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Synthesis of Natural Human Angiotensin I[1]

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The synthesis of a decapeptide, H-L-Asp-L-Arg-L-Val-L-Tyr-L-Ile-L-His-L-Pro-L-Phe-L-His-L-Leu-OH, which has an amino acid sequence assumed by Arakawa *et al.* for natural human angiotensin I, has been performed. The chemical and biological properties of the synthetic product have shown to be in agreement with those of the natural material, thus the structure of human angiotensin I has been confirmed by the present synthesis.

Angiotensin was found in 1939 during experiment with animals as a causal substance responsible for the experimental renal hypertension in animals.[2] Angiotensin I, decapeptides, have been isolated from horse[3] and bovine[4] materials; these peptides have been liberated from precursor molecule angiotensinogen contained in plasma protein by the proteolytic action of renin which derived from kidney. The decapeptides are then converted to angiotensin II, octapeptides, by the removal of a dipeptide, H-His-Leu-OH, from its carboxy terminal by the action of a specific enzyme, called converting enzyme,[5] which exists abundantly in plasma. The resulting angiotensin II are the most potent pressor substances ever known. The species difference resides in the fifth position which is occupied by isoleucine in horse[6] and valine in bovine.[7]

The menchanism of liberation of angiotensin, thus clarified only in animals except human, has been a working hypothesis in the pathogenesis of human hypertension. The fact that it had never been isolated nor elucidated in chemical terms in human has been the weekest point of the hypothesis in understanding the pathogenetic role of angiotensin in human hypertension. However, Arakawa *et al.* recently isolated pure human angiotensin from an incubation mixture of human serum protein and renin.[8] They reported that amino acid analysis of the natural peptide from human revealed the same composition as that known for horse angiotensin in which the structure has been established to be [Ile^5]-angiotensin I, *i. e.*, H-L-Asp-L-Arg-L-Val-L-Tyr-L-Ile-L-His-L-Pro-L-Phe-L-His-L-Leu-OH.[9]

Therefore, the amino acid sequence of human angiotensin has been plausibly anticipated to be same as that for [Ile^5]-angiotensin I. Since [Ile^5]-angiotensin I decapeptide had never been synthesized before, the present authors attempted to prepare the decapeptide (XVIII) mentioned above and to compare the identity of XVIII with the human angiotensin.

The sequence of reactions employed for the synthesis of the decapeptide (XVIII) is shown in Fig. 1. To obtain the final protected decapeptide *p*-nitrobenzyl ester (XVII), we selected a route involving the coupling of a mixed anhydride derived from benzyloxycarbonyl-β-benzylaspartic acid with the nonapeptide ester (XVI) which was derived from the benzyloxycarbonyl-nonapeptide ester (XV) by the action of hydrogen bromide in acetic acid. Similarly, the protected nonapeptide ester (XV) was prepared by coupling a mixed anhydride of benzyloxycarbonyl-nitroarginine with the octapeptide ester (XIV) which was obtained from the benzyloxycarbonyl-octapeptide ester (XIII). The acyl-octapeptide ester (XIII) was prepared by coupling the benzyloxycarbonyl-tripeptide azide derived from the hydrazide (XII) with the pentapeptide ester (VIII) which had been prepared from the benzyloxycarbonyl-pentapeptide ester (VII). Similarly, the acylpentapeptide ester (VII) was obtained by coupling the acyl-tripeptide azide from the corresponding hydrazide (VI)

1) A part of this work has been briefly communicated: H. Aoyagi, K. Arakawa and N. Izumiya, *J. Am. Chem. Soc.*, **89**, 4239 (1967).

2) I. H. Page and O. M. Helmer, *Proc. Central Soc. Clin. Invest.*, **12**, 17 (1939); E. Braun-Menéndez, J. C. Fasciolo, L. F. Leloir and J. M. Muñoz, *Rev. Soc. Argent. Biol.*, **15**, 420 (1939).

3) L. T. Skeggs, Jr., W. H. Marsh, J. R. Kahn and N. P. Shumway, *J. Exptl. Med.*, **102**, 435 (1955).

4) W. S. Peart, *Biochem. J.*, **62**, 520 (1956).

5) L. T. Skeggs, Jr., J. R. Kahn and N. P. Shumway, *J. Exptl. Med.*, **103**, 295 (1956).

6) L. T. Skeggs, Jr., K. E. Lentz, N. P. Shumway and K. R. Woods, *ibid.*, **104**, 193 (1956).

