Angiotensin II Analogues. 14. Roles of the Imidazole Nitrogens of Position-6 Histidine in Pressor Activity[1]

Kun-hwa Hsieh, Eugene C. Jorgensen,*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143

and Thomas C. Lee

Department of Human Physiology, School of Medicine, University of California, Davis, California 95616.
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Replacement of the position-6 histidine residue in [Asn[1],Ile[5]]angiotensin II produced analogues with pressor activities in the rat (compared to [Asn[1],Val[5]]angiotensin II = 100%) as follows: 2,4-diaminobutyric acid, 0.02%; 4-nitrophenylalanine, 0.02%; 4-aminophenylalanine, 0.05%; β-(2-imidazolyl)-L-α-alanine, 0.4%; β-(2-imidazolyl)-D-α-alanine, 0.04%; β-(2-pyridyl)-L-α-alanine, 3.5%; β-(2-pyridyl)-D-α-alanine, 0.1%. [Asn[1],Tyr(3-Bzl)[4],Ile[5],Phe(4-NO_2)[6]]AII was isolated as a side product in the HF-deprotection reaction and it was shown to possess 0.03% pressor activity. Extensive racemization (78%) of butyloxycarbonyl-β-(2-pyridyl)-L-α-alanine occurred during solid-phase synthesis, despite the use of conditions which minimized racemization of Boc-His(Bzl). The resultant diastereomeric peptides were separated by column chromatography and characterized. Incorporation of the racemic N^{α}-butyloxycarbonyl-N^{im}-benzyl-β-(2-imidazoyl)-DL-α-alanine into the peptide and separation of the resultant diastereomeric angiotensin II by countercurrent distribution eliminated the need for the laborious resolution and protection of the L isomer, which might racemize extensively during peptide synthesis. Correlation of the chemical structures with biological activities of position-6 analogues suggests that the heterocyclic nitrogens of histidine are important for angiotensin II to be recognized by the receptor, and the *pros*-pyridine nitrogen of histidine plays a minor role and the *tele*-pyrrole nitrogen a major role in this interaction. Since β-(2-imidazolyl)alanine (Ima) and β-(2-pyridyl)alanine (Pya) resemble histidine in steric environment and the ability to participate in hydrogen-bonding and nucleophilic interactions, they are generally useful for the study of structure–activity relationships in order to assess the importance of such effects in the molecular events of hormone–receptor interaction. Furthermore, tautomerization of these heterocyclic amino acids does not alter the spatial alignment of their heterocyclic nitrogens relative to the peptide backbone. Consequently, replacement of histidine by Ima and Pya can unambiguously delineate the roles of the pros and tele nitrogens of histidine in other biologically important peptides.

The imidazole group of histidine is essential for the biological activity of a variety of enzymes, including the serine and cysteine proteases, carbonic anhydrase, phosphotransferases, and hydrolases.[2] In the active center, the histidine residue may serve as both donor and acceptor in hydrogen-bonding interactions, and it can also promote proton transfer by a general acid–base catalysis, which is particularly facilitated by the neighboring acidic (pyrrole NH) and basic (pyridine N) groups in the imidazole side chain.[2] Because the imidazolyl nitrogen atoms are part of the aromatic ring, they are sterically exposed,[2] and histidine is an efficient nucleophile for acyl transfer[3] and for coordination with metal ions.[4] As a heterocyclic molecule, the imidazole group can tautomerize,[5] and both N^{τ}-H and N^{π}-H tautomers of histidine have been observed by ^{13}C NMR[6] in angiotensin,[7] bacitracin,[6] and thyroliberin (TRF).[8]

Modification of the histidine residue in peptides to alter the characteristics of the imidazole side chain can produce dramatic changes in biological activity. In thyroliberin, addition of a methyl group to histidine produced either the superactive [His(3-Me)[2]]TRF (800% activity) or the inactive [His(1-Me)[2]]TRF,[9,10] whereas deletion of the histidine residue in luliberin (LHRH) yielded an antagonist.[9,11] It is apparent from these results that the pyrrole and pyridine nitrogens of histidine are not equivalent, and elimination or enhancement of select features of histidine may reduce or increase the binding potency and the intrinsic activity of the analogue.

In angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, AII), histidine has a definite but poorly defined role in the recognition–activation of the receptor. Photooxidative destruction of the imidazole side chain resulted in parallel loss of biological activity.[12] The basic character of histidine was implicated in the tachyphylaxis of angiotensin II.[13,14]

Table I. Rat Pressor Activities of Some Position-6 Analogues of Angiotensin II

position-6 substit	pressor act., %	ref
His (AII)	100	
Pza	57, 34-50	15, 16
Thi	10-19, 1	16, 18
Phe	3-5, 1	16, 20
His(3-Me)	5	21
His(1-Me)	0.05	21
D-His	4	22
Ala	0.8	18
Met	0.2	16
Leu	0.02	16
Acpc	0	19
Lsy	0.1	17
Orn	0.03-0.04	16
Arg	0.01	16
Glu	0	16

Drastic reduction of this basicity in the analogue with β-(3-pyrazolyl)-L-alanine (Pza) in place of histidine resulted in the retention of substantial pressor activity (57%).[15,16] These results suggest that histidine is essential for the angiotensin response and that it may simultaneously cause the undesirable desensitization of the receptor. However, the structural features of histidine responsible for the two effects may be different.

In order to understand which potential interactions of the imidazole group might be involved in the pressor response, histidine was previously replaced by the basic,[16,17] neutral,[16-19] and acidic[16] aliphatic amino acids, which gave relatively inactive analogues. Substitution of histidine by the aromatic residues[16-22] also gave analogues of low activity (Table I). These findings suggest that an intact

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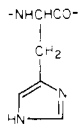
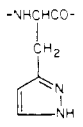
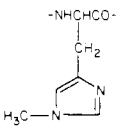
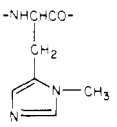
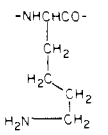
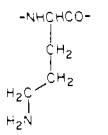
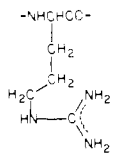
Table II. Structure and Activities of Some Position-6 Analogues of Angiotensin II

analogues	His^6 (AII)	Pza^6	His(3-Me)^6	His(1-Me)^6	Lys^6	Orn^6	Arg^6
pressor act. in rat	100	57	5	0.05	0.1	0.04	0
struct of position-6 substituents							
ref		15, 16	21	21	17	16	16

imidazole-like structure is necessary for the angiotensin response. However, when the histidine side chain was extended by a methylene group, preservation of the imidazole ring in the resultant homohistidine analogue, [Abu(γ-Im)^6]AII, exhibited little activity (0.05%),[23] suggesting that the angiotensin–receptor interaction may be very sensitive to steric interference. This interpretation is consistent with previous results in which many inactive analogues[16,17] have bulky substituents that can cause steric repulsion and reduce activity, whereas the β-(3-pyrazolyl)alanine residue in the only active position-6 analogue, [Pza^6]AII,[15,16] retains not only the structural features but also the steric environment of histidine (Table II). Thus, steric effects may play an important role in the biological response of angiotensin, and examination of structure–activity correlations of angiotensin II in position 6 with sterically unhindered and preferably heterocyclic analogues is indicated.

