tert-butyl), 1.47 (s, 9 H, *tert*-butyl), 3.72 (s, 3 H, OCH_3), 4.10–4.30 (m, 3 H, CH and CH_2), 4.46 (q, 1 H, CH, J = 7.0 Hz), 5.55 (br s, 1 H, NH), 6.60–6.80 (m, 1 H, CH=CH), 7.0–7.25 (m, 2 H, 2 × NH).

Compound **20** was prepared in the same manner.

Method D. N^2-L-Alanyl-N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic Acid Hydrochloride (21). N^2-(N-(*tert*-Butoxycarbonyl)-L-alanyl)-N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (0.77 g, 2 mmol) was dissolved in cold 2 N HCl in dioxane (19 mL) and left for 4 h. The solvent was removed in vacuo, and the residue crystallized from methanol–ethyl ether, affording peptide **21** (Table II). In this manner, peptides **22–38** were prepared, and their analytical data are collected in Table II.

Method E. N^3-(4-Methoxyfumaroyl)-L-2,3-diaminopropanoyl-L-alanine (39). The protected peptide **19** (0.886 g, 2 mmol) was dissolved in cold TFA (20 mL) and stirred for 2 h. The trifluoroacetic acid was removed in vacuo and the residue dissolved in water (20 mL), and passed through a column of Dowex 1 × 2 (acetate form) (5 mL), and eluted with water. The fractions containing peptide **39** were collected, evaporated to dryness, and crystallized from a water–methanol mixture. Similarly peptide **40** was also prepared. For analytical data of these peptides, see Table II.

Biological Assays. A. The minimum inhibitory concentration (MIC) of each peptide was determind by the serial twofold dilution method.

Determination for *C. albicans* was performed on ligand YNB medium (Difco) containing 1% glucose. The compounds were diluted from 200 to 0.2 μg/mL. The medium was inoculated with *C. albicans* AMB 25 cells from overnight culture on the same medium to a concentration of 10^4 cfu/mL and incubated at 30 °C for 48 h. Results were determined by using a turbidimetric method.

Determination for bacteria was performed on solid minimal agar Davis medium (Difco) containing 0.1% yeast extract (Difco) and 0.1% casamino amino (Difco). Twenty-milliliter portions of prewarmed medium containing tested compounds (from 200 to 0.2 μg/mL) were poured into petri dishes. Inocula of bacterial cells diluted to 10^5 cfu/mL were applied on the surface of agar plates by using a platinum loop. Plates containing inoculated agar were inoculated at 37 °C for 24 h. Results were read visually. The MIC was defined as the lowest concentration of peptide that resulted in complete inhibition of growth of yeasts or bacteria.

B. Disk diffusion assay for *C. albicans* strains was performed as follows: YNB medium containing 1% glucose and 1% agar was inoculated with 10^4 cfu/mL *C. albicans* cells from overnight culture on Sabouraud medium. Inoculated media were poured into petri dishes. The peptides were dissolved in water, and 6.35-mm Schleicher and Schuell disks were saturated with solutions containing 20 μg of the tested compound and placed on the agar surface. After 48-h incubation at 30 °C, zones of inhibition were measured.

C. Disk diffusion assay for bacteria was carried out as follows: Medium containing 20 mL of minimal agar Davis (Difco) and yeast extract (Difco) supplemented with 0.1% of casamino acids (Difco) was inoculated with 10^4 cfu/mL of bacteria cells from 18-h culture and incubated at 37 °C for 30 min. Twenty microliters of solution containing tested compound was poured onto 6.35-mm Schleicher and Schuell disks and placed on the prepared plates. After incubation at 37 °C for 24 h, the diameters of inhibition zones were determined.

D. For antagonism studies, dipeptides and GlcNAc solutions were applied on the same disks as FMDP dipeptides. Conditions for the disk diffusion assay were the same as described above.

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Registry No. **1**, 108232-97-9; **2**, 108232-98-0; **3**, 108232-99-1; **4**, 108233-00-7; **5**, 108233-01-8; **6**, 108233-02-9; **7**, 108233-03-0; **8**, 108233-04-1; **9**, 108233-05-2; **10**, 108233-06-3; **11**, 108233-07-4; **12**, 108233-08-5; **13**, 108233-09-6; **14**, 108233-10-9; **15**, 108233-11-0; **16**, 108233-12-1; **17**, 108233-13-2; **18**, 108233-14-3; **19**, 108233-15-4; **20**, 108233-16-5; **21**, 108233-17-6; **21** (free base), 108340-63-2; **22**, 108233-18-7; **22** (free base), 108340-64-3; **23**, 108233-19-8; **23** (free base), 108233-38-1; **24**, 108233-20-1; **24** (free base), 108340-65-4; **25**, 108233-21-2; **25** (free base), 108340-66-5; **26**, 108233-22-3; **26** (free base), 108340-67-6; **27**, 108233-23-4; **27** (free base), 108340-68-7; **28**, 108233-24-5; **28** (free base), 108340-69-8; **29**, 108233-25-6; **29** (free base), 108340-70-1; **30**, 108233-26-7; **30** (free base), 108340-71-2; **31**, 108233-27-8; **31** (free base), 108340-72-3; **32**, 108233-28-9; **32** (free base), 108233-39-2; **33**, 108233-29-0; **33** (free base), 108340-73-4; **34**, 108233-30-3; **34** (free base), 108340-74-5; **35**, 108233-31-4; **35** (free base), 108340-75-6; **36**, 108233-32-5; **36** (free base), 108340-76-7; **37**, 108233-33-6; **37** (free base), 108340-77-8; **38**, 108233-34-7; **38** (free base), 108340-78-9; **39**, 108233-35-8; **40**, 108233-36-9; FMDP, 96920-07-9; FMDP·HCl, 104371-44-0; BOC-Ala-ONSu, 3392-05-0; BOC-Met-ONSu, 3845-64-5; BOC-Gly-ONSu, 3392-07-2; BOC-Val-ONSu, 3392-12-9; BOC-Leu-ONSu, 3392-09-4; BOC-Phe-ONSu, 3674-06-4; BOC-Tyr-ONSu, 20866-56-2; BOC-Nva-ONSu, 108233-37-0; BOC-Nle-ONSu, 36360-61-9; BOC-Abu-ONSu, 103290-07-9; BOC-Lys(BOC)-ONSu, 30189-36-7; H-Gly-OH, 56-40-6; H-Val-OH, 72-18-4; H-Leu-OH, 61-90-5; H-Phe-OH, 63-91-2; H-Tyr-OH, 60-18-4; H-Nva-OH, 6600-40-4; H-Ala-OBu-*t*, 21691-50-9; Hh-Met-OBu-*t*, 4976-72-1; BOC-FMDP-ONSu, 108233-40-5; BOC-FMDP, 104302-84-3; *N*-hydroxysuccinimide, 6066-82-6; glucosamine-6-phosphate synthetase, 9030-45-9.

