reduced pressure and stirred into excess water. The still clear solution was acidified with dilute hydrochloric acid, and the resulting yellow precipitate was filtered, washed with water, and recrystallized to yield fluffy yellow crystals melting at 296–297°. The product appears to be a nitro-hydroxy-methoxy-benzoic acid.

Anal. Calcd. for $C_8H_7NO_6$: C, 45.08; H, 3.31. Found: C, 45.10; H, 3.32.

Inasmuch as Bolliger and Reuter[3] reported a melting point of 220° for 2-hydroxy-3-methoxy-5-nitrobenzoic acid, this compound is probably 3-hydroxy-2-methoxy-5-nitro-benzoic acid.

Acknowledgment.—The authors wish to thank Mr. Harold Willemsen for the analyses and Dr. R. Donovick of the Squibb Institute for Medical Research for the values of the inhibiting concentrations for the pathogenic microörganisms reported in this paper.

(3) A. Bolliger and F. Reuter, *J. Proc. Roy. Soc. N. S. Wales*, **72**, 329 (1939).

THE INSTITUTE OF PAPER CHEMISTRY
APPLETON, WISCONSIN

Azo Derivatives of Some Aromatic Poly-α-amino Acids

BY MICHAEL SELA AND EPHRAIM KATCHALSKI

RECEIVED DECEMBER 29, 1954

In this paper the synthesis and properties of some azo derivatives of poly-α-amino acids containing aromatic amino acid residues are described. These compounds may serve as models in the study of azo-proteins, which are widely used in immunological studies as well as in the investigation of the chemical and biological properties of proteins.[1,2] The colored azopolypeptides obtained represent a new group of polymeric dyes.[3] Their synthesis permits the attachment through an azo link of different compounds to polyamino acids containing residues of tyrosine, histidine, tryptophan or *p*-aminophenylalanine.

Poly-3-(*p*-nitrophenylazo)-L-tyrosine and poly-3,5-di-(*p*-bromophenylazo)-L-tyrosine were prepared by coupling poly-L-tyrosine[4] with *p*-nitrobenzene or *p*-bromobenzene diazonium salts, respectively. For comparison 3,3′-di-(*p*-nitrophenylazo)-DL-tyrosine anhydride was prepared. Poly-*p*-(1-hydroxynaphthyl-4-azo)-DL-phenylalanine was obtained by coupling α-naphthol with diazotized poly-*p*-amino-DL-phenylalanine.[5] The diazonium salt derived from poly-*p*-aminophenylalanine also was coupled with tyrosine and with polytyrosine.

The absorption spectrum of poly-3-(*p*-nitrophenylazo)-tyrosine (*n* average 30) was determined at *p*H 13 between 2500 and 6000 Å. Two characteristic peaks were found, at 3460 Å. (residue molar extinction coefficient, ε 10000) and at 5300 Å. (ε 6350). An identical spectrum was found for 3,3′-di-(*p*-nitrophenylazo)-tyrosine anhydride. Two peaks, at 3460 Å. (ε 7250) and at 5300 Å. (ε 6350) were found in the absorption spectrum of 3-(*p*-nitrophenylazo)-*p*-cresol,[6] determined for comparison. The absorption spectrum of poly-*p*-(1-hydroxynaphthyl-4-azo)-phenylalanine (*n* average 20) in ethanolamine shows, between 2500 and 6000 Å., one characteristic peak, at 5180 Å. (ε 10000).

A copolymer of L-tyrosine and L-lysine, in a molar ratio of 1:10, was coupled with *p*-nitrobenzene diazonium chloride. The azopolypeptide obtained resembled polylysine in its solubility, influence on blood clotting[7] and antibacterial activity.[8] A copolymer of *p*-aminophenylalanine and L-aspartic acid, in a molar ratio of 1:9, was diazotized and coupled with α-naphthol. The colored derivative thus obtained resembled polyaspartic acid in solubility and biological activity.[7,8] These findings indicate that azo derivatives of α-amino acid copolymers containing a small percentage of aromatic amino acids might be used as tagged compounds in certain biological investigations.

Experimental

All melting points are uncorrected. The coupling reactions were carried out in 1*N* sodium hydroxide or sodium carbonate at 0–5° in the usual way. Absorption measurements were made on a Beckman model DU spectrophotometer, at approximately 25°.

