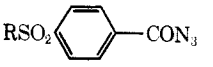
TABLE II



Compd	R	Mp, °C	Yield, %	Formula
1	CH_3	137	52	$C_8H_7N_3O_3S$
2	C_6H_5	110	84	$C_{13}H_9N_3O_3S$
3	p-FC_6H_4	112	90	$C_{13}H_8FN_3O_3S$
4	H_2N	130	45	$C_7H_6N_4O_3S$
5	$(CH_2)_4N$	125	52	$C_{11}H_{12}N_4O_3S$
6	$O(CH_2)_4N$	200	53	$C_{11}H_{12}N_4O_4S$

The growth inhibitions at 10 μg/ml were insignificant. Compds **2–6** were inactive. *P*-Morpholinosulfonyl carbanilic acid pentachlorophenyl ester (**13**) and *p*-piperidinosulfonyl carbanilic acid pentachlorophenyl ester (**14**) were found to be the most active compds in this series (Table III).

TABLE III

TESTING RESULTS[a]

	Candida albicans		*Aspergilus niger*		*Penicillium SP.*	
Compound	25 μg/ml	50 μg/ml	25 μg/ml	50 μg/ml	25 μg/ml	50 μg/ml
1						2+
7		+		2+		
8	2+	2+		2+		2+
9	2+	2+		2+		2+
10	+	2+				
11	+	+		2+		
12	2+	2+	+	2+		+
13	2+	2+	2+	2+	+	2+
14	2+	2+	2+	2+	+	2+
15	+	2+	+	2+		

[a] Blank equals no inhibition, 2+ = complete inhibition.

Experimental Section[5]

Substituted Benzoyl Azides.—To an ice-cold soln of 0.012 mole of NaN_3 in 25 ml of H_2O, a soln of 0.01 mole of the appropriate benzoyl chloride in 10 ml of cold Me_2CO was added with stirring. The ppt was filtered, washed with H_2O, and dried in air (see Table II).

Substituted Carbanilic Acid Esters.—A mixt of 0.01 mole of an appropriate benzoyl azide and 0.012 mole of pentachlorophenol (or 2,4,6-trichlorophenol) in 25 ml of dry PhMe was refluxed for 3 hr. After evapn of the solvent, the residue was crystd from EtOH (see Table I).

(5) Melting points were taken on a Kofler hot stage microscope. The ir spectra were detd with a Leitz Model III spectrograph (KBr). Nmr spectra were obtained on a Varian A60A instrument (Me_4Si).

Absence of Biochemical and Pharmacological Effects of the Trypsinogen Activation Peptide[1]

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This communication describes the solid phase synthesis of the peptide, Val-$(Asp)_4$-Lys, released on activation of bovine trypsinogen,[2] and its screening for possible biological activity, particularly with respect to the gastrointestinal tract.

Results and Discussion

The synthesis and purification of the trypsinogen activation peptide are described in the Experimental Section.

Table I shows the effect of Val-$(Asp)_4$-Lys on the activities of several of the enzymes normally occurring in the gastrointestinal tract. The trypsinogen activation peptide neither stimulates nor inhibits these enzymes.

TABLE I

EFFECT OF VAL-$(ASP)_4$-LYS ON THE ACTIVITIES OF VARIOUS ENZYMES[a]

	Peptide, m*M*	Enzyme alone, units/mg	Enzyme plus peptide, units/mg
Ribonuclease	1.0	380–450	350–440
Trypsin	0.7	185–200	190–215
Carboxypeptidase A	0.07	8.1–8.5	8.1–8.5
β-Amylase	0.2	270	270
Lipase	1.0	44–51	44–56

[a] Specific activities are given over the ranges found with at least 3 different concns of enzyme. The peptide was dissolved in the buffer used for assay of each enzyme, giving the final concns shown in the first column, when added to the enzyme solns. The mixts of enzyme and peptide were allowed to stand for 5 min at 27° before assay.

When the peptide was added to two preparations of guinea pig atria in concns of 4×10^{-5} and 8×10^{-4} *M*, neither significant chronotropic (change of rate varied from +8.5% to −11%) nor inotropic action were displayed. Cumulative full dose–response curves of isoproterenol were run at both concns of peptide with no significant change in the dose–response curve from controls. The peptide, therefore, possesses neither agonist nor antagonist action on β-adrenergic receptors.

