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Synthesis of the Vasoactive Intestinal Peptide (VIP) I. The C-Terminal Cyanogen Bromide Fragment

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The C-terminal cyanogen bromide fragment of VIP, Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (all L), was synthesized to provide evidence for the correctness of the sequence proposed by Mutt and Said (*1*). The synthesis of this hendecapeptide (VIP_{18-28}) was carried out by coupling Z-Ala-Val-(Z)Lys to (Z)Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2. After removal of the protecting groups and purification, the synthetic material was indistinguishable from the natural fragment on paper chromatograms and electropherograms. Their identity was further confirmed by comparison of the products formed on enzymatic hydrolysis.

A vasoactive peptide (VIP) was recently isolated from hog intestines by Said and Mutt (*2*, *3*). The whole sequence of the 28 amino acid residues constituting the single-chain molecule has not yet been completely elucidated. However, the presence of a methionine residue in the chain permitted selective cleavage with cyanogen bromide (*4*), resulting in the formation of two fragments. The sequence of the C-terminal fragment, a hendecapeptide, has been established (*1*) (Fig. 1); this paper reports its synthesis. The primary purpose of the synthesis was to provide independent evidence for the correctness of the sequence determined by degradation of the natural material. Some of the protected intermediates, however, are expected to serve also in the synthesis of the whole molecule of the biologically active peptide.

Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2
18 19 20 21 22 23 24 25 26 27 28

FIG. 1. Sequence of the C-terminal cyanogen bromide fragment of VIP.

The hendecapeptide VIP_{18-28} was obtained by coupling a protected tripeptide, VIP_{18-20}, to a partially protected octapeptide, VIP_{21-28}. The protected tripeptide, benzyloxycarbonyl-L-alanyl-L-valyl-N^{ϵ}-benzyloxycarbonyl-L-lysine (XVIII), was prepared through acylation of N^{ϵ}-benzyloxycarbonyl-L-lysine (*5*) with *t*-butyloxycarbonyl-L-valine-*N*-hydroxysuccinimide ester (*6*), removal of the α-amino protecting group with trifluoroacetic acid, and reaction of the resulting partially protected dipeptide with benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester.[2] Synthesis of the octapeptide derivative started with the ammonolysis of benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester (*7*, *8*), removal of the benzyloxycarbonyl group, and acylation of asparaginamide with benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester. The chain was

