Synthesis and Pharmacological Properties of 4-Decarboxamido-8-arginine-vasopressin and Its 1-Deamino Analog[a,b]

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4-Decarboxamido-8-arginine-vasopressin ([4-α-aminobutyric acid]-8-arginine-vasopressin), an analog of 8-arginine-vasopressin in which the carboxamide group of the glutamine residue at position 4 has been replaced by H, has an antidiuretic potency of approximately 760 units/mg, nearly twice the potency of 8-arginine-vasopressin, whereas it possesses a pressor potency of approximately 38 units/mg, about 0.1 that of the parent hormone. The antidiuretic potency of 1-deamino-4-decarboxamido-8-arginine-vasopressin ([1-β-mercaptopropionic acid,4-α-aminobutyric acid]-8-arginine-vasopressin) is approximately 1000 units/mg and its pressor potency is approximately 10 units/mg. Both analogs show low avian vasodepressor and oxytocic potencies.

In previous studies directed toward the evaluation of the extent to which the various chemical functional groups of the posterior pituitary hormone oxytocin contribute to its biological activities, it was shown that the carboxamide group on the glutamine residue at position 4 is not essential for the exhibition of pharmacological properties characteristic of this hormone. This fact was clearly demonstrated through the synthesis and pharmacological examination of 4-decarboxamido-oxytocin ([4-α-aminobutyric acid]-oxytocin),[2] an analog of oxytocin in which the carboxamide group of the glutamine residue is replaced by H. This structural modification was then extended to 8-lysine-vasopressin, the principal pressor-antidiuretic hormone of the hog posterior pituitary gland, by the synthesis of 4-decarboxamido-8-lysine-vasopressin ([4-α-aminobutyric acid]-8-lysine-vasopressin).[3] The antidiuretic potency of 4-decarboxamido-8-lysine-vasopressin was almost threefold higher and the pressor potency was about 25-fold lower than those of 8-lysine-vasopressin. Thus there was a dramatic change in the ratio of antidiuretic to pressor activity.

In an effort to determine whether the same structural modification of 8-arginine-vasopressin (Figure 1), the principal pressor-antidiuretic hormone of human and beef posterior pituitary glands, would result in a similar change of the activity profile, we have now synthesized 4-decarboxamido-8-arginine-vasopressin ([4-α-aminobutyric acid]-8-arginine-vasopressin) and investigated its pharmacological properties. We have also synthesized and tested the pharmacological behavior of 1-deamino-4-decarboxamido-8-arginine-vasopressin ([1-β-mercaptopropionic acid,4-α-aminobutyric acid]-8-arginine-vasopressin).

N-Benzyloxycarbonylasparaginyl-*S*-benzylcysteinylprolyl-N^G-tosylarginylglycinamide[4] served as the starting material for the preparation of 4-decarboxamido-8-arginine-vasopressin and 1-deamino-4-decarboxamido-8-arginine-vasopressin. The benzyloxycarbonyl group was removed, and the appropriate residues were added one by one by use of the *p*-nitrophenyl ester method,[5] as employed in the stepwise synthesis of oxytocin[6] and 8-lysine-vasopressin.[7]

The highly purified analogs gave the expected values in the amino acid and elementary analyses and appeared to be homogeneous when subjected to gel filtration on Sephadex G-25, paper electrophoresis, and tlc.

The analogs were tested for some of the biological activities exhibited by the posterior pituitary hormones. The 4-point assay design was used in the bioassays.[8] Assays for antidiuretic activity were performed on male rats according to the method of Jeffers, *et al.*,[9] as modified by Sawyer.[10] Pressor assays were carried out on anesthetized male rats as described in the USP.[11] Avian vasodepressor assays were performed on conscious chickens according to the procedure employed by Munsick, *et al.*[12] Oxytocic assays were performed on isolated uteri from rats in natural estrus according to the method of Holton,[13] as modified by Munsick,[14] with the use of Mg-free van Dyke–Hastings solution as the bathing fluid. All of the assays were carried out against the USP posterior pituitary reference standard.

The potencies of 4-decarboxamido-8-arginine-vasopressin and 1-deamino-4-decarboxamido-8-arginine-vasopressin with respect to these pharmacological activities, along with those of 8-arginine-vasopressin and 1-deamino-8-arginine-vasopressin, are presented in Table I. The potencies of the corresponding analogs of 8-lysine-vasopressin are also included for purposes of comparison.

