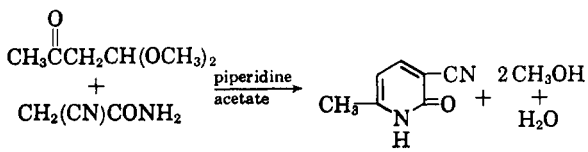
thetic programs centered around the B vitamins and other physiologically active pyridines. The starting materials employed in its preparation[1-4] have been difficult to handle. Recently, since we required kilogram quantities of 3-cyano-6-methyl-2(1H)pyridone, we evaluated the various synthetic methods. In the method of Matsukawa and Matsuno,[1] and, subsequently, that of Perez-Medina, Mariella and McElvain,[2] a condensation of sodium formyl acetone and cyanoacetamide was utilized. However, we found that the yield and quality varied widely when this method was employed. More recently, Kochetkov[3] reported an attractive method, wherein 3-ketobutyraldehyde-1-dimethyl acetal is condensed with cyanoacetamide under aqueous conditions with piperidine acetate as a catalyst. Furthermore, 3-ketobutyraldehyde-1-dimethylacetal[5] had recently become commercially available.[6]



Our experimental work has demonstrated that the desired pyridone is quickly obtained in good yield and high purity. A typical preparation is outlined below.

EXPERIMENTAL

A mixture of 650 g. (5.0 moles) of 3-ketobutyraldehyde-1-dimethylacetal (Henley & Co., n_D^{25} 1.4160), 462 g. (5.5 moles) cyanoacetamide (Eastman), 1 l. of water, and piperidine acetate solution[7] was prepared in a 3-l., three-necked, round bottom flask equipped with a mechanical stirrer and reflux condenser.

On gentle heating (about 80°), a clear solution was obtained. This was heated under reflux for 24 hr. in a Glas-Col mantle. The product began to precipitate after 1 hr. at reflux. After the reaction was complete, the reddish-tan slurry was cooled to 20°, filtered, and washed well with cold water. The light tan product was dried overnight in a steam oven at 50–60°; yield, 545 g. (81%), m.p. 285° dec.

This procedure has been extremely satisfactory on a 1- to 17-mole scale and has presented few operational problems. It obviates the need for organic solvents and difficultly handled starting materials. The product thus prepared was satisfactory for use in subsequent steps without further purification.

Organic Chemical Research Section
Lederle Laboratories Division
American Cyanamid Co.
Pearl River, N. Y.

(3) N. K. Kochetkov, *Doklady Akad. Nauk U.S.S.R.*, **84**, 289(1952), *Chem. Abstr.*, **47**, 3309a (1953).
(4) R. P. Mariella, *Org. Syntheses*, **32**, 32 (1952).
(5) A. C. Cope, *J. Am. Chem. Soc.* **59**, 2327 (1937).
(6) Henley & Co., New York, N. Y.
(7) The piperidine acetate was prepared by adding piperidine (about 25 ml.) to 100 ml. of 20% acetic acid solution until a *p*H of 9–10 was reached.

Synthesis of L-Valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine Methyl Ester Dihydrochloride

Edward Walton, John Otto Rodin, Charles H. Stammer and Frederick W. Holly

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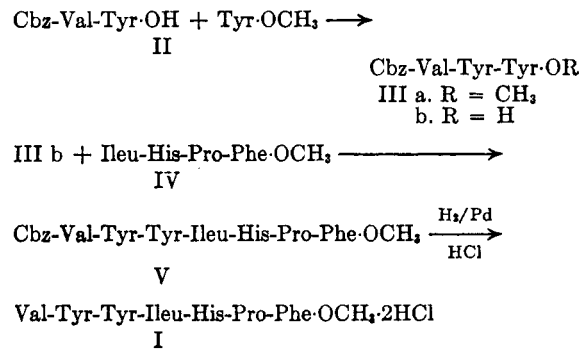
During the course of work related to the angiotensin peptides, the heptapeptide ester, L-valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrochloride (I), was synthesized. This paper reports the methods of synthesis and purification which were used. The scheme shown below indicates the order in which the amino acids were coupled together forming the final sequence I. *N*-Carbobenzyloxy-



L-valyl-L-tyrosyl-L-tyrosine methyl ester (III a) was obtained by coupling *N*-carbobenzyloxy-L-valyl-L-tyrosine[1] with L-tyrosine methyl ester[2] using dicyclohexylcarbodiimide[3] in dimethylformamide. Alkaline hydrolysis of the crystalline ester IIIa formed the acid IIIb which after adsorption on alumina and elution with methanol–formic acid was obtained crystalline. This acid was coupled with L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester[4] (IV) using dicyclohexylcarbodiimide, and chromatography of the crude product on alumina gave the crystalline *N*-carbobenzyloxy-heptapeptide ester V. Hydrogenation of V over a palladium catalyst followed by addition of hydrogen chloride yielded the heptapeptide ester I.