7) D. F. Elliott and W. S. Peart, *Biochem. J.*, **65**, 246 (1957).

8) K. Arakawa, M. Nakatani and M. Nakamura, *Nature*, **214**, 278 (1967).

9) K. Arakawa, M. Nakatani, A. Minohara and M. Nakamura, *Biochem. J.*, in press (1967).

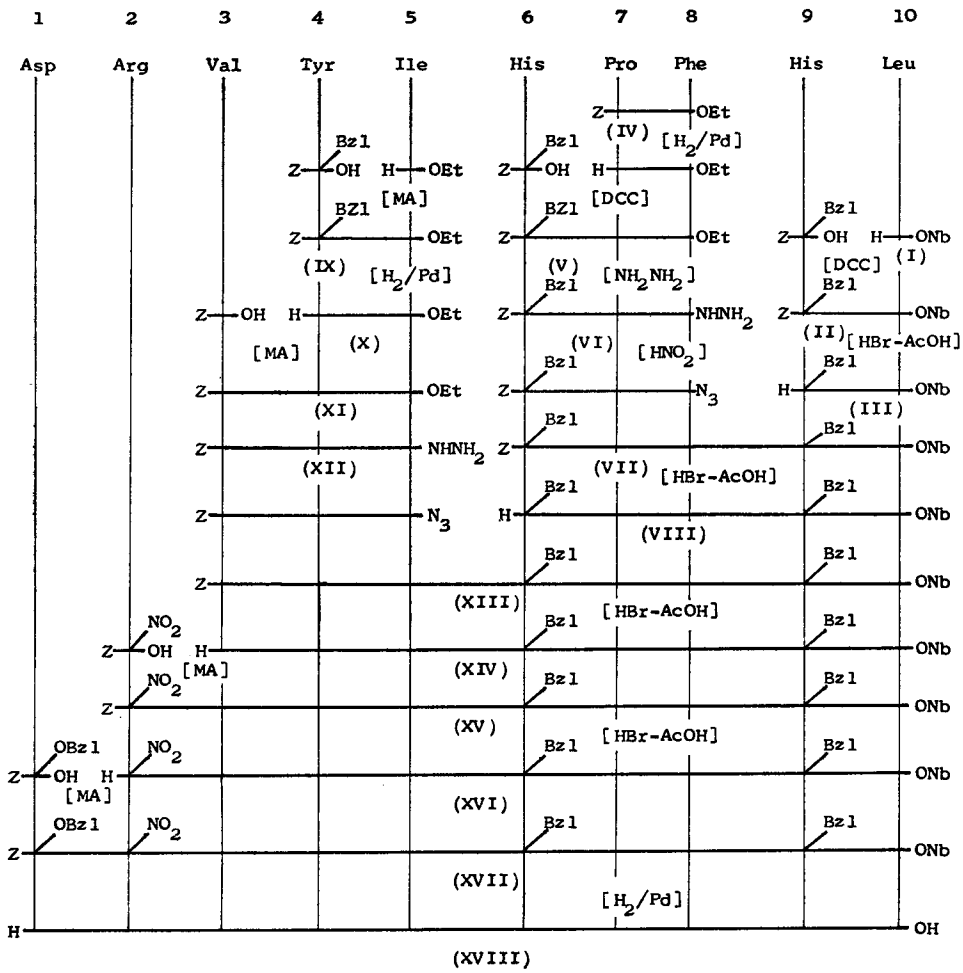

Fig. 1. Schematic diagram of synthesis of the decapeptide.
Z, benzyloxycarbonyl; Bzl, benzyl; $NHNH_2$, hydrazide; ONb, *p*-nitrobenzyl ester

with the dipeptide ester (III). The derivative (II, V or XI) of either the dipeptide or tripeptide was built up by stepwise elongation of the peptide chain from the carboxyl toward the amino end. Coupling of a benzyloxycarbonyl-amino acid with an amino component was mainly accomplished by carboxy activation through use of dicyclohexylcarbodiimide[10] or method of the mixed anhydride.[11]

The *p*-nitrobenzyl group was used for the protection of the carboxylic acid function of the *C*-terminal amino acid residue, and the benzyl group for the imidazoyl function of the histidine residue. It has been recognized that the action of hydrogen bromide in acetic acid removes the benzyloxycarbonyl group[12] without cleavage of the *p*-nitrobenzyl ester bond,[13] the imidazoyl-benzyl[14] or the nitro group;[13] many intermediate peptide ester hydrobromides (*e.g.*, XVI) were obtained in this manner.

