Raney nickel. Compound XV gave the known[18] thiosemicarbazone (XVI) and hydrazone (XVII) upon refluxing with solutions of thiosemicarbazide and hydrazine, respectively. Oxidation of XV with $KMnO_4$ afforded purine-6-carboxylic acid (XVIII).[18,19]

6-Mercaptopurine from 6-Hydrazinopurine.—6-Hydrazinopurine (XIX)[21] (0.20 g., 1.3 mmole) was dissolved in thiolacetic acid (2 ml.) and refluxed for 12 hr. The reaction product was washed with ether, dried, dissolved in dilute ammonia, treated with charcoal, and filtered. Upon neutralization with dilute acetic acid, 0.12 g. (60%), light cream-colored crystals were obtained, m.p. 298–301°, dec. The resulting product was identical with an authentic sample of 6-mercaptopurine (XXIII)[22] in ultraviolet spectral and chromatographic properties.

6-Mercaptopurine was also obtained by similar treatment from 6-N-hydroxylaminopurine (XX)[23] in 30% yield, from 6-chloropurine (XXI)[24] and 6-iodopurine (XXII)[25] in almost quantitative yield.

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Synthesis of a Renin Substrate

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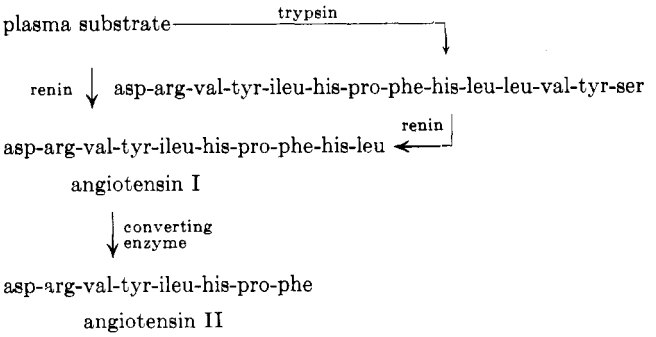
The synthesis of L-prolyl-L-phenylalanyl-L-histidyl-L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine, a substrate for the enzyme renin, is described. A significant improvement in the azide coupling procedure has been found.

A subject of intense study in recent years has been the renin–angiotensin system as it may be involved in normal homeostasis of blood pressure and in certain forms of hypertension.[1] One function of renin is to cleave a plasma substrate found in the α-globulin fraction to angiotensin I, a decapeptide.[2] The latter is then changed by a converting enzyme to the active hormone, angiotensin II, an octapeptide. Preliminary treatment of plasma substrate with trypsin gives a tetradecapeptide, asp-arg-val-tyr-ileu-his-pro-phe-his-leu-leu-val-tyr-ser,[3,4] which reacts with renin to liberate angiotensin I. The structure of the tetradecapeptide was confirmed by synthesis.[5] The anomalous reaction of trypsin to produce a peptide with C-terminal serine has never been explained. Chart I shows the

CHART I



sequence of reactions. Recent evidence indicates that the renin–angiotensin system may also control aldosterone synthesis.[6] If this is true, renin becomes an even more important enzyme than previously thought.

The present work stems from a program to find inhibitors of renin. The search for a renin inhibitor represents one rational approach to the discovery of antihypertensive drugs and possibly also to new types of antialdosterone compounds.[7,8] It is easily seen that an effective and selective renin inhibitor could have profound blood pressure effects. For purposes of reproducibility and control of variables, it was decided that an *in vitro* assay would be desirable and that a synthetic substrate would be preferable to one isolated from blood.[9] For a successful screening test, the substrate must be available in reasonable quantity and equally important its synthesis must permit repetition as new supplies are needed without each occasion requiring a major research effort.

Reports have appeared concerning a renin substrate, pro-phe-his-leu-leu-val-tyr-ser (**11**), which represents the C-terminal octapeptide from the tryptic tetradecapeptide.[10] Both substrates are cleaved by renin at the same position, at the leu–leu bond, although in the case of the octapeptide, the product, pro-phe-his-leu, has no pressor activity. Because of the same position of enzymic hydrolysis in the two peptides, it is reasonable to hope that renin inhibitors found by utilizing the octapeptide substrate might be active in intact animals. The octapeptide was first synthesized by coupling Z-pro-phe and his-leu-leu-val-tyr-ser-OMe with subsequent re-

(1) I. H. Page and F. M. Bumpus, *Physiol. Rev.*, **41**, 331 (1961).

(2) A. S. Plentl, I. H. Page, and W. W. Davis, *J. Biol. Chem.*, **147**, 143 (1943); A. S. Green and F. M. Bumpus, *ibid.*, **210**, 281 (1954).