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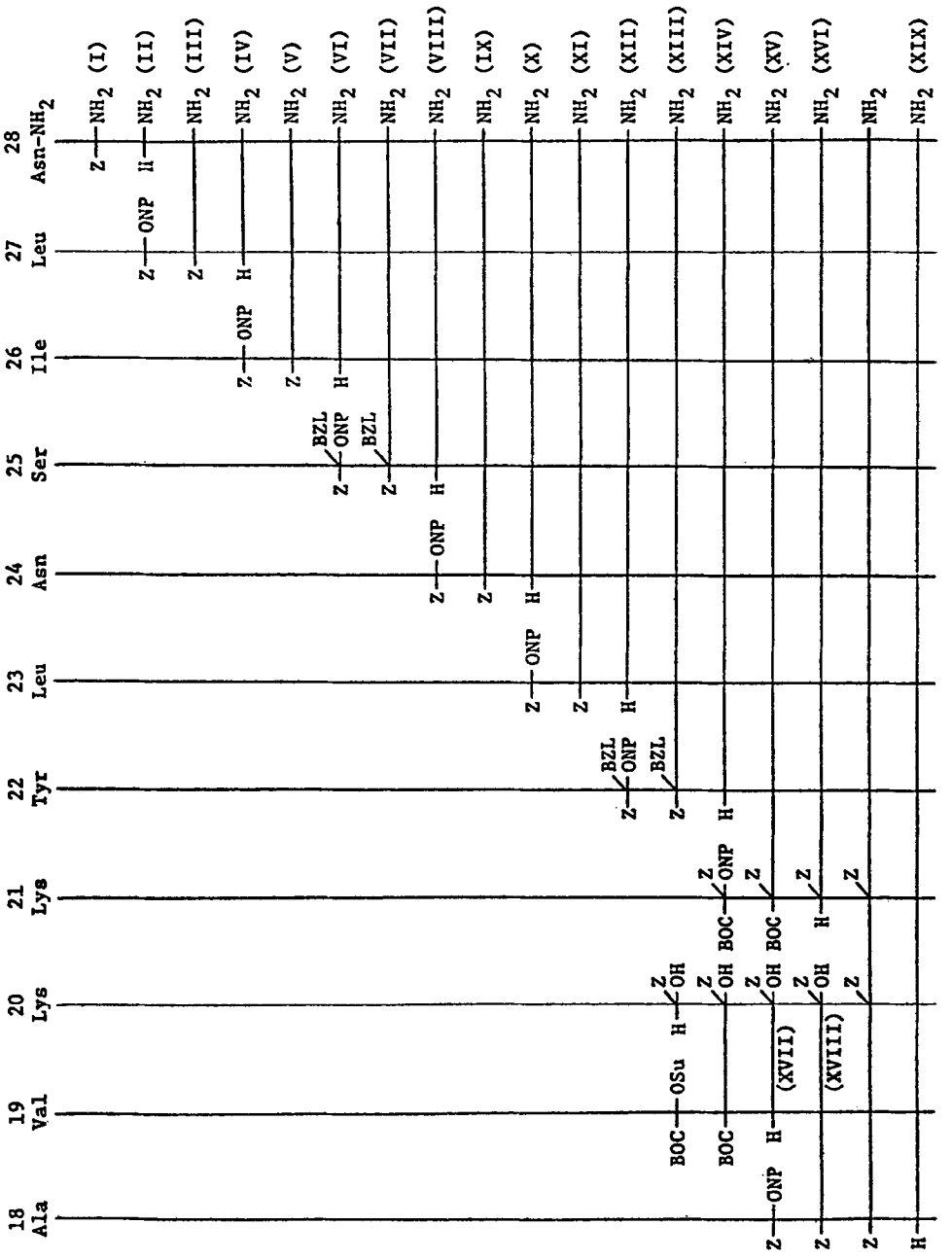
[2] Prepared according to the general procedure described in *Biochem. Prep.*, **9**, 110 (1962).


18 Ala
19 Val
20 Lys
21 Lys
22 Tyr
23 Leu
24 Asn
25 Ser
26 Ile
27 Leu
28 Asn-NH2
(I)
(II)
(III)
(IV)
(V)
(VI)
(VII)
(VIII)
(IX)
(X)
(XI)
(XII)
(XIII)
(XIV)
(XV)
(XVI)
(XVII)
(XVIII)
(XIX)
BOC
OSu
ONP
BZL
OH
Z
H
NH2

lengthened in this stepwise manner (*9*) until the protected peptide N^{α}-*t*-butyloxycarbonyl-N^{ϵ}-benzyloxycarbonyl-L-lysyl-L-tyrosyl-L-leucyl-L-asparaginyl-L-seryl-L-isoleucyl-L-leucyl-L-asparaginamide (XV) was secured. To avoid *O*-acetylation of the serine residue, hydrogenolysis rather than acidolysis was used for the removal of the protecting groups in the tetrapeptide intermediate and in the subsequent steps. Poor solubility of the protected intermediates rendered the synthesis of this octapeptide fairly difficult: suspensions rather than solutions of the amino components had to be used in the acylation steps; the products of these reactions also separated from the solution during the reaction. Even hydrogenations were started with suspensions of the protected peptides in 80% acetic acid.

The protected tripeptide XVIII was activated with dicyclohexylcarbodiimide-1-hydroxybenzotriazole (*10*, *11*) and coupled to the partially deprotected octapeptide XVI. No solvent system could be found for the purification of the protected hendecapeptide thus obtained. Therefore, it was deprotected by hydrogenolysis and the free peptide XIX was chromatographed on the ion-exchange resin Biorex 70 (H^+). The purified product was shown to be homogeneous on paper chromatograms, thin-layer chromatograms, and by electrophoresis. On quantitative amino acid analysis (*12*), it gave the expected ratios of the constitutent amino acids. The synthesis of XIX is summarized in Chart I.

