with [$^{14}$C]serotonin.[28] In experiments designed to assess the nature of inhibition, the shifts in the CRC's of U46619-induced aggregation and serotonin secretion by varying concentrations ($10^{-5}$, $3 \times 10^{-5}$, $7 \times 10^{-5}$, and $10^{-4}$ M) of **11** were evaluated.

**Data Analyses.** Effective concentration-50 ($EC_{50}$) values of U46619 on rat aorta were determined graphically from individual plots of percent response vs log concentration and expressed as a $pD_2$ value. Antagonists of U46619 responses were also quantified by calculating their $K_B$ or $pA_2$ values according to the methods of Furchgott and Bursztyn[29] and Arunlakshana and Schild.[30] A 5% level of significance was used to determine differences between control and drug-treated groups of data. Schild plots were analyzed by computer according to the method of Tallarida and Murray.[31]

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**Registry No.** (±)-**1**, 21650-42-0; (±)-**3**, 108925-23-1; (±)-**6**, 114185-24-9; (±)-**6**·HCl, 114185-17-0; (±)-**7**, 114185-25-0; (±)-**7**·HCl, 114185-18-1; (±)-**8**, 114185-26-1; (±)-**8**·HCl, 114185-19-2; (±)-**9**, 114185-27-2; (±)-**9**·HCl, 114185-20-5; (±)-**10**, 114185-28-3; (±)-**10**·HCl, 114185-21-6; (±)-**11**, 114185-30-7; (±)-**11**·2HCl, 114185-22-7; (±)-**16**, 114248-27-0; (±)-**16**·HCl, 114248-26-9; **17**, 104-81-4; **18**, 104-83-6; **19**, 824-94-2; **20**, 100-14-1; **21**, 102-47-6; (±)-**22**, 114185-12-5; (±)-**22**·oxalate, 114185-23-8; (±)-**23**, 114185-13-6; (±)-**23**·oxalate, 114197-85-2; (±)-**24**, 114185-14-7; (±)-**24**·oxalate, 114197-86-3; (±)-**25**, 114185-15-8; (±)-**25**·oxalate, 114197-87-4; (±)-**26**, 114185-16-9; (±)-**26**·oxalate, 114197-88-5; (±)-**27**, 114185-29-4; (±)-**27**·oxalate, 114197-89-6.

(28) Huzoor-Akbar; Navran, S. S.; Chang, J.; Miller, D. D.; Feller, D. R. *Thromb. Res.* **1982**, *27*, 405.
(29) Furchgott, R. F.; Bursztyn, P. *Ann. N. Y. Acad. Sci.* **1967**, *144*, 882.
(30) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
(31) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations with Computer Programs*; Springer-Verlag: New York, 1981; p 29.

# Ergolines as Selective 5-HT$_1$ Agonists

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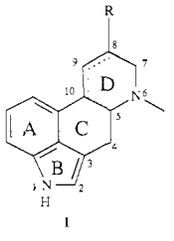
The synthesis and serotonin receptor subtype affinity of a series of ergolines are described. High selectivity for the 5-HT$_1$ subtype was found with a number of 8-substituted (3β,5β)-9,10-didehydro-6-methylergolines. The more potent and selective of these compounds increased the concentration of serotonin and decreased the concentration of 5-HIAA in rat brain and increased corticosterone concentration in rat serum. Oral administration of **13**, (3β)-2,3-dihydrolysergine, produced long-lasting decreases in serotonin turnover. Compound **13** lacked substantial dopaminergic activity as measured by its effects on dopamine turnover in whole brain or striatum and its affinity for α-adrenergic binding sites was significantly less than for 5-HT$_1$ binding sites. The increases in serum corticosterone concentrations produced by **13** were not blocked by the serotonin uptake inhibitor fluoxetine or by the serotonin synthesis inhibitor *p*-chlorophenylalanine, suggesting that **13** exerts its effects through direct stimulation of serotonin receptors.

Drugs that enhance central serotonergic function, such as serotonin-uptake inhibitors, monoamine oxidase (MAO) inhibitors, and direct acting serotonin agonists, have been shown to affect memory, depression, anxiety, pain, appetite, and other important centrally mediated functions in humans and animals.[1-5] Because serotonin-uptake and MAO inhibitors enhance serotonin function by increasing the availability of endogenous serotonin, these agents do not reveal if these effects are mediated through single or multiple serotonin receptor subtypes. The development of compounds that are selective agonists at a single serotonin receptor subtype could lead to the discovery of the role of these subtypes in central nervous system function and disease.

The receptors for serotonin in the central nervous system have been divided into two major subtypes, 5-HT$_1$ and 5-HT$_2$, on the basis of their relative affinities for [$^3$H]-serotonin and [$^3$H]spiperone, respectively.[6] Recently, the 5-HT$_1$ receptor has been subdivided into the 5-HT$_{1A}$,[7] 5-HT$_{1B}$,[7] 5-HT$_{1C}$,[8] and 5-HT$_{1D}$[9] subtypes on the basis of various biochemical evidence. The functional significance of these serotonin receptor subtypes and their possible roles in human disease is the subject of intensive current research and speculation.[1]

The tetracyclic structure of the ergolines, **1**, contains the essential features of the monoamine neurotransmitters dopamine, noradrenaline, and serotonin. Many naturally




**1**

occurring and synthetic ergolines have been shown to bind

(1) Glennon, R. A. *J. Med. Chem.* **1987**, *30*, 1.
(2) Stahl, M. S.; Palazidou, L. *Trends Pharmacol. Sci.* **1986**, 349.
(3) Dourish, C. T.; Hutson, P. H.; Curson, G. *Trends Pharmacol. Sci.* **1986**, 212.
(4) Fuller, R. W. *J. Clin. Psychiatry* **1986**, *47*(4) (Suppl), 4.
(5) Wong, D. T.; Reid, L. R.; Bymaster, F. P.; Threlkeld, P. G. *J. Neural Transm.* **1985**, *64*, 251.
(6) Peroutka, S. J.; Snyder, S. H. *Mol. Pharmacol.* **1979**, *16*, 687.
(7) Nelson, D. L.; Pedigo, N. W.; Yamamura, H. I. In *Psychopharmacology and Biochemistry of Neurotransmitter Receptors*; Yamamura, H. I.; Olsen, R. W.; Elsevier; North Holland, Amsterdam, 1980; p 325.
(8) Pazos, A.; Hoyer, D.; Palacios, J. M. *Eur. J. Pharmacol.* **1984**, *106*, 539.
(9) Heuring, R. E.; Peroutka, S. J. *J. Neurosci.* **1987**, *7*, 894.

0022-2623/88/1831-1512$01.50/0 

to receptors for these neurotransmitters[10-12] and to act as agonists or antagonists at receptors for these neurotransmitters.[13] This wealth of sympathomimetic and sympatholytic activity often means that the major challenge in developing therapeutically useful drugs from this class of compounds is the discovery of new compounds that are sufficiently selective for a single neurotransmitter.

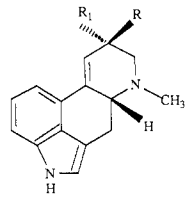
Molecular modification of the ergoline framework has, however, led to the development of biochemically selective and therapeutically useful drugs. For instance, the selective serotonin antagonists methylsergide and metergoline have been introduced for the treatment of migraine and, in more recent years, the dopamine agonists bromocriptine and lisuride have been introduced for the treatment of Parkinson's disease and hyperprolactinemia. A cursory review of the literature also reveals a number of other ergolines in advanced stages of therapeutic development.[14]

This paper describes our efforts to exploit the serotonergic properties of ergolines by trying to discover naturally occurring ergolines that show selective affinity for the 5-HT$_1$ receptor so that they might be evaluated as agents for enhancing central serotonin function. Molecular modification of some of these naturally occurring ergolines and their derivatives, particularly reduction of the indole ring, a modification known to reduce the dopaminergic activity of ergolines,[15] produces compounds with high selectivity for the 5-HT$_1$ receptor. The most selective and potent of these compounds produce profound effects on serotonin neurochemistry and other pharmacology associated with serotonergic activity.

