extracted with 5% hydrochloric acid solution. The aqueous solution was neutralized with 1 N sodium hydroxide solution and then extracted with methylene chloride. The extract was dried over sodium sulfate. Removal of solvent gave solid material that was recrystallized from methanol to give the title compound in 50% yield as an off-white solid: mp 179–181 °C; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3 H), 2.45–2.55 (t, 4 H), 3.68–3.8 (t, 4 H), 5.02 (s, 2 H), 6.01 (s, 1 H), 6.7–7.6 (m, 8 H); IR ($CHCl_3$) a strong C=N absorption peak at 1582 cm^{-1}; MS, *m/e* 330 (M^+). Anal. ($C_{21}H_{22}N_4$) C, H, N.

12-Ethoxy-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (5). A solution of 20 g (0.081 mol) of 6*H*-indolo[2,1-c][1,4]benzodiazepin-12(11*H*)-one (3) in 200 mL of methylene chloride was added to a mixture of triethyloxonium tetrafluoroborate in methylene chloride, prepared from 10 mL (0.12 mol) of epichlorohydrin and 20 mL (0.16 mol) of boron trifluoride etherate in 30 mL of anhydrous ether at room temperature. The reaction mixture was stirred at room temperature for 2 days and poured into 400 mL of ice water containing 7.5 g (0.18 mol) of sodium hydroxide. Extraction with chloroform, drying with sodium sulfate, and removal of solvent in vacuo yielded 22.0 g (98% yield) of a tan solid. Recrystallization from ethanol/chloroform yielded 18.6 g of the title compound as a white powder: mp 144 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3 H), 4.52 (q, 2 H), 5.07 (s, 2 H), 7.22 (m, 9 H); IR ($CHCl_3$) a C=N absorption at 1641 cm^{-1}. Anal. ($C_{18}H_{16}N_2O$) C, H, N.

12-(4-Methyl-1-piperazinyl)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (6a). Method B. A mixture of 10.5 g (0.038 mol) of 12-ethoxy-6*H*-indolo[2,1-c][1,4]benzodiazepine (5), 180 g (1.8 mol) of 1-methylpiperazine, and 2.0 mL of glacial acetic acid was heated at reflux temperature for 56 h. Excess amine was removed under vacuum, and the residue was purified by column chromatography (CH_2Cl_2). The obtained solid was recrystallized from EtOH to give an off-white solid (8.5 g, 68% yield) whose IR, NMR, and melting point were identical with that of the product prepared from method A.

Serotonin Release Assay. The procedure employed was as previously described by Chasin et al.[10] (1979). Briefly, rat peritoneal cells were harvested and incubated for 3 h in the presence of [^{3}H]-5-hydroxytryptophan to allow for conversion to [^{3}H]-serotonin. The labeled cells, compound 48/80, and the test compound were incubated together to determine the degree of inhibition of compound 48/80 induced serotonin release.

Passive Cutaneous Anaphylaxis (PCA) in the Rat. In this test, an IgE-mediated wheal was produced in male Sprague–Dawley rats by an intradermal injection of 0.05 mL of high-titer IgE rat antiovalbumin antiserum obtained from rats previously sensitized with ovalbumin.[11] Twenty-four hours postinjection, the rats were challenged intravenously with ovalbumin and Evans Blue dye in saline. Simultaneous intradermal injections of serotonin and histamine were made. Thirty minutes later, the animals were sacrificed and the skin was reflected for analysis of the response. Results are determined by measuring wheal sizes and scoring the color intensities relative to a standard color chart. Percent inhibition was calculated from the following formula: percent inhibition = (1 – mean experimental score/mean control score) × 100. Drugs were administered intraperitoneally 1 h prior to antigen, and the response was evaluated 30 min subsequent to antigen challenge.