(3) Abbreviations are based on the proposals of E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947). In addition, Z = carbobenzoxy, OMe = methyl ester, ONB = *p*-nitrobenzyl ester, and ONP = *p*-nitrophenyl ester.

(4) L. T. Skeggs, Jr., J. R. Kahn, K. Lentz, and N. P. Shumway, *J. Exptl. Med.*, **106**, 439 (1957).

(5) L. T. Skeggs, Jr., K. E. Lentz, J. R. Kahn, and N. P. Shumway, *ibid.*, **108**, 283 (1958).

(6) J. H. Laragh, *Circulation Res.*, **9**, 792 (1961); *Circulation*, **25**, 203 (1962). J. Mulrow and W. F. Ganong, *Yale J. Biol. Med.*, **33**, 386 (1961). J. O. Davis, C. C. J. Carpenter, and C. R. Ayers, *Circulation Res.*, **11**, 171 (1962). J. H. Laragh and W. G. Kelly in "Advances in Metabolic Disorders," Vol. 1, Academic Press, New York, N. Y., 1964, p. 218.

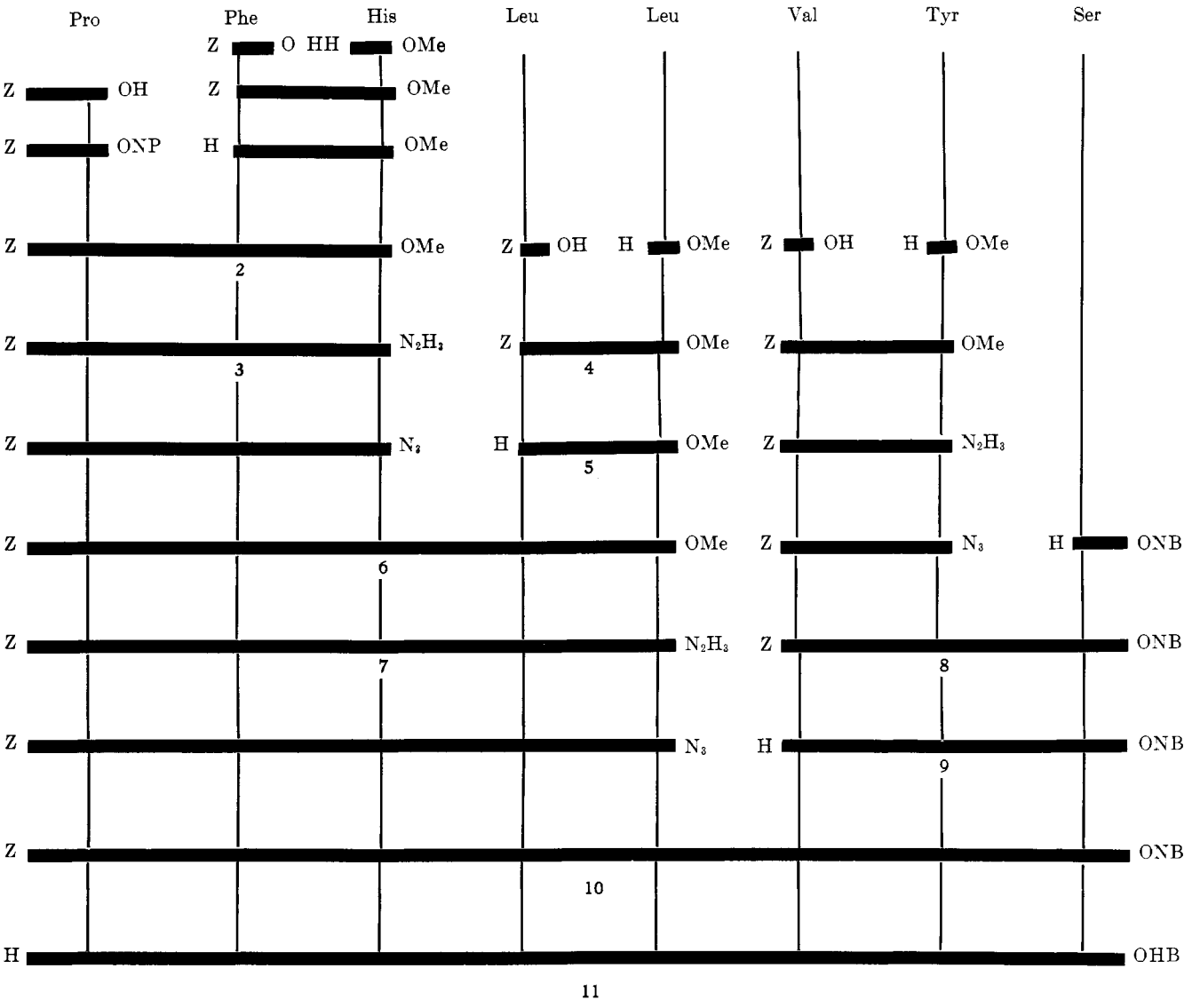
(7) J. A. Cella and R. C. Tweit, *J. Org. Chem.*, **24**, 1109 (1959).

