A sample was hydrolyzed in 6 *N* HCl at 110° for 22 hr and then analyzed in the 50–50° system on a Beckman-Spinco amino acid analyzer. The following molar ratios of amino acids and NH_3 were obtained with the value of glycine taken as 1.0: aspartic acid 1.0, proline 1.0, glycine 1.0, α-aminobutyric acid 1.0, tyrosine 0.9, phenylalanine 1.0, arginine 0.9, and NH_3 2.0. In addition, cystine (0.4) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.6) were present. These two sulfur compounds account for the half-cystine residue in this analog.[21]

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Synthesis of Analogs of the C-Terminal Octapeptide of Cholecystokinin–Pancreozymin. Structure–Activity Relationship

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A number of analogs of the C-terminal octapeptide of cholecystokinin–pancreozymin (CCK-PZ) were synthesized, using both stepwise and fragment condensation approaches. Their gallbladder-contractile activities were compared with those of the C-terminal octapeptide of CCK-PZ *in vivo* and *in vitro*.

Ivy and Oldberg[1] reported in 1928 the discovery of a new gallbladder-contracting principle of the small intestine, and named it cholecystokinin (CCK). Almost 15 years later, Harper and Raper[2] discovered in the mucosa of the upper intestine a substance that caused secretion of enzymes in pancreatic juice. This substance, which they also considered to be a specific hormone, was named pancreozymin (PZ). Finally, in 1964 Jorpes and Mutt[3] isolated from the same intestinal tissue a polypeptide that exhibited the properties of both cholecystokinin and pancreozymin. They have referred to it, at least temporarily, as cholecystokinin-pancreozymin (CCK-PZ). This hormone, for which a partial structure (I) has been proposed,[4–6] is a polypeptide of 33 amino acid residues:[7]

Lys-(Ala_1,Gly_1,Pro_1,Ser_1)-Arg-Val-(Ile_1,Met_1,Ser_1)-Lys-Asn-(Asx_1,Glx_1,His_1,Leu_2,Pro_1,Ser_2)-Arg-Ile-(Asp_1,Ser_1)-Arg-Asp-

SO_3H

(Gly_1,Met_2,Trp_1,Tyr_1)-Asp-Phe-NH_2

I

The sequence of the amino acids of the C-terminal octapeptide of I is as follows:[8]

SO_3H

Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2

1 2 3 4 5 6 7 8

II

This octapeptide II possesses the biological activities of the full hormonal molecule[9] and showed 8–10 times the gallbladder-contracting activity of CCK-PZ *in vivo* and *in vitro*.[10]

The C-terminal pentapeptide of I is structurally identical with both the C-terminal pentapeptide of gastrin,[11] a hormone that controls secretion of gastric acid, and with the C-terminal pentapeptide of caerulein,[12] a decapeptide isolated from the skin of an amphibian, *Hyla caerulea*.

The remarkable biological properties of II prompted us to study the importance of various structural features of the molecule, namely: (a) N-terminal amino group, (b) aspartic acid residues in positions 1 and 7, (c) methionine residues in positions 3 and 6, and (d) position of the *O*-sulfate-L-tyrosine residue. A selected number of analogs and derivatives of II, which are listed in Table I, were synthesized and evaluated biologically.

Chemistry.—Two synthetical approaches were used: (1) stepwise, starting from the C-terminal amino acid residue (analogs **6** and **7**), and (2) fragment condensation (**1, 2, 3, 4, 5, 8**). In the stepwise approach, two types of active esters, 2,4,5-trichlorophenyl[13] ester and *p*-nitrophenyl ester,[14] were used for the coupling steps. In the fragment condensation, the N-terminal dipeptide azide was coupled with the C-terminal hexapeptide. Both fragments were synthesized stepwise. The introduction of the sulfate ester group was carried out with concd H_2SO_4 in the preparation of **6** and **7**, and with pyridine–SO_3 complex[15,16] in that of the other analogs. Sulfation with H_2SO_4 was