The hendecapeptide XIX was indistinguishable from the C-terminal cyanogen bromide fragment of natural VIP on paper chromatograms and electropherograms. Moreover, an identical pattern of products was obtained from the synthetic and natural peptides on degradation with chymotrypsin (Fig. 2) and trypsin. Trypsin cleaves primarily between the two lysine residues in positions 20 and 21. This is the bond that was formed by fragment condensation. Its cleavage by trypsin shows that no noticeable racemization occurred in this step.

The unequivocal synthesis of compound XIX and its detailed comparison in side-by-side experiments with the C-terminal cyanogen bromide fragment of VIP support the sequence proposed by Mutt and Said (*1*).

EXPERIMENTAL

Capillary melting points are reported uncorrected. Thin-layer chromatograms (silica gel, Merck) were developed with the solvent systems A: *n*-butanol–acetic acid–water (4:1:1,) B: *n*-butanol–pyridine–acetic acid–water (30:24:6:20) and C: sec. butanol–3% ammonia (3:1). Spots were revealed with a modified Rydon-Zahn reagent (*13*). For amino acid analysis, samples were hydrolyzed with constant-boiling hydrochloric acid in evacuated, sealed ampules at 110°C for 16 hr, and analyzed by the method of Spackman, Stein, and Moore (*12*) on a Beckman–Spinco 120C instrument.

The following abbreviations are used: THF (tetrahydrofuran), TFA (trifluoroacetic acid), DMF (dimethylformamide), and DCC (dicyclohexylcarbodiimide).

Benzyloxycarbonyl-L-asparaginamide (*I*). Benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester (*8*) (3.1 g) was dissolved in THF (75 ml), and dry NH_3 was passed near the surface of the stirred solution. After a few minutes, the product started to precipitate. About 2 hr later, it was collected in a filter, washed with THF (60 ml) and ethyl acetate (60 ml), and dried in air to give 2.08 g (98%); mp 225–226°C dec (lit. (*14*) 219–223°C).

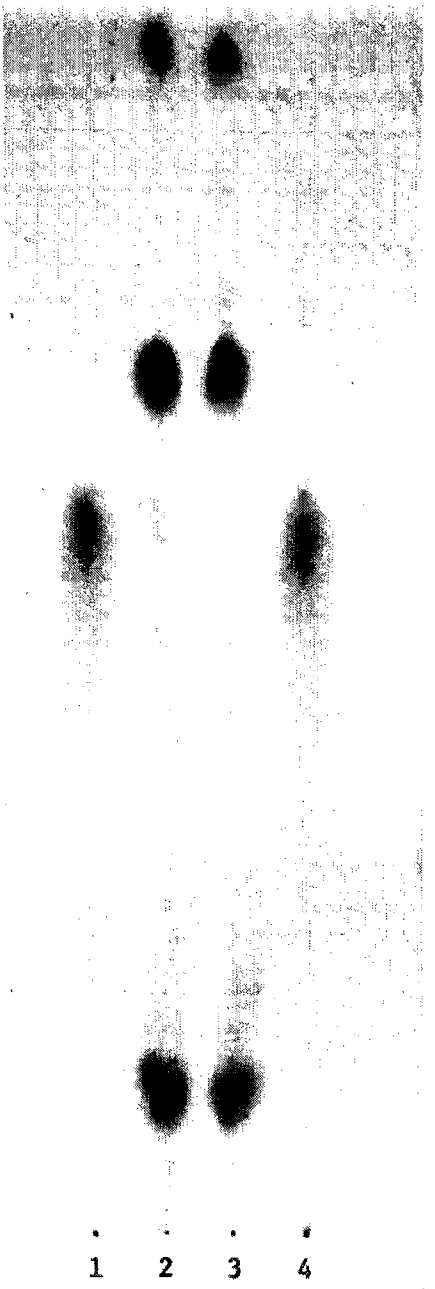


FIG. 2. Paper electrophoresis of the synthetic and natural hendecapeptides and their chymotryptic degradation products (cf. Experimental section): (1) natural CNBr fragment, (2) its chymotryptic digest, (3) chymotryptic cleavage of synthetic hendecapeptide, (4) undegraded synthetic hendecapeptide.