Active Lung Anaphylaxis (ALA) in the Guinea Pig. Guinea pigs were sensitized to egg albumin, 1 mg s.c., by *Bordetella pertussis* vaccine (0.5 mL) as an adjuvant.[12] Two weeks after sensitization, the animals were anesthetized with sodium pentobarbital, 65 mg/kg i.p. The trachea, jugular vein, and carotid artery were cannulated for monitoring airway resistance, intravenous administration of test compounds, and measuring mean arterial blood pressure, respectively. The animals were then challenged with egg albumin, 0.5 mg/kg iv, which produced an anaphylactic reaction characterized by severe bronchoconstriction. Compounds were administered either intravenously, orally, or intraperitoneally prior to the antigen challenge, and their effect on bronchoconstriction was evaluated. Compounds were also tested by using the oral route of administration.

Registry No. 1, 99384-70-0; 2, 99384-52-8; 3, 101226-22-6; 4, 101226-23-7; 5, 101226-24-8; 6a, 101226-25-9; 6b, 101226-26-0; 6c, 101226-27-1; 6d, 101226-28-2; 6e, 101226-29-3; 6f, 101226-30-6; 6g, 101226-31-7; 6h, 101226-32-8; 2-nitrobenzyl chloride, 612-23-7; ethyl indole-2-carboxylate, 3770-50-1; 1-methylpiperazine, 109-01-3; 1-(3-hydroxypropyl)piperazine, 5317-32-8; piperazine, 110-85-0; ethyl 1-piperazinecarboxylate, 120-43-4; 1-butylpiperazine, 5610-49-1; morpholine, 110-91-8; 4-hydroxypiperidine, 5382-16-1; piperidine, 110-89-4.

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Structure–Activity Relationships for the Competitive Angiotensin Antagonist [Sarcosine^1,*O*-methyltyrosine^4]angiotensin II (Sarmesin)

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Analogues of the competitive angiotensin antagonist [Sar^1,Tyr(Me)^4]ANG II (sarmesin) in which the sarcosine-1, *O*-methyltyrosine-4, and phenylalanine-8 residues were modified have been synthesized by the solid-phase method. The agonist and antagonist potencies of the 23 peptides synthesized were determined in the rat isolated uterus assay. At position 1, replacement of Sar with Asp, Ala, or Pro gave inactive analogues, and deletion of the N-terminal amino acid produced inactive heptapeptides for all analogues investigated. At position 4, substitution of Tyr with Tyr(Et), D-Tyr, D-Phe, Ile, Thr, or Hyp resulted in inactive analogues, whereas substitution of Phe gave a potent competitive antagonist (pA_2 = 7.9), which retained significant agonist activity (22%). For position 8, [Sar^1,Tyr(Me)^4,Ile^8]ANG II and [Sar^1,Phe^4,Ile^8]ANG II were weaker antagonists (pA_2 = 6.6 and 6.7, respectively) than [Sar^1,Ile^8]ANG II (pA_2 apparent = 8.1) and, moreover, were reversible competitive antagonists. These findings demonstrate that the structural requirements for receptor blockade by sarmesin are remarkably stringent—modifications at positions 1, 4, and 8 markedly reduce the antagonist activity of this peptide.

Antagonists of angiotensin II (ANG II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) are traditionally analogues of ANG II with amino acid substitutions at positions 1 and 8,[1] e.g., [Sar^1,Ala^8]ANG II (saralasin), [Sar^1,Ile^8]ANG II. A pre-

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Table I. Chemical Data on Synthetic Peptides