3,3′-Di-(*p*-nitrophenylazo)-DL-tyrosine anhydride was prepared by coupling DL-tyrosine anhydride[9] (1 mole) with *p*-nitrobenzene diazonium chloride (2 moles), precipitated from reaction mixture with dilute hydrochloric acid and recrystallized from *t*-butyl alcohol; m.p. 243–245°, yield 91%.

Anal. Calcd. for $C_{30}H_{24}N_8O_5$: C, 57.7; H, 3.9; N, 17.9. Found: C, 57.3; H, 3.9; N, 17.6.

The brown azo compound dissolves readily in acetone, pyridine, butylamine, ethanolamine and ethylenediamine, is sparingly soluble in ethanol, chloroform and dioxane, and is insoluble in water, ether, benzene and carbon tetrachloride. Its solutions in aqueous or alcoholic sodium hydroxide are red-violet.

The azo derivative of tyrosine anhydride moved as a single red-violet band on an activated alumina column, when 1 *N* sodium hydroxide was passed through the column. When the substance was reduced and hydrolyzed for 12 hours in a boiling solution of stannous chloride (10%) in 6 *N* hydrochloric acid, and an aqueous solution of the dried reaction mixture was run on a paper chromatogram developed with *n*-butyl alcohol–water–acetic acid (4:5:1), only one spot, with R_f 0.28, was obtained upon spraying with ninhydrin. One spot, with R_f 0.80, was obtained when the paper chromatogram was developed with *n*-propyl alcohol–0.6% aqueous ammonia (2:1). Paper electrophoresis of the above hydrolysate was carried out on Whatman No. 1 filter paper in acetate buffer of *p*H 3.6 and ionic strength 0.2, at a potential gradient of 10 *V*/cm. at room temperature. A single spot, at a distance of 8.7 cm. from the origin toward the cathode, was revealed, after two hours, with ninhydrin. Tyrosine did not move from the origin under the same conditions. The homogeneity of the azo derivative of the tyrosine anhydride, the lack of tyrosine in the hydrolysate of the reduction product, as well as the appearance of one spot, most probably corresponding to that of 3-aminotyrosine, in the chromatographic and electrophoretic experiments, support the symmetric formula of 3,3′-di-(*p*-nitrophenylazo)-DL-tyrosine anhydride suggested.

Poly-3-(*p*-nitrophenylazo)-L-tyrosine was prepared by coupling poly-L-tyrosine[4] (*n* average 30) with *p*-nitrobenzene-diazonium chloride (one mole for each mole of tyrosine residue); precipitated with dilute hydrochloric acid, purified by dissolving in aqueous ammonia and reprecipitating with acetic acid; yield 93%.

(1) K. Landsteiner, "The Specificity of Serological Reactions," Harvard University Press, Cambridge, Mass., 1945.

(2) R. Herriott, *Advances in Protein Chem.*, **3**, 169 (1947).

(3) For other examples of polymeric dyes (*cf.* Th. Lieser and G. Nischk, *Ann.*, **569**, 66 (1950); D. M. McQueen and R. W. Woodward, THIS JOURNAL, **73**, 4930 (1951); B. Eisler and A. Wassermann, *Nature*, **172**, 72 (1953).

(4) E. Katchalski and M. Sela, THIS JOURNAL, **75**, 5284 (1953).

(5) M. Sela and E. Katchalski, *ibid.*, **76**, 129 (1954).

(6) H. Mehner, *J. prakt. Chem.*, **65**, 453 (1901).

(7) A. DeVries, A. Schwager and E. Katchalski, *Biochem. J.*, **49**, 10 (1951).

(8) E. Katchalski, L. Bichowski-Slomnitzki and B. E. Volcani, *ibid.*, **55**, 671 (1953).

(9) E. Fischer and W. Schrauth, *Ann.*, **354**, 35 (1907).

Anal. Calcd. for poly-3-(*p*-nitrophenylazo)-L-tyrosine (*n* average 30): C, 57.6; H, 3.9; N, 17.9. Found: C, 57.5; H, 4.2; N, 17.7.

Poly-3-(*p*-nitrophenylazo)-L-tyrosine is soluble in aqueous and ethanolic alkali (red-violet color), ethanol (orange-red), butylamine and ethanolamine (deep blue), and is insoluble in water, benzene and carbon tetrachloride.

Paper chromatography and paper electrophoresis of the product of reduction and hydrolysis of the polymeric azo derivative (10% stannous chloride–6 *N* hydrochloric acid, 12 hours) yielded results identical with those obtained in the case of 3,3′-di-(*p*-nitrophenylazo)-DL-tyrosine anhydride.

