Biomedical Research Support Grant (NIH RR-5606), from the J. R. and Inez W. Jay Research Bequest at the University of Kansas, and from the Kansas Advanced Technology Commission made possible some of the hardware purchases for the University of Kansas Molecular Graphics and Modeling Laboratory in the Department of Medicinal Chemistry. In addition, we thank Dr. Michael Mokotoff (University of Pittsburgh) for a gift of compound 4. The Intersearch Program of the University of Kansas and the Victorian College of Pharmacy made possible a visit of the senior author to the Victorian College of Pharmacy (Melbourne, Australia), where the molecular orbital calculations in this manuscript were conducted in the laboratory of Dr. Peter Andrews. Discussions in his laboratory with Drs. Margaret Wong and Maruse Sadek were especially helpful. Research Grant HL 34193 from the USPHS, Grant-in-Aid 83-905 from the American Heart Association, and Grant-in-Aid KS-83-G-66 from the American Heart Association, Kansas Affiliate, Inc., are gratefully acknowledged.

Registry No. 4·HBr, 28446-49-3; 5·HCl, 5176-31-8; 6, 111634-87-8; 6·HCl, 111634-86-7; 7, 111634-88-9; 7·HCl, 111635-04-2; 8, 111634-89-0; 8·HCl, 111635-06-4; 9, 53921-73-6; 9·HCl, 111635-07-5; 10, 4965-09-7; 10·HCl, 111635-08-6; 11, 29726-60-1; 12, 110841-71-9; 12·HCl, 111661-47-3; 16, 3118-16-9; 17, 29427-69-8; 18, 29427-70-1; 19 (isomer 1), 111634-92-5; 19 (isomer 2), 111634-90-3; 20, 111634-91-4; 20·HCl, 111634-93-6; 21, 111634-94-7; 22, 111634-95-8; 23, 32499-64-2; 24, 111634-96-9; 25, 111634-97-0; 26, 111634-98-1; 27, 111634-99-2; 27·HCl, 111635-05-3; 28, 111635-00-8; 29, 53921-72-5; 30, 111635-01-9; 31, 111635-02-0; 32, 2412-58-0; 33, 111635-03-1; 34, 70079-42-4; PNMT, 9037-68-7; phenylsuccinic anhydride, 1131-15-3; tropinone, 532-24-1; dimethyl (methoxymethylene)malonate, 22398-14-7; *N*-vinyl-2-pyrrolidone, 88-12-0; 1,2,3,4-tetrahydroisoquinoline-1-acetic acid, 105400-81-5; 2-methyl-2-phenylethylamine, 582-22-9.

(8β)-Ergoline-8-carboxylic Acid Cycloalkyl Esters as Serotonin Antagonists: Structure–Activity Study

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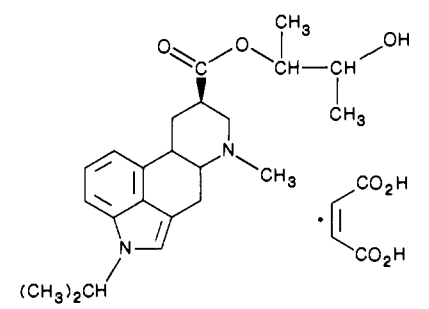
A series of (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid cycloalkyl esters were prepared and examined for blockade of vascular $5HT_2$ receptors. The antagonist in this series that had the highest $5HT_2$ receptor affinity was (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid cyclohexyl ester (**3**). This compound was therefore chosen as the basic backbone of a structure–activity study to determine what effect different N^1-substituents, N^6-substituents, and ester ring substituents had on $5HT_2$ receptor affinity. Maximal $5HT_2$ receptor affinity was obtained when the N^1-substituent was isopropyl, the N^6-substituent was methyl, and there was a hydroxy or keto substituent in the 4-position of the ester cyclohexyl ring.