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TABLE I

	Method of prepn	Activity, —CDU[a] mg— *in vivo*	*in vitro*
SO_3H | Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (II)[b]		30,000	26,000
SO_3H | *Boc*-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2[c]	D	7,000	30,000
SO_3H | β-*Asp*-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2	D	9,600	100,000
SO_3H | *Abu*-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2	D	12,800	5,000
SO_3H | *Ala*-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2	D	12,300	8,400
Asp-Tyr-Met-Gly-Trp-Met-*Abu*-Phe-NH_2 SO_3H |	D	1,000	200
Tyr-*Asp*-Met-Gly-Trp-Met-Asp-Phe-NH_2 SO_3H |	E	70	100
Asp-*Met*-*Tyr*-Gly-Trp-Met-Asp-Phe-NH_2 SO_3H |	E	200	100
Asp-Tyr-*Leu*-Gly-Trp-Met-Asp-Phe-NH_2 SO_3H |	D	33,000	22,700
Asp-Tyr-Met-Gly-Trp-*Leu*-Asp-Phe-NH_2 SO_3H |	D	3,400	8,200
Asp-Tyr-*Leu*-Gly-Trp-*Leu*-Asp-Phe-NH_2	D	2,400	4,400

[a] See A. C. Ivy and H. M. Janecek, *Acta Physiol. Scand.*, **70**, 250 (1959). [b] See ref 9. [c] See ref 10 and B. Rubin, *Pharmacologist*, **11**, 277 (1969). [d] Compounds are listed in numerical order (**1**–**10**) after compound II.

carried out after the removal of the protecting group from octapeptides XX and XXI (Table II). The temperature and the duration of reaction had to be carefully controlled to avoid sulfonation of tyrosine. The *t*-butoxycarbonyl octapeptides XVI, XVII, XVIII, XIX, XXII, and XXIV were sulfated with pyridine–SO_3 complex, with use of a large excess of the reagent and anhydrous conditions. The protecting group of these octapeptides was removed with trifluoroacetic acid to yield the free octapeptides. The extent of sulfation in both cases was determined by ir spectroscopy[16] and paper electrophoresis.[17] All the free sulfated octapeptides were purified by ion-exchange chromatography on DEAE-Sephadex A-25.

Table II lists the intermediates prepared for the synthesis of the octapeptide sulfates (Table I), which are not described in the literature.[18] Dipeptides were synthesized by use of *p*-nitrophenyl esters (for I, V, and VII) and 2,4,5-trichlorophenyl ester (for III). The pentapeptides VIII and IX were prepared by the reaction of *t*-butoxycarbonylglycine *p*-nitrophenyl ester with the required tetrapeptides, which had been obtained as described in the literature[18] (except that *t*-butoxycarbonylalanine *p*-nitrophenyl ester was used for the preparation of the required tetrapeptide). After removal of the protecting group (Boc), the free pentapeptides were allowed to react with the *t*-butoxycarbonyl amino acid *p*-nitrophenyl esters to yield hexapeptides X, XI, XII, and XIII. Similarly, the heptapeptide XIV was prepared from X. The heptapeptide XV was obtained from the reaction of tyrosyl glycyltrytophylmethionylaspartylphenylalanine amide[19] with *t*-butoxycarbonyl-methionyl-2,4,5-trichlorophenyl ester. The octapeptides XVI, XVII, XVIII, XIX, XXII, XXIII, and XXIV were prepared by condensing the dipeptides II, IV, VI, and *t*-butoxycarbonylaspartyltyrosine hydrazide,[9] respectively, with their corresponding hexapeptides, *i.e.*, X, XI, XII, XIII, and *t*-butoxycarbonylmethionylglycyltryptophylmethionylaspartylphenylalanine amide,[9,19] after removal of the protecting group (Boc) with trifluoroacetic acid. The octapeptide XX was prepared by reaction of the free heptapeptide obtained after removal of a *t*-butoxycarbonyl group from XIV with *t*-butoxycarbonyltyrosine 2,4,5-trichlorophenyl ester. The octapeptide XXI was obtained by coupling XV, after the removal of the protecting group, with *t*-butoxycarbonyl-β-*t*-butylaspartic acid α-*p*-nitrophenyl ester.

