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Conformationally Restricted Analogs of Histamine H_1 Receptor Antagonists. *trans*- and *cis*-1,5-Diphenyl-3-dimethylaminopyrrolidine

Patrick E. Hanna* and Ahmed E. Ahmed

Departments of Pharmacology and Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455.
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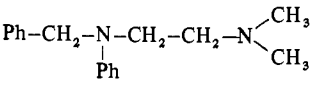
The syntheses of *trans*- and *cis*-1,5-diphenyl-3-dimethylaminopyrrolidine (**2** and **3**) are described. Compounds **2** and **3** were assayed for histamine H_1 receptor antagonist activity on the isolated guinea-pig ileum. The trans isomer **2** is a potent and selective long-acting histamine antagonist while the cis isomer **3** is a potent reversible antagonist. The results are discussed with regard to the conformational requirements for histamine antagonist activity.

The influence of stereochemical factors upon histamine antagonist activity is well recognized[1,2] and several authors have proposed specific conformational requirements for antihistamine activity at H_1 receptors.[3-9] The assumption is usually made that the competitive nature of histamine antagonism indicates that the antagonists interact directly with the histamine receptor area. However, in spite of both the observed stereoselectivity of antihistaminic agents at H_1 receptors and the apparent affinity of these drugs for the receptors, antihistamines exhibit a broad range of pharmacological actions.[1] In view of the fact that most of the commonly employed antihistamines are flexible molecules which would be expected to be capable of assuming conformations suitable for interaction with a variety of biological receptors, we initiated a study designed to examine the influence of conformational restrictions and the importance of steric and spatial relationships upon histamine antagonist activity.

The purpose of this report is to describe the synthesis and preliminary pharmacological evaluation of *trans*- and *cis*-1,5-diphenyl-3-dimethylaminopyrrolidine (**2** and **3**). Compounds



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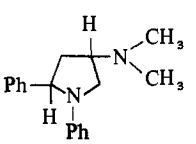

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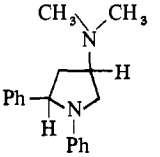
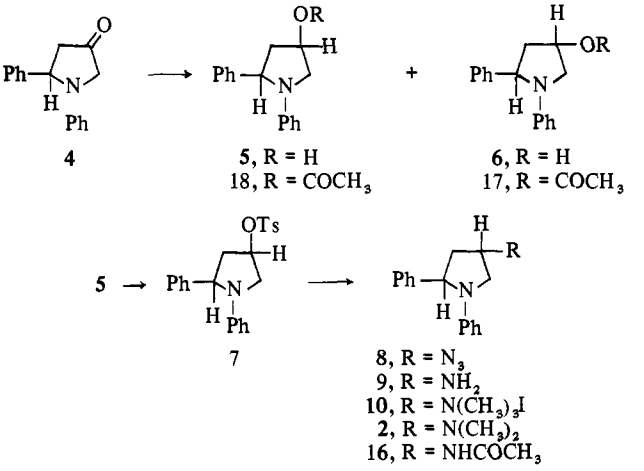


3

2 and **3** are semirigid cyclic analogs of the ethylenediamine antihistamine phenbenzamine (**1**). It was felt that the slight flexibility inherent in these substituted pyrrolidines would be advantageous since compounds possessing generally suitable steric orientations would be capable of a limited degree of adaptation to the requirements of the receptor surface while structures having unfavorable steric arrangements would be prohibited from undergoing large conformational adjustments in order to bind. In addition, the use of the pyrrolidine nucleus required the introduction of only a single CH_2 unit to the phenbenzamine structure in order to achieve conformational restrictions.

Synthesis. The key intermediate for the preparation of **2** and **3** was 1,5-diphenyl-3-pyrrolidone (**4**, Scheme I) which was synthesized according to the four-step sequence described by Southwick and Dimond.[10] Treatment of **4** with $NaBH_4$ afforded a mixture of *cis*- and *trans*-1,5-di-

Scheme I



phenyl-3-hydroxypyrrolidine (**5** and **6**). The cis isomer **5** was readily isolated in 73% yield by fractional crystallization from cyclohexane. Preparative tlc of the residue obtained by evaporation of the filtrate afforded an additional 17% of **5** and also the trans alcohol **6** in 5% yield. The trans isomer was converted directly to the crystalline acetate **17** for further characterization. There are two previous reports

of the preparation of 1,5-diphenyl-3-hydroxypyrrolidine, but the relative stereochemistry of the products was not determined.[10,11] Our results, discussed below, indicate that the previous workers isolated the cis alcohol **5**.