The protected decapeptide *p*-nitrobenzyl ester (XVII) was hydrogenated with palladium black in a solvent of methanol - acetic acid - water at room temperature. The proceeding of the hydrogenolysis was followed at various times with the assay of paper chromatographic technique and with the bioassay of the pressor activity, and the highest activity was observed after some 48 hr though small part of the imidazoyl-benzyl groups was still remained untouched. The crude material after the hydrogenation was purified by a set of the column chromatographies which have proved to be very effective in isolating natural human

10) J. C. Sheehan and G. P. Hess. *J. Am. Chem. Soc.*, **77**, 1067 (1955).
11) J. R. Vanghan, Jr., and R. L. Osato, *ibid.*, **74**, 676 (1952).
12) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).
13) K. Arakawa and F. M. Bumpus, *J. Am. Chem. Soc.*, **83**, 728 (1961).
14) K. O. Kopple and D. E. Nitecki, *ibid.*, **84**, 4457 (1962).

angiotensin.[8,9] The active fractions from the column were finally lyophilized leaving a white powder (acetate of XVIII, presumably); the homogeneity and stereospecific purity of the powder were ascertained by many chromatographic assays, the amino acid analysis and the digestion with the proteolytic enzymes.

The synthetic decapeptide obtained as the powder was then compared with natural human angiotensin I. In addition to having the same R_f values in paper and thin-layer chromatographies and affording a single peak in cochromatography of Bio-Gel column with the natural preparation, the synthetic and natural preparations were indicated to have the same specific activity on the bioassay of the rat pressor activity test. These results show that the synthetic product is identical to natural product, and the structure of human angiotensin I anticipated by Arakawa *et al.*[9] has been decided by the present synthesis.

Experimental

All the melting points are uncorrected. The optical rotations were measured on a Yanagimoto Photometric Polarimeter, OR-20 type.

L-Leucine *p*-Nitrobenzyl Ester *p*-Toluenesulfonate (I·TsOH). A solution of L-leucine (5.24 g, 40 mmol), *p*-nitrobenzyl alcohol (7.36 g, 48 mmol) and toluenesulfonic acid monohydrate (8.36 g, 44 mmol) in benzene (500 m*l*) was stirred under heating, and the water liberated was removed as an azeotropic mixture. The reaction mixture was concentrated *in vacuo*, and the residual oil was solidified by adding ether. The product was recrystallized from 80% methanol. Yield, 11.65 g (67%); mp 202°C; $[\alpha]_D^{22}$ +10.2° (*c* 1, ethanol); R_f 0.78.[15]

Found: C, 54.42; H, 5.98; N, 6.41%. Calcd for $C_{20}H_{26}O_7N_2S$: C, 54.78; H, 5.98; N, 6.39%.

Z-His(*im*-Bzl)-Leu-ONb (II). Into a solution of benzyloxycarbonyl-(*im*)benzyl-L-histidine[16] (3.80 g, 10 mmol) and I (4.36 g, 10 mmol) in dimethylformamide (30 m*l*) there were added triethylamine (1.40 m*l*) and dicyclohexylcarbodiimide[10] (2.08 g) at 0°C. After it had been stirred overnight at room temperature, the mixture was evaporated *in vacuo* and the residue was diluted with ethyl acetate; then the dicyclohexylurea thus precipitated was filtered off. The filtrate was washed successively with 3% hydrochloric acid, 4% sodium bicarbonate and water, and dried over sodium sulfate. The filtered solution was evaporated *in vacuo*. The product was obtained as an oil. Yield, 5.56 g (89%); R_f 0.76.[15]

H-His(*im*-Bzl)-Leu-ONb Dihydrobromide (III·2HBr). To a solution of II (5.00 g, 8 mmol) in acetic acid (8 m*l*), 3 N dry hydrogen bromide - acetic acid (16 m*l*) was added. After it had been stood for one hour, the solution was evaporated to dryness *in vacuo*. The residue was triturated with ether and washed repeatedly with ether by decantation. The product was collected by filtration with the aid of ether. Yield, 4.97 g (95%); mp 122—124°C; $[\alpha]_D^{22}$ +13.2° (*c* 1, ethanol); R_f 0.76.[15]

Found: C, 45.97; H, 5.23; N, 10.43%. Calcd for $C_{26}H_{35}O_5N_5Br_2 \cdot H_2O$: C, 46.37; H, 5.24; N, 10.40%.