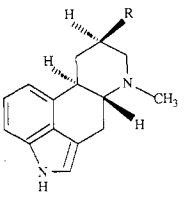
## Chemistry

Previously reported ergolines **2–12** were obtained as cited in the Experimental Section. The 2,3-dihydroergolines **13–23** and **25** were synthesized by treating the corresponding ergoline with triethylsilane in the presence of trifluoroacetic acid. Only dimeric material[16] and a single monomeric diastereomer could be isolated from these reactions.

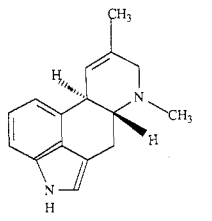
The stereochemistry of the 3- and 5-protons in these compounds was determined through $^1$H spectroscopy, the chemical shift data for representative compounds being provided in the supplementary material. For instance, the well-resolved chemical shift of 4-H$_{ax}$ in **13** was at 1.37 ppm. Homonuclear decoupling experiments showed couplings of approximately 12 Hz to both 3-H and 5-H, suggesting a cis-1,3-diaxial relationship between these latter two protons. Because 5-H was $\beta$ in **3** and other naturally occurring ergolines and this center was unaffected by the reduction of the indole ring, 5-H and 3-H were in a cis-$\beta$ relationship in **13**.[17] A comparable analysis of the remaining 2,3-dihydroergolines, **14–23**, **25**, and **26**, supported this same deduction about their stereochemistry. Similar



| | R | R$_1$ |
|---|---|---|
| 3 | $CH_3$ | H |
| 4 | H | $CH_3$ |
| 6 | $CH_2OH$ | H |
| 7 | $CH_2$= | |
| 9 | $CH_2SCH_3$ | H |
| 10 | $CH_2CN$ | H |
| 11 | $CH_2S$-Phenyl | H |
| 12 | $CH_2S$-2-Pyridyl | H |



| | R |
|---|---|
| 2 | $CH_3$ |
| 8 | $CH_2OH$ |



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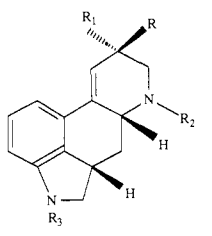
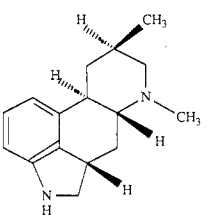


| | R | R$_1$ | R$_2$ | R$_3$ |
|---|---|---|---|---|
| 13 | $CH_3$ | H | $CH_3$ | H |
| 14 | H | $CH_3$ | $CH_3$ | H |
| 15 | $CH_2OH$ | H | $CH_3$ | H |
| 16 | $CH_2$= | | $CH_3$ | H |
| 17 | $CH_2SCH_3$ | H | $CH_3$ | H |
| 18 | $CH_2CN$ | H | $CH_3$ | H |
| 19 | $CH_2S$-Phenyl | H | $CH_3$ | H |
| 20 | $CH_2S$-2-Pyridyl | H | $-CH_3$ | H |
| 21 | $CO_2CH_3$ | H | $CH_3$ | H |
| 25 | $CH_3$ | H | *n*-Propyl | H |
| 26 | $CH_3$ | H | $CH_3$ | $CH_3$ |



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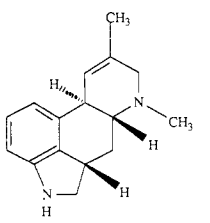

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conclusions were reached by Rebek et al.[18] and Ninomyia et al.[19] after analyzing the NMR spectra of analogous compounds.

The racemates of a number of these 2,3-dihydroergolines (**14**,[20] **15**,[21] **16**,[22] **21**,[19] and **23**[22]) have been synthesized. Because optically pure ergolines were used as substrates in these reduction reactions, the (3$\beta$)-2,3-dihydroergolines

(10) Closse, A.; Frick, W.; Dravid, A.; Bolliger, G.; Hauser, D.; Sauter, A.; Tobler, H. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 95.
(11) McPherson, G.; Beart, P. *Eur. J. Pharmacol.* **1983**, *91*, 363.
(12) Beart, P. M.; McDonald, D.; Cincotta, M.; De Vries, D. J.; Gundlack, A. L. *Gen. Pharmacol.* **1986**, *17*, 57.
(13) Berde, B., Schild, H. O. *Ergot Alkaloids and Related Compounds*; Springer-Verlag: Berlin, 1978.
(14) Stadler, P. A.; Giger, R. K. A. In *Natural Products and Drug Development*; Krogsgaard-Larsen, P., Christensen, S. B., Kofod, H., Ed.; Munksgaard: Copenhagen, 1983.
(15) Cassady, J. M.; Li, G. S.; Spitzner, E. B.; Floss, H. G.; Clemens, J. A. *J. Med. Chem.* **1974**, *17*, 300.
(16) Bach, N. K.; Kornfeld, E. C. *Tetrahedron Lett.* **1973**, 3315.
(17) Floss, H. G. *Tetrahedron* **1976**, *32*, 873.
(18) Rebek, J., Jr.; Tai, D. F.; Shue, Y. K. *J. Am. Chem. Soc.* **1984**, *106*, 1813.
(19) Ninomyia, I.; Hashimoto, C.; Kiguchi, T.; Naito, T. *J. Chem. Soc., Perkin Trans. 1* **1985**, 941.
(20) Kiguchrui, T.; Chiyomi, H.; Ninomiya, I. *Heterocycles* **1985**, *23*, 1925.
(21) Kiguchi, T.; Hashimoto, C.; Ninomiya, I. *Heterocycles* **1985**, *23*, 1377.
(22) Kiguchi, T.; Hashimoto, C.; Ninomiya, I. *Heterocycles* **1984**, *22*, 43.

Table I. 5-$HT_1$ and 5-$HT_2$ Binding Data

| compd | 5-$HT_1$ $IC_{50}$, nM | 5-$HT_2$ $IC_{50}$, nM | 5-$HT_1$/5-$HT_2$ selectivity ratio[a] |
|---|---|---|---|
| **2** | 360 | 440 | 1 |
| **3** | 5 | 110 | 22 |
| **4** | 80 | 130 | 1 |
| **5** | 140 | 270 | 2 |
| **6** | 15 | 160 | 11 |
| **7** | 20 | 140 | 7 |
| **8** | 120 | 450 | 4 |
| **13** | 17 | 1665 | 100 |
| **14** | 20 | >1000 | >50 |
| **15** | 26 | 2650 | 102 |
| **16** | 80 | 3820 | 48 |
| **17** | 20 | 1330 | 66 |
| **18** | 20 | 2120 | 106 |
| **19** | 8 | 210 | 26 |
| **20** | 3 | 110 | 28 |
| **21** | 40 | 1620 | 40 |
| **22** | 390 | 4220 | 11 |
| **23** | 130 | 1850 | 14 |
| **25** | 240 | >1000 | >5 |
| **26** | 160 | 1250 | 8 |

[a] The 5-$HT_1$/5-$HT_2$ selectivity ratio is $IC_{50}$ 5-$HT_2$/$IC_{50}$ 5-$HT_1$ site with values greater than 1 indicating selectivity for the 5-$HT_1$ receptors.

produced were expected to be optically active. Accordingly, all compounds tested for optical activity (**13**, **14**, and **18**) gave optical rotations.

The 6-*n*-propyl analogue **25** was derived from **3** by N-dealkylation with cyanogen bromide, hydrolysis, realkylation with 1-propyl iodide, and, finally, reduction with triethylsilane. The 1-methyl derivative **26** was obtained by carbamylation of **13** with ethyl chloroformate followed by reduction.

