Synthesis of Peptides with the Solid Phase Method. II. Octapeptide Analogues of Angiotensin II[1]

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Forty-six analogues of angiotensin II were obtained with the solid-phase method for peptide synthesis. The peptides were purified, using the conventional procedures; homogeneity and purity were established after paper, thin-layer chromatography, paper electrophoresis, amino acid analysis, elemental analysis, and enzymatic degradation by aminopeptidase. The biological activity of all compounds was compared with that of angiotensin II on the blood pressure of anesthetized rats. The same test was used to establish the antagonistic effect of several analogues against angiotensin II.

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Quarante-six analogues de l'angiotensine II furent obtenus avec la méthode de la synthèse des peptides sur phase solide. Les peptides furent purifiés en utilisant les procédures conventionnelles: l'homogénéité et la pureté des composés furent établies à l'aide de la chromatographie sur papier ou sur couche mince, l'électrophorèse sur papier, l'analyse des acides aminés, l'analyse élémentaire et la dégradation enzymatique par l'aminopeptidase. Les activités biologiques de tous les composés furent comparées avec celles de l'angiotensine II sur la pression artérielle du rat anesthésié. Le même test biologique fut utilisé pour établir l'action antagoniste de plusieurs composés vis-à-vis de l'angiotensine II.

Introduction

A large number of analogues of angiotensin, synthesized in our laboratory by the solid phase method of Merrifield (2), are reported in this paper.

The main purpose of synthesizing such a large number of compounds was to explore the structure–activity relationship of a potent peptide, angiotensin II (AT II), which has several physiological functions, such as vasoconstriction (3) and stimulation of release of aldosterone and catecholamines from the adrenal glands (4, 5). Unlike other peptide hormones, angiotensin is active on smooth muscles; this allows for precise pharmacological studies and for the application of receptor theories (6, 7).

The principal aims of this paper are to describe the chemical synthesis, the purification procedures, and the analyses performed to control the purity and homogeneity of the peptides. Results of biological assays *in vivo* are also given for all compounds, to allow the comparison of activities with those of a large series of angiotensin analogues reported in the literature (see reviews by Schröder and Lübke (8); Bumpus *et al.* (9), and more recently by Gross (10)). Results of *in vitro* studies have been presented in other papers (6, 7) and will not be repeated here.

Materials

Preparations of Amino Acid Derivatives for the Synthesis[3]

t-Butoxycarbonylamino (boc-amino) acids: alanine, nitroarginine, valine, isoleucine, leucine, glycine, proline, phenylalanine, norleucine, norvaline, D-alanine, α-amino-*n*-butyric acid, D-phenylalanine, and D-leucine were synthesized according to the procedure of Schwyzer *et al.* (11). *o*-Nitrobenzenesulfenyl amino acids, namely, 1-aminocyclopentanecarboxylic acid (Acpc) and L-phenylalanine mustard, were synthesized according to the procedure of Zervas *et al.* (12); *N*-carbobenzoxysarcosine was made by a modification of the method of Fox *et al.* (13). Boc-α-benzylaspartic acid, boc-β-benzylaspartic acid, *O*-benzyltyrosine, and

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[3]Abbreviations for amino acids residues and substituting groups and designation of synthetic peptides are given according to the rules of the IUPAC–IUB Commission on Biochemical Nomenclature (1972) (1).

imidazolbenzylhistidine were obtained from BACHEM Inc., Marina del Rey, Calif. Purity of all amino acid derivatives and peptides was determined as previously described (Park *et al.* (14)).

o-Nitrobenzenesulfenyl-melphalan (NBS-Melp)

Melphalan[4] (6.1 g, 0.02 mol) was dissolved in a mixture of 10 ml of 2 *N* NaOH and 25 ml of dioxane. During a period of 15 min, *o*-nitrobenzenesulfenyl chloride (4.17 g, 0.022 mol) was added in 10 equal portions, while 2 *N* NaOH (12 ml) was added drop by drop with vigorous stirring. The reaction mixture was diluted with 200 ml of water and filtered; the filtrate then was acidified with sulfuric acid (1 *N*) in an ice-water bath, and extracted twice with ethyl acetate (EtOAc) (200, 150 ml); the EtOAc phase was then washed three times with water (150 ml) and with saturated NaCl. After drying over anhydrous Na_2SO_4, the solvent was evaporated to an oil residue. The oily residue was dissolved in 15 ml of MeOH and evaporated to dryness in vacuum: yield 6.5 g (70%); R_f was measured using butanol : acetic acid : water (BAW) (4:1:5) as solvent.

o-Nitrobenzenesulfenyl-1-aminocyclopentane-carboxylic acid (NBS-Acpc)

NBS-Acpc was prepared as previously described by Park *et al.* (15). All peptides reported in this paper were synthesized with the solid-phase method of Merrifield (2), using a new reaction vessel developed in our laboratory (16). The synthesis was carried out in a stepwise manner according to the procedure of Park *et al.* (15).

