ethanol raised the melting point to 271–274° dec.[23] Similar results were obtained with the dipyridyl sulfide **2b**. When this compound was heated at reflux in absolute ethanol for 8 hr., it was converted to the dipyridylamine **8b** in 79% yield. On the other hand, the nonacetylated derivative **1b** rearranged only to the extent of about 10% during a 21-hr. reflux period in the same solvent.

In another experiment, 23.9 mg. (0.0785 mmole) of the dipyridyl sulfide **2c** was heated at reflux for 68 hr. in 10 ml. of dry benzene. Under these conditions no rearrangement occurred, as evidenced by the lack of a color change during the heating period and the isolation of 17.6 mg. (74%) of starting material, m.p. 145–147°. A second crop yielded an additional 5.1 mg. (21%) of less pure starting material, m.p. 131–141°.

Attempted Rearrangement of 3-Acetamido-3′-nitro-2,2′-dipyridyl Sulfide (2b) in Dimethyl Sulfoxide.—A solution of 76.7 mg. of the dipyridyl sulfide **2b** in 1.0 ml. of distilled dimethyl sulfoxide was heated for 11 hr. in an oil bath at 80°. Water was added and the mixture extracted with chloroform. The organic solvent was removed in a dry air stream and the residual solid was recrystallized from benzene–petroleum ether, yielding 61.0 mg. (80%) of unchanged (infrared spectrum) 3-acetamido-3′-nitro-2,2′-dipyridyl sulfide as pale yellow crystals, m.p. 162–165° dec. One recrystallization raised the melting point to 169–170° dec. A mixture melting point with an authentic sample showed no depression.

Rearrangement of 3-Acetamido-3′-nitro-2,2′-dipyridyl Sulfide (2b) in Water.—A mixture of 15.0 mg. (0.0517 mmole) of the dipyridyl sulfide and 5 ml. of distilled water was heated at reflux for 45 hr. During this time, the starting material dissolved, followed by the appearance of a red solid which deposited on the sides of the reaction vessel. This solid was collected by filtration, yielding 10.1 mg. (79%) of crude 2-mercapto-3′-nitro-3,2′-dipyridylamine (**8b**), m.p. 228–232° dec., the structure of which was confirmed by comparison of its infrared spectrum with that of an authentic sample. A recrystallization from acetone–water raised the melting point to 235–236° dec.[23]

Rearrangement of 3-Amino-3′-nitro-2,2′-dipyridyl Sulfide (1b) in Water.—A mixture of 57.0 mg. of the sulfide **1b** and 10 ml. of water was heated on a steam bath. After a short time the water became slightly yellow, but for the most part the sulfide appeared to remain insoluble. The color of the water slowly changed to a red, and after 0.5 hr. of heating, dark red needles of 2-mercapto-3′-nitro-3,2′-dipyridylamine (**8b**) began to appear. Additional water was added from time to time to replace that lost by evaporation. After 8.5 hr., no more undissolved starting sulfide **1b** could be detected among the red crystals of product. After cooling, 50.2 mg. (88%) of the dipyridylamine **8b** was collected by filtration as dark red needles, m.p. 236–238° dec. One recrystallization from acetone raised the melting point to 238–241° dec.[23]

Rearrangement of 3-Amino-5′-nitro-2,2′-dipyridyl Sulfide (1a), 3-Amino-3′-nitro-2,2′-dipyridyl Sulfide (1b), and 3-Amino-3′-methyl-5′-nitro-2,2′-dipyridyl Sulfide (1c) in the Solid State.—The dipyridyl sulfides **1a–c** were heated in an oven at 110° for 9 days, after which time their infrared spectra indicated that complete rearrangement to the corresponding dipyridylamines (**8a–c**) had taken place. Recrystallization in each case yielded pure rearranged product.

Hydrolysis of N-Acetyl-2-methylthio-5′-nitro-3,2′-dipyridylamine (7a).—A mixture of 309 mg. (1.02 mmoles) of the dipyridyl amine, 5 ml. of ethanol, and 5 ml. of 5 *N* aqueous sodium hydroxide was heated at the boiling point for 10 min. The dark mixture was diluted with a large amount of water, whereby it turned orange. The solid which formed was collected by filtration, washed with water, and recrystallized from ethanol, yielding 203 mg. (76%) of 2-methylthio-5′-nitro-3,2′-dipyridylamine (**6a**) as yellow crystals, m.p. 142–143°.

Anal. Calcd. for $C_{11}H_{10}N_4O_2S$: C, 50.37; H, 3.84; N, 21.36. Found: C, 50.13; H, 3.87; N, 21.59.

Hydrolysis of N-Acetyl-2-methylthio-3′-methyl-5′-nitro-3,2′-dipyridylamine (7c).—By the same procedure described above, 198 mg. (0.62 mmole) of the N-acetyldipyridylamine (**7c**) yielded 131 mg. (77%) of product **6c**, m.p. 143–144°.

