766. *Steps towards the Synthesis of the Histidine²-hypertensin* Analogue. Part I. Synthesis of an Intermediate Protected Heptapeptide.*

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Histidine, protected in the glyoxaline ring, has been used in the synthesis of the blocked heptapeptides, benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl and benzyl ester. The intermediate tetrapeptide, L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide has been synthesised by two routes, and the products compared by optical rotation.

A few years ago we applied 1(or 3)-benzyl-L-histidine [1] to the synthesis of histidyl-peptides,[2] finding that protection of the glyoxaline ring facilitated the incorporation of histidine into the chain and usually led to pure crystalline end products in high yield. Moreover protection of the glyoxaline ring decreases the polar character of the resulting

[1] du Vigneaud and Behrens, *J. Biol. Chem.*, 1937, **117**, 27.
[2] Theodoropoulos, *J. Org. Chem.*, 1956, **21**, 1550. * See footnote, p. 3862.

peptide and increases its solubility in organic solvents which is of practical importance in synthetical work.

In our studies of synthetic analogues of isoleucine hypertensin,[3] we used histidine protected in the glyoxaline ring for the synthesis of benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester, which is the key intermediate for the total synthesis of the histidine[2]-hypertensin analogue * and occupies positions 3—8 in isoleucine-hypertensin except that its *N*-terminal histidyl residue has replaced the arginine residue located at position 2 in the hormone.

Synthesis of the hypertensin analogue in which the arginine residue is replaced by histidine has been of special interest since the finding that the basicity of the side chain in vasopressin [4] influences the pressor activity of the hormone. Whether a similar result occurs with hypertensin seemed worthy of investigation.

Benzyloxycarbonyl-1(or 3)-benzyl-L-histidine [2] was condensed with L-valyl-L-tyrosine methyl ester by the carbodi-imide method,[5] to give crystalline benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosine methyl ester in high yield. Hydrolysis then afforded benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosine in 80% yield.

Benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidine (obtained by hydrolysis of its benzyl ester [6]) was coupled with L-prolyl-L-phenylalanine methyl ester in *NN*-dimethylformamide by means of dicyclohexylcarbodi-imide. The oily crude product was treated with hydrogen bromide,[7] giving L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide, a solid which appeared to be homogeneous on paper chromatography in two solvent systems and on hydrolysis by acid and paper chromatography gave spots corresponding to all the expected amino-acids as well as a weak spot corresponding to histidine. These results indicated the sequence isoleucyl-1(or 3)-benzylhistidylprolylphenylalanine but provided no information regarding the optical homogeneity.

The synthesis was therefore undertaken again, starting from the CO_2H-terminal end, one amino-acid being added at a time, a procedure which is presumed to proceed with little or no formation of diastereoisomers.[8] Benzyloxycarbonyl-1(or 3)-benzyl-L-histidine with L-prolyl-L-phenylalanine methyl ester gave, by the carbodi-imide method, oily benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester which with hydrogen bromide in acetic acid afforded 1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide † in crystalline form. The tripeptide ester was set free by triethylamine and then coupled with benzyloxycarbonyl-L-isoleucine [9] by the carbonic mixed anhydride procedure [10] in tetrahydrofuran. This tetrapeptide also was an oil, but with hydrogen bromide gave L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide identical in optical rotation with that obtained by the earlier route.

Attempts to saponify the protected tetrapeptide methyl ester with the equivalent amount of sodium hydroxide at room temperature did not give satisfactory results. At higher temperature (*e.g.*, at 60—65° for 2 min.) an appreciable amount of the acid, benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine, was obtained

* This terminology denotes that a histidine residue replaces the amino-acid residue normally present at position 2 of hypertensin [Schwyzer, *Chimia* (*Switz.*), 1957, **11**, 335].

† The sequence His.Pro.Phe. occurs also in valine-hypertensin isolated by Peart (*Biochem. J.*, 1956, **62**, 520).

