uptake procedure 500 μL of the synaptosome suspension was preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [^{3}H]GABA was added to give a final GABA concentration of 0.05 μM, and the incubation was continued for a further 10 min. The synaptosomes were isolated by rapid filtration through Whatman GF/C glass fiber filters, and the filters were washed with phosphate medium (10 mL). The filters were transferred to scintillation vials, and the radioactivity was measured by liquid scintillation counting after addition of Liposolve–Lipolume–water (1:10:0.2; 3 mL) (Lumac, Basel). The IC$_{50}$ values for inhibition of high-affinity neuronal (synaptosomal) GABA uptake at 0.05 μM GABA with preincubation of the tissue for 10 min in the presence of inhibitor were

determined as described elsewhere in detail.[41]

(41) Beart, P. M.; Johnston, G. A. R.; Uhr, M. L. *J. Neurochem.* **1972,** *19*, 1855.

Synthesis and Antidiarrheal Activity of N-(Aminoiminomethyl)-1H-pyrrole-1-acetamides Related to Guanfacine

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A series of N-(aminoiminomethyl)-1H-pyrrole-1-acetamides, related to guanfacine, were prepared and tested for antidiarrheal activity in castor oil dosed rats. trans-N-(Aminoiminomethyl)-2,5-dihydro-2,5-dimethyl-1H-pyrrole-1-acetamide (2), in which the dichlorophenyl ring of guanfacine is replaced by 2,5-dimethyl-2,5-dihydropyrrole, showed potent antidiarrheal activity but possessed only minimal cardiovascular activity in rats.

Most currently available antidiarrheal agents exert their activity by an action on cholinergic or opiate receptors.[1] The usefulness of anticholinergic agents is limited by their short duration of action and other anticholinergic side effects such as mydriasis, dry mouth, and blurred vision. Drugs acting at opiate receptors have the potential to produce undesirable CNS effects including addiction. In the search for more specific antidiarrheal agents our attention was directed toward the gastrointestinal actions of clonidine and related α$_2$-agonists.[2,3] In addition to its well-known cardiovascular actions, clonidine increases electrolyte absorption in the intestine[4] and inhibits castor oil[5] or naloxone[6] induced diarrhea in the rat. Antidiarrheal activity has also been reported for the clonidine-related compounds lofexidine[5] and lidamidine.[7] The latter compound has been shown to be an effective antidiarrheal agent in man,[8] recently launched as liderral in Mexico. In order to exploit the antidiarrheal activity of clonidine-like α-agonists, it is necessary to achieve a separation of their desired gastrointestinal effect from their cardiovascular activity. During the course of studies on a series of het-

erocyclic analogues of the clonidine-like antihypertensive agent guanfacine,[9] we synthesized an analogue that retained marked antidiarrheal activity but was free of significant cardiovascular effects.

Chemistry. The primary structural types desired were acylguanidines related to I, in which the dichlorophenyl ring of guanfacine (II) was replaced by a pyrrole or dihydropyrrole ring (Table I).
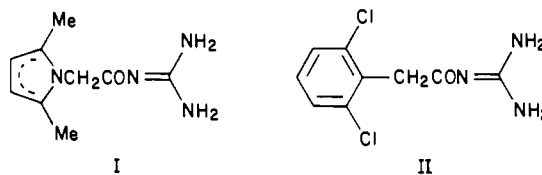

All of the acylguanidines listed in Table I, except compound 3, were prepared by reaction of an appropriate methyl or ethyl ester with a guanidine (Scheme I). a number of novel dihydropyrrole esters (Table II) were required as part of this work. These were prepared by alkylation of 2,5-dihydro-2,5-dimethylpyrrole[10] with halo acid esters and generally consisted of mixtures of cis and trans isomers in around 1:4 ratio. These mixed isomers were used for the preparation of the trans-acylguanidines (2 and 4–8), since the residual cis isomer was readily removed by crystallization at the final stage of the synthesis. The trans configuration of these acylguanidines was confirmed by examination of their NMR spectra in trifluoroacetic acid. Under these conditions protonation of the ring nitrogen inhibits its rate of inversion and results in nonequivalence of the trans-dimethyl groups that then

(1) Niemegeers, C. J. E. *Clin. Res. Rev.* **1981,** *1* (Suppl. 1), 125.