Hydrolysis of N-Acetyl-2-methylthio-3′-nitro-5′-methyl-3,2-dipyridylamine (7d).—By the procedure reported above, 214 mg. (0.67 mmole) of the N-acetyl derivative **7d** yielded 145 mg. (78%) of the deacetylated product **6d** as orange crystals, m.p. 149–150°.

Methylation of 2-Mercapto-3′-methyl-5′-nitro-3,2′-dipyridylamine (8c) and 2-Mercapto-5′-nitro-3,2′-dipyridylamine (8a).—Methyl iodide (1 ml.) was added to a hot solution of 169 mg. (0.64 mmole) of the dipyridylamine **8c** and 34 mg. of potassium hydroxide in methanol. The mixture was cooled and the yellow solid was collected by filtration, giving 124 mg. (70%) of the methylated derivative **6c** as yellow needles, m.p. 145–146°. Recrystallization from ethanol did not change the melting point.

By a similar procedure, the dipyridylamine **8a** gave the thiomethyl derivative **6a**, m.p. 139–140°, which showed no melting point depression on admixture with the thiomethyl product obtained by rearrangement of the dipyridyl sulfide **1a**.

The Synthesis of Polymers and Copolymers of β-(3-Pyridyl)-DL-alanine[1a,b]

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A reliable procedure for preparing β-(3-pyridyl)-DL-alanine in about 55% over-all yield, starting from 3-pyridylaldehyde, was developed. Methods for the preparation of synthetic polypeptides containing β-(3-pyridyl)-DL-alanine residues are described. These methods include the preparation of the carbobenzoxy derivative and the N-carboxy anhydride of pyridylalanine.

This paper describes the synthesis and polymerization of β-(3-pyridyl)alanine.[2] Polymers based on this amino acid are of interest because of their structural similarity to polypeptides of aromatic amino acids (phenylalanine, tyrosine, etc.) and because of the chemical similarity of pyridylalanine and histidine. The imidazole group of the latter amino acid is important for the catalytic activity of a number of enzymes.[6–9] In addition, both pyridyl and imidazole groups show interesting catalytic behavior.[10] Polypeptides described in this paper will subsequently be investigated as catalysts for ester hydrolysis and other reactions. Interesting catalytic behavior has already been reported for L-histidine containing polypeptides,[11–12] for poly(vinyl-

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(2) For leading references to peptide polymers, see E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958), and ref. 3–5.

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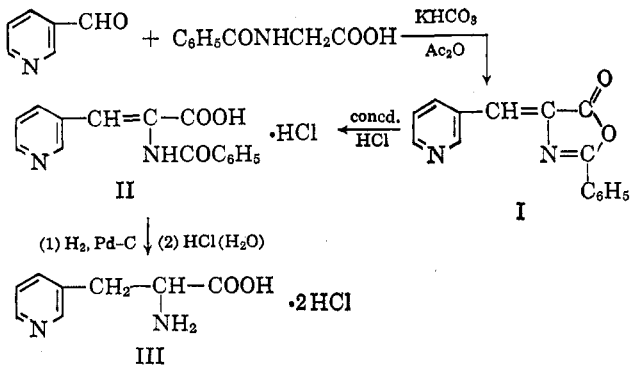
pyridine)[13] and for polymers containing vinylimidazole and vinylbenzimidazole units.[14]

Results

Synthesis of β-(3-Pyridyl)alanine.—The synthesis of β-(3-pyridyl)alanine has been previously described by Nieman, Lewis, and Hays[15] and by Wibaut, Wallingford, Rang, and Kettenes.[16] In both syntheses, however, the amino acid was prepared on a small scale and detailed experimental conditions were not given. The synthesis of β-(3-pyridyl)alanine employed in this work is outlined below.

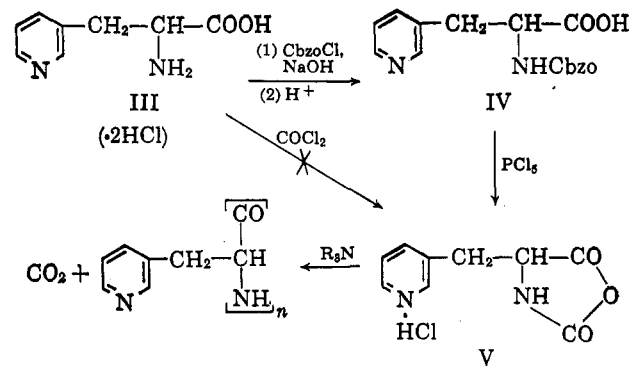


The first step in the synthesis, the condensation of 3-pyridylaldehyde with hippuric acid to yield the azlactone (I), was reported by Nieman and co-workers to produce low yields of a difficultly purified product, whereas Wibaut's group obtained the azlactone in 58–67% yield. The latter workers heated a mixture of the reactants and acetic anhydride for 0.5 hr. at 90–100° in the presence of fused sodium acetate. In our hands these comparatively severe conditions led to impure azlactone in only 14% yield. Lower reaction temperatures and shorter reaction times resulted in increased yields but the azlactone was usually accompanied by oils and tars which were difficult to remove completely and which had undesirable effects on subsequent steps. The use of potassium bicarbonate,[17] a more soluble catalyst for the condensation, permitted the reaction to occur at room temperature. In this way the azlactone was obtained in over 90% crude yield. Careful, but thorough, washing with ethanol purified the material adequately for subsequent reactions.