Esters of (8β)-6-methylergoline-8-carboxylic acids such as LY53857 (**1**) are potent and selective antagonists of $5HT_2$ receptors.[1,2] Compound **1** has been widely used to study $5HT_2$–receptor interactions[3-7] because of its high potency and greater selectivity for $5HT_2$ receptors (relative to α_1 receptors) than other potent $5HT_2$ receptor antagonists.[8] However, **1** is a mixture of isomers[1] which may possess different pharmacokinetic profiles complicating in vivo estimates of efficacy. With **1** as a prototype, it was our goal to search for even more potent and/or selective antagonists that were composed of a single isomer.

Affinities of these compounds for $5HT_2$ receptors were determined by 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.[9]



1

Previously, we established that maximal $5HT_2$ receptor affinity was obtained when the indole nitrogen of the ergoline (N^1) was alkylated with an isopropyl group.[2] We also determined that the stereochemical orientation of the ester side chain had only minimal influence on the $5HT_2$ receptor affinity.[10] It was important to identify further the effects of structural changes in the molecule on $5HT_2$ receptor affinity, in particular, to determine the influence of the N^6-substituent on $5HT_2$ receptor affinity and define what ester moiety gave the highest affinity for $5HT_2$ re-

(1) Cohen, M. L.; Fuller, R. W.; Kurz, K. D. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 327.
(2) Marzoni, G.; Garbrecht, W. L.; Fludzinski, P.; Cohen, M. L. *J. Med. Chem.* **1987**, *30*, 1823.
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(5) Sternberg, E. M.; Trial, J.; Parker, C. W. *J. Immunol.* **1986**, *137*, 276.
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(8) Cohen, M. L.; Schenck, K. W.; Kurz, K. D. *J. Cardiovasc. Pharmacol., Suppl.*, in press.
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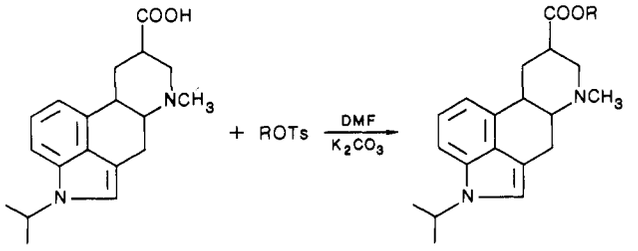
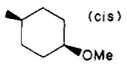
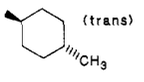
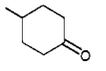
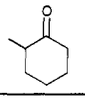
(10) Cohen, M. L.; Kurz, K. D.; Mason, N. R.; Fuller, R. W.; Marzoni, G. P.; Garbrecht, W. L. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 319.

0022-2623/88/1831-0444$01.50/0

Table I. Esterification by Displacement of Various Cyclohexyl Tosylates



no.	R	% theory yield
3		69%
17		91% (trans)
20		75%
22		84% (cis)
18		93%
19		83%

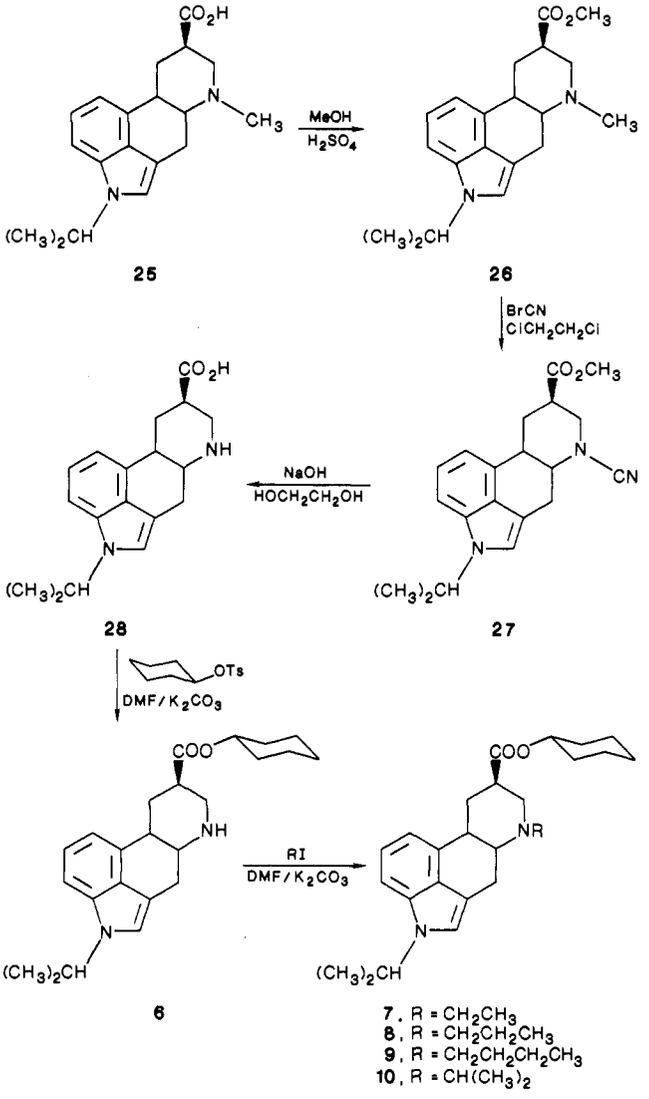
ceptors. In this paper, we report the results of this structure–activity study.

