3% aq AcOH with vigorous stirring. The precipitated octapeptide was filtered, washed several times with 3% aq AcOH, and dried *in vacuo*. The product was crystallized from EtOH: yield, 350 mg; R_f 0.75; amino acid analysis (acid hydrolysis): Asp(2.1), Tyr(1.0), Gly(1.0), Met(2.0), Phe(0.94), Trp(present).

Procedure D. β-Aspartyl-*O*-sulfatotyrosylmethionylglycyltryptophylmethionylaspartylphenylalanine Amide (21).—The octapeptide XVI (310 mg, 0.3 mmol) was dissolved in a mixture of anhydrous DMF (18 ml) and freshly distilled (over NaOH) pyridine (18 ml). To this solution, a solution of pyridine–SO_3 (936 mg, 6 mmol) in DMF (18 ml) was added. The resulting solution was kept at room temperature for 17 hr and then evaporated to dryness *in vacuo*. The crystalline residue was washed several times with H_2O (15 ml each time) and dried *in vacuo*; yield 395 mg. The crude compound was treated with trifluoroacetic acid (5 ml) for 20 min. The acid was removed *in vacuo* and the trifluoroacetate triturated with Et_2O and dried; yield, 380 mg. The crude octapeptide sulfate was dissolved in 0.1 *M* $(NH_4)_2CO_3$ and purified by chromatography on a DEAE-Sephadex A-25 column. The column (1.5 × 15 cm) was eluted with a linear gradient of $(NH_4)_2CO_3$ (350 ml of 0.1 *M* and 350 ml of 1.5 *M*) and fractions of 7 ml were collected and scanned by uv absorption at 253 mμ. The fractions (68–100) with the octapeptide sulfate were combined and lyophilized several times: yield, 87 mg; ir spectrum (KBr) 1050 and 1250 cm^{-1}; uv (0.1 *N* NaOH) λ_{max} 288 mμ (ε 4900); R_f 0.73; amino acid analysis (acid hydrolysis) Asp (2.0), Tyr (1.0), Met(2.0), Gly(1.0), Phe(1.0), Trp (present). Due to the β linkage of the N-terminal amino acid residue, leucine amino peptidase did not degrade the octapeptide.

Procedure E. *O*-Sulfatotyrosylaspartylmethionylglycyltryptophylmethionylaspartylphenylalanine Amide (6). The octapeptide trifluoroacetate (50 mg, 0.046 mmol, obtained from 50 mg of XX and 1 ml of trifluoroacetic acid) was added to precooled cooled H_2SO_4 (−5°) with stirring. After 15 min, it was poured into Et_2O (−70°), centrifuged, and washed several times with this ether. The residue was dissolved in 0.1 *M* $(NH_4)_2CO_3$ and purified as described under procedure D: yield, 18 mg; R_f 0.69; uv spectrum (0.1 *N* NaOH) λ_{max} 288 mμ (ε 4800); amino acid analysis (a) acid hydrolysis, Asp(2.1), Tyr(1.0), Met(2.0), Gly(0.97), Phe(1.0), Trp(present).

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Angiotensin II Analogs. III. Synthesis and Biological Evaluation of Some Des-aspartyl-angiotensins[1]

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Analogs of [des-Asp[1],Ile[5]]-angiotensin II were prepared by solid phase synthesis to test contributions of the α-amino and guanidino groups of arginine to the pressor activity of this heptapeptide. [Des-Asp[1],δAvl[2],Ile[5]]-angiotensin II and [des-Asp[1],εAcp[2],Ile[5]]-angiotensin II had lower pressor activities (7 and 5%, respectively) than would be predicted by the theory that the N-terminal α-amino group in related heptapeptides exerts an unfavorable effect. The high pressor activity of [des-Asp[1],D-Abu[2],Ile[5]]-angiotensin II (23%) compared with [des-Asp[1],L-Abu[2],Ile[5]]-angiotensin II and its desamino derivative (each 1%) showed that the α-amino group in the D series exerts a favorable effect which is absent in the L series. [Des-Asp[1],Gly[2], Ile[5]]-angiotensin II had approximately half the pressor activity (10%) and half the duration of action of [des-Asp[1],D-Abu[2],Ile[5]]-angiotensin II, perhaps because it lacked the orienting effect of a side chain or because it was more susceptible to aminopeptidases. [Des-Asp[1],Ac-Gly[2],Ile[5]]-angiotensin II had only 1% pressor activity indicating that the positive charge was probably the principal feature of the α-amino group which contributed to the biological activity.