Analysis of the structural features of histidine and of β-(3-pyrazolyl)alanine shows that, although both residues have five-membered π-electron-enriched rings, their most notable common feature is the location of the two heterocyclic nitrogens relative to the alanine backbone: a *pros*-pyridine N and a *tele*-pyrrole NH (Table II). For our study, we prepared angiotensin II analogues substituted in position 6 (Table III) in order to evaluate the respective contribution to the pressor activity by the structural features of histidine: namely, the π-electron-enriched ring, the two heterocyclic nitrogens, and their unique arrangement. While the *p*-nitrophenylalanine and *p*-aminophenylalanine have different degrees of π-electronic density and aromaticity, the γ-amino group of the aliphatic α,γ-diaminobutyric acid (A_2bu) may superimpose and mimick the pros nitrogen of histidine. In addition to having the pros nitrogen(s), the heterocyclic β-(2-pyridyl)-L-α-alanine (Pya) and β-(2-imidazolyl)-L-α-alanine (Ima) resemble histidine in molecular shape and size, although Pya is less enriched in π-electronic density than Ima and histidine. The isohistidine residue, Ima, is identical with histidine in all structural characteristics except the location of the alanine backbone.

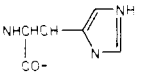
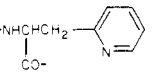
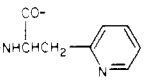
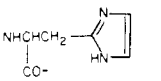
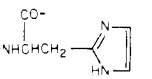
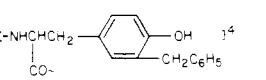
Biological Methods and Results. The compounds were tested for pressor activity in nephrectomized, pentolinium-treated male rats that had been anesthetized with pentobarbital.[24,25] The peptides were dissolved in normal saline containing 0.1% polyvinylpyrrolidone in siliconized glass containers. The molarities of peptide solutions, including the angiotensin standard, were based on peptide content found from amino acid analyses of the peptide hydrolysates. Pressor activities calculated on a molar basis for the compounds from the present study are listed in Table III.

Discussion

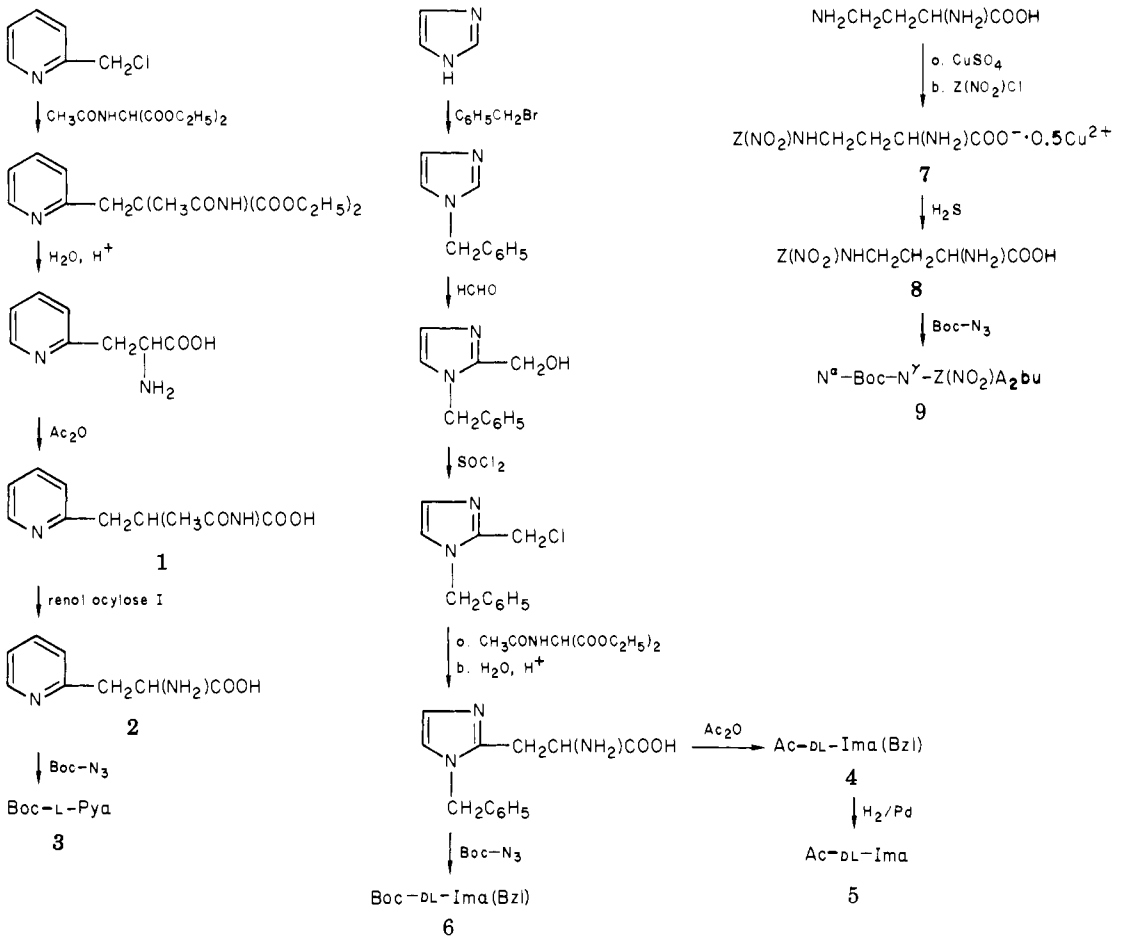
When the side-chain nitrogen was positioned one atom from the alanine backbone, the resultant pros nitrogen-containing analogues [Asn^1,Pya^6]AII, [Asn^1,Ima^6]AII, and [Asn^1,A_2bu^6]AII had, respectively, 3.5, 0.4, and 0.02% pressor activities. The significantly higher potency of the heterocyclic analogues than that of the aliphatic [Asn^1,A_2bu^6]AII analogue would imply an aromatic participation from position-6 histidine. However, enhancement or reduction of the π-electronic density in the aromatic analogues [Asn^1,Phe(4-NH_2)^6]AII and [Asn^1,Phe(4-NO_2)^6]AII did not give significantly different results (0.05 and 0.02% activity). Furthermore, the negligible pressor activities of the aromatic analogues were comparable to that of the aliphatic [Asn^1,A_2bu^6]AII analogue. This finding suggests a unique function for the heterocyclic nitrogens of histidine.

When the pressor activities of the heterocyclic analogues are compared, [Asn^1,Pya^6]AII (3.5%) is more active than [Asn^1,Ima^6]AII (0.4%), although β-(2-pyridyl)-L-α-alanine (Pya) differs from histidine in having only one heteroatom in a π-electron-deficient, six-membered ring, whereas the β-(2-imidazolyl)-L-α-alanine (Ima) is identical with histidine in every feature except the location of the alanine backbone relative to the ring nitrogens (Figure 1). Because the isohistidine analogue, [Asn^1,Ima^6]AII, is almost inactive, this result strongly suggests that the spatial

Table III. Relative Pressor Activities of [Asn^1,Ile^5]Angiotensin II Analogues in the Rat

angiotensin analogues	struct of position-6 substit	pressor act. in the rat
Asn^1,Val^5 (AII)		100
Asn^1,Ile^5,Pya^6		3.5
Asn^1,Ile^5,D-Pya^6		0.1
Asn^1,Ile^5,Ima^6		0.4
Asn^1,Ile^5,D-Ima^6		0.04
Asn^1,Ile^5,Phe(4-NH_2)^6	-NHCHCH$_2$-Ph-NH_2 (CO-)	0.05
Asn^1,Ile^5,Phe(4-NO_2)^6	-NHCHCH$_2$-Ph-NO_2 (CO-)	0.02
Asn^1,Tyr(3-Bzl)^{4}Ile^5,-Ph(4-NO_2)^6 and Ph(4-NO_2)^6		0.03
Asn^1,Ile^5,A_2bu^6	-NHCHCH$_2$CH$_2$ (CO- , NH_2)	0.02

Scheme I



alignment of the heterocyclic nitrogens is much more important than either retention of the imidazole structure or the degree of aromaticity for the position-6 analogues to elicit the angiotensin response. Moreover, the low but significant activity of the [Asn^1,Pya^6]AII analogue indicates that a pyridine N pros to the alanine backbone can contribute to the pressor activity. Since the [Asn^1,Ima^6]AII analogue has a *pros*-pyridine N and a *pros*-pyrrole NH, it is anticipated to be as active as [Asn^1,Pya^6]AII. The reduced potency of the [Asn^1,Ima^6]AII analogue suggests that the isohistidine ring may be perceived by the receptor as its energetically favorable tautomer, in which the two heterocyclic nitrogens share a proton and become equivalent, although this *pros*-pyrrole NH structure apparently cannot participate, or even may hinder, the angiotensin–receptor interaction.

