516. N-*Carboxy-anhydrides of* O-*Acetyl- and* O-*Carbobenzyloxy-serine.*

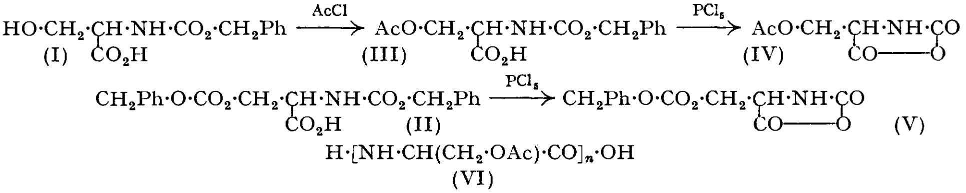
By Max Frankel and M. Halmann.

O-Acetyl-*N*-carbobenzyloxyserine, prepared from *N*-carbobenzyloxyserine and acetyl chloride, on reaction with phosphorus pentachloride yielded *O*-acetyl-*N*-carboxyserine anhydride. This when heated to 105° in a vacuum lost carbon dioxide and formed poly-(*O*-acetylserine) with an average degree of polymerization of ten. *O*-Carbobenzyloxy-*N*-carboxyserine anhydride was prepared from *ON*-dicarbobenzyloxyserine and phosphorus pentachloride; it reacted with atmospheric moisture to form *O*-carbobenzyloxyserine.

While *N*-carboxy-anhydrides of several neutral, basic, and acidic amino-acids have been prepared, and have been of considerable use as monomers in the synthesis of polypeptides, no *N*-carboxy-anhydrides of amino-acids containing an aliphatic hydroxylic function have been described. The present paper describes the preparation of *N*-carboxy-anhydrides of *O*-acetyl- and *O*-carbobenzyloxy-serine, in continuation of previous work (see Frankel and Berger, *J. Org. Chem.*, 1951, **16**, 1513).

DL-Serine was converted by the action of benzyl chloroformate into a mixture of *N*-carbobenzyloxy- (I) and *ON*-dicarbobenzyloxy-serine (II) by Bergmann and Zervas's (*Ber.*, 1932, **65**, 1194) and Levene and Schormueller's procedures (*J. Biol. Chem.*, 1934, **105**, 551). The mixture was separated by fractional crystallization.

N-Carbobenzyloxyserine (I) with acetyl chloride gave *O*-acetyl-*N*-carbobenzyloxyserine (III), from which, by the action of phosphorus pentachloride, *O*-acetyl-*N*-carboxyserine anhydride (IV) was obtained :



$$\underset{\text{(I)}}{\mathrm{HO{\cdot}CH_2{\cdot}\underset{CO_2H}{CH}{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph}} \xrightarrow{\mathrm{AcCl}} \underset{\text{(III)}}{\mathrm{AcO{\cdot}CH_2{\cdot}\underset{CO_2H}{CH}{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph}} \xrightarrow{\mathrm{PCl_5}} \underset{\text{(IV)}}{\mathrm{AcO{\cdot}CH_2{\cdot}\underset{CO{-}{-}O}{CH{\cdot}NH{\cdot}CO}}}$$

$$\underset{\text{(II)}}{\mathrm{CH_2Ph{\cdot}O{\cdot}CO_2{\cdot}CH_2{\cdot}\underset{CO_2H}{CH}{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph}} \xrightarrow{\mathrm{PCl_5}} \mathrm{CH_2Ph{\cdot}O{\cdot}CO_2{\cdot}CH_2{\cdot}\underset{CO{-}{-}O}{CH{\cdot}NH{\cdot}CO}} \quad \text{(V)}$$

$$\underset{\text{(VI)}}{\mathrm{H{\cdot}[NH{\cdot}CH(CH_2{\cdot}OAc){\cdot}CO]_{\mathit{n}}{\cdot}OH}}$$

Similarly *ON*-dicarbobenzyloxyserine (II) reacts with phosphorus pentachloride to form *O*-carbobenzyloxy-*N*-carboxyserine anhydride (V) (see Frankel and Katchalski, "Sci. Papers presented to Ch. Weizmann," 1944, Jerusalem, pp. 24, 74).