Poly-3,5-di-(*p*-bromophenylazo)-L-tyrosine was prepared by coupling poly-L-tyrosine (*n* average 28) with *p*-bromobenzenediazonium chloride (2 moles for each mole of tyrosine residue). After precipitation with dilute hydrochloric acid, the polymer was purified by dissolving in dilute ethanolic sodium hydroxide and reprecipitating with dilute sulfuric acid.

Anal. Calcd. for poly-3,5-di-(*p*-bromophenylazo)-L-tyrosine (*n* average 28): N, 13.2; Br, 30.1. Found: N, 13.0; Br, 29.9.

The azopolypeptide is soluble in dioxane and chloroform (yellow-greenish color), ethanolic alkali, aniline, pyridine and butylamine (dark red). It is sparingly soluble in acetone and carbon tetrachloride, and insoluble in water, ethanol, ether, aqueous acids and alkali.

Poly-*p*-(1-hydroxynaphthyl-4-azo)-DL-phenylalanine.—Poly-*p*-amino-DL-phenylalanine[5] (*n* average 20) was diazotized in the usual way, and the polymeric diazonium chloride coupled with α-naphthol. The product was precipitated with hydrochloric acid, dissolved in ethanolic sodium hydroxide, and reprecipitated with dilute hydrochloric acid.

Anal. Calcd. for poly-*p*-(1-hydroxynaphthyl-4-azo)-DL-phenylalanine (*n* average 20): C, 71.7; H, 4.8; N, 13.2. Found: C, 69.0; H, 4.9; N, 13.0.

Poly-*p*-(1-hydroxynaphthyl-4-azo)-DL-phenylalanine is soluble in ethanolic alkali (dark red color), dimethylformamide, pyridine, ethanolamine and dioxane–water (9:1 v./v.). It is sparingly soluble in aqueous alkali and in 90% ethanol, and it is insoluble in water, aqueous acids, absolute ethanol, anhydrous dioxane, benzene, acetone and chloroform.

Coupling of L-tyrosine with the diazonium salt derived from poly-*p*-aminophenylalanine (*n* average 20) yielded a **polymer of tyrosineazophenylalanine** which separated out. It was dissolved in hot dimethylformamide and precipitated with water.

Anal. Calcd. for $(C_{18}H_{18}N_4O_4)_{20}\cdot H_2O$: C, 61.0; H, 5.1; N, 15.8; equiv. wt., 354. Found: C, 60.9; H, 5.0; N, 15.9; neut. equiv., 370, determined in hot dimethylformamide by titration with 0.1 *N* sodium methoxide in benzene–methanol (1:1), using thymol blue as indicator.

The polymer is soluble in ethanolamine (red color) and dimethylformamide (yellow).

An insoluble product was obtained upon coupling poly-L-tyrosine (*n* average 30) with the diazonium salt derived from poly-*p*-aminophenylalanine (*n* average 20). On suspending the insoluble material in alkali it turned dark red.

Hydrobromide of a L-Lysine-L-tyrosine Copolymer.—ε,N-Carbobenzoxy-α,N-carboxy-L-lysine anhydride was mixed with O-carbobenzoxy-N-carboxy-L-tyrosine anhydride[4] in a molar ratio of 10:1. The mixture was polymerized in bulk[10] and the copolymer obtained was dissolved in dimethylformamide and precipitated with water.

Anal. Calcd. for a copolymer of ε,N-carbobenzoxy-L-lysine and O-carbobenzoxy-L-tyrosine in a molar ratio of 10:1 (*n* average 20): N, 10.2; amino N, 0.26. Found: N, 10.1; amino N, 0.26 (Van Slyke).

The N- and O-carbobenzoxy groups of the copolymer described above were removed with a 33% solution of anhydrous hydrogen bromide in glacial acetic acid[4,11] and the decarbobenzoxylated copolymer was precipitated with dry ether and purified by dissolving it in the minimum of water and precipitating with anhydrous ethanol and dry ether.

Anal. Calcd. for the hydrobromide of a copolymer of L-lysine and L-tyrosine in a molar ratio of 10:1 (*n* average 20): N, 13.0; amino N, 6.3; Br, 35.6; tyrosine residue, 7.2. Found: N, 13.1; amino N, 6.5 (Van Slyke); Br, 35.7; tyrosine residue, 7.3 (ultraviolet absorption).