At a bath concn of 10^{-4} *M*, Val-$(Asp)_4$-Lys displayed no agonist activity with strips of guinea pig ileum (no contraction). Antimuscarinic activity was not manifested as demonstrated by no significant change in the pD_2 of methylfurtrethonium (pD_2 values before, with, and after washout of the peptide were 7.3, 7.5, and 7.5, resp).

Val-$(Asp)_4$-Lys (7 mg, approximately 10^{-5} *M*) was injected into the perfused, denervated cephalic vein of a dog. The recordings showed no change in perfusion pressure or in systemic arterial blood pressure. The responses to epinephrine and ACh were unchanged in the presence of the peptide. These observations suggest that the peptide has neither activity as a muscarinic or adrenergic stimulant nor as a blocking agent for these two agonists. There was no evidence that the peptide released vasoactive materials.

The solid phase synthetic approach was used for the bovine peptide because of the similarity in the C-terminal portions of the trypsinogen activation peptides from other species:[3] Phe-Pro-Thr-$(Asp)_4$-Lys from pig; Phe-Pro-Val-$(Asp)_4$-Lys from sheep and goat; Ser-Ser-Thr-$(Asp)_4$-Lys from horse; and Ala-Pro-$(Asp)_4$-Lys from the spiny dogfish. Had the bovine peptide proved

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(2) E. W. Davie and H. Neurath, *J. Biol. Chem.*, **212**, 515 (1965).

(3) K. A. Walsh, *Methods Enzymol.*, **19**, 48 (1970).

to be active, these others would have been easily completed from a portion of $(Asp)_4$-Lys-resin. Other preparations of similar peptides of the N terminus of trypsinogen[4,5] have used synthetic reactions in soln.

Experimental Section

Solid Phase Synthesis of Val-$(Asp)_4$-Lys.—The resin used was chloromethyl resin (Schwarz Bioresearch, Inc.), containing 2.3 mmoles of Cl/g. The amino acid derivatives used were: α-BOC-ε-Cbz-Lys† (Schwarz Bioresearch, Inc.) and α-BOC-β-*O*-benzyl-Asp and BOC-Val (both from Fox Chemical Co., Los Angeles, Calif.). These compds were tested for purity by tlc in two different systems and all gave single spots. The synthesis was carried out at room temp according to the procedure described by Stewart and Young.[6] A mixt of the BOC-Cbz-Lys (4.05 g) and the chloromethyl resin (5 g) in 12 ml of abs EtOH plus 1.34 ml of Et_3N was refluxed for 24 hr. The substd resin contd 0.65 mmole of the lysine derivative/g by amino acid analysis. The substd resin (1 g) was placed in the reaction vessel and the following steps were used to add each new amino acid residue: (1) three washes with glacial AcOH (15 ml each); (2) removal of the BOC group by 30 min shaking with 1 *N* HCl in glacial AcOH (25 ml); (3) three washes with glacial AcOH, 3 washes with abs EtOH, and 3 washes with $CHCl_3$ (15 ml each); (4) neutralization of the hydrochloride by 10-min shaking with 15 ml of a 10% soln of Et_3N in $CHCl_3$; (5) three washes with $CHCl_3$ and 3 washes with CH_2Cl_2; (6) 5 min shaking with 1.7 mmoles (2.5-fold molar ratio) of BOC-amino acid in 10 ml of CH_2Cl_2; (7) 2–3 hr shaking after the addn of 0.7 ml of a 50% soln of dicyclohexylcarbodiimide in CH_2Cl_2; (8) three washes with CH_2Cl_2 (15 ml); (9) after the last amino acid, the process was contd to the EtOH wash of step 3.

The peptide-loaded resin was transferred to the cleavage vessel with EtOH, dried, washed twice with 10 ml of TFA, and suspended in 15 ml of TFA. Gaseous HBr was bubbled through the suspension for 90 min. The cleaved peptide was filtered off and the resin was washed 3 times with 15 ml of TFA. The combined filtrates were evapd to dryness. HBr was removed from the residue by dissolving 3 times in 7 ml of MeOH–water, 1:1, and evapg on a rotary evaporator. The freeze-dried product weighed 470 mg.

Purification and Analysis of the Synthetic Peptide.—The product was chromatogd on Dowex 50W-X2 (200–400 mesh) using a procedure similar to that developed for isolation of the natural activation peptide.[2] Several peaks were obtained (Figure 1).