O-Acetyl-*N*-carboxyserine anhydride (IV) behaved as a typical "Leuchs anhydride" (Leuchs, *Ber.*, 1906, **39**, 857 ; Leuchs and Geiger, *Ber.*, 1908, **41**, 1721 ; Leuchs and Manasse, *Ber.*, 1907, **40**, 3235); when heated in a vacuum to about 105° it evolved carbon dioxide with vigorous foaming. After 2 hours an amount of carbon dioxide approximately equivalent to the anhydride had been released; on the walls of the vessel a light-brown, almost transparent hard film had formed, which became sticky on exposure to the atmosphere. Its composition, as well as the ratio of " total nitrogen " to free " amino-mitrogen," showed it to be poly-(*O*-acetylserine) of an average degree of polymerization of ten (VI; $n = 10$). It was insoluble in water but easily soluble in dilute acids or bases, and gave a strong positive biuret reaction.

O-Carbobenzyloxy-*N*-carboxyserine anhydride (V) also lost carbon dioxide when heated; however, even after 8 hours' heating at 100—105° in a vacuum the decrease in " amino-nitrogen " showed it to have then a degree of polymerisation of only about five. The product was a dark brown glassy film, which apparently was a polypeptide of *O*-carbobenzyloxyserine. When hydrogen was passed through a dispersion of the compound in acetic acid, and phosphonium iodide added (Harington and Mead, *Biochem. J.*, 1935, **29**, 1605; Greenstein, *J. Biol. Chem.*, 1937, **118**, 321), carbon dioxide was evolved vigorously. On evaporation of the acetic acid, a white powdery substance remained which was easily soluble in water, giving positive ninhydrin and biuret reactions; it apparently contained a polypeptide of serine.

Wessely (*Z. physiol. Chem.*, 1925, **146**, 72) and Go and Tani (*Bull. Chem. Soc., Japan*, 1939, **14**, 510) have shown that freshly prepared crystalline *N*-carboxy-anhydrides of amino-acids, on exposure to moist air, undergo polymerization to poly-(α-amino-acids) even at room temperature. Miller, Fankuchen, and Mark (*J. Appl. Phys.*, 1949, **20**, 531), by observing the change in the *X*-ray spectrum of crystalline ε-carbobenzyloxy-α-*N*-carboxy-L-lysine anhydride, proved that the polymerization reaction took place in the solid state. It was therefore expected that *O*-carbobenzyloxy-*N*-carboxyserine anhydride, on contact with atmospheric moisture, would also polymerize. However, when the anhydride was exposed for 20 days in an open vessel, it was hydrolyzed to *O*-carbobenzyloxyserine.

EXPERIMENTAL

(M. p.s are uncorrected.)

Carbobenzyloxy-derivatives of Serine (Bergmann and Zervas, *loc. cit.*; Levene and Schormueller, *loc. cit.*; Fruton, *J. Biol. Chem.*, 1942, **146**, 465).—DL-Serine (6·4 g.; Merck) was dissolved in sodium hydroxide (60 ml.; N), and during ½ hour benzyl chloroformate (12 g.) and sodium hydroxide (20 ml.; 4N) were added alternately in small portions with constant shaking and cooling in ice-water. Concentrated hydrochloric acid was then added until the solution was acid (Congo paper). The precipitated oil was kept overnight in the ice-box, and the crystals were filtered off, dried in a vacuum-desiccator, and recrystallized from ethyl acetate, giving *N*-carbobenzyloxyserine (8 g.), m. p. 123°.

On concentration of the mother-liquor (Levene and Schormueller, *loc. cit.*) in a vacuum a yellowish crystalline mass remained; this was dissolved in a small amount of hot benzene, and after 1 day crystalline *ON*-dicarbobenzyloxyserine (2 g.), m. p. 94°, had formed.

O-*Acetyl*-N-*carbobenzyloxyserine*.—A suspension of *N*-carbobenzyloxyserine (3·0 g.) in dry benzene (300 ml.) was heated on a water-bath and acetyl chloride (6 ml.) was added; a vigorous evolution of hydrogen chloride ensued. Boiling was continued for an hour, and the benzene was then distilled off in a vacuum. The residual oil crystallized, giving colourless O-*acetyl*-N-*carbobenzyloxyserine* to which in ethyl acetate a little light petroleum was added. Overnight large prismatic crystals (2·97 g., 84%), m. p. 116—118°, developed (Found: C, 55·5; H, 5·6; N, 5·2; Ac, 15·7. $C_{13}H_{15}O_6N$ requires C, 55·5; H, 5·4; N, 5·1; Ac, 15·3%).

