1.04; Arg, 1.00; Ser, 0.80; Glu, 1.01; Pro, 0.95; Gly, 2.08; Leu, 1.00; Tyr, 0.85. *Anal.* ($C_{56}H_{77}N_{17}O_{13}\cdot 3CH_3COOH\cdot 4H_2O$) C, H, N.

pGlu-His-Trp-Ser(Bzl)-Phe(4-NO_2)-Gly-Leu-Arg(Tos)-Pro-Gly-NH_2 (II). The protected peptide was synthesized beginning with Boc-glycine resin (2.94 g, 1.0 mmol of Gly) in an analogous fashion to peptide I. However, tosyl group protection was used for His and Boc groups were removed by treatments (5 and 25 min) with 25% TFA in CH_2Cl_2. Acetic acid washes before and after deprotection were replaced by washes with CH_2Cl_2.

The dried peptide-resin weighed 4.55 g (118% incorporation) and 2.0 g of this material were ammonolyzed and extracted as described. The crude powder (700 mg) was reprecipitated from refluxing MeOH–EtOAc (3:1) to yield 285 mg (40%) of protected peptide II: single spot to Ehrlich and Pauly reagents and I_2 vapor; R_f[1] (silica), 0.41. Amino acid analysis gave Phe(4-NO_2), 0.93; Trp, 0.87; His, 0.99; NH_3, 1.10; Arg, 1.00; Ser, 0.83; Glu, 1.10; Pro, 0.97; Gly, 2.10; Leu, 1.00. *Anal.* ($C_{69}H_{86}O_{16}S\cdot 3H_2O$) C, H, N, S.

pGlu-His-Trp-Ser-Phe(4-NO_2)-Gly-Leu-Arg-Pro-Gly-NH_2 (IV). The protected peptide II (100 mg) and anisole (3 ml) were placed in the reaction vessel of an inert vacuum system (Peninsula Laboratories, San Carlos, Calif.) and thoroughly degassed. Anhydrous HF was condensed on top and the mixture stirred at 0° (1 hr). Excess HF was removed *in vacuo* and the peptide-anisole mixture distributed between 0.1 *M* AcOH and EtOAc. The aqueous layer (50 ml) was extracted with EtOAc (10 ml) and ether (three 10-ml portions) and lyophilized to yield crude IV (89 mg). This was dissolved in water (8 ml) and purified on the CM-cellulose column under the described conditions. Fractions eluted between 620 and 700 ml were pooled and lyophilized to constant weight from water to give peptide IV (46 mg, 62%): $[\alpha]^{26}D$ −58° (*c* 1.10, 0.1 *M* AcOH); single spot to Ehrlich, Pauly, and Cl-tolidine reagents; R_f[1] (cellulose), 0.61; R_f[2] (silica), 0.65; R_f[3] (cellulose), 0.53; single component moving in the direction of the cathode after tlc at pH 4.6 and 6.4. Amino acid analysis gave Phe(4-NO_2), 1.03; Trp, 1.00; His, 0.99; NH_3, 1.13; Arg, 0.92; Ser, 0.93; Glu, 1.08; Pro, 0.89; Gly, 2.20; Leu, 0.98. *Anal.* ($C_{55}H_{74}N_{18}O_{14}\cdot 2CH_3COOH\cdot 2H_2O$) C, H, N.

pGlu-His-Trp-Ser-Phe(4-NH_2)-Gly-Leu-Arg-Pro-Gly-NH_2 (V). The protected peptide II (150 mg) was dissolved in NH_3 (250 ml) and treated with small amounts of Na under conditions described above. During the addition the color of the reaction mixture changed progressively from pink to colorless to brown to colorless to permanent blue, the latter color being discharged immediately with 1 drop of AcOH.