Wibaut and co-workers hydrogenated the azlactone directly and then hydrolyzed the hydrogenation product to obtain the amino acid. Our attempts to hydrogenate the azlactone met with considerable difficulty. Catalyst poisoning, slow hydrogenation rates, incomplete hydrogenation, and solubility problems were encountered. These difficulties were overcome by treating the azlactone with concentrated HCl at room temperature to obtain relatively pure crystals of the hydrochloride of α-benzamido-β-(3-pyridyl)-acrylic acid (II), in 82% yield. The hydrochloride was readily soluble in water and it was easily hydrogenated using a palladium catalyst. Catalyst poisoning was not encountered and the catalyst could be reused over twenty times without loss of effectiveness.

The hydrogenated product, α,N-benzoyl-β-(3-pyridyl)-alanine, could be isolated from the hydrogenation mixture in 52% yield,[18] but in this work it was convenient to hydrolyze the hydrogenation mixture directly, using concentrated hydrochloric acid. The dihydrochloride of β-(3-pyridyl)-DL-alanine (III) could thus be obtained in 78% yield. The dihydrochloride was usually used in subsequent steps, but the free amino acid could be obtained in 93% yield by treating a suspension of the dihydrochloride in ethanol with excess triethylamine.

N-Carboxy Anhydride Preparation.—The preparation of 4-(3-pyridylmethyl)oxazolidine-2,5-dione (V), the N-carboxy anhydride of β-pyridylalanine, was made difficult by the presence of a pyridyl group in the amino acid. The pyridyl group complicated attempts to phosgenate the amino acid, since hydrogen chloride generated in the phosgenation reaction formed insoluble salts with the amino acid and with any anhydride which may have formed. A number of solvents were used in these studies, including pyridine and triethylamine, but in no case was a manageable amount of anhydride obtained.



The N-carboxy anhydride of β-(3-pyridyl)alanine (V) was eventually prepared *via* the N-carbobenzoxy derivative of the amino acid (IV). However, the preparation of IV was also complicated by the presence of the pyridyl group in the amino acid. It was only after an extensive study, involving more than 40 preparations, that a procedure yielding IV in 34% yield was developed. It is difficult to reconcile the difficulty[19–21] encountered in this step with the fact that the carbo-

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(19) The unfavorable effect of pyridine and pyridine-containing compounds on carbobenzyloxylation reactions was dramatically illustrated in studies on the reaction of phenylalanine with carbobenzoxy chloride. This reaction, which was relatively insensitive to reaction conditions, usually produced N-carbobenzoxyphenylalanine in high yield. However, when the reaction was conducted in the presence of an equivalent of pyridine or one-third equivalent of IV, the yield of carbobenzoxyphenylalanine fell essentially to zero. It was therefore concluded that *organic soluble pyridine derivatives facilitate the decomposition of carbobenzoxy chloride in aqueous alkali*. Similar effects are observed[18] in the benzoylation or carbobenzoxylation of glycine, DL-alanine, and L-phenylalanine.

(20) The low yields obtained in the preparation of IV are attributed to the ability of IV, once formed, to rapidly promote the destruction of unreacted carbobenzoxy chloride. Formation of additional quantities of IV are therefore hindered. Preparations affording IV in higher yield can probably be developed by using nonaqueous solvent systems or by removing IV from the reaction, as formed.

(21) Benzoylation of pyridylalanine also does not proceed by conventional techniques. The only satisfactory route to α,N-benzoyl-β-(3-pyridyl)-DL-alanine, at present, is through hydrogenation of II.[18]

benzoxy derivative of 1-benzyl-L-histidine,[22-23] a very similar compound, and also the dicarbobenzoxy derivative of histidine[24-27] have been prepared by many workers in 70–80% yields, apparently without difficulty. It should be noted that IV was stable to the conditions of the carbobenzyloxylation reaction.

Preparation of 4-(3-pyridylmethyl)-oxazolidine-2,5-dione hydrochloride (V) from the carbobenzoxy derivative IV was accomplished by a procedure analogous to that employed by Patchornik, Berger, and Katchalski[27] for preparing the N-carboxy anhydride of 1-benzyl-L-histidine. Treatment of IV with a solution of PCl_5 in dioxane afforded V in 92% yield. The anhydride salt was extremely sensitive to moisture and all work had to be done in a drybox. On exposure to the atmosphere, the crystalline anhydride hydrochloride was converted in seconds to an amorphous mass. The anhydride was characterized by elemental analysis, by its neutralization equivalent, by its quantitative (94%) evolution of CO_2 on treatment with water, and by its infrared absorption at 1850 and 1780 cm.$^{-1}$.