Synthesis of Angiotensin II Antagonists with Variations in Position 5†

Olga Nyéki,* Katalin Sz. Szalay,‡ Lajos Kisfaludy, Egon Kárpáti, László Szporny, Gábor B. Makara,‡ and Bertalan Varga‡

Chemical Works of Gedeon Richter, Ltd., H-1475 Budapest, Hungary, and Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, Hungary. *Received September 29, 1986*

Six angiotensin II antagonists containing cyclohexylglycine (Chg) or cyclopentylglycine (Cpg) in position 5 were synthesized by stepwise elongation in solution, using the pentafluorophenyl ester method. The influence of substitution on the inhibitory properties of the analogues was studied in four different bioassays. [Sar[1],Chg[5],Lac[8]]AII proved to be the most potent antagonist with low intrinsic activity in both the in vitro and in vivo tests.

Investigation of a large number of angiotensin II (AII) analogues affords the possibility of defining the role played by each amino acid residue in the biological activity of the hormone.[1-4] Analogues substituted in positions 1 and 8 are antagonists with high affinity to hormone receptors,

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‡ Hungarian Academy of Sciences.

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Table I. Characterization Data of Newly Synthesized AII Analogues[a]

no.	analogue	R_f^5	R_f^6	$[\alpha]^{24}_D$,[b] deg	amino acid analysis								
					Sar[c]	Arg	Val	Tyr[d]	Chg	Cpg	His	Pro	Ala[e]
1	[Sar^1,Chg^5,Ala^8]AII	0.10	0.27	−84.5	+	1.0	0.95	0.86	1.02	−	0.86	1.0	1.15
2	[Sar^1,Cpg^5,Ala^8]AII	0.10	0.13	−92.7	+	0.95	1.03	0.80	−	0.97	0.92	1.06	1.03
3	[Sar^1,Chg^5,Lac^8]AII	0.16	0.20	−86.5	+	0.95	1.04	0.49	1.09	−	0.92	1.01	−
4	[Sar^1,Cpg^5,Lac^8]AII	0.15	0.16	−94.6	+	0.97	1.10	0.58	−	1.02	0.98	0.93	−
5	[Lac^1,Cpg^5,Lac^8]AII	0.08	0.35	−100.7	−	0.97	1.03	0.62	−	1.05	0.97	1.05	−
6	[HOAc^1,Chg^5,Lac^8]AII	0.20	0.38	−106.4	−	0.98	1.05	0.67	1.05	−	0.90	1.08	−
7	[Lac^1,Ile^5,Lac^8]AII	0.08	0.30	−95.9	−	0.96	1.05	0.64	−	−	0.92	1.04	−

[a] Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations are as follows: Chg, L-cyclohexylglycine; Cpg, L-cyclopentylglycine; Lac, L-lactic acid; HOAc, hydroxyacetic acid. [b] *c* 0.3, 1 M AcOH. [c] Sar was detected but not quantified. [d] The content of Tyr in peptides containing acetic acid was determined with an OPTON PMQ-II UV spectrophotometer by measuring the maximum absorbance of Tyr at 294 nm, ϵ 2400 in 0.1 N NaOH solution. The Tyr content in peptides ranged from 90% to 95%. [e] ^{1}H NMR spectra were obtained with a Brucker WM 250 spectrometer in D_2O. CH_3 protons of Lac residue were observed as a doublet (1.44 ppm).

Table II. Yields and Characterization Data of the Protected Peptides[a]

compound	yield, %	mp, °C	R_f^3	$[\alpha]^{24}_D$,[b] deg
1a, Z-Sar-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Ala-ONB	58[c]	213 dec	0.80	−
2a, Z-Sar-Arg(NO_2)-Val-Tyr(Bzl)-Cpg-His(Dnp)-Pro-Ala-ONB	50[c]	178–181	0.77	−21.4
3a, Z-Sar-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl	54[d]	217 dec	0.63	−35.9
4a, Z-Sar-Arg(NO_2)-Val-Tyr(Bzl)-Cpg-His(Dnp)-Pro-Lac-OBzl	61[d]	192–199	0.60	−37.7
5a, Z-OAla-Arg(NO_2)-Val-Tyr(Bzl)-Cpg-His(Dnp)-Pro-Lac-OBzl	58[d]	180–184	0.76	−44.3
6a, Z-OGly-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl	61[d]	180–183	0.65	−26.6
7a, Z-OAla-Arg(NO_2)-Val-Tyr(Bzl)-Ile-His(Dnp)-Pro-Lac-OBzl	53[d]	187 dec	0.64	−43.4

[a] Abbreviations used are as follows: OAla, L-(aminooxy)propionic acid; OGly, (aminooxy)acetic acid. [b] *c* 1, DMF. [c] Overall yield calculated from Boc-Pro-OPfp. [d] Overall yield calculated from Boc-His(Dnp)-OPfp.

and they have characteristic transient agonistic activity.

