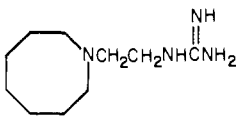
Conformational Analogues of Antihypertensive Agents Related to Guanethidine

R. F. Borne,* M. L. Forrester, and I. W. Waters

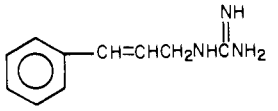
School of Pharmacy, University of Mississippi, University, Mississippi 38677. *Received October 21, 1976*

In an effort to clarify the conformational requirements, if any, of agents producing adrenergic neuronal blockade through mechanisms similar to guanethidine, the synthesis and pharmacological evaluation of 15 analogues of cinnamylguanidine are described. These analogues represent derivatives in which the distance between the center of the ring system and the guanidinium nitrogen atom varies from 3.9 to 6.2 Å. While conformational relationships could not be defined in this study, three analogues (**3**, **4**, and **5**) were apparently more potent than guanethidine in the in vitro assay employed.

Among the many classes of drugs which have been developed for the treatment and management of hypertension, guanethidine (**1**) has been widely used in cases of severe malignant hypertension. The characteristic action of guanethidine involves inhibition of responses to stimulation of adrenergic nerves but this action is not

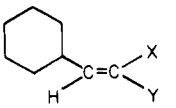


1

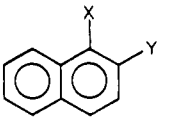


2

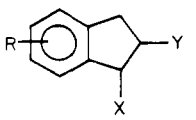
accompanied by the blockade of exogenous norepinephrine. Guanethidine is classified as an adrenergic neuron blocking agent since it appears to block transmission at adrenergic nerve terminals and depletes tissue stores of norepinephrine.[1] Several years ago Burn and Rand[2] proposed that the release of norepinephrine at sympathetic nerve endings is mediated by acetylcholine since xylocholine blocks the release of norepinephrine at the nerve endings.[3] In a review of evidence supporting the hypothesis that adrenergic neuron blocking drugs act by interfering with a cholinergic link of sympathetic transmission, Rand and Wilson postulated a hypothetical receptor for adrenergic neuron blocking agents.[4] It was assumed that the receptor was the same as that on which acetylcholine acts to release norepinephrine at sympathetic nerve endings and that guanethidine, and other neuron blockers, acts as an antagonist at this receptor preventing the release of norepinephrine by acetylcholine. The receptor was viewed as consisting of an area of van der Waals bonding, a hydrogen bonding site and an anionic site to which guanethidine could bind through the hydrocarbon portion of the heterocyclic ring, the heterocyclic nitrogen, and the guanidinium group, respectively. Prior to this postulation, it was reported that the cis and trans isomers of the sulfate salt of cinnamylguanidine (**2**) possess hypotensive activity and that the cis isomer is significantly more active than the trans isomer.[5,6] The cis isomer was proposed to act in a manner similar to guanethidine but was more potent, possessed a more rapid onset of action, but also possessed a shorter duration of action. The differences in activity between these geometric isomers may reflect a difference in the abilities of these isomers to "fit" the receptor proposed by Rand and Wilson. A recent study[7] regarding solution conformations of xylocholine and guanethidine, as determined by NMR, indicates preferred gauche conformations for both neuronal blockers (only 4% of the trans rotamer of xylocholine and 40% of the trans rotamer of guanethidine were observed to exist in solution). The possibility that *cis*-**2** may bind to the hypothetical receptor in a manner similar to that of guanethidine while *trans*-**2** may not prompted us to prepare analogues of **2** which would test this hypothesis. The importance of the aromatic ring of **2** could be determined through the synthesis of the cyclohexyl analogues **3** and **4** and the naphthalene analogues **5–7**. Compounds **4**, **5**, and **7–10** represent derivatives in which a cisoid relationship of the hydrocarbon center and the guanidine function exists. These functions are maximally translocated in **3**, **6**, **11**, and **12**. Cyclopropane derivatives **13–16** present a more direct opportunity to study the importance of the double bond in **2**.



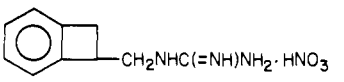
3, X = H; Y = $CH_2NHC(=NH)NH_2 \cdot HNO_3$
4, X = $CH_2NHC(=NH)NH_2 \cdot HNO_3$; Y = H



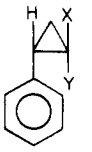
5, X = $CH_2NHC(=NH)NH_2 \cdot HNO_3$; Y = H
6, X = H; Y = $CH_2CH_2NHC(=NH)NH_2 \cdot HNO_3$
7, X = $CH_2CH_2NHC(=NH)NH_2 \cdot HNO_3$; Y = H



8, R = Y = H; X = $NHC(=NH)NH_2 \cdot HNO_3$
9, R = 5-OCH_3; X = $NHC(=NH)NH_2 \cdot H_2SO_4$; Y = H
10, R = 6-NO_2; X = $NHC(=NH)NH_2 \cdot HNO_3$; Y = H
11, R = X = H; Y = $NHC(=NH)NH_2 \cdot HNO_3$



12



13, X = $CH_2NHC(=NH)NH_2 \cdot HNO_3$; Y = H
14, X = H; Y = $CH_2NHC(=NH)NH_2 \cdot HNO_3$
15, X = $NHC(=NH)NH_2 \cdot HNO_3$; Y = H
16, X = H; Y = $NHC(=NH)NH_2 \cdot HNO_3$