Polymerization Studies.—The N-carboxyl anhydride of β-(3-pyridyl)alanine is an interesting monomer since it contains, within one molecule, a polymerizable group (NCA) and a group (pyridyl) capable of initiating polymerization.[28] The free anhydride was therefore expected to be an unstable material which would rapidly polymerize. When an equivalent of triethylamine was added to a suspension of V in dioxane, the free anhydride went into solution and triethylamine hydrochloride precipitated. The free anhydride slowly polymerized. Polymerization could also be initiated by diethylamine.

Polymers prepared by triethylamine- or diethylamine-initiated polymerizations were shown by end group analysis to have molecular weights ranging from 1500 to 3000. Polypeptides prepared with triethylamine were believed to have carboxyl groups at both chain ends, since they gave no nitrogen in Van Slyke determinations and since the neutralization equivalents of low molecular weight polymer fractions were approximately one-half of their molecular weights, as measured cryoscopically in *m*-dinitrobenzene. Polypeptides prepared with diethylamine had approximately equal amounts of carboxyl and amino end groups.

The polymers were characterized by elemental analysis, by their infrared and ultraviolet absorption, and by quantitative hydrolysis to β-(3-pyridyl)alanine dihydrochloride. Low values were obtained for the carbon and nitrogen contents of the polymers, although the C–N ratio was close to the expected ratio of 4. The analytical values obtained for the polymers were in reasonable agreement with those expected for a polymer containing one-half of a molecule of water per amino acid residue. Patchornik, Berger, and Katchalski[27] reported that poly(L-histidine) also retained approximately one-half a molecule of water per histidine residue, even after vigorous drying.

Copolymers of β-(3-pyridyl)-DL-alanine with γ-benzyl-L-glutamate and O-carbobenzoxy-L-tyrosine were prepared by copolymerizing mixtures of the corresponding anhydrides. The copolymers had molecular weights in the 4000–5000 range, as indicated by end-group analysis. They were characterized by chemical and spectroscopic analysis and by paper chromatographic examination of their hydrolysates. Treatment of the copolymers with anhydrous hydrogen bromide, in either dioxane or chloroform solutions, served to remove benzyl or carbobenzyloxy ester groups. In this way, pyridylalanine–tyrosine and pyridylalanine–glutamic acid copolymers were prepared.

Experimental

2-Phenyl-4-(3-pyridylmethylene)-2-oxazolin-5-one.—Hippuric acid (180 g., 1.0 mole) and potassium bicarbonate (40 g.) were dissolved in acetic anhyride (400 ml.) with stirring. The reaction vessel was cooled by a water bath and by two cold fingers placed within the flask to maintain a temperature near 20°. Pyridylaldehyde[29] (100 ml., 1.14 moles) was then added at once and the mixture was stirred for 1 hr. The semisolid, tan mixture was then poured into 2 l. of hot distilled water. The precipitate of 2-phenyl-4-(3-pyridylmethylene)-2-oxazolin-5-one, thus obtained, was filtered, washed with distilled water, and dried. The yield was 231 g. (92.4%), m.p. 157.5–160.5°. After being triturated with ethanol and redried, the product melted at 164.5° (lit.[16] m.p. 154–157°). The product showed the expected azlactone absorption at 1820 cm.$^{-1}$.

The compound could also be prepared using fused sodium acetate as a catalyst, according to the procedure of Wibaut. However, the product was obtained in lower yields (50–80%, depending on reaction conditions), and it was not so pure (m.p. after ethanol wash 158–160°) as the product obtained by the above procedure.

α-Benzamido-β-(3-pyridyl)acrylic Acid Hydrochloride.—Crude 2-phenyl-4-(3-pyridylmethylene)-2-oxazolin-5-one (160 g., 0.64 mole) was added to warm concentrated HCl with rapid stirring until no more would dissolve. The solution became bright red and, after a few minutes, began to deposit white needles. After cooling to room temperature, the mixture was filtered and the precipitate was washed with ethanol, then acetone and dried. The yield of α-benzamido-β-(3-pyridyl)acrylic acid hydrochloride, thus obtained, m.p. 208.5°, was 160 g. (82.5%).

Anal. Calcd. for $C_{15}H_{13}ClO_3$ (304.7): C, 59.12; H, 4.30; Cl, 11.63; N, 9.20. Found: C, 59.17, H, 4.52; Cl, 11.50, 11.40; N, 9.50.

The hydrochloride was readily soluble in water and in alkali. It was insoluble in ethanol, acetone, carbon tetrachloride, chloroform, and concentrated acids.

β-(3-Pyridyl)-DL-alanine Dihydrochloride.—A solution of α-benzamido-β-(3-pyridyl)acrylic acid hydrochloride (20 g., 0.067 mole) in 200 ml. of distilled water was shaken for 3–5 hr. with hydrogen at 50 p.s.i. in a Paar low-pressure hydrogenator, using 2.5 g. of 10% Pd–C as a catalyst. The uptake of hydrogen was essentially quantitative. The hydrogenation mixture was then filtered and concentrated *in vacuo* to about 30 ml.[30] The concentrate was then treated with about 10 ml. of concentrated HCl and the solution was refluxed overnight. After cooling and filtering to remove benzoic acid, the solution was again concentrated *in vacuo* to about 15 ml. The light brown concentrate was then added to a large amount of acetone with stirring to form a two-phase system. After 0.5–2.0 hr., a white precipitate formed. The precipitate was filtered, washed with ethanol, and dried to yield 12.4 g. (78.9%) of crude β-(3-pyridyl)-DL-alanine dihydrochloride, m.p. 190–200° dec. Recrystallization from hot ethanol yielded colorless needles, m.p. 238.5–241.5°.