AII antagonists are useful diagnostics, but their therapeutical use is limited by two factors: short biological half-life and the transient agonistic action mentioned above. Ever since the first potent antagonist of AII, saralasin, [Sar^1,Val^5,Ala^8]AII, was described,[5] the effect of lipophilicity, sterical requirements, basicity of the nitrogen at the N terminal, and the proton-accepting ability of the side chain in position 8 of the molecule have been taken into account in the design of the syntheses of new antagonists.[6-12] However, attempts to prepare an antagonist with strong inhibitory activity and smaller transient agonistic effect than that of saralasin have not yet succeeded.

On the basis of conformation–activity-relationship studies[13-15] and our previous results,[16] we have tried to improve the inhibitory properties of AII antagonists by modification of the hormone molecule in position 5. Now we report the synthesis of highly potent AII antagonists containing cyclohexylglycine (Chg) and cyclopentylglycine (Cpg) in position 5.

Table III. Antagonistic Activities of the AII Analogues

analogue	pA_2[a]	
	zona glomerulosa	rabbit aortic strip
1, [Sar^1,Chg^5,Ala^8]AII	9.981 (3)	11.18 (8)
2, [Sar^1,Cpg^5,Ala^8]AII	9.933 (3)	9.42 (7)
3, [Sar^1,Chg^5,Lac^8]AII	10.390 (3)	11.29 (7)
4, [Sar^1,Cpg^5,Lac^8]AII	9.649 (3)	11.09 (8)
5, [Lac^1,Cpg^5,Lac^8]AII	8.392 (2)	8.52 (6)
6, [HOAc^1,Chg^5,Lac^8]AII	8.614 (3)	9.53 (7)
7, [Lac^1,Ile^5,Lac^8]AII	7.999 (2)	9.5 (6)
[Sar^1,Val^5,Ala^8]AII (saralasin)	9.634 (3)	11.10 (21)

[a] Number of experiments is given in parentheses.

Results and Discussion

Chemistry. The new AII analogues shown in Table I were synthesized in solution by the stepwise elongation method. The well-proved pentafluorophenyl ester method[17] was used for preparing the analogues. Lactic acid was introduced into the molecule by acylation of zinc lactate with Boc-Pro-OSu followed by esterification of the dipeptolide with benzyl bromide. The hydroxy acids were introduced into position 1 of the molecule by means of Z-protected aminooxy acid pentafluorophenyl esters, with

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Table IV. Effects of AII Antagonists on the Mean Blood Pressure of Conscious Rats[a]

dose, μg/kg iv	maximal increase in mean blood pressure, mmHg							
	1	2	3	4	5	6	7	saralasin
1.25	10.9 ± 2.51	5.8 ± 1.54	3.6 ± 1.43	5.0 ± 1.83	5.8 ± 2.39	6.7 ± 1.05	4.2 ± 1.54	7.9 ± 1.49
2.5	17.9 ± 4.21	10.0 ± 2.24	5.7 ± 1.30	10.0 ± 2.24	5.0 ± 1.29	7.5 ± 1.12	5.8 ± 1.54	9.3 ± 1.30
5.0	16.9 ± 2.30	10.0 ± 1.83	12.1 ± 1.49	9.2 ± 2.39	5.0 ± 1.83	17.5 ± 2.81	11.7 ± 2.47	12.1 ± 1.84
10.0	16.4 ± 9.00	17.5 ± 2.14	8.6 ± 2.10	11.7 ± 3.80	10.0 ± 1.83	14.1 ± 2.39	10.0 ± 1.29	15.0 ± 2.89
20.0	–	14.2 ± 2.39	8.3 ± 2.11	11.7 ± 3.07	15.0 ± 2.89	15.8 ± 2.71	16.7 ± 4.01	20.0 ± 4.28

[a] Easch analogue was tested in six animals. Analogues were administered in a bolus for 10 s into the jugular vein.

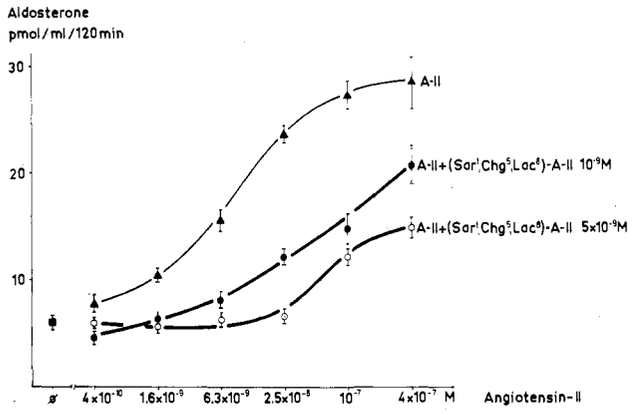

Figure 1. Effect of [Sar¹,Chg⁵,Lac⁸]AII on the AII-induced aldosterone secretion of isolated zona glomerulosa cells. (Data are those of a typical experiment. Each point is in triplicate.)