(2) Taylor, J. A.; Mir, G. N. *Drug Dev. Res.* **1982,** *2*, 105.

(3) Doherty, N. S.; Hanock, A. A. *J. Pharmacol. Exp. Ther.* **1983,** *225*, 269.

(4) Dharmsathaphorn, K.; Yamashiro, D. J.; Lindeborg, D.; Mandel, K. G.; McRoberts, J.; Ruffolo, R. R. *Gastroenterology* **1984,** *86*, 120.

(5) Lal, H.; Shearman, G. T.; Ursillo, R. C. *J. Clin. Pharmacol.* **1981,** *21*, 16.

(6) Nakaki, T.; Chang, P. C. J.; Tokunaga, Y.; Kato, R. *J. Pharm. Pharmacol.* **1981,** *33*, 397.

(7) Mir, G. N.; Alioto, R. I.; Aperow, J. W.; Eash, J. R.; Krebs, J. R.; Yelnosky, J. *Arzneim. Forsch. Drug Res.* **1978,** *28*, 1448.

(8) Leibach, J. R.; Sninsky, C. A.; Justus, P. G.; Riley, R. L.; Mathias, J. R. *Gastroenterology*, **1981,** *80*, 1207.

(9) Scholtysik, G.; Jerie, P.; Picard, C. W. In "Pharmacology of Antihypertensive Drugs"; Scriabine, A., Ed.; Raven Press: New York, 1980; pp 79.

(10) Evans, G. G. *J. Am. Chem. Soc.* **1951,** *73*, 5230.

Table I. Acylguanidines[a]

$$R_1AN=\!\!\!\!\!\!\begin{array}{c}NHR_2\\ \\NHR_3\end{array}$$
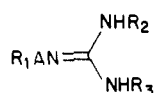
no.	R₁	A	R₂	R₃	cryst solv	mp, °C	yield,[b] %	formula[d]	dose, mg/kg po	% inhibn of caster oil induced diarrhea[c]	
										2 h	6 h
1	(2-methylpyrrolyl structure)	CH_2CO	H	H	EtOH	198.5–200	66	$C_9H_{14}N_4O \cdot HCl$	30	96	96
									10	100	75
									3	97	NS
2	(2,5-dimethylpyrrolidinyl structure)	CH_2CO	H	H	MeOH–EtOAc	215–217	49.7	$C_9H_{16}N_4O \cdot 2HCl$	30	100	99
									10	100	86
									3	100	NS
									1	92	NS
3	(2,5-dimethylpyrrolidinyl structure)	CH_2CO	H	H	EtOH–EtOAc		78.4	$C_9H_{18}N_4O \cdot 2HCl \cdot 0.5H_2O$	30	NS	NS
4	(2,5-dimethylpyrrolidinyl structure)	CH_2CO	H	Me	EtOH	199–200	23.8	$C_{10}H_{18}N_4O \cdot 2HCl$	30	88	NS
5	(2,5-dimethylpyrrolidinyl structure)	CH_2CO	$-(CH_2)_2-$		EtOAc	158–160	30	$C_{11}H_{18}N_4O$	30	35	NS
6	(2,5-dimethylpyrrolidinyl structure)	$(CH_2)_2CO$	H	H	IPA	126–127	23	$C_{10}H_{18}N_4O \cdot 2HCl \cdot H_2O$	30	NS	NS
7	(2,5-dimethylpyrrolidinyl structure)	$CH(Me)CO$	H	H	IPA	206–206.5	17.2	$C_{10}H_{18}N_4O \cdot 2HCl$	30	NS	NS
8	(2,5-dimethylpyrrolidinyl structure)	$COCO$	H	H	H_2O	>200[e]	47.5	$C_9H_{14}N_4O_2 \cdot 0.5H_2O$	30	NS	NS
9	(2,5-dimethylpyrrolidinyl structure)	$(CH_2)_2$	H	H	EtOH	237–239	47.2	$C_9H_{18}N_4 \cdot 2HCl$	30	NS	NS
10	(+)- (2,5-dimethylpyrrolidinyl NCH_2CON structure)				MeOH–EtOAc	209–211.5	21	$C_9H_{16}N_4O \cdot 2HCl$	30	100	100
									10	100	52
									3	100	NS
									1	NS	NS
11	(–)- (2,5-dimethylpyrrolidinyl NCH_2CON structure)				IPA	205–208	37.1	$C_9H_{16}N_4O \cdot 2HCl \cdot 0.25H_2O$	30	100	100
									10	100	45
									1	100	46
loperamide									30	98	97
									10	100	99
									3	96	77
lidamidine									30	100	100
									10	100	68
									3	100	NS
									1	NS	NS