The residue, after removal of NH_3, was desalted and subjected to chromatography on CM-cellulose. Peptide eluted between 565 and 640 ml weighed 46 mg (44%) after lyophilization: $[\alpha]^{26}D$ −50.6° (*c* 1.06, 0.1 *M* AcOH); single spot to Ehrlich, Pauly, and Cl-tolidine reagents; R_f[1] (cellulose), 0.39; R_f[2] (cellulose), 0.56; R_f[4] (silica), 0.18; single component moving in the direction of the cathode after tlc at pH 4.6 and 6.4. Amino acid analysis gave Phe(4-NH_2), 0.97; Trp, 1.03; His, 0.93; NH_3, 1.00; Arg, 0.99; Ser, 0.92; Glu, 1.07; Pro, 0.94; Gly, 2.20; Leu, 0.92. *Anal.* ($C_{55}H_{76}N_{18}O_{12}\cdot 3CH_3COOH\cdot 5H_2O$) C, H, N.

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Factors That Influence the Antagonistic Properties of Angiotensin II Antagonists

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Synthesis of [Suc[1],Ala[8]]-, [Ala[4],Ile[8]]-, and [D-*a*Ile[8]]angiotensin II was carried out by solid-phase procedure. Angiotensin II was synthesized as a model compound for separation of D-histidine containing octapeptide, formed as a side product during the synthesis, from the L-histidine containing parent hormone by ion-exchange chromatography followed by partition chromatography on Sephadex G-25. [D-His[6]]angiotensin II had 4% pressor response of angiotensin II. Antagonism to myotropic activity of angiotensin II on rabbit aortic strips was reduced when position 1 (Asp) in [Ala[8]]angiotensin II was substituted with succinic acid residue (log K_2 7.04) or when position 4 (Tyr) in [Ile[8]]angiotensin II was replaced with Ala (log K_2 6.55). Similar results were obtained when position 8 in [Ile[8]]angiotensin II was replaced with D-*allo*isoleucine (log K_2 7.33). The antagonistic activities of all the analogs were reduced when position 6 (L-histidine) was substituted with D-histidine. As compared to angiotensin II, pressor activity (vagotomized, ganglion-blocked rats) of all the analogs was less than 0.72%. These results indicate that although position 8 in angiotensin II is responsible for determining the agonistic or antagonistic properties of the compound, the degree of activity and duration of action are very much influenced by the nature and optical orientation of substituents in other positions of the molecule. Changes in positions other than in position 8 may exert their influence by affecting position 8 side group orientation with respect to other necessary side groups.

Lack of parallelism between the myotropic activity and the ability to release catecholamines from adrenal medulla[1] led to the discovery of the antagonistic potential of 8-substituted analogs of angiotensin II.[2,3] Subsequent investigations in our laboratories revealed that substitution of the aromatic ring in position 8 (Phe) by an aliphatic residue

Table I. Pressor and Antagonistic Activities of Analogs of Angiotensin II

Analog	Antagonistic activity (rabbit aortic strip) Log K_2[a]	Pressor activity (% of angiotensin II)
[Suc^1,Ala^8]-	7.04 ± 0.05	0.20
[Suc^1,D-His^6,Ala^8]-	7.01 ± 0.15	0.19
[Ala^4,Ile^8]-	6.55 ± 0.06	0.00
[Ala^4,D-His^6,Ile^8]-	6.32 ± 0.08	0.00
[D-*a*Ile^8]-	7.33 ± 0.09	0.72
[D-His^6,D-Ile^8]-	7.08 ± 0.06	0.014
[D-His^6]-		4

[a] The log K_2 has been calculated by the equation[36] log $(x - 1) = n \log B + \log K_2$. When $x = 2$, then $-\log B = pA_2$ value and if $n = 1$, $pA_2 = \log K_2$. The assumption that $n = 1$ has been made in previous reports on pA_2 values. The pA_2 values reported[4,5] for [Ala^8]- and [Ile^8]angiotensins II were 8.3 and 9.21, respectively.