(8) N. W. Atwater, *et al.*, *ibid.*, **26**, 3077 (1961).

(9) The assay is being developed in our laboratories by P. S. Cammarata and C. Hsu.

(10) F. M. Bumpus, R. R. Smeby, and I. H. Page, *Circulation Res.*, **9**, 762 (1961). The structure of the octapeptide was mentioned in the discussion of this paper. L. T. Skeggs, *Federation Proc.*, **20**, 465 (1961). Only the title of a symposium paper, "Peptides of the Renal Pressor System," is given in the journal. The paper described the octapeptide but an abstract seems not to have been published. H. Hochstrasser and J. R. Kahn, *Federation Proc.*, **22**, 542 (1963).

CHART II



moval of protecting groups.[11] The latter hexapeptide was available from the original synthesis of tetradecapeptide substrate.[5] Our purposes required a synthesis amenable to large-scale operations and to alteration in key positions to make analogs readily available. The scheme is outlined in Chart II. It may be seen that changes in both the N-terminal and C-terminal amino acids and in the structure at the reaction site (leu–leu) are particularly easily effected.

Carbobenzoxyphenylalanine and histidine methyl ester were coupled by the dicyclohexylcarbodiimide method[12] to carbobenzoxyphenylalanylhistidine methyl ester (**1**).[13] The latter was treated with hydrobromic acid in acetic acid[14] and the crude dihydrobromide, after neutralization, reacted with carbobenzoxyproline *p*-nitrophenyl ester to give carbobenzoxyprolylphenylalanylhistidine methyl ester (**2**).[13] The ester **2** yielded carbobenzoxyprolylphenylalanylhistidine hydrazide (**3**). Carbobenzoxyleucylleucine methyl ester (**4**) was prepared from carbobenzoxyleucine and leucine methyl ester hydrochloride using dicyclohexylcarbodiimide. The hydrobromide **5 HBr** was coupled with tripeptide **3** by the azide method to yield carbobenzoxyprolylphenylalanylhistidylleucylleucine methyl ester (**6**) which was converted to the corresponding hydrazide **7**.

Carbobenzoxyvalyltyrosine hydrazide and serine *p*-nitrobenzyl ester *p*-toluenesufonate[15] gave, using the azide procedure, carbobenzoxyvalyltyrosylserine *p*-nitrobenzyl ester (**8**). Treatment with hydrobromic acid in trifluoroacetic acid yielded the tripeptide ester hydrobromide which was isolated as the crystalline free base, valyltyrosylserine *p*-nitrobenzyl ester (**9**). The use of trifluoroacetic acid avoided acetylation of the serine hydroxyl group,[16] a rapid reaction under the usual conditions with hydrobromic in acetic acid.[17] Reaction of hydrazide **7** with ester **9** by the azide method gave the protected octapeptide **10** which was hydro-

(11) We would like to express our warmest appreciation to L. T. Skeggs, K. E. Lentz, and J. R. Kahn for supplying us with full experimental details of their synthesis.

(12) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(13) H. A. DeWald and E. D. Nicolaides, *J. Med. Chem.*, **7**, 50 (1964).

(14) D. Ben-Ishai, *J. Org. Chem.*, **19**, 62 (1954).

(15) R. H. Mazur and J. M. Schlatter, *ibid.*, **28**, 1025 (1963). The rotation of serine *p*-nitrobenzyl ester *p*-toluenesulfonate should be $[\alpha]^{25}D$ −5° (*c* 1, methanol). We would like to thank Dr. J. Jentsch of Munich for calling this to our attention.

(16) S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, **42**, 1257 (1959).

(17) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **28**, 1926 (1963).

genolyzed to the desired octapeptide prolylphenylalanylhistidylleucylleucylvalyltyrosylserine (**11**).