Hydrochloride of a Copolymer of L-Lysine and 3-(*p*-Nitrophenylazo)-L-tyrosine (in a molar ratio of 10:1) (*n* average 20).—The hydrobromide of the lysine–tyrosine copolymer was coupled with *p*-nitrobenzenediazonium chloride (one mole for each mole of tyrosine residue). The colored copolymer was precipitated with picric acid, and the polymeric picrate was transformed into the hydrochloride.

Anal. Calcd. for the hydrochloride of a copolymer of L-lysine and 3-(*p*-nitrophenylazo)-L-tyrosine (in a molar ratio of 10:1) (*n* average 20): *p*-nitrophenylazotyrosine residue, 13.0. Found: 13.2, as estimated colorimetrically at 5300 Å. and *p*H 13 (residue molar extinction coefficient ε 6350).

Hydrobromide of a Copolymer of L-Aspartic Acid and *p*-Amino-DL-phenylalanine.—β-Benzyl-N-carboxy-L-aspartate anhydride was mixed with *p*,N-carbobenzoxyamino-α,N-carboxy-DL-phenylalanine anhydride[5] in a molar ratio of 9:1. The mixture was polymerized in bulk[12] and the copolymer obtained was dissolved in dimethylformamide and precipitated with water.

Anal. Calcd. for a copolymer of β-benzyl L-aspartate and *p*,N-carbobenzoxyamino-DL-phenylalanine in a molar ratio of 9:1 (*n* average 50): N, 7.2; amino N, 0.13. Found: N, 7.0; amino N, 0.13 (Van Slyke).

The benzyl and carbobenzoxy groups of the copolymer described above were removed with a 33% solution of anhydrous hydrogen bromide in glacial acetic acid[5,11] and the debenzylated copolymer was precipitated with ether.

Anal. Calcd. for a hydrobromide of a copolymer of L-aspartic acid and *p*-aminophenylalanine in a molar ratio of 9:1 (*n* average 50) N, 12.1; α-amino N, 0.22; Br, 6.2; neut. equiv., 127.7. Found: N, 12.1; amino N, 0.23 (Van Slyke); Br, 5.9; neut. equiv., 126, as determined by dissolving the copolymer in an excess of sodium hydroxide and back titration with hydrochloric acid, using phenolphthalein as indicator.

Copolymer of L-Aspartic Acid and *p*-(1-Hydroxynaphthyl-4-azo)-DL-phenylalanine.—The above described copolymer was diazotized and coupled with α-naphthol.

Anal. Calcd. for a copolymer of L-aspartic acid and *p*-(1-hydroxynaphthyl-4-azo)-DL-phenylalanine in a molar ratio of 9:1 (*n* average 50): *p*-(1-hydroxynaphthyl-4-azo)-phenylalanine residue, 23.4. Found: *p*-(1-hydroxynaphthyl-4-azo)-phenylalanine residue, 19.7, as estimated colorimetrically at 5180 Å. in ethanolamine solution (residue molar extinction coefficient ε 10000).

(10) E. Katchalski, I. Grossfeld and M. Frankel, THIS JOURNAL, **70**, 2094 (1948).

(11) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(12) A. Berger and E. Katchalski, THIS JOURNAL, **73**, 4084 (1951).

DEPARTMENT OF BIOPHYSICS
WEIZMANN INSTITUTE OF SCIENCE
REHOVOT, ISRAEL

Imidocarboxylate Homologs of Phenylcarbamates

BY D. STEFANYE, W. L. HOWARD AND W. BEIDLER

RECEIVED DECEMBER 8, 1954

Work published at the close of World War II by Templeman and Sexton[1] which mentioned the herbicidal potentiality of phenylcarbamate esters led to the investigation of a homologous series for toxicity. The resulting discovery of the plant growth regulating properties of isopropyl phenylcarbamate by this group[2] and corroborating work by Allard, *et al.*,[3] opened up a new field to botanical research.[4-6] Templeman[2] tested ethyl phenylimido-

(1) W. Templeman and W. Sexton, *Nature*, **156**, 630 (1945).

(2) W. Templeman and W. Sexton, *Proc. Roy. Soc.* (*London*), **B133** 480 (1946).

(3) R. Allard, *et al.*, *Botan. Gazette*, **107**, 589 (1946).

(4) R. Weaver, *ibid.*, **109**, 72 (1947).

(5) R. Weaver, *ibid.*, **109**, 276 (1948).

(6) W. Ennis, *ibid.*, **109**, 473 (1948).