O-*Acetyl*-N-*carboxyserine Anhydride*.—*O*-Acetyl-*N*-carbobenzyloxyserine (1·2 g.) suspended in dry ether (15 ml.) was shaken with phosphorus pentachloride (1·5 g.), added in portions during 40 minutes with cooling to about −10°. The solution was filtered and the ether and phosphorus oxychloride were distilled off in a vacuum at 40°. Dry ethyl acetate was added and then evaporated and, after repetition of this operation, the temperature was kept for several hours at 50°. The residual yellow oil was dissolved in ethyl acetate and precipitated with light petroleum. When rubbed with small amounts of light petroleum the oil gradually became more viscous, and finally after storage overnight over phosphoric oxide in a vacuum desiccator in the ice-box the *anhydride* crystallized to a white, very hygroscopic powder (0·63 g., 85%)

(Found: C, 41·7; H, 4·2; N, 8·3; Ac, 24·6. $C_6H_7O_5N$ requires C, 41·6; H, 4·1; N, 8·1; Ac, 24·9%).

Poly-(O*-acetylserine*).—*O*-Acetyl-*N*-carboxyserine anhydride (0·47 g.) was dried by being heated in a high vacuum for 3 hours at 50°. The temperature was then raise to 105—110°, causing a sudden violent evolution of gas. After 2 hours' heating the film (0·34 g.) on the walls of the tube was dissolved in acetic acid and precipitated with dry ether. After three such purifications the *poly-*(O*-acetylserine*) was washed several times with ether (Found: C, 45·0; H, 5·1; N, 10·0; Ac, 33·0; amino-N, 0·98. $C_{50}H_{72}O_{31}N_{10}$ requires C, 45·9; H, 5·5; N, 10·7; Ac, 32·9%).

O*-Carbobenzyloxy-*N*-carboxyserine Anhydride* (cf. Frankel and Katchalski, *loc. cit.*).—*ON*-Dicarbobenzyloxyserine (1·5 g.) suspended in ether (28 ml.) was cooled to −10°, and phosphorus pentachloride (2·1 g.) was added portionwise during 20 minutes. The excess of phosphorus pentachloride was filtered off and the solution evaporated in a vacuum at 40° (1 hour). The residual oil was dissolved in ethyl acetate and the evaporation in a vacuum continued for a further 2 hours. The oil was then dissolved in hot ethyl acetate, and light petroleum added. When rubbed, O*-carbobenzyloxy-*N*-carboxyserine anhydride* (0·75 g., 71%) was obtained crystalline, m. p. about 95° (decomp.) (Found: C, 53·8; H, 3·9; N, 5·5. $C_{12}H_{11}O_6N$ requires C, 54·3; H, 4·1; N, 5·3%).

When the anhydride was heated in a high vacuum to 100—105°, it melted, and a strong evolution of gas started suddenly. After 2 hours' heating there remained a light-brown glassy film which was rather hard but brittle, and deliquescent in air. The substance, *poly*(*carbobenzyloxyserine*), was insoluble in water, but soluble in hot glacial acetic acid, from which it was precipitated on addition of water (cf. Breuer, Thesis, The Hebrew University, Jerusalem, 1951) [Found: C, 59·5; H, 5·0; N, 6·4. $(C_{11}H_{11}O_4N)_n$ requires C, 59·7; H, 5·0; N, 6·3%] (These analyses are by Drs. Weiler and Strauss, Oxford).

O*-Carbobenzyloxyserine*.—*O*-Carbobenzyloxy-*N*-carboxyserine anhydride was left in an open vessel for 20 days. Colourless O*-carbobenzyloxyserine* remained; it showed a strong blue reaction with ninhydrin, and had m. p. 132° (Found: N, 6·1; amino-N, 5·8. $C_{11}H_{13}O_5N$ requires N, 5·9; amino-N, 5·9%).

DEPARTMENT OF ORGANIC CHEMISTRY,
THE HEBREW UNIVERSITY, JERUSALEM, ISRAEL.

[*Received, April 7th,* 1952.]