(Ala, Leu, Val, Ile, etc.) changed the agonistic properties of the analogs into antagonistic properties.[3-9] These results were confirmed by several other investigators.[10-14] Antagonistic activity, both *in vitro* and *in vivo* studies, is further enhanced when aspartic acid in position 1 is replaced with sarcosine.[6,8,9,15] The present paper reports the synthesis of (a) [D-*a*Ile^8]angiotensin II to study the effect of spatial orientation in position 8 with respect to an aliphatic amino acid, (b) [Suc^1,Ala^8]angiotensin II to delineate the contribution of the N-terminal amino group for antagonistic activity, and (c) [Ala^4,Ile^8]angiotensin II to investigate the influence of the aliphatic side chain in position 4 on a potent antagonist.

The analogs were synthesized as reported earlier.[6] Comparative pressor activity of these analogs and their antagonism to myotropic activity of angiotensin II (*cf.* Experimental Section) are given in Table I.

Chemical and Stereochemical Purification of Angiotensin II and Its Analogs. Windridge and Jorgensen[16] observed that, compared to the solution method (~3%), solid-phase synthesis of angiotensin II analogs gave more racemization (36%) of histidine residue during dicyclohexylcarbodiimide (DCC) coupling of *tert*-butyloxycarbonyl-*im*-benzyl-L-histidine in DMF. The presence of *N*-hydroxysuccinimide[16] or 1-hydroxybenzotriazole[17] during coupling through DCC or protection of the imidazole moiety with tosyl or dinitrophenyl groups minimized the racemization of histidine residue but did not abolish it.[16,18] Since even small amounts of these stereochemical impurities can lead to considerable variation in biological activity,[19,20] it appeared of interest to find a procedure for separation of D-histidine containing octapeptides from the L-histidine analog of angiotensin II. This has been achieved by carrying out ion-exchange chromatography of the crude peptide mixture on AG-1 (AcO^-) ion-exchange resin using buffers of varying pH by a stepwise procedure, followed by partition chromatography of the octapeptide containing fractions on Sephadex G-25. Angiotension II was synthesized as a model compound for standardizing the procedure for synthesis and purification. Whereas L-His^6-octapeptide showed 100% biological activity, the D-His^6 analog gave 4% pressor response of the natural hormone; the latter activity is in accord with an earlier report for [Asn^1,Val^5,D-His^6]angiotensin II.[21]

The optical homogeneity of the components was determined by oxidation of the peptide hydrolysates with L-amino acid oxidase.[22] Corrections were applied for racemization occurring during hydrolysis in 6 *N* HCl or due to tris buffer; the latter gave an overlapping peak with that of histidine on the amino acid analyzer.

Hydrolysis of the peptide polymer in 12 *N* HCl-propionic acid (1:1)[23] promoted racemization of histidine and the extent of racemization was dependent upon duration of hydrolysis. Although no quantitation was carried out in this regard, this accounts for inconsistent or a comparatively higher percentage of racemization of histidine reported previously.[24] Hydrolysis of the peptide polymer in 6 *N* HCl gave very little racemization but quantitative cleavage of amino acids did not take place even after 36 hr. Unambiguous results were obtained when the peptide was first cleaved from the polymer and subsequently hydrolyzed (6 *N* HCl) for enzymic oxidation.

Structure-Activity Relationship. Since angiotensin II and its analogs are easily degraded by aminopeptidases, we synthesized [Suc^1,Ala^8]angiotensin II thus replacing position 1 of [Ala^8]angiotensin II with an unnatural des-amino residue (succinic acid) to increase the duration of antagonistic properties. However, this change invoked low antagonistic properties in the parent compound without enhancing its duration of action. Replacement of aspartic acid with sarcosine enhanced the antagonistic properties of several angiotensin II analogs.[6,8,9,15] These differences, due either to varying rates of enzymatic degradation or due to different affinities for the receptor, point out the importance of a nitrogen atom at the N terminus for antagonistic properties.

Drastic reduction in antagonistic activity of [Ile^8]angiotensin II by replacing tyrosine with alanine in position 4 indicates profound influence of position 4 on the antagonistic properties of the compound; it could be due to the phenolic hydroxyl or the aromatic moiety or both. Reduced pressor and antagonistic activities obtained by replacement of L-histidine in position 6 or L-isoleucine in position 8 by the corresponding D-amino acids indicate the importance of optical orientation in these positions.