The heptapeptide, [des-Asp[1],Ile[5]]-angiotensin II (Arg-Val-Tyr-Ile-His-Pro-Phe), was found to possess high pressor activity (15–35% of the activity of [Ile[5]]-angiotensin II), whereas the hexapeptide, Val-Tyr-Ile-His-Pro-Phe, had only slight pressor activity.[2,3] In an attempt to elucidate the contribution of arginine to the pressor activity of this peptide, Havinga, *et al.*,[3-7] synthesized a series of analogs in which arginine was replaced by other amino acids. Replacement of arginine by a D-amino acid always gave a peptide with higher pressor activity than the peptide containing the corresponding L-amino acid. In the two cases studied, the desamino compounds were found to be even more active than the corresponding D-α-amino compounds. Havinga and Schattenkerk[7] hypothesized that this was due to an unfavorable effect of the free N-terminal α-amino group in the heptapeptide which was less pronounced in the D isomer and absent in the desamino compounds. They concluded that the contribution of the arginyl residue to the pressor activity of [des-Asp[1],-Ile[5]]-angiotensin II was due to the H bonding capacity of the guanidinium group rather than its positive charge. This conclusion was based largely on the

(1) Part II: E. C. Jorgensen and W. Patton, *J. Med. Chem.*, **12**, 935 (1969). This investigation was supported in part by Public Health Service Research Grants AM 08066 and AM 06704 from the National Institutes of Arthritis and Metabolic Diseases and Training Grant No. 5 T01 GM 00728 from the National Institute of General Medical Sciences. The abbreviations used to denote amino acid derivatives and peptides are those recommended in *Biochemistry*, **5**, 2485 (1966). δAvl stands for δ-aminovaleric acid.

(2) F. M. Bumpus, P. A. Khairallah, K. Arakawa, I. H. Page, and R. R. Smeby, *Biochim. Biophys. Acta*, **46**, 38 (1961).

(3) E. Havinga, C. Schattenkerk, G. H. Visser, and K. E. T. Kerling, *Rec. Trav. Chim. Pays-Bas.*, **83**, 672 (1964).

(4) C. Schattenkerk, G. H. Visser, K. E. T. Kerling, and E. Havinga, *ibid.*, **83**, 677 (1964).

(5) G. H. Visser, C. Schattenkerk, K. E. T. Kerling, and E. Havinga, *ibid.*, **83**, 684 (1964).

(6) C. Schattenkerk and E. Havinga, *ibid.*, **84**, 653 (1965).

(7) E. Havinga and C. Schattenkerk, *Tetrahedron Suppl.*, **8**, 313 (1966).

low activities of the heptapeptides containing ϵ-trimethylamino-Nle (0.3%) or ϵ-trimethylaminocaproic acid (1.5%) in place of arginine compared with the higher activities of heptapeptides containing D-Cit (11%) or δ-nitroguanidinovaleric acid (7.7%). Since this conclusion was based upon D-amino acids and desamino compounds as well as L-amino acids, its validity is dependent upon the correctness of their hypothesis with regard to the unfavorable role of the free N-terminal α-amino group. As was discussed earlier,[8] there are reasons to doubt the validity of Havinga and Schattenkerk's hypothesis, and therefore a series of peptides was prepared to test it.

Havinga and Schattenkerk[7] had predicted, on the basis of their hypothesis, that des-aspartyl-angiotensins containing δ-aminovaleroyl or ϵ-aminocaproyl residues in place of arginine would show good activity. These analogs were therefore synthesized and tested for pressor activity. In order to test the alternate theory[8] that the D-α-amino group might be exerting a favorable influence, heptapeptides were prepared which had no side chain functional group in the N-terminal amino acid. These peptides had butyric acid, L-α-aminobutyric acid, D-α-aminobutyric acid, or glycine in place of arginine.

Chemistry.—The solid-phase synthesis was carried out as previously described[9] with the following variations. (1) Coupling reactions were allowed to proceed overnight. (2) Boc-His(Bzl) was coupled as a suspension in DMF–CH_2Cl_2 (1:1). (3) After incorporation of isoleucine, all subsequent deprotections were carried out by shaking the polymer with two portions of 1.5 *N* HCl–AcOH for 30 min each. This last variation was made necessary by the finding that the tripeptide had not been completely deprotected by shaking with 1.2 *N* HCl–AcOH for 30 min. This deprotection problem was probably due to dilution of the HCl by AcOH present in the polymer beads since the ratio of polymer to solvent was greater in this synthesis than that which is normally used.

After liberation from the polymer, the peptides were hydrogenated at 3 atm over 10% Pd-C. Hydrogenation for 48 hr was adequate for removal of the benzyl group from histidine, but under these conditions, the aromatic rings of tyrosine and phenylalanine were partially reduced. Model studies with tyrosine itself showed that some reduction occurred with this catalyst in 8 hr at atmospheric pressure; therefore, it seemed unlikely that conditions could be found which would permit quantitative debenzylation of histidine without concomitant reduction of tyrosine and phenylalanine. Accordingly, it was decided to use these excessive hydrogenation conditions to ensure that no benzylhistidine remained to further complicate the purification.