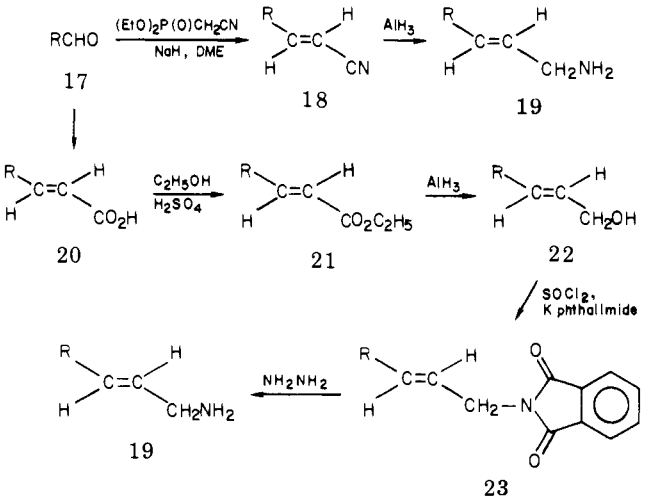
Chemistry. The preparation of the amines (Table I) used to prepare the guanidines **3–16** will be discussed first. (*E*)-3-Cyclohexyl-2-propenylamine (**19**) was prepared by two methods (Scheme I). The first method involved the phosphonate anion modification of the Wittig reaction[8] utilizing cyclohexanecarboxaldehyde (**17**) and diethyl cyanomethylphosphonate. The configuration of **18** was based upon NMR spectral data[9] which showed a doublet and a doublet of doublets, each set possessing $J = 11$ Hz, corresponding to a trans orientation of the olefinic protons. Previous studies have indicated that the use of phosphonate anions stabilized by electron-withdrawing α substituents furnishes the trans isomer stereoselectively.[10,11] Reduction of **18** with $LiAlH_4$ or Red-Al [sodium bis(2-methoxyethoxy)aluminum hydride] gave only the saturated amine. Use of aluminum hydride in THF[12,13]

Table I. Characteristics of Amines

Amine	% yield	Mp, °C (HCl salt)	Bp (mm), °C	Ref
19	56	212		*a*
25	56	167-169		*a*
26	32		110 (0.3)	15
27	41		142 (0.5)	28
28	52		178 (0.5)	29
33	72		88 (0.4)	30
34	24	228		31
35	74	40[b]		31
36	33	231		34
38	80	214		19
39	42	185		19
42	7		72 (0.5)	20
43	17		75 (0.2)	20
44	40	212		32
45	86		127 (25)	35

[a] See Experimental Section. [b] Free base.

Scheme I

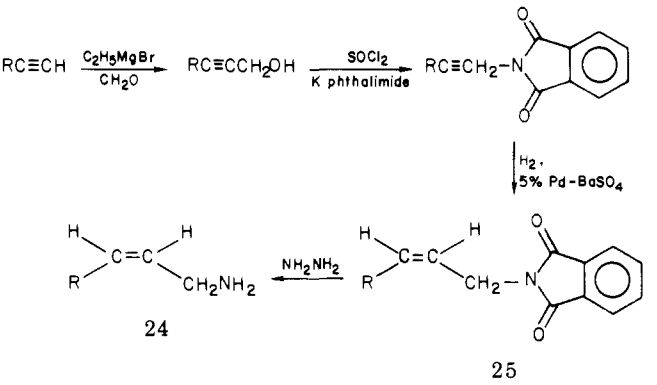


R = cyclohexyl

gave a mixture of **19** and the corresponding saturated amine which was separated by column chromatography. The second method employed the Dobner modification of the Knoevenagel reaction[14] to give the acid **20** which was established as the trans isomer on the basis of NMR data[9] in a manner similar to that previously described for **18**. The olefinic protons appeared as a doublet and a doublet of doublets, J = 16 Hz. The stereochemistry of the reaction also favors the formation of the trans isomer. The conformation of the intermediate enolate anion in which there is less steric interaction between the enolate anion and the β substituent is favored. Decarboxylation and dehydration would give the trans isomer. Esterification of the acid gave **21** which was reduced with aluminum hydride to give **22**. Treating **22** with thionyl chloride and subsequently with potassium phthalimide gave the imide **23**, hydrazinolysis of which gave **19**. The yield of **19** by this method was greater than the first method despite the greater number of steps in the second method. (*Z*)-3-Cyclohexyl-2-propenylamine (**25**) was prepared as shown in Scheme II. The phthalimide derivative **24** was prepared as indicated by catalytic reduction of the acetylene analogue and was cleaved with hydrazine to give **25**. Comparison of the NMR spectrum of **25** with that of **19** indicated a coupling constant of the olefinic protons of 10 Hz, smaller than that observed with **19** and thus consistent with cis stereochemistry.

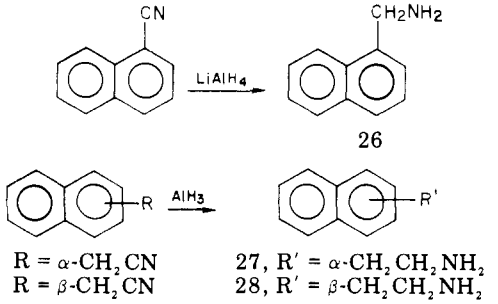
The synthesis of the aminonaphthalene derivatives necessary for the synthesis of **5**–**7** is shown in Scheme III.

