308. *The Structure of Native Poly-*D*-glutamic Acid. Part IV.* The Synthesis of Poly-*L*-glutamine and the Hofmann Degradation thereof.*

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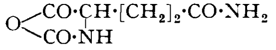
Methyl α-poly-L-glutamate was converted into poly-L-glutamine, which was degraded by Hofmann's method. Acid hydrolysis then gave αγ-diaminobutyric acid. This corroborates the previous conclusion that in native poly-D-glutamic acid α-glutamyl bonds cannot predominate, since analogous treatment of the polyamide prepared from native poly-acid gave no detectable αγ-diaminobutyric acid.

The isolation of β-formylpropionic acid but not of αγ-diaminobutyric acid from the acid hydrolysate of the Curtius-degraded polyhydrazide or the Hofmann-degraded polyamide, respectively, of *B. subtilis* poly-D-glutamic acid (Kovács and Bruckner, *Research*, 1952, **5**, 194; *J.*, 1952, 4255; Bruckner, Kovács, and Nagy, *J.*, 1953, 148) suggests the predominance of γ-glutamyl links in that polypeptide. This result appears to be further substantiated by the analogous degradation of the synthetically prepared polyamide of α-poly-L-glutamic acid (poly-L-glutamine), which affords αγ-diaminobutyric acid, as expected.

Our first attempt to prepare poly-DL-glutamine (V) directly from carbobenzyloxy-DL-glutamine (I) failed, because by the conventional method no intermediates (II and III) were isolated, although the resinous end product showed a distinct biuret reaction. However, treatment of a synthetic methyl α-poly-L-glutamate (IV) with liquid ammonia afforded a well-defined poly-L-glutamine (V).

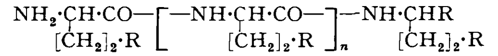
$NH_2{\cdot}CO{\cdot}[CH_2]_2{\cdot}CHR{\cdot}NH{\cdot}CO_2{\cdot}CH_2Ph$

(I; R = CO_2H) (II; R = COCl)



$$O\begin{cases}CO{\cdot}CH{\cdot}[CH_2]_2{\cdot}CO{\cdot}NH_2\\ CO{\cdot}NH\end{cases}$$

(III)



$$NH_2{\cdot}\underset{[CH_2]_2{\cdot}R}{CH}{\cdot}CO{-}\left[{-}NH{\cdot}\underset{[CH_2]_2{\cdot}R}{CH}{\cdot}CO{-}\right]_n{-}NH{\cdot}\underset{[CH_2]_2{\cdot}R}{CHR}$$

(IV; R = CO_2Me) (V; R = $CO{\cdot}NH_2$) (VI; R = NH_2)

$$(VI) \longrightarrow (n + 1)(H_2N)(CO_2H)CH{\cdot}[CH_2]_2{\cdot}NH_2 + NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CHO$$

(VII) (VIII)

For Hofmann degradation of the poly-L-glutamine sodium hypobromite or sodium hypochlorite was used. No attempt was made to isolate the primary product of the degradation (VI), although this may be of interest in connection with the structure of polymyxine. Rather, the solution was acidified with hydrochloric acid and then hydrolysed. From the hydrolysate αγ-diaminobutyric acid (VII) was isolated as the diflavianate and the dipicrate. Although the yield did not exceed 19·5%, the crucial point is that this treatment of the same small quantity of the material under exactly the same conditions as in the degradation of the polyamide of native poly-D-glutamic acid (Bruckner, Kovács, and Nagy, *loc. cit.*) readily afforded αγ-diaminobutyric acid, whence it follows that native poly-D-glutamic acid cannot contain an appreciable proportion of α-glutamyl bonds.

The other product (VIII) of the hydrolysis is unstable and is formed in such small quantity that it cannot be assayed.