Chemistry

N^1-Alkylated 6-methylergoline-8-carboxylic acids were prepared as reported previously.[2,11,12] Esters were prepared by one of three methods. The majority of the esters were synthesized by acid-catalyzed esterification of the parent acid with an alcohol (method A). The N^1-alkylated 6-methylergoline-8-carboxylic acid was heated in the appropriate alcohol overnight in the presence of *p*-toluenesulfonic acid. The resultant ester was generally isolated by extraction followed by precipitation as the maleate salt. In many instances, however, separation of the desired product from the alcohol solvent was difficult. In some cases preparative HPLC was required to isolate the ester in a pure state. As a result, isolated yields ranged from poor to good (<10% to 72%). A more convenient procedure was therefore developed to prepare those esters that could not be isolated easily, particularly the cyclohexyl and cyclohexyl-like esters.

While reaction of acids with alkyl tosylates or alkyl halides to give esters is known,[13-15] a successful esterification using cyclohexyl tosylate or a substituted cyclohexyl tosylate has never been reported. S_N2 displacements on cyclohexyl rings have generally been found to give mainly elimination products and little substitution.[16,17] We

Scheme I



discovered, however, that the potassium salt of 6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid could be alkylated with various cyclohexyl tosylates when heated under basic conditions in DMF (method B). Successful esterification required that both an excess of tosylate (3.6–5.0 equiv) and an excess of potassium carbonate (9–11 equiv) be present in the reaction mixture. The large excess of potassium carbonate keeps the carboxylic acid from being reprotonated by the *p*-toluenesulfonic acid formed during the competitive elimination side reaction of the tosylate. Isolated yields of product ranged from 69% to 93%, considerably better than obtained from the acid-catalyzed esterification. Workup was easier as well since the unreacted tosylate that remained at the end of the reaction did not interfere with isolation of the desired ester. Generally, the esters were isolated as either the maleate or the hydrochloride salt. Because of the S_N2 nature of the displacement reaction, cis-substituted tosylates gave trans-substituted esters and vice versa. The esters prepared in this manner are shown in Table I.

A third method (C) was used to make **4**, a cyclobutyl ester. The parent acid was activated by reaction with carbonyldiimidazole in DMF. Addition of cyclobutanol resulted in ester formation.

Variation of the substituents in the N^6-position required the synthesis of **6**. This was prepared according to Scheme I. Methyl ester **26** was prepared by dissolving (8β)-1-(1-methylethyl)-6-methylergoline-8-carboxylic acid (**25**) in

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(12) Marzoni, G.; Garbrecht, W. L. *Synthesis*, in press.
(13) Pfeffer, P. E.; Foglia, T. A.; Barr, P. A.; Schmeltz, I.; Silbert, L. S. *Tetrahedron Lett.* **1972**, 4063.
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(15) Liotta, C. L.; Harris, H. P.; McDermott, M.; Gonzalez, T.; Smith, K. *Tetrahedron Lett.* **1974**, 2417.
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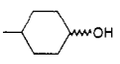
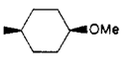
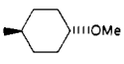
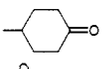
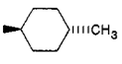
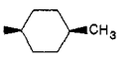
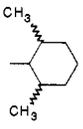
(17) Shaw, J. E.; Hsia, D. Y.; Parries, G. S.; Sawyer, T. K. *J. Org. Chem.* **1978**, *43*, 1017.