Scheme II



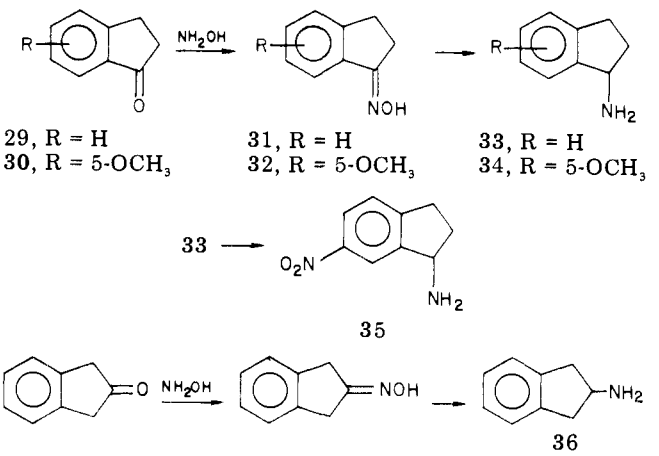
R = cyclohexyl

Scheme III

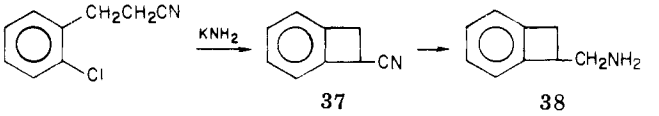


R = α-CH_2CN 27, R′ = α-$CH_2CH_2NH_2$
R = β-CH_2CN 28, R′ = β-$CH_2CH_2NH_2$

Scheme IV



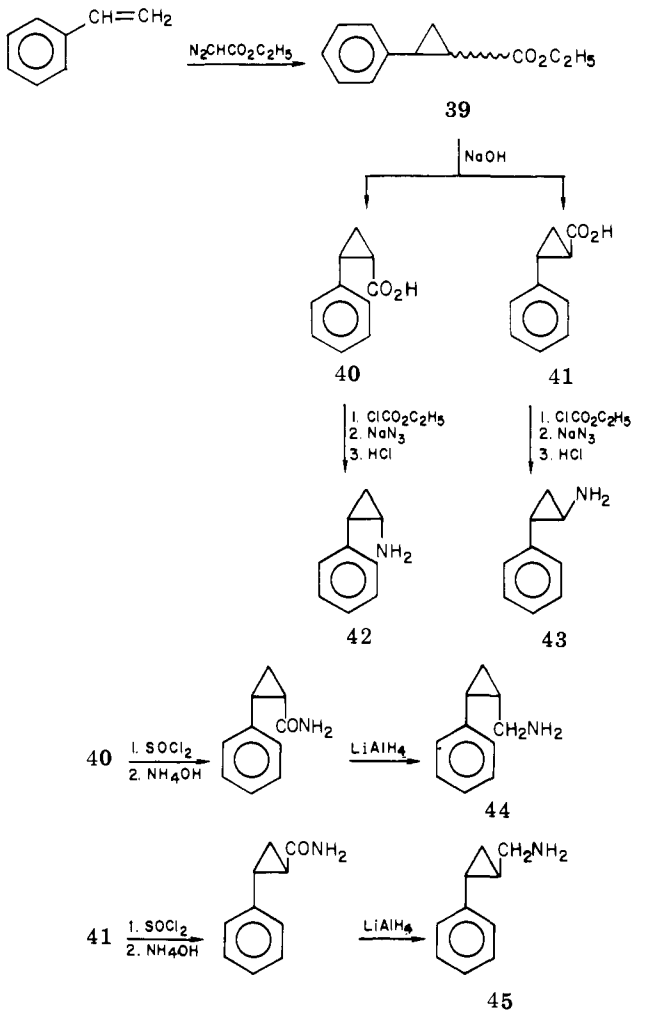
Scheme V



α-Naphthonitrile was easily reduced with $LiAlH_4$ to give **26**.[15] Amines **29** and **30** were prepared by aluminum hydride reduction of the commercially available α- and β-naphthylacetonitriles, respectively ($LiAlH_4$ reduction gave only 10% yields of **29** and **30**).

The amines required for the preparation of the indanylguanidines **8**–**11** were prepared by methods outlined in Scheme IV. The oxime **31** prepared from 1-indanone (**29**) was reduced with $LiAlH_4$ to give **33**. 5-Methoxy-1-indanone (**30**) was prepared by the methylation of 5-hydroxyindan and subsequent oxidation with chromic acid.[16] Catalytic hydrogenation of the corresponding oxime **32** yielded **34**. Nitration of **33** with cold nitric acid–sulfuric acid gave low yields of **35**. 2-Indanamine (**36**) was prepared

Scheme VI



Scheme VII

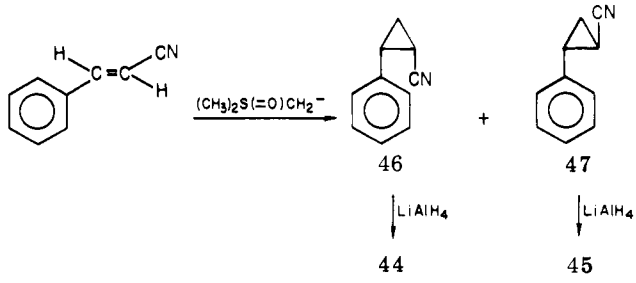
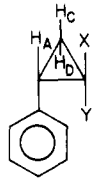


Table II. Multiplet Center and Coupling Constants for Cyclopropylmethine Protons



Compd	X	Y	δ, ppm (methine)	J, Hz
40	H	CO_2H	2.43	$J_{A,D} = 8$ $J_{A,X} = 8$ $J_{A,C} = 8$
42	H	NH_2	2.62	$J_{A,D} = 4$ $J_{A,X} = 8$ $J_{A,C} = 8$
46	H	CN	2.32	$J_{A,D} = 8$ $J_{A,X} = 7$ $J_{A,C} = 7$
41	CO_2H	H	2.37	$J_{A,D} = 4$ $J_{A,Y} = 6$ $J_{A,C} = 8$
43	NH_2	H	2.48	$J_{A,D} = 4$ $J_{A,Y} = 3$ $J_{A,C} = 8$
47	CN	H	2.54	$J_{A,D} = 6$ $J_{A,Y} = 6$ $J_{A,C} = 8$

by the catalytic reduction of the oxime derived from 2-indanone.