The purity and homogeneity of all intermediates was indicated by their analyses and small melting point ranges and by giving in each case a single spot on thin layer or paper chromatography. The free octapeptide **11** was not crystalline but was homogeneous in paper chromatography and countercurrent distribution. Optical purity was ensured by using the azide procedure every time the carboxyl component was a dipeptide or larger. Of all the procedures for peptide synthesis the azide method is the only one which, to date, has never given evidence of racemization.[18]

In view of the apparently clear superiority of the azide reaction in peptide synthesis, one may ask why it is not used more often or even exclusively for the preparation of large peptides since a few per cent of racemization in each of a number of steps can give a peptide that is extremely difficult to purify completely. The advantages and disadvantages of the azide synthesis have been reviewed[19] and in addition may be mentioned solvent problems in preparing larger azides. We have carefully studied the azide synthesis and would like to report a general procedure which seems to eliminate the difficulties usually encountered and (perhaps for that reason) also gives higher yields than the oridinary technique. Our method consists of using one principal solvent (dimethylformamide), forming the azide under nonaqueous conditions (hydrogen chloride and isoamyl nitrite), liberating the amino ester *in situ* from its salt and carrying out the reaction at -20 to $-40°$. The crude products are isolated simply by pouring the reaction solution into water. Triethylamine is used to neutralize excess acid after diazotization and to liberate peptide ester from its salt. Apparently, the presence of isoamyl nitrite, triethylamine salts, and free triethylamine has no deleterious effect on the course of the reaction at the temperatures employed. A very similar procedure has been reported[20] since the completion of our work.

The tripeptide **8** is a particularly good case for the detection of racemization since it has been reported that coupling carbobenzoxyvalyltyrosine by the carbodiimide procedure with valylhistidylprolylphenylalanine methyl ester[21] gave 30% D-tyrosine hexapeptide and with isoleucine methyl ester[22] by either the diimide or mixed anhydride procedure yielded a minimum of 11% valyl-D-tyrosylisoleucine. The crude tripeptide Z-val-tyr-ser-ONB obtained by our improved technique was hydrogenolyzed to remove protecting groups and the product (one spot on paper chromatography) was subjected to the action of leucine aminopeptidase.[23] Complete hydrolysis to valine, tyrosine, and serine occurred as determined by paper chromatography. Any valyl-D-tyrosylserine present would have reacted very slowly (approximately 1% the rate of hydrolysis of all L-val-tyr-ser) to give valine and D-tyrosylserine. The latter would presumably have been inert under the conditions used. The absence of unreacted tripeptide and of any spots not attributable to valine, tyrosine, or serine permits the conclusion that our homogeneous azide technique gave a product that was essentially all L.

Experimental

We would like to thank R. T. Dillon and associates for analyses, rotations, and spectra. Rotations were measured at $25 \pm 2°$ at a concentration of 1% in methanol except as indicated. Analytical samples were dried from 1 to 2 hr. under vacuum at 100–110° when the melting point permitted, otherwise at 60°. The paper chromatography solvent system was *n*-butyl alcohol–acetic acid–water, 7:1:2, ascending. Thin layer chromatography (t.l.c.) employed silica gel G. Spots were detected by ninhydrin (nin),[24] diazotized sulfanilamide (Pauly),[25] or *t*-butyl hypochlorite followed by starch–iodide (BuOCl).[26] The latter was the only reagent used for thin layer plates.[27] All amino acids have the L-configuration.

Quantitative amino acid ratios were determined by S. J. Eich and D. Malik.[28] Hydrogenations were carried out by J. D. Choi and W. M. Selby. We particularly appreciate the cooperation of G. Plume in the synthesis of some of the intermediates. Amino acid methyl ester hydrochlorides were prepared by the elegant method of Boissonnas.[29] The products were homogeneous by paper chromatography and had melting points agreeing well with literature values.

N-Carbobenzoxyphenylalanylhistidine Methyl Ester (**1**).—Histidine methyl ester dihydrochloride (14.2 g., 0.058 mole) was suspended in 100 ml. of chloroform and 16.8 ml. (0.12 mole) of triethylamine was added. The resulting solution was cooled and added to a cold solution of 15.0 g. (0.050 mole) of N-carbobenzoxyphenylalanine and 12.4 g. (0.060 mole) of dicyclohexylcarbodiimide in 100 ml. of chloroform. The mixture was stirred for 1 hr. in an ice bath and overnight at room temperature. After removal of dicyclohexylurea, the chloroform was washed three times with water, four times with 2 *M* potassium carbonate, and once with water, dried over magnesium sulfate, and distilled. Crystallization of the residue from methanol–ethyl acetate gave carbobenzoxyphenylalanylhistidine methyl ester as needles, 15.4 g. (68%), m.p. 130–133°, $[\alpha]^{26}D$ $-13°$, R_f 0.73 (Pauly +, BuOCl +); lit.[13] m.p. 121–124°, $[\alpha]^{23}D$ $-19°$ (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{24}H_{26}N_4O_5$: C, 63.99; H, 5.82; N, 12.44. Found: C, 64.03; H, 6.02; N, 12.65.