Since the highly active [Pza^6]AII and native AII have a *pros*-pyridine N and a *tele*-pyrrole NH, whereas the relatively inactive [Asn^1,Ima^6]AII and [Asn^1,Pya^6]AII have pros nitrogens only, it can be concluded that the *tele*-pyrrole NH of histidine has a predominant role in the pressor activity of angiotensin II and that the *pros*-pyridine N makes a minor contribution to this response. This conclusion is in good agreement with earlier results of the methylhistidine analogues[21] in which [His(3-Me)^6]AII has a *pros*-pyridine N and 5% pressor activity, while the inactive [His(1-Me)^6]AII (0.05% activity) has neither of the biologically active *tele*-pyrrole NH or *pros*-pyridine N structures but contains a *tele*-pyridine N (Table II).

In infusion studies, [Asn^1,Ima^6]AII did not antagonize the pressor effect of angiotensin II in the rat. Similarly, Needleman et al.[21] and Freer et al.[16] reported no inhibition of angiotensin response by the position-6 analogues in vivo or in vitro. Since isohistidine is identical with histidine in molecular size and shape, the possibility that steric interference may hinder the hormone–receptor interaction can be eliminated in the [Asn^1,Ima^6]AII analogue. As a result, the inactivity of this analogue as either agonist or antagonist can be attributed to the lack of the *tele*-pyrrole NH in the imidazole side chain, which may be important for angiotensin II to bind to the receptor.

Previous replacement of histidine by its D isomer[22] in angiotensin II reduced the pressor activity from 100 to 4%. Similarly, substitution of [Asn^1,Pya^6]AII and [Asn^1,-Ima^6]AII by their respective D isomers reduced the pressor activities to 0.1 and 0.04%.

Chemistry. The synthesis of DL-Pya[26] and its resolution to (+)-Pya and (−)-Pya by L-(+)-tartaric acid[27] were reported, although it was not known which was the desired L isomer. Moreover, derivatives of DL-Pya, like Z-DL-Pya,[26] Z-(+)-Pya, and Z-(−)-Pya,[27] and dipeptides[26] containing glycine and DL-Pya were prepared.

In this report, DL-Pya was synthesized according to a reported procedure[26] (Scheme I) by condensing 2-(chloromethyl)pyridine hydrochloride with sodium diethylacetamidomalonate, followed by acid hydrolysis. L-Pya (**2**) was obtained by hog renal acylase I resolution of Ac-DL-Pya (**1**), which was prepared by acetylation of DL-Pya with acetic anhydride in glacial acetic acid. The optical rotation of L-Pya corresponded to that of (+)-Pya.[27] Reaction of L-Pya with excess Boc-N_3 by the Schnabel procedure[28] gave Boc-L-Pya (**3**) in 98% yield. When incubated with the *C. adamanteus* L-amino acid oxidase, followed by amino acid analysis of the remaining D-amino

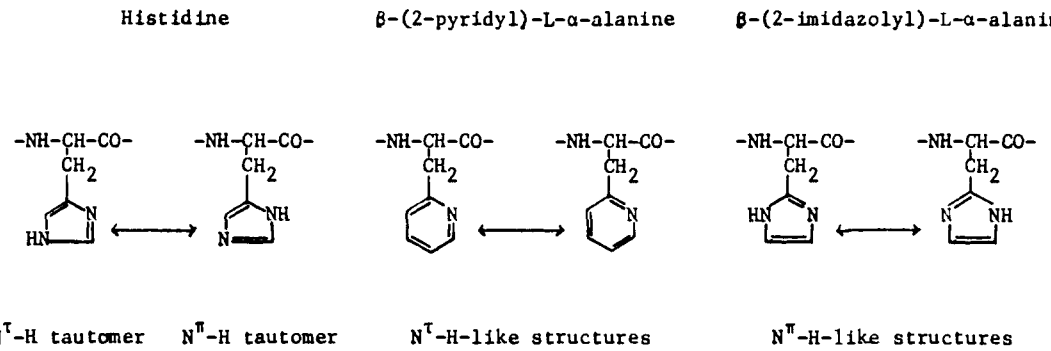


Figure 1. Tautomerization of the heterocyclic amino acids.

acid,[29] L-Pya and the deprotection product of Boc-L-Pya by HCl/AcOH contained over 99.8 and 99.6% of the L isomer. These results indicated that the optical purity of the L-Pya was preserved during introduction of the Boc group and its removal by HCl/AcOH.

Coupling of Z-DL-Pya with the amine component by the *N*-bromosuccinimide, the mixed anhydride, the azide, and the active *p*-nitrophenyl ester methods were reported[26] to give poor results. When Boc-L-Pya was incorporated into the Pro-Phe-resin in CH_2Cl_2 by DCC in the presence of equimolar 1-hydroxybenzotriazole (HOBt) to facilitate the coupling reaction and to suppress racemization,[29-31] instantaneous and intense coloration (dark brown) occurred. Hydrolysis of the resulting resin, followed by amino acid analysis, gave an equimolar ratio of all three amino acids, indicating quantitative incorporation of Boc-L-Pya to the Pro-Phe-resin in the stepwise solid-phase peptide synthesis. Incubation of the hydrolysate with L-amino acid oxidase, followed by amino acid analysis,[29] gave 43.5% undigestible D-Pya, which corresponded to 78% racemization[32] of the optically pure Boc-L-Pya during coupling, despite the addition of HOBt. No difficulty was observed in coupling the amine component of 2-pyridylalanine or in deprotection of the Pya-containing peptide by HBr/TFA and catalytic hydrogenation.

The diastereomeric mixture of [L-Pya^6]AII (**11**) and [D-Pya^6]AII (**12**) was separated by column chromatography on Sephadex G-25 eluted with 100:44 of *sec*-butyl alcohol–3% NH_4OH and purified further by ion-exchange chromatography. The isolated peptides were identified by amino acid analysis of an acid hydrolysate, followed by L-amino acid oxidase digestion.

Synthesis of the N^{im}-benzyl-β-(2-imidazolyl)-DL-α-alanine semihydrate, DL-Ima(Bzl), was carried out according to Jones,[33,34] with the exception of 1-benzylimidazole[35] being prepared directly from benzyl bromide and imidazole in Na/liquid NH_3. Hydroxymethylation of 1-benzylimidazole, followed by reaction with thionyl chloride, gave 1-benzyl-2-(chloromethyl)imidazole hydrochloride, which was condensed with sodium diethylacetamidomalonate and hydrolyzed.[32] DL-Ima(Bzl) is a convenient derivative of the amino acid 2-imidazolylalanine. The benzyl group protects the *imidazolyl* nitrogen from undesirable participation in reactions involving the α nitrogen.