The synthesis of the aminobenzocyclobutene analogues is outlined in Scheme V. Generation of a benzyne intermediate by the action of potassium amide on *o*-chlorohydrocinnamonitrile[17-19] and subsequent cyclization yielded **37** which was reduced with $LiAlH_4$ to give **38**.

The well-established procedure of Burger and Yost[20] was utilized for the preparation of the cyclopropylamines required for the synthesis of **13–16** (Scheme VI). The mixture of esters (**39**) obtained by the addition of ethyl diazoacetate to styrene was selectively hydrolyzed by a modification of the method of Walborsky and Plonsker.[21,22] The trans ester is more rapidly hydrolyzed than is the cis ester because of less steric hindrance of the carbonyl function to give a 3:1 ratio of **41** to **40**. Each acid was converted to the acyl azide by treating the acid first with ethyl chloroformate and then with sodium azide. The displacement of the mixed anhydride by azide leads to less isomerization of **40** to the trans isomer than if the acid halide is used. Rearrangement[23] of the acyl azides gave the cyclopropylamines **42** and **43**. The synthesis of the cyclopropylmethylamine derivatives **44** and **45** was achieved from the corresponding acids **40** and **41**. Treating **40** with thionyl chloride in refluxing benzene resulted in isomerization to the trans acid chloride[20] but stirring **40** and thionyl chloride in petroleum ether at room temperature gave the cis acid chloride. The trans acid was converted to its acid chloride in refluxing benzene. The acid chlorides were converted to the corresponding amides with ammonium hydroxide and subsequent $LiAlH_4$ reduction gave **44** and **45**. Since the amounts of cis acid obtained in the first step of this reaction sequence were relatively low, an alternate route to **44** was explored (Scheme VII). The methylide derived from trimethylsulfoxonium iodide[24] was added to *trans*-cinnamonitrile to give the nitriles **46** and **47** in a 22:78 ratio. No enhancement of the formation of the cis isomer was observed. Reduction of the nitriles with $LiAlH_4$ gave the cyclopropylmethylamines.

Stereochemical assignments of the cyclopropyl intermediates were made on the basis of NMR spectral studies which are summarized in Table II. In the cis series (compounds **40**, **42**, and **46**) the methine proton on the carbon atom bearing the carboxyl, amino, and cyano functions appeared as a four-line pattern while this proton in the trans series (compounds **41**, **43**, and **47**) appeared as a five-line pattern. First-order analysis of the signals was in agreement with NMR studies of other substituted cyclopropanes[9] and confirmed the observation that in this series $J_{cis} > J_{trans}$. It was also noted that the aromatic protons of the cis series appeared as singlets while those of the trans series appeared as multiplets.

The target guanidine derivatives were prepared by either of two methods. The initial method explored was the reaction of the appropriate amine with 2-methylpseudothiourea sulfate[25] in water or aqueous ethanol. Unfortunately, formation of the salt of the starting amine accompanied guanidine formation and the guanidinium sulfate salt could be obtained pure only after several re-

Table III. Characteristics of Guanidinium Salts

Guanidinium salt	Method[a]	% yield	Crystn solvent	Mp, °C	Formula	Analyses
3	B	40	EtOH	174-176	$C_{10}H_{20}N_4O_3$	C, H, N
4	B	43	EtOH	146-148	$C_{10}H_{20}N_4O_3$	C, H, N
5	B	20	EtOH	163-165	$C_{12}H_{14}N_4O_3$	C, H, N
6	B	46	MeOH	154-156	$C_{13}H_{16}N_4O_3$	C, H, N
7	B	70	MeOH	174-176	$C_{13}H_{16}N_4O_3$	C, H, N
8	B	30	MeOH	145-147	$C_{10}H_{14}N_4O_3$	C, H, N
9	A	8	H_2O	195-210	$C_{22}H_{32}N_6O_6S \cdot H_2O$	C, H, N
10	B	30	EtOH	190-193	$C_{10}H_{13}N_5O_5$	C, H, N
11	B	40	MeOH	178-179	$C_{10}H_{14}N_4O_3$	C, H, N
12	B	34	EtOH-Et_2O	119-121	$C_{10}H_{14}N_4O_3$	C, H, N
13	B	53	MeOH	144-147	$C_{11}H_{16}N_4O_3$	C, H, N
14	B	45	MeOH	153-155	$C_{11}H_{16}N_4O_3$	C, H, N
15	B	40	MeOH	145-146	$C_{10}H_{14}N_4O_3$	C, H, N
16	B	10	MeOH	165-166	$C_{10}H_{14}N_4O_3$	C, H, N

[a] See Experimental Section.

crystallizations. This method was useful only for the preparation of **9**. The second method involved the condensation of the amine with 3,5-dimethylpyrazole-1-carboxamidine nitrate.[26] The greater solubility of this nitrate in water and ethanol permitted the resulting guanidinium nitrate salts to be removed and purified in higher yields than in the first method discussed. The properties of the guanidinium salts prepared in this study are summarized in Table III. Nuclear magnetic resonance data of the guanidinium salts could be best obtained in methanol-d_4. The guanidinium protons rapidly exchanged and thus only the spectrum of the parent system was obtained in each case. Mass spectra of the salts were of little value since, in general, only the molecular ion of the parent amine was observed. In some cases, the molecular ion of the nitrate salt was obtained using low ionization potentials. The infrared spectra of the salts displayed characteristic guanidinium I and II bands at 1680 and 1640 cm^{-1}.