Benzyloxycarbonyl-L-leucyl-L-asparaginamide (III). A suspension of I (6.66 g) in acetic acid (25 ml) was treated with 4 *N* HBr in acetic acid (25 ml). After 1 hr at room temperature, dry ether (600 ml) was added; the hydrobromide (II) was collected by filtration, washed with ether, and dried *in vacuo* over NaOH and P_2O_5 for 1 hr. Benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester[2] (12.3 g) was added to a solution of II and triethylamine (3.5 ml) in DMF (125 ml). The reaction mixture was kept slightly alkaline by the addition of triethylamine (1.5 ml) in small portions. After standing overnight, the mixture was cooled with ice water and diluted with $CHCl_3$ (250 ml). The product was filtered, washed with $CHCl_3$ (125 ml) and EtOAc (40 ml), and dried in air to give 8.78 g of III (93%), mp 233–235°C. A sample was recrystallized from DMF–$CHCl_3$ and from DMF–H_2O: mp 238–239°C dec.; $[\alpha]_D^{25} - 7.5°$ (*c* 1, DMF); tlc R_fA 0.73, R_fB 0.69. Amino acid analysis: Leu, 1.0; Asp, 1.0; NH_3, 1.9.

Anal. Calcd for $C_{18}H_{26}N_4O_5$: C, 57.1; H, 6.9; N, 14.8. Found: C, 57.0; H, 6.8; N, 14.6.

Benzyloxycarbonyl-L-isoleucyl-L-leucyl-L-asparaginamide (V). To a suspension of III (756 mg) in acetic acid (3.5 ml) was added 4 *N* HBr in acetic acid (3.5 ml). After 1 hr at room temperature, the mixture was diluted with ether (100 ml), and the hydrobromide (IV) was collected on a filter, washed with ether, and dried *in vacuo*. Benzyloxycarbonyl-L-isoleucine *p*-nitrophenyl ester[2] (1.16 g) was added to a solution of IV and triethylamine (0.28 ml) in DMF (5 ml). After about 30 min, the product started to separate. The mixture was kept alkaline by the addition of triethylamine (0.6 ml). Next day $CHCl_3$ (20 ml) was added to the cooled mixture. The solid mass was disintegrated, filtered, washed with $CHCl_3$ (40 ml) and EtOAc (20 ml), and dried in air. The product, 865 mg (87%), melted at 267–268°C dec. Amino acid analysis: Ile, 1.0; Leu, 1.1; Asp, 0.9; NH_3, 2.2. For further characterization, a sample was hydrogenated in 80% AcOH and the product converted to the trifluoroacetate salt: mp 188–189°C dec; $[\alpha]_D^{25} - 4°$ (*c* 1.8, 80% AcOH); tlc R_fA 0.44, R_fB 0.58.

Anal. Calcd for $C_{18}H_{32}N_5O_6F_3 \cdot \frac{1}{2} H_2O$: C, 45.0; H, 6.9; N, 14.6. Found: C, 44.9; H, 6.7; N, 14.5. Loss of wt on drying at 110°C: calcd. 1.9, found 1.8.

N-Benzyloxycarbonyl-O-benzyl-L-seryl-L-isoleucyl-L-leucyl-L-asparaginamide (VII). The protecting group was removed from V (2.95 g) with 4 *N* HBr in AcOH (14 ml) added to the suspension of V in AcOH (14 ml). The hydrobromide VI was isolated by addition of ether (400 ml), as described for IV. The tripeptide hydrobromide (VI) was suspended in DMF (40 ml); diisopropylethylamine (*15*) (1.0 ml) was added, followed by a solution of *N*-benzyloxycarbonyl-*O*-benzyl-L-serine *p*-nitrophenyl ester[2] (3.8 g) in DMF (10 ml). The mixture was kept alkaline by the addition of diisopropylethylamine (1.6 ml) in small portions. After 48 hr, EtOH (50 ml) was added, the semisolid mass disintegrated, filtered, washed with EtOH (60 ml) and EtOAc (30 ml), and dried in air: 3.35 g (84%); mp 266–267°C dec. Amino acid analysis: Ser, 0.8; Ile, 1.0; Leu, 1.0; Asp, 1.0; NH_3, 2.0. For characterization, a small sample of VII was hydrogenated and converted to the trifluoroacetate: mp 235–236°C; $[\alpha]_D^{25} - 27.5°$ (*c* 1, 80% AcOH); tlc R_fA 0.32, R_fB 0.62. Amino acid analysis: Ser, 0.9; Ile, 1.0; Leu, 1.0; Asp, 1.0; NH_3, 2.0.