The peptides were purified by chromatography on sulfoethyl-Sephadex since the sulfoethyl-cellulose column used in earlier work[9] did not resolve the desired products from the des-isoleucine peptides.

Attempts to evaluate the steric homogeneity of some of the peptides with aminopeptidase-M were not satisfactory because the enzyme used did not attack the His–Pro bond at a practical rate; however, this enzyme did indicate the possibility of some racemization in histidine. Oxidation of acid hydrolysates with *Crotalus adamanteus* L-amino acid oxidase was useful for all of the amino acids except proline. This technique confirmed that approximately 10% of D-histidine was present in each peptide but all other amino acids were optically pure. Proline was demonstrated to be optically pure in one peptide by the chromatographic technique of Manning and Moore[10] after the other amino acids had been destroyed by HNO_2.[11] The Boc-His(Bzl) used in this work was optically pure as shown by the quantitative enzymatic oxidation of the histidine obtained from it by hydrogenolysis and acidolysis, so the presence of D-histidine in the peptides indicates that racemization occurred despite the use of a urethan protecting group. The cause of this racemization is not known at present.

Bioassay.—The compounds were tested for pressor activity in nephrectomized, pentolinium-treated male rats anesthetized with pentobarbital.[12] In most cases the slopes of the log dose–response curves differed slightly from that of the standard. The pressor activities listed in Table I were determined at response levels which were within the range of measured values for both the standard and the unknown. The figures for duration of response were obtained by measuring the widths of the blood pressure peaks at half-height for equipressor doses of standard and unknown. The molarities of the peptide solutions (including standard) were calculated from amino acid analyses and the biological results are expressed on a molar basis.

Structure–Activity Relationships.—The pressor activities of 5-aminovaleroyl-Val-Tyr-Ile-His-Pro-Phe (7%) and 6-aminocaproyl-Val-Tyr-Ile-His-Pro-Phe (5%) are much lower than one would expect from Havinga and Schattenkerk's hypothesis which predicts that these peptides, which lack the free N-terminal α-amino group, should be more active than the corresponding D-α-amino compounds (D-Orn-Val-Tyr-Ile-His-Pro-Phe, 23%[7] and D-Lys-Val-Tyr-Ile-His-Pro-Phe, 23%).[3] These peptides are, in fact, only slightly more active than the corresponding L-α-amino compounds (each 3%).[3] These data do not support Havinga and Schattenkerk's hypothesis that the α-amino group is deleterious.

The high pressor activity of D-Abu-Val-Tyr-Ile-His-Pro-Phe (23%) compared with L-Abu-Val-Tyr-Ile-His-Pro-Phe and butyryl-Val-Tyr-Ile-His-Pro-Phe (each 1%) is good evidence that the N-terminal D-α-amino group can exert a strong favorable effect in the heptapeptide. The magnitude of this effect suggests that the D-α-amino group might have been primarily responsible for the pressor activities of the analogs prepared by Havinga, *et al.*[3-7] Since the relative contributions of the D-α-amino group and the side chain functional group to the pressor activities of these analogs cannot be determined from existing data, it is impossible to draw any conclusions about the contribution of the guanidino group of arginine in [des-Asp[1],Ile[5]]-angiotensin II from the pressor activities of analogs containing a D-amino acid in place of arginine. Thus

(8) E. C. Jorgensen, G. C. Windridge, W. Patton, and T. C. Lee, *Proc. First Amer. Peptide Symp., Yale 1968*, in press.

(9) E. C. Jorgensen, G. C. Windridge, W. Patton, and T. C. Lee, *J. Med. Chem.*, **12**, 733 (1969).

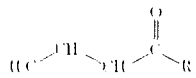
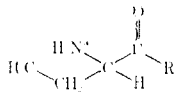
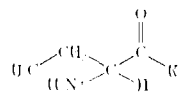
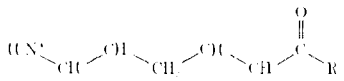
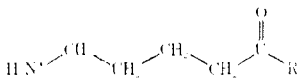
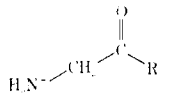
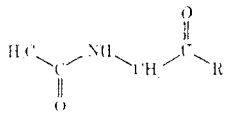
(10) J. M. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).

(11) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley & Sons, Inc., New York, N. Y., 1961, p 2184.

(12) R. Boucher, R. Veyrat, J. de Champlain, and J. Genest, *Can. Med. Assoc. J.*, **90**, 194 (1964).