## Binding Studies

Rat frontal cortex homogenates were employed in the serotonin binding site studies using [$^3$H]-5-HT and [$^3$H]-spiperone to label 5-$HT_1$ and 5-$HT_2$ sites, respectively. The results of the competition studies with the naturally occurring ergolines **2–8** and 2,3-dihydroergolines **13–23**, **25**, and **26** are shown in Table I.

All of the ergolines tested had higher affinity for 5-$HT_1$ binding sites than for 5-$HT_2$ sites. The most selective natural ergoline, lysergine, **3**, showed a 22-fold preference for 5-$HT_1$ sites. Reduction of the indole ring of these ergolines, a modification known to decrease dopaminergic activity,[15,23-25] diminished 5-$HT_2$ affinity while maintaining 5-$HT_1$ affinity (e.g., compare **3** and **13**, **5** and **23**, **2** and **22**, **6** and **15**) and produced compounds that are much more selective for 5-$HT_1$ binding sites.

The presence and position of unsaturation in the D ring of the ergolines and 2,3-dihydroergolines also influenced affinity for 5-$HT_1$ binding sites. Highest affinity was associated with a double bond in the 9,10-position and lowest affinity was obtained with compounds completely saturated in the D ring. (E.g., compare **3** and **5** and **2**, **13** and **23** and **22**.) A similar trend was seen for 5-$HT_2$ affinity, but the effect was less pronounced.

Small substituents in the 8-position of the 9,10-didehydro-2,3-dihydroergolines (**13–18** and **21**) had relatively minor effects on serotonin binding site affinity and selectivity. Even the stereochemistry at this position (e.g., compare **13** and **14**) had no effect on 5-$HT_1$ affinity. By contrast, larger and more lipophilic substituents, as found in **19** and **20**, increased affinity for both serotonin subtypes, but relatively more for the 5-$HT_2$ subtype, producing less selective compounds.

Increasing the length of the N-6 substituent as in the propyl derivative **25** or methylation of the N-1 position as in **26** lowered affinity for 5-$HT_1$ binding sites when compared to parent 2,3-dihydroergoline **13**.

Rat frontal cortex homogenates were employed in binding studies using [$^3$H]prazosin and [$^3$H]rauwolscine to label $\alpha_1$ and $\alpha_2$ binding sites, respectively.[26] The $IC_{50}$'s for **13** at the $\alpha_1$ and $\alpha_2$ binding sites were 10 500 ± 400 and 177 ± 5 nM, respectively, indicating that **13** had higher affinity for 5-$HT_1$ binding sites than for either of the $\alpha$-adrenergic binding sites.

Table II. Dose Dependence of the Effect of **13** on 5-Hydroxyindoles in Whole Brain and Corticosterone in Serum of Rats

| dose of **13**, mg/kg, sc | 5-hydroxyindoles, nmol/g: serotonin | 5-hydroxyindoleacetic acid | serum corticosterone, μg/100 mL |
|---|---|---|---|
| | Experiment 1 | | |
| 0 | 2.32 ± 0.04 | 1.89 ± 0.07 | 4.3 ± 0.3 |
| 0.03 | 2.80 ± 0.07[a] | 1.52 ± 0.05[a] | 6.5 ± 1.1 |
| 0.1 | 2.59 ± 0.08[a] | 1.45 ± 0.06[a] | 33.8 ± 8.7[a] |
| 0.3 | 2.95 ± 0.05[a] | 1.45 ± 0.03[a] | 46.6 ± 9.6[a] |
| 1 | 3.02 ± 0.06[a] | 1.47 ± 0.03[a] | 70.8 ± 3.2[a] |
| | Experiment 2 | | |
| 0 | 2.66 ± 0.09 | 2.20 ± 0.05 | |
| 0.01 | 2.67 ± 0.06 | 2.20 ± 0.06 | |
| 0.03 | 2.77 ± 0.07 | 1.90 ± 0.08[a] | |

[a] Significant difference from control group ($P < 0.05$). Compound **13** was injected 1 h before rats were killed. Mean values ± standard errors for five rats per group.

Table III. Minimum Effective Doses of Compounds **13–15**, **17**, and **18** Decreasing 5-HIAA Concentrations in Brain and Increasing Corticosterone in Serum of Rats[a]

| compd | minimum effective dose suppressing 5-HIAA, mg/kg, sc | minimum effective dose increasing serum corticosterone, mg/kg, sc |
|---|---|---|
| **13** | 0.03 | 0.1 |
| **14** | 0.001 | 0.003 |
| **15** | 1 | 0.1 |
| **17** | 0.1 | 1 |
| **18** | 0.1 | 0.1 |

[a] Minimum effective doses are the lowest doses producing significant differences from control group ($P < 0.05$). Compounds were injected 1 h before rats were killed. Five rats per group were used in each experiment.

## Pharmacological Studies

The functional consequences of the potent and selective 5-$HT_1$ binding of **13–15**, **17**, and **18** were explored by examining the effects of these 2,3-dihydroergolines on concentrations of serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in rat brain and corticosterone concentration in the serum of rats. The results of a representative experiment with compound **13** are shown in Table II. This compound decreased the concentration of 5-HIAA in hypothalamus and striatum and increased serotonin concentration in the hypothalamus. In similar

(23) Clemens, J. A.; Smalstig, E. B.; Shaar, C. J. *Acta Endocrinol.* **1975**, *79*, 230.
(24) Li, G. S.; Robinson, M.; Floss, H. G.; Cassady, J. M.; Clemens, J. A. *J. Med. Chem.* **1975**, *18*, 892.
(25) Crider, A. M.; Robinson, J. M.; Floss, H. G.; Cassady, J. M.; Clemens, J. A. *J. Med. Chem.* **1977**, *20*, 1473.
(26) Ruffolo, R. R.; Messick, K.; Horng, J. S. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, *329*, 244.

**Table IV.** Decreased Accumulation of 5-Hydroxytryptophan after Decarboxylase Inhibition in the Hypothalamus of Rats Treated with **13**

| dose of 13, mg/kg, sc | 5-hydroxytryptophan in hypothalamus, nmol/g |
|---|---|
| 0 | 1.92 ± 0.07 |
| 0.01 | 1.64 ± 0.07[a] |
| 0.03 | 1.19 ± 0.05[a] |
| 0.1 | 0.73 ± 0.02[a] |
| 0.3 | 0.71 ± 0.02[a] |

[a] Significant decrease ($P < 0.01$). NSD 1015 was given at 100 mg/kg, ip, 30 min before rats were killed and 30 min after **13**. Mean values ± standard errors for five rats in each treated group and six rats in the control group are shown.

**Table V.** Effects of **13** on Concentrations of Serotonin and 5-HIAA in Rat Brain Regions

| parameter measured | treatment group: control | treatment group: **13** (0.3 mg/kg, sc) |
|---|---|---|
| | Hypothalamus | |
| serotonin | 3.31 ± 0.07 | 4.78 ± 0.08[a] |
| 5-HIAA | 3.30 ± 0.11 | 2.18 ± 0.06[a] |
| | Striatum | |
| serotonin | 2.12 ± 0.09 | 1.94 ± 0.07 |
| 5-HIAA | 3.31 ± 0.10 | 2.38 ± 0.06[a] |

[a] Significant difference from control ($P < 0.05$). Compound **13** was injected 1 h before rats were killed. Mean values ± standard errors for five rats per group are shown.

experiments, all five compounds increased the concentration of serotonin and decreased the concentration of 5-HIAA in brain and increased serum corticosterone concentration. Significant decreases in 5-HIAA concentrations occurred at lower doses than increases in serotonin concentration. The minimum effective doses of the compounds producing significant decreases in brain 5-HIAA and significant increases in serum corticosterone concentrations are shown in Table III.