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(29) Aldrich Chemical Co., Milwaukee 10, Wis.
(30) No attempt was made to isolate the intermediate α-benzamido-β-(3-pyridyl)propionic acid at this point. However, a picrate prepared from the concentrate melted at 176°. Wibaut[16] reported the picrate of α-benzamido-β-(3-pyridyl)propionic acid to melt at 167–169°.

Anal.[31] Calcd. for $C_8H_{12}Cl_2N_2O_2$ (239.1): C, 40.18; H, 5.06; Cl, 29.66; N, 11.72; neut. equiv., 239.1. Found: C, 40.35; H, 5.11; Cl, 29.82; N, 11.93; neut. equiv., 239.0.

β-(3-Pyridyl)-DL-alanine.—A suspension of β-(3-pyridyl)-DL-alanine dihydrochloride (2.355 g., 0.0985 mole) in 10 ml. of ethanol was treated with 20 ml. of triethylamine. A mildly exothermic reaction took place in which the particles of amino acid dihydrochloride appeared to burst open, yielding smaller particles. After stirring 1 hr., an additional 10 ml. of ethanol was added and the precipitate was filtered, washed with ethanol and chloroform, and then dried *in vacuo*. The yield of β-(3-pyridyl)-DL-alanine was 1.529 g. (93.2%), m.p. 266.5° (lit.[16] m.p. 262–263°).

Anal. Calcd. for $C_8H_{10}N_8O_2$ (166.2); C, 57.82; H, 6.07; N, 16.86; neut. equiv., 166.2. Found: C, 57.98; H, 6.22; N, 16.63; neut. equiv., 159.5.

An aqueous solution of the amino acid gave a negative silver nitrate test. A solution of the amino acid in 50% ethanol showed ultraviolet absorption at 255 mμ (log ε 3.41), 261 (3.48), and 267 (3.33). The amino acid gave a positive ninhydrin test.

Its picrate melted at 188–189° (lit.[16] m.p. 187–189°). The amino acid was soluble in water, slightly soluble in dimethylformamide, and insoluble in methanol, ethanol, ethyl acetate, and pyridine.

A sample of β-(3-pyridyl)-DL-alanine (0.3287 g., 1.978 mmoles) was reacted with concentrated HCl. The reaction mixture was concentrated to dryness, yielding 0.4737 g. (theoretical yield, 0.4729 g.) of the dihydrochloride salt. The infrared spectrum of this product was identical with that of β-(3-pyridyl)-DL-alanine dihydrochloride.

α,N-Carbobenzoxy-β-(3-pyridyl)-DL-alanine.—A solution of β-(3-pyridyl)-DL-alanine dihydrochloride (13.7 g., 0.057 mole) in a mixture of 50 ml. of water and 38 ml. of 4.16 *N* NaOH was rapidly stirred in an ice bath while 4.16 *N* NaOH (17 ml.) and carbobenzoxy chloride[32] (10 ml.) were added through separate dropping funnels in four portions over 4 hr. Stirring was continued for 2 hr. after the final addition, whereupon the flask contents were shaken with ether to remove organic by-products. The light yellow aqueous solution was then adjusted to pH 4.2. After about 15 min., crystals of α,N-carbobenzoxy-β-(3-pyridyl)-DL-alanine began to separate. These were filtered, washed with water, and dried. The optimum product yield, which was extremely sensitive to changes in reaction conditions, was 5.81 g. (34%). The product was easily purified by recrystallization from ethanol, m.p. 155.5–156.0°, λ_{max} 260 mμ (log ε 3.40).

Anal. Calcd. for $C_{16}H_{16}N_2O_4$ (300.3): C, 63.99; H, 5.37; N, 9.33; neut. equiv., 300.3. Found: C, 64.23; H, 5.49; N, 9.30; neut. equiv., 303.5.

The product was slightly soluble in ethanol (1.090 g./100 ml. at 25°) and water (0.025 g./100 ml. at 25°). It was insoluble in ether, dioxane, chloroform, and ethyl acetate.

Decarbobenzoxylation of α,N-Carbobenzoxy-β-(3-pyridyl)-DL-alanine.—A solution of α,N-carbobenzoxy-β-(3-pyridyl)-DL-alanine in glacial acetic acid was treated with gaseous HBr. After standing for 24 hr., the mixture was swamped with ether to precipitate the dihydrobromide of β-(3-pyridyl)alanine, m.p. 235°. The product had the same infrared spectra and melting point as did dihydrobromide prepared from the amino acid directly.