As has been pointed out by us in an earlier publication,[6] position 8 in angiotensin II is responsible for determining the agonistic or antagonistic properties of the compound and any dislocation of the side chain in this position has drastic effects on the biological activity. Present results indicate that the degree of activity and duration of action in the antagonistic peptides are very much influenced by (a) substitution in position 1,4 or possibly other residues in the molecule and (b) optical orientation of residues in positions 6 and 8 which should be of the L configuration. These factors, which have profound effect on the biological activity of the analog, could exert their influence through a change in the conformation of the molecule. In an examination of the stepwise elongation of the fragments of angiotensin II from N to C terminal, in aqueous solution, Fermandjian, *et al.*,[25] found an unordered structure up to the hexapeptide (Asn-Arg-Val-Tyr-Val-His) stage. A more ordered structure, predominantly β-antiparallel, was found at the heptapeptide stage when proline was added at the C terminus. A further increase in the length of the peptide by one amino acid led to different CD spectra depending upon the nature of the amino acid added. With alanine in the 8 position ([Ala^8]angiotensin II), the peptide exhibited spectra typical of an unordered structure in aqueous solution but was helical in organic solvents and predominantly β-antiparallel in the dry state.[25,26] However, with L-phenylalanine in the 8 position, the octapeptide (angiotensin II), in aqueous solution, appeared to have a more ordered structure.[26-31] In the solid state, as well as at high concentrations in water, angiotensin II has been shown to adopt a β-antiparallel structure.[26,31] There are also indications that in

organic solvents angiotensin II assumes a cross β structure possibly due to the hydrophobic interactions between 3- and 5-valine and 8-phenylalanine side chains and to the cis conformation of the histidine-proline peptide bond.[26] These observations point out that these octapeptides exhibit polymorphism under different conditions and support our observations that the nature of the C-terminal amino acid plays an important role in determining the biological activity of the compound. Thus, one can speculate that for a peptide showing potent pressor and myotropic activity, it should be capable of adopting a suitable conformation so that it will have proper binding to the receptor site and convey the "biological message." The degree of variation in these properties could lead to the corresponding change in biological activities. A competitive type of antagonist, such as [Ala^8]-angiotensin II, possibly possesses many of the above characteristics but lacks the "message" carrying ability and appears to bind less firmly to the myotropic receptor. Polymorphism in these antagonistic peptides permits them to adopt a conformation conducive to binding with the macromolecules of the receptor site. It is perhaps due to this spontaneous adaptability that angiotensin II analogs with aliphatic side chains in position 8 are able to compete with angiotensin II and thus act as antagonists by occupying the receptor site.

Experimental Section

Solvents used for ascending paper chromatography (pc) on Whatman No. 1 filter paper and tlc were: (a) *n*-BuOH–AcOH–H_2O (BAW) (4:1:5); (b) *n*-BuOH–AcOH–H_2O–Pyr (BAWP) (30:6:24:20); (c) *n*-BuOH–AcOEt–AcOH–H_2O (BEAW) (1:1:1:1); (d) *n*-BuOH–Pyr–H_2O (BPW) (10:2:5); (e) MeCOEt–Pyr–H_2O (MPW) (40:20:16). Ascending tlc was conducted on silica gel or cellulose supported on glass plates (Brinkmann Silplate or Celplate). Ionophoresis was carried out on filter paper strips on S & S 2043A filter paper strips in Beckman electrophoresis cell (Durrum type) Model R, series D at 400 V, using HCO_2H–AcOH buffer prepared by diluting 60 ml of HCO_2H and 240 ml of AcOH to 2 l. with distilled H_2O (pH 1.9) and Beckman barbiturate buffer B-2 (pH 8.6). Glutamic acid was used as a reference compound and *E*(Glu) indicates the electrophoretic mobility relative to glutamic acid = 1.00. Detection of the compound on chromatograms was carried out with ninhydrin and/or with diazotized sulfanilic acid. The free peptides were hydrolyzed in sealed tubes under N_2 in 6 *N* HCl at 110° for 24 hr in the presence of phenol. Amino acid analyses were performed on Jeolco-5AH amino acid analyzer. Melting points were taken on a Leitz Wetzlar hot-stage apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. L-Amino acid oxidase from the venom of *Agkistrodon p. piscivorus* was purchased from General Biochemicals. Angiotensin analogs reported in this paper contain L-isoleucine in position 5.