Z-Pro-Phe-OEt (IV). *a) DCC Method.* Into a solution of benzyloxycarbonyl-L-proline (2.49 g, 10 mmol) and L-phenylalanine ethyl ester hydrochloride (1.93 g, 10 mmol) in chloroform (30 m*l*) there were added triethylamine (1.40 m*l*) and dicyclohexylcarbodiimide (2.06 g) at 0°C. After it had been stood overnight at 0°C, the desired product was obtained by the procedure described in the case of II. The residue obtained was solidified by adding ether and petroleum ether. The product was recrystallized from ethyl acetate - ether - petroleum ether. Yield, 3.30 g (78%); mp 65—68°C; $[\alpha]_D^{22}$ −33.8° (*c* 1, ethanol); R_f 0.87.[15] This compound was obtained previously as an oily product.[17]

Found: C, 67.72; H, 6.64; N, 6.61%. Calcd for $C_{24}H_{28}O_5N_2$: C, 67.90; H, 6.65; N, 6.60%.

b) MA Method. To a mixed anhydride prepared at −5°C from benzyloxycarbonyl-L-proline (2.4 g), isobutyl chloroformate (1.31 m*l*) and triethylamine (1.40 m*l*) in tetrahydrofuran (20 m*l*), a chilled solution of L-phenylalanine ethyl ester hydrochloride (1.93 g) dissolved in a mixture of triethylamine (1.40 m*l*) and chloroform (20 m*l*) was added. The reaction mixture was then allowed to stand overnight and then evaporated *in vacuo*. The oily residue was treated as described in the case of II. Yield, 3.00 g (71%); mp 65—68°C; R_f 0.87.[15]

Z-His(*im*-Bzl)-Pro-Phe-OEt (V). This compound was prepared by the same procedure as has been described for the preparation of II. One and ninety hundredths grams (5 mmol) of benzyloxycarbonyl(*im*)-benzyl-L-histidine and H-Pro-Phe-OEt·HCl[17] (1.64 g, 5 mmol), which was prepared from IV, gave 2.44 g (75%) of V as an oily product; R_f 0.73.[15]

Z-His(*im*-Bzl)-Pro-Phe-NHNH$_2$ (VI). A solution of V (3.00 g, 4.6 mmol) and hydrazine hydrate (4.6 m*l*, 92 mmol) in methanol (10 m*l*) was allowed to stand at room temperature for 2 days. The solution was then evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate, washed with water and the organic layer was dried over sodium sulfate and then evaporated *in vacuo*. The semi-solid residue weighed 2.50 g (85%); R_f 0.77.[15]

Z - His(*im* - Bzl) - Pro - Phe - His(*im* -Bzl)-Leu-ONb (VII). To a solution of VI (2.16 g, 3.4 mmol) dissolved in dimethylformamide (10 m*l*) cooled at 0°C, 3.8 N hydrogen chloride (1.80 m*l*, 6.8 mmol) in dioxane was added, followed by isoamyl nitrite (0.46 m*l*, 3.4 mmol).[18] After it had been allowed to stand for 6 min with occasional stirring, triethylamine (0.95 m*l*, 6.8 mmol) was added to the solution. To this, a chilled solution of III·2HBr (2.28 g, 3.4 mmol) dissolved in

15) The R_f value refers to the thin-layer chromatography with Merck silica gel G and to the *n*-butanol - acetic acid - pyridine - water (4 : 1 : 1 : 2, v/v) system. Compounds possessing a free amino group were detected by spraying them with ninhydrin, and those with a blocked amino group, by spraying them with 47% hydrobromic acid, and then with nihnhydrin.

16) B. G. Overell and V. Petrow, *J. Chem. Soc.*, **1955**, 232.

17) N. Izumiya and J. S. Fruton, *J. Biol. Chem.*, **218**, 59 (1956).

18) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).

a mixture of triethylamine (1.14 ml, 8.2 mmol) and dimethylformamide (7 ml) was added. The solution was stirred for 2 days at 0°C and then evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate, and the solution was washed successively with 3% hydrochloric acid, 4% sodium bicarbonate and water, dried over sodium sulfate, and then evaporated *in vacuo*. The residual oil weighed 1.72 g (46%); R_f 0.84.[15] All attempts to crystallize this oil failed.