Acetylation of DL-Ima(Bzl) by acetic anhydride in glacial acetic acid gave Ac-DL-Ima(Bzl) (**4**) in 97% yield. Reaction of DL-Ima(Bzl) with excess Boc-N_3 by the Schnabel procedure[28] gave Boc-DL-Ima(Bzl) (**6**) in 86% yield.

Preliminary enzymatic studies indicated that Ac-DL-Ima(Bzl) was not resolved by renal acylase I. When the N^{im}-benzyl protection group was removed by catalytic hydrogenation, the resulting Ac-DL-Ima was digested by acylase I to give L-Ima.

Incorporation of L-Ima into the peptide was not attempted, because extensive racemization of the heterocyclic amino acids, such as histidine[36] and β-(2-pyridyl)-L-alanine, was observed during DCC coupling, possibly through participation of the pros nitrogen, and β-(2-imidazolyl)alanine has two pros nitrogens. Furthermore, direct incorporation of Boc-DL-Ima(Bzl) into the peptide eliminated the need for the laborious enzymatic resolution and subsequent reprotection of the resolved L-Ima into Boc-L-Ima(Bzl) for peptide synthesis.

Stepwise solid-phase synthesis with Boc-DL-Ima(Bzl) gave the diastereomeric peptides. Separation of the mixture by countercurrent distribution between 5:2:5 of *n*-butyl alcohol–*tert*-butyl alcohol–0.4 M NH_4OAc gave the individual diastereomers.

The HF-resistant[37] *p*-nitrobenzyloxycarbonyl group, $Z(NO_2)$, was used to protect the γ-nitrogen of α,γ-diaminobutyric acid. This protection group is easily removed by catalytic hydrogenation. In order to introduce the $Z(NO_2)$ group selectively, the α-amino and carboxylic acid of α,γ-diaminobutyric acid (A_2bu) were chelated with copper,[38,39] which left the γ-amino group to react with *p*-nitrobenzyloxycarbonyl chloride. Treatment of the copper complex with H_2S removed the cupric ion and gave N^{γ}-$Z(NO_2)A_2bu$. Reaction of this γ-protected amino acid with excess Boc-N_3[28] gave N^{α}-Boc-N^{γ}-$Z(NO_2)A_2bu$, which was incorporated into the peptide. Stepwise solid-phase synthesis, followed by deprotection with HBr/TFA and catalytic hydrogenation, gave the [Asn^1,A_2bu^6]AII analogue.

Because *p*-nitrophenylalanine is reduced to *p*-aminophenylalanine by hydrogenation, [Asn^1,Phe(4-NO_2)^6]AII was prepared by stepwise solid-phase synthesis, followed by cleavage with liquid HF,[37] which removed the α-amino and side-chain protection groups simultaneously, such as the Z-group from Asn, the NO_2 group from Arg, and the Bzl group from Tyr. A side product, [Asn^1,Tyr(3-Bzl)^4,Phe(4-NO_2)^6]AII, which resulted from HF-catalyzed rearrangement of the benzyl ether of tyrosine,[40] was isolated in 23% yield. Catalytic hydrogenation of [Asn^1,-Phe(4-NO_2)^6]AII gave the corresponding *p*-aminophenylalanine analogue, [Asn^1,Phe(4-NH_2)^6]AII.

Conclusions. Our results suggest that the *tele*-pyrrole NH of histidine may make a major contribution to the pressor response of angiotensin II, in which the *pros*-pyridine N plays a minor role. Because replacement of the angiotension II sequence by D-amino acids usually gave inactive analogues,[41] the stereospecificity of the hormone–receptor interaction implies that there are at least three points of attachment or interaction between angiotensin II and the receptor. Our results indicate that position-6 histidine may be such a binding site; this is in addition to the previously identified recognition sites of the ionic carboxylate terminus[42] and the 4-tyrosine residue[1]

of angiotensin II. These findings suggest that enhancement of the *tele*-pyrrole NH structural feature in conjunction with elimination of the basic character in position 6 may provide highly active and tachyphylaxis-free angiotensin analogues. Introduction of such a structural feature may also be desirable in long-acting angiotensin inhibitors.[43-45]

Furthermore, our results indicate that the heterocyclic nitrogens of histidine in angiotensin II are different and nonexchangeable and that replacement of histidine by β-(2-imidazolyl)-L-α-alanine (Ima) and β-(2-pyridyl)-L-α-alanine (Pya) can unambiguously delineate the respective contribution by the *pros*-pyrrole NH and the *pros*-pyridine N of the histidine tautomers to the peptide–receptor interaction. This is significant in that, although spectroscopic methods like ^{13}C NMR have been valuable in elucidating the predominant tautomer of histidine in peptides,[6,46,47] in the event of a receptor-induced conformational change or a receptor-preferred minor conformer it would be difficult to relate the solution conformation of a linear and flexible peptide with its receptor-bound conformation, which is the biologically active species. Moreover, when general acid–base catalysis of histidine is not involved in the peptide–receptor interaction, it is conceivable that only a minor part of the preferred structure of histidine in the combination of the N^{τ}-H tautomer (*tele*-pyrrole NH–*pros*-pyridine N) and the N^{π}-tautomer (*tele*-pyridine N–*pros*-pyrrole NH) may be biologically significant, as shown in angiotensin (*tele*-pyrrole NH) and thyroliberin (*pros*-pyridine N),[9] even though both peptides show a preference for the N^{τ} structure of histidine.[7,8] In both situations, structure–activity correlation analysis with the novel amino acids Ima and Pya can verify a particularly favored structure of histidine in peptides at the receptor level and differentiate the specific roles of each heterocyclic nitrogen of histidine. Because β-(2-imidazolyl)-L-α-alanine is an isomer of histidine and retains the neighboring acid–base structure, it may also be an interesting substituent to evaluate the involvement of general acid–base catalysis of histidine in other peptides.

In this study, the synthesis of Ima and Pya derivatives and their incorporation into peptide by the solid-phase method did not present unusual problems. However, Boc-L-Pya racemized extensively (78%) upon C-terminal activation, despite the use of HOBt to suppress this undesirable reaction. Since heterocyclic amino acids like histidine are known to racemize easily via α-proton abstraction by the heterocyclic nitrogens,[48] racemization of Ima and Pya upon incorporation into a peptide can be a serious limitation for structure–activity correlation analysis using these amino acids. We found that both column chromatography and countercurrent distribution could separate the diastereomers of angiotensin II. Recent advances in high-performance liquid chromatography (LC) suggest that this technique may be especially suitable for semipreparative separation of peptide diastereomer pairs and for qualitative analysis to define the steric homogeneity of the peptide.[49,50] Further, the racemization of the heterocyclic amino acids may provide a useful and stringent system to evaluate the ability of various condensation agents and coupling additives to induce or to suppress racemization during peptide synthesis.

