standing for one hour, the phosphorus chlorides were distilled off under vacuum. The residue was poured over cracked ice and concentrated sodium hydroxide was added. The benzene extract was evaporated and distilled yielding 33 g. of material boiling 175–180° (6 mm.). The liquid had the following properties: $n^{20}D$ 1.5684 and d^{20}_4 1.311.

Anal. Found: C, 51.6, 52.1; H, 4.73, 4.72.

The liquid was undoubtedly a mixture of the dichloro- and trichloropyridine compounds. The calculated analytical data for a mixture which is 60% dichloro and 40% trichloro are: C, 52.2; H, 4.65. The molecular refraction figured on this same basis is: calcd.: 57.40; found, 57.26.

4-Cyclopentylpyridine.—The mixture of chloropyridines (36 g.) was shaken with hydrogen at 30 pounds pressure, using 8 g. of 5% palladium on charcoal as catalyst and 200 ml. of ethanol as solvent. After 36 hours, the catalyst was filtered off and washed with absolute ethanol. The ethanol solution was evaporated, and the residue was distributed between dilute hydrochloric acid and benzene. The water layer was basified and extracted with ether, and the combined ether extracts were evaporated. Distillation of the residue gave 4.3 g. of 4-cyclopentylpyridine. Distillation of the neutral benzene extract yielded 27.5 g. of the starting material. The yield of 4-cyclopentylpyridine on unrecovered starting material was 79%. The 4-cyclopentylpyridine was purified through the picrate, which melted 131–132°. Truitt reported m.p. 146°. The purified base had the properties: b.p. 129° (23 mm.), $n^{20}D$ 1.5306, and d^{20}_4 1.002.

Anal. Calcd. for $C_{10}H_{13}N$: N, 9.52. Found: N, 9.45.

A mixture melting point with the 4-cyclopentylpyridine picrate obtained from the Emmert synthesis showed no depression in melting point.

Acknowledgment.—The authors wish to express their appreciation to the Union Carbide and Carbon Corporation for the financial assistance obtained through the Union Carbide and Carbon Fellowship in Chemistry at The University of Texas for 1952–1953.

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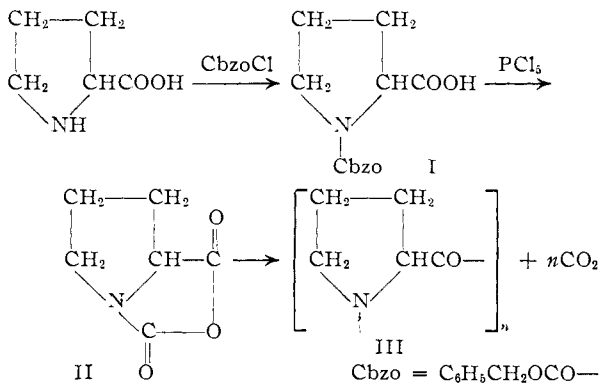
NOTES

Poly-L-proline

BY ARIEH BERGER, JOSEPH KURTZ AND EPHRAIM KATCHALSKI

RECEIVED MAY 21, 1954

A high molecular weight peptide composed exclusively of proline residues may serve as a useful model in physicochemical and biological studies of proteins, such as collagen, gelatin, casein and zein, which contain a high percentage of proline. The attempts of Astbury, *et al.*,[1] to synthesize polyproline by the polymerization of the corresponding N-carboxyamino acid anhydride were unsuccessful, probably due to the difficulties involved in the preparation and purification of N-carboxyproline anhydride.[2] The successful synthesis of N-carboxy-L-proline anhydride II enabled us to prepare poly-L-proline III according to the scheme



Carbobenzoxy-L-proline (I), obtained from L-proline and benzyl chloroformate in the usual way, was converted into N-carboxy-L-proline anhydride (II) by means of phosphorus pentachloride. II was purified by molecular distillation; it yielded on polymerization in bulk or in dioxane solution poly-L-proline (III). In the latter case diethylamine was used to initiate polymerization.