Degradation of AT_{II} and Analogues by Purified Aminopeptidases

Breakdown of several peptides by aminopeptidases from swine kidney (P-L Biochemicals Inc., Milwaukee) was measured by incubating 1 mg of each peptide with 0.1 mg of enzyme in bicarbonate buffer (0.01 *M* $NaHCO_3$ and 0.001 *M* $MgCl_2$) at pH 8.1. Incubations were carried out at 38 °C for 24 h. At the end of the incubaton, aliquots of 0.5 ml were taken out for amino acid analysis.

Results

The 46 analogues of angiotensin II presented in Table 1 can be divided into three groups: (*a*) single substituted analogues (Nos. 1–24), (*b*) double substituted analogues (Nos. 25–42), and (*c*) analogues in which one amino acid has been replaced by a D-amino acid derivative (Nos. 43–46). Forty-two of the forty-six compounds were synthesized by using a similar procedure as that described in Park *et al.* (14). The procedure is summarized here, using 1-[Ala]-angiotensin II (1-[Ala]-AT_{II}) as example. The synthesis of four other compounds (1-[*N*,*N*-dimethyl-glycine]-AT_{II} (1-[Me_2Gly]-AT_{II}), 1-[Betaine]-AT_{II} (1-[Bet]-AT_{II}), 1-[Me_2Gly],8-[Leu]-AT_{II}, and 1-[Bet],8-[Leu]-AT_{II}) required some modifications, which will be described taking as an example 1-[Me_2Gly]-AT_{II}.

(1) Synthesis of 1-[Ala]-AT_{II}

Boc-phenylalanine Polymer

Boc-phenylalanine polymer was prepared with the same method used for boc-*O*-benzyl-serine (Park *et al.* (14)). Analysis of the boc-phenylalanine polymer gave 0.67 mmol/g of polymer.

Boc-alanyl-nitro-arginyl-valyl-O-benzyl-tyrosyl-isoleucyl-imidazol-benzyl-histidyl-prolyl-phenylalanine Polymer

Boc-phenylalanine polymer (3.0 g) was placed in the reaction vessel and the desired sequence of the peptide was prepared as described in Park *et al.* (14). There were 6.0 mmol of boc-imidazol-benzyl-histidine and boc-nitro-arginine used for the respective cycles of synthesis.

Alanyl-arginyl-valyl-tyrosyl-isoleucyl-histidyl-prolyl-phenylalanine (1-[Ala],5-[Ile]-angiotensin II)

The free octapeptide was obtained with the procedure described in Park *et al.* (14). The yield averaged 65%.

(2) Synthesis of 1-[Me_2Gly]AT_{II}

N,N-dimethylglycyl-nitro-arginyl-valyl-O-benzyl-tyrosyl-isoleucyl-imidazol-benzyl-histidyl-prolyl-phenylalanine Polymer

N - ethyl - 5 - phenylisoxazolium - 3′ - sulfonate (Woodward's reagent K (30, 31), 1.27 g, 5.0 mmol) was dissolved in 40 ml of dimethylformamide (DMF) under vigorous stirring. At 0 °C, 0.7 g (5.0 mmol) of *N*,*N*-dimethylglycine HCl and 1.4 ml (10 mmol) triethylamine (Et_3N), dissolved in 40 ml of DMF, were added. Stirring was continued until the solution almost cleared (about 6 h). This was then added to a suspension of 5 g of heptapeptide polymer (nitro-arginyl - valyl - *O* - benzyl - tyrosyl - isoleucyl - imidazol-benzyl-histidyl-prolyl-phenyl-alanine polymer) suspended in 40 ml of DMF containing 0.7 ml (5.0 mmol) of Et_3N. The heptapeptide polymer was prepared with the usual procedure (Park *et al.* (14)). The reaction mixture (heptapeptide, Me_2Gly) was shaken in an ice bath for 2 h, then at room temperature overnight. The

[4] L-3-[*p*-[Bis(2-chloroethyl)amino]phenyl]alanine.