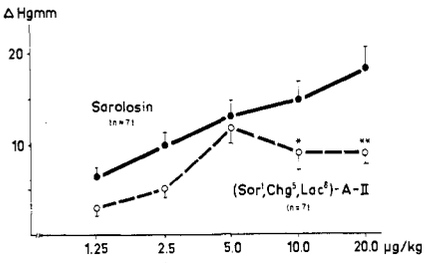

Figure 2. Intrinsic pressor activity of [Sar¹,Chg⁵,Lac⁸]AII and saralasin administered in a bolus for 10 s in the jugular vein of conscious rats. Increments of blood pressure after various doses of the analogues are plotted. (*) $p < 0.05$; (**) $p < 0.01$.

subsequent catalytic hydrogenation splitting not only the Z group but also the NH–O bond, resulting in the corresponding hydroxy acid derivatives.[16,18]

The protected peptides (Table II) were treated with 2-mercaptoethanol to remove the Dnp protecting group from the His imidazole and then hydrogenated. The deprotected peptides were purified by NH_4OAc gradient elution on a CMC column. The purity of peptides **1–7** was assessed in two different TLC systems and by analytical HPLC.

Biology. The analogues were examined in two in vitro tests to compare the influence of the same chemical modification on two different actions of the antagonists. Our results show that introduction of the β-branched cyclic amino acids into position 5 slightly enhanced the antagonistic activity of these analogues (Table III). This effect was more pronounced in the aldosterone inhibitory assay: the inhibitory action of the analogues **1–3** was higher than that of saralasin in this test.

On rabbit aortic strip only two analogues (**1**, **3**) containing Chg in position 5 exhibited stronger antagonistic activity than saralasin, whereas introduction of Cpg into the same position (compound **2**) resulted in slightly decreased inhibitory activity.

We suppose that the strong antagonistic effect observed is to be attributed to the substitutions in positions 1 and 8, yet introduction of Chg into position 5 results in additional antagonistic effect, probably due to an increase of the binding affinity to receptors. This effect is not strong enough to increase considerably the inhibitory properties of pure antagonists (compounds **5** and **6**). Our results are in good accordance with those of Jorgensen et al.,[19] who studied the influence of substitution in position 5 on the pressor activity of analogues, and also support Fermandjian's conformational studies.[14-15]

Of the three analogues that showed higher pA_2 values than saralasin, [Sar¹,Chg⁵,Lac⁸]AII (**3**) proved to be the best inhibitor in both tests. Figure 1 shows the inhibitory effect of **3** on AII-induced aldosterone secretion.

The analogues do not increase the aldosterone production of isolated zona glomerulosa cells at 10^{-10}–10^{-6} M concentrations; it means that they do not have any agonistic effect in this test (data not shown). A striking observation is that the analogues **6** and **7** inhibited aldosterone production of zona glomerulosa cells, probably due to inhibition of the 18-hydroxylase activity.

In our in vivo studies we measured the crucial intrinsic pressor activity in conscious rats. The maximal increment of the blood pressure after each dose is shown in Table IV.

The profiles of intrinsic activity of the analogues tested are very similar; however, compound **3** at 10 and 20 μg/kg doses induced significantly less blood pressure rise than did saralasin (Figure 2).

The inhibitory effect of the analogues was tested in vivo on the AII-induced rise of blood pressure. The inhibitory effect of **1** and **3** lasted longer than that of saralasin in a statistically significant manner. The inhibitory effect of saralasin was complete in 80 min, whereas compounds **1** and **3** produced this effect even after 180 min (Figure 3).

Our results indicate that replacement of Ile in position 5 by β-branched cyclic amino acids resulted in a slight but significant improvement of the antagonistic properties of analogues hitherto described in the literature. Furthermore, some of the new analogues, especially compound **3**, caused significantly less blood pressure rise, and the duration of the inhibitory effect on AII-induced elevated blood pressure was significantly longer than in the case of saralasin.

The increased duration of the inhibitory effect of compound **3** may arise from a longer half-life in the circulation or, less likely, from delayed absorption from the sc injection site.

It seems therefore that [Sar¹,Chg⁵,Lac⁸]AII is a potent AII antagonist that may find application in the diagnosis of hypertension.

Experimental Section

L-Cyclopentylglycine and L-cyclohexylglycine were prepared in our laboratory in the way described in the literature.[20-22]

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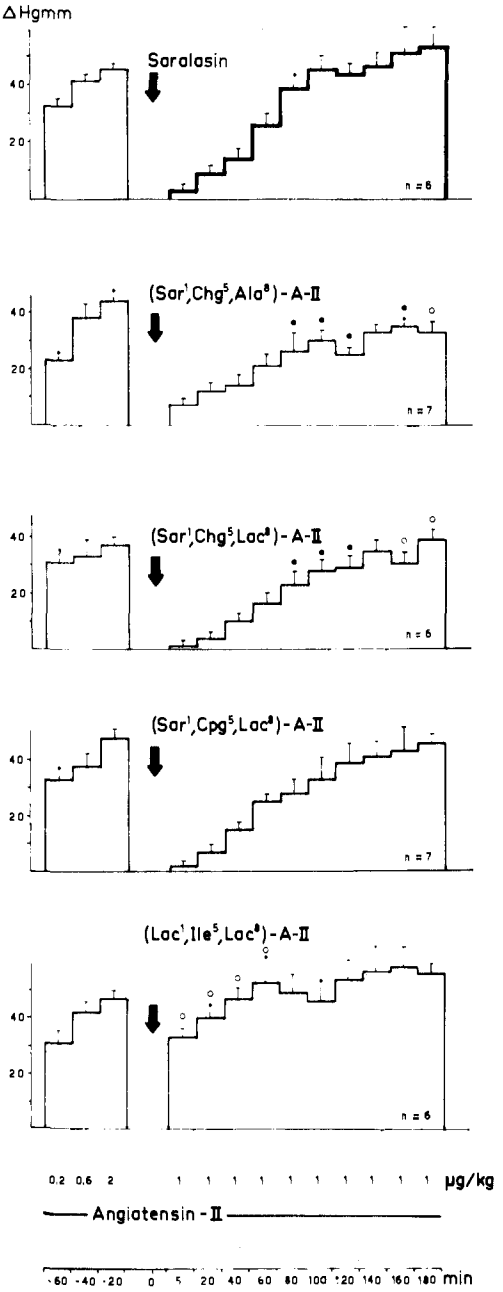