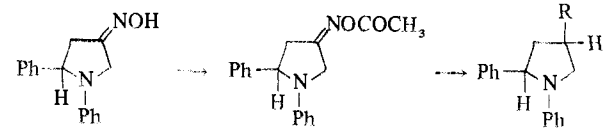
The nmr spectrum of **17** shows the expected methyl signal at δ 2.03 whereas the acetoxymethyl signal of the *cis*-acetate **18** appears at δ 1.76. This difference in the chemical shifts of the acetoxymethyl groups indicates a shielding effect by the aromatic π cloud of the 5-phenyl substituent in **18**. Apparently the 1-phenyl substituent, which might be expected to reside preferentially in a configuration trans to the 5-phenyl, does not exert a similar shielding effect on the acetoxymethyl protons of **17**. Resonance interaction between the ring nitrogen and the 1-phenyl substituent would tend to hold the 1-phenyl group in the plane of the pyrrolidine ring and thus reduce the probability of such a shielding effect in **17**. This reasoning supports our stereochemical assignments and is consistent with the uv data obtained with **2** and **3** (see below) as well as the stereoselectivity of the $NaBH_4$ reduction of **4**. A high degree of resonance interaction between the ring nitrogen and the 1-phenyl group reduces steric crowding on the side of the ring opposite the 5-phenyl substituent and permits greater ease of approach of hydride ions from the side trans to the 5-phenyl. This results in preferential formation of the cis alcohol **5**.

Also of interest are the differences in the nmr absorption patterns displayed by the C-2 methylene protons of **17** and **18**. The C-2 protons of the *cis*-acetate **18** appear as a narrow multiplet at δ 3.75 ($W_{1/2}$ = 6 Hz), whereas the corresponding C-2 protons of **17** appear as separate multiplets. One of the protons appears as a broadened doublet at δ 3.51 and the other appears as a four-line multiplet at δ 4.13. It is apparent that the conformation of the ring in the trans isomer **17** places the C-2 protons in distinctly different chemical environments in which one of the C-2 protons couples very weakly with the C-3 proton. An assumption may be made that the higher field proton is the one which is cis to the 1 phenyl substituent. However, the relative influences of the 1-phenyl, the nitrogen lone pair, and the 3-acetoxy group on the chemical shifts of the C-2 protons are difficult to assess and assignments regarding these two protons must remain tentative. The nmr spectra of the alcohols **5** and **6** are similar to those of the acetates in that the C-2 methylene protons of **5** appear as a narrow multiplet at δ 3.71 ($W_{1/2}$ = 6 Hz) and those of **6** appear as four-line multiplets at δ 3.38 and 3.71.

The cis alcohol **5** was transformed to the tosylate **7** which upon treatment with NaN_3 underwent SN2 displacement to yield the *trans*-azide **8**. Reduction of **8** with $NaBH_4$ in refluxing 2-propanol provided the *trans*-amine **9** in 79% yield. Compound **2** was obtained by conversion of **9** to the trimethylammonium derivative **10** according to the method of Sommer and coworkers[12] followed by treatment of **10** with $LiAlH_4$.[13]

For the preparation of **3** (Scheme II), the ketone **4** was transformed to its oxime derivative **11** which upon treatment with acetic anhydride in pyridine afforded **12**. The oxime acetate **12** was stereoselectively converted to the *cis*-amine **13** by reduction with B_2H_6. The stereoselectivity of the B_2H_6 reduction was confirmed by acetylation of a portion of the crude amine and integration of the two CH_3 signals in the nmr spectrum of the product. The spectrum of the crude acetamide exhibits singlets at δ 1.71 and 1.91 in a ratio of 85:15. These are assigned to the methyl groups of the *cis*- and *trans*-acetamides, respectively, by the same reasoning employed for the stereochemical designation of

Scheme II

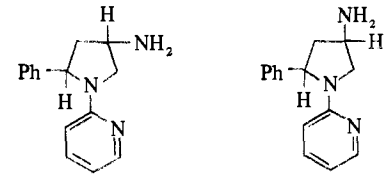


11 12 13, R = NH_2; 14, R = $N(CH_3)_3I$; 3, R = $N(CH_3)_2$; 15, R = $NHCOCH_3$

17 and **18** (see above). Acetylation of the *trans*-amine **9** yielded the acetamide **16** which shows a single methyl resonance at δ 1.91. The fact that the yield of the acetamide obtained by acetylation of **9** was comparable to that obtained by acetylation of the crude product from the B_2H_6 reduction of **12** shows that the 85:15 cis:trans ratio of acetamides is an accurate indication of the stereoselectivity of the diborane reduction. Thus, both the $NaBH_4$ reduction of **4** and the B_2H_6 reduction of **12** take place preferentially from the side of the ring trans to the 5-phenyl substituent.

When the crude product of the B_2H_6 reduction of **12** was allowed to stand in the cold, crystallization would sometimes occur and a sample of the *cis*-amine **13** which was apparently free (nmr) of trans isomer was collected. This was not, however, a practical procedure and the crude amine was usually treated directly with CH_3I to afford **14** after several recrystallizations. Treatment of **14** with $LiAlH_4$ yielded **3**. In the nmr spectrum of **3**, the C-5 benzylic proton appears as a four-line multiplet at δ 4.73 while the C-5 proton of **2** appears as a broadened doublet at δ 4.85. Additionally, the spectrum of **3** displays a multiplet at δ 3.75 ($W_{1/2}$ = 10 Hz) which is assigned to the C-2 protons while in the spectrum of **2**, the C-2 protons appear as two triplets at δ 3.85 and 3.30. These distinguishing spectral features permit ready differentiation of the cis and trans isomers **3** and **2**.