TABLE 1. Physical properties and pressor activity of analogues of angiotensin II (AT_{II})

No.	Name	Ref.	Chromatography p.c.(R_f) BAW	p.c.(R_f) BAPW	t.l.c.(R_f) BAW	t.l.c.(R_f) BAPW	E_G	m.p.	Yield, %	Pressor activity, %
1.	1-Ala-AT_{II}		0.41	0.30	0.44	0.84	1.30	228–230	65	37
2.	1-Sar-AT_{II}		0.52	0.57	0.38	0.63	1.29	209–212	65	70
3.	1-Pro-AT_{II}		0.54	0.70	0.48	0.80	1.28	220–224	50	50
4.	1-Me_2Gly-AT_{II}		0.38	0.44	0.30	0.57	1.18	224–226	40	88
5.	1-Bet-AT_{II}		0.34	0.60	0.51	0.69	1.27	150–152	40	10
6.	2-Ala-AT_{II}		0.67	0.39	0.39	0.70	1.02	220–222	60	7.7
7.	2-Pro-AT_{II}		0.66	0.47	0.48	0.66	1.00	192–195	45	1.3
8.	3-Ala-AT_{II}		0.46	0.37	0.32	0.63	1.10	255–257	55	80.5
9.	4-Ala-AT_{II}	(19)	0.28	0.19	0.36	0.66	1.24	194–197	55	0.37
10.	4-Gly-AT_{II}		0.36	0.21	0.37	0.72	1.25	198–211	50	0.7
11.	4-Leu-AT_{II}		0.52	0.47	0.46	0.70	1.26	193–196	60	0.2
12.	4-Phe-AT_{II}	(20)*	0.51	0.21	0.32	0.65	1.20	210–213	57	22
13.	4-Melp-AT_{II}		0.38	0.20	0.51	0.57	1.23	220–223	45	0.1
14.	5-Ala-AT_{II}	(21)	0.22	0.12	0.15	0.48	1.16	219–222 (decomp.)	58	4.9
15.	6-Ala-AT_{II}		0.51	0.44	0.53	0.79	0.99	225–228	54	0.9
16.	6-Gly-AT_{II}		0.52	0.41	0.53	0.76	0.99	211–214	57	0.03
17.	6-Leu-AT_{II}		0.63	0.35	0.48	0.80	0.91	230–232	55	0.04
18.	6-Tyr-AT_{II}		0.47	0.27	0.18	0.64	0.91	219–212	53	0.01
19.	7-Ala-AT_{II}	(22)	0.32	0.22	0.45	0.72	1.19	232–235	60	1.5
20.	7-Gly-AT_{II}		0.39	0.20	0.43	0.73	1.21	223–226	58	0.05
21.	8-Gly-AT_{II}	(23–24*)	0.32	0.25	0.22	0.43	1.29	209–212	55	0.035
22.	8-Nva-AT_{II}		0.35	0.18	0.20	0.53	1.19	213–215	60	0.3
23.	8-Nle-AT_{II}		0.37	0.23	0.22	0.55	1.20	235–238	62	0.9
24.	8-Melp-AT_{II}		0.28	0.22	0.25	0.68	1.20	222–225	50	0.3
25.	1-<Glu,8-Leu-AT_{II}		0.55	0.74	0.42	0.82	1.18	115–118	55	0.2
26.	1-<Glu,8-Gly-AT_{II}		0.38	0.53	0.29	0.65	1.30	175–180	53	0.04
27.	1-β-Asp,8-Leu-AT_{II}		0.46	0.57	0.32	0.47	1.16	233–237	50	0.7
28.	1-Sar,8-Gly-AT_{II}		0.32	0.42	0.18	0.58	1.37	227–230	52	0
29.	1-Sar,8-Abu-AT_{II}		0.35	0.44	0.25	0.59	1.24	209–211	55	0.05
30.	1-Sar,8-Val-AT_{II}		0.34	0.40	0.22	0.59	1.27	188–190	60	0.05
31.	1-Pro,8-Leu-AT_{II}		0.47	0.70	0.45	0.52	1.33	228–231	50	0.5
32.	1-Pro,8-Gly-AT_{II}		0.34	0.47	0.22	0.68	1.32	218–220	50	0.02
33.	1-Me_2Gly,8-Leu-AT_{II}		0.38	0.45	0.25	0.60	1.19	126–129	42	0.3
34.	1-Bet,8-Leu-AT_{II}		0.51	0.54	0.25	0.53	1.28	175–177	40	0.2
35.	4-Leu,8-Leu-AT_{II}		0.40	0.32	0.33	0.67	1.21	227–230	55	<0.01
36.	4-Acpc,8-Acpc-AT_{II}		0.25	0.23	0.37	0.67	1.26	200–202	52	0.003
37.	4-Ala,8-Tyr-AT_{II}		0.16	0.20	0.12	0.43	1.20	197–201	55	0.01
38.	4-Phe,8-Tyr-AT_{II}	(25)*	0.28	0.20	0.33	0.63	1.14	205–207	52	2.0
39.	6-Arg,8-Ala-AT_{II}		0.33	0.21	0.29	0.75	0.94	198–201	48	0
40.	4-Ala,6-Ala-AT_{II}		0.42	0.21	0.51	0.73	1.03	223–226	52	0.004
41.	4-Gly,6-Gly-AT_{II}		0.48	0.19	0.30	0.70	1.03	174–176	55	0.002
42.	4-His,6-Tyr-AT_{II}	(26)	0.37	0.22	0.27	0.65	1.10	213–216	53	0
43.	1-D-Ala-AT_{II}		0.52	0.63	0.45	0.80	1.16	230–232	50	100
44.	2-D-Abu-AT_{II}		0.68	0.52	0.81	0.78	1.05	232–234	45	0.9
45.	8-D-Ala-AT_{II}		0.34	0.47	0.34	0.78	1.26	210–212	55	0.025
46.	8-D-Leu-AT_{II}		0.34	0.20	0.28	0.68	1.18	240–243	53	0.05