[3] Skeggs, Lentz, Kahn, Shumway, and Woods, *J. Exp. Med.*, 1956, **104**, 193.
[4] Katsoyannis and du Vigneaud, *Arch. Biochem. Biophys.*, 1958, **78**, 555.
[5] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **75**, 1068.
[6] Theodoropoulos and Fölsch, *Acta Chem. Scand.*, 1958, **12**, 1955.
[7] Ben-Ishai and Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[8] Schwarz and Bumpus, *J. Amer. Chem. Soc.*, 1959, **81**, 890.
[9] Theodoropoulos and Craig, *J. Org. Chem.*, 1955, **20**, 1169.
[10] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547.

and hydrogenolysis [11] then afforded L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine which was isolated as the dihydrate. This tetrapeptide was readily converted into its benzyl ester on azeotropic distillation with benzyl alcohol and toluene-*p*-sulphonic acid in carbon tetrachloride; this esterification process, successfully used with amino-acids,[12] is well suited also for peptides also—a lysyl-tripeptide thus synthesised was completely digestible by trypsin.[13]

The free tetrapeptide benzyl ester was condensed with benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosine by the carbodi-imide method in *NN*-dimethylformamide, giving crystalline benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester.[14]

In a similar manner the free tetrapeptide methyl ester, condensed with the tripeptide derivative, afforded the crystalline protected heptapeptide, benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester in 60—65% yield. The elementary composition was correct and paper chromatography of a hydrolysed sample revealed the presence of valine, tyrosine, isoleucine, proline, phenylalanine, and 1(or 3)-benzylhistidine. The last acid gave a more intense spot and appeared to be stable under the usual conditions of hydrolysis with acid, since only a faint spot at the position of histidine could be detected. A sample of 1(or 3)-benzyl-L-histidine, hydrolysed under the same conditions, gave a weak spot at the position of histidine.

Experimental

All peptides tested were shown to contain the appropriate amino-acids by hydrolysis in 6N-hydrochloric acid at 105° for 24 hr. followed by paper chromatography. The solvent systems used were (*a*) butan-1-ol–acetic acid–water (4 : 1 : 5), (*b*) butan-2-ol–formic acid–water (1 : 3 : 2), and (*c*) butan-1-ol–acetic acid–pyridine–water (15 : 3 : 10 : 12). Whatman no. 1 paper was used and development was with 0·1% ninhydrin solution in ethanol. Tetrahydrofuran and ether were freshly distilled over sodium.

Benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidine.—A suspension of benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidine benzyl ester [6] (11·64 g.) in ethanol (30 ml.) was treated at room temperature with 2N-sodium hydroxide (11 ml.; 10% excess) in portions, with shaking, during 15 min. The ester, which at the beginning was partly in solution, dissolved as the hydrolysis proceeded. After 45 min. the solution was diluted with water (300 ml.), and the precipitate was acidified with acetic acid. The *product*, after cooling, was filtered off, washed with 1% acetic acid and water, and dried. Recrystallisation was effected by dissolving the *product* (7·8 g., 80%) in 96% ethanol and precipitating it with ether; it then had m. p. 173—174°, $[\alpha]_D^{26}$ −23·5° (*c* 1·1 in acetic acid) (Found: C, 65·4; H, 6·3; N, 11·0. $C_{27}H_{32}N_4O_5$ requires C, 65·8; H, 6·5; N, 11·3%).

L-Prolyl-L-phenylalanine Methyl Ester Hydrochloride.—Benzyloxycarbonyl-L-proline (2·49 g.) was condensed with L-phenylalanine methyl ester hydrochloride (2·12 g.) by the carbonic mixed anhydride procedure in tetrahydrofuran. The solvent was removed under reduced pressure and the oily residue was taken up in ether and washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water. After being dried (Na_2SO_4) the solvent was removed and the oily residue (2·9 g., 70%) was dissolved in absolute methanol (20 ml.) containing 0·23 g. of hydrogen chloride. It was hydrogenated in the presence of palladium black (0·5 g. of oxide) and then treated as previously described.[15] The product (1·8 g., 80%) had m. p. 158—159° (lit., 157—158°,[15] 162—164° [16]).

Benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine Methyl Ester.—A suspension of benzyloxycarbonyl-1(or 3)-benzyl-L-histidine (1·89 g.) in methylene chloride (20 ml.) was almost dissolved by addition of triethylamine (0·55 g.) and to this solution were added

[11] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[12] Cipera and Nicholls, *Chem. and Ind.*, 1955, 16.
[13] Theodoropoulos, Bennick, Fölsch, and Mellander, *Nature*, 1959, **184**, 187.
[14] Theodoropoulos, *Nature*, 1959, **184**, 1634.
[15] Rittel, Iselin, Kappeler, Rinniker, and Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 614.
[16] Schwarz, Bumpus, and Page, *J. Amer. Chem. Soc.*, 1957, **79**, 5697.

successively L-prolyl-L-phenylalanine methyl ester hydrochloride (1·56 g.) and *NN*-dicyclohexylcarbodi-imide (1 g.). The mixture was stirred at room temperature for 18 hr. and then dicyclohexylurea was removed. The filtrate was evaporated at 50°/1 mm. and the syrup was dissolved in ethyl acetate (100 ml.). This solution was washed with 10% aqueous lithium carbonate and water and dried (Na_2SO_4). The solvent was removed under reduced pressure, leaving an oil (2·1 g., 80%) which was used without purification.

1(*or* 3)-*Benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Methyl Ester Dihydrobromide.*—The preceding oily product was dissolved in glacial acetic acid (5 ml.) saturated with hydrogen bromide and set aside for ½ hr. Addition of dry ether precipitated the product, which was quickly filtered off, washed with ether, and placed immediately in a desiccator (P_2O_5). After two days the *product* (2·1 g., 80%) was purified from tetrahydrofuran–ether and had m. p. 208—209° (decomp.). A sample for analysis was dried in a high vacuum at 75° for 12 hr. (Found: N, 10·5; Br, 24·0. $C_{28}H_{33}N_5O_4$,2HBr requires N, 10·5; Br, 24·0%).

Benzyloxycarbonyl-L-*isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Methyl Ester.*—(*a*) Benzyloxycarbonyl-L-isoleucine (0·7 g.) and dried triethylamine (0·25 g.) were dissolved in dry tetrahydrofuran (5 ml.) at −10°. Ethyl chloroformate (0·27 g.) was added and after 5 min. a cooled solution of 1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide (1·66 g.) and triethylamine (0·5 g.) in tetrahydrofuran (10 ml.) was added. The temperature was allowed to rise and the mixture left for ½ hr. at room temperature. Then it was filtered and the filtrate evaporated to dryness. The oily residue was taken up in ethyl acetate (100 ml.) and washed with dilute sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Evaporation under reduced pressure gave the tetrapeptide derivative (1·1 g., 60%) as an oil.

(*b*) Benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidine (2·46 g.) was dissolved in *NN*-dimethylformamide (20 ml.) by addition of triethylamine (0·7 ml.). To this solution L-prolyl-L-phenylalanine methyl ester hydrochloride (1·5 g.) and dicyclohexylcarbodi-imide (1 g.) were successively added and the mixture was stirred for 18 hr. Dicyclohexylurea was filtered off and the solvent was removed at 50°/1 mm. The residual oil was taken up in ethyl acetate (150 ml.) and treated as above. The tetrapeptide methyl ester (2·9 g., 79%) was again oily.

L-*Isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Methyl Ester Dihydrobromide.*—A solution of benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester (3·65 g.) in acetic acid (10 ml.) was saturated with hydrogen bromide and set aside for ½ hr. Addition of dry ether precipitated the *product* which was dissolved in hot isopropyl alcohol and reprecipitated by ether. This process was repeated and the *product* (2·6 g.) placed in a desiccator (P_2O_5). A sample for analysis was dried under a high vacuum at 75° for 3 hr. (Found: N, 10·1; Br, 20·4. $C_{34}H_{44}N_6O_5$,2HBr requires N, 10·4; Br, 20·5%); it had $[\alpha]_D^{24}$ +24° (*c* 1 in acetic acid), R_F 0·85 in solvent (*a*), 0·77 in solvent (*b*).

