ance responses, (3) the number of escape responses, and (4) the number of trials in which no response occurred. These data were used to calculate the percent difference from control values previously determined. For active compounds, response counts were summed over all subjects at a given dose. The number of trials in which rats failed to exhibit an avoidance response (avoidance block, AB) was determined at each dose. This number was expressed as a percentage of the total trials. Control performance was arbitrarily set at 100% for avoidance responding responding (AB₅₀) was obtained from a dose–effect regression line fitted by the method of least squares.

Registry No. 3, 103096-52-2; 4, 109315-45-9; 4-maleate, 109315-46-0; 5, 58038-66-7; 6a, 6208-60-2; 6b, 19685-84-8; 6c, 39876-39-6; 8, 109839-59-0; 9, 107265-98-5; 9-2HCl, 107288-27-7; 10, 107266-04-6; 10-2HCl, 107266-16-0; 11, 107265-97-4; 11-2HCl, 107266-11-5; 12, 107266-01-3; 12·2HCl, 107266-14-8; 13, 107266-03-5; 13·2HCl, 107288-28-8; 14, 107265-96-3; 14·2HCl, 107266-10-4; 15, 107266-02-4; 15·2HCl, 107266-15-9; 16, 109839-60-3; 16·2HCl, 109839-61-4; 17, 107266-06-8; 17·2HCl, 107266-18-2; 18, 107266-

08-0; 18-2HCl, 107266-20-6; 19, 109839-62-5; 19-2HCl, 109839-63-6; 20, 109315-31-3; 20.2HCl, 109315-39-1; 21, 109315-29-9; 21.2HCl, 109315-38-0; 22, 109315-28-8; 22·2HCl, 109315-37-9; 23, 107266-00-2; 23·2HCl, 107266-13-7; 24, 107266-05-7; 24·2HCl, 107266-17-1; 25, 107265-99-6; 25.2HCl, 107266-12-6; 26, 109315-30-2; 26.2HCl, 109315-36-8; 27, 109862-74-0; 27.2HCl, 109862-75-1; 28, 109315-34-6; 28-2HCl, 109315-42-6; 29, 109315-35-7; 29-2HCl, 109315-43-7; 30, 109315-32-4; 30·2HCl, 109315-40-4; 31, 109839-64-7; 31·HCl, 109839-65-8; 32, 109839-66-9; 32·HCl, 109839-67-0; 33, 109839-68-1; 33.3HCl, 109839-69-2; 34, 109839-70-5; 34.3HCl, 109839-71-6; 35, 109839-72-7; **35**-3HCl, 109839-73-8; Br(CH₂)₃Br, 109-64-8; Br(C-H₂)₃Cl, 109-70-6; 4-(4-bromobutyl)pyridine, 109315-44-8; 3-(3bromopropyl)pyridine, 109839-74-9; 2-(2-bromoethyl)pyridine, 39232-04-7; 2-(2-bromoethyl)quinoline, 109839-75-0; 4-(2bromoethyl)pyridine, 39232-05-8; 4-(4-bromobutyl)quinoline, 109839-76-1; 2-vinylpyridine, 100-69-6; 4-vinylpyridine, 100-43-6; 2-vinylpyrazine, 4177-16-6; 2-vinylquinoline, 772-03-2; 8-chloro-2,3,4,5-tetrahydro-(2-(3-bromopropyl))-1H-pyrido[4,3-b]indole, 109839-77-2; 2,6-dimethylpiperazine, 108-49-6; 1-(2-pyrimidyl)piperazine, 20980-22-7; 4,4-bis(p-fluorophenyl)-1-bromobutane, 57668-61-8

6-Methylergoline-8-carboxylic Acid Esters as Serotonin Antagonists: N¹-Substituent Effects on 5HT₂ Receptor Affinity

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Three series of 6-methylergoline-8-carboxylic acid esters with various alkyl substituents in the N¹-position were prepared and their $5HT_2$ receptor affinities measured. Some overlap occurred in the $5HT_2$ receptor affinities of the different ester series, indicating that both the ester side chain and the indole substituent influenced $5HT_2$ receptor affinity. While $5HT_2$ receptor affinity was affected by the structure of the ester side chain, the N¹-substituent played a more crucial role in determining $5HT_2$ receptor affinity. When the ester side chain was held constant, maximal $5HT_2$ receptor affinity for that series of esters was obtained when the N¹-substituent was isopropyl. Smaller substituents in the N¹-position resulted in reduced $5HT_2$ receptor affinity. Groups C₄ or larger in the N¹-position resulted in a further decline in $5HT_2$ receptor affinity. The importance of the N¹-substituent in determining $5HT_2$ receptor affinity was further substantiated when several 2-methyl-3-ethyl-5-(dimethylamino)indoles with various N¹-substituents were tested. Again, maximal $5HT_2$ receptor affinity was obtained when the N¹-substituent was isopropyl.