The designation of the relative stereochemistry of the compounds described herein is further supported by characteristic similarities in the nmr spectra of **9** and **13** with certain features found in the spectra of *trans*- and *cis*-5-phenyl-3-amino-1-(2-pyridyl)pyrrolidine (**19** and **20**). The



19 20

relative stereochemistry of **19** and **20** has been established.† The C-4 methylene protons of **9** appear as a broadened triplet at δ 2.13 and the C-4 protons of **19** also appear as a broadened triplet at δ 2.11. However, in the case of the cis isomers **13** and **20**, each C-4 proton appears as a distinct broad multiplet. In the nmr spectrum of **13**, the C-4 protons appear at δ 1.90 and 2.75 and the corresponding C-4 protons of **20** are seen as multiplets at δ 1.85 and 2.68.

Biological Results. Compounds **2** and **3** were evaluated for histamine antagonist activity on the isolated guinea-pig ileum.[14] Preliminary reports of these studies have been presented.[15,16] The trans isomer inhibited the contractile re-

† The relative stereochemistries of **19** and **20** were determined by chemical methods which were not applicable to the 1-phenyl derivatives. This work will be the subject of a forthcoming paper. P. E. Hanna, unpublished results, University of Minnesota, 1973.

sponse to histamine at concentrations as low as $2 \times 10^{-9} M$, but the antagonistic effect could not be readily reversed by repeated washing of the tissue. Studies were then undertaken to compare the duration of histamine antagonist action of **2** on the guinea-pig ileum with the duration of action of the potent ethylenediamine antihistamine tripelennamine. Figure 1 shows the recovery of the response to histamine with time after a single exposure of the tissue to the antagonists followed by repeated washing (see Experimental Section). Following a 15-min exposure to **2** ($1 \times 10^{-8} M$), the response to $4 \times 10^{-6} M$ histamine was inhibited 85% while a 15-min exposure to a tenfold greater concentration ($1 \times 10^{-7} M$) of tripelennamine caused a 75% inhibition of the contractile response. The response to histamine did not exceed 45% of control 3 hr after treatment with **2**, whereas recovery after exposure to tripelennamine reached 70% of control within 10 min. The contractile response to $4 \times 10^{-7} M$ acetylcholine was not inhibited by **2** or by tripelennamine in these experiments. In other studies with the guinea-pig ileum, it was found that a concentration of $4 \times 10^{-6} M$ of **2** was required to produce an 85% inhibition of the contractile response to acetylcholine ($4 \times 10^{-7} M$).

These studies demonstrate that the trans isomer **2** is a potent, selective, and long-acting H_1 receptor[17] antagonist. Whether the long duration of action of **2** may be attributed exclusively to an "ideal" relative stereochemical configuration which produces a very high degree of affinity for the H_1 receptor cannot be deduced from the present evidence. However, it is reasonable to conclude that **2** contains the requisite structural features for an effective drug-receptor interaction.

The cis isomer **3** was found to be a potent reversible histamine antagonist which produced parallel shifts in the histamine dose-response curve at concentrations of 5×10^{-8}, 1×10^{-7}, and $2 \times 10^{-7} M$. The calculated pA_2 value[18] averaged 7.97 for three tissues. This value is comparable with the values reported for diphenhydramine (7.68–8.14) and it is somewhat lower than the values of 8.49–9.00 reported for tripelennamine.[3]

Conclusions

The results obtained with **2** and **3** do not support the suggestion that a fully extended trans conformation about the C-C bond of the dimethylaminoethyl grouping in antihistamines is essential to activity.[3,4,19] The requirement for a trans conformation was proposed on the basis of conformational similarities found in the crystal structures of histamine[20,21] and two antihistamines.[3,4,19] However, a search of the literature does not reveal pharmacological evidence that either histamine or histamine antagonists bind to their biological receptor(s) in the same conformations observed in the solid state. Indeed, Casy, Ison, and Ham have demonstrated that histamine in solution exists as approximately equal proportions of the trans and gauche rotamers and Ham has shown that several ethylenediamine antihistamines are not exclusively in the trans conformation in solution.[22,23] Since trans conformations are not necessarily retained exclusively in aqueous solution, there appears to be no compelling reason to assume that they would be retained in physiological media or when bound to receptors. Although both **2** and **3** are potent histamine H_1 receptor antagonists, neither compound is capable of attaining a fully extended *trans*-N-C-C-N conformation. The conformational rigidity of the pyrrolidine ring in **2** and **3** is enhanced by phenyl-nitrogen orbital overlap which restricts the ability of the nitrogen atoms to approach a fully extended conformation. Additionally, Witiak and coworkers recently published studies on the stereoselective antihistaminic properties of L-(*S*)- and D-(*R*)-3-ethylamino-1-phenylpyrrolidine in which pA_2 values of 5.92 and 4.92 were calculated for the L-*S* and D-*R* isomers, respectively.[24,25] These semirigid compounds also cannot achieve a fully extended *trans*-N-C-C-N conformation.