Biological Results.—The gallbladder-contractile activities of the analogs of II are listed in Table I. Analog **1**, whose N-terminal amino group is blocked with a *t*-butoxycarbonyl group, showed a potency similar to that of II *in vitro*, but was much less potent *in vivo*. When II is modified by replacing the aspartic acid residue in position 7 with alanine, as in **5**, the potency both *in vivo* and *in vitro* was markedly diminished. On the other hand, when the aspartic acid residue in position 1 was replaced with α-aminobutyric acid or alanine, as in **3** and **4**, moderate decreases in potencies occurred. More striking differences were observed between potencies *in vivo* and *in vitro* when the N-terminal aspartic acid was joined to the peptide chain through its β-carboxylic group, as in **2**. The alteration at the N-terminal position of the molecule may have hindered the transport of the peptide to the receptor site.

Analogs **6** and **7** show the effects of altering the distance of the *O*-sulfate-tyrosine residue from the C-terminal end of II. The surprisingly low potencies of these analogs suggest that the distance from the tyrosine-*O*-sulfate residue to some other portion of the molecule (perhaps to the aspartic acid in position 7), plays an important role in the interaction of II with the receptor site.

Although the replacement of methionine by leucine in the C-terminal tetrapeptide of gastrin causes no loss of activity,[20] the analog **10**, in which both methionine residues were replaced with leucine, is less potent than II. When only the methionine residue in position 6 is replaced with leucine, as in **9**, the potency is also lower than that of II. On the other hand, almost no difference in potency exists between II and **8**, in which only the methionine residue in position 3 is replaced with leucine. Therefore, the methionine residues do not seem to be of equal importance for biological activity of the C-terminal octapeptide of cholecystokinin (II).

From the structure–activity relationship discussed above, it seems that the presence of tyrosine *O*-sulfate in position 2 and of aspartic acid in position 7 is of

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TABLE II[a]