H-His(*im*-Bzl)-Pro-Phe-His(*im*-Bzl)-Leu-ONb Trihydrobromide (VIII·3HBr). To a solution of VII (3.85 g, 3.5 mmol) in acetic acid (10 ml), 3 N hydrogen bromide-acetic acid (20 ml) was added. After it has been allowed to stand for 1 hr, the solution was evaporated to dryness *in vacuo*. The oily residue was then treated as has been described in connection with the preparation of III. Yield, 4.02 g (95%); R_f 0.76.[15]

Z-Tyr(*O*-Bzl)-Ile-OEt (IX). Nine and ten hundredths grams (20 mmol) of benzyloxycarbonyl-*O*-benzyl-L-tyrosine[19] was coupled with L-isoleucine ethyl ester *p*-toluenesulfonate[20] (6.62 g, 20 mmol) by the mixed anhydride method, following the procedure described for the preparation of IV. Recrystallization from ethyl acetate-petroleum ether gave 8.58 g (82%); mp 128°C; $[\alpha]_D^{25}$ +10.7° (*c* 1, acetic acid); R_f 0.97.[15]

Found: C, 70.43; H, 7.03; N, 5.18%. Calcd for $C_{32}H_{38}O_6N_2$: C, 70.31; H, 7.01; N, 5.13%.

H-Tyr-Ile-OEt Hydrochloride (X·HCl). Three and forty hundredths grams (6.5 mmol) of IX dissolved in 0.5 N methanolic hydrogen chloride (14.3 ml) was hydrogenated in the presence of palladium black. The filtrate from the catalyst was evaporated to dryness *in vacuo*. Yield of an oily product, 2.40 g (94%); R_f 0.77.[15]

Z-Val-Tyr-Ile-OEt (XI). This compound was prepared by the mixed anhydride method as has been described for the preparation of IV. Two and fifty-one hundredths grams (9 mmol) of benzyloxycarbonyl-L-valine and X·HCl (3.53 g, 9 mmol) gave 4.40 g of the crude product. It was recrystallized from methanol-ether. Yield, 4.00 g (80%); mp 191°C; $[\alpha]_D^{23}$ −20.2° (*c* 1, acetic acid); R_f 0.97.[15]

Found: C, 64.68; H, 7.30; N, 7.75%. Cacld for $C_{30}H_{41}O_7N_3$: C, 64.84; H, 7.44; N, 7.56%.

Z-Val-Tyr-Ile-NHNH$_2$ (XII). To a solution of XI (5.56 g, 10 mmol) in dimethylformamide (30 ml), hydrazine hydrate (10 ml) was added; the solution was allowed to stand for 2 days at 30°C. The reaction mixture was evaporated *in vacuo*. The hydrazide which precipitated upon the addition of water was filtered, washed with water and dried. The product was recrystallized from dimethylformamide-ethyl acetate. Yield, 5.35 g (97%); mp 279—280°C.

Found: C, 60.98; H, 7.26; N, 12.59%. Calcd for $C_{29}H_{39}O_6N_5\cdot\frac{1}{2}H_2O$: C, 61.07; H, 7.32; N, 12.71%.

Z-Val-Tyr-Ile-His(*im*-Bzl)-Pro-Phe-His(*im*-Bzl)-Leu-ONb (XIII). To a solution of XII (0.54 g, 1 mmol) dissolved in a mixture of acetic acid (160 ml) and water (10 ml), N hydrochloric acid (3 ml) was added, followed by N sodium nitrite (1.1 ml). After it had stood for 5 min at 0°C, the solution was diluted with cold water (200 ml). The azide which precipitated was collected by filtration, washed with 4% sodium bicarbonate and water, and then dried in a desiccator *in vacuo* at 0°C. The dried azide was added to a chilled solution of VIII·3HBr (1.176 g, 1 mmol) dissolved in a mixture of triethylamine (0.50 ml, 3.6 mmol) and dimethylformamide (12 ml); the solution was stirred for 3 days at 0°C, and then evaporated *in vacuo*, The residual oil was dissolved in ethyl acetate, and the solution was washed successively with 3% hydrochloric acid, 4% sodium bicarbonate and water. Oily residue was solidified by adding ether and pertroleum ether. The product was recrystallized from ethanol-ether-petroleum ether. Yield, 0.506 g (34%); mp 125—134°C; $[\alpha]_D^{18}$ −41.0° (*c* 0.38, acetic acid); R_f 0.78.[15]

Found: C, 65.06; H, 7.15; N, 12.05%. Calcd for $C_{81}H_{95}O_{14}N_{13}\cdot H_2O$: C, 65.17; H, 6.55; N, 12.20%.

