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 and the dose calculated to produce a 50% block in avoidance responding (AB_{50}) 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; $Br(CH_2)_3Br$, 109-64-8; $Br(CH_2)_3Cl$, 109-70-6; 4-(4-bromobutyl)pyridine, 109315-44-8; 3-(3-bromopropyl)pyridine, 109839-74-9; 2-(2-bromoethyl)pyridine, 39232-04-7; 2-(2-bromoethyl)quinoline, 109839-75-0; 4-(2-bromoethyl)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))-1*H*-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: N^1-Substituent Effects on $5HT_2$ Receptor Affinity

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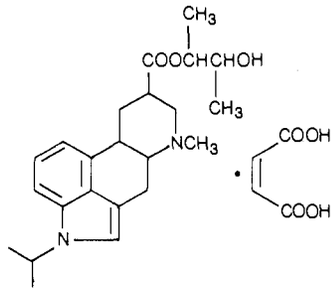
Received February 26, 1987

Three series of 6-methylergoline-8-carboxylic acid esters with various alkyl substituents in the N^1-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 N^1-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 N^1-substituent was isopropyl. Smaller substituents in the N^1-position resulted in reduced $5HT_2$ receptor affinity. Groups C_4 or larger in the N^1-position resulted in a further decline in $5HT_2$ receptor affinity. The importance of the N^1-substituent in determining $5HT_2$ receptor affinity was further substantiated when several 2-methyl-3-ethyl-5-(dimethylamino)indoles with various N^1-substituents were tested. Again, maximal $5HT_2$ receptor affinity was obtained when the N^1-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



1

of four diastereomers[2] 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 $5HT_2$ receptors.[2] This suggested that portions of the molecule other than the ester side chain may play an important role in determining $5HT_2$ receptor affinity. To test this hypothesis, three series of 6-methylergoline-8-carboxylic acid esters were prepared with various substituents in the N^1-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 N^1-substituent and not some other structural feature of the ergoline nucleus was crucial to $5HT_2$ 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 N^1-substitution has on $5HT_2$ receptor affinity for both 6-methylergoline-8-carboxylic acid esters and 2-methyl-3-ethyl-5-(dimethylamino)indoles.

Chemistry

Dihydrolysergic acid (**2**) was alkylated at the N^1-position prior to esterification. Two alkylation procedures were

(1) Cohen, M. L.; Fuller, R. W.; Kurz, K. D. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 327.
(2) 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.
(3) Cohen, M. L.; Fuller, R. W.; Wiley, K. S. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 421.

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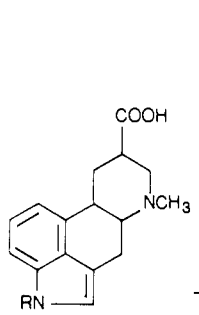
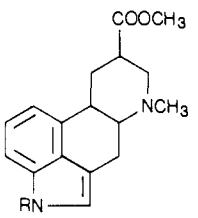
Table I. 6-Methylergoline-8-carboxylic Acid Esters





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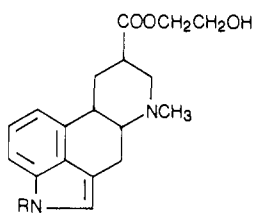

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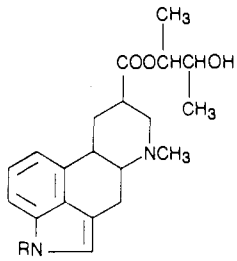
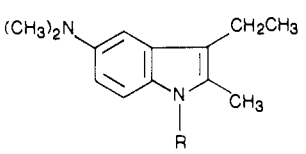


C

no.	R	A formula	A mp	A recrystn solvent	B formula	B mp	B recrystn solvent	C formula	C mp	C recrystn solvent
2	H	$C_{17}H_{20}N_2O_2$	182–184	MeOH/H_2O	$C_{18}H_{22}N_2O_3$	169.5–171	$HOCH_2CH_2OH$/H_2O	$C_{20}H_{26}N_2O_3$	242–244	DMF/H_2O
3	CH_3	$C_{18}H_{22}N_2O_2$	115–116.5	methylcyclohexane	$C_{19}H_{24}N_2O_3$	152–153	MeOH/H_2O	$C_{21}H_{28}N_2O_3$·maleate	112–113	EtOH/Et_2O
4	CH_3CH_2	$C_{19}H_{24}N_2O_2$	117–119	methylcyclohexane				$C_{22}H_{30}N_2O_3$·maleate	155–156	EtOAc
5	$CH_3CH_2CH_2$	$C_{20}H_{26}N_2O_2$·maleate	159–162	EtOAc/Et_2O				$C_{23}H_{32}N_2O_3$·maleate	132–135	MeOH/Et_2O
6	$CH_2{=}CHCH_2$	$C_{20}H_{24}N_2O_2$	101–103	methylcyclohexane	$C_{21}H_{26}N_2O_3$·maleate	112–114	CH_3CN/Et_2O			
7	$(CH_3)_2CH$	$C_{20}H_{26}N_2O_2$	109–110	MeOH/H_2O	$C_{21}H_{28}N_2O_3$	166–169	MeOH/H_2O	$C_{23}H_{32}N_2O_3$·maleate	187–188	MeOH/Et_2O
8	$(CH_3)_2CHCH_2$	$C_{21}H_{28}N_2O_2$·maleate	142–145	MeOH/Et_2O	$C_{22}H_{30}N_2O_3$·maleate	225–226.5	MeOH/Et_2O	$C_{24}H_{34}N_2O_3$·maleate	147–149.5	MeOH/Et_2O
9	$(CH_3CH_2)_2CH$	$C_{22}H_{30}N_2O_2$·maleate	162–163	MeOH/Et_2O				$C_{25}H_{36}N_2O_3$·HCl	155–158	EtOAc/Et_2O
10	cyclopentyl							$C_{25}H_{34}N_2O_3$·maleate	166.5–168.5	EtOH/Et_2O
11	cycloheptyl							$C_{27}H_{38}N_2O_3$·maleate	186–187	MeOH/Et_2O
12	benzyl	$C_{24}H_{26}N_2O_2$·maleate	193–194	EtOH/Et_2O	$C_{25}H_{28}N_2O_3$·maleate	132–134	MeOH/Et_2O	$C_{27}H_{32}N_2O_3$·maleate	84–88	EtOAc/Et_2O
13	$CH_3(CH_2)_6CH_2$							$C_{28}H_{42}N_2O_3$·maleate	103.5–106.5	EtOH/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. N^1-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 N^1-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-3-ethyl-5-(dimethylamino)indoles **14a–d** were prepared via the Fischer indole synthesis and have been reported recently.[6]