The chemical constitution of the products of polymerization of II was ascertained by elementary analysis and by the quantitative recovery of proline after acid hydrolysis. The specific rotation of the proline recovered from the hydrolyzate of III, proved that the polyproline synthesized from L-proline consists entirely of L-proline residues. Poly-L-proline shows a remarkably high levorotation in water and in formic acid. The specific rotation in water was practically independent of temperature (between 0° and 80°) and of *p*H (between *p*H 3 and 11). Since no gelation occurred on cooling aqueous polyproline solutions, the above observations seem to support the widely accepted view that the mutarotation of gelatin[3] is associated with gel formation.[4]

As it has been found that the polyproline preparations obtained either by bulk polymerization or by polymerization in solution, using an amine as a polymerization initiator, contain as terminal groups only carboxyl groups and no free imino groups, it seems likely that a termination reaction of the type discussed recently by Sela and Berger[5] took place in both cases. Such a termination reaction should lead to polymers containing one carboxyl group for each peptide chain in the case of amine initiated polymers and two carboxyl groups per peptide chain in the case of polymers prepared by bulk polymerization. Based on this assumption average chain lengths, n = 35 to 42 and n = 67 to 133, were

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(2) E. M. Petri and A. J. Staverman, *Rec. Trav. Chim.*, **71**, 385 (1952).

(3) C. R. Smith, THIS JOURNAL, **41**, 135 (1919).

(4) J. D. Ferry, *Advances in Protein Chem.*, **4**, 1 (1948).

(5) M. Sela and A. Berger, THIS JOURNAL, **75**, 6350 (1953).

calculated from titration data for polyproline preparations obtained by polymerization in solution and by bulk polymerization, respectively.

Poly-L-proline is water soluble and can be precipitated from its aqueous solutions by trichloroacetic acid and sodium chloride. Aqueous or acetous solutions of polyproline give after the evaporation of solvent coherent films, resembling in appearance gelatin flakes. The water solubility of poly-L-proline, as contrasted to the insolubility in water of other poly-α-amino acids derived from bifunctional α-amino acids (*e.g.*, polyglycine, poly-L-alanine, poly-L-leucine and poly-L-phenylalanine) may be explained by the absence of NH groups in polyproline. The intermolecular hydrogen bonds between the peptide (CONH) groupings of adjacent chains of normal polyamides are known to be responsible for the large association forces preventing their dissolution in water.[6] In its ability to dissolve in water poly-L-proline resembles polysarcosine,[7] whose amide links are also void of hydrogen.

It is thus possible to distinguish between two types of water-soluble poly-α-amino acids: (a) polyamino acids containing hydrophilic functional groups, such as polylysine, polyaspartic acid, polyglutamic acid[8] and polyserine,[9] and (b) polyamino acids such as polyproline and polysarcosine which do not contain hydrogen in their amide groups. The water-soluble poly-DL-alanine represents a special case. Its solubility might possibly be explained by the lack of regular steric configuration preventing the formation of the maximum number of intermolecular hydrogen bonds.

The infrared absorption spectrum (from 1000 to 3800 cm.$^{-1}$) of a film of poly-L-proline, cast from glacial acetic acid, has been determined. It shows a band at 1640 cm.$^{-1}$ presumably due to the stretching vibration of the C=O group, though its frequency is somewhat lower than that of a carbonyl group in a disubstituted amide.[10] Absorption bands at 1438, 2885 and 2955 cm.$^{-1}$ may be assigned to the CH– frequencies of the proline residues.[11] As expected, no absorption due to an NH deformation frequency at 1510 to 1580 cm.$^{-1}$ [11,12] was found. A band at 3495 cm.$^{-1}$ cannot be assigned easily. Polyproline does not contain OH or NH groups which absorb in this range[11] and since the proline residues retain their full optical activity, the formation of OH groups as a result of tautomerization involving the hydrogen atom bound to the α-carbon may be excluded. This absorption band may be a harmonic of the carbonyl frequency (coupled with a low skeleton frequency), similar to that of N,N-dibutyltrifluoroethanamide which absorbs at 3472 cm.$^{-1}$.[13] An absorption band at 3500 cm.$^{-1}$ was found by us in N-benzoyl-α-methylamino - isobutyryl - N,N - dimethylamide, where no enol tautomerization is possible. The infrared absorption spectrum of polysarcosine was found to resemble closely that of polyproline. This polymer also showed an absorption band at 3500 cm.$^{-1}$, as well as a strong CO band at 1660 cm.$^{-1}$, but did not absorb at 1510 to 1580 cm.$^{-1}$.