The question originally raised as to whether the presence of the carboxamide group at position 4 of arginine-vasopressin is essential for the exhibition of pharmacological activity is clearly answered by the data presented in Table I for 4-decarboxamido-8-arginine-vasopressin. As in the case of lysine-vasopressin, the

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Table I

Pharmacological Potencies[a] of Analogs of Arginine-vasopressin and Lysine-vasopressin

Vasopressin compd	Antidiuretic (A) (rat)	Pressor (B) (rat)	Vasodepressor (fowl)	Oxytocic (rat)	Ratio A:B
8-Arginine-vasopressin[b]	∽400	∽400	∽60	∽20	1
1-Deamino-8-arginine-vasopressin[c]	1300 ± 200	370 ± 20	150 ± 4	27 ± 4	3.5
4-Decarboxamido-8-arginine-vasopressin	∽760	∽38	∽25	∽5	20
1-Deamino-4-decarboxamido-8-arginine-vasopressin	1020 ± 67[d]	10.7 ± 1.3	9.9 ± 0.7	3.3 ± 0.4	95
8-Lysine-vasopressin[e]	240 ± 13	266 ± 18	50 ± 4	7.3 ± 0.2	1
1-Deamino-8-lysine-vasopressin[f]	301 ± 11	126 ± 2	61 ± 2	12 ± 0.5	2.4
4-Decarboxamido-8-lysine-vasopressin[e]	707 ± 107	10.2 ± 0.6	13.1 ± 0.7	1.54 ± 0.10	70
1-Deamino-4-decarboxamido-8-lysine-vasopressin[e]	729 ± 26	3.5 ± 0.2	12.6 ± 0.6	1.51 ± 0.05	200

[a] Expressed in units per milligram as mean potencies ± standard error. [b] R. A. Boissonnas, S. Guttmann, B. Berde, and H. Konzett, *Experientia*, **17**, 377 (1961). [c] R. L. Huguenin, E. Stürmer, R. A. Boissonnas, and B. Berde, *ibid.*, **21**, 68 (1965). [d] The antidiuretic action is considerably more prolonged than that of the USP posterior pituitary reference standard. [e] See D. Gillessen and V. du Vigneaud.[3] [f] R. D. Kimbrough, Jr., W. D. Cash, L. A. Branda, W. Y. Chan, and V. du Vigneaud, *J. Biol. Chem.*, **238**, 1411 (1963).

replacement of the carboxamide group of glutamine by H leads to an analog exhibiting the various activities characteristic of the hormone, although the profile of the activities is changed. The most striking change in going from arginine-vasopressin to the 4-decarboxamido analog is the twofold increase in antidiuretic activity accompanied by a tenfold decrease in pressor activity, leading to an antidiuretic pressor ratio of 20. The corresponding ratio for 4-decarboxamido-8-lysine-vasopressin is 70. The parent hormones possess a ratio of approximately 1. The carboxamido group of glutamine obviously plays an important role in the degree of pressor activity, whereas in the instances mentioned its absence enhances the antidiuretic activity.

The replacement of the amino group on the half-cystine residue at position 1 of 4-decarboxamido-8-arginine-vasopressin with H results in a further substantial increase in antidiuretic potency and a further decrease, approximately 3.5-fold, in the pressor potency, so that the ratio of antidiuretic to pressor activity has now become 95 for 1-deamino-4-decarboxamido-arginine-vasopressin in comparison to 20 for 4-decarboxamido-8-arginine-vasopressin. It will be noted in contrast that a similar modification of 4-decarboxamido-8-lysine-vasopressin did not lead to an increase in antidiuretic potency. However, there was a threefold decrease in pressor potency, so that the antidiuretic:pressor ratio of 1-deamino-4-decarboxamido-8-lysine-vasopressin is 200.

With their very high antidiuretic and their very low pressor and oxytocic potencies, both 1-deamino-4-decarboxamido-arginine-vasopressin and 1-deamino-4-decarboxamido-lysine-vasopressin offer attractive possibilities for clinical application as antidiuretic agents.

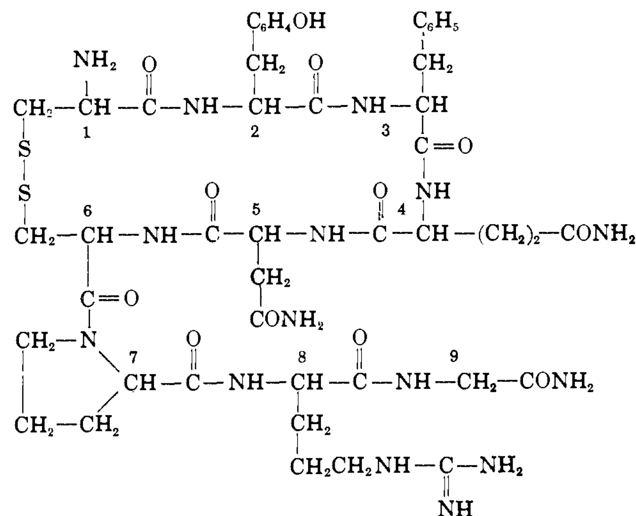

Figure 1.—Structure of 8-arginine-vasopressin. Numbers indicate the position of the individual amino acid residues.