TABLE I

PRESSOR ACTIVITIES OF NEW ANGIOTENSIN II ANALOGS

No.	Peptide	Structure	Activity,[a] %	Relative[a] duration
VII	Val-Tyr-Ile-His-Pro-Phe	H-R[b]	0.3	
VIII	Butyryl-Val-Tyr-Ile-His-Pro-Phe		1	70
IX	L-Abu-Val-Tyr-Ile-His-Pro-Phe		1	20–40
X	D-Abu-Val-Tyr-Ile-His-Pro-Phe		23	100
XI	δAcp-Val-Tyr-Ile-His-Pro-Phe		5	90
XII	δAvl-Val-Tyr-Ile-His-Pro-Phe		7	100
XIV	Gly-Val-Tyr-Ile-His-Pro-Phe		10	30–40
XV	Ac-Gly-Val-Tyr-Ile-His-Pro-Phe		1	100

[a] Relative to [Asn[1],Val[5]]-angiotensin II (Hypertensin–CIBA) = 100. [b] R = Val-Tyr-Ile-His-Pro-Phe.

the pressor activity which Havinga and Schattenkerk[7] found for [des-Asp[1],D-Cit[2],Ile[5]]-angiotensin II (11%) cannot be considered as valid support for their theory that the contribution of the arginyl residue in [des-Asp[1],Ile[5]]-angiotensin II is due to the H-bonding capacity of the guanidino group. A more valid evaluation of the contribution of the arginyl residue to the pressor activity of [des-Asp[1],Ile[5]]-angiotensin II would be *via* the analogs containing a free N-terminal L-α-amino group since this group is present in the arginyl residue and presumably influences the pressor activity. Of the analogs so far prepared, only the guanidino group of L-arginine itself conveys appreciable pressor activity to the peptide. Replacement of this guanidino group by nitroguanidino (1.3%),[6] ureido (0.5%),[3] or Me_3N^+ (0.3%) groups[7] gave compounds with essentially the same pressor activity as the peptide with no functional group in the side chain ([des-Asp[1],L-Abu[2],Ile[5]]-angiotensin II, 1%). This would indicate that the contribution of the guanidino group cannot be explained in terms of a single parameter such as cationic charge or hydrogen bonding capability.

The pressor activity of [des-Asp[1],Gly[2],Ile[5]]-angiotensin II (10%) indicated that its N-terminal α-amino group is capable of exerting the same effect as the D-α-amino group of [des-Asp[1],D-Abu[2],Ile[5]]-angiotensin II. Its lower activity might be ascribed to the lack of an alkyl side chain which sterically facilitates interactions of the amino group with the D-amino binding site. In addition, its lower activity could be due to its greater susceptibility to aminopeptidases since its duration of action was less than 0.5 that of the D-Abu compound. [Des-Asp[1],Gly[2],Ile[5]]-angiotensin II had the same duration of action and therefore presumably similar enzymatic susceptibility to that of [des-Asp[1],L-Abu[2],Ile[5]]-angiotensin II. The high activity of [des-Asp[1],Gly[2],-Ile[5]]-angiotensin II therefore showed that the difference in pressor activity between [des-Asp[1],D-Abu[2],Ile[5]]-angiotensin II and [des-Asp[1],L-Abu[2],Ile[5]]-angiotensin II was only partly due to differences in rate of attack by aminopeptidases. The most important contribution of the D-α-aminobutyryl residue was probably due to a direct effect of its α-amino group. This effect could be due to enhanced binding of the peptide to a receptor or to stabilization of a preferred conformation. When the terminal amino group of [des-Asp[1],Gly[2],Ile[5]]-angiotensin II was acetylated, the pressor activity dropped to the same low level (~1%) as the hexapeptide, Val-Tyr-Ile-His-Pro-Phe. It may be tentatively concluded from these data that the positive charge of the N-terminal D-α-amino group is the feature which is responsible for the group's contribution to the pressor activity of des-aspartyl angiotensins containing a D-amino acid in place of arginine.

If this N-terminal D-α-amino group functions through enhanced binding to the receptor, it probably interacts with a group in the receptor which is not normally involved in the binding of angiotensin II or des-aspartyl-angiotensin II. Such an "accidental" binding site need not occur in other species or in receptor sites associated with other physiological or pharmacological actions of angiotensin II so [des-Asp[1],D-Abu[2],Ile[5]]-angiotensin II may show a different spectrum of biological activities than does angiotensin II.

Experimental Section[13]

N-t-**Butyloxycarbonyl-D-α-aminobutyric Acid (I).**—To a solution of 2.06 g (20 mmol) of D-α-aminobutyric acid in 40 ml of H_2O were added 1.60 g (40 mmol) of MgO and 5.72 g (40 mmol) of *t*-butyloxycarbonyl azide. This mixture was stirred at 45° for 48 hr, extracted with Et_2O (2 × 40 ml), then acidified to pH 3.5 with citric acid. The oil which sepd was extracted into AcOEt (4 × 40 ml). The AcOEt washes were combined, washed with H_2O (4 × 40 ml), then dried ($MgSO_4$). Evaporation of AcOEt at 45° on a rotary evaporator gave 3.2 g (80%) of a colorless oil which could not be crystallized. Tlc revealed 3 Cl + spots,[14] R_f I: 0.00 (faint), 0.49 (strong), 0.65 (faint), and R_f II: 0.00 (faint), 0.81 (strong), 0.99 (faint).