Experimental

*Carbobenzyloxy-*DL*-glutamine* (I).—To an ice-cooled, stirred solution of DL-glutamine (10 g.) in 3·33% sodium hydrogen carbonate solution (180 ml.) benzyl chloroformate (25 g.) and sodium hydrogen carbonate (20 g.) were added portionwise (15 minutes). Stirring was continued for 1 hour with cooling, and for another 2 hours at room temperature. The excess of benzyl chloroformate was extracted with ethyl acetate (2 × 50 ml.), and the solution made acid to Congo-red, and then kept in the ice-box. The *product* (I) (12 g.; m. p. 142—143°), collected, washed

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* Part III, *J.*, 1953, 148.

with water, and recrystallized from ethyl acetate–petrol, formed colourless, glistening plates (9 g.), m. p. 144—145° (Found: C, 55·4; H, 5·75. $C_{13}H_{16}O_5N_2$ requires C, 55·7; H, 5·75%). Bergmann and Zervas (*Ber.*, 1932, **65**, 1192) report for carbobenzyloxy-L-glutamine m. p. 137°.

Attempted Synthesis and Polymerization of 4-2′-*Carbamoylethyloxazolid*-2 : 5-*dione* (III).—A mixture of carbobenzyloxy-DL-glutamine (5 g.), acetic anhydride (20 ml.), and purified thionyl chloride (2·5 ml.; freshly distilled from linseed oil and from quinoline) was refluxed on the steam-bath with exclusion of moisture. Gas was freely evolved and the substance dissolved in 20 minutes. Solvent and excess of reagent were removed *in vacuo*, and the remaining yellow oil was warmed (receiver cooled with liquid air) at 100°/0·001 mm. for 2 hours and then at 130° for another 2 hours (oil-bath). There was much foaming and a resin was formed [3·36 g. Calc. for formation of (III): 2·5 g.]. The product gave a distinct biuret reaction.

Poly-L-*glutamine* (V).—Finely powdered synthetic methyl α-poly-L-glutamate (12 g.) (Hanby, Waley, and Watson, *J.*, 1950, 3239; Coleman, *J.*, 1951, 2294; J. Kovács, Bruckner, and K. Kovács, *J.*, 1953, 145) was kept with liquid ammonia (200 ml.; distilled from metallic sodium) in a sealed tube at room temperature for 1 week, with occasional shaking, and gradually crumbled. The ammonia was removed, and the product washed five times with hot water (total, 200 ml.), then dried (P_2O_5) *in vacuo*. It (10·5 g.) was finely powdered, and the above treatment with ammonia was repeated. The *polymer*, washed with hot water and dried to constant weight at 100°/1 mm., was a colourless powder (9 g.), giving a strong biuret reaction [Found: C, 46·9; H, 6·25; total N, 20·4; amido- and ammonium N, 10·4; OMe, 1·3. $(C_5H_8O_2N_2)_n$ requires C, 46·9; H, 6·3; total N, 21·9; amido-N, 10·9; OMe, 0%]. The same product was obtained when reaction was effected at 60°.

Evaporation of the washings *in vacuo* gave an amorphous glass readily soluble in water and giving a strong biuret reaction. Analysis (Found: C, 43·0; H, 6·6; total N, 20·4; OMe, 0·4%) indicated partial hydrolysis of the polyamide extracted by the hot water on washing (cf. Schulze and Trier, *Ber.*, 1912, **45**, 257, concerning the hydrolysis of glutamine).

Degradation of Poly-L-*glutamine*.—(*a*) To a freshly prepared sodium hypobromite solution (0·6 ml. of bromine in 20 ml. of 3N-sodium hydroxide), finely powdered poly-L-glutamine (1·3 g.) was added. On being shaken, complete dissolution occurred (20 minutes), and the solution was then immersed in a bath at 90° for 15 minutes (slight odour of ammonia), acidified with concentrated hydrochloric acid, and evaporated *in vacuo*. After dissolution of the solid residue in concentrated hydrochloric acid (30 ml.), and removal of precipitated inorganic salts, the filtrate was refluxed for 20 hours, then evaporated *in vacuo*; the residue was redissolved in a small quantity of water, and the solution again evaporated. For complete removal of hydrochloric acid this double procedure was repeated. The residue was then dissolved in saturated aqueous picric acid (30 ml.), and next morning the solid was collected, washed with a little water and recrystallized from water (3 ml.), affording glistening yellow plates (0·4 g., 13·3%), m. p. 189° alone or mixed with an authentic specimen of αγ-diaminobutyric acid dipicrate (Found: C, 33·6; H, 3·0. Calc. for $C_{16}H_{16}O_{16}N_8$: C, 33·3; H, 2·8%).