Compounds **13** and **14** reduced brain 5-HIAA concentrations after oral administration. One hour after dosing, significant reductions in 5-HIAA concentrations were produced by both **13** and **14** with minimum effective doses of 1 and 0.3 mg/kg, respectively. A 1 mg/kg oral dose of either compound produced significant reductions in 5-HIAA concentrations for over 8 h.

Compounds **13** and **14** were differentiated by their ability to affect dopamine metabolite concentrations in rat brain. While **13**, when dosed at 1 mg/kg, po or sc, had no effect on dopamine metabolite concentrations, **14** significantly increased dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) at both 0.3 and 1 mg/kg, po. These increases in dopamine metabolite concentrations persisted for at least 5 h at the highest dose tested. The apparent lack of dopaminergic effects with **13** caused us to select this compound for further neurochemical study.

The decrease in 5-HIAA concentration is presumably the result of a lowering of serotonin turnover in rat brain. A more direct measure of turnover is the accumulation of the serotonin precursor 5-hydroxytryptophan (5-HTP) after amino acid decarboxylase inhibition by NSD 1015, (*m*-hydroxybenzyl)hydrazine.[27,28] Compound **13** produced a dose-related decrease in 5-HTP accumulation in the hypothalamus of NSD 1015 treated rats, significant effects being detected at all of the doses used. (See Table IV.)

(27) Euvrard, C.; Boissier, J. R. *Eur. J. Pharmacol.* **1980**, *63*, 65.
(28) Hamon, M.; Bourgoin, S.; Enjalbert, A.; Bockaert, J.; Hery, F.; Ternaux, J. P.; Glowinski, J. *Arch. Pharmacol.* **1976**, *294*, 99.

**Table VI.** Effects of Apomorphine and **13** on Dopamine Metabolite Concentrations in Rat Striatum

| treatment group | dose, mg/kg, sc | dopamine metabolites, nmol/g: DOPAC | dopamine metabolites, nmol/g: HVA |
|---|---|---|---|
| control | | 3.95 ± 0.40 | 2.88 ± 0.25 |
| apomorphine | 1 | 2.97 ± 0.28 | 1.59 ± 0.11[a] |
| | 3 | 2.33 ± 0.13[a] | 1.14 ± 0.06[a] |
| | 10 | 1.73 ± 0.11[a] | 0.91 ± 0.05[a] |
| **13** | 0.03 | 3.81 ± 0.36 | 2.89 ± 0.26 |
| | 0.1 | 4.44 ± 0.36 | 3.65 ± 0.29 |
| | 0.3 | 4.62 ± 0.45 | 3.27 ± 0.24 |

[a] Significant decrease ($P < 0.05$). Apomorphine hydrochloride or **13** was injected 1 h before rats were killed. Mean values ± standard errors for five rats per group are shown.

**Table VII.** Effects of Fluoxetine on the Elevation of Serum Corticosterone Produced by **13** and PCA

| treatment group | serum corticosterone, μg/100 mL: no pretreatment | serum corticosterone, μg/100 mL: fluoxetine pretreated |
|---|---|---|
| vehicle | 5.8 ± 0.4 | 6.5 ± 0.6 |
| **13** | 68.2 ± 4.0[a] | 61.2 ± 2.7[a] |
| PCA | 69.4 ± 1.3[a] | 29.5 ± 5.0[a,b] |

[a] Significant increase in corticosterone concentration ($P < 0.05$). [b] Significant difference from group with no pretreatment ($P < 0.05$). Compound **13** (0.3 mg/kg, sc) or (±)-*p*-chloroamphetamine hydrochloride (PCA) (2.5 mg/kg, ip) was injected 1 h before rats were killed and 16 h after fluoxetine hydrochloride (10 mg/kg, ip). Mean values ± standard errors for five rats per group are shown.

A maximal reduction of serotonin turnover was seen at 0.1 mg/kg.

The specificity of the effects of **13** on serotonin turnover in different brain regions was also studied. As shown in Table V, reductions in 5-HIAA concentrations were produced by 0.3 mg/kg, sc, doses of **13** in both the striatum and hypothalamus of rats. Serotonin concentration was significantly increased in the hypothalamus but not in the striatum.

Several experiments were performed to investigate the potential dopaminergic activity of **13**. A sensitive method for determining the dopaminergic activity of compounds is measurement of their effects on dopamine metabolite concentrations in brain regions containing large numbers of dopamine containing neurons such as the striatum of rat. As shown in Table VI, the dopamine agonist apomorphine decreased the concentrations of the dopamine metabolites DOPAC and HVA in rat striatum. By contrast, **13** had no effect on dopamine metabolite concentrations at any of the doses tested. These same doses of **13** decreased serotonin metabolite concentrations in whole rat brain and increased corticosterone concentration in rat serum. (Compare Tables II and VI.)

The mechanism by which **13** produces its serotonergic effects was studied by pretreating rats with drugs that alter serotonin neurochemistry in known ways. Table VII shows that the pretreatment of rats with the serotonin uptake inhibitor fluoxetine did not prevent the 10-fold elevation of serum corticosterone concentration in rats produced by a 0.3 mg/kg, sc, dose of **13**. This same pretreatment halved the effects of a 2.5 mg/kg, ip, dose of the serotonin releasing agent *p*-chloroamphetamine (PCA) on corticosterone, suggesting that **13** and PCA differ in their mechanism of action.

The effect of the pretreatment of rats with the serotonin synthesis inhibitor *p*-chlorophenylalanine on the elevation of serum corticosterone concentrations produced by **13** and PCA is shown in Table VIII. This pretreatment failed to affect the increases in corticosterone produced by **13**, but significantly reduced the increases in corticosterone

Table VIII. Effect of p-Chlorophenylalanine on the Elevation of Serum Corticosterone Produced by 13 and PCA

| | serum corticosterone, μg/100 mL | |
|---|---|---|
| treatment group | no pretreatment | PCPA pretreated |
| vehicle | 6.5 ± 0.6 | 7.7 ± 0.6 |
| 13 | 47.3 ± 3.1[a] | 42.0 ± 3.6[a] |
| PCA | 51.1 ± 3.3[a] | 14.9 ± 1.7[a,b] |

[a] Significant increase in corticosterone concentration ($P < 0.05$). [b] Significant difference from group with no pretreatment ($P < 0.05$). Compound 13 (0.3 mg/kg, sc) or p-chloroamphetamine hydrochloride (PCA) (2.5 mg/kg, ip) was injected 1 h before rats were killed and 24 h after p-chlorophenylalanine (PCPA) (300 mg/kg, ip). Mean values ± standard errors for five rats per group are shown.

Table IX. Effect of Metergoline on the Elevation of Serum Corticosterone Produced by 13

| | serum corticosterone, μg/100 mL | |
|---|---|---|
| dose of 13, mg/kg, sc | no pretreatment | metergoline pretreated |
| 0 | 4.3 ± 0.3 | 6.2 ± 0.7 |
| 0.03 | 6.5 ± 1.1 | 22.1 ± 3.7[a] |
| 0.1 | 33.8 ± 8.7[a] | 46.8 ± 5.2[a] |
| 0.3 | 46.6 ± 9.6[a] | 50.2 ± 6.8[a] |
| 1.0 | 70.8 ± 3.2[a] | 61.2 ± 3.9[a] |

[a] Significant elevation compared to corresponding control group ($P < 0.05$). Compound 13 was injected 1 h before rats were killed and 1 h after metergoline (3 mg/kg, ip). Mean values ± standard errors for five rats per group are shown.

produced by PCA. These results show that PCA but not 13 depends on endogenous stores of serotonin to produce these effects.