The oil was dissolved in 50 ml of Et_2O and 3.9 ml (20 mmol) of dicyclohexylamine was added. The Et_2O was removed on a rotary evaporator and the resulting oil was triturated with heptane giving a white powder. This was recrystd from heptane giving 2.3 g (30%) of colorless needles, mp 138–139°, $[\alpha]^{23}D$ −5.7° (*c* 2, MeOH). *Anal.* ($C_{21}H_{38}N_2O_4$) C, H, N.

The salt, 1.8 g (4.7 mmol) was dissolved in 5 ml of MeOH, diluted with 25 ml of H_2O, and basified with 5 ml of 1 *N* NaOH. The amine was removed by extraction with heptane (2 × 50 ml) then the aq solution was acidified with citric acid. The resulting oil was extracted into CH_2Cl_2 (4 × 50 ml). The CH_2Cl_2 washes were combined, washed with H_2O (2 × 100 ml), dried ($MgSO_4$), and then evapd on a rotary evaporator; yield 0.62 g (66%) of a colorless oil. Tlc showed one spot, R_f I: 0.49, R_f II: 0.81.

N-t-**Butyloxycarbonyl-L-α-aminobutyric Acid (II).**—This compd was prepared as described for the D isomer (I) and purified *via* the dicyclohexylammonium salt (mp 138–139°), $[\alpha]^{23}D$ +6.0° (*c* 2, MeOH). Tlc as for compd I. *Anal.* ($C_{21}H_{38}N_2O_4$) C, H, N.

N-t-**Butyloxycarbonyl-δ-aminovaleric Acid (III).**—This compd was prepared as described for I from 2.3 g (20 mmol) of δ-aminovaleric acid. An oil was obtained which crystallized when triturated with hexane; yield 2.0 g (45%), mp 49–52°. Tlc showed one Cl + spot, R_f I: 0.53, R_f II: 0.77 *Anal.* ($C_{10}H_{19}NO_4$) C, H, N.

N-t-**Butyloxycarbonyl-ε-aminocaproic Acid (IV).**—This compd was prepared as described for I from 2.6 g (20 mmol) of ε-aminocaproic acid. IV was obtained as an oil which crystallized when cooled to −20°: yield, 3.6 g (80%); mp 39–41°. Tlc showed one Cl + spot, R_f I: 0.54, R_f II: 0.80. *Anal.* ($C_{11}H_{21}NO_4$) C, H, N.

Boc-Tyr(Bzl)-Ile-His(Bzl)-Pro-Phe-polymer (V).—Boc-Phe-polymer (18 g, 3.6 mmol)[9] was placed in the reaction vessel,[15] deprotected, and neutralized as previously described,[9] then acylated overnight with 3.1 g (14.4 mmol) of Boc-Pro and 3.0 g (14.4 mmol) of DCCI in 50 ml of CH_2Cl_2. The peptide polymer was then analyzed and acetylated as before.[9] This same procedure was used with 5.0 g (14.4 mmol) of Boc-His(Bzl) suspended in 50 ml of DMF–CH_2Cl_2 (1:1). When acylation with 3.3 g (14.4 mmol) of Boc-Ile was carried out using the same procedure, approx 30% of the tripeptide [His(Bzl)-Pro-Phe] was detected by electrophoresis. This contaminant was reduced to approx 10% by repeating the acylation but the remaining contaminants could not be acetylated. Since this indicated that deprotection may have been incomplete, all subsequent deprotections were carried out by shaking the peptide polymer with 1.5 *N* HCl–AcOH for two 30-min periods. Incorporation of Tyr(Bzl) by this procedure using 5.4 g (14.4 mmol) of Boc-Tyr(Bzl) was satisfactory but analysis was complicated by the presence of Tyr-His(Bzl)-Pro-Phe.

Boc-Val-Tyr(Bzl)-Ile-His-(Bzl)-Pro-Phe-polymer (VI).—The protected pentapeptide polymer (V), 15.3 g (2.5 mmol), was deprotected and acylated with 2.2 g (10.0 mmol) of Boc-Val and 2.1 g (10.0 mmol) of DCCI in 50 ml of CH_2Cl_2.