4-(3-Pyridylmethyl)oxazolidine-2,5-dione Hydrochloride.—A suspension of triply recrystallized α,N-carbobenzoxy-β-(3-pyridyl)-DL-alanine (2.92 g., 9.7 mmoles) in 50 ml. of dry dioxane[33] was treated, in a drybox and with rapid stirring, with a solution of phosphorus pentachloride (3.06 g., 14.7 mmoles) in 125 ml. of dioxane. After a few minutes, a transient solution occurred, followed by cloudiness. In about 2 hr., precipitation began. After about 6 hr., the precipitate was collected, stirred overnight with dry ether or chloroform to remove occluded PCl_5, filtered, and dried to yield 2.05 g. (92.4%) of 4-(3-pyridylmethyl)oxazolidine-2,5-dione hydrochloride.

Anal. Calcd. for $C_9H_9ClN_2O_3$ (228.6): C, 47.28; H, 3.97; Cl, 15.51; N, 12.25; neut. equiv., 114.3. Found: C, 47.00 H, 4.05; Cl, 14.48; N, 12.20; neut. equiv.,[34] 116.

The compound did not melt but foamed at 115° if suddenly immersed in a bath at this temperature. At lower temperatures, only slow decomposition occurred. The compound showed N-carboxyl anhydride absorption at 1860 and 1780 $cm.^{-1}$. It was soluble in pyridine and N,N-dimethylformamide (DMF), but insoluble in ether, chloroform, ethyl acetate, and petroleum ether.

The anhydride was very sensitive to moisture and all the operations described above had to be done in a drybox. A sample of the anhydride, when dissolved in distilled water, evolved CO_2 vigorously. The CO_2 formed was measured to be 94% of the theoretical amount.

Poly[β-(3-pyridyl)-DL-alanine]. Polymerization Initiated with Triethylamine.—A suspension of 4-(3-pyridylmethyl)oxazolidine-2,5-dione hydrochloride (V, 4.94 g., 21.6 mmoles) in 515 ml. of dry dioxane was stirred in a drybox and treated with triethylamine (3.00 ml., 21.6 mmoles). Triethylamine hydrochloride (2.29 g., 77.5%), which formed in the reaction, was removed by filtration. The clear filtrate was allowed to stand for 11 hr., after which time infrared analysis of the reaction mixture indicated that the N-carboxy anhydride had completely reacted. After standing an additional 40 hr., the reaction mixture was filtered to remove polymer (fraction A) which had precipitated, yield 1.02 g. (28.1%). Concentration of the filtrate yielded additional precipitate (fraction B), 0.18 g. (5.6%). Finally, addition of ether to the filtrate separated from fraction B yielded a third fraction (fraction C), 0.27 g. (8.3%).

Titration of fractions A, B, and C with standard sodium methoxide in methanol using bromthymol blue as an indicator showed them to have neutralization equivalents of 1515, 684, and 598, respectively. In addition, cryoscopic molecular weights of fractions B and C, determined in *m*-dinitrobenzene, were found to be 1320 and 1150, respectively.

Fraction A was characterized more extensively than the other fractions. Although initially soluble in water and DMF and insoluble in acetonitrile, chloroform, pyridine, and dioxane, its solubility properties changed after a few days, and the material became insoluble in water but soluble in methanol and ethanol and partially soluble in acetonitrile. The polymer showed strong absorption in the infrared at 1650 and 1550 $cm.^{-1}$ and its absorption in the ultraviolet region occurred at 255 mμ (log ε 3.347), 261 (3.401), and 267 (3.204). The specific viscosity of the polymer in methanol (*c* 0.298) was 0.082. The polymer showed negative chloride and ninhydrin tests.

Anal. Calcd. for $(C_8H_8N_2O)_n$: C, 64.85; H, 5.44; N, 18.91. Calcd. for $(C_8H_8N_2O)_n \cdot n/2nH_2O$: C, 61.13; H, 5.77; N, 17.82. Found: C, 62.11; H, 5.91; N, 16.23; NH_2 (Van Slyke), 0.00, 0.00.

Polymerizations initiated by triethylamine in other solvents, such as in DMF or in dioxane–nitrobenzene mixtures, were not so efficient as the above procedure and lower molecular products were obtained. Polymerizations initiated with excess triethylamine led only to dimeric products.

Initiation with Diethylamine.—A suspension of 4-(3-pyridylmethyl)oxazolidine-2,5-dione hydrochloride (2.69 g., 1.18 mmoles in 100 ml. of dry dioxane was treated with stoichiometric amounts of dry diethylamine (0.861 g., 1.17 mmoles) with stirring. The precipitate of diethylamine hydrochloride which formed was immediately removed by filtration and dried, yield 1.074 g. (83.4%). The filtrate was permitted to stand for 2 hr. while polymerization proceeded. The precipitate (fraction A) which formed was then filtered and dried. An additional fraction (fraction B) was obtained by treating the filtered reaction mixture with an excess of ether.