Anal. Calcd for $C_{21}H_{37}N_6O_8F_3 \cdot H_2O$: C, 43.6; H, 6.8; N, 14.6; F, 9.9. Found: C, 43.7; H, 6.9; N, 14.4; F, 9.9. Loss of wt on drying at 110°C: 3.1. Calcd for 1 H_2O, 3.1.

Benzyloxycarbonyl-L-asparaginyl-L-seryl-L-isoleucyl-L-leucyl-L-asparaginamide (IX). A suspension of VII (2.2 g) in 80% AcOH (150 ml) was hydrogenated for 24 hr in the presence of a 10% Pd on charcoal catalyst (0.45 g). The catalyst was removed by filtration and the solvent evaporated *in vacuo* to leave a crystalline residue (VIII) that weighed 1.79 g after drying *in vacuo*. It was finely powdered, suspended in DMF (25 ml), and treated with benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester (*8*) (1.82 g). Within an hour, the reaction mixture turned into a semisolid mass. Two days later this mass was disintegrated with THF (75 ml); the product was filtered and then washed with THF (180 ml), with EtOH (100 ml), and once more with THF (60 ml). The air-dried material (IX), 1.8 g (79%), mp 270–271°C dec., was used as such in the next step. Amino acid analysis: Asp, 2.0; Ser. 0.9; Ile, 1.1; Leu, 1.0; NH_3, 3.0. For further characterization, a small sample was hydrogenated and converted to the trifluoroacetate: mp 210–211°C dec.; $[\alpha]_D^{25} - 32.5°$ (*c* 1, 80% AcOH); tlc R_fA 0.29, R_fB 0.47.

Anal. Calcd for $C_{25}H_{43}N_8O_{10}F_3 \cdot 2H_2O$: C, 42.4; H, 6.7; N, 15.8; F, 8.0. Found: C, 42.1; H, 6.5; N, 15.6; F, 8.3. Loss of wt on drying at 110°C: calcd 5.1, found 4.7.

Benzyloxycarbonyl-L-leucyl-L-asparaginyl-L-seryl-L-isoleucyl-L-leucyl-L-asparaginamide (XI). Hydrogenation of IX (1.25 g) in 80% AcOH (150 ml), in the presence of 10% Pd on charcoal (0.35 g), was carried out as described for IX. The pentapeptide acetate (X) was powdered and suspended in DMF (15 ml). After the addition of benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester[2] (1.05 g), the mixture solidified within an hour. Two days later the semisolid mass was disintegrated with THF (50 ml), and the solid was washed on a filter with THF (50 ml) and EtOH (50 ml), and then dried in air: 1.27 g (88%); mp 260–261°C dec. Amino acid analysis: Leu, 2.0; Asp, 2.1; Ser, 0.9; Ile, 1.0; NH_3, 3.2. A sample was hydrogenated and converted to the trifluoroacetate: mp 224–226°C; $[\alpha]_D^{25} - 30°$ (*c* 1, 80% AcOH); tlc R_fA 0.40, R_fB 0.62.

Anal. Calcd for $C_{31}H_{54}N_9O_{11}F_3 \cdot 2.5H_2O$: C, 44.8; H, 7.2; N, 15.2; F, 6.9. Found: C, 45.0; H, 6.9; N, 15.4; F, 6.3. Loss of wt on drying at 110°C: calcd 5.4, found 5.2.