Figure 3. Effect of the AII analogues on the AII-induced blood pressure rise. The analogues (1 mg/kg) were injected sc where the arrows indicate, and the doses of AII were administered in a bolus for 10 s into the jugular vein of conscious rats each 20 min. (○) $p < 0.05$; (●) $p < 0.01$.

Boc-amino acid pentafluorophenyl esters were prepared by known methods.[23,24] Melting points were determined with a Tottoli (Büchi) capillary apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 automatic readout polarimeter. HPLC was performed on a LABOR MIM apparatus coupled to an OE 308 UV detector set. The column (25 cm × 4.6 mm) was packed with Nucleosil 10 C_{18} (Chrompack), and acetonitrile/(2.5 µmol/L sodium tartrate, 2.5 µmol/L pentanesulfonic acid sodium salt, 25 µmol/L sodium sulfate, pH 3 buffer), 17:83, was used as eluent. For TLC, precoated plates (silica gel 60, 0.25 mm, Merck) were used in the following solvent systems (all v/v; solvent systems S_1–S_4 were made by mixing EtOAc and a stock solution, consisting of pyridine/AcOH/H_2O, 20:6:11, in the proportions shown): (S_1) EtOAc/stock, 19:1; (S_2) EtOAc/stock, 9:1; (S_3) EtOAc/stock, 4:1; (S_4) EtOAc/stock, 7:3; (S_5) *n*-BuOH/AcOH/H_2O, 4:1:5, upper phase; (S_6) *n*-BuOH/EtOAc/AcOH/H_2O, 1:1:1:1; (S_7) $CHCl_3$/MeOH, 9:1. Chromatograms were visualized by spraying with ninhydrin and then with *o*-tolidine/KI after chlorination.

The end products were purified by dissolving 0.5 g of the free peptide in 4 mL of 0.01 M NH_4OAc and pouring the solution onto a column filled with 0.5 L of (carboxymethyl)cellulose (CMC 52) that had been previously equilibrated with the above buffer. Elution with linear gradient between 0.01 M NH_4OAc and 0.5 M NH_4OAc was carried out for 4 days at a flow rate of 25 mL/h. The eluate was continuously registered by a LKB Uvicord-II instrument, and the main fraction was lyophilized. For amino acid analysis the peptides were hydrolyzed in 6 N HCl for 24 h at 110 °C, and the hydrolysates were analyzed on a Chinoin OE 975 amino acid analyzer.

Elemental analyses of the product agreed with the calculated values within 0.3%.

Except when otherwise stated, coupling with pentafluorophenyl esters was carried out in DMF at pH 8, adjusted with triethylamine. After coupling, DMF was evaporated and the residue dissolved in EtOAc, washed with 1 N HCl, 5% $NaHCO_3$, and water, and dried over Na_2SO_4.

Boc protecting groups were removed by treatment of the protected peptides with 8 N HCl/dioxane for 20 min at room temperature. After treatment, the peptide was precipitated with anhydrous ether, filtered off, washed with ether, and coupled with the next Boc-amino acid pentafluorophenyl ester.

Boc-Chg-OPfp (8). Boc-Chg-OH (3.0 g, 11.5 mmol) was dissolved in EtOAc (30 mL), and PfpOH (2.3 g, 12.7 mmol) and DCC (2.54 g, 12.7 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. The DCU was filtered off and the solution washed twice with 5% $NaHCO_3$ and water and dried over Na_2SO_4. After evaporation, the residue was recrystallized from *n*-hexane (20 mL) to yield 3.92 g (80%) of the product: mp 98–99 °C; $[\alpha]^{24}_D$ −20.2° (*c* 0.5, EtOAc). Anal. ($C_{19}H_{22}O_4NF_5$) C, H, N.

Boc-Cpg-OPfp (9). With Boc-Cpg-OH (3.5 g, 14.4 mmol) as starting material, **9** was prepared in the same way as described for **8**: yield 4.2 g (70%); mp 99–101 °C; $[\alpha]^{24}_D$ −31.8° (*c* 0.5, EtOAc). Anal. ($C_{18}H_{20}O_4NF_5$) C, H, N.

Boc-Pro-Lac-OH (10). A mixture of zinc lactate (7.3 g, 30 mmol), triethylamine (4.2 mL, 30 mmol), and Boc-Pro-OSu (9.36 g, 30 mmol) in DMF (40 mL) was stirred at room temperature for 24 h. The solution was evaporated and the residue dissolved in EtOAc (150 mL) and washed with 1 N HCl and water. After drying and evaporation, the residue solidified on rubbing with 50 mL of *n*-hexane. It was filtered off and recrystallized from 40 mL of ether/*n*-hexane, 1:1, to give 6.6 g (77%) of **10**: mp 108–110 °C; $[\alpha]^{24}_D$ −88.2° (*c* 2, EtOAc); R_f^5 0.30. Anal. ($C_{13}H_{21}O_6N$) C, H, N.