analogues	amino acid analysis											TLC, R_f	
	Ala	Arg	Asp	His	Hyp	Ile	Phe	Pro	Thr	Tyr	Val	BPAW[a]	CMAW[a]
[Sar[1],Tyr(Me)[4]]ANG II		1.12		1.05		0.88	1.08	0.99		0.90[b]	1.00	0.32	0.32
[Tyr(Me)[4]]ANG II		0.99	1.01	0.89		1.04	1.00	1.04		0.75[b]	1.00	0.40	0.46
[Ala[1],Tyr(Me)[4]]ANG II	1.07	1.05		0.99		1.03	1.05	1.04		0.58[b]	1.00	0.47	0.67
[Pro[1],Tyr(Me)[4]]ANG II		1.13		0.95		0.99	1.03	2.09		0.67[b]	1.00	0.44	0.44
[Sar[1],Tyr(Et)[4]]ANG II		1.15		1.05		0.98	1.09	1.10		0.74[b]	1.00	0.42 (0.38)	0.62 (0.70)
[Sar[1],D-Tyr[4]]ANG II		0.97		0.92		1.10	1.11	1.07		0.87	1.00	0.41 (0.48)	0.40 (0.49)
[Sar[1],Phe[4]]ANG II		0.96		0.93		0.94	2.08	0.99			1.00	0.32 (0.54)	0.51 (0.57)
[Sar[1],D-Phe[4]]ANG II		0.88		0.86		0.94	2.03	0.89			1.00	0.47 (0.49)	0.65 (0.74)
[Sar[1],Ile[4]]ANG II		1.04		1.10		2.03	1.11	1.06			1.00	0.63 (0.46)	0.82 (0.78)
[Sar[1],Thr[4]]ANG II		0.97		0.97		0.97	1.20	1.03	0.94		1.00	0.39 (0.42)	0.53 (0.65)
[Sar[1],Hyp[4]]ANG II		1.22		1.14	1.12	1.08	1.12	1.20			1.00	0.38 (0.55)	0.63 (0.73)
[Sar[1],Tyr(Me)[4]Ile[8]]ANG II		1.08		1.06		2.04		0.96		0.60[b]	1.00	0.35	0.38
[Sar[1],D-Tyr[4],Ile[8]]ANG II		1.10		0.98		2.06		0.97		1.01	1.00	0.44 (0.41)	0.44 (0.44)
[Sar[1],Phe[4],Ile[8]]ANG II		1.04		1.01		2.17	1.00	1.14			1.00	0.49 (0.69)	0.64 (0.73)
[Sar[1],D-Phe[4],Ile[8]]ANG II		1.09		1.07		2.12	1.06	1.13			1.00	0.44 (0.71)	0.75 (0.66)

[a] BPAW = 1-butanol–pyridine–acetic acid–water (15:10:3:6, v/v) and CMAW = chloroform–methanol–acetic acid–water (15:10:2:3, v/v); values in parentheses are for the des[1] analogues. [b] Tyr(Me) and Tye(Et) are partially converted to Tyr during acid hydrolysis.

vious report from this laboratory[2] has shown that the tyrosine residue occupying position 4 in ANG II can be modified to produce the type II antagonist sarmesin, [Sar[1],Tyr(Me)[4]]ANG II. The antagonist activity of sarmesin differs markedly from the antagonism produced by more classical type I antagonists such as [Sar[1],Ile[8]]ANG II, in that it is readily reversible and does not impose the long-term blockade characteristic of [Sar[1],Ile[8]]ANG II and related analogues.[3] With a view to synthesizing a more potent competitive angiotensin antagonist, and in order to explore the structural features of sarmesin that confer its antagonist properties, we have designed and synthesized several peptides of the type: X-Arg-Val-Y-Ile-His-Pro-Z, where X = Sar, Asp, Ala, Pro, or des (no amino acid); Y = D-Tyr, Tyr(Me), Tyr(Et), D-Phe, Ile, Thr, or Hyp; and Z = Phe or Ile.

The undertaking of a structure–activity study on sarmesin was prompted by the observation that [des-Sar[1]]-sarmesin is biologically inactive,[3] suggesting that the N-terminal residue in this peptide, unlike [Sar[1],Ile[8]]ANG II, is crucial for receptor binding. The present findings illustrate that the N-terminal amino acid does indeed play a unique role and that the structural requirements at position 4 are equally stringent. A structure–activity study on sarmesin should also provide data that can be helpful in illuminating aspects of the receptor conformation of ANG II. Recent conformational studies in this laboratory have suggested that the tyrosine hydroxyl group in ANG II may have its ionizable proton partially abstracted by the His N_3–this being a direct consequence of partial abstraction of the His N_1 proton by the C-terminal carboxylate.[4,5] This tripartite "charge delocalization network" (CDN), together with stacking of the His and Phe rings,[6] has been implicated in receptor activation.[7] Others have suggested[8] that the C-terminal carboxylate is involved in