[a] All compounds exhibited IR and ¹H NMR spectra consistent with the assigned structure.   [b] Yield of analytically pure material, yields not optimized.   [c] Mean percentages; all results analyzed for statistically significant differences from control values using analysis of variance; nonsignificant values ($p > 0.05$) indicated by NS.   [d] C, H, and N analyses were within ±0.4% of theoretical values for the formulas given.   [e] Decomposes.   [f] $[\alpha]^{22}_D$ +142.6° (c 0.94, MeOH).   [g] $[\alpha]^{24}_D$ −142.8° (c 1.3, MeOH).

appear as two distinct doublets separated by around 15 Hz. A similar nonequivalence of methyl groups would not be expected for the corresponding cis isomers. Compound

2 was subjected to further elaboration by reduction with hydrogen, to give the pyrrolidine 3, or with lithium aluminum hydride to give the alkylguanidine 9 (Scheme I).

Scheme I
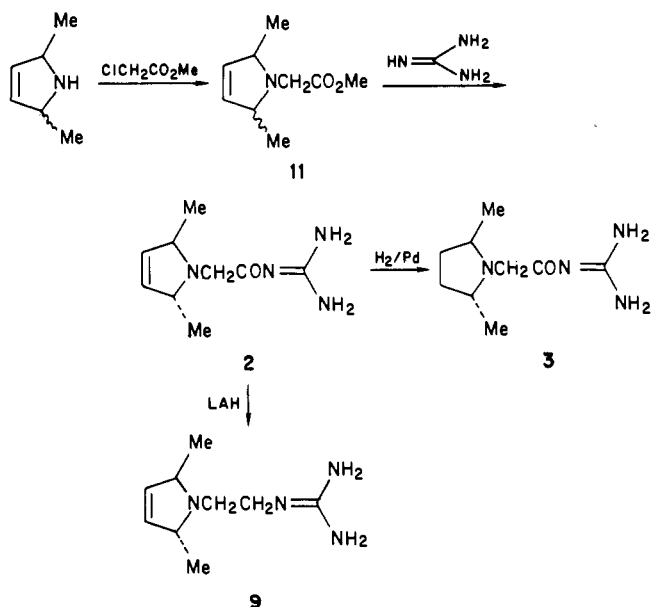
Table II. Intermediate Dihydropyrrole Esters[a]

no.	A	R	bp, °C (15 mm)	yield, %	formula	cis:trans[b] ratio
12	CH_2	Me	90–95	68.5	$C_9H_{15}NO_2$	1:3.5
13	$(CH_2)_2$	Et	102–105	49.4	$C_{11}H_{19}NO_2$[c]	1:4.5
14	CHMe	Et	97–101	30.4	$C_{11}H_{19}NO_2$	1:7.7
15	CO	Me	137	61.3	$C_9H_{13}NO_3$	1:6

[a] All compounds exhibited IR and H^1 NMR spectra consistent with the assigned structure. [b] Isomer ratios determined by GLC. [c] C: calcd, 66.97; found, 65.75.

Compound 2 was of particular interest, and accordingly its individual enantiomers were prepared. The intermediate ester 12 was resolved by crystallization as its dibenzoyl tartrate. The enantiomers of 12 were evaluated for their chiral purity by NMR measurements using the chiral shift reagent tris[3-(trifluoroacetyl)-d-camphorato]europium(III) (Eu-Opt). The best NMR separation on the racemate of 12 occurred on the olefinic signal, where a difference of 4 Hz was observed between the two enantiomers. The chiral purity of the two enantiomers was estimated to be greater than 98%. The individual enantiomers of 12 were then reacted with guanidine to give the corresponding enantiomers 10 and 11 of compound 2.