Pharmacology. The guanidinium salts prepared in this study were tested for adrenergic neuron blocking activity in vitro using the isolated guinea pig vas deferens preparation[27] and measuring the ability of these agents to inhibit electrically induced release of norepinephrine. Results are summarized in Table IV. To assist in the evaluation of these data Dreiding models of the test compounds were constructed and the average distance of the center of the ring system to the guanidinium carbon atom was calculated for each compound.

The data presented in Table IV represent interesting but, unfortunately, inconclusive results. Three analogues were more potent than guanethidine in this assay: **3**, **4**, and **5**. The order of potency **4** > **5** > **3** does not correlate with the distance of the center of the ring system to the guanidinium nitrogen atom. In general, compounds **4**, **5**, **8**–**10**, **14**, and **16** represent analogues of **2** in which a cis relationship of the ring system and guanidinium nitrogen exists while the remaining analogues represent trans analogues. In the most potent trans analogue **12**, the bond distance is very similar to that of the most potent cis analogue **4** (5.3 vs. 5.1 Å). However, **7** possesses the same interatomic relationship as exists in **4** but is some 120 times less active. Other relationships indicate that distance alone cannot account for the adrenergic neuron blocking activity of these analogues. For instance, while **8**, **9**, and **10** all possess interatomic distances of 3.9 Å, not all are equally effective, the 6-nitro derivative being three times less potent than the unsubstituted derivative and the 5-methoxy derivative being inactive in this preparation. The nature of the substituent effect in these derivatives merits further investigation. The cyclopropane derivatives

Table IV. Adrenergic Neuron Blocking Activity

Compd	ID_{50}, μg/mL[a]	Concn, M[b]	EPMR[c]	Distance, Å[d]
Guanethidine[e]	1.0	2.0×10^{-6}	1.0	4.0-6.5
3	0.19	7.7×10^{-7}	0.39	6.2
4	0.024	9.8×10^{-8}	0.049	5.1
5	0.05	1.9×10^{-7}	0.095	4.9
6	1.4	5.0×10^{-6}	2.5	5.6
7	3.5	1.2×10^{-5}	6.0	5.1
8	2.6	5.8×10^{-6}	2.9	3.9
9	Inactive[f]			3.9
10	5.7	2.0×10^{-5}	10.0	3.9
11	Inactive[f]			4.6
12	0.6	2.5×10^{-6}	1.25	5.3
13	1.85	7.3×10^{-6}	3.65	5.8
14	2.3	9.1×10^{-6}	4.55	4.5
15	1.2	5.05×10^{-6}	2.52	6.0
16	Inactive[f]			4.0

[a] Calculated as free base. [b] Final bath concentration at ID_{50} as free base. [c] Equipotent molar ratio. [d] Average intramolecular distance from the center of the ring to the guanidinium carbon atom. [e] Tested as the sulfate salt. [f] Inactive at 1×10^{-4} M.

13–**16** are particularly interesting. In both pairs of compounds, **13**, **14** and **15**, **16** the trans isomer was more potent than the cis isomer with the difference in activity of **15** and **16** being most dramatic. The reduced cyclohexane analogues of these derivatives have not been evaluated but would appear to be of interest. These results suggest that conformational factors alone do not account for interactions with the adrenergic neuronal receptor. Structural factors appear to be more important. While this study on an in vitro system did not conclusively demonstrate the dependence of conformation in receptor interactions of this class, the fact that compounds **3**–**5** were apparently more potent than guanethidine in this assay appears to warrant in vivo evaluation of these agents in antihypertensive assays.

Experimental Section

All melting points were determined on a Thomas-Hoover Unimelt or a Mel-Temp apparatus and are corrected. Infrared spectra were obtained on a Beckman IR 33 or a Perkin-Elmer 257 spectrophotometer. All NMR spectra were obtained on a Jeolco Model C-60-HL spectrometer and all values were reported in parts per million (ppm, δ) from Me_4Si or DSS. Elemental analyses were performed by Chemalytics, Inc., Tempe, Ariz., and Galbraith Laboratories, Inc., Knoxville, Tenn. Gas chromatography was performed on a Perkin-Elmer 900 gas chromatograph using 5.5-ft columns containing 4% 20M Carbowax on 80–100 mesh ABS Anakrom or 5% OV 17 on 110–120 mesh ABS Anakrom. Mass spectra were taken on a Du Pont Model 21-492

mass spectrometer. Column chromatography was performed on neutral alumina (Woelm).