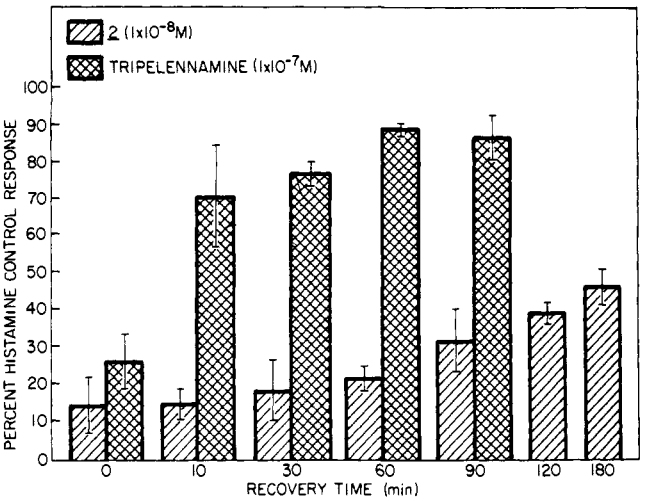
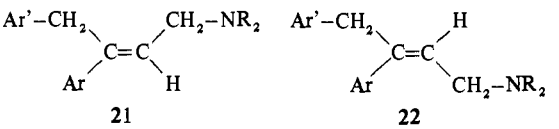


Figure 1. Duration of antihistamine action of **2** and tripelennamine on the isolated guinea pig ileum. Each bar represents the mean of three experiments with **2** or two experiments with tripelennamine. Vertical lines represent SE (**2**) and range (tripelennamine). 100% control response is defined as the mean response to $4 \times 10^{-6} M$ histamine in these experiments.

It is of interest to consider the results obtained with **2** and **3** in light of the conclusions of Casy and Ison regarding stereochemical influences upon antihistamine activity.[6] Casy and Ison evaluated the ability of a series of *cis*- and *trans*-1,2-diphenyl-4-amino-2-butenes (**21** and **22**) to inhibit histamine-induced contractions of isolated guinea-pig ileum. They found that in each instance a cis (H/Ar) (**21**) disposition of groups around the double bond is optimal



for activity and that a trans (H/Ar) arrangement (**22**) resulted in decreased activity. The authors proposed that structurally less rigid antihistamines may adopt biologically active conformations similar to **21** in which the Ar group and the carbon-carbon double bond are approximately coplanar. The plane of the second aryl group (Ar′) then lies at an approximate right angle to the Ar(C=C) plane with the $ArCH{=}CHCH_2N$ unit lying close to a mean plane. In the biologically active conformation proposed for the ethylenediamine antagonists (*e.g.*, **1**), a CH_2N grouping replaces the carbon-carbon double bond of **21** and maximal N-Ar resonance effects are invoked to maintain the required coplanarity.

Comparison of a Dreiding model of **21** with models of **2** and **3** shows that when the ArC=C unit of **21** is superimposed upon the 1-$PhNCH_2$ portion of **2** or **3**, the $Ar'CH_2$ moiety of **21** readily assumes an orientation which is essentially identical with that of the 5-PhCH of **2** and **3**. This superimposition of models permits the freely rotating amino group of **21** to acquire an orientation which is

identical with that of the dimethylamino groups of *either* 2 or 3. However, when the ArC=C and $Ar'CH_2$ portions of a model of 22 are superimposed upon the 1-$PhNCH_2$ and 5-PhCH groups of a model of 2 or 3, it becomes apparent that it is impossible for the amino substituent of the butene analog to approach the spatial orientation of the dimethylamino groups of either of the pyrrolidines.

Examination of molecular models of 2 and 3 also revealed the existence of steric interaction between the 1-phenyl and 5-phenyl substituents. It is possible that steric crowding might inhibit the development of coplanarity between the 1-phenyl and the pyrrolidine ring nitrogen. A near planar arrangement of these groups is critical to the overall molecular shape proposed by Casy and Ison.[6] In order to determine whether the 5-phenyl substituent exerts a measurable effect on the resonance interaction between the pyrrolidine nitrogen and the 1-phenyl substituent, the bands at 250 nm in the uv spectra of 2 and 3 were studied. The molar absorptivity of this band has been found to correlate well with the degree of phenyl-nitrogen resonance.[26] The molar absorptivity of 2 was found to be 19,750 while that of 3 is 17,000. This compares with a value of 19,100 found for 1-phenylpyrrolidine[26] and it indicates a high degree of Ph-N resonance overlap in 2 and 3.

It is concluded therefore that the structural and conformational requirements stipulated by Casy and Ison[6] represent a satisfactory approximation of the molecular conformation of H_1 receptor-bound histamine antagonists. It is further proposed that there is not a strict requirement for the fully extended *trans*-N-C-C-N conformation for H_1 receptor blockade by ethylenediamine antihistamines but rather that a range of values for this torsional angle is suitable for effective drug-receptor interaction.