LY53857 (1) has been shown to be a potent and selective antagonist of vascular $5HT_2$ receptors.¹ It was important to determine what structural features were most instrumental in bestowing this unusually high affinity at $5HT_2$ receptors to the molecule. Compound 1 is actually a mix



of four diastereomers² resulting from esterification of 1-(1-methylethyl)-6-methylergoline-8-carboxylic acid with racemic 2,3-butanediol. The four diastereomers of 1 are

all biologically active with nearly equal affinities for 5HT₂ receptors.² This suggested that portions of the molecule other than the ester side chain may play an important role in determining 5HT₂ receptor affinity. To test this hypothesis, three series of 6-methylergoline-8-carboxylic acid esters were prepared with various substituents in the N¹-position. The affinities of these compounds for $5HT_2$ receptors were then determined by measuring their ability to antagonize serotonin-induced contractions in the rat jugular vein, a tissue known to possess $5HT_2$ receptors that are responsible for serotonin-induced contractions.³ To further substantiate that the N1-substituent and not some other structural feature of the ergoline nucleus was crucial to 5HT₂ receptor affinity, several 2-methyl-3-ethyl-5-(dimethylamino)indoles with various substituents in the N^1 -position were also tested. The results of these experiments clearly demonstrate the dramatic influence N¹substitution has on $5HT_2$ receptor affinity for both 6methylergoline-8-carboxylic acid esters and 2-methyl-3ethyl-5-(dimethylamino)indoles.

Chemistry

Dihydrolysergic acid (2) was alkylated at the N¹-position prior to esterification. Two alkylation procedures were

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Table I. 6-Methylergoline-8-carboxylic Acid Esters

									CH3	
		COOCH3			COOCH2CH2OH			сооснснон		
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		formula	mn	recrystn	formula	mn	recrystn	formula	mn	recrystn
<u></u>	н	C H NO	182-184	MoOH/	C.H.N.O.	169 5-171	HOCH CH.OH/	C H NO	242-244	DMF/
-	11	$C_{17} R_{201} Q_2 C_2$	102 104	H ₂ O	$O_{18} I_{22} I_{2} O_{3}$	100.0 171	H ₂ O	$C_{20} 1_{26} 1_{2} C_{3}$	242 244	H ₂ O
3	CH_3	${\rm C}_{18}{\rm H}_{22}{\rm N}_{2}{\rm O}_{2}$	115 - 116.5	metĥyl-	$C_{19}H_{24}N_2O_3$	152 - 153	MeOH/	$C_{21}H_{28}N_2O_3$.	112 - 113	EtOH/
				cyclo-			H_2O	maleate		Et_2O
4	CH ₂ CH ₂	C10H24N2O2	117-119	methyl-				CaaHaaNaOa•	155 - 156	EtOAc
-		- 19 24- • 2 - 2		cyclo-				maleate		
		A M M A		hexane				a H N A		MOUL
5	$CH_3CH_2CH_2$	$C_{20}H_{26}N_2O_2$	159-162	EtOAc/ Et-O				C ₂₃ H ₃₂ N ₂ O ₃ -	132-135	MeOH/ Et.O
6	CH ₂ =CHCH ₂	$C_{20}H_{24}N_2O_2$	101-103	methyl-	$C_{21}H_{26}N_2O_3$.	112-114	CH ₃ CN/	maicate		11020
		50 51 5 5		cyclo-	maleate		Et_2O			
7	(CH) CH	CHNO	100 110	hexane	CHNO	166 160	Manu	СЧЛО	107 100	MOU
1	(CH ₃) ₂ CH	$C_{20}\Pi_{26}\Pi_2O_2$	109-110	H ₀ O	$C_{21}\Pi_{28}N_2O_3$	100-109	H ₂ O	$C_{23}\Pi_{32}\Pi_2O_3$ maleate	107-100	Et _o O
8	$(CH_3)_2CHCH_2$	$C_{21}H_{28}N_2O_2$.	142 - 145	MeOH/	$C_{22}H_{30}N_2O_3$.	225 - 226.5	MeOH/	$C_{24}H_{34}N_2O_3$.	147 - 149.5	MeOH/
0		maleate	100 100	Et_2O	maleate		Et_2O	maleate	155 150	Et_2O
y	$(CH_3CH_2)_2CH$	$C_{22}H_{30}N_2O_2$	162-163	MeOH/ Et.O				$C_{25}H_{36}N_2O_3$ HCl	155-158	EtOAC/ Et ₀ O
10	cyclopentyl	maleate		1120				$C_{25}H_{34}N_2O_3$	166.5 - 168.5	EtOH/
								maleate		Et_2O
11	cycloheptyl							C ₂₇ H ₃₈ N ₂ O ₃ ·	186-187	MeOH/ Et ₂ O
12	benzvl	CatHacNaOat	193-194	EtOH/	C25H28N2O2+	132-134	MeOH/	C ₂₇ H ₃₂ N ₂ O ₃ .	84-88	EtoAc/
	~	maleate		Et_2O	maleate		Et_2O	maleate		Et_2O
13	$CH_3(CH_2)_6CH_2$							$C_{28}H_{42}N_2O_3$	103.5-106.5	EtOH/
								maleate		Et_2O