Pharmacology Results and Discussion. The antidiarrheal activity of compounds in Table I was evaluated in the rat castor oil diarrhea test. All of the compounds were tested initially at an oral dose of 30 mg/kg. Total fecal output was measured at 2 and 6 h after dosing. The more active compounds were retested at lower doses as indicated.

The active ingredient in castor oil, ricinoleic acid, produces its laxative effect by parallel inhibition of water absorption and smooth muscle contractility.[11] This response of the gut to ricinoleic acid resembles its response to a wide variety of pathological stimuli including bacterially produced hydroxy fatty acids. The rat castor oil

test has been widely used for the identification of antidiarrheal activity in opiate-[1] and clonidine-related agents.[5,7] The test provides readily reproducible results and a reliable prediction of clinical efficacy.[1]

Initial interest in this series centered upon compound 1 in which the dichlorophenyl ring of guanfacine was replaced by dimethylpyrrole. Compound 1 showed marked antidiarrheal activity, but this was accompanied by marked cardiovascular activity (see later). Since the cardiovascular actions of 1 appeared to be centrally mediated, we reasoned that a hydrophilic analogue, incapable of crossing the hydrophobic blood–brain barrier, might show minimal cardiovascular activity while retaining the desired antidiarrheal activity. Transport of 1 into the CNS could be facilitated by its low basicity, pK_a 6.3, which ensures a substantial proportion of un-ionized molecules are available at physiological pH. Accordingly, we prepared the partially reduced form (2) in which a second basic center, pK_a 10.3, is introduced, thus ensuring complete ionization at physiological pH. In accord with expectation, compound 2 showed minimal cardiovascular activity. However 2 retained very marked antidiarrheal activity. Interestingly, the fully reduced pyrrolidine analogue (3) was devoid of both cardiovascular and antidiarrheal activity. Accordingly, further work was concentrated on analogues of 2. The nature of the linkage between the pyrroline and guanidine groups was critical for activity, and the modifications in 6–9 reduced or abolished activity. Similarly, substitution of the guanidine by alkyl groups (4 and 5) was also unfavorable. Finally, the individual enantiomers (10 and 11) of 2 were examined. Both enantiomers of 2 showed marked activity, although the levorotatory isomer (11) was marginally more potent. Of particular interest in this series is the separation achieved between gastrointestinal and cardiovascular activities in compounds 1 and 2. In normotensive rats (Table III), compound 1 produced a severe long-lasting bradycardia and transient hypertension, while compound 2, by contrast, produced only a modest short-lived bradycardia. Both lidamidine and loperamide produced more severe cardiovascular effects than 2. On the basis of these preliminary results, compound 2[12] was selected for more detailed pharmacological studies, to be reported elsewhere, and is currently undergoing toxicological evaluation.

Experimental Section

Melting points were obtained on a Reichert microscope heating stage and are uncorrected. IR spectra were obtained as Nujol mulls with a Perkin-Elmer Model 521 spectrophotometer. NMR spectra were determined on a Brucker WP200 instrument. C, H, and N analyses were within ±0.4% of theoretical values unless otherwise stated. GLC separations were carried out on a 10% MS200 column at 180 °C using a Pye 104 gas chromatograph. pK_a values were determined in water by potentiometric titration.

Methyl 2,5-Dihydro-2,5-dimethyl-1H-pyrrole-1-acetate (12). Methyl chloroacetate (32.4 g, 0.3 mol) was added dropwise over 10 min to a stirred solution of 2,5-dihydro-2,5-dimethylpyrrole[10] (29.1 g, 0.3 mol) and triethylamine (33 g, 0.033 mol) in 50 mL of DMF. The mixture was allowed to stand for 18 h and then diluted with 75 mL of brine. The organic phase was separated and the aqueous phase washed twice with Et_2O. The combined organic phases were dried and evaporated, and the residue was distilled under vacuum to give the title compound; 34.3 g (68.5%); bp 90–95 °C (15 mm).

trans-N-(Aminoiminomethyl)-2,5-dihydro-2,5-dimethyl-1H-pyrrole-1-acetamide (2). A solution of sodium methoxide was prepared from sodium (2.75 g, 0.12 mol) in 50 mL of methanol. Guanidine hydrochloride (12 g, 0.12 mol) was added to the above

(11) Stewart, J. J.; Gaginella, T. S.; Olsen, W. A.; Bass, P. J. Pharmacol. Exp. Ther. 1975, 192, 458.