Experimental Section

Melting points were obtained on a Thomas-Hoover Unimelt and a Mel-Temp apparatus and are uncorrected. Nmr spectra were recorded on a Varian Associates A-60-D spectrometer in $CDCl_3$ using tetramethylsilane as the internal standard. Ir spectra were recorded on Perkin-Elmer 237B and Perkin-Elmer Infracord spectrophotometers. All ir and nmr spectra were consistent with assigned structures. Uv spectra were recorded in hexane with a Beckman DB-GT spectrophotometer and microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind.

cis- and *trans*-1,5-Diphenyl-3-hydroxypyrrolidine (5 and 6). Sodium borohydride (0.95 g, 0.025 mol) was added in small portions to 2.37 g (0.01 mol) of 1,5-diphenyl-3-pyrrolidone (4) in 200 ml of 95% EtOH. The mixture was stirred at room temperature for 24 hr and cooled in an ice bath, and 25% AcOH was added until the pH was about 4. A 10% solution of NaOH was added to adjust the pH to 8 and the mixture was concentrated *in vacuo* to approximately 50 ml. The concentrate was extracted with $CHCl_3$, washed with water, dried ($MgSO_4$), and evaporated to afford 2.41 g of a yellow mass. Crystallization from cyclohexane afforded 1.74 g (73%) of 5, mp 91–92° (lit.[10,11] 92.5–94°, 88–90°). The filtrate was concentrated and purified by preparative tlc on alumina ($CHCl_3$). Extraction of the two principal bands with $CHCl_3$ yielded an additional 0.40 g (17%) of 5 and 0.17 g (5%) of 6 as a golden oil.

The trans alcohol 6 was further purified by chromatography on a 10-g column of Al_2O_3 (neutral). Elution with C_6H_6 containing increasing concentrations of $CHCl_3$ provided 0.12 g of 6. The nmr and ir spectra were in agreement with the assigned structure (see discussion).

trans-1,5-Diphenyl-3-acetoxypyrrolidine (17). Compound 6 (0.12 g, 0.0005 mol) was dissolved in pyridine (3 ml) and Ac_2O (3 ml) and the solution was stirred overnight at room temperature in a stoppered flask. The solution was poured into 20 ml of ice-cold dilute Na_2CO_3 and was extracted with $CHCl_3$, washed (H_2O), and dried (Na_2SO_4). Evaporation *in vacuo* and crystallization of the residue from MeOH afforded 0.035 g (20%) of 17, mp 145–146°. *Anal.* ($C_{18}H_{19}NO_2$) C, H, N.

cis-1,5-Diphenyl-3-acetoxypyrrolidine (18). The cis alcohol 5 (1.0 g, 0.004 mol) was dissolved in pyridine (10 ml) and Ac_2O (10 ml), and the solution was allowed to stand 24 hr at room temperature. The solution was poured onto ice, extracted with CH_2Cl_2, washed with 5% HCl and H_2O, and dried ($MgSO_4$). Evaporation afforded 0.90 g of a clear, brown viscous material which was purified by chromatography on 30 g of neutral Al_2O_3. Elution with C_6H_6–EtOAc mixtures provided 0.30 g (25%) of 18 as an oil. *Anal.* ($C_{18}H_{19}NO_2$) C, H, N.

cis-1,5-Diphenyl-3-hydroxypyrrolidine *p*-Toluenesulfonate (7). The cis alcohol 5 (2.39 g, 0.01 mol) was dissolved in 15 ml of pyridine and cooled to 0°, and *p*-toluenesulfonyl chloride (3.81 g, 0.02 mol) was added in one portion. After standing 48 hr in the cold, the mixture was poured into ice water with vigorous stirring. The solid was collected by filtration, washed with water, dried, and recrystallized from $CHCl_3$–petroleum ether (bp 30–60°) to yield 2.5 g (67%) of 7, mp 144–145°. *Anal.* ($C_{23}H_{23}NO_3S$) C, H, N.

trans-1,5-Diphenyl-3-azidopyrrolidine (8). The tosylate 7 (11.80 g, 0.03 mol) was dissolved in 120 ml of DMF and 3.25 g (0.05 mol) of NaN_3 in 16 ml of H_2O was added. The solution was stirred under N_2 at 70° for 5 hr, cooled, and poured into 2 l. of cold saturated NaCl. The aqueous mixture was extracted with ether, washed with saturated NaCl and H_2O, and dried ($MgSO_4$). Evaporation of the ether gave an oily residue (7.60 g, 97%) which was used without further purification.

trans-1,5-Diphenyl-3-aminopyrrolidine (9). The azide 8 (16.25 g, 0.06 mol) was dissolved in 285 ml of 2-propanol and 7.40 g (0.11 mol) of $NaBH_4$ was added in several portions. After 48 hr of stirring under reflux, the mixture was cooled and 10% HOAc was added dropwise to destroy excess $NaBH_4$. The mixture was made basic by the addition of 5% NaOH and 2-propanol was removed *in vacuo*. The aqueous mixture was then extracted with ether, dried ($MgSO_4$), and evaporated to yield 11.60 g (80%) of the crude amine 9, mp 83–86°. A sample of 9 was converted to the acetamide 16 for elemental analysis.

trans-1,5-Diphenyl-3-acetamidopyrrolidine (16). The amine 9 (0.88 g, 0.0037 mol) was dissolved in 45 ml of 5% HCl and 5% NaOH was added until the solution became slightly turbid. The turbidity was removed by addition of a few drops of 5% HCl. Ice chips were added followed by Ac_2O (9 ml) and then by 9.0 g of NaOAc in 9 ml of H_2O. After cooling in an ice bath a white solid was collected by filtration. After drying, the solid was recrystallized from $CHCl_3$–petroleum ether (bp 30–60°) to yield 0.85 g (85%) of 16, mp 165°. *Anal.* ($C_{18}H_{20}N_2O$) C, H, N.