Val-Tyr-Ile-His-Pro-Phe (VII).—A portion of the protected hexapeptide polymer (0.9 g, 0.15 mmol) was washed with CF_3COOH (3 × 20 ml) then suspended in CF_3COOH (10 ml) and anisole (1 ml). HBr (scrubbed with saturated resorcinol in C_6H_6 and with $CaCl_2$) was bubbled through the suspension for 1 hr. The suspension was filtered by suction and the polymer was washed with CF_3COOH (2 × 10 ml). The filtrate was evapd on a rotary evaporator at 30° and the residue lyophilized from AcOH. The resulting powder was washed with dry Et_2O and dried *in vacuo* giving 170 mg of off-white powder.

This powder was dissolved in 15 ml of 50% MeOH containing 2% AcOH, 150 mg of 10% Pd-C was added and the suspension was stirred under 3 atm of H_2 for 48 hr at room temperature. The catalyst was filtered off (filter aid) and washed with 95% AcOH. The filtrate was evapd *in vacuo* at 40° and the residue lyophilized from AcOH giving 120 mg of white powder. This was dissolved in 5 ml of 0.1 *M* NH_4OAc–1 *M* AcOH and the solution applied to a 2.5 × 100 cm column of Sephadex-C25-SE (NH_4^+) packed in the same buffer. The column was eluted at 34 ml/hr with a linear gradient of NH_4OAc in 1 *M* AcOH using a concentration change of 2.0 × 10^{-4} *M*/ml. The effluent was monitored at 280 mμ and 8-ml fractions were collected. Fractions which were homogeneous on tlc in solvents III and IV were pooled and lyophilized giving 46 mg (31%). After further purification *via* the picrate salt[9] there was 30 mg of chromatographically homogeneous material, R_f III: 0.67, R_f IV: 0.43, R_f V: 0.88, Pauly and ninhydrin +. A 72-hr acid hydrolysate had the following amino acids: Val 1.03, Tyr 0.97, Ile, 1.02, His 1.00, Pro 1.00, Phe 1.01; peptide content, 79%.

Butyryl-Val-Tyr-Ile-His-Pro-Phe (VIII).—To a solution of 15 mg (0.015 mmol) of the purified hexapeptide VII in 1 ml of purified DMF[16] were added 0.003 ml (0.045 mmol) of Et_3N and 0.007 ml (0.032 mmol) of *p*-nitrophenyl butyrate.[17] After 24 hr at 25°, electrophoresis showed a single Pauly +, ninhydrin − spot at E_H 0.27. The peptide was pptd by the addition of 10 ml of dry Et_2O, centrifuged, washed with 10 ml of Et_2O, and lyophilized from AcOH to yield 14 mg (91%). Tlc revealed a single Pauly +, ninhidrin − spot, R_f III: 0.65, R_f IV: 0.50, R_f V: 0.65. A 72-hr acid hydrolysate had the following amino acids: Val 1.04, Tyr 0.95, Ile 1.02, His 1.00, Pro 1.01, Phe 0.98; peptide content, 83%.

A 1-mg sample of the purified peptide was hydrolyzed under N_2 in constant boiling HCl at 110° for 48 hr. The solution was evapd in a vacuum desiccator over NaOH pellets, the residue was dissolved in 0.2 ml of H_2O and 5 mg of Tris was added. The solution was adjusted to pH 7.2 with 1 *N* HCl and 0.04 ml (2.25 units) of *C. adamanteus* L-amino acid oxidase (Worthington) was added. The solution was incubated at 37° for 24 hr, another 0.04 ml of enzyme solution was added and the solution was incubated for another 24 hr. The solution was diluted with 1.0 ml of 0.2 *N* sodium citrate, pH 2.2, and 0.5-ml portions were subjected to amino acid analysis. The following amino acids remained:[18] Val 0.00, Tyr 0.00, Ile 0.00, His 0.11, Pro 1.00, Phe 0.00.

Abu-Val-Tyr-Ile-His-Pro-Phe (IX).—A sample of the protected hexapeptide polymer VI (0.9 g, 0.15 mmol) was deprotected and acylated with 122 mg (0.60 mmol) of Boc-Abu (II) and 125 mg (0.60 mmol) of DCCI in 20 ml of CH_2Cl_2. After acetylation, the peptide was cleaved and hydrogenated, as described for VII, giving 155 mg. The crude product was purified on a 2.5 × 100 cm column of Sephadex-C25-SE (NH_4^+) packed in 0.05 *M* NH_4OAc–1 *M* AcOH. The column was eluted at 60 ml/hr with a linear gradient of NH_4OAc in 1 *M* AcOH using a concentration change of 3.3 × 10^{-4} *M*/ml. The effluent was monitored at 280 mμ and 8-ml fractions were collected. The fractions from the center of the main peak were lyophilized to give 75 mg of an off-white powder. After further purification *via* the picrate salt,[9]

