N, 6.8. Calcd. for $C_{11}H_7O_4N$ (VII): C, 60.8; H, 3.2; N, 6.4%.

Hydrazinolysis of VII.—The polymer VII (1.5 g.) was dissolved in hydrazine hydrate (15 ml.) and the solution was boiled for about 3 hr. The hydrazinolyzate was concentrated to drynes and the residue was redissolved in aqueous 0.1 N potassium hydroxide (5 ml.) and the phtaloyl hydrazide formed, precipitated on addition of enough acetic acid to reduce the pH to 5. The white precipitate formed was centrifuged off, and washed with water; 1.0 g. of phthaloyl hydrazide was recovered. The supernatant was concentrated to 5 ml. and poured into ethanol (about 100 ml.) to precipitate polymer VIII, which was centrifuged off, washed with ethanol, and dried in vacuo at room temperature; slightly brown powder (80 mg.) which colored gradually above 250°C.

Found: C, 43.9; H, 4.5; N, 17.5. Calcd. for $C_3H_3ON\text{-}0.7\cdot H_2O$: C, 44.1; H, 5.4; N, 17.2%; equiv. wt./N=81.6.

Copolymerization of II with Vinyl Acetate.—A mixture of II (5 g.), vinyl acetate (20 g.) and azobisisobutylonitrile (0.5 g.) in methanol (20 ml.) was heated in a sealed tube at 60°C for about 100 hr. The gelatinous precipitates formed were centrifuged off, washed with methanol, and powdered from dimethyl formamide and ether (IX-a); m. p. 220~230°C, wt. 4.0 g.

Found: C, 56.9; H, 5.2; N, 5.0. Calcd. for $C_{11}H_7O_4N\text{-}0.625\cdot C_4H_6O_2\text{-}H_2O$ (IX-a): C, 56.1; H, 4.5; N, 4.9%; eq. wt./N=289.

From the methanolic mother liquor, polymer IX-b was precipitated by addition of ether. It was centrifuged off, washed with ether and dried; sintered at 160~170°C and melted at 200°C, wt. 4.6 g.

Found: C, 56.6; H, 6.3; N, 2.2. Calcd. for $C_{11}H_7O_4N\text{-}4.8\cdot C_4H_6O_2\text{-}0.67\cdot H_2O$ (IX-b): C, 56.5; H, 5.8; N, 2.2%; equiv.wt./N=642.

Hydrazinolysis of IX-a and IX-b.—The polymer IX-a (3 g.) was dissolved in hydrazine hydrate (50 ml.) and the solution was boiled for about 3 hr. The hydrazinolyzate was concentrated to dryness, and the residue was redissolved in aqueous N potassium hydroxide (5 ml.) and the phthaloyl hydrazide formed precipitated on additon of acetic acid. The white precipitates were centrifuged off, and washed with water; about 1.6 g. of phthaloyl hydrazide was recovered. The supernatant and the washings were combined and after adding a few drops of N potassium hydroxide, the solution was dialyzed against distilled water for about 2 days. The dialyzed solution was lyophilized to yield a spongy material X-a; colored gradually above 250°C, wt. 0. 5 g.

Found: C, 48.5; H, 7.6; N, 13.5. Calcd. for $C_3H_3ON\text{-}0.625\cdot C_2H_4O\text{-}0.5\cdot H_2O$: C, 48.5; H, 6.2; N, 13.3%; equiv.wt./N=105.5.

The polymer IX-b (4 g.) was similarly treated with hydrazine hydrate (50 ml.) as described above, and material X-b) (1.1 g.) was obtained as a white spongy material; m. p. 200~210°C.

Found: C, 51.2; H, 8.1; N, 6.0. Calcd. for $C_3H_3ON\text{-}3.5\cdot C_2H_4O\text{-}0.67\cdot H_2O$ (X-b): C, 51.1; H, 7.8; N, 6.0%; equiv.wt./N=235.

pH-Titrations.—Each polyampholyte (VI, VIII, X-a or X-b) was weighed (50~150 mg.) and dissolved in 0.1 N potassium chloride (20.00 ml.) containing 0.30 ml. of 1.00 N potassium hydroxide. The solution was titrated with 1.00 N hydrochloric acid using an Agla micrometer-syringe under stirring in an atmosphere of nitrogen. Determination of pH-values was carried out by using a Beckman, GS pH-meter. The curves shown in Figs. 1, 2, 3 and 4 were obtained by Parke and Davis's method[6].

Summary

Homopolymers of *N*-carboxy-dehydroalanine anhydride (I) and of *N*-phthaloyl-dehydroalanine (II), and copolymers of II with vinyl acetate were synthesized. Hydrolysis or hydrazinolysis was carried out to obtain polymers containing α-amino acid structures. Actual structures of these polymers were discussed.

The author wishes to express his thanks to Professor Shiro Akabori and Professor Shunsuke Murahashi for their valuable advice and encouragement.

Institute for Protein Research
Osaka University
Kita-ku, Osaka

[*4] Another example of analysis.

[6] T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).