trans-1,5-Diphenyl-3-trimethylammoniopyrrolidine Iodide (10). The *trans*-amine 9 (0.50 g, 0.0021 mol) and tributylamine (0.778 g, 0.0042 mol) were dissolved with stirring in EtOAc (25 ml). CH_3I (1.19 g, 0.0084 mol) was added in several portions and the mixture was stirred overnight at room temperature in a stoppered flask. The mixture was diluted with 25 ml of EtOAc and the white precipitate was collected by filtration, washed with ether, and dried. Recrystallization from EtOH afforded 0.35 g (41%) of 10, mp 249–250°. *Anal.* ($C_{19}H_{25}N_2I$) C, H, N.

trans-1,5-Diphenyl-3-dimethylaminopyrrolidine (2). A solution of 10 (5.50 g, 0.0125 mol) in 200 ml of dry THF was cooled in an ice bath and $LiAlH_4$ (1.90 g, 0.05 mol) was added in several portions. The reaction mixture was then stirred and heated under reflux in a N_2 atmosphere for 18 hr. After cooling in an ice bath, excess $LiAlH_4$ was destroyed by addition of 10% NaOH and H_2O. The white precipitate was removed by filtration and washed with ether. The combined organic fractions were evaporated *in vacuo* and 10% NaOH was added to the residue which was then extracted with ether and dried ($MgSO_4$). Evaporation afforded 3.1 g (90%) of a solid, mp 112–116°. An analytically pure sample was recrystallized from 2-propanol, mp 115.5–117.5°. *Anal.* ($C_{18}H_{22}N_2$) C, H, N.

trans-1,5-Diphenyl-3-dimethylaminopyrrolidine hydrochloride was prepared in quantitative yield by passing dry HCl through a cold, stirring solution of 2 in anhydrous ether for 1 hr. After standing in the cold overnight, the solid hydrochloride was collected by filtration and recrystallized from EtOH–ether to give a white crystalline solid, mp 263–264°. *Anal.* ($C_{18}H_{23}N_2Cl$) C, H, N.

1,5-Diphenyl-3-pyrrolidone Oxime (11). Hydroxylamine hydrochloride (19.00 g, 0.27 mol) was dissolved in 115 ml of H_2O and 75 ml of 10% NaOH. The ketone 4 (7.50 g, 0.032 mol) was added along with sufficient 95% EtOH (approximately 800 ml) to produce a clear solution. After heating for 15 min on a steam bath, the solution was concentrated *in vacuo* which resulted in precipitation of the oxime 11 (7.25 g, 88%), mp 137–140° (lit.[10] mp 136–138°).

1,5-Diphenyl-3-pyrrolidone Oxime Acetate (12). Acetic anhydride (15 ml) was added to a solution of the oxime 11 (2.00 g,

0.0079 mol) in 20 ml of pyridine and the solution was heated on a steam bath for 15 min. The hot solution was poured onto 400 ml of ice water and was stirred vigorously for 15 min. A precipitate formed which was collected by filtration, washed with H_2O and 10% Na_2CO_3, and dried under vacuum. The dried residue (mp 95–100°) was recrystallized from EtOH–H_2O to yield 1.35 g (58%) of **12**, mp 105–106°. *Anal.* ($C_{18}H_{18}N_2O_2$) C, H, N.

An alternative procedure for the preparation of **12** involved pouring the reaction mixture onto ice and making the mixture basic (pH 8) with 10% Na_2CO_3. The mixture was then extracted with ether, washed with 2.5% HCl and water, and dried ($MgSO_4$). Evaporation afforded a material which was pure enough for use in the next reaction. The product did not crystallize in every instance.

cis-1,5-Diphenyl-3-aminopyrrolidine (13). To a cold solution of **12** (3.0 g, 0.01 mol) in 50 ml of THF was added dropwise 100 ml (0.1 mol) of 1 *M* BH_3 in THF. The solution was stirred under N_2 at room temperature for 20 hr and again cooled in ice. There was then added dropwise 100 ml of 5% HCl. THF was removed *in vacuo* and the residue was extracted with ether. The aqueous residue was treated with 120 ml of 5% NaOH and extracted with several portions of ether. The combined ether extracts were washed with H_2O and 5% HCl. The acidic phase was made basic with 5% NaOH and extracted with ether and dried ($CaSO_4$). Evaporation *in vacuo* afforded a residue which solidified upon standing in the cold. The solid residue was washed with a 1:5 solution of cyclohexane and petroleum ether (bp 30–60°) and collected to give 1.95 g (80%) of crystalline material, mp 62–66°. A sample of **13** (mp 64–66°) free of the trans isomer (see discussion) was prepared by recrystallization from cyclohexane–petroleum ether (bp 30–60°). The amine **13** was converted to the acetamide **15** for elemental analysis.