Experimental Section

Melting points (Thomas-Hoover Uni-Melt) are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif. Rotations were measured with a Bendix-NPL automatic polarimeter, Type 143A, equipped with a digital readout and printer. Precoated silica gel 60 F254 on glass plates (E. Merck) were used for TLC with the following solvents: I, *sec*-butyl alcohol–3% NH_4OH (100:44); II, butanol–AcOH–H_2O (4:1:5, upper phase); III, xylene–pyridine–AcOH (100:15:5); IV, butanol–AcOH–H_2O (3:1:1); V, *i*-Pr_2O–$CHCl_3$–AcOH (6:3:1); VI, Et_2O; VII, $CHCl_3$–MeOH (4:1). Electrophoresis was carried out on Whatman no. 1 paper (0.16-mm thick) at 5000 V using AcOH–HCOOH buffer, pH 1.85, in a Savant apparatus. E_H indicates electrophoretic mobility relative to histidine = 1.00. Peptides were hydrolyzed for 48 h under N_2 in constant-boiling HCl containing D-alanine as an internal standard. Hydrolyses were carried out in the presence of phenol to protect tyrosine from degradation. Amino acid analyses (Spinco Model 116 analyzer) were obtained using the standard 4-h method. Peptide content was calculated in terms of free peptide. The 2-(chloromethyl)pyridine hydrochloride was from Research Organic/Inorganic Chemical Corp., Sun Valley, Calif. The L-α,γ-diaminobutyric acid dihydrochloride was from Sigma Chemical Co., St. Louis, Mo. *p*-Nitrobenzyloxycarbonyl chloride was from Fox Chemical Co., Los Angeles, Calif.

N^{α}-Acetyl-β-(2-pyridyl)-DL-α-alanine (Ac-DL-Pya; 1). The β-(2-pyridyl)-DL-α-alanine[26] (19.9 g, 120 mmol) was dissolved in 150 mL of glacial AcOH, and Ac_2O (14.1 mL, 150 mmol) in 50 mL of glacial AcOH was added. The mixture was heated under reflux for 3 min and then treated with H_2O and charcoal for 30 min. The suspension was filtered, and the filtrate was lyophilized. Recrystallization of the residue from MeOH–Me_2CO–$CHCl_3$–anhydrous Et_2O gave 21.8 g (yield 87%; mp 161.5–163.5 °C), which contained a trace of unreacted DL-Pya. A 18.1-g portion was chromatographed on a 5 × 35 cm column of Dowex Ag 1-X2 (acetate, 50–100 mesh). Elution of the column with H_2O gave 0.55 g of DL-Pya. The column was washed further with 750 mL of H_2O and eluted with 1 N AcOH. The appropriate fractions were combined and then repeatedly evaporated from H_2O to dryness. Recrystallization of the residue from MeOH–Me_2CO–anhydrous Et_2O gave 11.4 g (63%): TLC R_f (II) 0.085, R_f (VII) 0.1; E_H 0.38; mp 165–166 °C. Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

Previous preparations of Ac-DL-Pya in scales from 3 to 20 mmol under the same conditions did not result in unreacted Pya, and gave a higher overall yield (76%) of the product. At elevated temperature or prolonged reaction duration, the reaction mixture darkened and the yield of the product was reduced.

β-(2-Pyridyl)-L-α-alanine (Pya; 2). N^{α}-Acetyl-β-(2-pyridyl)-DL-α-alanine (1; 11.4 g, 55 mmol) was dissolved in 250 mL of H_2O, and the solution was adjusted to pH 7.4 with dilute AcOH and dilute NH_4OH. Hog renal acylase I (140 mg, activity 20 mmol/mg for acetylmethionine) was added to the solution, and the suspension was incubated for 12 h at 37 °C. Fresh acylase I (80 mg) was added, and the suspension was incubated for another 24 h, then concentrated in vacuo, and acidified to pH 5 with glacial AcOH. The suspension was treated with charcoal at 80 °C for 10 min and filtered. The filtrate was lyophilized repeatedly from H_2O. The residue was chromatographed on a 1.25 × 30 cm column of Dowex AG 1-X2 (acetate, 50–100 mesh) and eluted with H_2O. The appropriate fractions were combined, concentrated, and crystallized from H_2O–EtOH–Me_2CO to give 1.57 g (34%): TLC R_f (II) 0.1, R_f (VII) 0.02; mp 199.5–200.5 °C; $[\alpha]^{23}_D$ +48.9° (*c* 2.1, 1 N HCl) [lit.[27] yield 82%; mp 199–200 °C; $[\alpha]^{23}_D$ +36° (c 1, 1 N HCl)]. Anal. ($C_8H_{10}N_2O_2$) C, H, N.

N^{α}-(*tert*-Butyloxycarbonyl)-β-(2-pyridyl)-L-α-alanine (Boc-Pya; 3). The β-(2-pyridyl)-L-α-alanine (2; 1.57 g, 9.5 mmol) was dissolved in 15 mL of H_2O, *tert*-butyloxycarbonyl azide (2.72 g, 19 mmol) in 5 mL of dioxane was added, and the mixture was stirred at pH 10 (pH stat, 3 N NaOH) for 24 h. More *tert*-butyloxycarbonyl azide (2.72 g, 19 mmol) in 5 mL of dioxane was added, and the reaction was continued for another 24 h. The mixture was washed with Et_2O, acidified to pH 4 with 6 N HCl, and extracted with $CHCl_3$. The $CHCl_3$ layers were combined, dried (Na_2SO_4), and evaporated to dryness. Recrystallization of the residue from $CHCl_3$–Me_2CO–heptane gave 2.48 g (98%): TLC R_f (II) 0.33; $[\alpha]^{23}_D$ −6.92 (*c* 2.01, 1 N HCl); mp 146–146.5 °C. Anal. ($C_{13}H_{18}N_2O_4$) C, H, N.

N^{α}-Acetyl-N^{im}-benzyl-β-(2-imidazolyl)-DL-α-alanine Monohydrate [Ac-DL-Ima(Bzl); 4]. The N^{im}-benzyl-α-(2-imidazolyl)-DL-β-alanine semihydrate[33] (320 mg, 1.2 mmol) was

dissolved in 1.4 mL of glacial AcOH and Ac_2O (0.13 mL, 1.5 mmol) in 1 mL of glacial AcOH was added. The solution was stirred at 100 °C for 2 h and then heated under reflux for 3 min. The mixture was treated with H_2O and then lyophilized. The residue was decolorized with charcoal and recrystallized from MeOH–$CHCl_3$–Me_2CO–Et_2O to give 355 mg (97%): TLC R_f (I) 0.03, R_f (II) 0.29; mp 136.5–138 °C. Anal. ($C_{15}H_{19}N_3O_4$) C, H, N.

N^{α}-Acetyl-β-(2-imidazolyl)-DL-α-alanine (Ac-DL-Ima; 5). Compound 4 (305 mg, 1.05 mmol) was hydrogenated with 150 mg of Pd/C under 2 atm of H_2 for 18 h. The suspension was filtered, and the filtrate was concentrated and lyophilized. Recrystallization of the residue from MeOH–$CHCl_3$–Me_2CO gave 160 mg (80%): TLC R_f (I) 0, R_f (II) 0.09; mp 234.5 °C dec. Anal. ($C_8H_{11}N_3O_3$) C, H, N.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{im}-benzyl-β-(2-imidazolyl)-DL-α-alanine [Boc-DL-Ima(Bzl); 6]. The N^{im}-benzyl-β-(2-imidazolyl)-DL-α-alanine semihydrate[33] (1.52 g, 6 mmol) was suspended in the mixture of dioxane–H_2O, and *tert*-butyloxycarbonyl azide (1.72 g, 12 mmol) in dioxane was added. The mixture was stirred at pH 10 (pH stat, 3 N NaOH) for 24 h. More *tert*-butyloxycarbonyl azide (1.72 g, 12 mmol) was added, and the reaction was continued for 6 days. The mixture was washed with $CHCl_3$–Et_2O, acidified with 6 N HCl to pH 4.0, and extracted with $CHCl_3$. The $CHCl_3$ solution was combined, dried (Na_2SO_4), and evaporated to dryness. Recrystallization of the residue from MeOH–$CHCl_3$–Et_2O–heptane gave 1.48 g (86%): TLC R_f (I) 0.37, R_f (II) 0.47; mp 198–199 °C. Anal. ($C_{18}H_{23}N_3O_4$) C, H, N.