Table IX compares the effect of a number of doses of 13 on serum corticosterone concentrations in control rats and rats pretreated with the serotonin antagonist metergoline. Metergoline (3 mg/kg, ip) failed to block the corticosterone elevations produced by any dose of 13. In another experiment, (±)-pindolol (3 mg/kg, sc), when injected 1 h before 13 (0.3 mg/kg, sc), antagonized the increase in corticosterone concentration. Basal corticosterone concentrations were 4.1 ± 0.3 μg/100 mL in control rats and 8.2 ± 2.6 μg/100 mL in rats pretreated with pindolol. Compound 13 increased serum corticosterone concentrations to 46.3 ± 2.1 μg/100 mL, and pindolol pretreatment reduced that value to 28.6 ± 3.9 μg/100 mL ($P < 0.05$).

## Discussion

Ergolines 2–8 show high affinity for 5-$HT_1$ receptors and somewhat lower affinity for 5-$HT_2$ receptors. The slight selectivity of these compounds is dramatically increased by reduction of the 2,3-double bond in the indole nucleus. The higher selectivity is achieved by a diminution in 5-$HT_2$ receptor affinity and not by an increase in the affinity for 5-$HT_1$ receptors.

The configurational changes induced by reduction of the indole ring were studied to determine why only the 5-$HT_2$ affinity was affected by this modification. Examination of models of these structurally rigid ergolines reveals that when the D ring is in a chair or pseudochair configuration, the 6-nitrogen is near or above the plane defined by the phenyl ring of the indole. By contrast, reduction of the indole ring causes this D-ring nitrogen to fall below the plane of the phenyl ring. When this nitrogen drops significantly below this plane, it may be denied some important interaction with the receptor that normally confers high 5-$HT_2$ affinity.

Serotonin, whose elementary (aminoethyl)indole structure is easily seen in the ergolines, also shows much higher affinity for the 5-$HT_1$ receptor than for the 5-$HT_2$ receptor.[1] The X-ray crystal structure of serotonin picrate shows that the side chain amino group is well below the plane defined by the indole ring of the molecule and supports the contention that high affinity for the 5-$HT_2$ receptor may depend on coplanarity of the side chain amino groups and the aromatic rings of both serotonin and the 2,3-dihydroergolines.[29]

Changes in the steric features, acidity, and dipole of the B ring of the ergolines could also be produced by the reduction of the pyrrole portion of the molecule. The 5-$HT_2$ binding site should be insensitive to increases in steric demand in the area of the B ring because a number of ergolines that have relatively large alkyl groups attached to the 1-nitrogen are extremely potent 5-$HT_2$ ligands.[30] Therefore, the changes in steric demands caused by the indole reduction should have little effect on 5-$HT_2$ affinity. Changes in acidity and dipole, however, could introduce new molecule–binding site interactions that may also account for the changes in 5-$HT_2$ affinity.

The ability of the potent and selective 5-$HT_1$ ligands 13–15, 17, and 18 to decrease serotonin metabolite, 5-HIAA, concentration in rat brain suggests that these dihydroergolines decrease serotonin turnover. Compounds 13 and 14 are the most potent of the compounds in suppressing serotonin turnover when administered subcutaneously and both are also orally effective. Unlike 13, however, 14 increases the concentration of dopamine metabolites in rat brain, suggesting that 14 also increases dopamine turnover. Although some serotonin agonists and serotonin releasing agents have been reported to increase dopamine turnover,[31-34] these effects are also characteristic of dopamine antagonists. To avoid interactions with dopaminergic systems, 13 has been selected for further mechanistic evaluation.

Compound 13 decreases serotonin turnover in rat hypothalamus as measured by decreases in the accumulation of serotonin precursor 5-hydroxytryptophan in the presence of the amino acid decarboxylase inhibitor NSD 1015. However, 13 does not appear to be particularly specific for any of the brain regions studied. A dose of 13 producing a maximal suppression of serotonin turnover in whole brain (0.3 mg/kg, sc) produces similar reductions in serotonin turnover in the striatum and hypothalamus. Increases in serotonin concentrations are seen only in whole brain and hypothalamus.

All of these results show that 13 has dramatic effects on serotonin turnover, but they do not exclude potential interactions with the dopaminergic and α-adrenergic neurotransmitter systems that have been associated with other ergolines. Compound 13 does not appear to interact with dopamine receptors because it did not affect dopamine turnover in whole brain. Further evidence that 13 lacks dopaminergic activity is obtained by measuring the effects of 13 on dopamine metabolite concentrations in rat striatum. In the striatum, the brain region richest in dopaminergic nerve terminals in the rat, large changes in dopamine turnover, as measured by changes in dopamine metabolite concentrations, are produced by dopamine

(29) Thewalt, U.; Bugg, C. E. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1972**, *B28*, 82.
(30) Cohen, M. L.; Fuller, R. W.; Kurz, K. D. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 327.
(31) Fuller, R. W.; Snoddy, H. D.; Molloy, B. B. *J. Pharmacol. Exp. Ther.* **1986**, *239*, 454.
(32) Fuller, R. W.; Snoddy, H. D.; Mason, N. R.; Molloy, B. B. *Eur. J. Pharmacol.* **1978**, *52*, 11.
(33) Fuller, R. W.; Snoddy, H. D. *Res. Commun. Chem Pathol. Pharmacol.* **1977**, *17*, 551.
(34) Crunelli, V.; Bernasconi, S.; Samanin, R. *Pharmacol. Res. Commun.* **1980**, *12*, 215.

agonists and antagonists. By contrast, **13** has no effect on dopamine metabolite concentrations in this brain region. These results corroborate those found for dopamine turnover in whole brain and show that **13** lacks substantial dopaminergic activity.

The decrease in serotonin turnover produced by **13** apparently was not due to interactions with $\alpha_2$ receptors. In other experiments (unpublished data), we have found that clonidine, a classical $\alpha_2$-receptor agonist, did not affect hypothalamic 5-HIAA concentrations. Piperoxan, an $\alpha_2$-receptor antagonist, increased 5-HIAA concentration in rat hypothalamus. Thus neither agonists nor antagonists of $\alpha_2$ receptors mimicked the effect of **13**.

Agents that enhance serotonin function either directly or indirectly will produce many of the effects seen with **13**. For instance, serotonin turnover is suppressed by serotonin-uptake inhibitors[35,36] and serotonin-releasing agents[35,37] as well as by direct-acting serotonin agonists.[27,28,31,32,38,39] In addition, serotonin-releasing agents and serotonin agonists increase serum corticosterone concentrations in rats.[31,39-42] To investigate the mechanism by which **13** increases corticosterone concentration, the effect of **13** on corticosterone was measured in rats pretreated with the serotonin uptake inhibitor fluoxetine and with the serotonin synthesis inhibitor *p*-chlorophenylalanine.

*p*-Chloroamphetamine releases serotonin and produces increases in serum corticosterone concentrations secondary to activation of brain serotonin receptors by the released serotonin.[43,44] This release of serotonin is dependent on the serotonin uptake pump and can be blocked with serotonin-uptake inhibitors. By contrast, the elevation of corticosterone concentrations in rats produced by direct-acting serotonin agonist quipazine is not prevented by pretreatment with the serotonin uptake inhibitor fluoxetine.[44] Fluoxetine blocks the elevation of corticosterone produced by PCA but fails to prevent the increase in serum corticosterone produced by **13**. This shows that **13** is not a serotonin-releasing agent that depends on the serotonin-uptake pump to produce its effects and is consistent with the idea that **13** may be a direct-acting serotonin agonist.

*p*-Chlorophenylalanine inhibits the synthesis of serotonin and markedly reduces brain serotonin concentration in rats.[44,45] Pretreatment of rats with *p*-chlorophenylalanine attenuates the increase in serum corticosterone concentrations produced by the serotonin releasing agent PCA, indicating that serotonin depletion will block the effects of agents dependent on endogenous serotonin. By contrast, increases in corticosterone concentrations produced by the direct-acting serotonin agonist quipazine are not prevented by *p*-chlorophenylalanine pretreatment.[44] Similar to the results obtained with quipazine, the elevation of corticosterone concentrations produced by **13** are not blocked by *p*-chlorophenylalanine pretreatment. These results indicate that **13** does not depend on endogenous stores of serotonin to elevate corticosterone concentrations and that the compound is a direct-acting serotonin agonist.