Phenylalanylhistidine Methyl Ester Dihydrobromide.—Compound **1** (26.3 g., 0.058 mole) was dissolved in 100 ml. of acetic acid, the solution was cooled to 10°, and 48 ml. of 4.9 *M* hydrogen bromide in acetic acid was added. The solution was stirred for 1 hr. at room temperature and poured into 1 l. of ether. The resulting precipitate was collected by filtration, washed five times with ether, and dried under vacuum at 50°, yielding 27.9 g. (100%), R_f 0.29 (nin +, Pauly +). The ester hydrobromide was too hygroscopic to be analyzed.

N-Carbobenzoxyprolylphenylalanylhistidine Methyl Ester (**2**).—Phenylalanylhistidine methyl ester dihydrobromide (10.0 g., 0.021 mole) and 7.8 g. (0.021 mole) of carbobenzoxyproline *p*-nitrophenyl ester[30] were dissolved in 60 ml. of methylene chloride and 6.2 ml. (0.044 mole) of triethylamine was added. The solution was stored for 24 hr. at room temperature, washed twice with water, three times with cold 1 *N* potassium hydroxide, and three times with water, and dried over magnesium sulfate; the methylene chloride was distilled. The residue, on triturating with ethyl

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(25) D. Bolling, H. A. Sober, and R. J. Block, *Federation Proc.*, **8**, 185 (1949).

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(29) R. A. Boissonnas, S. Guttmann, P. A. Jaquenoud, and J. P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955); *ibid.*, **39**, 1421 (1956).

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acetate, yielded the desired tripeptide **2**, 10.0 g. (88%), m.p. 150–153°. Reprecipitation from methanol–ethyl acetate gave a powder, m.p. 155–159°, $[\alpha]^{26}D$ −72°, R_f 0.79 (Pauly +, BuOCl +); lit.[13] m.p. 160–162°, $[\alpha]^{23}D$ −38° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{29}H_{33}N_5O_6$: C, 63.60; H, 6.07; N, 12.79. Found: C, 63.32; H, 5.93; N, 13.09.

N-Carbobenzoxyprolylphenylalanylhistidine Hydrazide (3).—To tripeptide **2** (22.6 g., 0.041 mole) in 300 ml. of methanol was added 20 ml. of 95% hydrazine and the solution was allowed to stand overnight at room temperature. The solution was concentrated to a small volume and the product was precipitated with water. The yield of tripeptide hydrazide **3** was 19.0 g. (84%), m.p. 140–150°, R_f 0.71 (Pauly +, BuOCl +). Recrystallization from methanol–ethyl acetate by concentrating to the cloud point gave a granular solid, m.p. 153–157°, $[\alpha]^{26}D$ −67°.

Anal. Calcd. for $C_{28}H_{33}N_7O_5$: C, 61.41; H, 6.08; N, 17.50. Found: C, 61.37; H, 6.12; N, 17.29.

N-Carbobenzoxyleucylleucine Methyl Ester (4).—Carbobenzoxyleucine (142.5 g., 0.50 mole) and 96.4 g. (0.53 mole) of leucine methyl ester hydrochloride were dissolved in 1.5 l. of methylene chloride. The solution was stirred in an ice bath and 77.0 ml. (0.55 mole) of triethylamine was added followed by 113.3 g. (0.55 mole) of dicyclohexylcarbodiimide in 500 ml. of methylene chloride at such a rate that the temperature remained below 15°. The mixture was stirred for 2 hr. more in the ice bath and allowed to stand overnight at room temperature. The work-up was essentially as described for compound **1**. The crude product was crystallized from 800 ml. of cyclohexane to yield carbobenzoxyleucylleucine methyl ester as needles, 176.9 g. (90%), m.p. 93–95°, t.l.c. (50% ethyl acetate–benzene) R_f 0.57, $[\alpha]^{26}D$ −40°. The melting point was unchanged on recrystallization.

Anal. Calcd. for $C_{21}H_{32}N_2O_5$: C, 64.26; H, 8.22; N, 7.14. Found: C, 64.58; H, 8.18; N, 7.08.

Leucylleucine Methyl Ester Hydrobromide (5 HBr). Dipeptide **4** (10.0 g., 0.025 mole) in 10 ml. of acetic acid was treated with 30 ml. of 3.6 *M* hydrogen bromide in acetic acid; the solution was allowed to stand for 1 hr. at room temperature and concentrated to dryness. The residue was rubbed under ether until it crystallized. The crude product was washed thoroughly with ether to give the ester hydrobromide **5 HBr** as needles, 7.7 g. (89%), m.p. 175–180°, R_f 0.72 (nin +, BuOCl +). Crystallization from ether–isopropyl alcohol (1:1) raised the melting point to 181–184°, $[\alpha]^{26}D$ −40°.