Table II. Biological Activities of Sarmesin Analogues in the Rat Isolated Uterus Assay[a]

analogue	agonist act., %	antagonist act. (pA_2)
[Sar[1],Tyr(Me)[4]]ANG II	<0.1	7.5 ± 0.1 (14), 8.1[b]
[Tyr(Me)[4]]ANG II	<0.1	<6
[Ala[1],Tyr(Me)[4]]ANG II	<0.1	<6
[Pro[1],Tyr(Me)[4]]ANG II	0.3 ± 0.1 (3)	<6
[Sar[1],Tyr(Et)[4]]ANG II	0.2 ± 0.1 (3)	<6
[Sar[1],D-Tyr[4]]ANG II	2.0 ± 0.5 (4)	<6.5
[Sar[1],Phe[4]]ANG II	22 ± 4 (9)	7.9 ± 0.1 (4)
[Sar[1],D-Phe[4]]ANG II	<0.1	<5.5
[Sar[1],Ile[4]]ANG II	0.2 ± 0.1 (4)	<6
[Sar[1],Thr[4]]ANG II	2.0 ± 0.3 (3)	<6
[Sar[1],Hyp[4]]ANG II	<0.1	<5
[Sar[1],Tyr(Me)[4],Ile[8]]ANG II	<0.1	6.6 ± 0.1 (4)
[Sar[1],D-Tyr[4],Ile[8]]ANG II	<0.1	<5
[Sar[1],Phe[4],Ile[8]]ANG II	<0.1	6.7 ± 0.2 (4)
[Sar[1],D-Phe[4],Ile[8]]ANG II	<0.1	<5
[Sar[1],Ile[8]]ANG II	<0.1	8.1 ± 0.2 (3)[c]

[a] The corresponding ANG III analogues of the peptides listed in the table, i.e., heptapeptide analogues lacking an amino acid in position 1, were inactive in every case except [des[1],Ile[8]]ANG II. [b] From ref 2, using an isometric bioassay system. [c] [Sar[1],Ile[8]]ANG II is a slowly reversing antagonist and, as such, cannot be regarded as a truly competitive antagonist.[3,5] Values are given as mean ± SEM (number of experiments), using [Asp[1],Ile[5]]ANG II (human angiotensin II) as the standard.

two alternative interactions: (i) interaction of the carboxylate carbonyl with the Phe ring and (ii) interaction of the other carboxylate oxygen with the amide NH of Phe. Since our proposed interaction of the Phe ring with the His ring[6] could in essence include partial overlap of the Phe ring with (and π-electron contribution from) the contiguous carboxylate in the CDN, interation (i) is not necessarily precluded by our CDN model. The major discrepancy derives from the O^-··· HN interaction, which involves the His N_1H in our CDN model[5,7] compared to the Phe NH in the other model.[8] However, the latter model does not explain the importance of the Tyr hydroxyl group in receptor activation whereas the CDN model invokes a role for the phenoxyl in ANG II. The CDN model predicts that the absence of a phenoxyl proton should reduce the efficacy of ANG II analogues without necessarily disrupting binding affinity.