Table II. $5HT_2$ Receptor Affinities and Physical Properties of Ergoline-8-carboxylic Acid Esters

no.	R	R^1	R^2	$-\log K_B \pm SE$ (n)	formula	mp, °C recrystn solvent	anal.[a]	meth of prepn
1	$(CH_3)_2CH$	CH_3	$CH(CH_3)CH(CH_3)OH$	10.27 ± 0.13 (17)	$C_{23}H_{32}N_2O_3$·maleate	203–205 dec MeOH/Et_2O	C,H,N	A
2	$(CH_3)_2CH$	CH_3		8.91 ± 0.18 (9)	$C_{24}H_{32}N_2O_2$·maleate	216–217 dec MeOH/Et_2O	C,H,N	A
3	$(CH_3)_2CH$	CH_3		8.67 ± 0.12 (6)	$C_{25}H_{34}N_2O_2$·maleate	214–215 dec MeOH/Et_2O	C,H,N	A, B
4	$(CH_3)_2CH$	CH_3		8.22 ± 0.24 (3)	$C_{23}H_{30}N_2O_2$·maleate·H_2O	190–192 dec MeOH/H_2O	C,H,N	C
5	$(CH_3)_2CH$	CH_3		7.73 ± 0.21 (3)	$C_{26}H_{36}N_2O_2$·maleate	220–222 dec MeOH/Et_2O	C,H,N	A
6	$(CH_3)_2CH$	H		<6	$C_{24}H_{32}N_2O_2$·HCl	194–196 EtOAc/Et_2O	C,H,N	A, B
7	$(CH_3)_2CH$	CH_2CH_3		7.81 ± 0.17 (3)	$C_{26}H_{36}N_2O_2$·HCl	225–230 dec EtOAc	C,H,N	alkylation of 6
8	$(CH_3)_2CH$	$CH_2CH_2CH_3$		7.07 ± 0.05 (3)	$C_{27}H_{38}N_2O_2$·HCl	252–257 dec acetone	C,H,N	alkylation of 6
9	$(CH_3)_2CH$	$(CH_2)_3CH_3$		7.40 ± 0.19 (4)	$C_{28}H_{40}N_2O_2$·HCl	252–258 dec 2-propanol	C,H,N	alkylation of 6
10	$(CH_3)_2CH$	$CH(CH_3)_2$		6.42 ± 0.06 (3)	$C_{27}H_{38}N_2O_2$·HCl	255–262 dec EtOH/Et_2O	C,H,N	alkylation of 6
11	CH_3	CH_3		8.12 ± 0.12 (4)	$C_{23}H_{30}N_2O_2$·maleate	175–177 MeOH/Et_2O	C,H,N	A
12	H	CH_3		8.05 ± 0.14 (4)	$C_{22}H_{28}N_2O_2$·maleate	196–197 dec MeOH/Et_2O	C,H,N	A
13	$(CH_3CH_2)_2CH$	CH_3		7.31 ± 0.11 (3)	$C_{26}H_{38}N_2O_2$·HCl·H_2O	159–163 EtOH/H_2O	C,H,N	A
14	$C_6H_5CH_2$	CH_3		6.52 ± 0.37 (3)	$C_{29}H_{34}N_2O_2$·maleate	100–101 MeOH/Et_2O	C,H,N	A
15	$(CH_3)_2CH$	CH_3		10.18 ± 0.12 (10)	$C_{25}H_{34}N_2O_3$	194.5–197.5 MeOH/H_2O	C,H,N	A
16	$(CH_3)_2CH$	CH_3		9.54 ± 0.11 (3)	$C_{26}H_{36}N_2O_3$·maleate	196–198 MeOH/Et_2O	C,H,N	A
17	$(CH_3)_2CH$	CH_3		8.96 ± 0.07 (18)	$C_{26}H_{36}N_2O_3$·maleate	172–173 EtOH/Et_2O	C,H,N	B
18	$(CH_3)_2CH$	CH_3		10.01 ± 0.08 (10)	$C_{25}H_{32}N_2O_3$·maleate·H_2O	184–185 MeOH/Et_2O	C,H,N	B
19	$(CH_3)_2CH$	CH_3		9.31 ± 0.28 (3)	$C_{25}H_{32}N_2O_3$·maleate·H_2O	203–205 MeOH/Et_2O	C,H,N	B
20	$(CH_3)_2CH$	CH_3		9.86 ± 0.14 (7)	$C_{24}H_{32}N_2O_3$·maleate	191–193 EtOAc/Et_2O	C,H,N	A, B
21	$(CH_3)_2CH$	CH_3		7.21 ± 0.08 (3)	$C_{26}H_{36}N_2O_2$·maleate	185–188 MeOH/Et_2O	C,H,N	A
22	$(CH_3)_2CH$	CH_3		7.69 ± 0.12 (4)	$C_{26}H_{36}N_2O_2$·maleate	197–199 MeOH/Et_2O	C,H,N	A, B
23	$(CH_3)_2CH$	CH_3		7.32 ± 0.17 (3)	$C_{27}H_{38}N_2O_2$·maleate	190–193 dec MeOH/Et_2O	C,H,N	A
24	$(CH_3)_2CH$	CH_3		9.47 ± 0.08 (4)	$C_{25}H_{34}N_2O_3$·maleate	197–199 dec MeOH/Et_2O	C,H,N	A