The serotonin antagonist metergoline has been shown to block some central serotonergic responses including the elevation of corticosterone concentrations by the serotonin agonist quipazine.[46,47] However, the pretreatment of rats with metergoline (3 mg/kg, ip) fails to block the elevation of serum corticosterone concentrations by **13** (0.3 mg/kg, sc). Metergoline does not antagonize all serotonin receptors or block all central serotonergic responses.[48-50] The same dose of metergoline used in our experiment has also been shown to be ineffective in antagonizing the elevation of corticosterone concentrations by other serotonin agonists.[31,40] In addition, other serotonin antagonists fail to antagonize a number of effects of both direct and indirect serotonin agonists.[48,51-54] Therefore, the failure of metergoline to block the elevation of serum corticosterone produced by **13** does not mean that this effect of **13** is not serotonergically mediated or that **13** is not a serotonin agonist. In fact, the ability of pindolol to antagonize the effect of **13** suggests that activation of a 5-HT$_1$ receptor subtype mediates the increase in serum corticosterone concentration. Koenig et al. had reported that pindolol antagonized the elevation of serum corticosterone concentration in rats by 8-hydroxy-2-(di-*n*-propylamino)tetralin, a selective 5-HT$_{1A}$ receptor agonist, but not the increase elicited by the 5-HT$_2$ receptor agonist MK-212.[55]

The ability of **13** to bind with high affinity to 5-HT$_1$ binding sites in rat cortex, to suppress the turnover of serotonin in the striatum, hypothalamus, and whole brain of rats, and to elevate serum corticosterone concentrations in rats suggest that **13** activates serotonin receptors. These serotonergic effects appear to be due to a direct action on serotonin receptors because the elevation of serum corticosterone produced by **13** does not depend on the serotonin-uptake pump or on endogenous stores of serotonin. The compound does not affect dopaminergic neurotransmission as measured by its lack of effect on dopamine turnover in whole brain or striatum. Compound **13** has low affinity for $\alpha_1$ binding sites and affinity for $\alpha_2$ binding sites 10-fold lower than its affinity for 5-HT$_1$ binding sites. Therefore, the major effects produced by **13** appear to be those of a direct-acting serotonin agonist substantially free of direct catecholaminergic activity. Further studies are in progress to substantiate this view and to evaluate the usefulness of **13** in diseases in which enhancements in serotonin function would be therapeutic.

## Summary

Reduction of the indole ring of some naturally occurring

(35) Fuller, R. W. In *Neuropharmacology of Serotonin*; Green, A. R., Ed.; Oxford University Press: Oxford, 1985.
(36) Marco, E. J.; Meek, J. L. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1979**, *306*, 75.
(37) Fuller, R. W.; Perry, K. W. *J. Pharm. Pharmacol.* **1983**, *35*, 597.
(38) Jacoby, J. H.; Howd, R. A.; Levin, M. S.; Wurtman, R. J. *Neuropharmacology* **1976**, *15*, 529.
(39) Fuller, R. W. *Monogr. Neural Sci.* **1984**, *10*, 158.
(40) Fuller, R. W.; Snoddy, H. D. *Endocrinology* **1979**, *105*, 923.
(41) Fuller, R. W.; Snoddy, H. D.; Clemens, J. A. *Endocr. Res. Commun.* **1978**, *5*, 161.
(42) Fuller, R. W.; Clemens, J. A. *IRCS Med. Sci.* **1979**, *7*, 106.
(43) Trulson, M. E.; Jacobs, B. L. *Eur. J. Pharmacol.* **1976**, *36*, 149.
(44) Fuller, R. W.; Snoddy, H. D. *Neuroendocrinology* **1980**, *31*, 96.
(45) Koe, B. K.; Weissman, A. *J. Pharmacol. Exp. Ther.* **1966**, *154*, 499.
(46) Fuller, R. W.; Snoddy, H. D. *Endocrinology* **1979**, *105*, 923.
(47) Mawson, C.; Whittington, H. *Br. J. Pharmacol.* **1970**, *39*, 223.
(48) Haigler, H. D.; Agjahanian, G. K. *J. Neural Trans.* **1974**, *35*, 257.
(49) Beretta, C.; Ferrini, R.; Glasser, A. H. *Nature (London)* **1965**, *207*, 421.
(50) Barrett, R. J.; Blackshear, M. A.; Sanders-Bush, E. *Psychopharmacology* **1982**, *76*, 29.
(51) Renyi, L.; Archer, T.; Minor, B. B.; Tandberg, B.; Fredricksson, A.; Ross, S. B. *J. Neural Trans.* **1986**, *65*, 193.
(52) Wang, R. Y.; Aghajanian, G. K. *Brain Res.* **1977**, *120*, 85.
(53) Lakoski, J. M.; Aghajanian, G. K. *Neuropharmacology* **1985**, *24*, 265.
(54) Ahlenius, S.; Larsson, K. *Eur. J. Pharmacol.* **1984**, *99*, 279.
(55) Koenig, J. I.; Gudelsky, G. A.; Meltzer, H. Y. *Eur. J. Pharmacol.* **1987**, *137*, 1.

ergolines produces compounds with high affinity and selectivity for 5-$HT_1$ binding sites. Some in vivo pharmacological consequences of this high affinity are a reduction in serotonin turnover and an elevation of serum corticosterone concentrations in rats. Because of its long-lasting oral efficacy in decreasing serotonin turnover, **13** was selected for further pharmacological evaluation. Several tests show that this 2,3-dihydroergoline lacks substantial dopaminergic activity and its affinity for $\alpha$-adrenergic binding sites is significantly less than for 5-$HT_1$ binding sites. The failure of serotonin-uptake inhibitors or serotonin-synthesis inhibitors to block the effect of **13** on corticosterone elevation suggests that the compound exerts its effects through direct stimulation of serotonin receptors.

## Experimental Section

**Synthesis.** Elemental analysis are indicated only by symbols of the elements after the empirical formula and are within 0.4% of the theoretical values. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Mass spectra were determined on a Varian MAT731 spectrometer and were consistent with the assigned structures. Optical rotations were taken on a Perkin-Elmer 241 polarimeter at 28 °C in pyridine with a 5-cm path length at the wavelength specified. HPLC separations were performed on a Waters PrepLC/500A using PrepPAK-500 silica gel cartridges with the solvents specified. TLC was carried out on Merck F254 silica gel plates. All reactions, exclusive of extraction procedures, were conducted under a $N_2$ atmosphere. NMR measurements were made in $CDCl_3$ with a QE300, WM250, or WM270 instrument. Spectral data were consistent with the assigned structures. The ergolines **2**,[56] **3**,[57] **4**,[57] **6**,[58] **7**,[59] **8**,[60] **9**,[61] **10**,[62] **11**,[61] and **12**[63] were obtained as described in the references cited.

**Reduction of Ergolines with Triethylsilane. (3$\beta$)-2,3-Dihydrolysergine (13).** A mixture of 25 mL of trifluoroacetic acid (TFA) and 6.81 g (58.6 mmol) of triethylsilane was vigorously stirred as 3.38 g (14.2 mmol) of lysergine was added in portions. The mildly exothermic reaction was stirred at ambient temperture for 16 h. The solvent was evaporated in vacuo and the residue dissolved in water and extracted with ether. The aqueous portion was made basic (5 N NaOH, pH > 10) and extracted with ether (3 × 200 mL). The combined organic extracts were dried ($Na_2SO_4$) and evaporated in vacuo to give a solid residue. The desired material (2.36 g) was separated by HPLC with an 8 L gradient beginning with hexane/EtOAc (1:1) and going to EtOAc/$NH_4OH$ (99:1). Recrystallization of this material (EtOAc/hexane) gave 1.66 g of **13** (49%), mp 199–201 °C dec, $[\alpha]_{589}$ +18°, $[\alpha]_{365}$ +239°. Anal. ($C_{16}H_{20}N_2$) C, H, N.