Fractions A and B exhibited neutralization equivalents of 2560 and 1435, respectively, when titrated with sodium methoxide in methanol against bromthymol blue. Fraction A showed strong peptide absorption at 1550 and 1650 $cm.^{-1}$. Fraction B showed, in addition, absorption at 1760 and 1710 $cm.^{-1}$, indicating the presence of hydantion-3-acetic acid residues. The polymers showed negative chloride and ninhydrin tests.

A sample of fraction A gave the following analysis.

Anal. Calcd. for $(C_8H_8N_2O)_n$: C, 64.85; H, 5.44; N, 18.91. Calcd. for $(C_8H_8N_2O \cdot 0.5\ H_2O)_n$: C, 61.13; H, 5.77; N, 17.82. Found: C, 62.29; H, 6.04; N, 17.32; NH_2 (Van Slyke), 0.76.

Initiation with Other Materials.—Solubility difficulties prevented the use of sodium methoxide or sodium hydroxide as initiators and the use of pyridine led only to low molecular weight polymer and appreciable quantities of free amino acid. Thermal decomposition of a suspension of the anhydride hydrochloride in

(31) This substance loses HCl on vacuum drying. Satisfactory analytical values are obtained only if vacuum-dried material is exposed to gaseous hydrogen chloride and then stored under N_2.

(32) A. C. Farthing, *J. Chem. Soc.*, 3213 (1950). Purity of the preparations used was always above 98%.

(33) K. Hess and H. Frahm, *Ber.*, **71**, 2627 (1938).

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

dioxane yielded appreciable amounts of pyridylalanine hydrochloride, neut. equiv. 199 (calcd., 202).

Acidic Hydrolysis of Poly[β-(3-pyridyl)-DL-alanine].—A sample of poly[β-(3-pyridyl)-DL-alanine] (58.2 mg., 0.393 mmole) was refluxed with 10 ml. of concentrated HCl for 2 days and the solution was then evaporated to dryness, yielding 90.5 mg. (96.4%) of pyridylalanine dihydrochloride. Identification of the product was by infrared analysis and by paper chromatography using a water–butanol–acetic acid (2.5:2.5:0.60) mixture as the partitioning solvent. Identical R_f values (0.11) were obtained for the product and for authentic pyridylalanine dihydrochloride.

Poly[β-(3-pyridyl)-DL-alanine–O-carbobenzoxy-L-tyrosine].—A suspension of 4-(3-pyridylmethyl)oxazolidine-2,5-dione hydrochloride (1.775 g., 7.77 mmoles) in 100 ml. of dioxane (drybox) was treated with triethylamine (1.06 ml., 7.65 mmoles) while being stirred. After a few minutes, the precipitate of triethylamine hydrochloride was filtered. The clear filtrate was mixed with a solution of O-carbobenzoxy-N-carboxy-L-tyrosine anhydride (4-benzyloxycarbonyloxybenzyl-2,5-oxazolidinedione, 2.462 g., 7.22 mmoles) in 50 ml. of dioxane, and the mixture was treated with 0.05 ml. of triethylamine (0.36 mmoles) to initiate polymerization. After 24 hr., infrared analysis showed polymerization to be complete. The reaction mixture was treated with excess ether and the precipitated polymer was filtered, extracted successively with boiling water, cold dimethylformamide, and ether. The polymer, 1.626 g. (49.3%), was then dried under vacuum.

Concentration of the filtered reaction mixture yielded 0.345 g. (10%) of material which showed no peptide absorption but which showed absorption at 1700 and 1750 $cm.^{-1}$, suggesting the presence of hydantoin-3-acetic acid derivatives. The neutralization equivalent of this fraction was 443. This fraction was soluble in methanol, chloroform, and dimethylformamide, but insoluble in ethanol, ether, and benzene.

The polymer fraction was insoluble in ether, ethanol, benzene, acetonitrile, and water. It showed amide absorption in the infrared at 1640 and 1550 $cm.^{-1}$ and ester absorption at 1770 $cm.^{-1}$. The polymer showed negative chloride and ninhydrin tests.

A sample of the polymer (71.6 mg.) was refluxed for 2 days with concentrated hydrochloric acid and the solution was evaporated to dryness leaving 69.4 mg. of residue. Ultraviolet analysis of the residue, using absorption at 267 and 292 mμ (pH 12.3) as a measure of the tyrosine and pyridylalanine present, indicated that the ratio of tyrosine to pyridylalanine in the copolymer was 1.14. Elemental analysis of the original copolymer was consistent with this result.

Anal. Calcd. for $C_8H_8N_2O$–1.14($C_{17}H_{15}NO_4$): C, 67.53; H, 5.19; N, 9.03. Found: C, 66.86; H, 5.48; N, 8.87; neut. equiv., 4200; NH_2 (Van Slyke), 0.24.

When a portion of the copolymer hydrolysate was submitted to paper chromatography using a water–butanol–acetic acid (2.5:2.5:0.60) solvent system, two spots were observed. These were identified by comparison with reference chromatograms as being due to L-tyrosine hydrochloride (R_f 0.41) and β-(3-pyridyl)-DL-alanine dihydrochloride (R_f 0.11).