Copper Complex of N^{γ}-(*p*-Nitrobenzyloxycarbonyl)-L-α,γ-diaminobutyric Acid (7). To the aqueous copper complex[38,39] solution of α,γ-diaminobutyric acid dihydrochloride (4.78 g, 25 mmol) *p*-nitrobenzyloxycarbonyl chloride (6.5 g, 30 mmol) in dioxane was added. The reaction mixture was stirred at pH 10 (pH stat, 3 N NaOH) for 19 h and filtered. The precipitate was washed with H_2O, Me_2CO, and Et_2O and dried to give 5.1 g (62%), mp 227–229 °C.

N^{γ}-(*p*-Nitrobenzyloxycarbonyl)-L-α,γ-diaminobutyric Acid [N^{γ}Z(NO_2)A_2bu; 8]. Compound 7 (5.1 g, 15.5 mmol) was dissolved in a mixture of concentrated HCl–H_2O–MeOH and filtered. The filtrate was saturated with H_2S and centrifuged. The supernatant and hot H_2O washings of the precipitate were combined and evaporated to dryness. The residue was dissolved in MeOH, neutralized to pH 7 with Et_2NH, and cooled to 5 °C. The precipitate was washed with MeOH and Et_2O and recrystallized from H_2O to give 3.3 g (77%): TLC R_f (II) 0.36, R_f (IV) 0.44; mp 210–212 °C; $[\alpha]^{23}_D$ +1.23° (*c* 1.265, AcOH). Anal. ($C_{12}H_{15}N_3O_6$) C, H, N.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{γ}-(*p*-nitrobenzyloxycarbonyl)-L-α,γ-diaminobutyric Acid [N^{α}-Boc-N^{γ}-Z(NO_2)A_2bu; 9]. To the suspension of compound 8 (2.98 g, 10 mmol) in 20 mL of dioxane, *tert*-butyloxycarbonyl azide (2.14 g, 15 mmol) in dioxane was added. The mixture was stirred at pH 10 (pH stat, 3 N NaOH) for 36 h, washed with EtOAc–Et_2O, acidified with concentrated HCl to pH 1, and extracted with EtOAc and Et_2O. The extract was dried (Na_2SO_4) and evaporated to dryness. Recrystallization of the residue from Me_2CO–H_2O gave 2.2 g (55%): TLC R_f (II) 0.73, R_f (IV) 0.72; mp 131–133 °C; $[\alpha]^{23}_D$ −16.8° (c 2.0, Me_2CO). Anal. ($C_{17}H_{23}N_3O_8$) C, H, N.

Asn-Arg-Val-Tyr-Ile-DL-Pya-Pro-Phe (10). The Boc-Phe-polymer (2.8 g, 0.56 mmol) was prepared and acylated in a stepwise manner[43] with 2.2 mmol of Boc-Pro, Boc-Pya in the presence of equimolar HOBt·H_2O, Boc-Ile, Boc-Tyr(Bzl), Boc-Val, Boc-Arg(NO_2), and 3.3 mmol of Z-Asn-ONp. Cleavage of the octapeptide-polymer by HBr/TFA gave 571 mg of peptide. Hydrogenation of a 170-mg portion in 15 mL of 4% AcOH in 1:1 MeOH–H_2O with 87 mg of 10% Pd/C under 1 atm of H_2 for 17 h gave 140 mg of the diasteriomeric peptides **10**.

The peptide was chromatographed on a 2.5 × 90 cm column of Sephadex G-25 (fine, 20–89 mesh) and eluted with the mixture of 100:44 of *sec*-butyl alcohol–3% NH_4OH at 12 mL/h. The eluate was monitored at 280 nm and was analyzed by TLC and electrophoresis. The fractions from 82 to 126 mL and from 170 to 218 mL were identical by electrophoresis. The fractions from 82–126 mL had higher R_f values in TLC than the fractions from 170–218 mL, and each fraction was devoid of the other in both TLC solvent systems I and II. The fraction from 170–218 mL was chromatographed on a 2.5 × 90 cm column of carboxymethylcellulose (Whatman CM-52, NH_4^+) and eluted with an NH_4OAc gradient (7 × 10^{-5} M NH_4OAc/mL of H_2O starting from 0.03 M NH_4OAc; flow rate 42 mL/h). The eluate was monitored at 280 and 254 nm and was analyzed by TLC. The appropriate fractions were combined, and the peptide was further purified through the picrate salt[1] to give 15 mg of [Asn[1],Ile[5],L-Pya[6]]angiotensin II (**11**): TLC R_f (I) 0.13, R_f (II) 0.17; E_H 0.69. An acid hydrolysate gave the following amino acid ratios: Asp, 0.98; Arg, 1.00; Val, 1.01; Tyr, 0.98; Ile, 0.99; Pya, 0.98; Pro, 0.97; Phe, 1.02; peptide content was 90%. When eluted with pH 4.30 (0.2 N) sodium citrate buffer for 1 h followed by pH 5.26 (0.35 N) sodium citrate buffer for 1 h, the β-(2-pyridyl)-α-alanine emerged from the short column at 20 mL (34.5 min) with a color value of 75% of that of arginine (59 mL, 103 min). A 48-h acid hydrolysate incubated with L-amino acid oxidase for 48 h showed the following amino acid ratios: Asp, 0.80; Arg, 0.04; Val, 0.02; Tyr, 0.04; Ile, 0.02; Pya, 0.22; Pro, 1.00; Phe, 0.04. A mixture of amino acids subjected to the same hydrolytic and enzymatic procedures had the following amino acid ratios: Arg, 0.04; Val, 0.02; Tyr, 0.04; Ile, 0.02; Phe, 0.04; Pya, 0.20; A_2bu, 0.04; Phe(4-NO_2), 0.04; Phe(4-NH_2), 0.00. Asp is attacked too slowly by L-amino acid oxidase to be checked by this method.

Chromatography of the fraction of 82–126 mL on CM-52 (NH_4^+), followed by picrate treatment as described for **11**, gave 15 mg of [Asn[1],Ile[5],D-Pya[6]]AII (**12**): TLC R_f (I) 0.21, R_f (II) 0.20; E_H 0.69. An acid hydrolysate had the following amino acid ratios: Asp, 0.98; Arg, 1.00; Val, 1.01; Tyr, 0.98; Ile, 0.99; Pya, 0.98; Pro, 0.97; Phe, 1.02; peptide content was 90%. A 48-h acid hydrolysate incubated with L-amino acid oxidase for 48 h showed the following amino acid ratios: Asp, 0.82; Arg, 0.03; Val, 0.01; Tyr, 0.03; Ile, 0.01; Pya, 0.76; Pro, 1.00; Phe, 0.03.

Asn-Arg-Val-Tyr-Ile-DL-Ima-Pro-Phe (13). Synthesis was carried out on 1 mmol of Pro-Phe-polymer as described previously,[43] and Boc-DL-Ima(Bzl) was incorporated in the presence of equimolar HOBt·H_2O. Acidolysis of the peptide-polymer by HBr/TFA gave 750 mg of peptide, which was hydrogenated in a mixture of 2:5:5 HOAc–MeOH–H_2O with 600 mg of Pd/C under 2 atm of H_2 for 48 h to give 445 mg of the diasteriomeric peptides **13**.