14a. R = H
b. R = CH_3
c. R = CH_2CH_3
d. R = $CH(CH_3)_2$

Results and Discussion

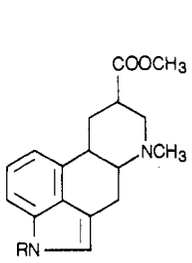
Biological activities of the prepared esters were measured by their ability to antagonize serotonin-induced contractions in the rat jugular vein, a tissue known to contain $5HT_2$ receptors.[3] The affinity of each of these esters for $5HT_2$ receptors was calculated from these data and is presented 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.

(4) Garbrecht, W. L. U.S. Patent 3 580 916, 1971; *Chem. Abstr.* 1971, *75*, 35986*b*.
(5) Marzoni, G.; Garbrecht, W. L.; *Synthesis*, in press.
(6) Fludzinski, P.; Wittenauer, L. A.; Schenk, K. W.; Cohen, M. L. *J. Med. Chem.* **1986**, *29*, 2415.

Table II. Affinity of 6-Methylergoline-8-carboxylic Acid Esters at $5HT_2$ Receptors



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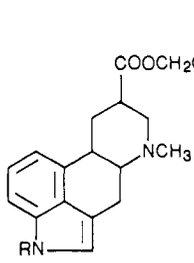

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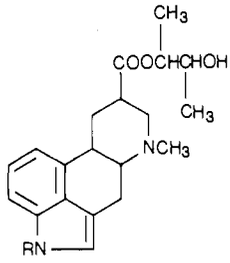


C

no.	R	A: $-\log K_B$ (n)	B: $-\log K_B$ (n)	C: $-\log K_B$ (n)
2	H	8.10 ± 0.11 (3)	7.62 ± 0.04 (3)	8.42 ± 0.08 (3)
3	CH_3	8.23 ± 0.12 (3)	8.63 ± 0.22 (3)	9.26 ± 0.16 (4)
4	CH_3CH_2	8.12 ± 0.13 (3)		8.90 ± 0.11 (4)
5	$CH_3CH_2CH_2$	7.72 ± 0.09 (4)		8.34 ± 0.09 (4)
6	$CH_2{=}CHCH_2$	7.89 ± 0.12 (3)	8.17 ± 0.07 (4)	
7	$(CH_3)_2CH$	8.29 ± 0.19 (3)	9.13 ± 0.08 (3)	9.76 ± 0.15 (8)
8	$(CH_3)_2CHCH_2$	7.57 ± 0.09 (3)	7.42 ± 0.11 (4)	7.53 ± 0.09 (4)
9	$(CH_3CH_2)_2CH$	<7		<7
10	cyclopentyl			<7
11	cycloheptyl			7.45 ± 0.05 (3)
12	benzyl	7.64 ± 0.07 (3)	8.12 ± 0.21 (3)	7.37 ± 0.11 (3)
13	$CH_3(CH_2)_6CH_2$			<7

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.

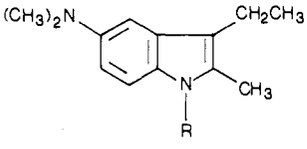
The $5HT_2$ receptor affinity of the esters, however, can be greatly altered by changing the alkyl substituent on the indole nitrogen. Modifying the indole substituent (R) affects the $5HT_2$ receptor affinity in a predictable pattern within a given series of esters. When R is hydrogen, moderate affinity for the $5HT_2$ receptor is observed. Changing R to C_1–C_3 straight chain alkyl increases $5HT_2$ 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 $5HT_2$ 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 $5HT_2$ 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 N^1-isopropyl methyl ester **7a** has a lower $5HT_2$ receptor affinity than does the N^1-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 $5HT_2$ Receptors



no.	R	$-\log K_B$ (n)
14a	H	4.93 ± 0.08 (3)
b	CH_3	6.51 ± 0.17 (3)
c	CH_3CH_2	6.45 ± 0.09 (7)
d	$(CH_3)_2CH$	7.28 ± 0.15 (3)

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 CH_3CN/0.1 M NH_4OAc, flow rate = 2 mL/min, UV detector set at 254 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-8-carboxylic 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 NH_4OH. 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*-(*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 *p*-toluenesulfonic 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 NH_4OH. 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; $CaCl_2 \cdot 2H_2O$, 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 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:

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

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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