Polytryptophan

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In continuation of experiments on poly-α-amino acids,[1,2] the synthesis of polytryptophan has been carried out. Poly-DL-tryptophan with a number average degree of polymerization n = 80, as determined by amino-N end-group analysis,[3] and poly-L-tryptophan (n = 90) were prepared by bulk polymerization of α,N-carboxy-DL- and α,N-carboxy-L-tryptophan anhydride, respectively. The synthesis of poly-DL-tryptophan through the Lossen rearrangement of sodium α-carboxy-β-3-indolylpropiono-(benzoylhydroxamate) has been mentioned by Hurd, *et al.*,[4] although experimental details have not yet been published.

α,N-Carboxytryptophan anhydride was obtained from tryptophan and phosgene according to the general procedure of Farthing.[5] Its constitution was proved by the analytical results, the quantitative evolution of carbon dioxide on heating and the quantitative yield of tryptophan on treatment with hydrochloric acid. Despite the known acidity of the NH group of indoles, the possibility of reaction between this group and phosgene was ruled out by the negative reaction of skatole with this reagent under similar experimental conditions.

On alkaline hydrolysis poly-DL- and poly-L-tryptophan yield tryptophan quantitatively. No attempt was made to recover the optically active tryptophan from the hydrolysate of poly-L-tryptophan as the hydrolytic conditions used are known to favor racemization.[6] The marked dextrorotation (in dimethylformamide) of the poly-L-tryptophan, and the fact that no racemization occurs during the polymerization of N-carboxy-α-amino acid anhydrides,[1,2] suggest that the polymer derived from α,N-carboxy-L-tryptophan anhydride contains L-tryptophan residues exclusively.

As expected, no cross-links between α-carboxyl and indole–NH groups occurred during polymerization. The formation of such links would cause a significant increase in the content of α-amino groups of the polymer. The solubility of the L- and DL-tryptophan polymers in various organic solvents supports the linear structure of these preparations.

The infrared absorption in the region 2.6 to 14 μ of films of poly-L-tryptophan and poly-DL-tryptophan cast from pyridine and acetone, respectively, were found identical with that reported for poly-DL-tryptophan prepared by an independent method.[4] The typical strong absorption at 2.9 μ indicates the presence of free indole NH groups in both polymers.

The absorption spectrum of poly-DL-tryptophan in butylamine–water (9:1 by volume) was measured between 2600 and 3000 Å. Two characteristic peaks, at 2840 Å. (molar extinction coefficient per tryptophan residue, ϵ 5470) and at 2920 Å. (ϵ 5030) are present, slightly lower than the peaks at 2840 Å. (ϵ 5780) and at 2920 Å. (ϵ 5280) found in the absorption spectrum of DL-tryptophan in the same solvent.

Experimental

All melting points are uncorrected. L-Tryptophan and DL-tryptophan from Nutritional Biochemicals Corporation were used throughout.

α,N-Carboxytryptophan Anhydride.—The DL-anhydride was prepared by passing phosgene for 45 minutes through a suspension of DL-tryptophan in dry dioxane maintained at 40°, from which oxygen had been removed by a stream of nitrogen (*cf.* Farthing)[5]; yield 84%; m.p. 142° (from ethyl acetate–petroleum ether) (dec. with carbon dioxide evolution).

Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.6; H, 4.4; N, 12.2; mol wt., 230.2. Found: C, 62.5; H, 4.5; N, 12.0; equiv. wt., 228, determined by titration with sodium methoxide in benzene using thymol blue as indicator.[7]

α,N-Carboxy-DL-tryptophan anhydride (100 mg.) yielded upon treatment with hot N hydrochloric acid 89.5 mg. of tryptophan (101% of the theoretical), as calculated from the extinction of the solution at 2805 Å and *p*H 12, using the molar extinction coefficient of tryptophan ϵ 5430.[8] When a drop of the neutralized solution was run on a descending paper chromatogram using 1-butanol–glacial acetic acid–water (4:1:5) as the mobile phase, one spot was obtained after spraying with ninhydrin, with R_f 0.46, identical with that of an authentic sample of DL-tryptophan run on the same strip.

α,N-Carboxy-DL-tryptophan anhydride is soluble in ethyl acetate, dioxane and benzene.