Boc-Pro-Lac-OBzl (11). A mixture of **10** (2.87 g, 10 mmol), triethylamine (1.7 mL, 12 mmol), and benzyl bromide (1.42 mL, 12 mmol) in 20 mL of EtOAc was refluxed for 8 h, then diluted with 20 mL of EtOAc, and washed as described above. After drying and evaporation, **11** (3.05 g, 81%) was obtained as an oil: R_f^1 0.67; $[\alpha]^{24}_D$ −90.0° (*c* 2, EtOH).

Z-Sar-Arg(NO$_2$)-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl (3a). General Procedure. Compound **11** (2.65 g, 7 mmol) was dissolved in 10 mL of 8 N HCl/dioxane. After being stirred for 15 min, the soltuion was diluted with 20 mL of dry ether and evaporated. The resulting H-Pro-Lac-OBzl·HCl (R_f^4 0.2) was dissolved in DMF (15 mL) and coupled with Boc-His(Dnp)-OPfp (3.5 g, 6 mmol) under the conditions described above. DMF was evaporated and the oily residue dissolved in 60 mL of EtOAc and washed as described above. After drying and evaporation of the oil residue, Boc-His(Dnp)-Pro-Lac-OBzl (R_f^2 0.8) was deprotected and H-His(Dnp)-Pro-Lac-OBzl·HCl (R_f^3 0.1) was precipitated with anhydrous ether, filtered off, and acylated with Boc-Chg-OPfp (2.1 g, 5 mmol). After being stirred for 30 min, the solution was

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evaporated and the oily residue dissolved in 30 mL of EtOAc and washed. After drying and evaporation, the protected tetrapeptide (R_f^2 0.6) solidified on rubbing with 40 mL of *n*-hexane. It was filtered off, washed twice with *n*-hexane/ether, 3:1 and then deprotected. H-Chg-His(Dnp)-Pro-Lac-OBzl·HCl (R_f^4 0.25) was precipitated with 100 mL of dry ether and coupled with Boc-Tyr(Bzl)-OPfp (2.54 g, 4.7 mmol). After 15 min, DMF was evaporated, the residue was dissolved in 30 mL of EtOAc, 0.11 mL of 2-(*N*,*N*-dimethylamino)ethylamine was added, and, after 15 min, the solution was washed, dried, and evaporated. The residue was rubbed with ether and the solidified Boc-Tyr-(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl (R_f^2 0.5) was filtered off and deprotected. H-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl·HCl (R_f^4 0.63) was precipitated with ether, isolated by filtration, dissolved in DMF (15 mL), and acylated with Boc-Val-OPfp (1.8 g, 4.5 mmol). The resulting hexapeptide (R_f^2 0.7) was solidified by rubbing with ether, filtered off, and deprotected. After precipitation, H-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl·HCl (R_f^4 0.45) was dissolved in DMF (15 mL) and coupled with Boc-Arg(NO_2)-OPfp (2.35 g, 4.8 mmol). After being stirred for 40 min, the reaction mixture was diluted with $CHCl_3$ (50 mL), washed with 1 N HCl and water, and then evaporated. The protected heptapeptide (R_f^3 0.8) was precipitated with EtOH/ether, 1:4 (50 mL), filtered off, and washed with ether. Boc-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His(Dnp)-Pro-Lac-OBzl was treated with 30 mL of 8 N HCl/dioxane, and then the deprotected heptapeptide (R_f^3 0.15) was isolated, dissolved in DMF (20 mL), and coupled with Z-Sar-OPfp (2.0 g, 5 mmol) for 30 min. The reaction mixture was diluted with $CHCl_3$ (70 mL), washed with 1 N HCl and water, and then evaporated. Compound **3a** solidified on rubbing with EtOH/ether, 1:4 (50 mL). It was filtered off and washed twice with this mixture to yield 4.0 g (54%) of product: mp 217 °C dec; R_f^3 0.8; $[\alpha]^{24}_D$ − 35.9° (*c* 1, DMF).

Deprotection. H-Sar-Arg-Val-Tyr-Chg-His-Pro-Lac-OH (3). To a solution of **3a** (3.6 g, 2.64 mmol) in DMF (10 mL) was added 8 mL of 2-mercaptoethanol (106 mmol). After the reaction mixture was stirred for 1 h at room temperature, Z-Sar-Arg-(NO_2)-Val-Tyr(Bzl)-Chg-His-Pro-Lac-OBzl (R_f^3 0.24) was precipitated with 200 mL of dry ether, filtered off, and washed with ether. The product was treated with hot EtOH (30 mL), then filtered off, and washed with EtOH to yield 2.8 g (82%) of the partially deprotected peptide. It was dissolved in 40 mL of a MeOH/AcOH/H_2O, 5:1:1, mixture, 1.2 g of 10% palladium on charcoal was added, and hydrogen gas was allowed to bubble through the mixture for 25 h. The catalyst was filtered off and the solution evaporated to dryness. The residue solidified on rubbing with EtOH/ether, 1:1. It was filtered off and washed with the same mixture to yield 1.44 g (72%) of **3**. Purification was carried out as described above. The HPLC profile of the substance (flow rate 1 mL/min) showed a single peak at 10.3 min and revealed the compound purity of 98%. Amino acid analysis data are given in Table I.