Poly[β-(3-pyridyl)-DL-alanine–L-tyrosine].—Anhydrous hydrogen chloride was bubbled into a suspension of the above described [β-(3-pyridyl-DL-alanine–O-carbobenzoxy-L-tyrosine] copolymer in dry chloroform. Anhydrous hydrogen bromide was then introduced for 1 hr., followed by a nitrogen stream overnight. After filtration, the polymer was washed with chloroform and water. It was then treated several times with triethylamine, followed each time by washing with chloroform. Finally, the polymer was washed with acetone and dried. The ninhydrin test and tests for chloride ion were negative.

Acid hydrolysis of a sample of the copolymer, followed by quantitative ultraviolet analysis, as described above, established the tyrosine–pyridylalanine ratio to be 1.11 in the copolymer.

Anal. Calcd. for $C_8H_8N_2O$–1.11($C_9H_9NO_2$): C, 65.63; H, 5.51; N, 13.22. Found: C, 63.13; H, 6.06; N, 10.12. neut. equiv., 5000.

Poly[β-(3-pyridyl)-DL-alanine–γ-benzyl-L-glutamate].—A solution of 4-(3-pyridylmethyl)oxazolidine-2,5-dione hydrochloride (V, 2.268 g., 9.92 mmoles) in dry dioxane (150 ml.) was treated with 1.375 ml. (9.92 mmoles) of triethylamine. The precipitate of triethylamine hydrochloride, weighing 1.16 g. (85%), which formed was separated by filtration and a solution of γ-benzyl-N-carboxy-L-glutamic acid anhydride (2.566 g., 9.76 mmoles) in dioxane (150 ml.) was then added to the clear pyridylalanine NCA solution. Within a few hours, the solution developed a yellow-green color and showed fluorescence under ultraviolet light.

After five days, the reaction mixture was concentrated *in vacuo* to dryness. The brown residue was extracted with 100 ml. of benzene and the benzene was evaporated, yielding 1.362 g. of material (fraction A). The benzene insoluble fraction (fraction B) weighed 2.26 g. Neither fraction gave positive chloride or ninhydrin tests. Sodium methoxide titrations indicated fractions A and B to have neutralization equivalents of 2245 and 1018, respectively.

Portions of the two fractions were hydrolyzed with hydrochloric acid and the hydrolysates were submitted to paper chromatographic and spectrophotometric analysis. The paper chromatographic studies, using the butanol–water–acetic acid system, established the presence of glutamic acid (R_f 0.21) and pyridylalanine (R_f 0.13) in the hydrolysates. Ultraviolet study of the hydrolysates was made difficult by the presence of a highly colored impurity whith interferred with the analysis. This difficulty was encountered with all glutamic acid copolymer hydrolysates. A consideration of the ultraviolet results, in conjunction with C–N ratio and mass balance considerations led to the conclusion that fractions A and B contained approximately 30 and 60 mole % pyridylalanine residues, respectively.

Anal. of fraction A. Calcd. for $C_8H_8N_2O$–2.33($C_{12}H_{13}NO_3$): C, 65.54; H, 5.87; N, 9.19. Found: C, 65.55; H, 6.17; N, 9.37; NH_2 (Van Slyke), 0.00. Fraction B. Calcd. for ($C_8H_8N_2O$–0.67($C_{12}H_{13}NO_3$): C, 65.33; H, 5.71; N, 12.68. Found: C, 62.10; H, 6.01; N, 11.56; NH_2 (Van Slyke), 0.00.

Poly[β-(3-pyridyl)-DL-alanine–L-glutamic acid].—Portions of the pyridylalanine–γ-benzyl-L-glutamate copolymer fractions described above, 0.6421 g. and 0.9322 g. of fractions A and B, respectively, were dissolved in dioxane and the solutions were treated with anhydrous hydrogen bromide for 1 hr. At the end of this time, the solutions were swamped with ether, the supernatant liquids were decanted and the residues were treated with triethylamine in chloroform. Since the polymers dissolved in chloroform, the solutions were evaporated to dryness and the residues were dried under vacuum at 90° for several days. This treatment removed triethylamine hydrochloride, for the products did not react with silver nitrate solution. The yields obtained were very close to those expected, based on the estimated composition of the original polymers. Thus, the yield of product from fraction A was 0.483 g. (calcd., 0.436 g.) and the yield of product from fraction B was 0.734 g. (calcd., 0.742 g.)

These products were not submitted to elemental analysis, but analytical results were obtained on a similar product.

A sample of a pyridylalanine–γ-benzyl-L-glutamate copolymer containing about 30 mole % pyridylalanine residues (*Anal.* Calcd: C, 65.54; H, 5.87; N, 9.19. Found: C, 61.46; H, 6.34; N, 10.05.) was hydrolyzed by a procedure similar to that described above, except that chloroform was used as the initial solvent. The product contained about 35% pyridylalanine residues. Its analysis was reasonably consistent with this result.

Anal. Calcd. for $C_8H_8N_2O$–2($C_5H_7NO_3$)·H_2O: C, 50.94; H, 6.07; N, 13.20. Found: C, 50.80; H, 6.03; N, 12.07.

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