Studies on Dehydroalanine Derivatives. IV. Synthesis of N-Phthaloyl-Dehydroalanine by Thermal Decomposition of Cysteine and Serine in the Presence of Phthalic Anhydride

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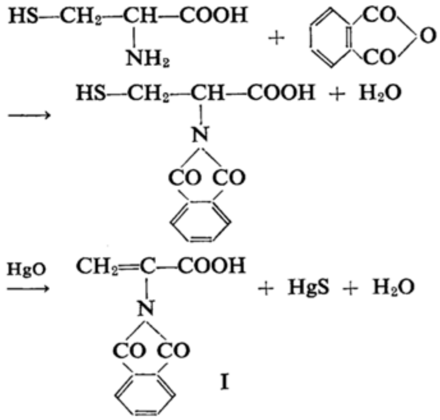
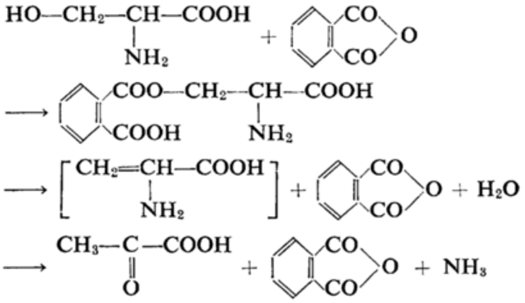
Bergmann et al.[1] reported, that in alkaline solution, serine- or cysteine- diketopiperazine (or peptide) was converted to dehydroalanine-diketopiperazine (or peptide). Weygand and Rinno[2] pointed out that *N*-trifluoroacetyl-serine was converted into *N*-trifluoroacetyl dehydroalanine, when it was distilled under high vacuum. In the present investigation, the thermal decomposition of cysteine and of serine in the presence of phthalic anhydride were studied.

Usually α-amino acids are easily *N*-phthaloylated when heated with phthalic anhydride at 180°C for about half an hour[3]. However, cystine has not yet been tested owing to its poor solubility and reactivity. On the contrary, cysteine and serine were both very unstable to heat, and their *N*-phthaloyl derivatives have not been separated from the respective reaction mixtures after the thermal treatment. When a mixture of cysteine and phthalic anhydride was heated at or above 150°C, formation of hydrogen sulfide was detected by its characteristic odor and by using the lead acetate paper test. The thermal decomposition of cysteine at about 180°C was, however, not so great that its complete decomposition might not be expected within a short time. It was found experimentally that an equimolar amount of yellow mercuric oxide greatly accelerated the desulfhydration reaction and the reagent was rapidly converted into mercuric sulfide at 180°C. After purification of the reaction mixture, *N*-phthaloyl dehydroalanine (I) was obtained as colorless prisms (m. p. 171°C). Use of more than twice the molar equivalent of phthalic anhydride resulted in a better yield, because during the reaction, water is formed and the excess phthalic anhydride may compensate for the partial rehydrolysis of the newly-formed phthaloylimino groups.



When a mixture of serine and phthalic anhydride was heated at 180°C, vigorous evolution of water was observed, and the mixture changed to a dark brown syrup within a short time. At the same time, the formation of pyruvic acid was detected by its order. The purification of the reaction product was very difficult and the yield of substance I was poor. Perhaps part of the serine might have been converted into its *O*-phthalic acid monoester, which was then decomposed to pyruvic acid and ammonia at high temperature before the *N*-phthaloylation reaction occurred. Therefore it was necessary to establish conditions in which the *N*-phthaloylation of serine was complete before such a dehydration reaction could occur. Sheehan et al. have shown[4] that usual amino acids could be *N*-phthaloylated in



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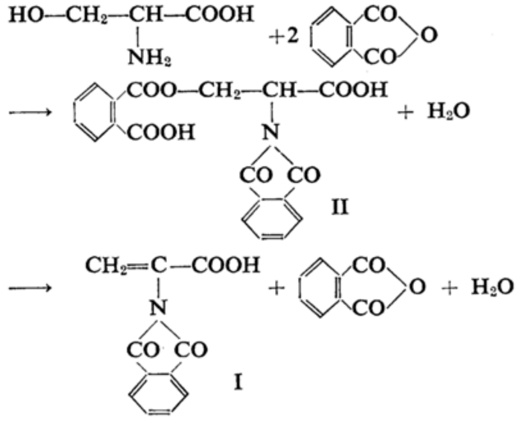
[1] M. Bergmann and A. Miekeley, *Ann.*, **458**, 40 (1927); B. H. Nicolet, *J. Am. Chem. Soc.*, **53**, 3066 (1931); B. H. Nicolet, *J. Biol. Chem.*, **140**, 685 (1941).

[2] F. Weygand and H. Rinno, *Chem. Ber.*, **92**, 517 (1959).

[3] J. H. Billman and W. F. Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948).