H-Val-Tyr-Ile-His(*im*-Bzl)-Pro-Phe-His(*im*-Bzl)-Leu-ONb Trihydrobromide (XIV·3HBr). XIII (472 mg, 0.3 mmol) was treated with 3 N hydrogen bromide-acetic acid (3 ml) as has been described in connection with the preparation of III. Yield of an oily product, 494 mg (97%); R_f 0.60.[15]

Z-Arg(NO$_2$)-Val-Try-Ile-His(*im*-Bzl)-Pro-Phe-His-(*im*-Bzl)-Leu-ONb (XV). To a mixed anhydride prepared from of benzyloxycarbonyl-nitro-L-arginine[21] (170 mg, 0.48 mmol), isobutyl chloroformate (0.062 ml, 0.48 mmol) and triethylamine (0.068 ml, 0.48 mmol) in tetrahydrofuran (2 ml), a chilled solution of XIV·3HBr (508 mg, 0.32 mmol) dissolved in a mixture of triethylamine (0.162 ml, 1.15 mmol) and dimethylformamide (2 ml) was added. The reaction mixture was then allowed to stand overnight. The precipitate which formed upon the addition of 3% hydrochloric acid to the solution was collected, washed with 3% hydrochloric acid, 4% sodium bicarbonate and water, and dried. It was recrystallized from ethanol-ether. Yield, 448 mg (78%); mp 135—142°C; $[\alpha]_D^{18}$ −32.5° (*c* 0.4, acetic acid); R_f 0.75.[15]

Found: C, 58.19; H, 6.57; N, 14.54%. Calcd for $C_{87}H_{106}O_{17}N_{18}\cdot 6H_2O$: C, 58.57; H, 6.67; 14.13%.

H-Arg(NO$_2$)-Val-Tyr-Ile-His(*im*-Bzl)-Pro-Phe-His(*im*-Bzl)-Leu-ONb Trihydrobromide (XVI·3HBr). XV (428 mg, 0.24 mmol) in 3 N hydrogen bromide-acetic acid (3 ml) was treated as has been described in connection with the preparation of III. Yield of a hygroscopic powder, 412 mg (96%); R_f 0.68.[15]

Z-Asp(OBzl)-Arg(NO$_2$)-Val-Tyr-Ile-His(*im*-Bzl)-Pro-Phe-His(*im*-Bzl)-Leu-ONb (XVII). This compound was prepared by the mixed anhydride method as has been described for the preparation of XV. One hundred and twenty two milligrams (0.35 mmol) of benzyloxycarbonyl-β-benzyl-L-aspartic acid[22] and XVI·3HBr (410 mg, 0.23 mmol) gave 362 mg (79%) of XVII; mp 138—144°C; $[\alpha]_D^{18}$ −30.0° (*c* 0.3, acetic acid); R_f 0.90.[15]

Found: C, 58.82; H, 6.34; N, 13.38%. Calcd for $C_{98}H_{117}O_{20}N_{19}\cdot 6H_2O$: C, 59.17; H, 6.54; N, 13.39%.

H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH (XVIII). Two hundred milligrams (0.1 mmol) of

19) E. Wünsch, G. Fries and A. Zwick, *Chem. Ber.*, **91**, 5421 (1958).

20) T. Kato, S. Makisumi, M. Ohno and N. Izumiya, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **83**, 1151 (1962).

21) M. Bergmann, L. Zervas and H. Rinke, *Z. Physiol. Chem.*, **224**, 40 (1934).

22) N. Izumiya, H. Uchio and T. Yamashita, *Nippon Kagaku Zassi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **78**, 420 (1958).