Experimental Section[15]

***N*-Benzyloxycarbonyl-α-aminobutyrylasparaginyl-*S*-benzylcysteinylprolyl-*N*^G^-tosylarginylglycinamide.**—*N*-Benzyloxycarbonylasparaginyl-*S*-benzylcysteinylprolyl-N^G-tosylarginylglycinamide[4] (5.6 g) was dissolved in 20 ml of glacial AcOH, and 20 ml of a solution of HBr in glacial AcOH (40%, w/w) was added. The resulting precipitate dissolved during the next 15 min. After 1.5 hr at room temperature the solution was poured into 400 ml of dry Et_2O. The precipitated hydrobromide of the free pentapeptide was washed by decantation with three 150-ml portions of Et_2O. After being dried *in vacuo* over NaOH and $CaCl_2$ overnight, the hydrobromide was dissolved in 120 ml of dry MeOH and passed through a column of Rexyn RG1 (OH) (Fisher). The column was washed with 350 ml of MeOH and, after removal of the solvent from the combined eluate and washing, the residue was dried for several hours *in vacuo* and then dissolved in 11 ml of DMF. To this solution 2.2 g of *p*-nitrophenyl *N*-benzyloxycarbonyl-α-aminobutyrate[2] was added at 5°. The reaction mixture partially solidified after it was allowed to stand for 2 days at room temperature. EtOAc, 50 ml, and EtOH, 10 ml, were added and the resulting gel was kept for 1 hr at 2° before it was filtered off and then washed with a total of 150 ml of EtOAc and 120 ml of EtOH. After being dried over P_2O_5 *in vacuo* the product weighed 4.4 g; mp 196–199° (sintering at 189°), $[\alpha]^{23}D$ −36.6° (*c* 1, DMF). *Anal.* ($C_{46}H_{61}N_{11}O_{11}S_2$) C, H, N.

***N*-Benzyloxycarbonylphenylalanyl-α-aminobutyrylasparaginyl-*S*-benzylcysteinylpropyl-N^G-tosylarginylglycinamide.**—The protected hexapeptide described in the preceding section (4.0 g) was dissolved in 10 ml of glacial AcOH and treated with 10 ml of HBr in glacial AcOH (40%, w/w) by the procedures described in the preceding section. The hydrobromide was isolated, dried, dissolved in 80 ml of dry MeOH, and passed through Rexyn RG1 (OH), and the column was washed with 150 ml of MeOH. The residue obtained after evaporation of the MeOH from the eluate and washing was dried *in vacuo*, dissolved in 8 ml of DMF, and then treated with *p*-nitrophenyl *N*-benzyloxycarbonylphenylalaninate[16,17] (1.7 g) at 0°. After 3 days at room tempera-

(15) All melting points are corrected capillary melting points. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

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ture 100 ml of EtOAc were added. The precipitate was collected and washed with several portions of EtOAc and of EtOH until the washing was free of p-nitrophenol. After being dried over P_2O_5 *in vacuo* the compound weighed 3.2 g; mp 191–193°, $[\alpha]^{23}D$ −33.8° (c 1, DMF). *Anal.* ($C_{55}H_{70}N_{12}O_{13}S_2$) C, H, N.

N-Benzyloxycarbonyl-O-benzyltyrosylphenylalanyl-α-aminobutyrylasparaginyl-S-benzylcysteinylpropyl-N^G-tosylarginylglycinamide.—The preceding protected heptapeptide (2.6 g) was dissolved by being heated in 15 ml of glacial AcOH, cooled to room temperature, and converted into the hydrobromide by treatment with 15 ml of HBr in glacial AcOH (40%, w/w) as already described. The salt was then dissolved in 80 ml of dry MeOH and passed through a column of Rexyn RG1 (OH). After removal of the solvent the residue was dissolved in 5 ml of DMF and allowed to react with 1.2 g of p-nitrophenyl N-benzyloxycarbonyl-O-benzyltyrosinate[8] for 3 days at room temperature. After addition of 50 ml of EtOAc to the semisolid material, the precipitate was collected, and washed with small portions of EtOAc and of EtOH until the washings were free of p-nitrophenol. After being dried *in vacuo* over P_2O_5 the compound weighed 2.7 g; mp 221–223°, $[\alpha]^{23}D$ −36.2° (c 1, DMF). *Anal.* ($C_{71}H_{85}N_{13}O_{14}S_2$) C, H, N.