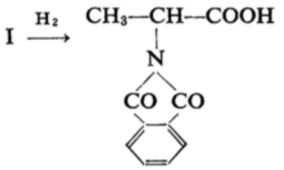
[4] J. C. Sheehan, M. Goodman and G. P. Hess, ibid., **78**, 1367 (1956).

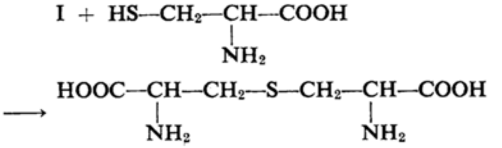
a gentle reaction in boiling dioxane. However, they did not mention such a reaction for serine. When serine and an equimolar amount of phthalic anhydride were boiled in dioxane for about five hours, a part of the serine remained in suspension, but twice the molar amount of phthalic anhydride readily solubilized the remaining serine in dioxane. Perhaps ***O*, *N***-diphthaloylated serine (II) is formed under these conditions.



After the solvent had been distilled off, the residual syrup was heated gently in an oil bath at 200°C for about one hour under reduced pressure, and as in the case of cysteine, *N*-phthaloyl dehydroalanine (I) was obtained from the reaction mixture. The melting points of substance I from cysteine and serine were the same (m. p. 171°C), and no depression of the melting point was observed, when equal amounts of product I from cysteine and from serine were mixed. However, the yield of substance I from serine was inferior to that from cysteine. When substance I was recrystallized from dimethylformamide, crystals were obtained which melted at 84～88°C and contained one mole of dimethylformamide as crystallization solvent. Since the dimethylformamide complex was readily crystallizable, this procedure was suitable for purification of the product.

Hydrogenation of substance I gave rise to *N*-phthaloyl-DL-alanine, and lanthionine could also be formed from substance I by an addition reaction with L-cysteine under the conditions described by Schöberl5).

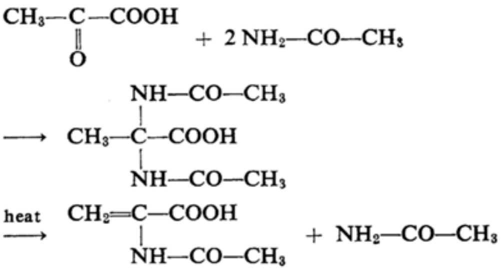




$$\mathrm{I} + \mathrm{HS{-}CH_2{-}\underset{NH_2}{CH}{-}COOH} \longrightarrow \mathrm{HOOC{-}\underset{NH_2}{CH}{-}CH_2{-}S{-}CH_2{-}\underset{NH_2}{CH}{-}COOH}$$

The properties of substance I all supported structure I.

Bergmann6) first prepared *N*-acetyl dehydroalanine from pyruvic acid and acetamide as follows:



$$\mathrm{CH_3{-}\underset{\underset{O}{\|}}{C}{-}COOH} + 2\,\mathrm{NH_2{-}CO{-}CH_3} \longrightarrow \mathrm{CH_3{-}\overset{NH{-}CO{-}CH_3}{\underset{NH{-}CO{-}CH_3}{C}}{-}COOH} \xrightarrow{\text{heat}} \mathrm{CH_2{=}\underset{NH{-}CO{-}CH_3}{C}{-}COOH} + \mathrm{NH_2{-}CO{-}CH_3}$$

Many investigators utilized this procedure for preparing other *N*-acyl-dehydroalanine derivatives7). However, an attempt in the present work to prepare substance I by this procedure failed, because of the poor reactivity of phthalimide with pyruvic acid.

Substance I can polymerize to a vinylidene-type poly-α-phthalimino acrylic acid, which may be converted into poly-α-amino acrylic acid by hydrazinolysis.

Experimental

***N*-Phthaloyl Dehydroalanine (I) from Cysteine.** —A mixture of freshly prepared cysteine (12 g.) and phthalic anhydride (37 g.) was placed in a round bottomed flask (300 ml.) fitted with a distillation adaptor. The flask was heated in an oil bath at 180°C (for about 20 min.) until a clear syrup was obtained, and then the water formed was distilled off under vacuum, using a water pump for about 10 min. more. An intimate mixture of yellow mercuric oxide (20 g.) and phthalic anhydride (22 g.) was added to the reaction mixture in one portion and then the flask was kept under vacuum for about 10～15 min. at 180～190°C. After being cooled to 50～60°C, the reaction mixture was extracted with hot ethyl acetate (300 ml.). The black precipitate was centrifuged off, and extracted three times with 40 ml. each of ethyl acetate. The combined extracts were concentrated to dryness under reduced pressure. The excess phthalic anhydride was separated by sublimation at 140°C in vacuo. The residue was dissolved in ethyl acetate (about 50 ml.) and the resinous products were precipitated by adding ether (about 150～200 ml.). The precipitates were centrifuged off and the supernatant was concentrated to dryness. The

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5) A. Schöberl, *Chem Ber.*, **80**, 379 (1947).

6) M. Bergmann and K. Grafe, *Z. Phys. Chem.*, **187**, 187 (1930).