(6) K. H. Meyer, "Natural and Synthetic High Polymers," 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1950, page 264.

(7) S. G. Waley and J. Watson, *Proc. Royal Soc. London*, **A199**, 499 (1949).

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(10) R. E. Richards and H. W. Thompson, *J. Chem. Soc.*, 1248 (1947); *cf.* also S. I. Mizushima, *et al.*, *Nature*, **169**, 1058 (1952).

(11) *Cf.* F. A. Miller in "Organic Chemistry," edited by H. Gilman, Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 143.

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(13) H. Letaw, Jr., and A. H. Gropp, *J. Chem. Phys.*, **21**, 1621 (1953).

Experimental

All melting points are uncorrected. L-Proline obtained from Nutritional Biochemicals Corporation was used without further purification.

N-Carbobenzoxy-L-proline (I).—Benzyl chloroformate (4 g.) and 2 *N* sodium hydroxide (15 ml.) were added simultaneously during 15 minutes with vigorous shaking to an ice-cooled solution of L-proline (2.3 g.) in 2 *N* sodium hydroxide (10 ml.). The reaction mixture was extracted twice with 25-ml. portions of ether and the ethereal extracts discarded. The aqueous solution was acidified to congo red with 6 *N* hydrochloric acid. The oil which separated was taken up in ethyl acetate (100 ml.), the solution washed with water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the oily residue dissolved in boiling carbon tetrachloride (25 ml.). On the addition of petroleum ether (50 ml.) an oil separated which crystallized on standing overnight at room temperature. The crystals (5 g.) were collected and recrystallized from carbon tetrachloride; m.p. 76–77°, $[\alpha]^{20}D$ −61.7° (*c* 5.30, in glacial acetic acid).

Anal. Calcd. for $C_{13}H_{15}O_4N$: C, 62.6; H, 6.0; N, 5.6; equiv. wt., 249. Found: C, 63.1; H, 6.3; N, 5.8; equiv. wt., 250, determined by titration with 0.1 *N* sodium methoxide in dioxane using thymol blue as indicator.[14]

N-Carboxy-L-proline Anhydride (II).—N-Carbobenzoxy-L-proline (4 g.) was dissolved in anhydrous benzene (30 ml.) and 10 ml. of solvent were distilled off to remove azeotropically traces of water. Phosphorus pentachloride (4 g.) was added to the ice-cooled solution and the reaction mixture shaken for 15 minutes. The solution was decanted from excess of phosphorus pentachloride and concentrated *in vacuo* (25 mm.) with rigorous exclusion of moisture. The oily residue was redissolved in anhydrous benzene (15 ml.) and the solvent again distilled off *in vacuo*. The procedure was repeated and the final residue transferred with the aid of a few ml. of anhydrous xylene into a Hickman molecular still[15] equipped with a magnetic stirrer. The solvent and the benzyl chloride formed during cyclization were removed at room temperature and at a pressure of 2×10^{-2} mm. over a period of three hours. The condenser of the still was then cooled with ice and the N-carboxyproline anhydride distilled at a bath temperature of 65–70° and a pressure of 5×10^{-3} mm. The colorless, liquid distillate (1.0 g.) was dissolved in ethyl acetate (10 ml.), and petroleum ether (50 ml.) was added. The anhydride (0.7 g.) crystallized out as long colorless needles, either at once or, occasionally only, after standing in the freezer overnight; recrystallized from ethyl acetate and petroleum ether before analysis, m.p. 45°; carbon dioxide evolution starts at 105 to 115°.