Procedure for Synthesis and Purification. Angiotensin II and other analogs were synthesized by the solid-phase procedure[32] as previously described by Khosla, *et al.*[6] The crude products were purified on a column (5 × 100 cm) of AG-1x2 (200–400 mesh) in acetate form by eluting with ammonium acetate buffers of varying pH containing 5% *n*-PrOH. The resin was conditioned by washing with a solution of 1 *M* in both AcOH and AcONa (1 l.), deionized H_2O (2 l.), and ammonium acetate buffer of pH 8.5 (1 l.). Crude peptide (*ca.* 500 mg) was dissolved in ammonium acetate buffer of pH 8.5 (8 ml), filtered, and applied to the column. The column was eluted with pH 8.5 buffer (2 l.) at the rate of 70 ml/hr. If necessary, this was followed successively with 1.5 l. each of buffers with pH 8.0, 7.5, 7.0, etc. The effluent was monitored at 280 mμ; fractions (15 ml) giving Pauly-positive reaction were chromatographed on cellulose tlc using BPW as the solvent. Components with the same R_f values were pooled, evaporated to a small volume, and lyophilized; 1 mg of each fraction was hydrolyzed for amino acid analysis. The peptide giving correct amino acid analysis for the desired octapeptide was then rechromatographed on a column (2.5 × 100 cm) of Sephadex G-25 using *n*-BuOH–Pyr–H_2O (10:2:5, upper phase) as the solvent at a flow rate of 15 ml/hr. In general, D-histidine containing octapeptide was eluted first. For determining the optical purity, 1 mg of each octapeptide was hydrolyzed (6 *N* HCl), solvent was removed *in vacuo*, the residue, dried over KOH pellets *in vacuo*, was dissolved in 2 ml of tris buffer (pH 7.6), and the pH of the solution was adjusted to 7.2 with 0.5 *N* HCl. The solution was treated with a few drops of toluene and 0.2 ml of 1% buffered L-amino acid oxidase solution. The mixture was incubated for 48 hr at 37° with shaking and diluted to 10 ml of lithium citrate buffer (pH 2.6), and an aliquot of this solution was applied directly to the amino acid analyzer column. Proline did not oxidize under these conditions and served as an internal standard. Aspartic acid and valine were oxidized very slowly with *Agkistrodon p. piscivorus* L-amino acid oxidase, and the extent of oxidation differed with each batch. This was confirmed by subjecting a mixture of these amino acids to the same hydrolytic and enzymic oxidation procedures. Jorgensen, *et al.*,[33] also reported that aspartic acid was attacked too slowly by *C. adamanteus* L-amino acid oxidase to be checked by this method. All the other amino acids reported in this paper were oxidized within the range of experimental error.

[Ile^5]**angiotensin** II had the following amino acid ratio in the acid hydrolysate, Asp 0.90, Arg 0.98, Val 1.00, Tyr 1.04, Ile 1.05, His 1.03, Pro 0.96, Phe 1.06, and after incubation with L-amino acid oxidase, His 0.06, Pro 1.00. *Anal.* ($C_{50}H_{71}N_{13}O_{12}\cdot 1AcOH\cdot 0.5H_2O$) C, H, N.