XVII dissolved in a mixture (30 ml) of methanol : acetic acid : water (60 : 20 : 15, v/v) was subjected to hydrogenolysis in the presence of palladium black with vigorous stirring for 48 hr.[23] After removal of the catalyst by filtration the solvent was removed *in vacuo*. Evaporation was repeated with the addition of dioxane. The crude residue, 130 mg, was collected with the aid of a mixture of dioxane and ether. The paper chromatography of the residue indicated the presence of a major spot, R_f 0.53,[24] positive with ninhydrin, Sakaguchi and Pauly reaction, besides several weak spots positive with ninhydrin. A material in the major spot revealed strong pressor activity when it was extracted with dilute acetic acid. The crude residue (120 mg) was divided to six portions, and each portion was purified by subsequent column chromatography using carboxymethyl cellulose (2.5×50 cm; solvent, ammonium acetate with a gradient from 0.05 to 0.4 M), DEAE-Sephadex A-25 (1.5×50 cm; solvent, pyridine-collidine-acetate buffer with a gradient from pH 8.1 to 7.0), and Bio-Gel P-2 (0.8×100 cm; solvent, 0.1 N acetic acid) which have proved to be most effective in isolating natural human angiotensin I.[8,9] The six active fractions from the Bio-Gel column were pooled and lyophilized, leaving a white powder (acetate of XVIII, presumably), 8.6 mg; $[\alpha]_D^{26}$ $-63\pm3°$ (*c* 0.08, 0.05 N acetic acid;[25] amino acid ratios in acid hydrolysate, aspartic acid 0.98, arginine 1.03, valine 1.12, tyrosine 0.88, isoleucine 0.92, histidine 1.98, proline 1.10, phenylalanine 1.03, leucine 1.06. The homogeneity of the powder was ascertained by paper, thin-layer and column chromatography as described later. Furthermore, the stereospecific purity of each amino acid residue of the powder was confirmed by the enzymic method; with leucine aminopeptidase[7] the sample was degraded completely to nine amino acid residues leaving no decapeptide, while incubation of the sample with carboxypeptid ase A[26] caused the liberation of leucine, histidine and phenylalanine leaving H-Asp-Arg-Val-Try-Ile-His-Pro-OH as an unhydrolyzable core.

Comparison of Synthetic XVIII and Natural Human Angiotensin I. The powder (acetate of XVIII) obtained above was chromatographed on Toyo Roshi No. 51 paper affording R_f 0.53[24] and 0.51,[27] and on Serva TLC thin-layer affording R_f 0.71;[28] these values were exactly identical with those of natural human angiotensin I. The chemical identy was confirmed further by the cochromatography of a mixture of the preparations from the synthesis and the human origin with the Bio-Gel column (0.8×100 cm; solvent, 0.1 N acetic acid). Bioassay with the rat pressor activity was performed as has been described in the paper;[9] the specific pressor activity of the synthetic decapeptide was identical with that of the natural material within the limit of the experimental error. Oxytocic activity of the synthetic material was nil, also as has been observed in natural material.[9]

We wish to thank Mr. Kosaku Noda for amino acid analysis and Miss Junko Yamada for skillful technical assistance.

23) The reaction was followed at various times with a paper chromatography, and the bioassay of the pressor activity; the highest activity observed after the hydrogenation of some 48 hr. However, the chromatography indicated that a small part of the imidazoyl-benzyl groups was remained untouched; this fact might be one of the reasons that the yield (8.6 mg) of pure product was poor. Treatment of the hydrogenated material of XVII with sodium-liquid ammonia did not give a product bearing stronger pressor activity.

24) The R_f value refers to the paper chromatography with the solvent system of *n*-butanol - acetic acid - pyridine - water (4 : 1 : 1 : 2 v/v).

25) We could not determine the specific rotation of natural human angiotensin I because of the scantity of material. Neither Skeggs *et al.* nor Peart *et al.*, also, did not determine the specific rotation of natural horse and bovine angiotensin I.[3,4] It would be of noteworthy that the specific rotations of synthetic [Val^5]-angiotensin I are reported as value of close resemblance with that of the powder (acetate of XVIII); $[\alpha]_D$ $-67.5\pm2°$ (*c* 1.69, H_2O) (R. Schwyzer *et al.*, *Helv. Chim. Acta*, **41**, 1273 (1958)) and $[\alpha]_D^{22}$ $-74\pm3°$ (*c* 1.6, 0.05 N acetic acid) (St. Guttmann, *ibid.*, **44**, 721 (1961)) for the synthetic [Val^5]-angiotensin I as an acetate salt.

26) F. Sanger and E. O. P. Thompson, *Biochem. J.*, **53**, 366 (1953).

27) Solvent, *n*-butanol - acetic acid - water (7 : 1 : 2, v/v).

28) Solvent, *s*-butanol - 3% ammonium hydroxide (100 : 44; v/v).