α,N-Carboxy-L-tryptophan anhydride was prepared from L-tryptophan analogously to the DL-derivative; yield 95%; m.p. 135° (from ethyl acetate–petroleum ether).

Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.6; H, 4.4; N, 12.2; mol. wt., 230.2. Found: C, 62.9; H, 4.8; N, 12.2; neut. equiv., 225.[7]

DL-Tryptophan methyl ester hydrochloride was prepared from the N-carboxy anhydride in the usual way (*cf.* preparation of ϵ-N-carboxylysine methyl ester hydrochloride[9]); m.p. 225° (from methanol–ethyl acetate). No depression in melting point was obtained on admixture with an authentic sample of DL-tryptophan methyl ester hydrochloride.[10]

Anal. Calcd. for $C_{12}H_{15}N_2O_2Cl$: C, 56.6; H, 5.9; N, 11.0; Cl, 13.9; CH_3O, 12.2. Found: C, 56.8; H, 5.8; N, 10.9; Cl, 13.9; CH_3O, 12.2.

Polytryptophan.—The DL-polymer was prepared by the polymerization of a twice recrystallized α,N-carboxy-DL-tryptophan anhydride at 150° in a high vacuum (10^{-4} mm.).[1] The polymer obtained was dissolved in hot glacial acetic acid and was precipitated with water slightly acidified with hydrochloric acid; quantitative yield.

Anal. Calcd. for poly-DL-tryptophan (n average 80): C, 70.9; H, 5.4; N, 15.0; amino-N, 0.094. Found: C, 69.0; H, 5.3; N, 15.0; amino-N, 0.094.[3]

In a preliminary experiment it was found that the carbon dioxide evolved during polymerization amounted to 96% of the theoretical.

Poly-DL-tryptophan (n average 80) is soluble in dioxane, acetone, butylamine, pyridine, dimethylformamide and hot glacial acetic acid. It is sparingly soluble in methanol, ethanol and ethyl acetate. It is insoluble in benzene, carbon tetrachloride and water.

Poly-L-tryptophan was prepared analogously to the DL-polymer; $[\alpha]^{25}D$ +147° (c 6 in dimethylformamide).

Anal. Calcd. for poly-L-tryptophan (n average 90): C,

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(9) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935).

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70.9; H, 5.4; N, 15.0; amino-N, 0.083. Found: C, 69.6; H, 5.5; N, 15.3; amino-N, 0.083.[3]

Poly-L-tryptophan differs in its solubility from the DL-polymer. It is soluble in dimethylformamide and pyridine, but insoluble in dioxane, acetone, butylamine, hot glacial acetic acid, methanol, ethanol and ethyl acetate.

An aqueous suspension of polytryptophan turns deep violet when treated with Hopkins–Cole reagent; it gives a positive ninhydrin reaction and a negative picric acid test.[11]

Hydrolysis of Polytryptophan.—Poly-DL-tryptophan (22.3 mg.) was dissolved in a mixture of dioxane (1 ml.), 4 *N* methanolic sodium methoxide (2 ml.) and water (0.2 ml.). Oxygen was removed by a stream of nitrogen and the hydrolysis carried out by heating in a sealed tube at 110–120° for 72 hours.

The amount of tryptophan in the hydrolysate was determined by its ultraviolet absorption[8] and colorimetrically.[12]

Anal. Calcd. for a hydrolysate of 100 mg. of poly-DL-tryptophan (*n* average 80): tryptophan, 109 mg. Found: tryptophan 105 mg. (ultraviolet absorption[8]); 107 mg. (colorimetric ninhydrin determination[12]).

Poly-L-tryptophan was hydrolyzed analogously to the DL-polymer; as it is insoluble in dioxane, pyridine was used instead. A quantitative yield of tryptophan was obtained also in this case.

A chromatographic analysis of the neutralized hydrolysate of poly-DL- and poly-L-tryptophan carried out as above yielded one spot with R_f 0.46 identical with that of authentic samples of DL- and L-tryptophan.

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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Nicotinyl and Isonicotinyl Hydrazones of Pyridoxal

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Nicotinyl and isonicotinyl hydrazones of pyridoxal, two new compounds that show interesting biological properties,[1] may be prepared easily by the following procedure.