Benzyloxycarbonyl-L-*valyl*-L-*tyrosine Methyl Ester.*—Benzyloxycarbonyl-L-valine [17] was condensed with L-tyrosine methyl ester by the mixed anhydride procedure in tetrahydrofuran; the dipeptide derivative, which was precipitated by addition of water, had m. p. 143—145° and after recrystallisation from ethyl acetate–light petroleum ether, m. p. 146—148° (lit., 144—147°,[15] 155·5—156·5° [16]) (Found: C, 64·45; H, 6·5; N, 6·6. Calc. for $C_{23}H_{28}N_2O_6$: C, 64·5; H, 6·5; N, 6·5%).

L-*Valyl*-L-*tyrosine Methyl Ester Hydrochloride.*—Benzyloxycarbonyl-L-valyl-L-tyrosine methyl ester (4·5 g.) in absolute ethanol (10 ml.) containing 0·3 g. of hydrogen chloride was hydrogenated in the presence of 0·3 g. of palladium oxide. When evolution of carbon dioxide ceased hydrogenation was discontinued, the catalyst filtered off, and the solution evaporated *in vacuo* at 35°. The remaining oil was dissolved in the minimum amount of acetone and by addition of dry ether a wax was precipitated. This wax was washed with more ether and on trituration under ether solidified. The hygroscopic product (3·3 g.) had $[\alpha]_D^{25}$ +32·5° (*c* 1 in MeOH), R_F 0·91 in solvent (*c*).

Benzyloxycarbonyl-1(*or* 3)-*benzyl*-L-*histidyl*-L-*valyl*-L-*tyrosine Methyl Ester.*—Benzyloxycarbonyl-1(or 3)-benzyl-L-histidine (3·79 g.) was almost dissolved in *NN*-dimethylformamide (20 ml.) by addition of triethylamine (1·01 g.) and then were added L-valyl-L-tyrosine methyl ester hydrochloride (2·58 g.) and dicyclohexylcarbodi-imide (2 g.). The mixture was stirred for 18 hr., then dicyclohexylurea was removed. The filtrate was diluted with water (800 ml.). The precipitate was filtered off, washed with water, and dried. Purification was by dissolution in ethanol and precipitation with ether–light petroleum (1 : 1). The *product* (4·4 g., 73%)

[17] Synge, *Biochem. J.*, 1948, **42**, 99.

had m. p. 193—195°, $[\alpha]_D^{24}$ −8·2° (*c* 2 in acetic acid) (Found: C, 65·8; H, 6·25; N, 10·6. $C_{36}H_{41}N_5O_7$ requires C, 65·9; H, 6·3; N, 10·7%).

Benzyloxycarbonyl-1(*or* 3)-*benzyl*-L-*histidyl*-L-*valyl*-L-*tyrosine*.—To a suspension of the above ester (1·63 g.) in 96% ethanol (10 ml.) was added N-sodium hydroxide (3 ml.) and the mixture was stirred at room temperature for 1 hr. The ester dissolved. On addition of water (200 ml.) a gelatinous precipitate was deposited. After acidification with acetic acid the precipitate was filtered off, washed with 1% acetic acid and water, dried (P_2O_5), dissolved in boiling ethanol–ethyl acetate (1 : 2), and reprecipitated with light petroleum. The *product* (1·28 g., 80%), m. p. 185—187°, was a white solid (Found: C, 65·45; H, 6·1; N, 11·0. $C_{35}H_{39}N_5O$ requires C, 65·5; H, 6·1; N, 10·9%).

Benzyloxycarbonyl-L-*isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine*.—To a solution of benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester (4 g.) in methanol (10 ml.) was added sodium hydroxide (0·3 g.). The solution was heated to 60° for 1 min. and set aside for ½ hr. This was repeated and finally the solution was diluted with water (100 ml.) and acidified with acetic acid. A sticky *compound* was produced which slowly solidified. After recrystallisation from the minimum amount of ethanol, it (1·9 g.) had m. p. 192—193°, $[\alpha]_D^{24}$ −24·2° (*c* 0·9 in acetic acid) (Found: C, 66·7; H, 6·5; N, 11·2. $C_{41}H_{48}N_6O_7$ requires C, 66·8; H, 6·6; N, 11·4%).

