Poly-(DL-leucylglycylglycine) and Some Poly-α-amino Acids

Y. Liwschitz, A. Zilkha, H. Borensztain, and
Max Frankel

Since our last publications on the preparation of polymeric peptides through the acyl chlorides of amino acids and oligopeptides,[1] some additional methods for the production of polypeptides, the recurring unit of which consists of an oligopeptide residue, have been published.[2-4]

Further work on the acyl chloride hydrochloride polymerization has again corroborated the obvious fact that the chain length of polypeptides produced is greatly dependent on the purity of the monomers used. Thus, in the case of DL α-amino-n-butyric acid, the chloride of which could be prepared in great purity, the polymer obtained was composed, on the average, of 45 units. When the amino acids did not undergo complete chlorination, the resulting polymers showed a much lower degree of polymerization, e.g., poly-L-proline hydrochloride consisted of six units and the polymer obtained from the chloride hydrochloride of DL-leucylglycylglycine had an average chain length of five and consisted of 15 amino acid residues.

Polymerization was carried out by heating the monomers in bulk in a high vacuum, at temperatures varying from 115–200°. Since all polymers contained one mole of hydrochloric acid, their average chain length was determined by end group assay. The Van Slyke nitrogen of the water soluble poly-tripeptide corresponded to the chlorine value, but in the case of the insoluble poly-DL-α-amino-n-butyric acid and the poly-L-proline such corroborating determinations could not be made. Determinations of the Van Slyke nitrogen after hydrolysis were in complete agreement with the respective total nitrogen values, as regards poly-α-amino-n-butyric acid and the poly-tripeptide. The properties of the poly-proline were similar to those of the polymer derived by the N-carboxy anhydride route.[5] Poly-α-amino-n-butyric acid hydrochloride was insoluble in water dilute acids or alkali and the usual organic solvents. Poly-leucylglycylglycine hydrochloride was water-soluble.

In the course of experiments to improve the method for preparing pure chloride hydrochlorides of amino acids, it was found that the modification

of E. Fischer's method recently reported by Levine,[6] in which recrystallization of amino acids, e.g., glycine and alanine, is omitted when conducting the chlorination in purified carbon tetrachloride, did not yield satisfactory results. Omission of the recrystallization in the case of alanine gave incompletely chlorinated products and the reaction time generally required in this medium was considerably higher than in acetyl chloride.

EXPERIMENTAL[7]

DL-α-*Amino-n-butyryl chloride hydrochloride.* DL-α-Amino-n-butyric acid (2 g.), freshly reprecipitated from its aqueous solution by absolute ethanol and dried at 110°, was finely ground, driven through a hair-sieve,[8] and placed in a glass-stoppered bottle with 40 ml. of freshly distilled acetyl chloride. After addition of 4.4 g. of phosphorus pentachloride the bottle was shaken for 3 hours. The substance then was quickly filtered off, with careful exclusion of moisture, washed with acetyl chloride and petroleum ether, and dried in a vacuum desiccator. Yield 2 g. (66%).

Anal. Calc'd for $C_4H_9Cl_2NO$: Cl, 44.9. Found: Cl, 44.1.

Polymerization of DL-α-amino-n-butyryl chloride hydrochloride. DL-α-Amino-n-butyryl chloride hydrochloride (2 g.) was heated in high vacuum at 200° for three hours. Despite the high temperature the substance was not appreciably discolored. The resulting polymer was insoluble in dilute alkali, mineral acids, and glacial acetic acid. It was purified by boiling first in water and then in ethanol. Particles of the substance developed a bluish color on heating with an aqueous solution of ninhydrin. A positive reaction resulted on prolonged contact with the biuret reagent. Because of the low solubility of this polymer, no Van Slyke amino nitrogen determination was made, but according to the chlorine content, the chain length was 45 on the average. The substance commenced to decompose at 300°.

Anal. Calc'd for $C_{180}H_{318}ClN_{45}O_{46}$: C, 55.5; H, 8.2; N, 16.2; Cl, 0.93. Found: C, 53.2; H, 8.5; N, 14.9; Cl, 0.93.

Hydrolysis of poly-DL-α-amino-n-butyric acid hydrochloride. The above polymer of chain length n = 45 (0.0147 g.) was boiled under reflux with 12 ml. of 6 N hydrochloric acid for 20 hours. The solution then was quantitatively transferred to a 25-ml. volumetric flask, 2.4 g. of solid sodium hydroxide was added, and then the mixture was accurately neutralized with 6 N sodium hydroxide solution and finally made up to the mark with distilled water. Van Slyke amino nitrogen determinations made from aliquot parts of this solution gave values of N = 15.3, 15.2. Paper-partition chromatography by the ascending method of the hydrolyzate (phenol-water) gave the characteristic spot for α-amino-n-butyric acid.