The stereochemical composition of the crude product from B_2H_6 reduction of **12** was determined in the following manner. A sample (0.78 g, 0.003 mol) of the crude reduction product was dissolved in 45 ml of 5% HCl and 5% NaOH was added until the solution became slightly turbid. Sufficient 5% HCl was added to remove the turbidity and a few ice chips were added followed by 9 ml of Ac_2O. The mixture was stirred vigorously and NaOAc (9.0 g) in 9 ml of H_2O was added immediately. After cooling in an ice bath 0.75 g (84%) of an 85:15 mixture (nmr) of the *cis*- and *trans*-acetamides was collected, mp 176–181°

cis-1,5-Diphenyl-3-acetamidopyrrolidine (15). A purified sample of *cis*-amine **13** (1.77 g, 0.007 mol) was dissolved in 90 ml of 5% HCl and small portions of 5% NaOH were added until the solution became slightly turbid. Addition of several drops of 5% HCl removed the turbidity and ice chips were added followed by 18.0 ml of Ac_2O. The mixture was stirred vigorously and a solution of NaOAc (18.0 g) in 18 ml of H_2O was added in one portion. After cooling in an ice bath, a precipitate formed which was collected and recrystallized from EtOH–H_2O to yield 1.50 g (78%) of **15**, mp 193°. *Anal.* ($C_{18}H_{20}N_2O$) C, H, N.

cis-1,5-Diphenyl-3-trimethylammoniopyrrolidine Iodide (14). The unpurified amine (2.38 g, 0.01 mol) obtained by BH_3 reduction of **12** was dissolved in 100 ml of EtOAc to which tributylamine (3.70 g, 0.02 mol) was added. CH_3I (5.68 g, 0.04 mol) was added in several portions and the mixture was stirred overnight in a stoppered flask at room temperature. An additional 2.0 g of CH_3I was added 2 hr prior to termination of the reaction. The white precipitate was collected and washed with EtOAc and ether. Three recrystallizations from EtOAc–MeOH afforded 0.98 g (24%) of **14**, mp 242–243°. *Anal.* ($C_{19}H_{25}N_2I$) C, H, N.

cis-1,5-Diphenyl-3-dimethylaminopyrrolidine (3). To a cold solution of **14** (5.50 g, 0.013 mol) in 200 ml of dry THF was added $LiAlH_4$ (1.90 g, 0.05 mol) in several portions. The mixture was heated under reflux in a N_2 atmosphere for 18 hr, cooled in ice, and treated with 10% NaOH and H_2O. The precipitate was filtered and washed with ether and the filtrate and ether washings were combined and evaporated *in vacuo*. The residue was made basic (pH 10) with aqueous NaOH and extracted with ether and dried ($CaSO_4$). Evaporation *in vacuo* afforded 2.90 g (83%) of **3**, mp 98–103°. An analytically pure sample (mp 101–103°) was prepared by recrystallization from *n*-heptane. *Anal.* ($C_{18}H_{22}N_2$) C, H, N.

cis-1,5-Diphenyl-3-dimethylaminopyrrolidine hydrochloride was prepared in quantitative yield by passing dry HCl through a cold, stirring ether solution of **3** for 1 hr. After standing overnight in the cold, the solid hydrochloride was collected and recrystallized from EtOH–ether to provide a white crystalline solid, mp 266°. *Anal.* ($C_{18}H_{23}N_2Cl$) C, H, N.

Pharmacology. The experiments in which the duration of action of **2** was compared with the duration of action of tripelennamine were carried out on the isolated guinea-pig ileum which was prepared according to a standard method.[14] The ileum was bathed in Tyrodes solution at 37° and bubbled with air. The antagonists (as the hydrochloride salts) were kept in contact with the ileal tissue for 15 min prior to the addition of agonists. The tissue was then washed at 5-min intervals with fresh Tyrodes solution, and after each wash either histamine (4×10^{-6} *M*) or acetylcholine (4×10^{-7} *M*) was added. The concentration of histamine employed in these experiments produced a near maximal contraction prior to the introduction of antagonists. The response to acetylcholine was not inhibited by **2** or by tripelennamine in the concentrations employed and the response to acetylcholine did not fall below control levels during the period of the experiments. The final bath concentration of **2** was 1×10^{-8} *M* and the final bath concentration of tripelennamine was 1×10^{-7} *M* in each set of experiments.

The antihistaminic activity of **3** was measured on the isolated guinea-pig ileum suspended in Tyrodes solution at 37° [14] A stream of air was bubbled through the medium. Tissues were allowed to stabilize for a minimum of 15 min before introduction of agonists. Histamine was introduced at regular intervals until reproducible responses were obtained and a dose-response curve was then constructed by giving histamine at 3-min intervals. Concentrations of 5×10^{-8}, 1×10^{-7}, and 2×10^{-7} *M* of **3** produced parallel shifts in the dose-response curve. The drug was washed out of the tissue and the original standard dose-response curve was reproduced before proceeding to the next concentration of drug. Washing the tissue at 5-min intervals for 30 min was usually sufficient to terminate the antagonistic effect of **3**.