*Reference to articles in which the synthesis of the 5-[Val] derivative has been described.
NOTE: Parentheses give reference to articles in which the synthesis of the same compound or of the 5-[Val] derivative has been described.

resulting protected peptide polymer was collected by filtration, washed three times with DMF (80 ml) and ethanol (EtOH) (80 ml), and dried over P_2O_5 under vacuum to yield 1.5 g of protected peptide polymer.

N,N-dimethylglycyl-arginyl-valyl-tyrosyl-isoleucyl-histidyl-prolyl-phenylalanine (1-[Me_2Gly],5-[Ile]-angiotensin II (Compound 4 of Tables 1 and 2)

The protected polymer was suspended in 100

TABLE 2. Ratio of amino acids (on an acid hydrolysate), chemical formula, and molecular weight of analogues of angiotensin II

No.	Name	Ratio of amino acids: Asp 1	Arg 2	Val 3	Tyr 4	Ile 5	His 6	Pro 7	Phe 8	Chemical formula and molecular weight
1.	1-Ala-AT_{II}	1.02	1.05	1.00	0.93	1.03	1.06	0.98	1.00	$C_{49}H_{70}N_{13}O_{10}$ (1001.15) 2(A,W)
2.	1-Sar-AT_{II}	1.04	1.03	1.00	0.95	1.06	1.02	1.01	1.00	$C_{49}H_{70}N_{13}O_{10}$ (1001.15) 2(A,W)
3.	1-Pro-AT_{II}	——	1.02	1.00	0.92	1.05	1.03	1.96	1.00	$C_{51}H_{72}N_{13}O_{10}$ (1027.19) 2(A,W)
4.	1-Me_2Gly-AT_{II}	——	1.03	0.98	0.95	1.00	1.02	1.05	1.00	$C_{50}H_{72}N_{13}O_{10}$ (1015.18) 2(A,W)
5.	1-Bet-AT_{II}	——	0.98	1.02	0.92	1.00	0.96	1.02	1.00	$C_{51}H_{76}N_{13}O_{10}$ (1031.22) 2(A,W)
6.	2-Ala-AT_{II}	1.02	1.00	1.03	0.98	1.04	0.94	1.01	1.00	$C_{47}H_{64}N_{10}O_{12}$ (961.06) A,W
7.	2-Pro-AT_{II}	0.98	——	1.00	0.96	1.02	1.04	2.04	1.00	$C_{49}H_{66}N_{10}O_{12}$ (991.13) A
8.	3-Ala-AT_{II}	1.01	1.03	1.02	0.94	1.00	1.04	0.98	1.00	$C_{48}H_{69}N_{13}O_{12}$ (1018.12) A,W
9.	4-Ala-AT_{II}	1.02	1.03	1.05	1.00	1.01	0.96	0.98	1.00	$C_{44}H_{67}N_{13}O_{11}$ (954.08) A,W
10.	4-Gly-AT_{II}	0.98	1.02	1.00	1.03	1.03	0.98	1.01	1.00	$C_{43}H_{65}N_{13}O_{11}$ (940.05) A,W
11.	4-Leu-AT_{II}	1.01	1.02	1.00	1.00	0.98	1.03	0.96	1.00	$C_{47}H_{73}N_{13}O_{11}$ (996.15) A,W
12.	4-Phe-AT_{II}	0.98	1.04	1.00	——	1.02	1.03	0.97	2.03	$C_{50}H_{71}N_{13}O_{11}$ (1030.17) A,W
13.	4-Melp-AT_{II}	1.02	1.01	1.00	—*	1.00	0.98	1.03	1.00	$C_{54}H_{78}N_{14}O_{10}Cl_2$ (1154.18) 2(A,W)
14.	5-Ala-AT_{II}	0.95	1.02	1.00	0.94	1.02	1.03	0.96	1.00	$C_{47}H_{65}N_{12}O_{12}$ (990.08) A,W
15.	6-Ala-AT_{II}	1.02	1.01	1.00	0.96	1.00	1.03	1.03	1.00	$C_{47}H_{71}N_{11}O_{12}$ (982.12) A,W
16.	6-Gly-AT_{II}	0.96	1.02	1.00	0.95	1.03	1.00	0.98	1.00	$C_{46}H_{67}N_{11}O_{12}$ (966.08) A,W
17.	6-Leu-AT_{II}	0.98	1.03	1.01	0.98	1.00	1.02	1.01	1.00	$C_{50}H_{75}N_{11}O_{12}$ (1022.18) A,W
18.	6-Tyr-AT_{II}	1.01	1.03	1.00	1.94	1.02	——	0.98	1.00	$C_{53}H_{73}N_{11}O_{13}$ (1072.19) A,W
19.	7-Ala-AT_{II}	0.96	0.98	1.00	0.94	1.00	1.03	1.00	1.00	$C_{48}H_{69}N_{13}O_{12}$ (1020.13) A,W