L-*Prolyl chloride hydrochloride.* L-Proline (4 g.) was suspended in 20 ml. of freshly distilled acetyl chloride and after cooling in a freezing mixture, 8.8 g. of phosphorus pentachloride was added. The mixture was shaken at room temperature for two hours. Part of the substance, which precipitated during the chlorination, was filtered off and treated like the α-amino-n-butyryl chloride hydrochloride. Yield 1.2 g. (20%). The filtrate was kept in an ice box for 24 hours and on addition of 3 ml. of acetyl chloride, 2 g. (34%) of a second fraction of the L-prolyl chloride hydrochloride was obtained. Since this substance proved to be more completely chlorinated than the first fraction, it was used in the subsequent polymerization. It decomposed at

(1) Max Frankel, Y. Liwschitz, and A. Zilkha, *J. Am. Chem. Soc.*, **76**, 2814 (1954); *Bull. Research Council Israel*, **4**, 36 (1954).

(2) J. Noguchi and T. Hayakawa, *J. Am. Chem. Soc.*, **76**, 2846 (1954).

(3) V. Bruckner, *et al.*, *Acta Chim. Hung.*, **6**, 219 (1955).

(4) F. J. Weymouth, *Chemistry & Industry*, R. 34 (1956).

(5) A. Berger, J. Kurtz, and E. Katchalski, *J. Am. Chem. Soc.*, **76**, 5552 (1954).

(6) S. Levine, *J. Am. Chem. Soc.*, **76**, 1382 (1954).

(7) Micro-combustion analyses were by Drs. Weiler and Strauss, Oxford.

(8) Proline and the tripeptide were also treated in this way prior to chlorination.

80° in contrast to acylchloride hydrochlorides of amino acids dealt with by us hitherto.

Anal. Calc'd for $C_5H_9Cl_2NO$: Cl, 41.6. Found: Cl, 40.8.

Polymerization of L-prolyl chloride hydrochloride. L-Prolyl chloride hydrochloride (2 g.) was heated in a high vacuum at 115° for 10 hours. The polymer thus obtained was soluble in water, slightly soluble in ethanol, and insoluble in benzene, dioxane, etc. Purification was effected by dissolving in the minimum amount of water and precipitation with absolute ethanol. Ninhydrin and biuret reactions were negative. According to the chlorine content the polymer was composed of 6 units on the average. It decomposed at 165°.

Anal. Calc'd for $C_{30}H_{45}ClN_6O_7$: C, 56.5; H, 7.1; N, 13.2; Cl, 5.6. Found: C, 53.6; H, 7.5; N, 13.0; Cl, 5.5.

DL-*Leucylglycylglycyl chloride hydrochloride.*[9] The tripeptide (0.9 g.) was suspended in 20 ml. of freshly distilled acetyl chloride, and after cooling in an ice-bath, 1 g. of phosphorus pentachloride was added in two portions and the mixture was shaken at room temperature for four hours. Then 0.5 g. of phosphorus pentachloride was added and shaking was resumed for another 13 hours. Finally, after addition of 0.3 g. of the chlorinating agent, shaking was continued for 7 more hours. The substance was treated and isolated in the usual way. Yield 0.85 g. (77.5%).

Anal. Calc'd for $C_{10}H_{19}Cl_2N_3O_3$: Cl, 23.7. Found: Cl, 21.4.

Polymerization of DL-*leucylglycylglycyl chloride hydrochloride.* The tripeptide chloride hydrochloride (0.8 g.) was heated in a high vacuum at 135° for seven hours. The polymer thus obtained was soluble in water and ethanol. It gave positive biuret and ninhydrin reactions. It was purified by dissolving in ethanol and reprecipitation with dry ether. According to the chlorine content and free amino end groups (Van Slyke's manometric method), it was composed of 5 tripeptide residues.

Anal. Calc'd for $C_{50}H_{88}ClN_{15}O_{16}$: C, 50.1; H, 7.4; N, 17.6; Cl, 3.0; Van Slyke nitrogen, 1.2. Found: C, 46.7; H, 7.5; N, 16.8; Cl, 3.1; Van Slyke nitrogen, 1.4. Amino nitrogen after hydrolysis 16.3.