Results and Discussion

Tables I and II give the chemical data and biological

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activities for the various sarmesin analogues synthesized in this study. As is evident from the data for the first four peptides listed in Table II and also for the various desaspartyl derivatives (see legend to Table II), the requirement for Sar in position 1 is obligatory. Neither the naturally occurring Asp residue nor an Ala residue at position 1 gives biologically active analogues ($pA_2 < 6$); even the secondary amino acid Pro is not acceptable at the N-terminus. This contrasts with data on these same substitutions in ANG II, where Ala[1] and Pro[1] only reduce the pressor activity to 37% and 50%, respectively.[9] It is well known that the presence of Sar in position 1 increases the potencies of both agonist and antagonist analogues of ANG II, although sarmesin may be the most striking example of this phenomenon. Possibly the conformation of sarmesin that results in its high binding affinity for angiotensin receptors is dependent on steric influences imposed by the *N*-methyl group of Sar. Whatever the reason, it is clear that the Sar residue in sarmesin has a powerful influence concerning receptor recognition for this particular peptide.

At position 4, replacement of the *O*-methyl group of sarmesin with a *O*-ethyl moiety severely reduces the binding affinity, suggesting that steric constraints resulting from the presence of the bulkier ethyl group do not permit [Sar[1],Tyr(Et)[4]]ANG II to occupy the receptor sites. Apparently the receptor binding "pocket" that accommodates the Tyr side chain of ANG II has limited dimensions. A precise orientation of the Tyr side chain is also important as evidenced by the low biological activities of [Sar[1],D-Tyr[4]]ANG II and [Sar[1],D-Phe[4]]ANG II. [Sar[1],Phe[4]]ANG II represents an interesting situation— this analogue can be an agonist or an antagonist depending on the dosage. The data suggest that [Sar[1],Phe[4]]ANG II has very high affinity for angiotensin receptors in smooth muscle, but the lack of a phenolic hydroxyl group impairs its ability to elicit the contractile response mechanism. It is another example, in addition to sarmesin, of the importance of the phenolic hydroxyl group in ANG II for agonist activity. The pressor activity of [Phe[4]]ANG II is about 20% of that of ANG II.[9,10]

[Sar[1],D-Tyr[4]]ANG II retains low but significant agonist activity in the rat uterus assay (Table II). By comparison, [D-Tyr[4]]ANG II has 0.025% pressor activity.[11] NMR studies on [Sar[1],D-Tyr[4]]ANG II (Moore and Goghari, unpublished data) indicate that this peptide takes up two distinct conformations in dimethyl sulfoxide—it is possible that in one of these two conformers the Tyr side chain can correctly position itself to activate the receptors and that the low level of agonist activity observed with this peptide results mainly from a low binding affinity in this distorted conformation. The low biological activity of [Sar[1],Ile[4]]-ANG II demonstrates the importance of an aromatic side chain in the L configuration in position 4 for receptor binding. This is confirmed by these low activities of [Sar[1],Thr[4]]ANG II and [Sar[1],Hyp[4]]ANG II—these peptides also suggest that an aliphatic hydroxyl group (although incorrectly positioned) cannot assume the function of a phenolic hydroxyl group in ANG II.

When Ile is substituted in position 8 of sarmesin and [Sar[1],Phe[4]]ANG II, the binding affinities of the resulting peptides are significantly decreased (Table II). The affinities (pA_2) are also decreased compared to the potent antagonist [Sar[1],Ile[8]]ANG II. In our hands [Sar[1],Ile[8]]ANG II is a slowly reversing antagonist,[3] and since receptor blockade by either [Sar[1],Tyr(Me)[4],Ile[8]]ANG II or [Sar[1],Phe[4],Ile[8]]ANG II is readily reversible, the decreased pA_2's observed for the latter peptides may result primarily from an increased rate of receptor dissociation compared to [Sar[1],Ile[8]]ANG II. This being the case, one would conclude that the phenolic hydroxyl group is somehow responsible for the slow dissociation rate of [Sar[1],Ile[8]]ANG II. Recent data from this laboratory have suggested an interaction between the Tyr and His residues of ANG II which can be explained by the occurrence of a Tyr-His-carboxylate charge delocalization network (CDN) in the ANG II molecule.[4,5] The present findings are not inconsistent with a biologically active conformation for ANG II in which the phenoxyl group interacts with the His N_3. We have suggested[7] that the CDN may "charge up" an intrinsic molecular microcapacitor formed by stacking of the His and Phe rings[6] in ANG II. This model stipulates that methylation or elimination of the phenoxyl group would prevent CDN formation and full charging of the microcapacitor, thereby reducing agonist activity (efficacy) but not necessarily the ability to bind to the receptors. Similarly, the presence of Ile[8] would eliminate the microcapacitor and thereby limit agonist activity. A slow rate of dissociation for [Sar[1],Ile[8]]ANG II, compared to [Sar[1],Tyr(Me)[4],Ile[8]]ANG II and [Sar[1],Phe[4],Ile[8]]ANG II, might possibly result from a tendency of the receptor to acylate this peptide as a direct consequence of the increased nucleophilicities[4,5,7] of the Tyr and His side chains in the proposed CDN. Alternatively microcapacitor discharge, which could not occur with [Sar[1],Ile[8]]ANG II, might provide the impetus (change in receptor conformation) required for rapid dissociation of the ligand from its receptors.