(12) Compound 2 (code designation Wy 25021) has been assigned the BAN name "rolgamidine".

Table III. Cardiovascular Activity in Normotensive Rats[a]

no.	n^b	heart rate, beats/min					systolic bp, mmHg				
		predose	1 h	2 h	4 h	6 h	predose	1 h	2 h	4 h	6 h
1	6	493	175[c]	218[c]	218[c]	242[c]	153	193[c]	159	163	138
2	5	430	395	373[d]	418	443	141	133	137	133	134
loperamide	5	438	278[c]	290[c]	324[d]	360[d]	142	119	117	124	125
lidamidine	5	393	263[d]	241[d]	261[d]	298	140	123	132	126	131

[a] Mean values for systolic blood pressure and heart rate observed after oral administration of 30 mg/kg of test compound. [b] Number of rats used in each experiment. [c] Significantly different from predose value, $p < 0.001$. [d] Significantly different from predose value, $p < 0.05$.

solution and the mixture stirred for 1 h. The ester 12 (13.4 g, 0.08 mol) was then added and the mixture allowed to stand for 18 h. The solvent was evaporated and the residue diluted with 30 mL of H_2O and 60 mL of Et_2O. After stirring and ice-cooling the precipitated base was collected, washed with cold H_2O, and dried. The base was suspended in 50 mL of IPA and acidified with ethanolic HCl to precipitate the hydrochloride. The hydrochloride was dissolved in 30 mL of hot methanol and diluted with 30 mL of EtOAc. On cooling, the title compound crystallized: 10.7 g (49.7%); mp 215–217 °C; IR (Nujol) 3400, 3220, 1730, 1695 cm^{-1}; NMR (D_2O) δ 1.43 (d, 6 H, 2,5-CH$_3$), 4.43 (dd, 2 H, CH$_2$), 4.7 (q, 2 H, 2,5-H), 5.99 (s, 2 H, 3,4-H); NMR (CF$_3$CO$_2$H) δ 1.5 (d, 3 H, CH$_3$), 1.65 (d, 3 H, CH$_3$), 4.5 (dd, 2 H, CH$_2$), 5.15 (m, 2 H, 2,5-H). Anal. (C$_9$H$_{18}$N$_4$O·2HCl) C, H, N.

***trans*-*N*-(Aminoiminomethyl)-2,5-dimethylpyrrolidine-1-acetamide (3).** A solution of 2·2HCl (1.08 g, 0.004 mol) in 15 mL of ethanol was hydrogenated at atmospheric pressure over 10% Pd/C (0.08 g). The catalyst was filtered off, the solvent was evaporated, and the residue was crystallized from ethanol–ethyl acetate (1:1) to give 0.85 g (78.4%): mp 206–209 °C; IR (Nujol) 3640, 3430, 1735, 1605cm^{-1}; NMR (D_2O) δ 1.3 (br s, 6 H, 2,5-CH$_3$), 1.83 (m, 2 H, 3,4-H), 2.35 (m, 2 H, 3,4-H), 3.75 (br m, 1 H, 2/5-H), 4.25 (br m, 1 H, 2/5-H), 4.3 (dd, 2 H, CH$_2$); NMR (CF$_3$CO$_2$H) δ 1.53 (d, 3 H, CH$_3$), 1.68 (d, 3 H, CH$_3$), 2.05 (m, 2 H, 3,4-H), 2.6 (m, 2 H, 3,4-H), 3.9 (m, 1 H, 2/5-H), 4.45–4.65 (m, 3 H, CH$_2$ + 2/5-H). Anal. (C$_9$H$_{18}$N$_4$O·2HCl) C, H, N.