4-Decarboxamido-8-arginine-vasopressin ([4-α-Aminobutyric acid]-8-arginine-vasopressin).—The preceding protected octapeptide (1.4 g) was suspended in 35 ml of trifluoroethanol and HBr was bubbled through the suspension for 75 min. The solvent was removed *in vacuo* and the residue was dissolved in 20 ml of dry MeOH, precipitated with 350 ml of dry Et_2O, washed repeatedly with Et_2O, and dried over NaOH and $CaCl_2$. The material was then dissolved in 40 ml of dry MeOH, and the solution was cooled to −10° before the pH was adjusted to 8.5 (Gramercy indicator) by the addition of TEA. The solvent was removed *in vacuo* and the residue was suspended in 30 ml of H_2O, stirred for 1 hr at 0°, filtered off, washed with H_2O, and dried over P_2O_5 *in vacuo*. This material, 1.15 g, was dissolved in 10 ml of DMF and allowed to react with 550 mg of p-nitrophenyl N-benzyloxycarbonyl-S-benzylcysteinate.[6,18] After 5 days at room temperature the solution was concentrated to a volume of 5 ml before the addition of 50 ml of EtOAc. The resulting precipitate was collected and washed in the usual manner with EtOAc and with EtOH. After being dried *in vacuo* over P_2O_5 the compound weighed 1.25 g; mp 201–204° (sintering at 189°), $[\alpha]^{23}D$ −35.3° (c 1, DMF).

This protected nonapeptide (307 mg) was dissolved in approximately 200 ml of liquid NH_3 (freshly distilled from Na) and treated at the boiling point with Na until a blue color persisted throughout the solution for about 30 sec.[19,20] The NH_3 solution was subsequently concentrated, and the final 25 ml were removed by lyophilization. The loose powder was dissolved in 800 ml of H_2O containing a few drops of AcOH. The pH was adjusted to 6.8 with NaOH and was kept between 6.5 and 7.0 by the addition of NaOH while the solution was titrated with 45 ml of a 0.01 N $K_3Fe(CN)_6$ solution.[21] After 0.5 hr the ferro- and ferricyanide ions were removed by passage of the solution through a column of AG3-X4 (Calbiochem) in the Cl^- form. After the solution was acidified with AcOH and concentrated to approximately 20 ml, a mixture of BuOH–pyridine (10:7) was added dropwise until a second phase started to separate. This solution was applied to a Sephadex G-25 column (2.15 × 110 cm) that had been equilibrated with the aqueous phase of the system BuOH–pyridine–0.2 N AcOH (10:7:24). The column was eluted[22,23] with the organic phase at a flow rate of 13 ml/hr, and 90 5-ml fractions were collected. A plot of the Folin–Lowry color values[24] of aliquots from alternate fractions indicated a major peak with a maximum at fraction 48. The central fractions of the main peak (fractions 35–55) were combined, diluted with twice the volume of H_2O, and concentrated to 30 ml. After the addition of 100 ml of H_2O and subsequent concentration to a volume of approximately 30 ml, the solution was filtered and lyophilized, and 133 mg of a fluffy, white powder was obtained. This material was dissolved in 7 ml of the organic phase of the system BuOH–EtOH–pyridine–0.2 N AcOH (8:1:2:14) and applied to a Sephadex G-25 column (2.83 × 58 cm) that had been equilibrated with both phases of this system. Elution with the organic phase was performed and fractions were collected at a flow rate of 22.5 ml/hr. A plot of the Folin–Lowry color values showed a major symmetrical peak having an R_f of 0.25. The amount of 4-decarboxamido-8-arginine-vasopressin isolated from the main peak was 55 mg, $[\alpha]^{26}D$ −24.0° (c 0.5, 1 N AcOH). When the analog was subjected to paper electrophoresis in a pyridine–AcOH buffer (pH 5.6) and to tlc on Silica Gel G in the upper phase of the system BuOH–AcOH–H_2O (4:1:5), it traveled as a single spot. Upon gel filtration of the compound on Sephadex G-25 in 0.2 N AcOH it emerged as a single peak. For analysis a sample was dried *in vacuo* at 100° over P_2O_5 for 6 hr, and a weight loss of 8.7% was observed. *Anal.* ($C_{46}H_{64}N_{14}O_{11}S_2 \cdot C_2H_4O_2$) C, H, N.