20.	7-Gly-AT_{II}	1.01	0.96	1.00	0.94	1.00	0.98	1.00	1.02	$C_{47}H_{67}N_{13}O_{12}$ (1006.11) A,W
21.	8-Gly-AT_{II}	0.98	1.02	1.00	0.95	1.00	1.03	0.96	1.00	$C_{43}H_{65}N_{13}O_{12}$ (956.05) A,W
22.	8-Nva-AT_{II}	1.01	1.00	1.03	0.98	1.00	1.02	1.05	1.02	$C_{46}H_{71}N_{13}O_{12}$ (998.13) A,W
23.	8-Nle-AT_{II}	1.02	1.03	0.98	0.94	1.00	1.01	0.96	1.00	$C_{47}H_{73}N_{13}O_{12}$ (1012.12) 2(A,W)
24.	8-Melp-AT_{II}	1.03	0.96	1.00	0.94	1.00	0.98	1.02	—*	$C_{54}H_{78}N_{14}O_{12}Cl_2$ (1186.18) A,W
25.	1 Glu,8-Leu-AT_{II}	0.96	1.02	1.00	0.95	1.00	1.03	1.01	1.00	$C_{48}H_{73}N_{13}O_{11}$ (1008.16) A,W
26.	1 Glu,8-Gly-AT_{II}	0.98	1.02	1.00	0.94	1.00	1.03	0.97	1.02	$C_{44}H_{65}N_{13}O_{11}$ (952.06) A,W
27.	1-β-Asp,8-Leu-AT_{II}	1.02	0.98	1.04	0.95	1.00	1.01	1.03	1.00	$C_{47}H_{73}N_{13}O_{12}$ (1012.15) A,W
28.	1-Sar,8-Gly-AT_{II}	1.00	1.02	1.00	0.94	1.00	1.03	0.98	1.03	$C_{42}H_{65}N_{13}O_{10}$ (912.04) 2(A,W)
29.	1-Sar,8-Abu-AT_{II}	1.02	1.03	1.00	0.96	1.04	0.98	1.05	1.00	$C_{44}H_{69}N_{13}O_{10}$ (940.10) 2(A,W)
30.	1-Sar,8-Val-AT_{II}	1.00	1.02	2.04	0.98	1.00	1.03	1.05	——	$C_{45}H_{71}N_{13}O_{10}$ (954.12) 2(A,W)
31.	1-Pro,8-Leu-AT_{II}	——	0.98	1.00	0.95	1.02	1.02	2.05	1.00	$C_{48}H_{75}N_{13}O_{10}$ (994.18) 2(A,W)
32.	1-Pro,8-Gly-AT_{II}	——	1.02	1.03	0.94	1.00	1.04	1.98	1.00	$C_{44}H_{67}N_{13}O_{10}$ (938.08) 2(A,W)
33.	1-Me_2Gly,8-Leu-AT_{II}	——	1.03	1.00	0.96	1.02	0.98	1.04	1.00	$C_{47}H_{73}N_{13}O_{10}$ (980.15) A,2W
34.	1-Bet,8-Leu-AT_{II}	——	1.02	1.00	0.94	1.00	1.03	0.98	1.00	$C_{48}H_{79}N_{13}O_{10}$ (998.21) A,2W
35.	4-Leu,8-Leu-AT_{II}	0.98	1.03	1.00	——	1.00	1.02	0.96	2.05	$C_{44}H_{75}N_{13}O_{11}$ (962.14) 2(A,W)
36.	4-Acpc,8-Acpc-AT_{II}	0.96	0.98	1.00	——	1.00	0.97	1.01	1.96	$C_{44}H_{71}N_{13}O_{11}$ (958.11) 2(A,W)
37.	4-Ala,8-Tyr-AT_{II}	1.02	1.03	1.05	0.96	1.00	1.04	0.98	1.00	$C_{44}H_{67}N_{13}O_{12}$ (970.08) 2(A,W)
38.	4-Phe,8-Tyr-AT_{II}	0.94	1.03	1.00	0.96	1.00	1.02	1.00	0.98	$C_{50}H_{71}N_{13}O_{12}$ (1046.17) 2(A,W)
39.	6-Arg,8-Ala-AT_{II}	1.03	2.05	1.00	0.94	1.01	——	1.02	1.00	$C_{44}H_{72}N_{14}O_{12}$ (989.13) 2(A,W)
40.	4-Ala,6-Ala-AT_{II}	1.03	1.04	1.00	——	1.00	1.96	0.98	1.02	$C_{41}H_{65}N_{11}O_{11}$ (888.01) 2(A,W)
41.	4-Gly,6-Gly-AT_{II}	0.98	1.06	1.00	——	1.03	2.05	1.02	1.00	$C_{39}H_{61}N_{11}O_{11}$ (859.96) 2(A,W)
42.	4-His,6-Tyr-AT_{II}	1.02	0.96	1.00	0.94	1.00	1.02	0.98	1.00	$C_{50}H_{71}N_{13}O_{12}$ (1046.17) 2(A,W)
43.	1-D-Ala-AT_{II}	1.00	0.98	1.00	0.93	1.03	1.01	0.95	1.00	$C_{49}H_{70}N_{13}O_{10}$ (1001.15) A,W
44.	2-D-Abu-AT_{II}	0.98	1.03	1.05	0.96	1.00	1.01	1.00	1.02	$C_{48}H_{66}N_{10}O_{12}$ (975.09) 2(A,W)
45.	8-D-Ala-AT_{II}	1.01	1.03	1.00	0.94	1.00	1.05	1.04	1.03	$C_{44}H_{67}N_{13}O_{12}$ (970.08) 2(A,W)
46.	8-D-Leu-AT_{II}	0.96	1.02	1.00	0.94	1.00	1.05	0.98	1.00	$C_{47}H_{73}N_{13}O_{12}$ (1012.15) 2(A,W)