Anal. Calcd. for $C_{13}H_{27}BrN_2O_3$: N, 8.26; Br, 23.56. Found: N, 8.29; Br, 23.34.

N-Carbobenzoxyprolylphenylalanylhistidylleucylleucine Methyl Ester (6).—Carbobenzoxyprolylphenylalanylhistidine hydrazide (5.00 g., 0.0091 mole) in 25 ml. of dimethylformamide was cooled to −40° and 18.3 ml. of 2.0 *M* hydrogen chloride in tetrahydrofuran was added followed by 1.42 ml. (0.0105 mole) of isoamyl nitrite. After 30 min. at −40°, 6.9 ml. (0.049 mole) of triethylamine was added followed by 3.26 g. (0.0096 mole) of leucylleucine methyl ester hydrobromide in 25 ml. of dimethylformamide. The mixture was stirred for 30 min. at −40° and stored for 2 days at −18°. Dilution with 500 ml. of water yielded the desired pentapeptide **6** as an amorphous solid, 6.18 g. (89%), t.l.c. (20% methanol–chloroform) R_f 0.67. A trace of impurity was present at R_f 0.79. Reprecipitation from isopropyl alcohol–ether removed the fast-running impurity and gave a powder, m.p. 110–115°, $[\alpha]^{26}D$ −72°.

Anal. Calcd. for $C_{41}H_{55}N_7O_8$: C, 63.63; H, 7.16; N, 12.67. Found: C, 63.44; H, 7.12; N, 12.45.

N-Carbobenzoxyprolylphenylalanylhistidylleucylleucine Hydrazide (7).—The above crude ester was dissolved in 50 ml. of methanol, 8.0 ml. of hydrazine hydrate was added and the solution was allowed to stand overnight at room temperature. Dilution with 500 ml. of water gave the crude hydrazide (5.3 g.) which was crystallized from methanol to yield 4.6 g. (65% based on tripeptide hydrazide **3**) of compound **7** as a granular solid, m.p. 190–202°. Crystallization from methanol raised the melting point to 203.5–206°, $[\alpha]^{26}D$ −66°, t.l.c. (20% methanol–chloroform) R_f 0.28.

Anal. Calcd. for $C_{40}H_{55}N_9O_7$: C, 62.07; H, 7.16; N, 16.29. Found: C, 62.18; H, 7.17; N, 16.44.

N-Carbobenzoxyvalyltyrosylserine *p*-Nitrobenzyl Ester (8).—Carbobenzoxyvalyltyrosine hydrazide[31] (4.28 g., 0.010 mole) was dissolved in 30 ml. of dimethylformamide and 25 ml. of 2.0 *M* hydrogen chloride in tetrahydrofuran. The solution was cooled to −40°, 1.61 ml. (0.012 mole) of isoamyl nitrite was added, and the solution was kept for 10 min. at −45 to −30°. Triethylamine (14.0 ml., 0.10 mole) was added followed by 4.95 g. (0.012 mole) of serine *p*-nitrobenzyl ester *p*-toluenesulfonate in 10 ml. of dimethylformamide. The mixture was stored for 3 days at −18° and diluted with 500 ml. of ice–water to give 5.33 g. (84%) of crystalline tripeptide **8**, m.p. 185–190°; t.l.c. (10% methanol–chloroform) R_f 0.58. Recrystallization from 100 ml. of methanol yielded needles, 4.30 g. (68%), m.p. 196–198°, $[\alpha]^{26}D$ −28°.

Anal. Calcd. for $C_{32}H_{36}N_4O_4$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.37; H, 5.74; N, 8.43.

Enzymatic Hydrolysis.—Crude, protected tripeptide **8** (10 mg.) was hydrogenated in 10 ml. of 90% acetic acid over 10 mg. of palladium black for 5 hr. The filtrate after removal of the catalyst was concentrated to dryness; the residue was concentrated twice with water to dryness and dissolved in 2.0 ml. of 0.1 *M* tris buffer, pH 8.1, which was 0.002 *M* in magnesium chloride. The solution showed only one spot (val-tyr-ser) on paper chromatography, R_f 0.39 (nin +, Pauly +). To 1.0 ml. of peptide solution was added 0.10 ml. of a 0.10 mg./ml. aqueous solution of leucine aminopeptidase (Worthington) and the reaction was allowed to proceed for 2 days at room temperature. The solution was acidified to pH 1 with hydrochloric acid and examined by descending paper chromatography. In the absence of hydrochloric acid, the buffer interfered with the detection of serine. Only three spots were observed corresponding to serine (R_f 0.11), tyrosine (R_f 0.34), and valine (R_f 0.44). Authentic amino acids were run simultaneously. There was no evidence of unreacted starting material or partially hydrolyzed peptide.