[a] All compounds gave satisfactory analyses.

methanol containing sulfuric acid. Reaction of **26** with cyanogen bromide in dichloroethane gave cyanamide **27**. Hydrolysis of **27** in ethylene glycol containing sodium hydroxide at 130 °C gave **28**.[18] Esterification of **28** using the displacement procedure described above yielded **6**. Recrystallization of **6** was required prior to subsequent reactions; hence a lower than desired yield (41%) was obtained. Alkylation of **6** with the appropriate alkyl iodide in DMF gave esters **7–10**.

Results and Discussion

Cycloalkyl esters **2–5** (see Table II) were prepared and studied for $5HT_2$ receptor affinity with the long-term goal of developing agents with a more sterically hindered ester linkage than **1**, hence even greater in vivo biological sta-

(18) Misner, J., personal communication.

bility. Cyclopentyl ester **2** and cyclohexyl ester **3** showed high affinity for vascular $5HT_2$ receptors. Cycloalkyl esters with either smaller (**4**) or larger (**5**) rings resulted in diminished $5HT_2$ receptor affinities. Compounds **2** and **3** were therefore singled out for further investigation. Subsequent testing of **2** and **3** in pithed spontaneously hypertensive rats indicated that, in vivo, **3** was more potent as an antagonist of serotonin-induced pressor responses mediated by $5HT_2$ receptors than **2**.[1,19] Because of this, **3** was chosen as the parent compound for the study of further structure-modification effects.

All the ergoline cycloalkyl esters studied to this point had a methyl substituent in the N^6-position. Compounds **6–10** were therefore prepared to determine the effect of alkyl group chain length at the N^6-position on $5HT_2$ receptor affinity. Clearly, maximal $5HT_2$ receptor affinity is obtained when the N^6-substituent is methyl. Affinity decreases as the length or volume requirement of the N^6-alkyl substituent increases. Perhaps surprisingly, the least active of the compounds was **6**, which had no alkyl substituent in the N^6-position and therefore no volume requirement. This suggests that more than steric effects are involved in determining $5HT_2$ receptor affinity.

It having been determined that a methyl group in the N^6-position gave maximal $5HT_2$ receptor affinity, it was important to confirm that an isopropyl group in the N^1-position would also give the cyclohexyl ester with the highest $5HT_2$ receptor affinity. As was seen previously with methyl and hydroxyalkyl esters,[2] maximal $5HT_2$ receptor affinity in the cyclohexyl ester series was obtained when the N^1-substituent was isopropyl. Substitution with hydrogen or methyl resulted in moderate $5HT_2$ receptor affinities while groups larger than three carbons resulted in greatly reduced $5HT_2$ receptor affinity.