(*E*)-3-Cyclohexyl-2-propenylamine (19). To a solution of AlH_3 prepared from 3.3 g (0.08 mol) of $LiAlH_4$ and 4.3 g (0.04 mol) of H_2SO_4 in 250 mL of THF[13] was added 5.9 g (0.04 mol) of **18** prepared[33] from **17**.[36] The cooled mixture was stirred for 30 min and decomposed by the addition of 15 mL of THF–H_2O (1:1) and 4.5 g of NaOH in 40 mL of H_2O. The precipitate was removed by filtration and washed with THF. The filtrate was dried (K_2CO_3) and evaporated to yield 4.0 g of a viscous oil which was chromatographed on 70 g of neutral alumina with chloroform–benzene (3:7). The first fraction eluted was the saturated amine (2.0 g) followed by **19** (1.0 g, 16%): IR (liquid film) 3250 cm^{-1} (NH_2); NMR ($CDCl_3$) δ 5.83 (m, 2, HC=CH). The HCl salt was prepared in the normal manner: mp 212 °C dec. Anal. ($C_9H_{18}ClN$) C, H, N.

(*Z*)-3-Cyclohexyl-2-propenylamine (25). A solution of cyclohexylacetylene (10.8 g, 0.10 mol) was added to a mixture of EtMgBr [prepared from 2.5 g (0.11 g-atom) of Mg and 12.0 g (0.11 mol) of EtBr] in 50 mL of Et_2O. The mixture was refluxed for 1 h and CH_2O generated from 60.0 g of paraformaldehyde was bubbled through the mixture. After cooling, saturated NH_4Cl was added, and the Et_2O layer was separated and dried over K_2CO_3. Evaporation of the Et_2O and distillation of the residue gave an oil [8.1 g (60%); bp 122–126 °C (20 mm)]. To a solution of 2.0 g (0.02 mol) of this oil in 20 mL of $CHCl_3$ was added a cold solution of 1.3 g of pyridine and 1.9 g (0.02 mol) of $SOCl_2$ in 20 mL of $CHCl_3$. The mixture was stirred in an ice bath for 1 h and poured onto ice. The $CHCl_3$ layer was separated, dried ($CaCl_2$), and evaporated to give an oil which was dissolved in 50 mL of DMF and combined with 2.2 g of potassium phthalimide and a crystal of KI. The mixture was refluxed for 5 h, cooled, poured onto ice, and extracted with $CHCl_3$. The $CHCl_3$ layer was removed, washed with 10% NaOH, and dried (Na_2SO_4). The organic layer was evaporated and the residue triturated with hexane to give a solid which, when recrystallized from hexane, gave 1.0 g of **24** as yellow crystals, mp 104 °C. A solution of 1.0 g of the phthalimide in 20 mL of EtOAc was hydrogenated (1 atm) over 20 mg of 5% Pd/$BaSO_4$ (containing 1 drop of quinoline) using a Brown[2] hydrogenator. After 1 equiv of H_2 was absorbed, the solution was filtered over Celite and evaporated to give 0.8 g of an oil which was combined with 0.6 mL of 85% hydrazine in 20 mL of MeOH. The mixture was refluxed for 2 h, cooled, acidified with concentrated HCl, and refluxed an additional 30 min. The precipitate was removed and the filtrate concentrated to yield a residue which was basified with 20% NaOH and extracted with Et_2O. The Et_2O layer was dried (Na_2SO_4) and evaporated to give **25** as an oil (0.3 g, 56%): IR (liquid film) 3220 cm^{-1} (NH_2); NMR ($CDCl_3$) δ 5.22 (m, 2, HC=CH). The HCl salt was prepared in the normal manner: mp 167–169 °C. Anal. ($C_9H_{18}ClN$) C, H, N.

General Synthesis of Guanidinium Salts. Method A. A mixture of 0.012 mol of amine and 0.006 mol of 2-methylpseudothiourea sulfate in 20 mL of 50% EtOH was refluxed for 12 h. The solvent was removed and the residue refluxed in absolute EtOH. The resulting precipitate was collected by filtration and recrystallized from H_2O.

Method B. A solution of 0.01 mol of amine and 0.01 mol of 3,5-dimethylpyrazole carboxamidine nitrate in 10 mL of absolute EtOH was refluxed for 2.5 h. The resulting precipitate was collected and recrystallized from either EtOH, MeOH, or EtOH–Et_2O.

Pharmacological Methods. The method of Hukovic[27] utilizing the isolated guinea pig ileum was employed to measure adrenergic neuronal blocking activity. Adult male guinea pigs were stunned by a blow on the head. The abdomen was opened and the testes retracted into the abdominal cavity. The vasa deferentia were carefully removed and placed in a Petri dish containing Krebs solution. The mesentery was then trimmed and a single vas was mounted in a 60-mL organ bath in continuously oxygenated Krebs solution maintained at 31–32 °C. Contractions were recorded by a "B" myograph connected to a Narco-DMP-4A physiograph (Narco Bio-Systems, Inc., Houston, Texas).

The vas deferens was suspended between two parallel platinum electrodes connected to a Grass S 44 stimulator. Stimuli were applied transmurally for periods of 15 s at 4-min intervals at a frequency of 25–35 shocks/s with a pulse duration of 0.1 ms and at a supramaximal voltage (110–120 V). A dose–response curve for guanethidine was determined with one of the vas deferens from each guinea pig, since preliminary data indicated that there was no difference between the two organs from the same animal. The solution was drained from the organ bath and replaced with fresh warm Krebs solution. A fresh preparation was used for testing each drug. A solution of the nitrate or sulfate salt of each of the test compounds was prepared in distilled water. Measurements were made at 10-min intervals between drug administration and dose–response curves constructed. The ID_{50} was calculated from the dose–response curve of the test compound at no less than three concentrations and represents the dosage causing 50% inhibition of the response. The equipotent molar ratio (EPMR) was calculated using the ratio of the ID_{50} of the test compound as compared to guanethidine. The ID_{50} of guanethidine was determined from a standard curve obtained by averaging the dose–response curves of guanethidine obtained from each guinea pig used. The active compounds were checked for adrenergic neuron blocking activity by administering 0.2 mL of exogeneous norepinephrine. The final bath concentration of the norepinephrine was 1.97×10^{-5} M. The preparation gave normal contractile responses to the norepinephrine indicating no receptor blockade. The solution containing the test compound was drained from the organ bath and the preparation was washed 10–20 times with fresh Krebs solution over a 5-min period. The preparation was stimulated to determine if normal response remained. The test compounds were easily removed by washing.