used. The procedure most useful for alkylation of 2 with small groups involved reaction of 2 with sodium amide in liquid ammonia, followed by addition of an appropriate C_1-C_3 alkyl halide.⁴ This alkylation procedure was not successful with larger groups, so another process was used for introducing alkyl groups C_3 or larger.⁵ This second process required addition of the appropriate alkyl tosylate to a solution of 2 in dimethyl sulfoxide containing excess potassium hydroxide. N¹-Substituted acids 3–13 were prepared by using these two routes in 77–94% theory yield.

The acids made were then esterified in turn with methanol, ethylene glycol, and 2(R),3(R)-butanediol to give three different series of esters (Table I). The methyl esters were prepared by dissolving the acid in methanol containing sulfuric acid and stirring at ambient temperature for 24-48 h. The hydroxyethyl esters (from ethylene glycol) and the (R,R)-2-hydroxy-1-methylpropyl esters (from 2(R),3(R)-butanediol) were prepared by dissolving the desired N¹-substituted acid in the appropriate alcohol in the presence of *p*-toluenesulfonic acid and heating at 60-80 °C for 4-24 h. The esters were isolated as either a maleate salt, a hydrochloride salt, or the free base. Yields of analytically pure material ranged from 48% to 86% theory for the methyl esters, from 27% to 77% theory for the hydroxyethyl esters, and from 20% to 86% theory for the (R,R)-2-hydroxy-1-methylpropyl esters. 2-Methyl-3ethyl-5-(dimethylamino)indoles 14a-d were prepared via the Fischer indole synthesis and have been reported recently. 6



Results and Discussion

Biological activities of the prepared esters were measured by their ability to antagonize serotonin-induced contractions in the rat jugular vein, a tissue known to contain $5HT_2$ receptors.³ The affinity of each of these esters for $5HT_2$ receptors was calculated from these data and is presented in Table II. Several conclusions about the effect of the ester moiety and the indole substituent on $5HT_2$ receptor affinity can be made.

Ergoline acids 2-13 (all with $-\log K_B < 7$) had no appreciable $5HT_2$ receptor affinity when compared to their derivative esters. Esterification of the acids greatly improves the compounds' affinity for $5HT_2$ receptors. Generally, the methyl esters are 100 times more active than the parent acid.

With respect to the esters, $5HT_2$ receptor affinity also increases as branching on the ester side chain increases.

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The (R,R)-2-hydroxy-1-methylpropyl esters are in some cases 10 times as active as the methyl esters, with the activity of the hydroxyethyl esters generally lying between the two.

The $5HT_2$ receptor affinity of the esters, however, can be greatly altered by changing the alkyl substituent on the indole nitrogen. Modifying the indole substituent (R) affects the $5HT_2$ receptor affinity in a predictable pattern within a given series of esters. When R is hydrogen, moderate affinity for the 5HT₂ receptor is observed. Changing R to C_1 - C_3 straight chain alkyl increases 5HT₂ receptor affinity by 1–10 times. Maximal $5HT_2$ receptor affinity for that ester series is observed when R is isopropyl (C_3 branched). Such compounds show an increase in 5HT₂ receptor affinity 1–26 times greater than that shown by compounds with C_1 – C_3 straight chain substitution. Further increasing the number of carbons in R results in a large reduction in $5HT_2$ receptor affinity. Changing R to isobutyl caused the 5HT₂ receptor affinity to be lower than when R was hydrogen. Addition of even more carbons to R results in the ester becoming as inactive as the acids themselves. Some $5HT_2$ receptor affinity returns when R is converted to benzyl; however, the $5HT_2$ receptor affinity is not nearly as great as when R is C_1-C_3 alkyl.