The two constituent amino acids were identified on paper chromatograms made from the hydrolyzate.

DEPT. OF ORGANIC CHEMISTRY
THE HEBREW UNIVERSITY
JERUSALEM, ISRAEL

(9) E. Fischer, *Ber.*, **38**, 2914 (1905).

Partial Degradation of the Benzene Ring of Estradiol-17β

HAROLD WERBIN

The biosynthesis of carbon-14 labeled estrone and estradiol-17β from acetate-C^{14} has been reported recently under both *in vitro* and *in vivo* conditions.[1-4] Insight into the mechanisms involved in the biosynthesis may be forthcoming when methods become available for the total degrada-

tion of the aromatic and alicyclic parts of the estrogen molecules enabling a determination of the total distribution of the radioactive carbon atoms in the labeled estrogens. Recently, the partial degradation of the aromatic ring of estrone was reported.[5] This was accomplished by applying the bromopicrin split to several nitroestrones.[6]

This note describes the synthesis of 2,4-dinitroestradiol-17β and its partial degradation to tribromonitromethane (representing both carbon atoms 2 and 4 of the benzene ring) by a similar application of the bromopicrin split. The 2,4-dinitroestradiol-17β was characterized by its ultraviolet and infrared absorption spectra. The steroid had absorption bands at 741 cm.$^{-1}$, 759 cm.$^{-1}$, and 904 cm.$^{-1}$ which had been found previously to be characteristic of dinitrophenolic pentasubstituted compounds.[5] The ultraviolet spectrum of the dinitrosteroid showed absorption maxima in absolute ethanol at 277 mμ (ε 6655), 352-354 mμ (ε 3340), and a plateau of 424-426 mμ (ε 910). These values are in excellent agreement with those recorded for similar pentasubstituted aromatic compounds.[5] The 2,4-dinitroestradiol-17β was also characterized by conversion to a colorless 3,17-diacetate which was purified by filtration in ether over charcoal. The diacetate was hydrolyzed readily to a yellow 17-monoacetate by elution from a column of aluminum oxide. The bromopicrin resulting from the cleavage of the benzene ring of the dinitrosteroid was identified by infrared analysis.

EXPERIMENTAL[7]

2,4-Dinitroestradiol-17β. Estradiol-17β (100 mg.) was dissolved in 3 ml. of glacial acetic acid, and 60 μl of concentrated nitric acid was added slowly at room temperature with good mixing. After standing for 24 hours, the solution was poured into 50 ml. of water and was extracted several times with ether. The ether solution was concentrated to dryness *in vacuo.* The yellow residue was treated with 5 ml. of 5% methanolic potassium hydroxide for 2 hours to hydrolyze any ester that may have formed, after which the solution was acidified and centrifuged. The precipitate was washed several times with water and was crystallized from aqueous ethanol, m.p. 240-250° (dec.).

Anal. Calc'd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 60.12; H, 6.50; N, 7.35.

2,4-Dinitroestradiol-3,17β-diacetate. 2,4-Dinitroestradiol-17β (20 mg.) was mixed with 100 μl. of acetic anhydride and 50 μl. of dry pyridine. After standing at room temperature overnight, the mixture was evaporated to dryness with a stream of nitrogen. The residue was taken up in ether, 70 mg. of activated charcoal was added, and the solution was filtered. Evaporation of the ether left a colorless solid that was crystallized from aqueous methanol, m.p. 161.5-164.5°.

Anal. Calc'd for $C_{22}H_{26}N_2O_8$: N, 6.28. Found: N, 6.30.

2,4-Dinitroestradiol-17β-acetate. The diacetate was taken up in benzene and was placed on a column of 5 g. of aluminum oxide (Merck acid-washed). Benzene containing 0.5%

(1) Heard and O'Donnell, *Endocrinology*, **54**, 209 (1954).

(2) Rabinowitz and Dowben, *Biochim. Biophys. Acta*, **16**, 96, (1955).

(3) Levitz, Condon, and Dancis, *Fed. Proc.*, **14**, 245 (1955).

(4) Wotiz, Davis, and Lemon, *J. Biol. Chem.*, **216**, 677 (1955).

(5) Werbin and Holoway, *J. Biol. Chem.*, in press.

(6) Since estriol can be converted to estrone, the partial degradation of the benzene ring of the three most abundant estrogens found in human urine now is possible.

(7) Melting points were taken on a Fisher-Johns melting block and are uncorrected.