Acknowledgment. The authors gratefully acknowledge the mass spectral data supplied by Drs. John K. Baker and Stephen Billets. This research was supported in part by a Faculty Research Grant, University of Mississippi, and in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

References and Notes

(1) A. L. A. Boura and A. F. Green, *Annu. Rev. Pharmacol.*, **5**, 183 (1965).
(2) J. H. Burn and M. J. Rand, *Nature (London)*, **184**, 163 (1959).
(3) P. Hey and G. L. Willey, *Br. J. Pharmacol.*, **9**, 471 (1954).
(4) M. J. Rand and J. Wilson, *Circ. Res.* (Suppl. III to Vol. XX and XXI), 89 (1967).
(5) W. E. Barrett, R. P. Mull, and A. J. Plummer, *Experientia*, **21**, 506 (1965).
(6) W. E. Barrett, R. Rutledge, R. Povalski, and A. J. Plummer, *Pharmacologist*, **8**, 182 (1966).
(7) N. S. Ham, *J. Pharm. Sci.*, **65**, 612 (1976).
(8) W. S. Wadsworth, Jr., and W. D. Emmons, *J. Am. Chem. Soc.*, **83**, 1733 (1961).
(9) L. M. Jackman and S. Shernhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, New York, N.Y., 1969, pp 227, 323.
(10) D. H. Wadsworth, O. E. Schupp, III, E. J. Seus, and J. A. Ford, Jr., *J. Org. Chem.*, **30**, 680 (1965).
(11) H. O. House, V. K. Jones, and G. Frank, *J. Org. Chem.*, **29**, 3327 (1964).
(12) H. C. Brown and N. M. Yoon, *J. Am. Chem. Soc.*, **88**, 1464 (1966).
(13) N. M. Yoon and H. C. Brown, *J. Am. Chem. Soc.*, **90**, 2927 (1968).
(14) J. Koo, M. S. Fish, G. N. Walker, and J. Blake, "Organic Syntheses", Collect. Vol. IV, Wiley, New York, N.Y., 1963, p 327.
(15) F. F. Blicke and C. E. Maxwell, *J. Am. Chem. Soc.*, **61**, 1781 (1939).
(16) C. A. Panetta and S. C. Bruce, *J. Org. Chem.*, **26**, 4859 (1961).
(17) J. F. Bunnett and J. A. Skorcz, *J. Org. Chem.*, **27**, 3836 (1962).
(18) J. A. Skorcz, J. T. Suh, C. I. Judd, M. Finkelstein, and A. C. Conway, *J. Med. Chem.*, **9**, 656 (1966).
(19) J. A. Skorcz and J. E. Robertson, *J. Med. Chem.*, **8**, 255 (1965).
(20) A. Burger and W. L. Yost, *J. Am. Chem. Soc.*, **70**, 2198 (1948).

(21) H. M. Walborsky and L. Plonsker, *J. Am. Chem. Soc.*, **83**, 2138 (1961).
(22) C. Kaiser, J. Weinstock, and M. P. Olmstead, *Org. Synth.*, **50**, 94 (1970).
(23) J. Weinstock, *J. Org. Chem.*, **26**, 3511 (1961).
(24) C. Kaiser, B. M. Trost, J. Beeson, and J. Weinstock, *J. Org. Chem.*, **30**, 3972 (1965).
(25) B. Rathke, *Ber.*, **14**, 1774 (1881).
(26) F. L. Scott, D. G. O'Donovan, and J. Reilly, *J. Am. Chem. Soc.*, **75**, 4053 (1953).
(27) S. Hukovic, *Br. J. Pharmacol.*, **16**, 188 (1961).
(28) E. E. Smissman, A. C. Makriyannis, and E. J. Walaszek, *J. Med. Chem.*, **13**, 640 (1970).
(29) F. Meyer and O. Schnecko, *Ber.*, **56**, 1413 (1923).
(30) A. Konig, *Justus Liebigs Ann. Chem.*, **275**, 348 (1893).
(31) C. K. Ingold and H. A. Piggott, *J. Chem. Soc.*, **123**, 1469 (1923).
(32) C. Dupin and R. Fraisse-Jullien, *Bull. Soc. Chim. Fr.*, 1993 (1966).
(33) G. Zweifel, J. T. Snow, and C. C. Whitney, *J. Am. Chem. Soc.*, **90**, 7139 (1968).
(34) N. Levin, B. E. Graham, and H. G. Kolloff, *J. Org. Chem.*, **9**, 380 (1944).
(35) C. Kaiser, B. Lester, C. Zirkle, A. Burger, C. Davis, T. Delia, and L. Zirngibl, *J. Med. Chem.*, **5**, 1243 (1962).
(36) H. C. Brown and A. Tsukamoto, *J. Am. Chem. Soc.*, **86**, 1095 (1964).