Valyltyrosylserine *p*-Nitrobenzyl Ester (9).—Tripeptide **8** (7.92 g., 0.012 mole) was dissolved in 100 g. of trifluoroacetic acid, the solution was cooled to 0°, and hydrogen bromide was bubbled through for 1 hr. The solution was concentrated to dryness; the residue was dissolved in 40 ml. of water and washed twice with ether. The solution was concentrated somewhat to remove dissolved ether and the pH was brought to 8.5 with 0.5 *M* potassium carbonate. The tripeptide ester crystallized, yielding 6.0 g. (96%), m.p. 78–90°, t.l.c. (30% methanol–chloroform) R_f 0.51 (one spot only), $[\alpha]^{26}D$ +22°. Recrystallization from 50% methanol did not raise the melting point. Prolonged heating in methanol caused decomposition as shown by t.l.c.

Anal. Calcd. for $C_{24}H_{30}N_4O_8$: C, 57.36; H, 6.02; N, 11.15. Found: C, 57.24; H, 6.00; N, 11.22.

N-Carbobenzoxyprolylphenylalanylhistidylleucylleucylvalyltyrosylserine *p*-Nitrobenzyl Ester (10).—Pentapeptide hydrazide **7** (3.97 g., 0.005 mole) in 23 ml. of dimethylformamide and 10 ml. of 2.0 *M* hydrogen chloride in tetrahydrofuran was cooled to −40° and 0.81 ml. (0.006 mole) of isoamyl nitrite was added. After 0.5 hr. of stirring at −40°, 2.80 ml. (0.020 mole) of triethylamine was added followed by 2.76 g. (0.0055 mole) of tripeptide ester **9**. The solution was stirred for 2 hr. at −40°, allowed to stand for 2 days at −18°, and filtered; 200 ml. of ice–water was added to give the desired octapeptide **10**, 5.56 g. (90%), t.l.c. (20% methanol–chloroform) R_f 0.69 (one spot only). Crystallization from 50 ml. of methanol gave a microcrystalline powder, 4.83 (78%), m.p. 217–220° dec., $[\alpha]^{26}D$ −26°.

Anal. Calcd. for $C_{84}H_{81}N_{11}O_{15}$: C, 61.77; H, 6.56; N, 12.38. Found: C, 61.75; H, 6.78; N, 12.52.

When a conventional procedure of diazotization in 1 *N* hydrochloric acid, basification, and extraction of the azide into chloroform (bad emulsions but no better solvent could be found) was used, the yield of analytical quality octapeptide, m.p. 219° dec., was 41%.

Prolylphenylalanylhistidylleucylleucylvalyltyrosylserine (11).—Protected octapeptide **10** (2.30 g., 0.00185 mole) in 250 ml. of 90% acetic acid was hydrogenated over 1.0 g. of palladium black at room temperature and atmospheric pressure. Hydrogen uptake ceased after 2 hr. The catalyst was removed by filtration and the filtrate was concentrated to dryness. Stirring the residue with methanol yielded the octapeptide **11**, 1.75 g. (93%), m.p. 228–230° dec., $[\alpha]^{26}D$ −21° (*c* 1, acetic acid), $\lambda_{max}^{75\%\ HOAc}$ 275 mμ (ε 1430), R_f 0.60 (Pauly +, BuOCl+); quantitative amino acid analysis showed pro 0.83, phe 1.14, his 1.12, leu 2.00, val 0.98, tyr 0.87, and ser 0.81.

Anal. Calcd. for $C_{49}H_{70}N_{10}O_{17}\cdot 2H_2O$[11]: C, 58.20; H, 7.38; N, 13.66. Found: C, 58.33; H, 7.64; N, 13.66; residue, 0.66.

(31) H. Schwarz, F. M. Bumpus, and I. H. Page, *J. Am. Chem. Soc.*, **79**, 5697 (1957).

The analytical sample contained approximately 0.5% palladium as determined by spectral analysis.[32] Countercurrent distribution in *n*-butylalcohol–acetic acid–isopropyl acetate–water, 10:10:10:27 (400 transfers), gave a slightly skewed curve (K = 0.43) indicating a decrease in partition coefficient with increasing solute concentration. The reisolated material contained less than 0.05% palladium.

(32) Spectral analyses were by Spectro-Chemical Research Laboratories, Inc., Chicago, Ill.