(13) Melting points were measured in a Thomas–Hoover Uni-Melt apparatus and are corrected. Amino acid analyses were performed on a Spinco Model 116 amino acid analyzer using the standard 4-hr methodology. Peptides were hydrolyzed under N_2 at 110° in constant boiling point HCl containing aspartic acid or alanine as internal standards. Peptide content was calculated in terms of free peptide rather than the hydrated salt. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif. Where analyses are indicated only by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values. Rotations were measured with a Bendix-NPL automatic polarimeter, Type 143A, equipped with a digital readout and printer. Precoated silica gel G plates (E. Merck) were used for tlc. The following solvent systems were used: I, xylene–pyridine–AcOH (100:15:5); II, *i*-Pr_2O–$CHCl_3$–AcOH (6:3:1); III, *n*-BuOH–AcOH–H_2O (3:1:1); IV, *sec*-BuOH–3% NH_3 (100:44); V, pyridine–H_2O (4:1); VI, *n*-BuOH–AcOH–H_2O (4:1:5), upper phase. Electrophoresis was carried out on Whatman No. 1 paper at 5000 V using AcOH–HCOOH buffer, pH 1.85, in a Savant apparatus. E_H indicates the electrophoretic mobility relative to histidine = 1.00.

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(18) These values are corrected for the amount of racemization occurring when a mixture of these amino acids was subjected to the same hydrolytic conditions.

there was 50 mg (33%) of white powder. Tlc revealed a single Pauly + spot, R_f III: 0.64, R_f IV: 0.50, R_f V: 0.91. A 72-hr acid hydrolysate had the following amino acids: Abu 1.01, Val 1.02, Tyr 0.91, Ile 1.02, His 1.05, Pro 1.00, Phe 0.93; peptide content, 88%. Abu emerged from the long (56 cm) column of the analyzer 20 ml before valine and had a color value which was 97% of that of leucine. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: Abu 0.00, Val 0.00, Tyr 0.00, Ile 0.00, His 0.11, Pro 1.00, Phe 0.00. A 27-hr aminopeptidase-M digest[19] had Abu 1.00, Val 0.96, Tyr 0.88, Ile 0.81, His 0.00, Pro 0.00, Phe 0.71.

D-Abu-Val-Tyr-Ile-His-Pro-Phe (X).—This compd was prepared in the same manner as the L isomer IX except that 125 mg of Boc-D-Abu (I) was used. The crude product (175 mg) was purified in the same manner as the L isomer giving 55 mg (36%) of white powder. Tlc showed one Pauly + spot, R_f III: 0.64, R_f IV: 0.50, R_f V: 0.91, R_f VI: 0.50. A 72-hr acid hydrolysate had the following amino acids: Abu 1.01, Val 1.00, Tyr 0.98, Ile 0.98, His 1.02, Pro 0.99, Phe 1.00; peptide content, 84%. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: Abu 1.03, Val 0.00, Tyr 0.00, Ile 0.00, His 0.10, Pro 1.00, Phe 0.00.

εAcp-Val-Tyr-Ile-His-Pro-Phe (XI).—This was synthesized as described for IX from 900 mg (0.15 mmol) of the protected hexapeptide polymer (VI) and 138 mg (0.60 mmol) of Boc-εAcp (IV). Purification of the crude peptide (115 mg) on Cellex-SE (NH_4^+)[9] did not give a chromatographically homogeneous product. The partially purified product (40 mg) was further purified on Sephadex-C25-SE as described for compd VII giving 8 mg. After further purification *via* the picrate salt there was 5.5 mg (3%). Tlc revealed a single Pauly + spot, R_f III: 0.65, R_f IV: 0.34. A 72-hr acid hydrolysate had the following amino acids: εAcp 1.00, Val 1.07, Tyr 0.95, Ile 1.04, His 1.00, Pro 1.05, Phe 1.05; peptide content, 78%. εAcp emerged from the short (5.3 cm) column of the analyzer 3 ml before lysine and had a color value which was 27% of that of leucine. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: εAcp 1.02, Val 0.00, Tyr 0.00, Ile 0.00, His 0.12, Pro 1.00, Phe 0.00.

δAvl-Val-Tyr-Ile-His-Pro-Phe (XII).—This was synthesized as described for IX from 900 mg (0.15 mmol) of the protected hexapeptide polymer (VI) and 130 mg (0.60 mmol) of Boc-δAvl (III). Purification of the crude peptide (125 mg) on Cellex-SE (NH_4^+) or on Sephadex-C25-SE (NH_4^+) using an NH_4OAc concentration change of 3.3×10^{-4} *M*/ml did not give a chromatographically homogeneous product. The partially purified material (45 mg) was purified on Sephadex-C25-SE (NH_4^+) as described for compd VII using an NH_4OAc concentration change of 1.75×10^{-4} *M*/ml from 0.1 *M*. After picrate treatment there was 13 mg (8%), one Pauly + spot on tlc, R_f III: 0.54, R_f IV: 0.33. A 72-hr acid hydrolysate had the following amino acids: δAvl 0.98, Val 1.02, Tyr 0.97, Ile 1.00, His 0.99, Pro 0.99, Phe 1.01; peptide content, 84%. δAvl emerged from the short (5.3 cm) column of the analyzer 10 ml before lysine and had a color value which was 60% of that of leucine. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: δAvl 1.05, Val 0.00, Tyr 0.00, Ile 0.00, His 0.07, Pro 1.00, Phe 0.00.