5-Aryl-1,5-dihydro-2*H*-1,4-benzodiazepin-2-one Derivatives as Antianxiety Agents

Masaru Ogata,* Hiroshi Matsumoto, and Katsumi Hirose

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan. *Received December 16, 1976*

A new series of 7-chloro- and 7-nitro-5-methoxy-5-phenyl-1,5-dihydro-2*H*-1,4-benzodiazepin-2-ones (**7a**,**c**–**e**) was synthesized and found to have potent antipentylenetetrazole activity. These compounds were also employed as intermediates in the synthesis of 3-substituted 1,3-dihydro-1,4-benzodiazepin-2-ones (**8f**–**v**).

A vast number of 1,4-benzodiazepines have been synthesized by a variety of methods and extensive data on their pharmacological activity have been accumulated. Most of the marketed 5-aryl-1,4-benzodiazepines have a double bond at C-4,5 (1,3-dihydro type) and not a C-3,4 (1,5-dihydro type). In connection with this structural problem, Bell et al.[1] showed that 7-chloro-1,5-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**1**)[1,2] is less potent than its isomer 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**2a**). These observations prompted us to extend the synthetic research to other 1,5-dihydro-1,4-benzodiazepines which might have potent actions on the central nervous systems and led to the discovery that 1,5-dihydro-5-methoxy-5-phenyl-2*H*-1,4-benzodiazepin-2-ones (**7a**,**c**–**e**) are potent agents.

Chemistry. The addition reaction of acetyl nitrate has been frequently employed on the olefinic bond.[3] We examined the addition reaction of acetyl nitrate to the C=N bond in 1,4-benzodiazepines **2a**–**e**. Reaction of 7-chloro-1,4-benzodiazepine (**2a**) with fuming nitric acid (d = 1.50) in acetic anhydride at ca. 40 °C resulted in the recovery of starting material as its nitric acid salt. However, when **2a** was converted into the known 1-chloro-1,4-benzodiazepine (**2b**),[4] before subjecting conditions similar to those for **2a**, an insoluble adduct (**3b**) was obtained in 76% overall yield. That the addition reaction had occurred was indicated by the IR spectrum which showed a strong acetoxy carbonyl band at 1723 cm^{-1}. Compound **3b** is unstable when heated in aqueous dioxane leading to formation of the benzophenone derivative **4a** which cyclized to the carbostyril derivative **5a** with aqueous potassium carbonate.

When **3b** was treated with an equimolar amount of methylamine in dichloromethane at room temperature, the dechlorinated product **3a** was obtained in 94% yield. Refluxing **3a** with methanol afforded the methoxy compound **6a** (81%), which was allowed to stand in dichloromethane in the presence of triethylamine at room temperature, giving 1,5-dihydro-1,4-benzodiazepine (**7a**) in 68% yield (see Scheme I).

We also studied the reaction of 7-nitro-1,4-benzodiazepine (**2c**) with acetyl nitrate under similar conditions. Treatment of **2c** afforded the insoluble adduct **3c** in 75% yield, without precipitation of the nitric acid salt of the starting material. When **3c** was heated under reflux with aqueous acetic acid, the benzophenone derivative **4c** was obtained, analogous to the reaction of **4a**. To obtain 1,5-dihydro-1,4-benzodiazepine (**7c**), methanolysis was applied to **3c** to give the methoxy compound **6c** (88%), which was treated with triethylamine at room temperature to give 1,5-dihydro-1,4-benzodiazepine (**7c**) in 92% yield.

This reaction could be used to prepare other 5-methoxy-1,5-dihydro-1,4-benzodiazepines as shown by the synthesis of the 1-methyl analogues **7d**,**e**. Namely, the 1,5-dihydro-1,4-benzodiazepines **6d** and **6e** were prepared from **2d** and **2e** in satisfactory yield via addition of acetyl nitrate followed by methanolysis of the resulting adduct (**3d** and **3e**, respectively). Elimination of nitrous acid from **6d** with triethylamine was markedly slow under similar conditions as for **6a** and resulted in formation of 5-methoxy-1,5-dihydro-1,4-benzodiazepine (**7d**) contaminated with the starting material (**6d**). Treatment of **6e** with triethylamine gave not the analogous 1,5-dihydro-1,4-benzodiazepine (**7e**) but the starting material, although a long reaction time was allowed.

Treatment of **6d** with sodium hydride in dimethylformamide at −30 °C resulted in a 44% yield of **7d**. N-Methylation of **7a** and **7c** as another synthetic route to **7d** and **7e** was tried. Treatment of **7a** and **7c** with methyl iodide in the presence of sodium hydride in dimethylformamide at low temperature afforded **7d** and **7e** in 61 and 74% yield, respectively.

We found that the 1,5-dihydro-1,4-benzodiazepines **7a**,**c**–**e** could be used as intermediates in the synthesis of 3-substituted 1,3-dihydro-1,4-benzodiazepines **8f**–**v**. When **7a** was allowed to stand at room temperature in aqueous dioxane containing a trace amount of hydrochloric acid or *p*-toluenesulfonic acid, the known 3-hydroxy-1,3-dihydro-1,4-benzodiazepine (**8f**, oxazepam)[5] was obtained in 66% yield. By analogy, **7c**, **7d**, and **7e** underwent hydroxylation to form the corresponding 3-hydroxy-1,3-dihydrobenzodiazepines (**8g**,[6] **8h**,[5] and **8i**[6]) as shown in Table I. An analogous nucleophilic reaction of **7a** and **7e** at the 3 position proceeded easily upon treatment with methanol, ethylene glycol, ethylene chlorohydrin, hydroxylamine, sodium cyanide, and methylamine to give the corre-