N-Benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-leucyl-L-asparaginyl-L-seryl-L-isoleucyl-L-leucyl-L-asparaginamide (XIII). A suspension of XI (966 mg) in 80% AcOH (120 ml) was hydrogenated for 7 hr in the presence of a 10% Pd on charcoal catalyst. The product (XII, acetate, 870 mg) was powdered and suspended in DMF (10 ml). After the addition of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine *p*-nitrophenyl ester[2] (948 mg), a semisolid mass was formed within an hour. The next morning, this mass was disintegrated with THF (25 ml) and washed on a filter with THF (40 ml), EtOAc (30 ml), and dried: 1.23 g (88%); mp 263–264°C dec. A small sample of XIII was hydrogenolyzed in 80% AcOH and the resulting acetate (100 mg) was dissolved in a mixture of methanol (50 ml) and H_2O (50 ml). The solution was applied to a column of Biorex 70 (H^+ form, 1.1 × 40 cm). The column was washed with the same solvent mixture (500 ml), and the purified material was eluted with a 1:1 mixture of methanol and 8% AcOH. The ninhydrin-positive fractions were pooled, concentrated *in vacuo*, and lyophilized. After a second lyophilization from H_2O, 30 mg of XIV were secured: mp 234–236°C; $[\alpha]_D^{25} - 13.5°$ (*c* 1.8, 80% AcOH); tlc R_fA 0.46, R_fB 0.62. Amino acid analysis: Tyr, 1.0; Leu, 2.2; Asp, 2.0; Ser, 1.1; Ile, 1.0; NH_3, 3.0.

Anal. Calcd for $C_{38}H_{62}N_{10}O_{11}\cdot 2CH_3COOH\cdot 4H_2O$: C, 49.1; H, 7.7; N, 13.6. Found, C, 48.9; H, 7.1; N, 13.6. Loss of wt on drying at 130°C: calcd 12.9, found 12.4.

N^{α} - t - Butyloxycarbonyl - N^{ϵ} - benzyloxycarbonyl - L - *lysyl* - L - *tyrosyl* - L - *leucyl*-L-*asparaginyl*-L-*seryl*-L-*isoleucyl*-L-*leucyl*-L-*asparaginamide* (*XV*). Hydrogenation of XIII (1.04 g) overnight in 80% AcOH (100 ml), in the presence of a 10% Pd on charcoal catalyst, resulted in XIV (950 mg). A suspension of this material in DMF (8 ml) was treated with N^{α}-*t*-butyloxycarbonyl-N^{ϵ}-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester[3] (750 mg). Within 1 hr the reaction mixture solidified. Two days later, ethanol (50 ml) was added, and the disintegrated solid was washed on a filter with EtOH (50 ml), EtOAc (20 ml) and dried in air: 890 mg (83%); mp 254–255°C dec. For characterization, a sample (100 mg) was dissolved in TFA (1 ml), the TFA removed *in vacuo* after 10 min, and the residue triturated with ether (50 ml). The trifluoroacetate was washed with ether and dried *in vacuo* over NaOH and P_2O_5. It was dissolved in a mixture of methanol (75 ml) and H_2O (75 ml), and applied to a column of Dowex 1-X8 (acetate form, 2 × 19 cm). The eluate was applied directly to a column of Biorex 70 (H^+ form, 1.1 × 40 cm). The column was washed with the same solvent mixture. The purified material (XVI) was secured as described for XIV except that it was lyophilized twice from AcOH. Compound XVI, 40 mg, mp 210–212°C, $[\alpha]_D^{25} - 19.0°$ (*c* 1, 80% AcOH), gave a single spot on tlc, R_fA 0.60, R_fB 0.70. Amino acid analysis: Lys, 1.0; Tyr, 0.9; Leu, 2.0; Asp, 2.2; Ser, 0.9; Ile, 1.0; NH_3, 3.3.

Anal. Calcd for $C_{52}H_{80}N_{12}O_{14}\cdot CH_3COOH$: C, 56.0; H, 7.3; N, 14.5. Calcd for $C_{52}H_{80}N_{12}O_{14}\cdot 4CH_3COOH$: C, 53.9; H, 7.2; N, 12.6. Found: C, 54.1; H, 7.2; N, 12.3. Loss of wt on drying at 130°C: calcd 13.5, found *ca.* 10.