Samples of each peak were examd by high-voltage paper electrophoresis in pyridine–acetate buffer, pH 6.4, using the natural peptide prepd from trypsinogen as a marker. Only peak 3 migrated identically to the natural peptide. Peak 3 was partially desalted by acidifying the soln with concd formic acid to pH 2.75 and extg 5 times with dry Et_2O. The aq layer was lyophilized; the residue was dissolved in 2 ml of 0.02 *M* HCO_2H and acidified to pH 2.75 with concd HCO_2H. The desalting was completed by 2 passages through a column (2 × 98 cm) of Sephadex G-10, equilibrated with 0.02 *M* HCO_2H. The peptide and citrate were detected in sep peaks by their absorbance at 220 nm. The salt-free peptide was lyophilized; the yield was 98 mg (21%).

Amino acid analysis gave ratios of 3.95 Asp and 1.03 Val residues based on Lys = 1.00. The $[\alpha]^{24}D$ was −43° in H_2O (*c* 0.85). The peptide moved as a single spot on paper chromatography in *n*-BuOH–AcOH–pyridine–H_2O(4:1:1:2) with R_f 0.102, exactly equal to the natural peptide. Yoshida, *et al.*,[5] found $[\alpha]^{22}D$ −38° and R_f 0.11. It was shown that the amino acids were present in α linkage by sequencing the peptide by the 3-stage Edman degradation.[7] The cyclization step (conversion of the PTC-amino acid to the thiazolinone) was done twice each time. The PTH derivs were identified by tlc. The yield at each step was detd by absorbance at 269 nm. The results, expressed as moles of PTH-amino acid per mole of peptide, were as follows: step 1, Val 0.71; step 2, Asp 0.48; step 3, Asp 0.36; step 4, Asp 0.20; step 5, Asp 0.08. The yields for the four steps of removal of aspartic acid, although low, fell on a straight line;[8] aspartic acid is known to be cyclized with difficulty. No other spots were detected on the chromatograms.

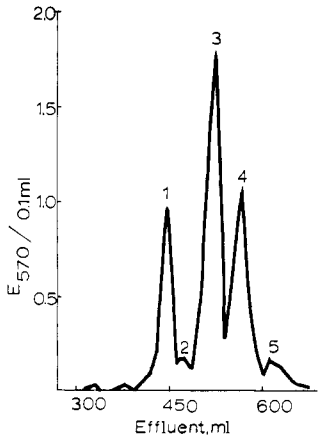

Figure 1.—Purification of the synthetic product (470 mg) on a column of Dowex 50W-X2 (2 × 31 cm) using a linear gradient of pH and concn at room temp; 3-ml fractions were collected. Starting buffer: 300 ml of 0.1 *M* sodium citrate, pH 3.0. Gradient buffer: 300 ml of 0.21 *M* sodium citrate, pH 5.0 (buffer concns based on Na^+).

Enzymic Assays.—Ribonuclease was assayed by the method of Kalnitsky, *et al.*,[9] in which the hydrolysis of RNA at pH 5.0 is measured by the liberation of acid-sol oligonucleotides. Trypsin was assayed by measuring the change in absorbance at 247 nm on hydrolysis of the substrate Ts-L-ArgOMe.[10] Carboxypeptidase A was detd by following the change in absorbance at 254 nm on hydrolysis of hippuryl-L-Phe, after the method of Folk and Schirmer.[11] Pancreatic amylase was assayed by the rate of liberation of maltose from starch, as measured by its ability to reduce 3,5-dinitrosalicylic acid.[12] Pancreatic lipase was detd by a modification of the turbidimetric method of Grossberg, *et al.*[13] Lipostrate-CB was used as substrate in place of Lipo-Mul-Oral vegetable oil emulsion. Activity was expressed in lipase units (L.U.) as calcd from the equation, L.U. = 2.05 $K_0^{0.6\ 37}$, defined by Grossberg, *et al.*[13]

Studies on Vascular Smooth Muscle Action.—In a mongrel dog, anesthetized with Na pentobarbital (35 mg/kg, iv), the accessory cephalic vein was isolated downstream from its confluence with the cephalic vein, as described by Rice and Long,[14] denervated,[15] and pump-perfused at a const flow rate with blood obtd from a femoral artery. Cephalic vein perfusion pressure and arterial blood pressure were recorded using P23AC Statham transducers and a Grass polygraph.[16] Compds under study were injected into the cannula close to the perfused vein.

Studies on Cardiac Action (β-Adrenergic).—Isolated, spontaneously beating guinea pig atria were prepd by a std method.[17] The Ringer–Locke soln at 30° was gassed with 95% O_2–5% CO_2. Inotropic and chronotropic actions were recorded from a Grass Model FT.03C force transducer on a Grass Model 5C polygraph. Basal tension was 1 g. Cumulative dose–response curves of isoproterenol·HCl were obtd by adding amts increased by 3-fold at each 3-min interval.