Anal. Calcd. for $C_6H_7O_3N$: C, 51.0; H, 5.0; N, 9.95; equiv. wt., 141. Found: C, 50.6; H, 5.2; N, 10.1; equiv. wt., 140, determined by titration with 0.1 *N* sodium methoxide in anhydrous dioxane using thymol blue as indicator.[16]

II is soluble in benzene, ethyl acetate, carbon tetrachloride and dimethylformamide. It is insoluble in petroleum ether.

Poly-L-proline (III). (a) By Bulk Polymerization.—Twice-recrystallized N-carboxy-L-proline anhydride (II) was heated to 130–135° *in vacuo* (25 mm.) for two hours. The transparent solid material obtained was dissolved in the minimal amount of glacial acetic acid and precipitated by the addition of five volumes of ether; yield quantitative.

Anal. Calcd. for $(C_5H_7NO)_n$: C, 61.8; H, 7.2; N, 14.4. Found: C, 60.5, 62.5 (wet combustion[17]); H, 7.4; N, 14.3.

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(15) K. C. D. Hickman and C. R. Sanford, *J. Phys. Chem.*, **34**, 637 (1930).

(16) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

(17) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

In preliminary experiments it was found that the carbon dioxide which evolved during the polymerization amounted to 97% of the theoretical.

Various preparations of polyproline obtained as above were found to contain no free imino groups, as determined by titration with 0.1 *N* perchloric acid in glacial acetic acid, using crystal violet as indicator.[18] Proline required one equivalent of perchloric acid under similar conditions. The amount of free carboxyl groups in the various preparations of III, obtained by bulk polymerization, was determined by titration with 0.1 *N* sodium methoxide using thymol blue as indicator.[14] Values of 0.030 to 0.015 carboxyl groups per one proline residue were obtained. These values correspond to an average chain length n = 67 to 133, assuming the presence of two terminal carboxyl groups per each peptide chain.

A polyproline preparation with an average degree of polymerization n = 67 showed an optical rotation of $[\alpha]^{20}D$ −372° (*c* 2.0 in glacial acetic acid), $[\alpha]^{20}D$ −483° (*c* 1.5 in formic acid) and $[\alpha]^{20}D$ −353° (*c* 2.0 in water).

The various preparations of poly-L-proline (n 67 to 133) dissolved readily in formic acid, glacial acetic acid and phenol. They dissolved partially in methanol and ethanol and were practically insoluble in dioxane, petroleum ether, acetone, ether and nitrobenzene. The solubility of polyproline in water was found to depend on the way in which the solutions were prepared. When 200 mg. of polyproline (n 80) was stirred with 10 ml. of water for 48 hours at room temperature, only 100 mg. went into solution. The material, obtained from the aqueous solution on drying, dissolved readily in water. Complete dissolution in water of the original polymer was achieved when it was dissolved in a minimal amount of hot formic acid, or glacial acetic acid, and diluted with water to the required concentration. No precipitate formed on neutralization with sodium hydroxide. When the polyproline was dissolved in cold formic acid or acetic acid, a slight precipitate formed on the addition of water. An excess of water precipitated the polymer from its solution in phenol, either hot or cold. Polyproline may be precipitated from its aqueous solution with trichloroacetic acid or with a concentrated sodium chloride solution. From its solution in glacial acetic acid it may be precipitated by ether, acetone or perchloric acid (at a final concentration of about 0.1%).

(b) **By Polymerization in Solution.**—A solution of N-carboxy-L-proline anhydride (0.30 g.) and diethylamine (0.003 g.) in anhydrous dioxane (7.0 ml.) was heated under reflux to 80° for 12 hours. The reaction mixture was protected from moisture by means of a calcium chloride tube. The polymer which separated out was filtered, washed with dioxane and dried *in vacuo* over concentrated sulfuric acid, yield 0.2 g.

The elementary analysis of the polyamino acid obtained agreed closely with that given for the poly-L-proline obtained by bulk polymerization.

Various preparations of polyproline obtained by polymerization in solution, contained no free imino groups. The free carboxyl groups were determined as described previously. From the values obtained (0.029 to 0.024 carboxyl group per one proline residue), average degrees of polymerization of n = 35 to 42 were calculated on the assumption that there is one free carboxyl group per each peptide chain.