*Melphalan decomposed very rapidly on acid hydrolysis and the amino acid could not be detected.
NOTE: A = CH_3·COOH, W = H_2O. Chemical formula and molecular weight do not include acetate or water.

ml of trifluoroacetic acid (TFA), and hydrogen bromide (HBr) was bubbled through the suspension for 50 min under anhydrous conditions. The polymer was removed by filtration, washed three times with 15-ml portions of TFA, and the combined filtrate was evaporated at room tem-

perature under vacuum. The residual syrup was triturated with anhydrous ether and the amorphous white powder was collected by filtration and washed several times with anhydrous ether; it was then dried over NaOH, paraffin, and P_2O_5 under vacuum.

This partially protected octapeptide was dissolved in methanol – acetic acid – water (5:2:2) and hydrogenated over palladium black (Pd) for 3 days. The peptide was isolated in the usual manner to yield 970 mg of solid powder. This was purified by chromatography on a Sephadex G-25 (coarse) column by elution with BAW solvent and 10-ml fractions were collected. Fractions 76–110 were collected together and evaporated to give 0.63 g of octapeptide 1-[Me_2Gly]-AT_{II} (approximately 40% yield based on 1.5 mmol of N-terminal nitroarginyl heptapeptide polymer). *N,N*-dimethylglycine was identified after hydrolysis on paper chromatography, according to Dawson *et al.* (17), using 0.05% bromophenol blue in 0.2% citric acid.

(3) Physical Constants, Biological Activities, Amino Acid, and Elemental Analyses

The results of a series of analysis, performed to establish the homogeneity and purity of the 16 analogues of angiotensin II are reported in Tables 1 and 2. Purification procedures (column chromatography on Sephadex G-25, coarse, and CM cellulose) as described in Park *et al.* (14), using BAW or BAPW (1-BuOH:AcOH:pyridine:H_2O (30:6:20:24)) solvent systems and gradient elution for the CM column, were repeated if necessary to obtain single, well-delimited spots after paper, thin-layer chromatography, and paper electrophoresis. Details of these techniques as well as of the solvents or buffer used have been given in Park *et al.* (14).