The role of the Sar[1] residue in sarmesin and [Sar[1],Phe[4]]ANG II in conferring high binding affinity to these peptides still requires explanation. It has been suggested that the role of the N-terminus of ANG II might be to repel the Tyr side chain toward the His side chain and thus facilitate the interaction of these two aromatic residues.[5] When there is no free phenolic hydroxyl group available to stabilize the interaction with His (as would be the case for sarmesin and [Sar[1],Phe[4]]ANG II), a more pronounced repelling influence on the Tyr side chain may be required in order to correctly orient this side chain toward the His residue. Such considerations might explain the requirement for an added steric influence from the *N*-methyl group of Sar[1] in permitting receptor binding of sarmesin and [Sar[1],Phe[4]]ANG II. Recent NMR studies from this laboratory (Matsoukas, Goghari, and Moore, manuscript in preparation) illustrate that the *N*-methyl group in these analogues is indeed subject to a shielding influence, which could be imposed in part by the Tyr side chain. The contention, for illustrative purposes, that the ANG II molecule may take up a "scorpion-like" receptor conformation in which the "sting" residues at the N-terminus[5] could turn out to be a more realistic description of [the role of] the N-terminus than was originally intended.

Experimental Section

tert-Butyloxycarbonyl-blocked amino acids were purchased from Peninsula Laboratories, Bachem. Inc. or Chemical Dynamics Corp. Protected-amino acid-resins, ANG II and [Sar[1],Ile[8]]ANG II were purchased from Peninsula Laboratories. Solid-phase peptide synthesis was carried out with Beckman 990 or 990B peptide synthesizers essentially by procedures described previously.[3] Two coupling steps with 2.5 equiv of Boc-protected amino

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acid and coupling reagent were employed for each amino acid; the first coupling was mediated by dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HBT) for 3 h and the second was mediated by *N*-(ethoxycarbonyl)-2-ethoxyl-1,2-dihydroquinoline (EEDQ) for 8 h. Deprotection steps (2 and 25 min) utilized 25% CF_3CO_2H in $CHCl_3$ containing indole (0.05%) and ethyl methyl sulfide (0.005%). Neutralization steps (2 and 15 min) utilized 10% triethylamine in $CHCl_3$. Peptides were synthesized on a 0.5-mmol scale up to the heptapeptide stage, and thereafter the protected resin was divided in half and the next Boc-amino acid was added to one half. Yields of fully protected peptide–resins were in excess of 90%. The completed peptide was removed from the resin and simultaneously deprotected by reaction with anhydrous HF (20 mL) in the presence of *p*-cresol (1 g) for 30 min at 0 °C. The peptide was dissolved in CF_3CO_2H (100 mL), and the resin was removed by filtration. The solvent was removed by rotary evaporation, and the crude product was isolated by trituration with ether and filtration. Yield 200–300 mg.