		Method of prepn[b]	Mp, °C	Recrystn solvent	$[\alpha]^{25}D$, deg (c 1, DMF)	Analyses
Dipeptides						
	Bz |					
I	Boc-(α-OBzl)-β-Asp-Tyr-NHNHZ	A	166–168	EtOAc–hexane	−3.7	($C_{40}H_{44}N_4O_9$) C, H, N
II	Boc-β-Asp-Tyr-NHNH$_2$	B	193–195	i-Pr_2O[c]	−5.5	($C_{18}H_{26}N_4O_7$) C, H, N
III	Boc-Abu-Tyr-NHNHZ	A	70–72	i-Pr_2O[c,d]	−32	($C_{26}H_{34}N_4O_7$) C, H, N
IV	Boc-Abu-Tyr-NHNH$_2$	B	158–161	70% EtOH	−34[e]	($C_{18}H_{28}N_4O_5 \cdot 0.5H_2O$) C, H, N
V	Boc-Ala-Tyr-NHNHZ	A	68	i-PrOH–i-Pr_2O	−30.8	[$C_{25}H_{32}N_4O_7 \cdot 0.5(C_3H_7)_2O$] C, H, N
VI	Boc-Ala-Tyr-NHNH$_2$	B	175–177	70% EtOH	−35.5[e]	($C_{17}H_{26}N_4O_5$) C, H, N
VII	Boc-Ala-Phe-NH$_2$	A	163–164	EtOAc	−36.3	($C_{17}H_{25}N_3O_4$) C, H, N
Pentapeptides						
VIII	Boc-Gly-Trp-Met-Ala-Phe-NH$_2$	A	190–192	MeOH	−15.5	($C_{35}H_{47}N_7O_7S$) C, H, N, S
IX	Boc-Gly-Trp-Leu-Asp-Phe-NH$_2$	A	190–192	70% EtOH	−28.5[e]	($C_{37}H_{49}N_7O_9$) C, H, N
Hexapeptides						
X	Boc-Met-Gly-Trp-Met-Ala-Phe-NH$_2$	A	202–204	EtOH	−17.8	($C_{40}H_{56}N_8O_8S_2$) C, H, N, S
XI	Boc-Leu-Gly-Trp-Met-Asp-Phe-NH$_2$	A	199–201	70% EtOH	−29.8	($C_{42}H_{58}N_8O_{10}S \cdot 1.5H_2O$) C, H, N, S
XII	Boc-Met-Gly-Trp-Leu-Asp-Phe-NH$_2$	A	195–197	70% EtOH	−30	($C_{42}H_{58}N_8O_{10}S \cdot 1.5H_2O$) C, H, N, S
XIII	Boc-Leu-Gly-Trp-Leu-Asp-Phe-NH$_2$	A	185–186	70% EtOH	−24.4	($C_{43}H_{60}N_8O_{10}$) C, H, N
Heptapeptides						
	O-t-Bu |					
XIV	Boc-Asp-Met-Gly-Trp-Met-Asp-Phe-NH$_2$	A	207–209	EtOH	−25.5	($C_{49}H_{69}N_9O_{13}S_2 \cdot H_2O$) C, H, N, S
XV	Boc-Met-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$	A	204–206	EtOH	−36.5	($C_{50}H_{68}N_9O_{12}S_2$) C, H, N
Octapeptides						
XVI	Boc-β-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH$_2$	C	195–196	EtOH	−29.2	($C_{54}H_{70}N_{10}O_{15}S_2 \cdot 2H_2O$) C, H, N
XVII	Boc-Abu-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH$_2$	C	197–199	EtOH	−24	($C_{54}H_{72}N_{10}O_{13}S_2 \cdot H_2O$) C, H, N, S
XVIII	Boc-Ala-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH$_2$	C	198–200	EtOH	−24.6	($C_{53}H_{70}N_{10}O_{13}S_2 \cdot H_2O$) C, H, N
XIX	Boc-Asp-Tyr-Met-Gly-Trp-Met-Ala-Phe-NH$_2$	C	204–205	EtOH	−23.7	($C_{53}H_{70}N_{10}O_{13}S_2$) C, H, N
XX	Boc-Tyr-Asp-Met-Gly-Trp-Met-Asp-Phe-NH$_2$	A	188–190	EtOH	−24.3	($C_{54}H_{70}N_{10}O_{15}S_2 \cdot H_2O$) C, H, N
	O-t-Bu |					
XXI	Boc-Asp-Met-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$	A	215	EtOH abs	−27.8	($C_{58}H_{78}N_{10}O_{15}S_2$) C, H, N
XXII	Boc-Asp-Tyr-Leu-Gly-Trp-Met-Asp-Phe-NH$_2$	C	168–170	70% EtOH	−32.5	($C_{55}H_{72}N_{10}O_{15}S \cdot 2H_2O$) C, H, N
XXIII	Boc-Asp-Tyr-Met-Gly-Trp-Leu-Asp-Phe-NH$_2$	C	192–193	EtOH–H_2O	−41	($C_{55}H_{72}N_{10}O_{15}S \cdot H_2O$) C, H, N
XXIV	Boc-Asp-Tyr-Leu-Gly-Trp-Leu-Asp-Phe-NH$_2$	C	188–190	EtOH–H_2O	−29.1[f]	($C_{56}H_{74}N_{10}O_{15} \cdot H_2O$) C, H, N

[a] For the synthesis of intermediates which are not described, see ref 9, 11, 12, 15; J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc. C*, 555 (1966); H. Gregory, J. S. Morley, J. M. Smith, and M. J. Smithers, *ibid.*, 715 (1968); J. C. Anderson, G. W. Kenner, J. K. MacLeod, and R. C. Sheppard, *Tetrahedron Suppl.*, **8**, Part 1, 39 (1966); and J. S. Morley and J. M. Smith, *J. Chem. Soc. C*, 726 (1968). [b] See Experimental Section. [c] Suspended in i-Pr_2O and filtered. [d] The crude dipeptide was purified by countercurrent distribution (K = 1.9) in the system PhMe–$CHCl_3$–MeOH–H_2O (5:5:8:2). [e] MeOH. [f] EtOH.

paramount importance for effective interaction between the peptide and the gallbladder tissue. Changes in any other position do not severely impair the biological activity of the C-terminal octapeptide of CCK-PZ (II).

Experimental Section

Melting points are not corrected. Ir spectra were measured in KBr disks in a Perkin-Elmer spectrophotometer; uv spectra were recorded on a Cary-15 spectrophotometer; nmr spectra were obtained at 60 Mc on a Varian Associates A-60 instrument (with Me_4Si); the Stein–Moore procedure of quantitative amino acid analysis, as modified by Technicon, Inc., and paper electrophoresis (apparatus by Misco) operated at 10 V/cm, were used. All amino acids described in this paper have L-configuration and a Perkin-Elmer 141 polarimeter was used to measure the optical rotation.