7) Cf. S. Sakakibara, This Bulletin, **32**, 13 (1959).

residue was recrystallized from toluene (about 30 ml.) to yield light-brown prisms (15 g.), which melt at 150~165°C. The small amount of phthalic anhydride remaining was sublimed out at 140°C in vacuo, and the remaining crystals were recrystallized from ethyl acetate after treatment with a small amount of active charcoal. The yield of slightly colored prisms with a melting point of 164~167°C was 60% (13.2 g.).

For final purification, the product was recrystallized from dimethyl formamide. The dimethyl formamide-complex (plates) formed melted at 84~88°C.

Found: C, 58.70; H, 4.98; N, 9.11. Calcd. for $C_{11}H_7O_4N\cdot C_3H_7ON$: C, 58.00; H, 4.84; N, 9.65%.

The complex was dried at 120°C in vacuo for about 3 hr. and recrystallized from ethyl acetate to yield colorless prisms with m. p. 169~171°C. The over-all yield was about 50~55%.

Found: C, 60.80; H, 3.17; N, 6.37. Calcd. for $C_{11}H_7O_4N$: C, 60.82; H, 3.24; N, 6.46%.

I from Serine.—A mixture of DL-serine (10.5 g.) and phthalic anhydride (37 g.) in dioxane (100 ml.) was refluxed until a clear solution was obtained (for about 5 hr.). The solution was concentrated to dryness in a flask, as shown in Fig. 1, under reduced pressure, and the residue was heated to 190~200°C under reduced pressure for about 20 min., during which time about 22 g. of phthalic anhydride distilled over. The residue was dissolved in ethyl acetate (50 ml.) and resinous products were precipitated by adding ether (150~200 ml.). The precipitates were centrifuged off, and the supernatant was concentrated to dryness. The residue was heated at 190~200°C for about 20 min. in vacuo and then the same procedures for separating the resinous products formed were repeated. The residue obtained was recrystallized from toluene (30 ml.) to yield light-brown prisms (9 g.) which melted at 150~160°C. The crystals were recrystallized from butanol-toluene (1 : 1) and then from dimethylformamide. The dimethylformamide complex (8.5 g., m. p. 82~88°C) was dried at 120°C in vacuo for about 3 hr. and recrystallized from ethyl acetate to yield colorless prisms (5 g.) with m. p. 169~171°C. The yield was 23%. The product was identified exactly with that obtained from cysteine by the mixed melting point test.

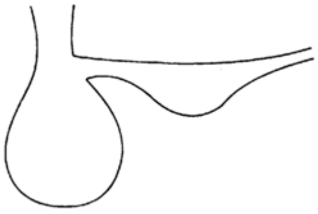

Fig. 1.

***N*-Phthaloyl DL-Alanine (II).** — A solution of I (1 g.) in ethanol (30 ml.) was hydrogenated in the presence of silk-palladium catalyst[8] (150 mg.) in an autoclave. The initial hydrogen pressure was 60 atm. The mixture was heated at 40°C for about 3 hr. and then at 70°C for additional 3 hr. After the reaction, the mixture was cooled to room temperature, and the catalyst was filtered off. The filtrate concentrated to dryness under reduced pressure. The residue was recrystallized from water-ethanol, and prisms with m. p. 159~161°C (650 mg.) and with m. p. 157~160°C (280 mg.) were obtained; the total yield was 93%. No depression of the melting point was observed, when the substance with m. p. 159~161°C was mixed with an authentic sample with m. p. 161~161.5°C. The infrared absorption spectrum of the product was also exactly the same as that of an authentic sample.

Lanthionine (III). — Substance I (3.3 g.) and L-cysteine hydrochloride (1.6 g.) were dissolved in aqueous N sodium hydroxide (24 ml.), and the solution was heated at 50°C for 1 hr. and then at 80°C for 2 hr. in an atmosphere of hydrogen. Concentrated hydrochloric acid (30 ml.) was added to the reaction mixture and the solution was boiled for about 6 hr. After colling to room temperature, the phthalic acid formed was filtered off, and the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in water, and recrystallized by neutralizing the solution with aqueous ammonia. After being kept overnight in a refrigerator, the crystals were filtered off and recrystallized from 6 N hydrochloric acid and aqueous ammonia as described above. The crystals formed were filtered off, washed with water, and alcohol, and dried; wt. 1.8 g., the yield was 86% (calcd. from cysteine).

Found: C, 34.18; H, 6.02; N, 13.44; S, 15.39. Calcd. for $C_6H_{12}O_4N_2S$: C, 34.61; H, 5.81; N, 13.45; S, 15.40%.

Summary

N-Phthaloyl dehydroalanine (I) was synthesized by heating cysteine or serine in the presence of phthalic anhydride. Yellow mercuric oxide greatly accelerated the desulfhydration reaction. The yield of I from serine was inferior to that from cysteine. *N*-Phthaloyl-DL-alanine and lanthionine were derived from I.

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8) Y. Izumi, This Bulletin, 32, 932 (1959).