(Carboxymethyl)cellulose Column Chromatography. The crude product obtained after the reaction with HF was dissolved in 7% acetic acid (10 mL), clarified by centrifugation, and chromatographed on a column (70 × 1.6 cm) of Whatman CM 23 (carboxymethyl)cellulose with use of a linear gradient of ammonium acetate from 0.01 M, pH 5.0 to 0.5 M, pH 8.0. Fractions of the major peak detected from the absorbance at 280 nm were collected (cut for purity rather than yield), lyophilized, and relyophilized from 1% acetic acid. Yield 40–100 mg.

Semipreparative Reversed-Phase HPLC. Final purification of peptides was accomplished with use of a Varian HPLC system equipped with a Vista 401 microprocessor controller. Separations were achieved on a Bio-Rad Hi-Pore 318 reversed-phase preparative column (25.0 × 2.15 cm) at 25 °C with a stepped linear gradient of acetonitrile in 0.1% CF_3CO_2H at a flow rate of 7.5 mL/min. Automated repetitive injections of peptides (5 × 5 mg) were made from a nitrogen pressurized Rheodyne injector with a 2.0-mL sample loop. One-fifth of the total sample was injected during each run by lowering the flow rate to 4.0 mL/min for a 0.1-min "inject" period. One cycle consisted of the following events: 0 → 10 min, 7.5 mL/min, 90% H_2O/10% of 1% aqueous CF_3CO_2H; 10 → 11 min, → 4.0 mL/min; 11 → 11.1 min, "inject"; 11.1 → 13 min, → 7.5 mL/min, → 70% H_2O/20% CH_3CN/10% of 1% CF_3CO_2H; 13 → 30 min, → 45% H_2O/45% CH_3CN/10% of 1% CF_3CO_2H; 30 → 42 min, → 90% CH_3CN/10% of 1% CF_3CO_2H; 42 → 50 min, → 100% H_2O.

Fractions were collected at 0.1-min intervals with a Gilson Model 201 fraction collector programmed to collect for a 5-min period centered around the elution time (22–27 min) of the major product. The fraction collector was restarted by the Vista 401 at the beginning of each HPLC run so that material eluting with the same retention time was repeatedly deposited in the same tubes. Elution of the peptide was detected simultaneously from the absorbances at 254 nm (Varian UV-1) and 230 nm (Kratos SF769Z). Fractions containing the required peptide were pooled; after removal of CH_3CN on a rotary evaporator at 40 °C, the fractions were lyophilized and stored at −20 °C. Yield 10–40 mg. Overall yield from starting Boc-amino acid–resin ranged from 10% to 50%. Amino acid analyses (Beckman 121 M) were carried out after acid hydrolysis in 6 N HCl (containing 1% cresol to prevent loss of tyrosine) at 110 °C for 18 h in vacuo.

Thin-Layer Chromatography (TLC). TLC was carried out with precoated silica gel on glass (Merck Kiesel gel 60 F254) TLC plates. The two solvent systems used were 1-Butanol–pyridine–acetic acid–water (15:10:3:6) (BPAW) and chloroform–methanol–acetic acid–water (15:10:2:3) (CMAW). Peptides were detected by fluorescence quenching under UV light followed by development with ninhydrin (0.3%) in 1-butanol/acetic acid (100:3, v/v).

Rat Uterus Bioassay. Defatted uterine horns from diethylstilbestrol-primed virgin Sprague–Dawley rats (150–250 g) were cut in half, and each tissue was suspended under 1 g of tension in a 3-mL tissue bath containing 150 mM NaCl, 5.6 mM KCl, 0.18 mM $CaCl_2$, 1.8 mM $NaHCO_3$, and 1.4 mM glucose at pH 7.0 gassed with oxygen. Contractions were monitored with Gould Metripak 763341-4042 isotonic transducers coupled to Gould 13-4615-50 transducer amplifiers housed in a Gould 2600S recorder. Agonist activities of peptides were determined by matching the response with an equivalent response to ANG II (human). Antagonist activities (pA_2) were determined as the negative logarithm of the concentration of antagonist required to reduce the response to an EC_{50} dose of ANG II to the response to half the EC_{50} dose.

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