Reactions of Metal Chelates. VII. Dimethylaminomethylation and Chloromethylation of Metal Acetylacetonates[1]

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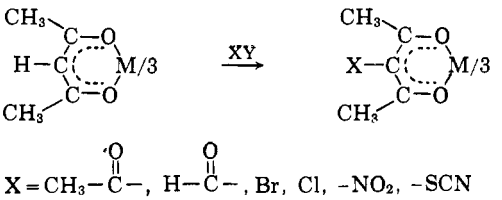
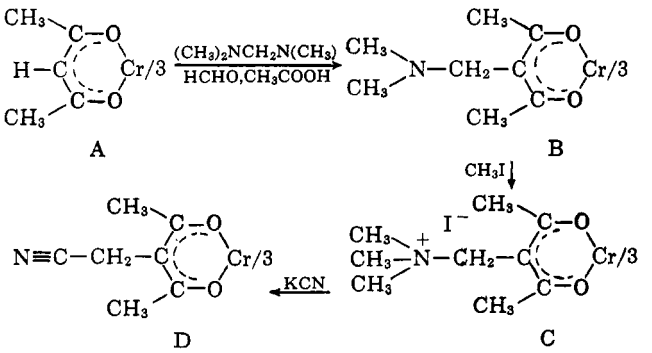
Several of the pseudo-aromatic trivalent metal acetylacetonates have been found to undergo a modified Mannich-type condensation. The resulting triamines are readily quaternized by treatment with methyl iodide to produce salts which are amenable to attack by some nucleophiles. This provides a new route to reactive molecular chelates and constitutes the first reported instance of the direct introduction of an uncoordinated amino function into such a species. A method has also been devised for the synthesis of the analogous monoamine in the chromium(III) series by use of a chelate substrate having two of its three active sites blocked by inert functions. After quaternization, this monofunctional analog seemed to be more reactive toward nucleophiles than the corresponding triamine. Another type of reactive molecular chelate was produced when the parent acetylacetonates were subjected to chloromethylation conditions. In this case the intermediate, presumably the expected chloromethyl chelate, could not be isolated except in the form of derivatives. All attempts to isolate the chloromethyl chelate itself resulted only in polymeric products. As in the case of the chelate amines, the best results were obtained by studying the monofunctional species.

We previously reported that metal acetylacetonate rings undergo a number of substitution reactions which are usually associated with reactive aromatic systems.[3-7] It has now been found that these chelates are also susceptible to a modified Mannich-type condensation. All three of the active sites in the chelate molecule have been substituted by dimethylaminomethyl functions to produce products with drastically altered properties. This provides a new route to reactive molecular chelates and constitutes the first reported instance of the direct introduction of an uncoordinated amino function into such a species.



Although the conventional Mannich conditions were unsuccessful in producing the desired amines, it was found that good results could be obtained from a modification of the procedure which Lindsay and Hauser[8] had previously used in the ferrocene series. The unsubstituted chromium(III) acetylacetonate (A) was allowed to react with paraformaldehyde and N,N,N′,N′-tetramethyldiaminomethane in glacial acetic acid solution. The product B, a dark, low-melting glass, exhibited a strong infrared absorption peak at 1565 cm.$^{-1}$, but no other absorption in the 1500–1600-cm.$^{-1}$ region. This is as expected from the empirical rule of Dryden[9] who observed that a series of metal acetylacetonates bearing functions other than hydrogen on the central carbon atom of the ring had a single absorption peak in this region of their infrared spectra. However, in the compounds bearing a hydrogen on this position, characteristic doublets appeared in this region of the spectra. Also, a small peak in the 1200-cm.$^{-1}$ region of the spectra of the unsubstituted chelates, usually associated with a wagging mode of the ring hydrogen, characteristically disappears upon substitution of the ring. No 1200-cm.$^{-1}$ peak could be detected in the spectrum of the Mannich product.



Also indicative of the assigned structure was the nuclear magnetic resonance spectrum of the analogous product in the cobalt(III) series. This showed the presence of three types of hydrogen atoms with peaks at τ 6.92, 7.72, and 7.85 and integrated intensities of

(1) (a) Initially reported at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April, 1963; (b) previous paper by J. P. Collman and M. Yamada, *J. Org. Chem.*, **28**, 3017 (1963); (c) portions of this work were supported by the National Science Foundation, U. S. Army Research Office, Grant Number DA-ORD-D-31124-G-185, and the Petroleum Research Fund; (d) abstracted in part from the Ph.D. Dissertations of R. H. Barker and R. L. Marshall, University of North Carolina, 1962.

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(6) J. P. Collman and E. T. Kittleman, *ibid.*, **83**, 531 (1961).

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