L-*Valyl-N^{ϵ}-benzyloxycarbonyl*-L-*lysine* (*XVII*). N^{ϵ}-Benzyloxycarbonyl-L-lysine (*6*) (3.4 g) was suspended in water (20 ml); *N* NaOH (12 ml) and dimethoxyethane (20 ml) were added, followed by *t*-butyloxycarbonyl-L-valine *N*-hydroxysuccinimide ester (*6*) (3.5 g). The alkalinity of the solution was maintained at about pH 8.5 by the slow addition of *N* NaOH (12 ml). When no more NaOH was required, the major part of the solvent was removed with a stream of N_2. The mixture was acidified with hydrochloric acid to pH 4 and then with a 20% solution of citric acid to pH 3. The gummy precipitate was dissolved in ether, the aqueous layer extracted with ether, and the combined ether solutions (*ca.* 150 ml) were washed with water, dried over Na_2SO_4, and the solvent removed *in vacuo*. The white foamy residue was treated with 95% TFA (15 ml). About 10 min later, the TFA was removed *in vacuo*, the residue dissolved in ethyl acetate and the solution neutralized (wet indicator paper) with triethylamine. The crystalline precipitate was collected, washed with ethyl acetate, and dried to give 2.9 g. The crude material was purified by recrystallization from hot H_2O (15 ml) to give chromatographically homogeneous product: 1.75 g (48%); mp 155°C dec. (sinters at 100–110°C); $[\alpha]_D^{25} + 19°$ (*c* 1.8, AcOH); tlc R_fA 0.53, R_fB 0.60. For analysis, a sample was recrystallized once more from H_2O (same mp).

Anal. Calcd for $C_{19}H_{29}N_3O_5\cdot \frac{1}{2}H_2O$: C, 58.9; H, 7.5; N, 10.8. Found: C, 58.7; H, 7.5; N, 10.9. Loss of wt on drying at 110°C: calcd 2.3, found 2.6.

Benzyloxycarbonyl-L-*alanyl*-L-*valyl-N^{ϵ}-benzyloxycarbonyl*-L-*lysine* (*XVIII*). NaOH (1 *N*) was added to a suspension of XVII (0.76) g in H_2O (8 ml) and pyridine (10 ml), until solution occurred (1.5 ml). Benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester[2]

[3] Purchased from Fox Chemical Co., Los Angeles, CAL.

(0.69 g) was added and complete solution was observed almost immediately. The pH of the mixture was maintained at 9 by the addition of *N* NaOH (3.5 ml). When the pH remained constant, the solution was acidified with 2 *N* HCl. The oily precipitate solidified under H_2O. It was triturated with ether, filtered, washed with ether and dried. The protected tripeptide, 0.70 g (58%), mp 187–189°C, $[\alpha]_D^{25} -2°$ (*c* 3, DMF), gave analytical values for a monohydrate.

Anal. Calcd for $C_{30}H_{40}N_4O_8 \cdot H_2O$: C, 59.8; H, 7.0; N, 9.3. Found: C, 59.7; H, 7.0; N, 9.5. Loss of wt on drying at 110°C: calcd 3.1, found 2.6.

A sample of XVIII (0.25 g) was hydrogenated for 2 hr at room temperature in ethanol (10 ml) and acetic acid (1 ml) in the presence of a 10% Pd on charcoal catalyst. After removal of the catalyst and the major part of the solvent, the solution was diluted with ether. The free tripeptide, Ala-Val-Lys (acetate, monohydrate), was collected by filtration. The dried material, 82 mg, mp 215–218°C dec., gave a single spot on tlc: R_fA 0.80, R_fB 0.61.

Anal. Calcd for $C_{14}H_{28}N_4O_4 \cdot CH_3COOH \cdot H_2O$: C, 48.7; H, 8.7; N, 14.2. Found: C, 48.8; H, 8.4; N, 14.5. Loss of wt on drying at 110°C: calcd 4.6, found 4.1.