Table 1 shows the R_f values on paper and thin-layer chromatography as well as uncorrected values of melting points, measured on a Kofler hot stage apparatus, and the yield for each compound.

Biological activities *in vivo* were measured on rats weighing 250–280 g, nephrectomized 24 h before, anesthetized with urethane, and prepared according to the method published elsewhere (8). The residual pressor activity, in percentage of the activity of standard AT_{II} (1-[Asp],5-[Ile]-AT_{II}) was evaluated as described in Park *et al.* (14).

Table 2 summarizes the results of the amino acid analysis on an acid hydrolysate, performed with a Technicon amino acid analyzer*. Molecular weights and results of elemental analysis are given in the same table. Elemental analysis (C, N, and H) was performed for most of the compounds and values, which did not differ from the calculated ones by more than 0.4%, were found Compounds 1, 6, and 8–24 of Table 1 were incubated with aminopeptidase for 24 h. The samples were submitted to amino acid analysis (Technicon) to estimate the concentrations of the first five amino acids. In all cases, the amino acid ratio was similar to that found in acid hydrolysates.

Discussion

The solid-phase method for peptide synthesis (2) and the purification by Sephadex or CM column chromatography have been carried out successfully in various laboratories (6, 15, 21, 24, 25, 27) to obtain angiotensin and analogues. In fact, relatively short peptides such as angiotensin can be made quickly and in good yield, and can be purified easily enough with this method to obtain products useful for pharmacological purposes. It has therefore become relevant to inquire about problems of drug design and structure–activity relations and to develop specific antagonists for this peptide hormone (7).

Reliable data on structure–activity relations and success in the discovery of antagonists can be obtained only if the compounds are perfectly homogeneous and pure. Jorgensen (27) has recently pointed out that analogues of angiotensin synthesized by the solid-phase technique must undergo the following sequential purification: "countercurrent distribution, Sephadex chromatography, and CM-cellulose chromatography. The products are tested by: nuclear magnetic resonance, thin-layer chromatography in six solvent systems, high voltage electrophoresis at two different pH values, amino acid analysis and hydrolysis, and L-amino acid oxidase studies to detect racemization."

This series of analyses, particularly the use of countercurrent distribution and of nuclear magnetic resonance, can, however, be performed only in a few specialized laboratories. In general, the purification through Sephadex G-25 repeated several times if necessary and, for some compounds, the use of CM-cellulose column as the

second step of purification are sufficient to obtain homogeneous products. The analysis by nuclear magnetic resonance is usually limited to a few compounds. With the large series of analogues of angiotensin reported in this paper, all criteria suggested by Jorgensen (27), except countercurrent distribution and nuclear magnetic resonance, were used. In addition, a large number of compounds were digested with purified leucine aminopeptidase and the ratios of the first five amino acids were estimated with an amino acid analyzer. The octapeptides were degraded up to 99%, thus indicating that racemization did not occur. Racemization of histidine could not be excluded; however, the synthesis of analogues was carried out in parallel with that of angiotensin II and of some fragments reported in Park *et al.* (14). The biological activities (pressor and myotropic) of angiotensin II and heptapeptide (2–8), synthesized simultaneously with the analogues were found to be the same as those of hypertensin (Ciba) and heptapeptide (2–8) (kindly supplied by Dr. B. Riniker). This suggests that racemization of histidine did not occur, because 6-[D-His]-AT_{II} has only 1% of the activity of AT_{II} (28).

Imidazol-benzyl-histidine was used in all compounds, except those substituted in position 6. In spite of the objections raised by Stewart *et al.* (29) to the use of imidazol-benzyl-histidine, we found this method fully satisfactory; however, the catalytic hydrogenation was prolonged for several days and the absence of imidazol-benzyl-histidine was established on the tripeptide His-Pro-Phe, resulting from the leucine aminopeptidase degradation. This tripeptide was colored with ninhydrin and Pauly's reagent; the two spots had the same location in the paper. This indicates that the catalytic hydrogenation had removed the imidazol-benzyl group. All peptides were tested on several biological preparations and the results have been reported in recent publications (6, 15, 16, 18, 23) or will be published (7).

Several compounds reported in this paper were synthesized by other groups, either with the solution or the solid-phase method; biological activity of the present compounds was very similar to that reported by other workers (see references mentioned in Table 1).