**(3$\beta$)-2,3-Dihydroisolysergine (14).** A mixture of 12 mL of TFA, 3.27 g (28.1 mmol) of triethylsilane, and 1.62 g (6.8 mmol) of **4** on stirring overnight gave 0.4 g of **14** (24%) after HPLC (see **13**) and recrystallization from pentane, mp 85–87 °C, $[\alpha]_{589}$ +126°, $[\alpha]_{365}$ +25°. Anal ($C_{16}H_{20}N_2$) C, H, N.

**(3$\beta$)-2,3-Dihydrolysergol (15).** A mixture of 7 mL of TFA, 1.78 g (15.28 mmol) of triethylsilane, and 0.508 g (2.0 mmol) of **6** was stirred 3 h and gave 0.13 g of **15** (25%) after column chromatography (50 g of silica gel, $CHCl_3$/MeOH/$NH_4OH$, 8:2:0.5), mp 177–178.5 °C. Anal. ($C_{16}H_{20}N_2O$) C, H, N.

**(3$\beta$)-2,3-Dihydrolysergene (16).** A mixture of 15 mL of TFA, 2.4 g (20.7 mmol) of triethylsilane, and 1.18 g (5.0 mmol) of **7** provided 0.34 g of **16** (28%) after HPLC (see **13**) and recrystallization from $Et_2O$/pentane, mp 153–155 °C. Anal. ($C_{16}H_{18}N_2 \cdot 0.25\ H_2O$) C, H, N.

**(3$\beta$,5$\beta$,8$\beta$)-9,10-Didehydro-2,3-dihydro-6-methyl-8-[(methylthio)methyl]ergoline (17).** A mixture of 20 mL of TFA, 5.24 g (45.1 mmol) of triethylsilane, and 3.1 g (10.9 mmol) of **9** on stirring 5 h gave 1.34 g of **17** (42%) after HPLC (see **13**) and recrystallization from $Et_2O$/hexane, mp 93–97 °C. Anal. ($C_{17}H_{22}N_2S \cdot 0.25\ H_2O$) C, H, N.

**(3$\beta$,5$\beta$,8$\beta$)-9,10-Didehydro-2,3-dihydro-6-methylergoline-8-acetonitrile (18).** A mixture of 10 mL of TFA, 1.82 g (15.7 mmol) of triethylsilane, and 1.0 g (3.8 mmol) of **10** on stirring overnight gave 0.37 g of **18** (37%) after recrystallization from EtOAc/$Et_2O$, mp 180–182 °C, $[\alpha]_{589}$ +14°. Anal. ($C_{17}H_{19}N_3$) C, H, N.

**(3$\beta$,5$\beta$,8$\beta$)-9,10-Didehydro-2,3-dihydro-6-methyl-8-[(phenylthio)methyl]ergoline (19).** A mixture of 12 mL of TFA, 3.0 g (26.1 mmol) of triethylsilane, and 2.32 g (6.7 mmol) of **11** on stirring overnight provided 0.81 g of **19** (35%) after HPLC (5% MeOH/$CH_2Cl_2$), mp 54–58 °C. Anal. ($C_{22}H_{24}N_2S$) C, H, N.

**(3$\beta$,5$\beta$,8$\beta$)-9,10-Didehydro-2,3-dihydro-6-methyl-8-[(2-pyridylthio)methyl]ergoline (20).** A mixture of 12 mL of TFA, 2.9 g (25.0 mmol) of triethylsilane, and 2.23 g (6.4 mmol) of **12** on stirring overnight gave 0.85 g of **20** (38%) after HPLC (5% MeOH/$CHCl_3$/0.5% $NH_4OH$) and recrystallization from $Et_2O$/hexane, mp 68–71 °C. Anal. ($C_{21}H_{23}N_3S$) C, H, N.

**Methyl (3$\beta$)-2,3-Dihydrolysergate (21).** A mixture of 13 mL of TFA, 3.4 g (29.3 mmol) of triethylsilane, and 2.0 g (7.1 mmol) of methyl lysergate on stirring overnight gave 1.3 g of **21** (66%) after recrystallization from $Et_2O$/hexane, mp 80–85 °C. Anal. ($C_{17}H_{20}N_2O_2$) C, H, N.

**(3$\beta$)-2,3-Dihydrofestuclavine (22).** A mixture of 18 mL of TFA, 4.8 g (41.0 mmol) of triethylsilane, and 2.4 g (10 mmol) of **2** on stirring overnight gave 0.72 g of **22** (30%) after recrystallization from $Et_2O$/hexane, mp 173–175.5 °C. Anal. ($C_{16}H_{22}N_2$) C, H, N.

**(3$\beta$)-2,3-Dihydroagroclavine (23).** A mixture of 15 mL of TFA, 4.5 g (38.8 mmol) of triethylsilane, and 2.0 g (8.4 mmol) of **5** on stirring 4 h gave 0.95 g of **23** (47%) after recrystallization from $Et_2O$, mp 176–178 °C. Anal. ($C_{16}H_{20}N_2$) C, H, N.

**(5$\beta$,8$\beta$)-9,10-Didehydro-8-methylergoline (24).** A mixture of 6.96 g (29.20 mmol) of **3**, 4.65 g (43.90 mmol) of cyanogen bromide, and 88 mL of dry DMF was stirred for 71 h at ambient temperature. After evaporation of the solvent in vacuo, the residue was dissolved in 200 mL of $CHCl_3$ and washed with $H_2O$. The organics were dried ($Na_2SO_4$), and the solvent was evaporated in vacuo to give 6.1 g of solid. The NMR of this material was consistent with that expected for 9,10-didehydro-8-methyl-6-cyanoergoline although impurities, including **3**, were also present. A mixture of this material (6.1 g, 24 mmol), 74 mL of HOAc, 15 mL of $H_2O$, and 14.7 g (226 mmol) of Zn dust was heated to reflux for 3 h. The reaction was cooled, diluted with 75 mL of $H_2O$, and made basic ($NH_4OH$, pH >10). The mixture was extracted with $CHCl_3$ (3 × 100 mL). The organic extracts were dried ($Na_2SO_4$), and the solvent was evaporated in vacuo. HPLC (25% MeOH/$CH_2Cl_2$) of the residue gave 4.4 g of an impure fraction that was dissolved in dilute HCl and extracted with ether. The aqueous fraction was made basic ($NH_4OH$, pH >10) and extracted with $CHCl_3$ (3 × 100 mL). The organic extracts were dried ($Na_2SO_4$) and evaporated in vacuo to give 3.8 g of a solid (58%) which was one spot by TLC (MeOH/$CHCl_3$, 1:9). A 0.5-g sample of this material was treated with ethanolic HCl to precipitate the HCl salt. Digestion of the salt with acetone gave 0.44 g of the HCl salt of **24**, mp 276–278 °C. Anal. ($C_{15}H_{16}N_2 \cdot HCl$) C, H, N.