Due to the combined influences of the ester side chain and the indole substituent on $5HT_2$ receptor affinity, some overlap did occur in the $5HT_2$ receptor affinities of the different ester series. For example, the N¹-isopropyl methyl ester **7a** has a lower $5HT_2$ receptor affinity than does the N¹-methyl (*R*,*R*)-2-hydroxy-1-methylpropyl ester **3c**.

The importance of the alkyl substituent on the indole nitrogen in determining $5HT_2$ receptor affinity is further demonstrated with the series of 2-methyl-3-ethyl-5-(dimethylamino)indoles 14a-d. As shown in Table III, parent 14a has low affinity for $5HT_2$ receptors. Addition of a methyl or ethyl group to the indole nitrogen increases $5HT_2$ receptor affinity by a factor of 30-40. However, maximal $5HT_2$ receptor affinity is again observed when the indole substituent is isopropyl. Compound 14d is 224 times more active than the unsubstituted 14a.

On the basis of data obtained with 1, we previously suggested that the stereochemistry of the ester side chain was not crucial in determining $5HT_2$ receptor affinity.²

Table III. Affinity of

2-Methyl-3-ethyl-5-(dimethylamino)indoles at 5HT₂ Receptors



The data presented above now confirms that the $5HT_2$ receptor affinity of 6-methylergoline-8-carboxylic acid esters is not solely determined by the ester moiety. Clearly the indole substituent has a major role in determining the $5HT_2$ receptor affinity of these particular compounds. Maximal $5HT_2$ receptor affinity for a particular ester side chain is always obtained when there is an isopropyl group on the indole nitrogen.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Identities of all compounds were confirmed by ¹H NMR, mass spectra, and combustion analysis, except for acids 3–13. Compounds 3–13 were characterized by ¹H NMR and mass spectra, and their purity was determined by HPLC (Zorbax CN, mobile phase: 25:75 CH₃CN/0.1 M NH₄OAc, flow rate = 2 mL/min, UV detector set at 25-4 nm). Water content of compounds 3–13 was determined by Karl Fischer titration. All esterification reactions were followed by TLC carried out on Merck F254 silica gel plates using chloroform/methanol/acetic acid, 18:6:1, as developing solvent. Microanalyses were provided by the Physical Chemistry Department of the Lilly Research Laboratories. The experimental procedures described below are representative of the procedures used to prepared the esters listed in Table I.

 (8β) -6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic Acid (7). A mixture of sodium amide in liquid ammonia was prepared by the addition of 31 g (1.348 mol) of sodium to 4 L of anhydrous ammonia. A small amount of ferric nitrate was added to catalyze the formation of sodium amide. After all the blue color was discharged, 108 g (0.366 mol, water content = 8.3%) of dihydrolysergic acid was added in portions and then the mixture was stirred for 30 min. To this mixture was added 272 g (1.6 mol) of 2-iodopropane over a 30-min period. The reaction mixture was stirred for 1 h and then the ammonia allowed to evaporate. The residue was taken up in 1400 mL of methanol and treated with Darco and the pH adjusted to 6 with glacial acetic acid. The resultant crystals were collected and reslurried in water. The product was collected and dried in vacuo to yield 93.8 g of 7 (97.9% assay, water content 1.0%, 79% theory yield).

 (8β) -1-Cyclopentyl-6-methylergoline-8-carboxylic Acid (10). A mixture of 10 g (33.88 mmol) of dihydrolysergic acid, 12.05 g (86% pure, 185 mmol) of powdered potassium hydroxide, and 75 mL of dimethyl sulfoxide was stirred until all of 2 dissolved, and then a solution of 12.21 g (50.81 mmol) of cyclopentyl tosylate in 25 mL of dimethyl sulfoxide was added dropwise over a 2-h period. After 2 h, the reaction mixture was poured into 500 mL of ice water and filtered to remove turbidity. The product was precipitated from the filtrate by adjusting the pH to 5-6 with glacial acetic acid and then was collected and dried in vacuo yielding 11.43 g of 10 (92.5% assay, water content 0.9%, 91% theory yield). Purer material could be obtained, if necessary, by reprecipitating the product from dilute ammonia solution.