The homogeneity and purity of the octapeptide sulfates (Table I) were proved by the following techniques: (a) tlc (Eastman Chromagram Sheet–silica gel 0.1 mm) in a MeOH–$CHCl_3$ (1:3) system, only for 1; (b) descending paper chromatography using a n-BuOH–pyridine–AcOH:H_2O (30:20:6:24) system; (c) paper electrophoresis at five different pH values (3.3, 4.7, 7.2, 8.0, and 9.3);[17] and (d) quantitative amino acid analysis after acid and enzymatic[21] hydrolysis. The uv spectra (0.1 N NaOH) showed the typical indole absorption [λ_{max} 288 mμ (ε 4900); shoulder at 282 mμ (ε 4980)] and the ir spectra showed peaks at 1050 and 1250 cm^{-1}.[16]

Procedure A. N-(t-Butoxycarbonyl-α-benzyl-β-aspartyl-O-benzyltyrosyl)-N'-benzyloxycarbonylhydrazine (I).—This compound was prepared by a method described for its isomer.[9] N-(O-Benzyltyrosyl)-N'-benzyloxycarbonylhydrazine (2.5 g, 5.8 mmol, obtained from 2.7 g of N-(t-butoxycarbonyl-O-benzyl)-N'-benzyloxycarbonylhydrazine and 25 ml of trifluoroacetic acid) was dissolved in DMF (20 ml) and the pH was adjusted to 7.5–8 with NEt_3 at 0°, followed by the addition of t-butoxycarbonyl-α-benzylaspartic acid β-p-nitrophenyl ester (2.8 g, 6.3 mmol). The reaction was carried on at room temperature until the ninhydrin reaction was negative. The solution was diluted with EtOAc (100 ml), washed (20% solution of citric acid, H_2O), and dried (Na_2SO_4). After removal of solvents *in vacuo*, the crystalline product was obtained from EtOAc by addition of hexane: yield, 2.7 g; mp 166–168°; nmr spectra ($CDCl_3$) 1.38 [singlet, 9 H, $(CH_3)_3$]. *Anal.* ($C_{40}H_{44}N_4O_9$) C, H, N.

Procedure B. t-Butoxycarbonyl-β-aspartyltyrosine Hydrazide (II).—A solution of I (2.6 g, 3.6 mmol) in 300 ml of a mixture of MeOH–AcOH–H_2O (2:1:1) was hydrogenated at room temperature and atmospheric pressure over Pd–C (10%, 500 mg) until the evolution of CO_2 ceased (4–5 hr). After removal of the catalyst, the solution was evaporated to dryness and the crystalline residue was suspended in i-Pr_2O, filtered, and dried: yield, 1.4 g; mp 193–195°; nmr spectra (D_2O) showed no phenyl protons. *Anal.* ($C_{15}H_{26}N_4O_7$) C, H, N.

Procedure C. t-Butoxycarbonyl-β-aspartyltyrosylmethionylglycyltryptophylmethionylaspartylphenylalanine Amide (XVI).—A solution of II (310 mg, 0.77 mmol) in DMF (2.3 ml) was treated with concd HCl (0.37 ml) in a Dry Ice–acetone bath at −20° and stirred mechanically. After 3 min, an aq solution of $NaNO_2$ (14%, 0.38 ml) was added while the temperature of the bath remained unchanged. The reaction mixture was kept at this temperature for an additional 3 min. N-Ethylpiperidine (0.61 ml) was added, followed by the addition of a solution of methionylglycyltryptophylmethionylaspartylphenylalanine amide (556 mg, 0.61 mmol, prepared from 600 mg of t-butoxycarbonylmethionylglycyltryptophylmethionylaspartylphenylalanine amide[9] with trifluoroacetic acid) in DMF (1.5 ml). The reaction mixture was kept at 4° for 24 hr, after which a second portion of the dipeptide azide (prepared from 160 mg of II) was added. When the ninhydrin reaction was negative (after another 24 hr), the mixture was poured into 30 ml of

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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 (2).—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

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