(*b*) Finely powdered poly-L-glutamine (325 mg.) was treated with a sodium hypobromite solution (one-quarter of above quantities) and worked up as under (*a*) except that refluxing with concentrated hydrochloric acid was reduced to 8 hours. The hydrolysate was evaporated *in vacuo*, and the residue redissolved in water (4 ml.) in which flavianic acid (1·5 g.) was then dissolved with gentle warming. After cooling, the crystalline precipitate was collected and washed with water (two portions, total 4 ml.), methanol, and ether. The yellow needles of the product (370 mg., 19·5%; m. p. 232°) were once recrystallized from water, and then melted at 239° alone or mixed with an authentic specimen of αγ-diaminobutyric acid diflavianate.

(*c*) Finely powdered polyamide (100 mg.) was treated with 3·2% sodium hypochlorite solution (prepared from 3·7 ml. of 2·5N-sodium hydroxide) as under (*a*), and hydrolysis was similarly effected. The residue of the evaporated hydrolysate was dissolved in water (3 ml.), the solution again evaporated under diminished pressure, and this treatment repeated. The residue was dissolved in water (0·5 ml.), and saturated aqueous flavianic acid solution (2 ml.) was added. After two days the precipitate was collected, washed, and dried as usual, the yield being 95 mg. (16·3%). The m. p. (233°) was raised by one recrystallization from water to 239° and remained undepressed on admixture with αγ-diaminobutyric acid diflavianate, m. p. 239°.

(*d*) Poly-L-glutamine (50 mg.) was treated with alkaline sodium hypochlorite solution as under (*c*), and the subsequent acid hydrolysis was effected in exactly the same way as after the Hofmann degradation of the polyamide of native poly-D-glutamic acid (Bruckner, Kovács, and Nagy, *loc. cit.*). To the alkaline solution (2·1 ml.) resulting from the reaction, concentrated hydrochloric acid (10 ml.) was added, and the solution was refluxed for 30 minutes, cooled to room temperature, and filtered from deposited inorganic salts; the filtrate was evaporated *in vacuo*, the residue redissolved in water, the solution again evaporated under diminished pressure, and saturated aqueous flavianic acid solution (1 ml.) added. After two days the crystals were collected and washed as usual; yield, 10 mg., m. p. 202—210°, raised by recrystallization from four drops of water to 233°, undepressed on admixture with authentic αγ-diaminobutyric acid diflavianate, m. p. 239°.

(*e*) The following experiment shows that under conditions in which degradation of the amide of native poly-D-glutamic acid affords the maximal yield of β-formylpropionic acid, poly-L-glutamine does not produce this degradation product. Finely powdered poly-L-glutamine (50 mg.) was shaken with 1·40% alkaline sodium hypochlorite solution (2·1 ml.) for 1 hour, and the mixture warmed to 50° for 10 minutes. Complete dissolution did not occur, and no evolution of ammonia was observed (contrast behaviour of the amide of native poly-D-glutamic acid). The mixture was then refluxed with concentrated hydrochloric acid (10 ml.) for 30 minutes, and evaporated, the residue taken up in a small quantity of water, and the solution again evaporated. The residue completely dissolved in 12 drops of a freshly prepared saturated solution of *p*-nitrophenylhydrazine in N-hydrochloric acid. Neither on subsequent warming, nor on cooling and seeding with β-formylpropionic acid *p*-nitrophenylhydrazone was any separation of solid observed, whereas degradation of the amide of native poly-D-glutamic acid (50 mg.) under exactly identical conditions afforded 32 mg. of β-formylpropionic acid *p*-nitrophenylhydrazone.

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[*Received, December* 11*th*, 1952.]