EXPERIMENTAL

Melting points were taken on a Kofler Micro Hot Stage. Radial paper chromatograms[5] were done on 32-cm. Whatman No. 1 circles. The developing solvent mixtures are desig-

(1) H. Schwarz and F. M. Bumpus, *J. Am. Chem. Soc.*, **81**, 890 (1959).
(2) E. Fischer and W. Schrauth, *Ann.*, **354**, 34 (1907).
(3) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
(4) W. Rittel, B. Iselin, H. Kappeler, B. Riniker, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 614 (1957).
(5) E. Lederer and M. Lederer, *Chromatography*, Second Ed., Elsevier Publishing Company, New York, N. Y., 1957, p. 134.

nated as below: *BAW*—butanol: acetic acid: water—4:1:5. The upper phase was used: *BAm*—butanol: 1.5*N* ammonium hydroxide—1:1. The upper phase was used. *MPW*—methyl ethyl ketone: pyridine: water—4:1:1.6. The compounds were located on the paper by means of ninhydrin (N) or diazotized sulfanilic acid (P). A compound which has an R_5 value of 0.5 in the MPW system and was located with ninhydrin reagent is reported as R_f^{MPW} 0.5 (N).

*N-Carbobenzyloxy-*L*-valyl-*L*-tyrosyl-*L*-tyrosine methyl ester* (IIIa). A solution of 12.5 g. of L-tyrosine methyl ester[2] in 92 ml. of *N,N*-dimethylformamide was cooled to about 0° in an ice bath and 13.5 g. of *N,N'*-dicyclohexylcarbodiimide was added. A cold solution of 26.6 g. of *N*-carbobenzyloxy-L-valyl-L-tyrosine[1] in 180 ml. of ethyl acetate was added dropwise during a period of 30 min. while the reaction mixture was stirred. After 2 hr. at 0°, the mixture was kept at room temperature for 16 hr. The *N,N'*-dicyclohexylurea (12 g.; 82%) was removed and the filtrate was concentrated at reduced pressure. The residual oil (55 g.) was dissolved in 150 ml. of warm ethyl acetate and kept at 0° for 2 hr. About 0.2 g. of additional *N,N'*-dicyclohexylurea was removed. The ethyl acetate solution was washed with 100 ml. of saturated sodium bicarbonate solution and 100 ml. of saturated sodium sulfate solution. The product (23 g.) crystallized rapidly from the ethyl acetate layer. Recrystallization from 75 ml. of methanol gave 16.5 g. of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosine methyl ester, m.p. 173–176°. A 0.5-g. sample of the product, recrystallized from 5 ml. of methanol, gave 0.38 g. of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosine methyl ester, m.p. 175–177°; $[\alpha]_D^{25}$ −6.0° (*c*, 2.02 in *N,N*-dimethylformamide).

Anal. Calcd. for $C_{32}H_{37}N_3O_8$ (591.6). C, 64.96; H, 6.30; N, 7.10. Found: C, 65.27; H, 6.73; N, 6.97.

*N-Carbobenzyloxy-*L*-valyl-*L*-tyrosyl-*L*-tyrosine* (IIIb). *N*-Carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosine methyl ester (9.4 g.; 15.9 mmoles) was dissolved in a mixture of 100 ml. of methanol, 50 ml. of acetonitrile and 32.7 ml. of 1.0*N* sodium hydroxide. An additional 32.7 ml. of 1.0*N* sodium hydroxide and 50 ml. of water were added and the solution was kept at room temperature for 1.5 hr. The reaction mixture was diluted with 250 ml. of water and acidified (*p*H 1) with concentrated hydrochloric acid. After being cooled in an ice bath for 1 hr., the mixture was filtered. The dried product, m.p. 175–205°, weighed 9.7 g.

The product above was combined with similar material from another preparation giving a total of 11.9 g. which was dissolved in 250 ml. of boiling acetone. The cooled solution was chromatographed on a column of 300 g. of acid-washed alumina. Elution with 1200 ml. of acetone gave 1.5 g. of recovered starting ester. Elution with 1 l. of methanol–formic acid (9:1) gave, after concentration of the eluate to a volume of 100 ml., 7.2 g. of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosine, m.p. 214–217°.

A 0.5-g. sample was dissolved in 15 ml. of acetone and 50 ml. of water was added; 0.45 g. of crystalline product was obtained, m.p. 215–217°, $[\alpha]_D^{25}$ +1.7° (*c*, 2.33 in *N,N*-dimethylformamide); R_f^{BAm} 0.5–0.7(P).

Anal. Calcd. for $C_{31}H_{35}N_3O_8$ (577.61): C, 64.46; H, 6.11; N, 7.28. Found: C, 64.57; H, 6.26; N, 7.15.

