All samples were analyzed for their metal content by standard methods. The org matter was first destroyed by oxidn with a mixt of HNO_3–H_2SO_4. Complexometric titrations with standard EDTA soln were performed with murexide indicator in some cases and in others the Hg–EDTA electrode[6] was used as the indicator.

Antitumor Tests.—The methods used in this investigation for the evaluation of antitumor activity in mice have been described elsewhere.[7] The finely ground drugs were suspended in sterile distd H_2O with the addn of a small drop of Tween 80. Doses were chosen on the basis of preliminary toxicity tests. The drugs were given fresh daily ip or by gavage at approximately the max tolerated dose starting 24 hr after tumor inoculation. The index of evaluation for the ascitic tumors is T/C [(mean survival time of treated mice)/(mean survival time of control mice)] × 100. For L1210 a $T/C \geq 125$ is considered positive; for S-180 ascites a % $T/C \geq 150$ is required. In the solid tumor Lewis lung carcinoma % T/C is [(mean tumor weight of treated mice)/(mean tumor weight of control mice)] × 100. A %T/C value ≤ 30 is considered pos.

Spectroscopic Studies.—Schiff bases of salicylaldehyde and arom amines in EtOH have characteristic uv absorptions at 300–360 and at 250–290 nm.[8] The Schiff bases corresponding to the 4 sol Co derivatives show absorbances in anhyd PhH in these regions also. The Co derivatives of these compds in anhyd PhH absorb in the 380- to 420-nm region in addn to the 300- to 360-nm and 250- to 290-nm regions. However, when the Co chelates of these Schiff bases were placed in anhyd dioxane, the spectra changed within a few min. In the case of II an absorption peak at 390 nm decreased and in the case of I a peak at 340 nm increased in intensity. These changes, which were not observed with anhyd PhH solns, could have been caused by reaction with a small amt of H_2O left in the dioxane or by complexation with the dioxane itself.

Solubilities.—The solubilities of I, II, VII, and VIII were detd in anhyd PhH. The other compds did not dissolve to any measurable extent in this solvent. The solvent was dried for 5 days by passing it repeatedly through a Soxhlet extractor filled with 4-A Molecular Sieves;[9] care was then taken to avoid absorption of H_2O from the atm.

Solubilities in PhH (given in Table III) were detd spectroscopically by use of Beer's law plots on solns of known concns.

TABLE III

SOLUBILITIES AND RATES OF HYDROLYSIS

Compound	R	Solubilities of chelates in benzene, g/100 ml	Half-times of hydrolysis, min: Schiff base	Half-times of hydrolysis, min: Cobalt deriv
I	*o*-CH_3	0.143	7.1	5.8
II	*p*-CH_3	0.166	6.5	5.4
VII	*p*-CH_3O	0.041	17	22
VIII	*p*-$N(CH_3)_2$	0.031	1.8	3.0

These solubilities differ widely and are related to the melting points; the compds with the higher melting points being less sol.

For the study of the rates of hydrolysis, each of the 4 sol compds was dissolved in 1 ml of anhyd dioxane and at time zero this soln was mixed rapidly with 100 ml of pH 7.0 phosphate buffer. An aliquot of the soln was placed in a quartz cell and the uv spectrum was scanned from 220 to 400 nm in about 4 min with a Cary 14 recording spectrophotometer. The spectrum was recorded at various times until no further changes were seen.

The absorption peak at 380–390 nm, characteristic of the Co chelates, disappeared in the first 5 min and was replaced by a peak at 330–340 nm which is characteristic of the Schiff bases. The latter decreased at a measurable rate and so was used for detn of the rate of hydrolysis. A peak at 255 nm, characteristic of salicylaldehyde,[10] increased slowly and was also used for studies on the rate of hydrolysis.

The half-times of approach to equil, calcd by the method of Reeves,[11] are shown in Table III for the Co chelates and the Schiff bases. The values obtd for the Schiff bases are in agreement with the values found by Reeves.[11]

Acknowledgment.—Grateful acknowledgment is made of the assistance of the staff of the Research Foundation of Oklahoma State University in preparing this report.

(6) C. N. Reilley, R. W. Schmid, and D. W. Lawson, *Anal. Chem.*, **30**, 953 (1958).

(7) F. A. French, E. J. Blanz, Jr., J. R. DoAmaral, and D. A. French, *J. Med. Chem.*, **13**, 1117 (1970).