Boc-Gly-Val-Tyr(Bzl)-Ile-His(Bzl)-Pro-Phe-polymer (XIII).—The protected hexapeptide polymer (VI), 5.4 g (0.9 mmol), was deprotected and acylated with 630 mg (3.6 mmol) of Boc-Gly and 750 mg (3.6 mmol) of DCCI in 30 ml of CH_2Cl_2.

Gly-Val-Tyr-Ile-His-Pro-Phe (XIV).—A portion of the protected heptapeptide polymer (XIII) (0.6 mmol) was cleaved and hydrogenated as described for VII. A 250-mg portion of the crude product was purified on Sephadex-C25-SE (NH_4^+) as described for IX giving 145 mg. Another 300-mg portion was purified in the same manner giving 200 mg. These products were combined and further purified *via* the picrate salt giving 270 mg (44%) of white powder. Tlc revealed a single Pauly + spot, R_f III: 0.53, R_f IV: 0.40, R_f VI: 0.41. A 72-hr acid hydrolysate had the following amino acids: Gly 0.97, Val 1.01, Tyr 0.97, Ile 1.01, His 1.01, Pro 1.04, Phe 1.01; peptide content, 82%. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: Gly 0.92, Val 0.00, Tyr 0.00, Ile 0.00, His 0.11, Pro 1.00, Phe 0.00. A 23-hr aminopeptidase-M digest had Gly 0.98, Val 1.00, Tyr 0.96, Ile 0.83, His 0.00, Pro 0.00, Phe 0.85. Further incubation with an additional portion of enzyme produced no significant change in composition.

A 72-hr acid hydrolysate of 3 mg of this peptide was evapd to dryness on a rotary evaporator at 60°. The residue was dissolved in 1 ml of 5% $NaNO_2$ and 0.5 ml of AcOH was added. After 1 hr at 25°, 10 ml of 12 *N* HCl was added and the solution was coned to ~2 ml over a low flame. Another 10 ml of 12 *N* HCl was added, the solution was again coned, and the remaining solvent removed on a rotary evaporator at 60°. The residue was dissolved in 0.4 ml of 0.45 *M* sodium borate, pH 10.2, the pH was adjusted to ~10.5 with 1 *N* NaOH, and the solution transferred to a 7.5-cm test tube. The solution was cooled to 0° and stirred with a vortex stirrer and 1.2 mg (7 μmol) of L-leucine *N*-carboxyanhydride was added to the swirling solution. After stirring for 2 min, 0.1 ml of 2 *N* HCl was added; all of the solution was applied to the long column of the amino acid analyzer (packed with Beckman UR-30 resin), and eluted with 0.2 *M* sodium citrate, pH 4.30. Four peaks emerged, Pro, 39 ml; Leu, 61.5 ml; L-Leu-D-Pro, 106 ml; and L-Leu-L-Pro, 135.5 ml. Using the constants published by Manning and Moore,[10] it was found that the hydrolysate contained 7.5% of D-proline. This value is consistent with that expected from racemization during hydrolysis since Manning and Moore[10] found 2.0–2.3% racemization of L-proline during 22-hr acid hydrolyses.

Ac-Gly-Val-Tyr-Ile-His-Pro-Phe (XV).—To a solution of 25 mg (0.025 mmol) of the purified heptapeptide XIV in 1 ml of purified DMF were added 0.005 ml (0.075 mmol) of Et_3N and 9 mg (0.050 mmol) of *p*-nitrophenyl acetate. After 24 hr at 25°, the acetylated peptide was pptd with 10 ml of AcOEt, centrifuged, and washed with 5 ml of AcOEt. After lyophilizing from AcOH there was 16 mg (63%) of white powder. Electrophoresis revealed a single Pauly +, ninhydrin − spot at E_{Tu} 0.27. No free heptapeptide was detected. Tlc showed a single Pauly + compound, R_f III: 0.53, R_f IV: 0.44, R_f V: 0.66. A 72-hr acid hydrolysate had: Gly 1.00, Val 1.01 Tyr 0.98, Ile 0.99, His 1.03, Pro 1.00, Phe 0.98; peptide content, 86%. A 48-hr acid hydrolysate incubated with L-amino acid oxidase had the following amino acids: Gly 0.94, Val 0.00, Tyr 0.00, Ile 0.00, His 0.09, Pro 1.00, Phe 0.00.

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