Bioassay. Adrenocortical Zona Glomerulosa Cell Incubation. Male CFY rats of 250–300 g were used. After decapitation, the adrenal glands were dissected free of fat and placed, till processed, in ice-cold Krebs–Ringer buffer containing 2 g/L glucose and 40 g/L bovine serum albumin with a potassium concentration of 3.7 mequiv/L (KRBGA). The capsular layer consisting predominantly of zona glomerulosa cells was separated from the zona fasciculata, reticularis, and medulla under a dissecting microscope, according to the method of Tait et al.[25] For cell dispersion, the capsules were minced with scissors and incubated at 37 °C under carbogen atmosphere in a shaking incubator for 2 × 25 min with collagenase (2 mg/mL) and DNAse (0.1 mg/mL) dissolved in KRBGA, 1 mL for two capsules. The supernatant containing the dispersed cells was decanted, filtered through nylon gauze (60-μm pore size), washed with KRBGA, and centrifuged for 10 min. Then the cell pellets were resuspended in KRBGA containing 5 g/L albumin. The volume of the final suspension was adjusted to contain glomerulosa cells from two adrenals in 1 mL of KRBGA, corresponding usually to 3.0–3.5 × 10^5 cells/mL. Serial 10-fold dilutions of the AII antagonists (2×10^{-12}–2×10^{-6} M) were added to the tubes alone or together with 2×10^{-8} M AII (Medical Research Council, U.K.) in duplicate or triplicate. AII and AII antagonists were dissolved in water containing 0.9% NaCl and 5 g/L bovine serum albumin. The aldosterone content of the incubation media was determined by RIA[26] after extraction with $CHCl_3$. Potency of the compounds tested was expressed as pA_2 values (Table III).

Rabbit Aorta Preparation. White New Zealand rabbits of either sex weighing 2–3 kg were used. After pentobarbital anesthesia and killing of the animals by exsanguination from the carotid arteries, the thorax was opened. The aorta segment possibly most proximal to the heart was excised and placed into a modified Krebs–Henseleit solution of the following composition (g/L): NaCl, 6.92; KCl, 0.35; $CaCl_2 \cdot 2H_2O$, 0.32; KH_2PO_4, 0.16; $MgSO_4 \cdot 7H_2O$, 0.29; $NaHCO_3$, 2.1; and glucose, 2.0. The aorta was cleaned from adherent tissue, and three spiral strips, 4 mm in width and 30 mm in length, were cut helically. Each strip was suspended in an organ bath of 10 mL containing the above-described solution. The bath medium was aerated with a mixed gas of 95% oxygen and 5% carbon dioxide and maintained at 37 °C. A resting tension of 2 g was applied, and the preparations were allowed to equilibrate for 60 min. The contractile response was measured isotonically by a strain gauge (K-30, HSE) and recorded on a compensograph recorder (RADELKISZ). AII and AII antagonists were dissolved in Krebs–Henseleit solution. AII was added to the bath medium cumulatively. The results were obtained by comparing the AII dose–response curves in the presence and absence of the AII analogue. Potency of the compounds was characterized by the pA_2 values.

Blood Pressure Test on Conscious Rats. In superficial pentobarbital anesthesia, specifically bent polyethylene cannulas were fixed in the left carotid artery and the right jugular vein. The cannulas were filled with physiological saline containing heparin and fixed on the backside of the neck. The rats were kept in separate cages at standard temperature and humidity. Water and food were given ad lib.

Twenty-four hours after the operation, the carotid artery was connected (with a long joint) to a pressure transducer (Bell and Howell) and the blood pressure of the free-moving animals was registered continuously on a Hellige multiscriptor. For measuring intrinsic pressor activity, the analogues dissolved in 0.2 mL of water containing 0.9% NaCl and 5 g/L bovine serum albumin (one analogue/rat) were injected (in a bolus) for 10 s into the jugular vein in 1.25, 2.5, 5.0, 10, and 20 μg/kg doses (random) each 20th min. The rise of the blood pressure after the injection was recorded. Each analogue was tested in 6 rats. The increments of the blood pressure were compared by analysis of variance and Dunnet's multiple test.[27]

For measuring the inhibitory effect of the analogues on the AII-induced blood pressure increase, first we determined the dose–response curve of AII (Hypertensin, CIBA) by injecting 0.2, 0.6, and 2.0 μg/kg AII into the jugular vein each 20 min. Subsequently 10 mg/kg of the tested analogue was injected sc. Five minutes after inection of the analogue, and subsequently every 20th min through 3 h, 1.0 μg/kg AII was given into the jugular vein. Each analogue was tested in 6–7 rats. The effects of the analogues were compared with that of saralasin. The increments of blood pressure were compared by analysis of variance and Dunn contrasts.[28]

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Registry No. **1**, 84992-83-6; **1a**, 109279-93-8; **2**, 109279-88-1; **2a**, 109279-94-9; **3**, 109279-89-2; **3a**, 109279-95-0; **4**, 109279-90-5; **4a**, 109281-39-2; **5**, 109279-91-6; **5a**, 109279-96-1; **6**, 109279-92-7; **6a**, 109279-97-2; **7**, 109183-68-8; **7a**, 109183-73-5; **8**, 109183-69-9; **9**, 109183-70-2; **10**, 98694-84-9; 11, 81000-64-8; AII, 11128-99-7; BOC-chg-oh, 109183-71-3; BOC-Cpg-OH, 109183-72-4; BOC-Pr-OSu, 3392-10-7; $PhCH_2Br$, 100-39-0; H-Pro-Lac-OBzl·HCl, 81000-65-9; BOC-His(DNP)-OPFp, 57866-88-3; BOC-His-(DNP)-Pro-Lac-OBzl, 81000-66-0; H-His(DNP)-Pro-Lac-OBzl·