(8) B. M. Krasovitskii, B. M. Bolotin, and R. N. Nurmukhametov, *Zh. Obshch. Khim.*, **34**, 3786 (1964).

(9) The water content can be reduced to less than 0.0005%. P. Arthur, W. M. Haynes, and L. P. Varga, *Anal. Chem.*, **38**, 1630 (1966).

(10) "UV Atlas of Organic Compounds," Vol. 3, Butterworth, London, 1967, p 35.

(11) R. L. Reeves, *J. Org. Chem.*, **30**, 3129 (1965).

Synthesis and Antifungal Activity of Substituted Carbanilic Acid Esters

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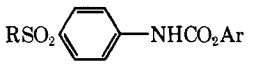
As part of a continuing study of the chemical and pharmacological properties of substituted carbamate esters,[1-4] new compds listed in Table I were prepared by Curtius degradation of appropriate azides. New azides prepared are tabulated in Table II.

TABLE I



Compd	R	Ar	Mp, °C	Yield, %	Formula[a]
1	CH_3	TCP[b]	185	32	$C_{14}H_{10}Cl_3NO_4S$
2	C_2H_5[c]	TCP	208	41	$C_{15}H_{12}Cl_3NO_4S$
3	C_6H_5	TCP	195	44	$C_{19}H_{12}Cl_3NO_4S$
4	H_2N	TCP	235	49	$C_{13}H_9Cl_3N_2O_4S$
5	$(CH_2)_4N$	TCP	190	44	$C_{17}H_{16}Cl_3N_2O_4S$
6	$O(CH_2)_4N$	TCP	260	52	$C_{17}H_{16}Cl_3N_2O_5S$
7	CH_3	PCP[d]	145	45	$C_{14}H_8Cl_6NO_4S$
8	C_2H_5[c]	PCP	127	77	$C_{15}H_{10}Cl_6NO_4S$
9	*p*-FC_6H_4	PCP	170	75	$C_{19}H_9Cl_6FNO_4S$
10	H_2N	PCP	230	31	$C_{13}H_7Cl_6N_2O_4S$
11	$(CH_3)_2N$[e]	PCP	174	83	$C_{15}H_{11}Cl_6N_2O_4S$
12	$(CH_2)_4N$	PCP	135	80	$C_{17}H_{12}Cl_6N_2O_4S$
13	$O(CH_2)_4N$	PCP	172	39	$C_{17}H_{12}Cl_6N_2O_5S$
14	$(CH_2)_5N$[e]	PCP	180	72	$C_{18}H_{14}Cl_6N_2O_4S$
15	Cyclo $C_6H_{11}NH$[e]	PCP	155	65	$C_{19}H_{17}Cl_5N_2O_4S$

[a] All compds were analyzed for C, H, and the results were satisfactory. Ir and nmr spectra were as expected. [b] TCP = 2,4,6-trichlorophenyl. [c] Azide was prepd according to ref 1. [d] PCP = pentachlorophenyl. [e] Azide was prepd according to ref 2.

All compds prepared were tested *in vitro* for antifungal activity against *Candida albicans*, *Aspergilus niger*, and *Penicillum sp.* Concentrations of 10 and 25 μg/ml of each compd in BBL Sabouraud dextrose agar medium were used. Compds were dissolved in acetone (1.5 mg/ml), diluted with hot culture medium to the desired concn, and autoclaved at 120° for 1 hr.

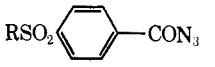
(1) N. Sharghi, I. Lalezari, Gh. Niloufari, and F. Ghabgharan, *J. Med. Chem.*, **13**, 1248 (1970).

(2) I. Lalezari and H. Golgolab, *J. Med. Chem.*, **14**, 1017 (1971).

(3) H. Mirshamsi, I. Lalezari, M. Kamali, G. Niloufari, and N. Rezvani, *Arch. Virusforsch.*, **29**, 267 (1970).

(4) I. Lalezari and A. Vahdat, *J. Med. Chem.*, **14**, 59 (1971).

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]

Compound	*Candida albicans* 25 μg/ml	*Candida albicans* 50 μg/ml	*Aspergilus niger* 25 μg/ml	*Aspergilus niger* 50 μg/ml	*Penicillium SP.* 25 μg/ml	*Penicillium SP.* 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).