A sample was hydrolyzed in 6 N HCl at 110° for 22 hr and then analyzed by the method of Spackman, *et al.*,[25] 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, cystine 1.0, tyrosine 1.0, phenylalanine 1.0, arginine 1.0, and NH_3 2.2.

S-Benzyl-β-mercaptopropionyltyrosylphenylalanyl-α-aminobutyrylasparaginyl-S-benzylcysteinylprolyl-N^G-tosylarginylglycinamide.—N-Benzyloxycarbonyl-O-benzyltyrosylphenylalanyl-α-aminobutyrylasparaginyl-S-benzylcysteinylprolyl-N^G-tosylarginylglycinamide (1.1 g) was suspended in 35 ml of trifluoroethanol and HBr was bubbled through the suspension for 1.5 hr. The solvent was removed *in vacuo* and the residue was dissolved in 20 ml of dry MeOH, precipitated with 200 ml of Et_2O, and washed twice with small portions of Et_2O. After being dried over NaOH the material was dissolved in 3 ml of DMF. The pH was adjusted to 8.5 with TEA and 390 mg of p-nitrophenyl-S-benzyl-β-mercaptopropionate[21] was added. After 10 days at room temperature the material was precipitated by addition of 50 ml of EtOH–H_2O (1:1), collected, and washed twice with 25-ml portions of EtOH and with small portions of EtOAc until the washings were free of p-nitrophenol. After being dried over P_2O_5 *in vacuo* the compound weighed 900 mg; mp 192–194°, $[\alpha]^{23}D$ −33.7° (c 1, DMF). *Anal.* ($C_{65}H_{81}N_{13}O_{13}S_3$) C, H, N.

1-Deamino-4-decarboxamido-8-arginine-vasopressin ([1-β-Mercaptopropionic acid,4-α-aminobutyric Acid]-8-arginine-vasopressin).—The removal of the protecting groups from the preceding protected polypeptide and the oxidation of the resulting disulfhydryl compound to the cyclic disulfide were carried out by the procedures already described for the synthesis of 4-decarboxamido-8-arginine-vasopressin. After removal of the ferri- and ferrocyanide ions, the aqueous solution of the 1-deamino-4-decarboxamido-8-arginine-vasopressin prepared from 400 mg of the protected polypeptide was concentrated to 20 ml, equilibrated with the organic phase of the solvent system BuOH–pyridine–0.2 N AcOH (6:1:8), and applied to a Sephadex G-25 column (2.15 × 110 cm) that had been equilibrated with the aqueous phase of the system. Elution with the organic phase was performed, and 5.0-ml fractions were collected at a flow rate of 12.5 ml/hr. A plot of the Folin–Lowry color values for aliquots of selected fractions indicated a major peak with a maximum at fraction 56. The material isolated from fractions 47–60 weighed 145.2 mg. This material was submitted to chromatography in the solvent system BuOH–C_6H_6–pyridine–0.2 N AcOH (12:1:2:14). The sample was dissolved in 12 ml of the organic phase of this system and chromatographed on a 2.83 × 58 cm column with the collection of 5-ml fractions at a flow rate of 23 ml/hr. Plotting of the Folin–Lowry color values showed a major peak having an R_f of 0.36. From fractions 46–59, 120.9 mg of 1-deamino-4-decarboxamido-8-arginine-vasopressin, having an $[\alpha]^{26.5}D$ −109.9° (c 0.5, 1 N AcOH), was isolated. When a sample of this compound was subjected to electrophoresis and tlc under conditions already described, it traveled as a single spot. It also emerged as a single peak from a gel filtration column. For analysis a sample was dried *in vacuo* at 100° over P_2O_5 for 6 hr and a weight loss of 7.9% was observed. *Anal.* ($C_{45}H_{63}N_{13}O_{10}S_2 \cdot C_2H_4O_2$) H, N; C: calcd, 52.0; found, 51.4.

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(25) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

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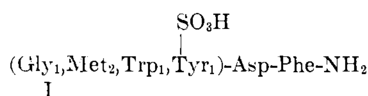
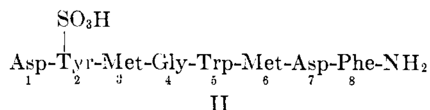
A number of analogs of the C-terminal octapeptide of cholecystokinin–pancreozymin (CCK-PZ) were synthesized, using both stepwise and fragment condensation approaches. Their gallbladder-contractile activities were compared with those of the C-terminal octapeptide of CCK-PZ *in vivo* and *in vitro*.

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-



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



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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