Substitution on the cyclohexyl ring was the next variable studied. With alkyl esters, inclusion of oxygen in the alkyl side chain improved $5HT_2$ receptor affinity.2 Introduction of a hydroxy or keto group onto the cyclohexyl ring also greatly increased $5HT_2$ receptor affinity. Compounds **15**, **18**, **19**, and **24** are substantially more active than the parent **3**. The position of substitution is also important. For a given substituent, higher $5HT_2$ receptor affinity occurs when substitution is in the 4-position than when it is in the 2-position. The importance of having an oxygen atom in or near the 4-position of the ring is demonstrated with **20**. Replacing a carbon atom in the cyclohexyl ring with an oxygen atom increases $5HT_2$ receptor affinity by a factor of 15.

Other varieties of ring substitution have less influence on $5HT_2$ receptor affinity. Introduction of a methoxy group onto the 4-position of the cyclohexyl ring (**16** and **17**) increases $5HT_2$ receptor affinity moderately, while placing a methyl group in the same position (**21** and **22**) lowers $5HT_2$ receptor affinity. This again highlights the importance of the oxygen in the 4-position for $5HT_2$ receptor affinity. Methyl groups in the 2- and 6-positions (**23**) further lowered $5HT_2$ receptor affinity.

Comparing the $5HT_2$ receptor affinity of **16** and **17** and **21** with **22** also reveals the importance of the stereochemical orientation of the substituents in determining activity. Compounds that have a cis relationship across the cyclohexyl ring (such as **16** and **22**) have higher $5HT_2$ receptor affinities than their isomers (such as **17** and **21**), which have a trans relationship across the cyclohexyl ring.

With regard to other serotonergic receptors, compound **1** has been most extensively studied and shown to possess approximately 100-fold lower affinity at $5HT_1$ relative to $5HT_2$ binding sites in brain cortical membranes.[10] Although not all compounds synthesized were examined at $5HT_1$ brain binding sites, in general the ergoline esters in this series possessed lower $5HT_1$ binding affinity relative to $5HT_2$ receptor affinity.

Thus, these efforts to utilize cycloalkyl esters of ergoline-8-carboxylic acid as serotonin receptor antagonists indicate that such agents retain potent $5HT_2$ receptor affinity and that structural modification resulted in improved $5HT_2$ receptor affinity. Furthermore, as a result of this and previous studies,[2] several factors can now be identified that enhance $5HT_2$ receptor affinity of ergoline-8-carboxylic acid esters: (1) the N^1-position substituted with an isopropyl group, (2) the N^6-position substituted with a methyl group, and (3) the ester side chain containing an alkyl portion with an oxygen three to four carbons away from the ester oxygen.

Experimental Section

All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. All alcohols used in the esterification reactions were purchased from Aldrich Chemical Co., except for 4-methoxycyclohexanol, which was prepared by the procedure of Eliel and Brett.[20] The tosylates used in the displacement reactions were prepared from the corresponding alcohols by using standard literature procedures,[21] except for 4-ketocyclohexyl tosylate, which was prepared from the 2,2-dimethyl-1,3-dipropylene ketal of 4-ketocyclohexyl tosylate. Identities of all compounds were confirmed by ^1H NMR, mass spectra, and combustion analysis except **28**, which was characterized by ^1H NMR and exact mass spectra. Karl Fischer analysis (KF) of **28** was performed by using a Fischer Automatic K-F Titrimeter system. Esterification reactions were followed by TLC carried out on Merck F254 silica gel plates with chloroform/methanol/acetic acid, 18:6:1, as developing solvent. Displacement reactions were followed by HPLC using a Zorbax CN HPLC column (mobile phase: 25:75 0.1 M NH_4OAc/CH_3CN, flow rate at 1 mL/min) on a Waters M-45 liquid chromatography pump with a Waters 440 absorbance detector set at 254 nm. 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 prepare the esters listed in Table II.