[2-(*trans*-2,5-Dihydro-2,5-dimethyl-1*H*-pyrrol-1-yl)-ethyl]guanidine (9). A solution of 2 base (1.95 g, 0.01 mol) in 50 mL of dry THF was added to a stirred suspension of lithium aluminum hydride (0.8 g, 0.02 mol) in 50 mL of dry THF. The mixture was stirred at reflux for 4.5 h and then quenched by cautious addition of 3 mL of H_2O. The precipitated solid was removed by filtration and washed with 50 mL of ethanol. The combined filtrates were evaporated, and the residue was dissolved in 5 mL of ethanol and acidified with ethanolic HCl to precipitate the dihydrochloride: 1.2 g (47.2%); mp 237–239 °C; IR (Nujol) 2640, 1660, 1630, 1360, 755 cm^{-1}; NMR (D_2O) δ 1.43 (d, 6 H, 2,5-CH$_3$), 3.35–3.75 (m, 4 H, CH$_2$CH$_2$), 4.5 (q, 2 H, 2,5-H), 5.91 (s, 2 H, 3,4-H). Anal. (C$_9$H$_{18}$N$_4$O·2HCl) C, H, N.

Resolution of *trans*-Methyl 2,5-Dihydro-2,5-dimethyl-1*H*-pyrrole-1-acetate (12). The racemic ester (12) (13.36 g, 0.08 mol) was added with stirring to a solution of (–)-dibenzoyl-L-tartaric acid monohydrate (15 g, 0.4 mol) in 40 mL of MeOH and 40 mL of IPA. The solution was set aside to crystallize at 4 °C overnight. The crystalline precipitate, crop A (17.2 g), was collected and recrystallized from methanol to give (+)-12 acid (–)-dibenzoyl-L-tartrate: 13.7 g (65%); mp 163–164 °C; $[\alpha]^{23}_D$ –1.8° (c 8.68, MeOH). Anal. (C$_9$H$_{15}$NO$_2$·C$_{18}$H$_{14}$O$_8$) C, H, N. The salt was basified with aqueous NaHCO$_3$ and extracted with ether. The ether extract was dried and evaporated, and the residual oil was dried at 0.1 mmHg to give (+)-12: 3g (45%); $[\alpha]^{22}_D$ + 287° (c 0.94, CHCl$_3$). The filtrate obtained after collection of crop A was acidified further with (–)-dibenzoyl-L-tartaric acid monohydrate (15 g) and again set aside to crystallize overnight. The second crop of crystals was collected, washed with IPA, and recrystallized from methanol to give (–)-12 acid (–)-dibenzoyl-L-tartrate: 14.9

g (70.6%); mp 144–145 °C; $[\alpha]^{25}_D$ –142.5° (c 1.94, MeOH). Anal. (C$_9$H$_{15}$NO$_2$·C$_{18}$H$_{14}$O$_8$) C, H, N. The salt was basified as previously described to give (–)-12: $[\alpha]^{25}_D$ –269° (c 1.9, CHCl$_3$).

Chiral Purity of the Enantiomers of 12. The above resolved enantiomers of 12 were examined by NMR in CDCl$_3$ in the presence of 1.4 mol of Eu-Opt/mol of 12. The best separation of signals was found for the olefinic protons, which occurred at δ 5.90 and 5.92 for (+)-12 and (–)-12, respectively. The chiral purity was determined by integration and estimated to be greater than 98% for each isomer.

Blood Pressure and Heart Rate Measurements in Conscious Rats. Cannulae were implanted under anaesthesia into the left carotid artery of female normotensive rats. The following day they were dosed orally with the test substance in 0.5% hydroxymethylcellulose/0.9% saline vehicle (10 mL/kg) or vehicle alone. Blood pressure was measured from the cannulae, using a Statham P23dB pressure transducer, before dosing and at 1, 2, 4, and 6 h post-dosing. The blood pressure signal triggered a tachometer, and heart rate and blood pressure were displayed on a Grass recorder.

Inhibition of Castor Oil Induced Diarrhea in Rats. Male rats were housed individually in cages with wide-mesh grids to prevent coprophagy, deprived of food, but allowed free access to water, overnight. On the following day the rats were divided into groups of six and dosed orally with the test substance in 0.3% hydroxymethylcellulose/0.9% saline (10 mL/kg) or with vehicle alone. One hour later, each rat was given 1 mL of castor oil, orally, to induce diarrhea. Fecal output was collected on preweighed papers beneath each cage and weighed 2 h and 6 h after castor oil administration. The fecal output in the drug group was expressed as a percentage of the output in the group dosed with vehicle.