[Ile^5,D-**His**^{6}]**angiotensin** II had mp 236–238° dec; pc R_f 0.38 (BAW), R_f 0.55 (BAWP); tlc (silica gel) R_f 0.17 (BAW), R_f 0.37 (BAWP); tlc (cellulose) R_f 0.42 (BPW); *E*(Glu) 1.23 (pH 1.95), *E*(Glu) 0.74 (pH 8.6). *Anal.* ($C_{50}H_{71}N_{13}O_{12}\cdot 1AcOH\cdot 1.5H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Asp 0.97, Arg 0.97, Val 0.99, Tyr 0.95, Ile 1.02, His 1.03, Pro 0.99, Phe 1.00, and after incubation with L-amino acid oxidase, His 1.00, Pro 1.00.

[Ala^4,Ile^8]**angiotensin** II had mp 244–246° dec; pc R_f 0.42 (BAW), R_f 0.57 (BAWP); tlc (silica gel) R_f 0.05 (BAW), R_f 0.24 (BAWP), R_f 0.06 (BPW), R_f 0.17 (BEAW); tlc (cellulose) R_f 0.51 (BAW), R_f 0.54 (BAWP); *E*(Glu) 1.10 (pH 1.95), *E*(Glu) 0.86 (pH 8.6). *Anal.* ($C_{41}H_{69}N_{13}O_{11}\cdot 1.5AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Asp 0.99, Arg 1.02, Val 1.01, Ala 1.00, Ile 1.96, His 0.99, Pro 0.99, and after incubation with L-amino acid oxidase, His 0.09, Pro 1.00.

[Ala^4,D-**His**^{6},Ile^8]**angiotensin** II had mp 242–244° dec; pc R_f 0.50 (BAW), R_f 0.62 (BAWP); tlc (silica gel) R_f 0.07 (BAW), R_f 0.27 (BAWP); tlc (cellulose) R_f 0.12 (BPW), R_f 0.17 (MPW); *E*(Glu) 1.11 (pH 1.95), *E*(Glu) 0.86 (pH 8.6). *Anal.* ($C_{41}H_{69}N_{13}O_{11}\cdot 1.5AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Asp 0.98, Arg 0.97, Val 1.00, Ala 1.00, Ile 1.96, His 0.97, Pro 0.97, and after incubation with L-amino acid oxidase, His 0.97, Pro 1.00.

[D-*a*Ile^8]**angiotensin** II had mp 244–245° dec; pc R_f 0.35 (BAW), R_f 0.50 (BAWP); tlc (silica gel) R_f 0.2 (BAW), R_f 0.41 (BAWP), R_f 0.20 (BPW); tlc (cellulose) R_f 0.11 (BPW), R_f 0.54 (MPW); *E*(Glu) 1.11 (pH 1.95), *E*(Glu) 0.69 (pH 8.6). *Anal.* ($C_{47}H_{73}N_{13}O_{12}\cdot 0.5AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Asp 0.96, Arg 0.94, Val 1.00, Tyr 0.80, Ile 1.98, His 0.94, Pro 0.95, and after incubation with L-amino acid oxidase, His 0.02, Pro 1.00, Ile 1.06.

[D-**His**^{6},D-*a*Ile^8]**angiotensin** II had mp 243–245° dec; pc R_f 0.37 (BAW), R_f 0.50 (BAWP); tlc (silica gel) R_f 0.14 (BAW), R_f 0.44 (BAWP), R_f 0.25 (BPW); *E*(Glu) 1.11 (pH 1.95), *E*(Glu) 0.70 (pH 8.6). *Anal.* ($C_{47}H_{73}N_{13}O_{12}\cdot 1AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Asp 0.97, Arg 1.00, Val 1.00, Tyr 0.97, Ile 2.02, His 0.99, Pro 1.00, and after incubation with L-amino acid oxidase, His 1.06, Pro 1.00, Ile 1.00.