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HCl, 81000-67-1; BOC-Chg-His(DNP)-Pro-Lac-OBzl, 109183-74-6; H-Chg-His(DNP)-Pro-Lac-OBzl·HCl, 109183-75-7; BOC-Tyr-(Bzl)-OPFp, 55878-15-4; BOC-Tyr(Bzl)-Chg-His(DNP)-Pro-Lac-OBzl, 109183-76-8; H-Tyr(Bzl)-Chg-His(DNP)-Pro-Lac-OBzl·HCl, 109183-77-9; BOC-Val-OPfp, 50903-49-6; BOC-Val-Tyr(Bzl)-Chg-His(DNP)-Pro-Lac-OBzl, 109183-78-0; H-Val-Tyr(Bzl)-Chg-His(DNP)-Pro-Lac-OBzl·HCl, 109183-79-1; BOC-Arg(NO_2)-OPFp, 57866-90-7; BOC-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His(DNP), 109183-80-4; Pro-lac-OBzlH-Arg(NO_2)-Val-Tyr-(Bzl)-Chg-His(DNP)-Pro-Lac-OBzl·HCl, 109183-81-5; Z-Sar-OPFp, 80909-58-6; Z-Sar-Arg(NO_2)-Val-Tyr(Bzl)-Chg-His-Pro-Lac-OBzl, 109183-82-6; zinc lactate, 16039-53-5.

Cardiotonic Agents. 6. Synthesis and Inotropic Activity of 2,4-Dihydro-5-[4-(1*H*-imidazol-1-yl)phenyl]-3*H*-pyrazol-3-ones: Ring-Contracted Analogues of Imazodan (CI-914)

Ila Sircar,*† Glenn C. Morrison,† Sandra E. Burke,‡ Richard Skeean,† and Ronald E. Weishaar‡

Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received January 12, 1987*

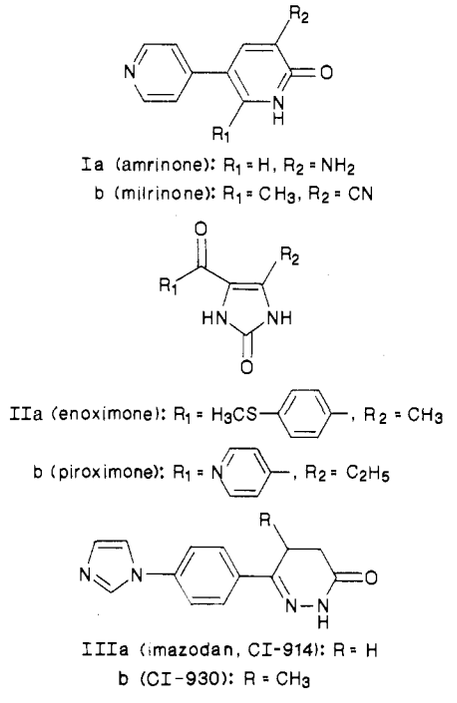
A series of 2,4-dihydro-5-[4-(1*H*-imidazol-1-yl)phenyl]-3*H*-pyrazol-3-ones was synthesized and evaluated for positive inotropic activity. Only compounds with two small alkyl groups at C-4 showed significant activity. The structure–activity relationships for optimal inotropic activity are presented and compared with those of the 4,5-dihydro-3(2*H*)-pyridazinone series. The phosphodiesterase inhibitory activity is also reported and correlated with the substitution pattern at C-4 in the pyrazolone ring.

Recently several noncatecholamine, nonglycoside cardiotonic agents have been discovered that possess both inotropic and vasodilator activities.[1,2] Prototypical compounds have emerged from three different classes of potent inotropes. These are (i) 5-aryl-2(1*H*)-pyridinones, such as amrinone (Ia, Chart I)[3,4] and milrinone (Ib),[5,6] (ii) 5-aroyl-1,3-dihydro-2*H*-imidazol-2-ones, such as enoximone (IIa),[7,8] and piroximone (IIb),[9,10] and (iii) 6-aryl-4,5-dihydro-3(2*H*)-pyridazinones, namely, imazodan (CI-914, IIIa) and CI-930 (IIIb).[11,12] Although the pharmacological profiles of these newer agents appear to be similar in animal models, there exists a difference in the potency and relative balance of the inotropic and vasodilator activities.[1,2]

The positive inotropic and vascular relaxant actions of these agents are apparently due to their selective inhibitory effects on the cyclic AMP specific form of phosphodiesterase (PDE III) present in cardiac and vascular muscle.[13,14] The potent activity of 4,5-dihydro-6-[(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones led us to investigate the corresponding five-membered analogues, namely, 2,4-dihydro-3*H*-pyrazol-3-ones.

The potential therapeutic utility of several 2,4-dihydro-5-phenyl-3*H*-pyrazol-3-ones (IV) in the treatment of hypertension, inflammation, arrhythmia, and analgesia has recently been reported.[15,16] It has also been shown that 4-unsubstituted (or monosubstituted) pyrazol-3-ones exist in several tautomeric forms (Chart II, A–D).[17,18] By contrast, 4,4-disubstituted analogues can only exist in the keto form (E). The essential features required for inotropic activity in several series of PDE inhibitors have been identified both from qualitative and quantitative structure–activity relationships.[12,19] Two key elements necessary for inotropic activity are (i) a polar group and (ii) an acidic hydrogen adjacent to the polar group (for example, CO–NH). On the basis of these observations, we reasoned that the inotropic activity in the 5-(substituted phenyl)-3*H*-pyrazol-3-one series would reside in the 4,4-disubstituted compounds that only exist in the keto form (E) To test this hypothesis, we prepared a number of 5-(substituted phenyl)-3*H*-pyrazol-3-ones having different substituents at the 4-position of the pyrazolone ring[20] and evaluated the pharmacological activity. This paper reports

Chart I



† Department of Chemistry.
‡ Department of Pharmacology.

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