L-*Alanyl*-L-*valyl*-L-*lysyl*-L-*lysyl*-L-*tyrosyl*-L-*leucyl*-L-*asparaginyl*-L-*seryl*-L-*isoleucyl*-L-*leucyl*-L-*asparaginamide* (*XIX*). The protected octapeptide XV (420 mg) was dissolved in 95% TFA (3 ml). After 10 min at room temperature, the TFA was removed *in vacuo* and ether was added to the residue. The precipitate was washed on a filter with ether (50 ml) and dried over NaOH and P_2O_5 *in vacuo* to give 398 mg (94%) of XVI. A suspension of this material in DMF (3 ml) was treated with triethylamine (1 equivalent) and then with an ester of XVIII prepared in the following manner. XVIII (349 mg), 1-hydroxybenzotriazole (78 mg) and DCC (103 mg) were dissolved in a mixture of DMF (1.5 ml) and THF (2.5 ml). After ½ hr at 0°C and 3 hr at room temperature, the ir spectrum indicated the disappearance of DCC and the presence of the desired active ester. The mixture was used as such for the acylation of XVI. The next day, a second portion of similarly prepared active ester (⅓ of the former amount) was added. After standing overnight, the reaction mixture was diluted with EtOH (30 ml); the precipitate was collected by filtration and washed with EtOH (40 ml), THF (10 ml), and again with EtOH (20 ml). The air-dried crude product weighed 450 mg. An aliquot of this material (250 mg) was suspended in 80% AcOH (50 ml) and hydrogenated for 7 hr in the presence of a 10% Pd on charcoal catalyst (75 mg). After removal of the catalyst and the solvent, the residue (240 mg) was dissolved in a mixture of methanol (150 ml) and H_2O (150 ml) and applied to a column of Biorex 70 (H^+ form, 1.1 × 40 cm). The column was washed with the same solvent mixture (750 ml), and the product eluted with a mixture of equal volumes of methanol and 8% AcOH (500 ml). After concentration *in vacuo* to a small volume, the product was secured by lyophilization from acetic acid and water: 118 mg, mp > 300°C, $[\alpha]_D^{52} -37°$ (*c* 1, 80% AcOH). Amino acid analysis: Asp, 2.1; Ser, 0.9; Ala, 1.0; Val, 1.0; Ile, 1.0; Leu, 1.9; Tyr, 1.0; Lys, 2.0; NH_3, 3.3.

Anal. Calcd for $C_{58}H_{100}N_{16}O_{15} \cdot 4CH_3COOH \cdot 2H_2O$: C, 51.5; H, 7.9; N, 14.6. Found: C, 51.5; H, 7.5; N, 14.6 (dried at 130°C for 1.5 hr). Calcd for $C_{58}H_{100}N_{16}O_{15} \cdot 2\frac{1}{2}CH_3COOH$: C, 53.6; H, 7.9; N, 15.9. Found: C, 53.8; H, 7.9; N, 15.9 (dried at 130°C for 4 hr). The elemental analysis of compound XIX is complicated by its tendency to retain acetic acid and water.

COMPARISON OF THE SYNTHETIC AND NATURAL HENDECAPEPTIDES

The two preparations were indistinguishable on chromatography (*16*) on Whatman 42 paper, *n*-butanol/acetic acid/pyridine/water (30:6:20:24), both in parallel and in mixture. The R_f value was 0.25. They were likewise indistinguishable on high-voltage paper electrophoresis, performed for 90 min at 50 V/cm in pyridine/acetic acid/water (300:11.5:2700 by vol, pH 6.4) on Whatman 3MM paper, as were the products obtained on their degradation products with chymotrypsin (Fig. 2). α-Chymotrypsin CDS (Worthington) was treated with TLCK (*17*) (Cyclo Chemicals Co.). The degradation was carried out as follows: 500 μg of peptide was dissolved in water (125 μl). A 2% solution of NH_4HCO_3 in water (125 μl) was added, immediately followed by a 0.2% solution of chymotrypsin in 10^{-3} *M* AcOH (5 μl). The mixture was kept at room temperature for 2 hr, whereupon the same amount of the enzyme was added again. After 2 more hr, the clear solution was lyophilized. In the case of the natural substance, the split products have been shown (*1*) to be, from the origin, Leu-Asn-Ser-Ile-Leu, Ala-Val-Lys-Lys-Tyr and Asn-NH_2. The pentapeptide Ala-Val-Lys-Lys-Tyr was isolated from the digests of the natural and synthetic materials, and tryptic hydrolysis of the two samples led to the indistinguishable pattern in paper electrophoresis described above. No uncleaved peptide could be found.

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