[Suc^1,Ala^8]**angiotensin** II had mp 210–212° dec; pc R_f 0.45 (BAW), R_f 0.63 (BAWP); tlc (cellulose) R_f 0.67 (BAW), R_f 0.72 (BAWP), R_f 0.73 (BEAW); *E*(Glu) 1.03 (pH 1.95), *E*(Glu) 0.703 (pH 8.6). *Anal.* ($C_{44}H_{66}N_{12}O_{12}\cdot 3.5H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Arg 0.96, Val 0.98, Tyr 1.03, Ile 1.02, His 0.98, Pro 0.99, Ala 1.02, and after incubation with L-amino acid oxidase, His 0.025, Pro 1.00.

[Suc^1,D-**His**^{6},**Ala**^{8}]**angiotensin** II had mp 218–220° dec; pc R_f 0.47 (BAW), R_f 0.57 (BAWP); tlc (cellulose) R_f 0.67 (BAW), R_f 0.72 (BAWP), R_f 0.73 (BEAW); *E*(Glu) 1.06 (pH 1.95), *E*(Glu) 0.68 (pH 8.6). *Anal.* ($C_{44}H_{66}N_{12}O_{12}\cdot 1AcOH\cdot 1H_2O$) C, H, N. Amino acid ratio in the acid hydrolysate gave Arg 0.98, Val 1.00, Tyr 0.90, Ile 1.03, His 1.04, Pro 1.01, Ala 1.04, and after incubation with L-amino acid oxidase, His 0.90, Pro 1.00.

Biological Results. The pressor activity was determined by pressor assay in vagotomized, ganglion-blocked rats.[34] Inhibition of myotropic activity of angiotensin II with the analogs was studied on isolated, spirally cut rabbit aortic strips, prepared according to the

method of Furchgott and Bhadrakom.[35] They were mounted in a 5-ml organ bath containing Krebs solution (NaCl, 11 mmol; $NaHCO_3$, 25 mmol; KCl, 5 mmol; NaH_2PO_4, 1 mmol; $MgCl_2$, 0.5 mmol; $CaCl_2$, 2.5 mmol, and dextrose, 11.5 mmol) at 37° and were aerated with a mixture of 95% O_2 and 5% CO_2. The strips were placed under 1 g passive tension and allowed to equilibrate for 1.5–2.0 hr. Isometric contractions were recorded using a Grass force displacement transducer (FT-03) on a Grass polygraph (Model 7).[4]

In order to evaluate the mechanism of antagonism, the criteria used by Schild were used.[36] First a log dose-response curve for angiotensin II was determined on aortic strips, before and in the presence of the analogs. Next, the dose ratio of angiotensin II was calculated in the presence of the various analogs at several molar concentrations. The calculated log K_2 values and pressor activities are given in Table I.

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Uptake of Androgen Analogs by Prostate Tissue†

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A study of the *in vitro* binding of the three radioactive androgens 1,2-[^{3}H]-5α-dihydrotestosterone (**3**), 17α-[^{14}C]methyl-5α-androstan-17β-ol (**2**), and 17α-[^{14}C]methyl-5α-androst-2-en-17β-ol (**10**) to minced rabbit ventral prostate is described. The competitive effect of several other steroids on the binding is also outlined. The resulting data are explained most readily by an assumption of the presence of three different binding sites for such steroids: the "classical" dihydrotestosterone site, as well as separate sites for the hydrocarbon and olefin derivatives. The implications of this for structure-activity relationships in androgen analogs are discussed.

In previous papers we evaluated the structural characteristics of ring A in steroidal androgens which have importance in eliciting the characteristic biological response to these substances.[1] We concluded that a six-membered or equivalent A ring, flattened at C-2 and/or C-3, is required for activity. However, certain steroids having an unsubstituted A ring or a single C-3 substituent, such as androstan-17β-ol[2] (**1**) and 5α-androstane,[3,4] represent androgens which do not fit into this hypothesis. One reason for this could be metabolic conversion, for example, oxygenation of an A-ring hydrocarbon to an active 3-keto derivative. Recently, we have investigated this possibility[5] and found that 17α-methyl-5α-androstan-17β-ol (**2**), which has clinically useful[6] anabolic

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