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† Abbreviations used are: BOC, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; TFA, trifluoroacetic acid, PTH, phenylthiohydantoin of, PTC, phenylthiocarbamyl.

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(14) A. J. Rice and J. P. Long, *J. Pharmacol. Exp. Ther.*, **151**, 423 (1966).

(15) A. J. Rice, C. R. Leeson, and J. P. Long, *ibid.*, **154**, 539 (1966).

(16) P. J. Kadowitz and A. C. Yard, *Eur. J. Pharmacol.*, **13**, 281 (1971).

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Studies on Gastrointestinal Action.—Strips of isolated guinea pig ileum were suspended in Tyrode's soln according to the method of Magnus.[18] The soln at 37° was gassed with 95% O_2–5% CO_2. Contractions were recorded with a Brush isotonic muscle transducer on a Heath Model EU-20B servorecorder. 5-Methylfurtrethonium was used as a muscarinic std.[19]

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Antimalarials. 8[1]. 2,3-Trimethylene-4-quinoline Amino Alcohols. 5,7-Dichloro-2,3-dihydro-1*H*-cyclopenta[*b*]quinoline-9-(α-di-*n*-butylaminomethyl)methanol

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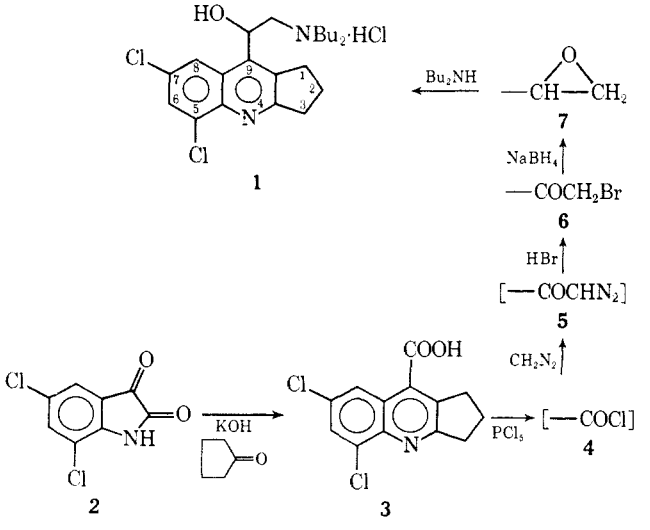
The title compound (**1**) was synthesized to provide, for antimalarial testing, an example of a 4-quinoline amino alcohol in which position 2 was blocked by the CH_2 group of the rigid 2,3-trimethylene ring.[3] It was hoped that this arrangement would prevent rapid biodegradation,[4] and, through lack of conjugation of the type involved in the 2-aryl series, would minimize phototoxicity.[5]



The synthesis started from 5,7-dichloroisatin (**2**) and proceeded by the classical route,[6] namely, Pfitzinger condensation with cyclopentanone to 6,8-dichloro-2,3-trimethylenecinchoninic acid (**3**),[7] followed by diazomethylation of the acid chloride **4** to **5**, hydrobromination to bromo ketone **6**, reduction by $NaBH_4$–NaOH to the epoxide **7**, and aminolysis with Bu_2NH.

Biological Activity.[1d,8]—Target compound **1** proved to be only moderately active against *Plasmodium berghei* in mice, doubling survival time at a dosage of 320 mg/kg, and trebling it at 640 mg/kg.

Experimental Section[9]

6,8-Dichloro-2,3-trimethylenecinchoninic Acid (5,7-Dichloro-2,3-dihydro-1*H*-cyclopenta[*b*]quinoline-9-carboxylic Acid) (3) (*Cf.* the Unchlorinated Acid[7]).—The purple slurry from addition of 21.6 g (0.1 mole) of **2** to 16.8 g (0.3 mole) of KOH in 125 ml of H_2O was added under stirring to 20 g (0.238 mole) of cyclopentanone in 150 ml of abs EtOH. After refluxing (25 hr) and evapn *in vacuo*, the residue was dissolved in 700 ml of H_2O. Acidification with AcOH gave **3**; this was dissolved in KOH–H_2O, repptd by AcOH, and washed successively with dil AcOH, H_2O, and cold EtOH: 23 g (81.6%); mp 272–274° dec. *Anal.* ($C_{13}H_9Cl_2NO_2$) C, H, N.[9b]

3·Potassium Salt (8).—A hot soln of 5 g of KOH in 20 ml of abs EtOH was added with stirring to a suspension of 21.9 g of **3** in 150 ml of warm EtOH. Chilling, filtering, and washing with cold EtOH and with 250 ml of Et_2O gave 21.47 g: unchanged at 325°; ir (cm^{-1}) 2975, 2930, 1580 (C=O). *Anal.* ($C_{13}H_8Cl_2KNO_2$) C, H, N.