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1. IUPAC–IUB Commission on Biochemical Nomenclature (1972) *Symbols for Amino-Acid Derivatives and Peptides. Recommendations (1971) J. Biol. Chem. 247*, 323–325
2. Merrifield, R. B. (1963) *J. Am. Chem. Soc. 85*, 2149–2152
3. Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1956) *J. Expt. Med. 103*, 295–299
4. Biron, P., Koiw, E., Nowaczynski, W., Brouillet, J. & Genest, J. (1961) *J. Clin. Invest. 40*, 338–347
5. Feldberg, W. & Lewis, G. P. (1964) *J. Physiol. 171*, 98–108
6. Regoli, D., Rioux, F. & Park, W. K. (1972) in *Chemistry and Biology of Peptides* (Meienhofer, J., ed), pp. 495–499, Ann Arbor Science Publishers, Ann Arbor, Michigan
7. Regoli, D., Park, W. K. & Rioux, F. (1974) Pharmacology of angiotensin. *Pharmacol. Rev.* submitted for publication
8. Schröder, E. & Lübke, K. (1966) in *The Peptides*, vol. II, Academic Press Inc., New York, N.Y.
9. Bumpus, F. M. & Smeby, R. R. (eds) (1968) in *Renal Hypertension*, chapt. 2, pp. 62–98, Year Book Medical Publishers, Chicago
10. Gross, F. (1971) in *Pharmacology of Naturally Occurring Polypeptides and Lipid-Soluble Acids, International Encyclopedia of Pharmacology and Therapeutics*, section 72, vol. 1, pp. 73–286, Pergamon Press Inc., Oxford
11. Schwyzer, R., Sieber, P. & Kappeler, H. (1959) *Helv. Chim. Acta 42*, 2622–2624
12. Zervas, L., Borovas, D. & Gazis, E. (1963) *J. Am. Chem. Soc. 85*, 3660–3666
13. Fox, S. W., Fling, M., Wax, H. & Pettinga, C. W. (1950) *J. Am. Chem. Soc. 72*, 1862
14. Park, W. K., Choi, C., Rioux, F. & Regoli, D. (1974) *Can. J. Biochem. 52*, 106–112
15. Park, W. K., Asselin, J. & Berlinguet, L. (1970) *Progress Peptide Res.* vol. II, pp. 49–58
16. Park, W. K. & Regoli, D. (1972) *Can. J. Biochem. 50*, 755–757
17. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1959) *Data for Biochemical Research*, p. 212, Clarendon Press, Oxford
18. Regoli, D., Park, W. K., Rioux, F. & Chan, C. S. (1971) *Rev. Can. Biol. 30*, 319–329
19. Seu, J. H., Smeby, R. R. & Bumpus, F. M. (1962) *J. Am. Chem. Soc. 84*, 4948–4950
20. Riniker, B. & Schwyzer, R. (1961) *Helv. Chim. Acta 44*, 677–684
21. Khosla, M. C., Chaturvedi, N. C., Smeby, R. R. & Bumpus, F. M. (1968) *Biochemistry 7*, 3417–3421
22. Seu, J. H., Smeby, R. R. & Bumpus, F. M. (1962) *J. Am. Chem. Soc. 84*, 3883–3887
23. Rioux, F., Park, W. K. & Regoli, D. (1973) *Can. J. Physiol. Pharmacol. 51*, 108–113
24. Fessler, D. C., Sipos, F., Denning, G. S., Pals, D. T. & Masucci, F. D. (1972) *J. Med. Chem. 15*, 1015–1016
25. Marshall, G. R., Vine, W. & Needleman, P. (1970) *Proc. Natl. Acad. Sci. U.S. 67*, 1624–1630

26. Khosla, M. C., Kumar, S., Smeby, R. R. & Bumpus, F. M. (1971) *J. Med. Chem. 15*, 627–629
27. Jorgensen, E. C. (1972) in *Chemistry and Biology of Peptides* (Meienhofer, J., ed), pp. 579–580, Ann Arbor Science Publishers Inc., Ann Arbor, Michigan
28. Riniker, B. & Schwyzer, R. (1968) in *Renal Hypertension* (Page, I. H. & McCubbin, J. W., eds), pp. 80–82, Year Book Medical Publishers, Chicago
29. Stewart, J. M., Knight, M., Paiva, A. C. M. & Paiva, T. (1972) in *Progress in Peptide Research* (Lande, S., ed), pp. 10-1–10-6, Gordon and Breach, New York, N.Y.
30. Woodward, R. B. & Olofson, R. A. (1961) *J. Am. Chem. Soc. 83*, 1007–1009
31. Woodward, R. B., Olofson, R. A. & Mayer, H. (1961) *J. Am. Chem. Soc. 83*, 1010–1012