*N-Carbobenzyloxy-*L*-valyl-*L*-tyrosyl-*L*-tyrosyl-*L*-isoleucyl-*L*-histidyl-*L*-prolyl-*L*-phenylalanine methyl ester* (V). A solution of 4.0 g. of L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanyl methyl ester dihydrobromide[4] in 100 ml. of water was washed with 50 ml. of ethyl acetate. The aqueous phase plus 200 ml. of chloroform was cooled and adjusted to *p*H 10 with saturated potassium carbonate solution. The chloroform layer was separated and the aqueous layer was extracted with an additional 100 ml. of chloroform. The dried chloroform solution was concentrated at reduced pressure. About 2.2 g. of L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester was obtained as an oil.

This tetrapeptide methyl ester was dissolved in 20 ml. of ethyl acetate; 0.9 g. (4.3 mmoles) of *N,N'*-dicyclohexylcarbodiimide was added. The mixture was cooled to about 0° and a solution of 2.4 g. (4.2 mmoles) of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosine in 15 ml. of *N,N*-dimethylformamide was added dropwise. Stirring was continued at room temperature for 16 hr.

The precipitated *N,N'*-dicyclohexylurea (0.42 g.) was removed and the filtrate was concentrated at reduced pressure. The residue was dissolved in 100 ml. of ethyl acetate-*n*-butyl alcohol (3:1) and the solution was washed with two 50-ml. portions of 1*N* hydrochloric acid, two 50-ml. portions of saturated aqueous sodium bicarbonate and 25 ml. of water. The organic phase was concentrated at reduced pressure and a 4.7-g. residue was obtained. This was dissolved in 50 ml. of warm acetonitrile. When the solution was cooled, 2.2 g. of crude substituted heptapeptide precipitated. For further purification, it was dissolved in 20 ml. of isopropyl alcohol and chromatographed on 150 g. of acid-washed alumina. Elution with isopropyl alcohol containing increasing amounts of methanol gave a total of 0.46 g. of product containing impurities. Finally, elution with methanol gave 1.3 g. of product. This material was dissolved in 100 ml. of acetone, 400 ml. of water was added, and the solution was concentrated to 250 ml., giving 0.95 g. of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester, m.p. 205–210°, $[\alpha]_D^{25}$ −59° (*c*, 2.0 in methanol). A sample after treatment with hydrogen bromide in acetic acid showed R_f^{BAW} 0.78 (N).

Anal. Calcd for $C_{58}H_{71}N_9O_{12}$ (1086.2). C, 64.13; H, 6.59; N, 11.61. Found. C, 63.85; H, 6.79; N, 12.27.

L-Valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrochloride (I). A mixture of 817 mg. of *N*-carbobenzyloxy-L-valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester, 400 mg. of 10% palladium on charcoal and 50 ml. of acetic acid was shaken with hydrogen at low pressure for 16 hr. The catalyst was removed and the filtrate was concentrated at reduced pressure. The product showed R_f^{BAW} 0.80 (N) and R_f^{MPW} 1.0 (N), while L-valyl-L-tyrosyl-L-tyrosine showed R_f^{BAW} 0.70 (N) and R_f^{MPW} 0.80 (N) and L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine showed R_f^{BAW} 0.70 (N) and R_f^{MPW} 0.90 (N).

The product was dissolved in 6 ml. of methanol and treated with dry hydrogen chloride. When ether was added, the hydrochloride precipitated. This material was reprecipitated from 3 ml. of methanol with 50 ml. of acetone. The yield of L-valyl-L-tyrosyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrochloride, $[\alpha]_D^{25}$ −34.3° (*c*, 1.05 in 0.1*N* hydrochloric acid) was 630 mg.

Anal. Calcd. for $C_{50}H_{67}N_9O_{10}Cl_2$ (1024.1). C, 58.60; H, 6.59; Cl, 6.92. Found: C, 58.66; H, 6.59; Cl, 7.27; ash, 1.4.

The ratios of the amino acids in this product relative to phenylalanine were Val, 1.04; Tyr, 1.91; Ileu, 0.98; His, 0.96; Pro, 1.05; Phe, 1.00.[6]

MERCK SHARP & DOHME RESEARCH LABORATORIES
DIVISION OF MERCK & CO., INC.
RAHWAY, N. J.

(6) Amino acid analyses were carried out in the laboratories of Prof. M. Brenner of the Organisch-Chemische Anstalt der Universität, Basel, Switzerland by the Moore and Stein method.

Improved Preparation of Phosphorylethanolamine and Phosphorylcholine

JOHN H. WEISBURGER AND WALTER C. SCHNEIDER

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The cytidine and deoxycytidine diphosphates of choline and ethanolamine occur in living matter