L-*Isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine*.—A solution of benzyloxycarbonyl-L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine (1·83 g.) in absolute ethanol (20 ml.) was hydrogenated in the presence of palladium (0·3 g. of oxide). When the evolution of carbon dioxide ceased, the catalyst was removed and the filtrate evaporated under reduced pressure. The remaining oil was dissolved in acetone and by addition of dry ether the *product* (1·3 g.) was precipitated. It was at once filtered off and placed in a desiccator (P_2O_5). A sample for analysis, dried under a high vacuum at 60° for 1 hr. (Found: C, 62·0; H, 7·3; N, 13·2. $C_{33}H_{42}N_6O_5,2H_2O$ requires C, 62·0; H, 7·3; N, 13·15%), had $[\alpha]_D^{26}$ +19° (*c* 1 in acetic acid), R_F 0·76 in solvent (*b*).

L-*Isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Benzyl Ester Ditoluene*-p-*sulphonate*.—The above tetrapeptide (0·5 g.), toluene-*p*-sulphonic acid monohydrate (0·33 g., 10% excess), and benzyl alcohol (5 ml.) were heated on a steam bath, the liberated water being removed azeotropically with carbon tetrachloride. After ½ hr. no more water was removed and addition of ether precipitated the *product*. This was washed with ether (yield, 0·9 g.) and used without purification in the next step (Found: N, 7·85. $C_{54}H_{64}N_6O_{11}S_2,H_2O$ requires N, 8·2%); it had R_F 0·95 in solvent (*c*).

Benzyloxycarbonyl-1(*or* 3)-*benzyl*-L-*histidyl*-L-*valyl*-L-*tyrosyl*-L-*isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Methyl Ester*.—A solution of benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosine (0·9 g.) in *NN*-dimethylformamide (5 ml.) was mixed with L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester dihydrobromide (1·09 g.) in *NN*-dimethylformamide (10 ml.) and triethylamine (0·4 ml.). Then dicyclohexylcarbodiimide (0·3 g.) was added and the mixture was stirred at room temperature for 18 hr. Next ethyl acetate (100 ml.) was added and, after cooling to 0°, dicyclohexylurea was removed. The solvent was evaporated under reduced pressure and the residue was taken up in ethyl acetate (100 ml.). This solution was washed with 10% aqueous lithium carbonate and water, dried (Na_2SO_4), and evaporated under reduced pressure. Addition of ether started crystallisation. On cooling and filtration 1·25 g. of *product* were obtained. The material was dissolved in absolute ethanol (30 ml.), decolorised with charcoal, and precipitated with ether–light petroleum (2 : 1); it (1 g.) had m. p. 158—160°, $[\alpha]_D^{24}$ −28·6° (*c* 1 in acetic acid) (Found: C, 66·45; H, 6·7; N, 12·3. $C_{69}H_{81}N_{11}O_{11}$ requires C, 66·8; H, 6·6; N 12·4%).

Benzyloxycarbonyl-1(*or* 3)-*benzyl*-L-*histidyl*-L-*valyl*-L-*tyrosyl*-L-*isoleucyl*-1(*or* 3)-*benzyl*-L-*histidyl*-L-*prolyl*-L-*phenylalanine Benzyl Ester*.—The *benzyl ester*, prepared similarly in 60% yield from benzyloxycarbonyl-1(or 3)-benzyl-L-histidyl-L-valyl-L-tyrosine and L-isoleucyl-1(or 3)-benzyl-L-histidyl-L-prolyl-L-phenylalanine benzyl ester di-toluene-*p*-sulphonate, had m. p. 145—147°, $[\alpha]_D^{25}$ −27° (*c* 1 in acetic acid) (Found: C, 68·2; H, 6·35; N, 11·6. $C_{75}H_{85}N_{11}O_{11}$ requires C, 68·5; H, 6·5; N, 11·7%).

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