Hydrolysis of Poly-L-proline.—Poly-L-proline (n 67, 16.6 mg.) was dissolved in 2 ml. of 6 *N* hydrochloric acid and hydrolyzed in a sealed tube at 110° for 24 hours. The hydrolyzate was concentrated in a vacuum desiccator over solid sodium hydroxide and concentrated sulfuric acid, and the dry residue dissolved in water (50 ml.). A chromatographic analysis of the hydrolyzate on paper using *n*-butyl alcohol–acetic acid–water (4:1:5) as the mobile phase, yielded with ninhydrin one spot with R_f 0.22 identical with that of an authentic sample of L-proline. The amount of proline in the aqueous solution was determined colorimetrically.[19]

Anal. Calcd. for hydrolyzate of 100 mg. of poly-L-proline $(C_5H_7ON)_n$: proline, 118 mg. Found: proline, 120 mg.

From the optical rotation of the total hydrolyzate of poly-L-proline, $[\alpha]^{20}D$ −51.5 (*c* 5.75 in 0.5 *N* hydrochloric acid), was calculated for the proline liberated. An authentic sample of proline showed under identical conditions $[\alpha]^{20}D$ −52.6°.

N-Benzoyl-α-methylaminoisobutyryl-N,N-dimethylamide.—N-Benzoyl-α-methylaminoisobutyryl chloride[20] was added to an excess of dimethylamine in anhydrous ether and left at room temperature overnight. The dimethylamine hydrochloride formed was filtered off and the ethereal solution extracted with water. The residue after the removal of the ether was crystallized from dibutyl ether; m.p. 127°, yield 75%.

Anal. Calcd. for $C_{14}H_{20}N_2O_2$: C, 67.6; H, 8.1; N, 11.2. Found: C, 67.1; H, 7.7; N, 11.2.

Acknowledgment.—This investigation was supported by a research grant (PHS G-3677) from the National Institutes of Health, Public Health Service.

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The Conversion of Primary Amines to Carbonyl Compounds by a Chloromine Degradation[1]

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RECEIVED JUNE 3, 1954

The oxidation of a primary amine to the corresponding aldehyde or ketone can be a useful conversion, particularly for degradative purposes. This change has been effected by chlorination of an amine with hypochlorous acid to an N-chloramine, followed by dehydrochlorination to an imine, and hydrolysis of the latter to a carbonyl compound.[4] One adaptation of this method which has been described qualitatively[5] is the conversion of the primary steroidal amine, 3β-acetoxy-20-amino-5-pregnene to 5-pregnenolone.

We have found that *t*-butyl hypochlorite, a fairly stable and readily obtainable material, is a much more convenient reagent for the N-chlorination of amines than is hypochlorous acid itself. A series of five different primary amines has been treated with *t*-butyl hypochlorite. The N-chloramines were converted directly to the imines by reaction with sodium ethoxide, and hydrolysis with dilute mineral acid gave the carbonyl compound, which was isolated either directly or as its 2,4-dinitrophenylhydrazone.

Experimental

5-Pregnenolone.—To a suspension of 621 mg. of 3β-acetoxy-20-amino-5-pregnene acetate[6] (m.p. 200–204°) in 10 ml. of dry ether at 0° was added 120 mg. of sodium bicarbonate, followed by a solution of 165 mg. of *t*-butyl hypochlorite in 10 ml. of dry ether. After allowing the mixture to stand in the cold for 20 minutes, 5 ml. of ethanol was added, followed by a cold solution of sodium ethoxide made by dissolving 350 mg. of sodium in 15 ml. of ethanol. The mixture was heated without reflux on a steam-bath and boiled until a drop of the solution no longer gave a positive reaction with acidified starch-iodide paper. Water was added until the precipitated sodium chloride just dissolved,

(1) Abstracted in part from the Ph.D. dissertation of Michael P. Cava, University of Michigan, 1951.

(2) Deceased March 22, 1951.

(3) Department of Chemistry, The Ohio State University, Columbus, Ohio.

(4) A. Berg, *Ann. chim. phys.*, [3] **7**, 289 (1894).

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