Partition of the peptides between 4:2:5 of *n*-butyl alcohol–*tert*-butyl alcohol–0.4 M NH_4OAc by countercurrent distribution for 400 transfers gave two fractions. The fraction of lower partition coefficient (tubes 200–277, K = 1.5) was chromatographed on a 2.5 × 100 cm column of CM-52 (NH_4^+) and eluted with an NH_4OAc gradient as for compound **10**. The appropriate fractions were combined, and the peptide was further purified through the picrate salt to give 52 mg of [Asn[1],Ile[5],L-Ima[6]]AII (**14**): TLC R_f (I) 0.16, R_f (II) 0.15; E_H 0.69. An acid hydrolysate had the following amino acid ratios: Asp, 1.00; Arg, 1.00; Val, 1.01; Tyr, 1.01; Ile, 0.97; Ima, 1.02; Pro, 1.01; Phe, 1.00; peptide content was 91%. When eluted with pH 5.26 (0.35 N) sodium citrate buffer for 60 min, β-(2-imidazolyl)-α-alanine emerged from the short column at 19 mL (32.5 min) with a color value of 66% of that of arginine (31 mL, 54 min). A 60-h acid hydrolysate incubated with L-amino acid oxidase for 48 h showed the following amino acid ratios: Asp, 0.90; Arg, 0.05; Val, 0.02; Tyr, 0.02; Ile, 0.01; Ima, 0.12; Pro, 1.00; Phe, 0.02.

The fraction of higher partition coefficient (tubes 309–375, K = 5.9) gave 213 mg of peptide. A portion (99 mg) was chromatographed on a 2.5 × 100 cm column of CM-52 (NH_4^+) and eluted with an NH_4OAc gradient as for compound **10**. The appropriate fractions were combined, and the peptide was further purified through the picrate salt to give 25 mg of [Asn[1],Ile[5],D-Ima[6]]AII (**15**): TLC R_f (I) 0.21, R_f (II) 0.14; E_H 0.69. An acid hydrolysate had the following amino acid ratios: Asp, 0.98; Arg, 1.02; Val, 1.02; Tyr, 1.01; Ile, 0.98; Ima, 1.04; Pro, 1.00; Phe, 1.01; peptide content was 92%. A 60-h acid hydrolysate incubated with L-amino acid oxidase for 48 h showed the following amino acid ratios: Asp, 0.84; Arg, 0.05; Val, 0.02; Tyr, 0.05; Ile, 0.01; Ima, 0.39; Pro, 1.00; Phe, 0.04.

Previous attempts to resolve [Asn[1],Ile[5],DL-Ima[6]]AII into the individual diastereomers by use of a Sephadex G-25 column eluted with the mixture of 100:44 of *sec*-butyl alcohol–3% NH_4OH gave partial but incomplete separation. Further chromatography of the partially resolved diastereomeric peptides on CM-52 (NH_4^+) did not improve the resolution.

Asn-Arg-Val-Tyr-Ile-A_2bu-Pro-Phe (16). Synthesis was carried out on 0.25 mmol of Pro-Phe-polymer as described previously,[43] and α,γ-diaminobutyric acid was incorporated as N^{α}-Boc-N^{γ}-Z(NO_2)A_2bu (**9**). Acidolysis of the peptide-polymer by HBr/TFA gave 240 mg of peptide. A portion (130 mg) was hydrogenated with 180 mg of Pd/C under 3 atm of H_2 for 48 h to give 95 mg of **16**.

The peptide was chromatographed on a 1.2 × 80 cm column of sulfoethylcellulose (Cellex SE, NH_4^+) and eluted with an NH_4OAc gradient (6.5×10^{-5} M NH_4OAc/mL of 1 N HOAc starting from 4.6×10^{-2} M NH_4OAc in 1 M HOAc, flow rate 19 mL/h). Fractions containing the peptide were combined to give 49 mg, which was not homogeneous to TLC. Rechromatography of the peptide (49 mg) on Cellex SE (NH_4^+) gave 20 mg, which was further purified on a 2.5 × 100 cm column of CM-52 (NH_4^+) with an NH_4OAc gradient (7×10^{-5} M NH_4OAc/mL starting from 0.03 M NH_4OAc, 42 mL/h). The appropriate fractions were combined, and the peptide was further purified through the picrate salt to give 6 mg of **16**: TLC R_f (I) 0.13, R_f (II) 0.12; E_H 0.71. An acid hydrolysate had the following amino acid ratios: Asp, 1.00; Arg, 1.02; Val, 1.04; Tyr, 1.00; Ile, 1.00; A_2bu, 1.03; Pro, 0.97; Phe, 0.98; peptide content was 78%. When eluted with pH 5.26 (0.35 N) sodium citrate buffer, α,γ-diaminobutyric acid emerged from the short column at 17 mL (28.5 min) with a color value of 96% that of arginine (31 mL, 54 min). A 48-h acid hydrolysate incubated with L-amino acid oxidase showed the following amino acid ratios: Asp, 0.84; Arg, 0.05; Val, 0.02; Tyr, 0.06; Ile, 0.02; A_2bu, 0.15; Pro, 1.00; Phe, 0.04.

Asn-Arg-Val-Tyr-Ile-Phe(4-NO_2)-Pro-Phe (17). Synthesis was carried out on 0.8 mmol of Pro-Phe-polymer as described previously[43] with 3.2 mmol of Boc-Phe(4-NO_2), Boc-Ile, Boc-Tyr(Bzl), Boc-Val, Aoc-Arg(Tos), and 4.8 mmol of Z-Asn-ONp. Cleavage of the peptide-polymer with liquid HF at 0 °C for 1 h in the presence of anisole gave 552 mg.

Partition of the peptide between 4:1:5 of 1-butanol–AcOH–H_2O by countercurrent distribution for 200 transfers gave two fractions. The fraction of K = 1.3 was combined to give 166 mg of peptide. A portion (100 mg) was chromatographed on a 2.5 × 76 cm column of Sephadex C-25 (NH_4^+) and eluted with an NH_4OAc gradient (6×10^{-5} M NH_4OAc/mL of 3 N HOAc starting from 0.12 M NH_4OAc in 3 M HOAc). The appropriate fractions were combined, and the peptide (31 mg) was further purified through the picrate salt to give 21 mg of **17**: TLC R_f (I) 0, R_f (II) 0; E_H 0.40. An acid hydrolysate had the following amino acid ratios: Asp, 1.02; Arg, 1.01; Val, 1.01; Tyr, 0.97; Ile, 1.01; Phe(4-NO_2), 1.01; Pro, 1.00; Phe, 0.97; peptide content was 82%. When eluted with pH 5.26 (0.35 N) sodium citrate buffer, Phe(4-NO_2) emerged from the short column at 11 mL (18.5 min) with a color value of 82% that of arginine (31 mL, 54 min). A 48-h acid hydrolysate incubated with L-amino acid oxidase for 48 h showed the following amino acid ratios: Asp, 0.71; Arg, 0.04; Val, 0.02; Tyr, 0.03; Ile, 0.04; Phe(4-NO_2), 0.09; Pro, 1.00; Phe, 0.03.