 (8β) -1-(1-Ethylpropyl)-6-methylergoline-8-carboxylic Acid, Methyl Ester (Z)-2-Butenedioate (1:1) (9a). A solution of 1.50 g (4.40 mmol) of (8β) -1-(1-ethylpropyl)-6-methylergoline-8carboxylic acid, 50 mL of methanol, and 0.75 g (7.65 mmol) of sulfuric acid, 98%, was stirred overnight at ambient temperature. The solvent was evaporated, and the residue was partitioned between dichloroethane and saturated aqueous sodium bicarbonate solution. The organic layer was dried over sodium sulfate, filtered, and evaporated, yielding 1.6 g of crude ester. This material was dissolved in 15 mL of methanol, and 0.59 g (5.08 mmol) of maleic acid was added. The ester salt was precipitated from solution by the dropwise addition of 150 mL of diethyl ether. The product was collected by filtration and dried in vacuo to give 1.66 g of 9a (80%), mp 162–163 °C.

 (8β) -6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic Acid, 2-Hydroxyethyl Ester (7b). A mixture of 5.00 g (16.00 mmol) of (8β) -6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 5.00 g (26.30 mmol) of p-toluenesulfonic acid, and 50 mL of ethylene glycol was stirred at 80 °C overnight. The reaction mixture was cooled to ambient temperature and added to 350 mL of ice water containing 25 mL of concentrated NH₄OH. The resultant mixture was chilled at 0 °C. The precipitated crude ester was collected by filtration and dried in vacuo. The dried filter cake was dissolved in 125 mL of warm methanol and filtered. Purified 7b was precipitated from the filtrate by the dropwise addition of 250 mL of water. The product was collected by filtration and dried in vacuo to yield 4.50 g of 7b (79%), mp 166–169 °C.

 (8β) -1,6-Dimethylergoline-8-carboxylic Acid, $[R \cdot (R^*, R^*)]$ -2-Hydroxy-1-methylpropyl Ester (Z)-2-Butenedioate (1:1) (3c). A mixture of 2.16 g (7.60 mmol) of (8β) -1,6-dimethylergoline-8-carboxylic acid, 2.16 g (11.36 mmol) of ptoluenesulfonic acid, and 20 mL (222 mmol) of 2(R),3(R)-butanediol was stirred at 80 °C overnight. The mixture was cooled to ambient temperature and added to 150 mL of ice water containing 10 mL of concentrated NH₄OH. This mixture was extracted with 200 mL of dichloroethane. The dichloroethane layer was back-extracted with 100 mL of water and then evaporated. The crude residue was taken up in 100 mL of ethyl acetate, and 0.85 g (7.29 mmol) of maleic acid was added. The precipitated crude **3c** was collected by filtration, dried, then reprecipitated from ethanol solution by the addition of diethyl ether, which yielded on drying 2.0 g (68%) of **3c**, mp 112–113 °C.

Isolation of Tissue for Receptor Antagonist Studies. Male Wistar rats (150–300 g) (Harlan Sprague–Dawley, Inc.) were killed by cervical dislocation. External jugular veins from the rats were dissected free of connective tissue, cannulated in situ with polyethylene tubing (PE-50, o.d. = 0.97 mm), and placed in Petri dishes containing Krebs bicarbonate buffer (see below). The tips of two 30-gauge stainless steel hypodermic needles bent into an L-shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was attached with thread to a stationary glass rod and the upper one was tied with thread to a transducer. This procedure for ring preparations (circular smooth muscle) of blood vessels has been described previously.⁷

Tissues were mounted in organ baths containing 10 mL of modified Krebs solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; CaCl₂·2H₂O, 1.6; KH₂PO₄, 1.2; MgSO₄, 1.2; dextrose, 10.0; and NaHCO₃, 24.8. Tissue bath solutions were maintained at 37 °C and aerated with 95% $O_2/5\%$ CO₂. An initial optimum resting force of 1 g was applied to the jugular vein. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and a microscale accessory attachment. Tissues were allowed to equilibrate for 1–2 h before exposure to drugs.

Determination of Apparent Dissociation Constants. After control cumulative contractile responses to serotonin in the jugular vein were obtained, vessels were incubated with appropriate concentrations of antagonist for 1 h. Responses to serotonin were then repeated in the presence of antagonist. Contraction to serotonin was evaluated in the jugular vein as this tissue produced marked responses to serotonin in the absence of α receptors.⁸

Apparent antagonist dissociation constants (K_B) were determined for each concentration of antagonist according to the following equation:

$$K_{\rm B} = [{\rm B}]/({\rm dose \ ratio} - 1)$$

where [B] is the concentration of the antagonist and dose ratio is the ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50} . These results were then expressed as the negative logarithm of the $K_{\rm B}$ (i.e., -log $K_{\rm B}$).

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