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Angiotensin II. Synthesis and Biological Activity of 6-(1-Methylhistidine) and 6-(3-Methylhistidine) Analogs

Philip Needleman,* Garland R. Marshall, and Jean Rivier

Departments of Pharmacology and of Physiology and Biophysics, Washington University Medical School, St. Louis, Missouri 63110.
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The synthesis and purification of [1-MeHis^6]angiotensin II and [3-MeHis^6]angiotensin II are described. The [3-MeHis^6] analog was about 5% as active as angiotensin II in contracting isolated smooth muscle preparations, as a vasopressor agent, and in releasing prostaglandin from isolated perfused rabbit kidneys. [1-MeHis^6]angiotensin II had little or no biological activity. Neither of the analogs had any antagonistic activity. The effect of pH on the uterine sensitivity to histidine analogs and to *N*-acetyl angiotensin II indicates that neither the state of protonation of the α-amino or imidazole groups nor a conformation charge associated with titration of these groups accounts for the increased sensitivity of the uterus to angiotensin at alkaline pH.

The histidine moiety of angiotensin II (AII) has been implicated in the pH-dependent sensitivity of uterine strips to angiotensin analogs[1-3] and to the development of *in vitro* tachyphylaxis.[2] Substitution of the imidazole ring of histidine by a methyl group has been reported for the hypothalamic thyrotropin releasing factor[4] (pGlu-His-Pro-NH_2, TRF) and the hypothalamic luteinizing hormone releasing factor[5] (pGlu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, LRF). [3-MeHis^2]-TRF was found to have 800% of the biological activity of TRF whereas its isomer [1-MeHis^2]-TRF is essentially inactive. [1-MeHis^2]-LRF and [3-MeHis^2]-LRF were found to have 3 and 6%, respectively, of LRF biological activity. Thus, the role of nitrogen 1 and nitrogen 3 of the imidazole ring of histidine in TRF and LRF seems to be different. This is substantiated by the fact that substitution by Gly or elimination of His^2-residue in LRF yielded two analogs exhibiting definitive inhibitory biological properties.[6] In order to examine further the role of histidine in biologically active peptides, we have synthesized [1-MeHis^6]- and [3-MeHis^6]angiotensin II and report here their biological properties.

Results†

The two octapeptides [1-MeHis^6]-AII and [3-MeHis^6]-AII were synthesized by the solid-phase methodology; these two analogs were evaluated for their biological activity.

Oxytocic Effects of Angiotensin Analogs. In isolated rat uterine strips, at pH 6.8, [3-MeHis^6]-AII was about 10% as active as AII, whereas [1-MeHis^6]-AII had only low levels of biological activity (0.1%) (Table I). Incubation of uterine strips with 2 μg/ml of [1-MeHis^6]-AII did not alter the AII oxytocic response. Under more alkaline conditions (pH 8.1) the AII and [3-MeHis^6]-AII dose-response curves were shifted to the left indicating a twofold increase in sensitivity. There was no change in [1-MeHis^6]-AII response at pH 8.1.

Pressor Effects. [3-MeHis^6]-AII was about 5% as active as AII in elevating rat blood pressure and the [1-MeHis^6]-AII was only 0.05% as active (Table II). Thus, the relative activity in the intact animal and uterine strips was about the same. Neither MeHis analog inhibited AII pressor responses nor lowered renal hypertension in rats (not shown).

Prostaglandin Release. For release of a half-maximal amount of prostaglandin-like substance (PLS) and for a half-maximal contraction of a rat stomach strip, a dose of 5-10 μg of [3-MeHis^6]-AII was required (1.5-3% of AII), whereas the [1-MeHis] analog was inactive in both systems (Table III). The [3-MeHis] analog was somewhat more effective in releasing PLS from the kidney than in directly contracting rat stomach strips. Infusion of [1-MeHis^6]-AII did not inhibit the AII induced release of PLS.

Table I. Comparison of the Spasmogenic Action of Angiotensin Analogs on Isolated Rat Uterine Strips at pH 6.8 and 8.1[a]

	Amt of analog needed to produce a half-maximal uterine contraction, ng/ml	
Peptide analogs	pH 6.8	pH 8.1
AII	25	7
N-Acetyl-AII	25	8
[3-MeHis^6]-AII	100	48
[1-MeHis^6]-AII	10,000	10,000

[a]The maximal response achieved with the angiotensin analogs was 2.5 g of uterine tension. The values represent the means determined on strips isolated from five animals. The standard errors were 15% or less. Each strip served as its own control, so that AII dose-response curves were tested before and after the MeHis^6 analogs. The oxytocic effects of the analogs were completely reversible. None of the analogs possessed AII inhibitory activity at either pH.

Table II. Pressor Response of Rats to AII Analogs[a]

Peptide analogs	Δ_{25}, μg/kg
AII	0.1 (8)
[3-MeHis^6]-AII	2.0 (4)
[1-MeHis^6]-AII	200 (4)

[a]Δ_{25} indicates the dosage of analog required to produce a 25% increase in mean blood pressure. The Δ_{25} was obtained from the graphic plot of the dose-response curves for each analog. These curves were parallel to the pressor curve for angiotensin II. The standard errors were 10% or less. The number in parentheses indicates the number of animals tested.

†The following abbreviations are utilized in this manuscript: PLS, prostaglandin-like substance; P_4T_8, [Phe^4,Tyr^8]angiotensin II; and AII, angiotensin II.