The fraction of K = 2.8 was combined to give 72 mg of peptide. A portion (30 mg) was chromatographed on a 2.5 × 80 cm column of Cellex SE (NH_4^+) and eluted with an NH_4OAc gradient (3.3×10^{-5} M NH_4OAc/mL of 1 N HOAc, flow rate 48 mL/h). The appropriate fractions were combined, and the peptide (9 mg) was further purified through the picrate salt to give 3 mg of [Asn[1],Tyr(3-Bzl)[4],Ile[5],Phe(4-NO_2)[6]]AII (**18**): R_f (I) 0, R_f (II) 0.25; E_H 0. An acid hydrolysate had the following amino acid ratios: Asp, 1.00; Arg, 1.00; Val, 1.00; Tyr(3-Bzl), 0.92; Ile, 1.00; Phe(4-NO_2), 0.98; Pro, 0.98; Phe, 1.02; peptide content was 76%. A 48-h acid hydrolysate incubated with L-amino acid oxidase for 60 h showed the following amino acid ratios: Asp, 0.65; Arg, 0.05; Val, 0.02; Tyr(3-Bzl), 0.21; Ile, 0.03; Phe(4-NO_2), 0.07; Pro, 1.00; Phe, 0.04.

Asn-Arg-Val-Tyr-Ile-Phe(4-NH_2)-Pro-Phe (19). A portion (66 mg) of the peptide (**17**), which was partially purified by countercurrent distribution (K = 1.3), was hydrogenated in 1:1 MeOH–H_2O containing a drop of HOAc with 30 mg of Pd/C under 1 atm of H_2 for 12 h to give 60 mg.

Partition of the peptide between 4:1:5 1-butanol–AcOH–0.15 M NH_4OAc by countercurrent distribution for 300 transfers gave 38 mg (K = 1.4). The peptide was chromatographed on a 2.5 × 80 cm column of Cellex SE (NH_4^+) and eluted with an NH_4OAc gradient (5×10^{-5} M NH_4OAc/mL of 3 N AcOH). The appropriate fractions were combined, and the peptide (39 mg) was further purified through the picrate salt to give 30 mg of **19**: R_f (I) 0.16, R_f (II) 0.16; E_H 0.63. An acid hydrolysate had the following amino acid ratios: Asp, 0.99; Arg, 1.00; Val, 1.01; Tyr, 1.00; Ile, 1.01; Phe(4-NH_2), 0.98; Pro, 0.98; Phe, 1.01; peptide content was 88%. A 48-h acid hydrolysate incubated with L-amino acid oxidase showed the following amino acid ratios: Asp, 0.04; Arg, 0.02; Val, 0.01; Tyr, 0.04; Ile, 0.03; Phe(4-NH_2), 0.09; Pro, 1.00; Phe, 0.05.

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References and Notes

(1) (a) The abbreviations used to denote amino acids and peptides are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature: *J. Biol. Chem.*, **247**, 911 (1972), and *Biochemistry*, **6**, 362 (1967). Other abbreviations are: AII, angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe); Pya, β-(2-pyridyl)alanine; Ima, β-(2-imidazolyl)alanine; Phe(4-NO_2), 4-nitrophenylalanine; Phe(4-NH_2), 4-aminophenylalanine; A_2bu, 2,4-diaminobutyric acid; Z(NO_2), *p*-nitrobenzyloxycarbonyl; Tyr(3-Bzl), 3-benzyltyrosine; HOBt, 1-hydroxybenzotriazole; DCC, *N,N*-dicyclohexylcarbodiimide. A *pros*-pyridine N represents a pyridine-like nitrogen atom, –N=, proximal to the alanine side chain, and a *tele*-pyrrole NH represents a pyrrole-like nitrogen, –NH–, distal to the alanine side chain. (b) For paper 13 in this series, see K. H. Hsieh, I. C. Kiraly-Olah, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, **22**, 1044 (1979). (c) This report was presented in part at the 3rd American Peptide Symposium, 1972.

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(32) When L-Pya was treated under regular hydrolytic conditions for peptides, 20% of D-Pya resulted. If the percent of racemization of the peptide is expressed as two times the percent of D isomer found in the reaction product, no racemization occurs when no D isomer is found, and total racemization occurs when the reaction product gives 50% D and 50% L isomer. When 43.5% D-Pya was found in the hydrolysate of Boc-Pya-Pro-Phe-polymer as the sum of the racemization during DCC coupling and acid hydrolysis, $43.5\% = (1 - 0.2)D + 0.2L$. D and L represent the percent of the D- and L-Pya-containing tripeptide resulting from the coupling. Therefore, D and L total 100% of the product tripeptide, and 20% of either D or L is converted to the opposite isomer by acid hydrolysis. The amount of D-Pya-containing tripeptide can be calculated from $43.5\% = (1 - 0.2)D + 0.2(1 - D)$, where $0.6D = 0.235$. The amount of D-Pya-containing peptide was $0.235/0.6 = 39\%$ of the total tripeptide; thus, DCC coupling caused 78% of the Boc-L-Pya to racemize.
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Biologically Active Polycycloalkanes. 6.[1] Antiviral 1-Tricyclo[4.3.1.1^{2,5}]undecyl Derivatives

Yoshiaki Inamoto,* Koji Aigami, Naotake Takaishi, Yoshiaki Fujikura, Motoyoshi Ohsugi, Hiroshi Ikeda, Kiyoshi Tsuchihashi,

Tochigi Research Laboratories, Kao Soap Company, Ltd., Ichikaimachi, Tochigi 321-34, Japan

Akira Takatsuki, and Gakuzo Tamura

Department of Agricultural Chemistry, The University of Tokyo, Tokyo 113, Japan. *Received January 17, 1979*

Functionalization reactions via cationic intermediates of tricyclo[4.3.1.1^{2,5}]undecane (**2**) were investigated to prepare derivatives with potential antiviral activities. Bromination of **2** took place regiospecifically at C-1, and the resulted bromide **5** was converted into the hydroxide **9**, the carboxylic acid **12**, and the amine **22**, from which were synthesized a variety of secondary derivatives, including homologous esters **10** and **20**, amides **14** and **19**, carbamates **24**, and ureas **17** and **25**. The hydroxide **9**, the acid **12**, and the acetamide **21** were also obtainable directly from tricyclo[5.2.1.0^{2,6}]dec-*endo*-2-ylcarbinol (**1**), the precursor for the synthesis of the hydrocarbon **2**. Success in these functionalization–rearrangements was attributed to the inability of the intermediate 2-1-yl cation (**2**$^+$) for further skeletal isomerizations. Among the 1-substituted derivatives of **2** prepared, the amine hydrochlorides (**16** and **23**), a few esters (**20b** and **20d**), and some *N*-alkylamides (**19c**, **19d**, and **19e**) exhibited marked antiviral activities as compared to amantadine hydrochloride, when tested in vitro on a monolayer culture of chick embryo fibroblasts against Newcastle disease virus.

Since the discovery in 1964 of the antiviral activity of amantadine (1-aminoadamantane)[2] which has a characteristic bridged tricyclic structure, a number of efforts have been devoted for the synthesis of a wide variety of its analogues having larger activities with broader antiviral spectra and less CNS effects. While the modifications of the 1-amino group to form substituted amines mainly effected variations in specificity to individual virus, as well as extent of side effects,[3] entire replacements of the amino by other functional groups only slightly modified the activity, as measured in vitro on chick embryo cells against NDV.[4] In contrast to this, a change in the tricyclic alkyl residue from 1-adamantyl to 4-homoisotwistyl (tricyclo[5.3.1.0^{3,8}]undecyl) resulted in a drastic enhancement of the activity for amino derivatives.[5] The evidence suggested the possibility of access to still more potent compounds by appropriate choice of the bridged polycyclic structure. Tricyclo[4.3.1.1^{2,5}]undecane (**2**), another tricycloalkane recently prepared by us,[6] was now functionalized, and the resulting derivatives, as well as their secondary derivatives,

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