**(3$\beta$,5$\beta$,8$\beta$)-9,10-Didehydro-2,3-dihydro-8-methyl-6-propylergoline (25).** A mixture of 1.5 g (6.7 mmol) of **24**, 1.8 g (13 mmol) of $K_2CO_3$ (anhydrous), 2.2 g (13 mmol) of 1-iodopropane, and 50 mL of dry DMF was stirred for 42 h. The solvent was evaporated in vacuo and the residue digested with ether (2 × 75 mL) and filtered. The ether extracts were evaporated, and the solid residue was suspended in 15 mL of cold ether and collected by filtration to give 0.72 g (40%) of one spot material by TLC (MeOH/$CHCl_3$, 1:9). The NMR of the material was fully consistent with that expected for 6-propyllysergine. A mixture of 5 mL of TFA, 1.3 g (11.1 mmol) of triethylsilane, and 0.72 (2.7 mmol) of 6-propyllysergine on stirring 6 h followed by the usual workup gave 0.05 g of **25** (7%) after HPLC (see **13**) and recrystallization from

(56) Spilsbury, J. F.; Wilkinson, S. *J. Chem. Soc.* **1961**, 2085.
(57) Schreier, E. *Helv. Chim. Acta* **1958**, *41*, 1984.
(58) Stoll, A.; Hofmann, A.; Schlientz, W. *Helv. Chim. Acta* **1949**, *32*, 1949.
(59) Nakahara, Y.; Niwaguchi, T.; Ishii, H. *Chem. Phar. Bull.* **1977**, *25*, 1756.
(60) Semonsky, M.; Kucharczyk, N. *Collect. Czech. Chem. Commun.* **1968**, *33*, 577.
(61) Kornfeld, E. C.; Bach, N. J. U.S. Patent 3901894, 1975; *Chem. Abstr.* **1975**, *83*, 193578q.
(62) Troxler, F.; Stadler, P. A. *Helv. Chim. Acta* **1968**, *51*, 1060.
(63) Stutz, P. L.; Stadler, P. A.; Vigouret, J.-M.; Jaton, A. *J. Med. Chem.* **1978**, *21*, 754.

MeOH/pentane, mp 135–137 °C. Anal. ($C_{18}H_{24}N_2$) C, H, N.

**(3β)-2,3-Dihydro-1-methyllysergine (26).** A mixture of 0.56 g (2.3 mmol) of **13**, 0.29 g (2.9 mmol) of triethylamine, and 50 mL of toluene was stirred as 0.32 g (2.9 mmol) of ethyl chloroformate was added dropwise. After 117 h the mixture was filtered and the solvent evaporated in vacuo. HPLC (10% MeOH/$CH_2Cl_2$–25% MeOH/$CH_2Cl_2$ gradient, 4 L) of the residue gave 0.47 g (65%) of the desired carbamate. A suspension of 0.20 g (5.3 mmol) of $LiAlH_4$ in 15 mL of dry THF was cooled to −10 °C as 0.41 (1.3 mmol) of the carbamate in 10 mL of dry THF was added dropwise. Cooling was removed and the reaction stirred for 24 h. The reaction mixture was cooled as 0.25 mL of $H_2O$ was added dropwise followed by 0.2 mL of 5 N NaOH. The reaction mixture was heated to reflux 1.5 h and the solvent decanted. The residue was triturated with 25 mL of hot THF and the combined decanted liquids filtered and evaporated in vacuo. HPLC ($CH_2Cl_2$–10% MeOH/$CH_2Cl_2$ gradient, 8 L) of the residue gave 0.09 g of **26** (27%), mp 94 °C dec. Anal. ($C_{17}H_{22}N_2 \cdot 0.25\ H_2O$) C, H; *m/e* calcd 254.1783, found 254.1808.

**Binding Studies. 5-HT$_1$ and 5-HT$_2$ Binding Sites.** Male Wistar rats, 140–160 g, were used as the source of brain tissue. The rat brain frontal cortex was dissected, homogenized, and prepared according to the method described by Nelson et al.[64] The pellet from frontal cortex homogenates was suspended in 0.05M Tris buffer containing 10 μM pargyline and 4 mM $Ca^{2+}$ to give a final protein concentration of 250–350 μg/sample. The effect of compounds on [$^3$H]-5-HT binding (5-HT$_1$ site) was measured as described by Bennett and Snyder using 2 nM [$^3$H]-5-HT.[65] Nonspecific binding was measured with 10 μM 5-HT. The method of Peroutka and Snyder was used for determining binding to 5-HT$_2$ sites with 0.7–0.9 nM [$^3$H]spiperone as the radioactive ligand and 1μM LSD for nonspecific binding.[6]

Specific binding was determined as the difference between total binding and nonspecific binding. Eleven concentrations of the test compound were used between 0.1 and 10000 nM. All samples were run in duplicate. By use of the ALLFIT curve-fitting computer program of Munson and Rodbard, $IC_{50}$ values were determined as the amount of test compound that reduced the specific binding of the radioactive ligand by 50%.[66] For 5-HT, the $IC_{50}$ at 5-HT$_1$ and 5-HT$_2$ binding sites was 4 and 5000 nM, respectively, and for spiperone, the $IC_{50}$ at 5-HT$_1$ and 5-HT$_2$ sites was 400 and 1.0 nM, respectively. Radioactive ligands were obtained from New England Nuclear, Boston, MA.

**α-Adrenergic Binding Sites.** Receptor membranes were prepared from fresh frontal cortex of adult male Sprague–Dawley rats. Aliquots containing approximately 1 mg of protein were used to determine the effect of **13** on [$^3$H]rauwolscine binding ($\alpha_2$ site) as described by Ruffolo et al., using 0.5 nM [$^3$H]rauwolscine.[26] Nonspecific binding was measured with 10 μM yohimbine. Similarly, the effect of **13** on [$^3$H]prazosin binding ($\alpha_1$ site) was measured with 0.5 nM [$^3$H]prazosin and 10 μM unlabeled prazosin was used to define nonspecific binding. Radioactive ligands were obtained from New England Nuclear, Boston, MA.

Specific binding was determined as the difference between total binding and nonspecific binding. Displacement of the radioligand from specific binding sites was quantified by expressing binding as percentage of the specific binding that occurred in the absence of **13**. Log–probit analysis was used to linearize plots and to obtain an estimate of the $IC_{50}$. The $IC_{50}$ values were determined from a minimum of four experiments. For UK-14,304, pergolide, and prazosin, the $IC_{50}$'s at $\alpha_2$-binding sites were 65, 30, and 140 nM, respectively, and the $IC_{50}$'s at $\alpha_1$-binding sites were 14000, 8200, and 0.6 nM, respectively.

**Neurochemical Studies and Serum Corticosterone Determinations.** Male Wistar rats (HSD/[WI]BR) weighing about 150 g were kept in a temperature-controlled (24 °C) and light-controlled (lights on 7 a.m. to 7 p.m.) room with food and water freely available. Compounds were dissolved in dilute hydrochloric acid. After drugs were injected, rats were decapitated at the times specified in the tables and their brains were quickly excised. Whole brains or dissected brain regions were frozen on dry ice and stored at −15 °C before analysis. Serotonin, 5-HTP, 5-HIAA, dopamine, DOPAC, and HVA were determined by liquid chromatography with electrochemical detection.[67,68] Corticosterone in serum was determined spectrofluorometrically by the method of Solem and Brinck-Johnsen.[69]

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**Supplementary Material Available:** NMR chemical shift data for compounds **13**, **14**, **17**, **18**, **20**, and **21** (1 page). Ordering information is given on any current masthead page.

(64) Nelson, D. L.; Herbet, A.; Bourgoin, S.; Glowinski, J.; Hamon, M. *Mol. Pharmacol.* **1978**, *14*, 983.
(65) Bennett, J. P.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 373.
(66) Munson, P. J.; Robard, D. *Anal. Biochem.* **1980**, *107*, 220.
(67) Fuller, R. W.; Perry, K. W. *Biochem. Pharmacol.* **1977**, *26*, 2087.
(68) Perry. K. W.; Fuller, R. W. *Soc. Neurosci. Abstr.* **1979**, *5*, 348.
(69) Solem, J. H.; Brinck-Johnsen, T. *Scand. J. Clin. Lab. Invest.* **1965**, *17* (Suppl. 80), 1.