Method A. (8β)-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic Acid, Cyclohexyl Ester (*Z*)-2-Butenedioate (1:1) (3). A mixture of 23.4 g (74.9 mmol) of 8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 14.20 g (74.9 mmol) of *p*-toluenesulfonic acid, and 100 mL of cyclohexanol was heated at 90 °C with stirring overnight. The reaction mixture was partitioned between dichloroethane and dilute ammonia water. The organic layer was washed twice with water and then evaporated. The residue was dissolved in methanol, and excess maleic acid was added. The product was precipitated from solution by the dropwise addition of diethyl ether. The product was collected by filtration and dried in vacuo to yield 27.8 g of **3** (73% theory yield): mp 214–215 °C dec.

Method B. [*trans*-(8β)]-6-Methyl-1-(1-methylethyl)-ergoline-8-carboxylic Acid, 4-Methoxycyclohexyl Ester (*Z*)-2-Butenedioate (1:1) (17). A mixture of 10.05 g (32.1 mmol) of (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 39.75 g (288 mmol) of potassium carbonate, anhydrous, and 150 mL of DMF was heated to 70 °C. The mixture was stirred until no further solids dissolved, and then 45.5 g (160 mmol) of *cis*-4-methoxycyclohexyl tosylate was added in one portion. The resultant mixture was heated at 70 °C for 20 h. The reaction mixture was then partitioned between ethyl acetate and water. The organic layer was washed twice with water, dried over sodium sulfate, and then acidified with HCl(g). After cooling, the resultant precipitate was collected and dried in vacuo, yielding 13.5 g of crude HCl

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salt. The dried filter cake was stirred in 340 mL of 0.2 N sodium hydroxide solution and extracted three times with ethyl acetate. The combined ethyl acetate extracts were dried over sodium sulfate and evaporated, yielding 12.4 g of free base. This residue was taken up in ethanol, and 3.87 g (33.3 mmol) of maleic acid was added. The product was precipitated from solution by the addition of diethyl ether. After cooling, the precipitate was collected and dried in vacuo, yielding 15.8 g of **17** (91% theory yield): mp 172–173 °C.

Method C. (8β)-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic Acid, Cyclobutyl Ester (*Z*)-2-Butenedioate (1:1) Monohydrate (4). A mixture of 5.16 g (18.0 mmol) of (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 4.0 g (24.0 mmol) of carbonyldiimidazole, and 15 mL of DMF was prepared and stirred at room temperature under a nitrogen atmosphere. A mixture of 1.0 g (14.0 mmol) of cyclobutanol, 5 mL of DMF, and 0.1 g (2 mmol) of 50% sodium hydride was then added to the reaction solution. The resultant mixture was stirred overnight. The reaction mixture was partitioned between dichloroethane and dilute ammonia water. The organic layer was evaporated, the residue taken up in methanol, and the solution acidified with excess maleic acid. The product was precipitated from solution by the addition of water. The crude product was collected and reprecipitated from methanol by the addition of water. The product was collected and dried in vacuo to yield 2.10 g of **4** (31% theory yield based on cyclobutanol): mp 190–192 °C dec.

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic Acid, Methyl Ester (26). A solution of 15.6 g (50.0 mmol) of (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 500 mL of methanol, and 9.8 g (100 mmol) of sulfuric acid was stirred at room temperature for 20 h. The solution was partially evaporated until crystals formed, the pH of the mix was adjusted to 8 by the addition of concentrated ammonium hydroxide, and then the mixture was diluted by the dropwise addition of 400 mL of water. After cooling, the product was collected and dried in vacuo to yield 12.4 g of **26** (76% theory yield): mp 109–110 °C.

(8β)-6-Cyano-1-(1-methylethyl)ergoline-8-carboxylic Acid, Methyl Ester (27). A solution of 10.0 g (30.6 mmol) of **26**, 100 mL of dichloroethane, and 4.9 g (46.0 mmol) of cyanogen bromide was stirred overnight at room temperature. The solution was evaporated and the residue dissolved in refluxing methanol. This solution was filtered hot and then cooled. The precipitated product was collected and dried in vacuo, yielding 8.6 g of **30** (84% theory yield): mp 136–137 °C.