Pyridoxal hydrochloride (product of Nutritional Biochemicals Corporation, 2 g.) in water (20 ml.) was treated with isonicotinic acid hydrazide[2] or nicotinic acid hydrazide[3] (1.4 g.) in 50% ethanol (20 ml.). Sodium acetate (1 g.) in water (10 ml.) was then added and the reactants were heated on the steam-bath for 10 minutes and allowed to stand for 24 hours at room temperature. The crude crystalline product was recrystallized from a mixture of methanol and benzene (1:2). The yield of the purified product was between 2.4 and 2.6 g.

Equally satisfactory results were obtained by using an alternate procedure which consisted of heating equivalent amounts of the reactants in pyridine and removing the solvent by steam distillation with the addition of sodium acetate.

Pyridoxal isonicotinyl hydrazone forms pale-yellow, small prisms either from dilute ethanol or from a mixture of methanol and benzene, m.p. 261–262° dec. (cor.).

Anal. Calcd. for $C_{14}H_{14}O_3N_4$: C, 58.72; H, 4.94; N, 19.57. Found: C, 58.55; H, 5.03; N, 19.77.

Pyridoxal nicotinyl hydrazone forms practically colorless, fine needles from dilute ethanol or thick platelets from a mixture of methanol and benzene, m.p. 235–236° dec. (cor.).

Anal. Calcd. for $C_{14}H_{14}O_3N_4$: C, 58.72; H, 4.94; N, 19.57. Found: C, 58.68; H, 4.87; N, 19.63.

These two compounds are very slightly soluble in cold water, slightly soluble in cold methanol or ethanol but soluble in hot; soluble in cold 10% sodium hydroxide; very soluble in dilute mineral acids; but insoluble in benzene or petroleum ether. A mixture of methanol and petroleum ether may also be used for recrystallization. Quantitatively, pyridoxal nicotinyl hydrazone is considerably more soluble than pyridoxal isonicotinyl hydrazone in most of the solvents tested.

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(1) Pyridoxal isonicotinyl hydrazone was found by W. B. Sutton of the Lilly Research Laboratories, Indianapolis, Indiana, to possess significant antitubercular activity *in vitro* as well as *in vivo;* its isomer, pyridoxal nicotinyl hydrazone, however, is much less active *in vitro* and inactive *in vivo*. Following this observation, both derivatives were found by Dr. Louis Greenberg of the University of California School of Medicine to be equal to pyridoxine in their vitamin B_6 activity. Recently, both were found by Dr. B. Freedlander of Mount Zion Hospital, San Francisco and Dr. A. Furst of Stanford University School of Medicine to show distinct activity against mammary cancer in mice and certain leukemia in mice. The details of these biological results will be reported by these investigators elsewhere.

(2) H. Meyer and J. Mally, *Monatsh.*, **33**, 393 (1912).

(3) Prepared by Dr. C. T. Peng of University of California College of Pharmacy according to the method described in the literature; "Beilsteins Handbuch der organischen Chemie," Bd. XXII, 41 (1935); Th. Curtius and E. Mohr, *Ber.*, **31**, 2493 (1898). It formed white, stout needles or rods from a mixture of benzene and dioxane, m.p. 163–164° (cor.).

A New Synthesis of Perfluoroaldehydes

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The preparation of perfluoroaldehydes has been described by reduction of the corresponding acid[1] or nitrile[2] with lithium aluminum hydride, by oxidative nitration of 1,1,1-trifluoropropane,[3] and by the Rosemund reduction of the corresponding acid chloride.[4] In this Laboratory it has been found that perfluoroaldehydes can be prepared by reduction of the corresponding perfluoroacid esters with lithium aluminum hydride at −70° in good yield (70–80%). The method employs a reverse addition technique and only small amounts of the by-product fluorine-containing alcohol are formed.

An explanation of the ready formation of aldehydes, in contrast to the usual alcohol formation from esters,[5] is not apparent at this time and is under investigation. Supporting work in this Laboratory has indicated that esters containing halogen atoms in the α- and β-positions will yield aldehydes on similar reductions. This would indicate that a strong inductive effect is a determining factor in the reaction mechanism.

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(4) Central Research Department, Minnesota Mining and Manufacturing Co., private communication.

(5) W. G. Brown, "Reductions by Lithium Aluminum Hydride," in Adams, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, pp. 469–509.