3·Methyl ester (9) was prepd by CH_2N_2–Et_2O on **3**; crystd from EtOH–hexane: mp 177–178°; ir (cm^{-1}) 1720 (C=O); nmr ($CDCl_3$), δ 8.30 (1 H, doublet), 7.30 (1 H, d), 4.13 (3 H, s), 3.31 (4 H, triplet), 2.25 (2 H, quintuplet). *Anal.* ($C_{14}H_{11}Cl_2NO_2$) C, H, N.

3·Amide (10) was prepd from **4** by aq NH_3; crystd from Et_2O–hexane: mp 285–287° dec; ir (cm^{-1}) 3350, 3160, 1680. *Anal.* ($C_{13}H_{10}Cl_2N_2O$) C, H, N.

6,8-Dichloro-4-bromoacetyl-2,3-trimethylenequinoline (6).—A C_6H_6 soln of **3·acid chloride, 4**,[10] was prepared from 13.8 g of 3·HCl by reaction with PCl_5 (100°, 30 min) and extg with dry C_6H_6[11] (quenching of an aliquot in ice–NH_3 gave **10**). This was added (below 10°, over 0.5 hr) to 5.61 g of dry CH_2N_2 in 700 ml of Et_2O (KBr pellets; H_2O present at this point readily converts **4** through **3** and CH_2N_2 to **9**). After warming to room temp (2 hr) 48% HBr–H_2O was added (stirring, 40 min). The Et_2O layer was washed successively with 48% HBr, H_2O, and NaCl–H_2O, dried ($MgSO_4$), and evapd *in vacuo*. The residual oil in 700 ml of petr ether (bp 65–110°) was decolorized (charcoal, reflux) and successively concd and cooled giving **6**: recrystd (hexane), mp 125–127° (still impure); ir (cm^{-1}) 3090, 3000, 2970, 2940, 1720; nmr ($CDCl_3$), 7.80 (1 H, d), 7.60 (1 H, d), 4.38 (2 H, s), 3.21 (4 H, overlapping triplets), 2.37 (2 H, quintuplet).

(1) (a) This work was supported by the U. S. Army Medical Research and Development Command, Office of the Surgeon General; Contract No. DA-49-193-MD-2955, R. E. Lutz, Responsible Investigator. (b) Contribution No. 934 of the Army Research Program on Malaria. (c) Presented in part at the Southeast Regional Meeting of the American Chemical Society, Richmond, Va., Nov 1969, Abstract 255. (d) Antimalarial test results were supplied by the Walter Reed Army Institute of Research.
(2) Postdoctoral Research Associates.
(3) (a) Cf. reported antimalarial properties of derivatives of β-quininclene: (b) M. S. Chadha, K. K. Chakravarti, and S. Siddiqui, *J. Sci. Indian Res.*, **10B**, 1 (1951); *Chem. Abstr.*, **46**, 4545 (1952).
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(7) *cf.* V. Q. Yen, N. P. Buu-Hoi, and N. D. Xuong, *J. Org. Chem.*, **23**, 1858 (1958).
(8) The method of T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(9) Instruments: (a) Thomas-Hoover apparatus for mp; (b) ir, Perkin-Elmer 337; (c) nmr, Hitachi-Perkin Elmer R-20; (d) anal. (Gailbraith Lab, Inc.) were correct within ±0.4%.
(10) First attempted prepns of **4** using PCl_5 were frustrated by facility of hydrolysis. Use of $SOCl_2$ (with or without DMF), and oxalyl chloride [J. Szmuszkovic, *J. Org. Chem.*, **29**, 843 (1964)], gave amorphous orange products, except in one of the latter experiments using **3·K salt (8)** (not successfully repeated) where MeOH quench gave **3·Me ester (9, 87%)**.
(11) *Cf.* the tetrahydroacridine analogs; G. K. Patnaik, M. M. Vohra, J. S. Bindra, C. P. Garg, and N. Arnand, *J. Med. Chem.*, **9**, 483 (1966).