(8β)-1-(1-Methylethyl)ergoline-8-carboxylic Acid (28). A mixture of 25.0 g (74.1 mmol) of **27**, 8.89 g (222 mmol) of sodium hydroxide pellets, and 250 mL of ethylene glycol was heated with stirring at 130 °C for 2.75 h. Heat was removed, and the reaction mixture was diluted with 750 mL of water. The pH of the solution was adjusted to 5 with glacial acetic acid. The resultant mix was cooled, and then the product was collected and dried in vacuo, yielding 19.6 g of **28** (88% theory yield): MS (70 eV) *m/e* theory 298.1681, found 298.1679; HPLC assay (Zorbax CN, 25:75 CH_3CN/0.1 M NH_4OAc, flow rate 2 ml/min) 97.3%, KF < 1%.

(8β)-1-(1-Methylethyl)ergoline-8-carboxylic Acid, Cyclohexyl Ester (*Z*)-2-Butenedioate (1:1) (6). A mixture of 2.00 g (6.70 mmol) of **28** (KF = 15%), 8.32 g (60.32 mmol) of potassium carbonate, anhydrous, and 35 mL of DMF was heated to 65 °C. To this mixture was added in one portion 8.52 g (33.51 mmol) of cyclohexyl tosylate. The reaction mixture was stirred at 65 °C for 20 h and then partitioned between ethyl acetate and water. The ethyl acetate layer was washed twice with water, then dried over sodium sulfate, and evaporated. The residue was taken up in 25 mL of fresh ethyl acetate, and 0.78 g (6.70 mmol) of maleic acid was added. The crude product was precipitated from solution by the addition of diethyl ether. After collection, the product was recrystallized from ethyl acetate to yield, on drying, 1.17 g of **6** (41% theory yield): mp 190–191 °C.

(8β)-6-Butyl-1-(1-methylethyl)ergoline-8-carboxylic Acid, Cyclohexyl Ester Hydrochloride (9). A suspension of 2.0 g (4.02 mmol) of **6** in 100 mL of saturated aqueous sodium bicarbonate solution was extracted with 100 mL of dichloroethane. The dichloroethane solution was evaporated, and to the residue were added 15 mL of DMF, 0.67 g (4.83 mmol) of potassium carbonate, and 0.89 g (4.83 mmol) of butyl iodide. This mixture was stirred at ambient temperature for 72 h and then was partitioned between ethyl acetate and water. The ethyl acetate layer was washed twice with water, then dried over sodium sulfate, and evaporated. This residue was taken up in fresh ethyl acetate and acidified with HCl(g). The resultant precipitate was collected and dried to yield 1.76 g of crude **9**. The dried filter cake was recrystallized from 2-propanol to yield 1.45 g of pure **9** (76% theory yield): mp 252–258 °C dec.

4-Ketocyclohexyl Tosylate. The tosylate of 4-ketocyclohexanol 2,2-dimethyl-1,3-dipropylene ketal was prepared from the ketalized alcohol[22] by using standard procedures.[21] A solution of 25.08 g (70.75 mmol) of the 2,2-dimethyl-1,3-dipropylene ketal of 4-ketocyclohexyl tosylate in 400 mL of glacial acetic acid was prepared. The solution was diluted with 100 mL of 0.1 N H_2SO_4. The resultant turbid solution was heated on a steam bath until clear. This solution was stirred at ambient temperature for 15 min and then added to 1500 mL of ice water. The resultant precipitate was collected and dried in vacuo to yield 17.7 g of crude 4-ketocyclohexyl tosylate. Recrystallization from petroleum ether/ethyl acetate yielded 11.5 g of pure 4-ketocyclohexyl tosylate (60% theory yield): mp 99–101 °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 rats were dissected free of connective tissue, cannulated in situ with polyethylene tubing (PE-50, outside diameter = 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.[23]

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; $CaCl_2 \cdot 2H_2O$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; and $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37 °C and aerated with 95% O_2/5% CO_2. 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 antagonists 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.[24]

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

$$K_B = [B]/(\text{dose ratio} - 1)$$

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_B (i.e., $-\log K_B$).

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